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FLORAL SCENT AND POLLINATORS OF THE HOLOPARASITE *PILOSTYLES THURBERI* (APODANTHACEAE)

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Abstract—Floral scent is likely important to the pollination of parasitic plants, despite that it has not been well-studied. We studied the pollination ecology of the North American stem holoparasite *Pilostyles thurberi* (Apodanthaceae) at two field sites in Texas. To identify effective pollinators, we collected floral visitors to *P. thurberi* flowers, observed their foraging behavior, and looked for *P. thurberi* pollen on their bodies. *Augochloropsis metallica* bees (Halictidae) and eumenine potter wasps (Vespidae) were pollinators. *P. thurberi* flowers are visually inconspicuous but produce a strong fruity fragrance. GC/MS analysis of whole floral extracts and dynamic headspace samples revealed the fragrance to be an unusually simple bouquet of raspberry ketone and several eugenols. Comparison of scent profiles to those from uninfected host plants (*Dalea formosa*) allowed putative separation of parasite and host volatiles. This is the first report of the constituents of floral fragrance in Apodanthaceae.

Keywords: Floral scent, dioecy, endoparasite, bee pollination, raspberry ketone, eugenol

INTRODUCTION

Darwin (1859) coined the term "tangled bank" to refer to the intricate networks present among flowering plants and other species in their communities, interactions that include both mutualists and antagonists. Owing to their direct physical connection to another plant, parasitic angiosperms present additional life history complexities. Recently, reproductive traits were reviewed across all parasitic plants (Bellot & Renner 2013) and two conclusions emerged: about 10% of parasitic plants are dioecious (higher than the angiosperm average of 6%; Renner and Ricklefs 1995) and the vast majority of parasitic plants are pollinated by animals. Across the 12 clades of parasitic plants the most frequent pollinating agents are insects such as beetles, butterflies, bees, wasps, and flies.

This paper addresses pollinators and floral scent in the holoparasite *Pilostyles* Guillemin (Apodanthaceae), a genus of ca. 14 species with widely disjunct distributions from North to South America, western Asia, and Australia. If one includes *Berlinianche* within *Pilostyles*, the distribution also includes tropical Africa. The holoparasites of Apodanthaceae were formerly included within Rafflesiaceae, which was shown via molecular phylogenetic analyses to be a polyphyletic assemblage of parasites belonging to four different orders (Nickrent et al. 2004). Apodanthaceae was first shown to be a member of Cucurbitales by Nickrent et al. (2004), later confirmed by Filipowicz and Renner (2010). Apodanthaceae are dioecious (and some possibly monoecious) endoparasites whose only externally visible parts are their unisexual flowers.

Flower visitors have been recorded for several species in Apodanthaceae, although in many instances, the efficacy of these as pollinators has not been determined. *Trigona* spp. (stingless honeybees) have been recorded visiting flowers of the neotropical genus *Apodanthes* in Costa Rica (Gomez 1983). In Panama, Croat (1978) reported bees, small butterflies and even mosquitoes visiting *Apodanthes caseariae*. *Pilostyles hamiltonii* in Australia is possibly wasp pollinated (Dell & Burbidge 1981) whereas in the South American *P. berteroi* unidentified solitary bees are reported to be pollinators (Kummerow 1962). Recently, Bellot & Renner (2013) recorded floral visitors on flowers of *Pilostyles haussknechtii* in Iran and *P.* [*Berlinianche*] *aethiopica* in Zimbabwe. In addition to ants, which are unlikely pollinators, calliphorid flies visited *P. aethiopica* and ulidiid flies visited *P. haussknechtii*; the fly taxa were determined to be effective pollinators based on their foraging behavior and pollen carriage.

Despite this diversity of pollinators documented for *Pilostyles* spp., the pollination relationships of these parasitic plants may be constrained by the limited visual cues produced by the plant: the flowers are small (< 2 mm) and inconspicuous due to their placement on interior branches of the host plant. We hypothesize that, for plants such as these, chemical cues may be especially important in attracting pollinators. At the same time, floral chemistry in stem parasites may be constrained by a number of factors related to their parasitic lifestyle, such as influences of host chemistry and overall anatomical and morphological reductions (Kuijt 1969).

Floral scents are chemically complex cues that are involved in pollination (Vainstein et al. 2001, Raguso

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2008), and major contributions to pollination ecology have been made with the analysis of floral fragrances using techniques such as dynamic headspace sampling combined with gas chromatography and mass spectrometry (Raguso & Pellmyr 1998, Tholl & Röse 2006). To date, only subjective descriptions (i.e. by the human nose) have been recorded for members of Apodanthaceae, despite that floral scent may be of special importance in these plants. Here we report observations and identities of floral visitors, assessment of their potential as pollinators, and analysis of floral scent chemistry of *P. thurberi* Gray, a parasite of *Dalea* and *Psorothamnus* species in the southwestern United States.

MATERIALS AND METHODS

Study sites

We conducted field studies June 23-26, 2007 at two populations in north central Texas: one near Post in Garza county, and one near Benjamin in Knox county. At these sites, the host plant of *Pilostyles thurberi* is *Dalea formosa* Torr. Both locations were semi-arid rocky escarpments with a plant community dominated by *Juniperus ashei*, *Chrysothamnus nauseosus*, *Prosopis glandulosa*, and *D. formosa*. *P. thurberi* flowers in early summer at these sites, and its flowering does not overlap with that of its host. The Post site was at or near peak flower (most infected plants with ~10-20 open *P. thurberi* flowers), while the Benjamin site was earlier in relative phenology (about half the infected plants had some open *P. thurberi* flowers while the remainder had unopened buds). Population size is difficult to determine precisely because endophytes may be present in host plants but not visible prior to flowering. Based on hosts with visible *P. thurberi* flowers/buds, we estimate that at least 100 infected host plants were present at both sites.

Assessment of floral visitors

We observed and collected floral visitors throughout the day for 1-2 sunny days at each site. During these days, visitor observations and collections were conducted between the hours of 9:00 and 16:00 by 1-3 observers. Observations filled all available time within these hours except the times required to start/stop the scent collections. Observations consisted of at least one person walking slowly among infected host plants looking for visitors, and one person sitting near an infected host plant for ~15-30 minute intervals watching carefully for visitors to *P. thurberi* flowers. To discriminate between pollinators and non-pollinating visitors, the behavior and constancy of insects were noted in the field. Collected insects were examined for placement of *P. thurberi* pollen on the body. These slides were compared to voucher slides of *P. thurberi* pollen. Floral visitors were

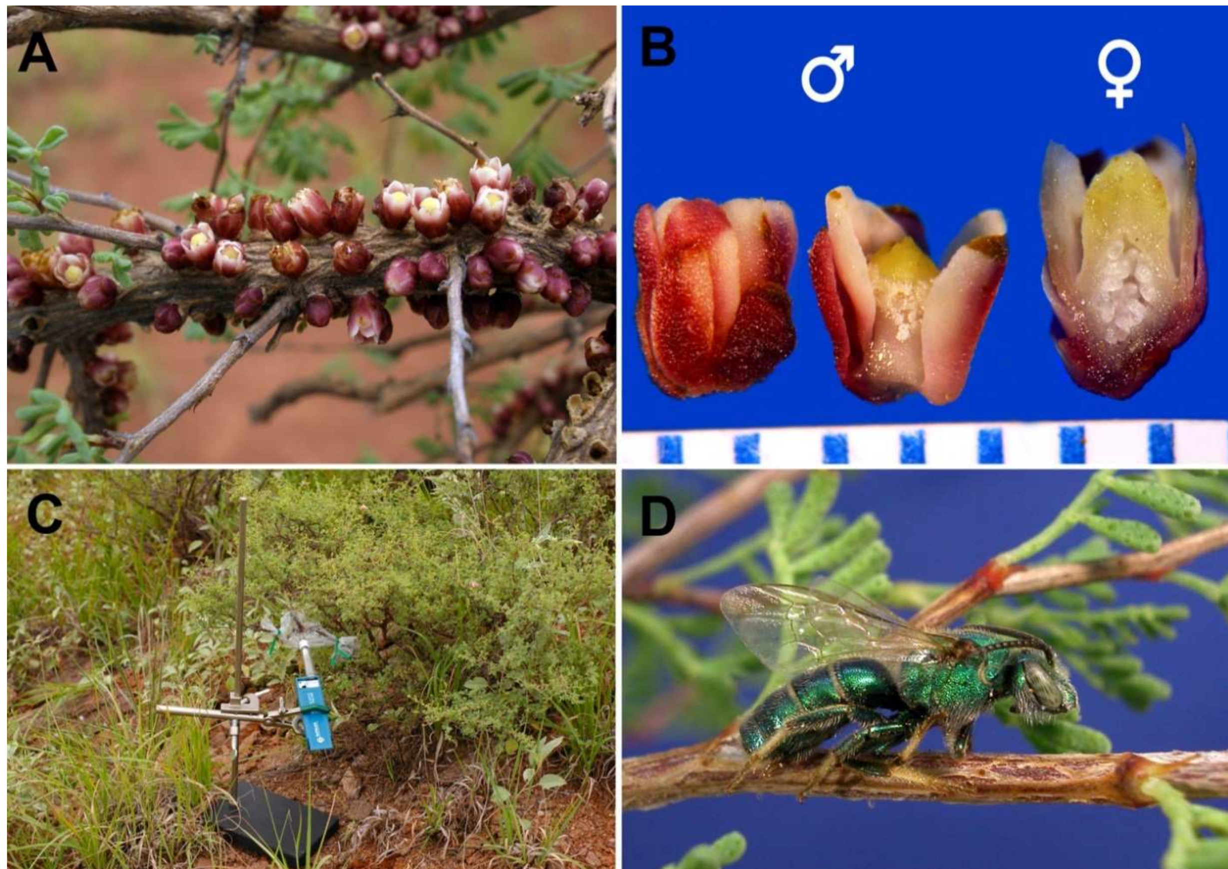

FIGURE 1. A) Male flowers of *Pilostyles thurberi* emerging from the stem of *Dalea formosa*. B) Male flower intact (left), flower with petals removed to show central column with anthers dehiscing pollen (center), and female flower in longitudinal section showing ovules (right). C) Dynamic headspace sampling apparatus. D) A male *Augochloropsis metallica* bee (dead) posed on a *Dalea formosa* stem.

identified by SDS and deposited in the Southern Illinois Pollinating Insect Collection, Department of Plant Biology, SIUC.

Analysis of floral scent

Dynamic headspace sampling was used to sample floral scents at the Post and Benjamin study sites, following established protocols for collection of volatiles (Raguso & Pellmyr 1998). We collected scent samples from six male and four female flower clusters, each on a different host individual. Flower clusters were bagged using Reynolds oven bags (a clean, inexpensive plastic that emits minimal volatiles) cut to size and formed into tubes using a food sealer. These tubes were slipped over infected *D. formosa* stems with *P. thurberi* flower clusters and sealed on each end with twist ties (Fig.1C). Leaves of the host *D. formosa* were excluded (trimming when necessary) from the bag to minimize the amount of host volatiles collected. Volatiles were collected for 1 hr using Supelco Personal Air Samplers (PAS-500) calibrated to 200 ml/min flow rate, and scent traps made from 10 mg Super Q adsorbent (Alltech Associates, Deerfield, IL) placed in glass pipettes and sandwiched between small balls of glass wool. Samples were eluted with 300 µl of hexane in the field and stored on ice until they were transported back to SIUC, where they were stored at -80°C until analysis. To help distinguish volatiles produced by *P. thurberi* from those of its host, or other environmental contaminants, we collected two ambient samples as well as scents from leaves of five putatively uninfected *D. formosa* individuals. A *D. formosa* individual was considered putatively uninfected if it had neither *P. thurberi* flowers nor flower scars on its basal stems from previous years' flowering. The flowering time of *D. formosa* does not overlap with that of *P. thurberi*, so we needed only to sample vegetative headspace from the former as a control. We collected and analyzed headspace scent from a total of 10 stems with *P. thurberi* flowers (6 with male flowers and 4 stems with female flowers), 2 uninfected *D. formosa* individuals, and 2 ambient controls.

In addition to the head space samples, whole flower extracts were also analyzed by gas chromatography/mass spectrometry (GC/MS). The whole flower extracts were obtained by sonicating 100 mg of air dried flowers (which were still strongly scented), with 1.0 ml of *n*-hexane for 5 minutes. The extract was analyzed by GC/MS.

In the lab, both the whole flower extracts and the headspace samples were concentrated down to a volume of approximately 100 µl using nitrogen gas, and analyzed using a cylindrical ion trap MS (Saturn 2100T, Varian) equipped with a 3900 GC. 1.0 to 3.0 µl of each scent extract was injected and analyzed using a method adapted from Adams (2007). Samples were injected in splitless mode with an injector temperature of 220°C. The extracts were separated using a capillary column (5% phenyl 95% dimethylpolysiloxane, VF-5MS, Varian Factor Four, 30 m x 0.25 mm x 0.25 µm) at a flow rate of 1.0 ml/min using helium (Airgas Mid America, 99.999%) as carrier gas. The initial oven temperature was 60°C, and the temperature was ramped at a rate of 3°C /min to a final temperature of 246°C, giving a total analysis time of 62 minutes. The analytes were detected in the electron ionization (70 eV) mode scanning from 10-650 m/z. The temperature parameters for the mass spectrometer were as follows: trap temperature: 170°C, manifold temperature: 40°C and transfer line temperature: 240°C. The GCMS data were collected and processed using the Varian Workstation software (ver. 6.9).

To identify peaks originating from *P. thurberi* floral scents, the resulting chromatograms of the extracts were compared to chromatograms of *n*-hexane solvent blanks, ambient samples collected from the field sites, and headspace samples from vegetative branches of uninfected *D. formosa*. Only peaks with a signal-to-noise ratio greater than 3 were considered, and selective ion monitoring was used to reduce the background noise level. The analytes were identified by retention time and mass spectrum in comparison to authentic standards. Because we replicated the analytical conditions of Adams (2007), we used Adams's (2007) retention times and an NIST05 library search of mass spectra when authentic standards were not available.

Several polar compounds that were prominently present in the whole flower extracts (eugenols and raspberry ketone) were not detected in the headspace samples. To confirm the presence of these, we split some of the samples that had sufficient amount left after analysis (1 whole flower extract, 3 headspace samples from male flowers, 3 headspace samples from female flowers, and 2 samples from uninfected *D. formosa*) and derivatized the second fraction in an attempt to improve detection of these polar compounds. Derivatization is routinely used to improve GC/MS detection of alcohols (e.g. Casaña-Giner et al. 2003). The second fraction was treated with N,O-*bis*(trimethylsilyl)trifluoroacetamide (BSTFA) which replaces the –OH moiety with $–OSi(Me)_3$. This derivatization reaction converts phenol and alcohol groups to trimethylsilyl ethers and improves the peak efficiencies and the detection limits of the analytes with an –OH moiety. To derivatize the extracts, we added 125 µl of the pre-dried extract (using anhydrous Na_2SO_4, Sigma) to 100 µl of BSTFA in 1% trimethylchlorosilane (Thermo Pierce) and 15 µl of pyridine (99%, ACS grade, ACROS). The solution was mixed and incubated at 65 °C for 2 hours and analyzed within 24 hours after derivatization. The chromatograms for the silylated headspace and whole flower *P. thurberi* samples were compared to silylated *n*-hexane blanks for identification, as well as a silylated authentic standard of raspberry ketone (Sigma).

RESULTS

Assessment of floral visitors

Both male and female *P. thurberi* flowers were visited by male *Augochloropsis metallica* bees (Halictidae) (Fig. 1D) as well as several unidentified taxa of potter wasps (Eumeninae: Vespidae). We collected four *A. metallica* and two potter wasps at Post, and one *A. metallica* and three potter wasps at Benjamin; however, more individuals were observed than were collected. *A. metallica* especially was difficult to collect

TABLE 1. Floral scent compounds detected in *P. thurberi* whole flower extract. Compounds were identified by comparison to authentic standards.

Retention time, minutes	Chemical Species
21.85	Eugenol
27.93	Methyl isoeugenol
30.10	Raspberry ketone

with nets, given the location of the *P. thurberi* flowers within the host plant branches. The individuals collected were taken using a double armed glass flask with tubing attached to both arms; one tube was used to aspirate the pollinators into the flask. These taxa were observed at both sites collecting nectar from flowers of both sexes and appeared to be foraging with constancy on *P. thurberi* (i.e. visiting numerous *P. thurberi* flowers sequentially). Small amounts of *P. thurberi* pollen were carried on the ventral surface of their bodies, which would potentially contact the stigmatic surface of female flowers as the bees crawled across the flower clusters. Therefore, both taxa are probably serving as pollinators of *P. thurberi*. We did not observe these visitors go to flowers of any other plant species, and we did not detect pollen of other species in the samples taken from these visitors.

Analysis of floral scent

Both fresh and dried flowers of *P. thurberi* had a strong fruity scent similar to raspberries. Constituents of floral scent in *P. thurberi* detected by GC/MS included raspberry ketone (4-(4-hydroxyphenyl)-2-butanone), eugenol, methyl isoeugenol, and isoeugenol isomers; these were all found from both male and female flowers (Tables 1-2, Figs. 2-4). Raspberry ketone and the eugenols were detected by GC/MS analysis in whole-flower extraction but not in the underivatized headspace samples.

Derivatization greatly improved detection of the eugenols in the headspace and whole flower samples. One peak (RT 25.5 min.) was found in each scent trap extract and *Pilostyles* extract. A NIST database search showed a good match to isoeugenol trimethylsilyl ether. However, isoeugenol has two isomers (*cis-* and *trans-*) as well as a structural isomer (eugenol) and two other peaks (27.5 min. and 29.5 min.) with similar mass spectra were observed in some of the other scent trap extracts. Pure authentic standards of isolated eugenol isomers (i.e. eugenol, *cis*-isoeugenol, and *trans*-isoeugenol) were not available, so we were not able to determine by comparison which isomer corresponded to each peak. However, based on the known relative order of retention times of silylated eugenol isomers (Saitta et al. 2009), it is most likely that the first peak is eugenol, the second peak is *cis*-isoeugenol and the third peak is *trans*-isoeugenol.

The trimethylsilyl ether of raspberry ketone, identified by comparison of the retention time and mass spectrum to the derivatized authentic standard, was detected in the whole flower derivatized sample (Table 2, Figs. 3-4), but not in the derivatized (or underivatized) headspace samples. Headspace sampled in the lab from dried whole flowers also lacked raspberry ketone, suggesting that this compound was present in *P. thurberi* flowers but either not captured by or not eluted from our scent traps.

Derivatized samples also contained a peak (RT 36.7 minutes) putatively identified via NIST database match as hydrocinnamic acid *p*-(trimethylsiloxy)-, trimethylsilyl ester, which was not detected in any of the underivatized samples. We did not detect any eugenol-related compounds, raspberry ketone, or hydrocinnamic acid in uninfected *D. formosa* headspace samples, suggesting that these compounds do indeed originated from *P. thurberi*.

Headspace samples also contained a suite of other compounds, including numerous terpenoids (Table 3). Limonene and α-pinene were the most common monoterpenes and these were found in each of the extracts that contained monoterpenes. Camphene, α-thujene, β-pinene, and a monoterpene ester, bornyl acetate, were found in nearly all of the headspace samples of *P. thurberi* and uninfected *D. formosa*. Additionally, 22 sesquiterpenoid peaks were found in the 20-35 minute time frame by their characteristic fragment ions (m/z 105, 161), and molecular weight (204). Authentic standards for all potential NIST05 matches in this time range were not available to confirm their identity. However, we were able to assign putative IDs to these by comparing NIST05 matches, Adams's (2007) retention times, and a quantitative list of compounds found in *Dalea formosa* by Lucero et al. (2005). The monoterpene and sesquiterpene peaks were found in the uninfected *D. formosa* headspace samples as well as some of the *P. thurberi*

TABLE 2. Compounds found in derivatized (silylated) headspace samples and whole flower extract from *P. thurberi*. Letters in parentheses correspond to labeled peaks and mass spectra in Figs. 3 and 4. None of these compounds were detected in the uninfected *D. formosa* vegetative samples. [1]Putative identification by comparison to retention times in Saitta et al 2009. [2]Identification by comparison to authentic standard. [3]Putative identification using NIST05.

Retention Time, minutes	Chemical Species	# Samples Detected
17.6	Unidentified compound MW 192 (A)	3 male headspace, whole flower extract
25.5	Eugenol trimethylsilyl ether[1] (B)	3 male & 3 female headspace, whole flower extract
27.5	*cis*-Isoeugenol trimethylsilyl ether[1] (C)	1 male headspace
29.5	*trans*-Isoeugenol trimethylsilyl ether[1] (D)	2 male & 3 female headspace
31.32	Raspberry ketone trimethylsilyl ether[2] (E)	Whole flower extract only
36.65	Hydrocinnamic acid, *p*-(trimethylsiloxy)-, trimethylsilyl ester[3] (F)	Whole flower extract only

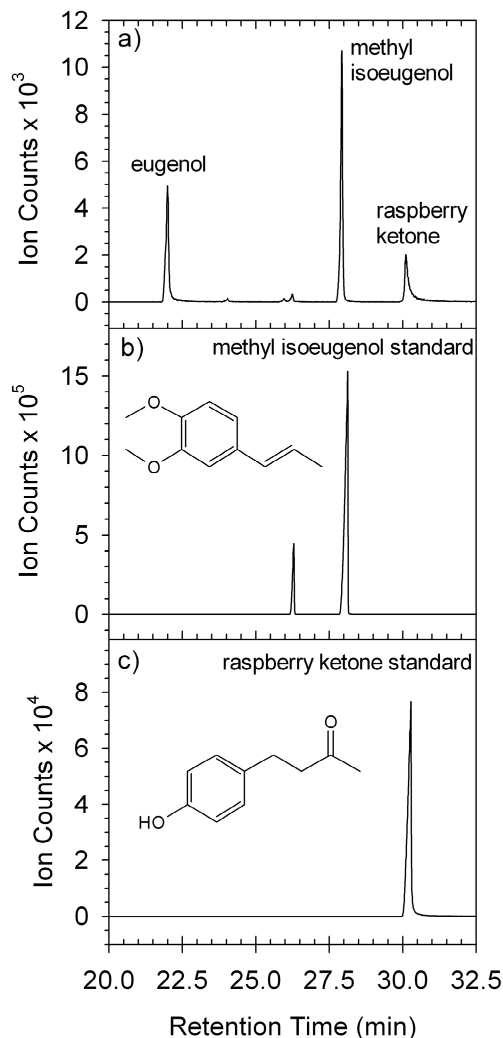

FIGURE 2. Gas chromatogram of a) underivatized whole flower extract of P. thurberi, b) methyl isoeugenol standard, and c) raspberry ketone standard. Chromatogram is filtered to show only 164 and 178 ions in order to remove several artifactual peaks arising from siloxane contaminants.

headspace samples, but were not detected in the *P. thurberi* whole flower extracts.

DISCUSSION

The foraging behavior of both *Augochloropsis metallica* and the eumenine wasps, along with the placement of pollen on their bodies suggests that these visitors are effective pollinators of *P. thurberi*. Additionally, both were foraging with at least temporary constancy on *P. thurberi* during our observation times. Most eumenine wasps are predators of lepidopteran larvae (O'Neill 2001). The eumenines at our study sites appeared to be visiting *P. thurberi* opportunistically while searching the host plant *D. formosa* for prey. If the wasps' preferred prey are host-specific herbivores of *Dalea*, the wasps may be consistent visitors to *P. thurberi* simply because these flowers are convenient nectar sources in close vicinity to their prey. This multi-trophic interaction is worthy of more study: if *P. thurberi* flowers attract predators of *D. formosa* herbivores, infected host plants could actually experience increased fitness, much like plants that attract ant guards by offering extrafloral nectaries (Beattie & Hughes 2002). Under such a scenario, the parasite could potentially function either as an antagonist, commensalist, or mutualist of its host plant, depending on the abundance of herbivores and herbivore predators in the community.

Augochloropsis metallica a polylectic bee, i.e. females collect pollen provisions for larvae from many plant species (Hurd 1979). Because we did not observe or collect any foraging *A. metallica* females, we do not know if *P. thurberi* is used as a pollen host by this bee species. The male bees we observed were collecting nectar. However, even though male bees do not collect pollen for nest provisioning, they are often as good or better pollinators than female bees (e.g. McIntosh 2005). Female bees groom their bodies during foraging, moving pollen from various body parts to the hind leg or abdominal scopae for transport back to the nest or

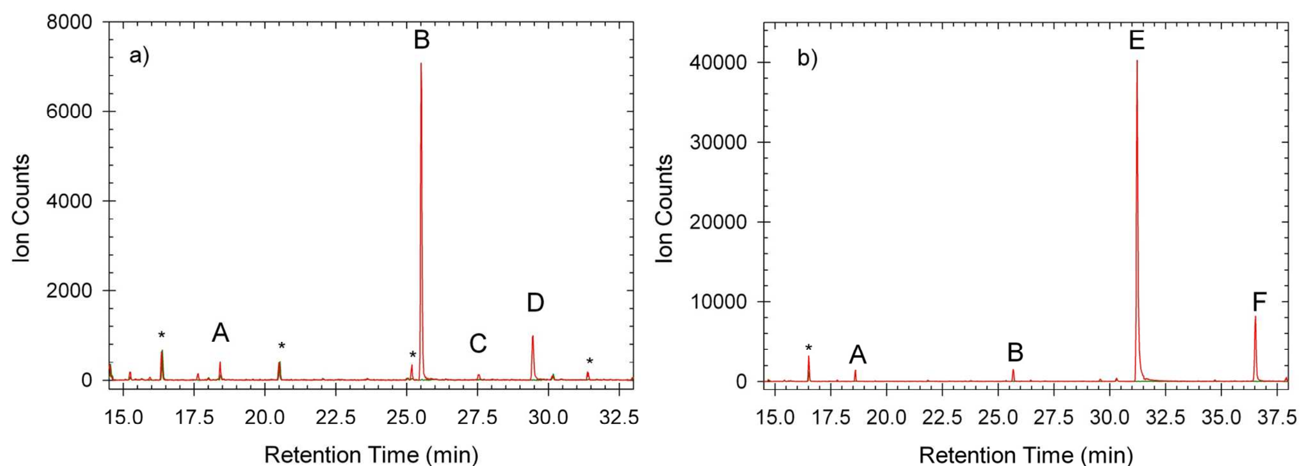

FIGURE 3. Chromatograms from derivatized samples of a) headspace sample from male *P. thurberi*) and whole flower *P. thurberi*, showing ions m/z 179 and 236. Letters correspond to putative identifications listed in Table 2. Floral samples shown in red and derivatized hexane blank in green. Peaks marked by * are contaminants due to the extraction solvent.

colony. Grooming is known to reduce pollen carryover and therefore pollinator efficacy (e.g. Barrett et al. 1994). Male bees, including those observed in this study, do not groom which may account for their high efficacy as pollinators in several studies. *A. metallica* males flew from one *Dalea* plant to the next, diving directly down towards the stems where the *P. thurberi* flowers were located, without spending much time searching.

Peak flowering in *Dalea* is April and May but some flowers can be found during June when peak flowering occurs in *Pilostyles*. It is also possible that some pollinators are active across the flowering times of both plant species. For this reason, it is possible that the two species could share pollinators. *Dalea* species are primarily bee pollinated and, attract a rich fauna of native bees (Epple 2005, Cane 2006, 2012). However, *Dalea* is not among the 75 genera of plants in North America known to be visited by *A. metallica* (Hurd 1979). Moreover, *D. formosa* flowers do not resemble those of *P. thurberi* and do not smell of raspberries or fruit (DLN and SDS, pers. obs.), so it is unlikely that host and parasite consistently share pollinators due to shared floral characteristics.

Our findings, combined with others in the literature, suggest that *Pilostyles* species are pollinated by a variety of insects, including bees, wasps, and flies. Our limited observation period leaves us with the question of how representative our data are across time. However, floral visitors described as "metallic green bees" have been observed visiting *P. thurberi* in several previous years by an amateur naturalist and landowner of the Post site (Z. Kirkpatrick, pers. comm.). *Augochloropsis metallica* is not the only metallic green bee at these field sites (we collected *Agapostemon texana* from contemporaneously-flowering Asteraceae at the Post site), but these observations at least suggest *A. metallica* may be a consistent visitor to *P. thurberi*.

The flowers of *P. thurberi* do not present visually conspicuous floral displays to attract these or other floral visitors (Fig. 1A). Flowers are tiny (< 2 mm) and often occur on stems within the interior of the host plants, where they are largely obscured from view by the host's leafy shoots. It is very likely that floral scent plays an especially important role in the pollination relationships of this plant species. However, unlike most entomophilous angiosperms that produce complex floral bouquets consisting of dozens of compounds, *P. thurberi*'s floral odors are relatively simple,

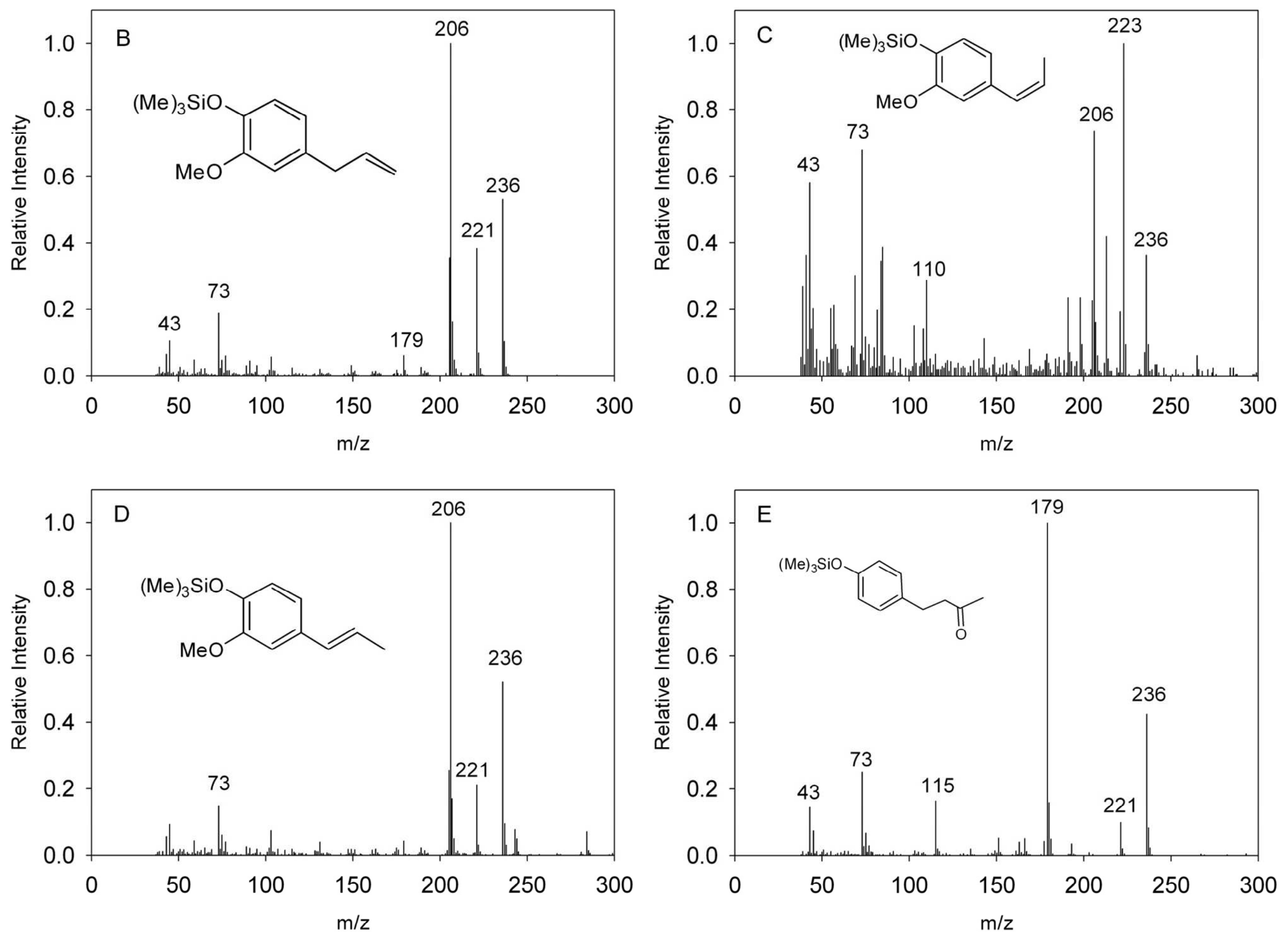

FIGURE 4. Mass spectra of selected peaks from gas chromatograms of derivatized samples; letters refer to peaks labeled in in Fig. 3. Structures of silylated derivatives are shown, where B is eugenol trimethylsilyl ether, C is *cis*-isoeugenol trimethylsilyl ether, D is *trans*-isoeugenol trimethylsilyl ether, and E is raspberry ketone trimethylsilyl ether.

TABLE 3. Compounds detected in underivatized headspace samples of both *P. thurberi* and uninfected *D. formosa*. These all are considered to be originating from *D. formosa*. Unidentified sesquiterpenes are listed with most abundant mass spectral fragments. [1]Putative identification using NIST05, Adams's (2007) retention times, and Lucero et al. (2005). [2]Identification by comparison to authentic standard.

Retention Time, minutes	Chemical Species	# Samples Detected	
		P. thurberi	*D. formosa*
5.15	α-Thujene[1]	5 (3 male & 2 female)	2
5.36	α-Pinene[2]	7 (4 male & 3 female)	2
5.83	Camphene[2]	5 (3 male & 2 female)	2
6.61	β-Pinene[2]	6 (3 male & 2 female)	1
6.83	Myrcene[1]	4 (2 male & 2 female)	1
7.44	α-Phellandarene or 3-Carene	3 (2 male & 1 female)	1
7.78	α-Terpinene[2]	-	1
8.17	Limonene[2]	7 (4 male and 3 female)	2
8.73	*trans*-Ocimene[1]	2 (2 male)	2
9.17	γ-Terpinene[2]	2 (2 male)	2
10.22	Terpinolene[2]	2 (2 male)	2
18.41	Bornyl acetate[2]	6 (4 male & 2 female)	1
19.74	m/z 136, 131, 93	3 (2 male & 1 female)	-
21.01	α-terpinyl acetate[1]	3 (2 male & 1 female)	1
21.85	Unidentified sesquiterpenoid m/z 204, 161, 133, 105	1 female	1
22.121	α-copaene[1]	3 (2 male & 1 female)	1
23.41	Unidentified sesquiterpenoid m/z 204, 189, 161,133,119,105	1 female	1
23.93	β-Caryophyllene[2]	4 (2 male & 2 female)	1
24.37	Unidentified sesquiterpenoid m/z 204, 161, 105	1 female	1
25.41	α-Humulene[2]	1 female	1
26.08	γ-Muurolene[1]	1 female	1
26.22	γ-Curcumene[1]	1 female	1
26.43	Germacrene D[1]	3 (2 male & 1 female)	1
26.91	Unidentified sesquiterpenoid m/z 204, 161, 133, 119, 105, 91	3 (2 male & 1 female)	-
27.08	Unidentified sesquiterpenoid m/z 204, 189, 161, 119, 105, 81	2 (1 male & 1 female)	1
27.18	Unidentified sesquiterpenoid m/z 204, 161, 105	1 female	1
27.34	β-Curcumene[1]	1 female	1
27.74	γ-Cadinene[1]	2 (1 male, 1 female)	1
28.00	Unidentified sesquiterpenoid m/z 204, 161, 13, 119, 105	5 (2 male, 3 female)	1
28.12	Cadina-1(2),4-diene[1]	4 (2 male, 2 female)	1
28.52	Unidentified sesquiterpenoid m/z 204, 161, 119, 105	2 (1 male, 1 female)	1
28.69	Unidentified sesquiterpenoid m/z 204, 161, 133, 105	2 (1 male, 1 female)	1
28.84	Unidentified sesquiterpenoid m/z 204, 161, 122, 107	4 (2 male, 2 female)	1
29.51	Unidentified sesquiterpenoid m/z 204, 161, 133, 121, 105	1 female	1

consisting of raspberry ketone, methyl isoeugenol, and several isomers of eugenol. All of these are known to be general insect attractants and male bee attractants (e.g. Nogueira et al. 2001). The sweet raspberry scent of *P. thurberi* flowers is quite strong to the human nose, and raspberry ketone is detectable by humans at thresholds as low as 1 ppb (Larsen & Poll 1990). The raspberry ketone fragrance remains strong from air-dried flowers, even months or years after collection. Another holoparasitic plant, *Cytinus visseri* (Cytinaceae), has an aliphatic ketone-dominated scent a combination of 3-hexanone and 1-hexen-3-one, that attracts its rodent pollinator, *Elephantulus brachyrhynchus* (Johnson et al. 2010).

Most literature accounts of raspberry ketone as a floral scent

constituent deal with the specialized relationships between certain orchids and male tephritid flies, who use both raspberry ketone and methyl isoeugenol collected from flowers as sex pheromones (Tan and Nishida 1995, 2005). Bellot and Renner (2013) did not report the sex of the calliphorid and ulidiid fly pollinators of *P. aethiopica* and *P. haussknechtii*. Although there is nothing in the literature to suggest that male halictid bees display similar chemically-mediated behaviors, similar behaviors are found in males of the distantly-related orchid bees (Euglossini) (Dodson et al. 1969). The pattern is intriguing and worthy of more detailed studies.

The terpenoids detected in the *P. thurberi* headspace samples, all of which were also detected in uninfected *D. formosa* individuals, are most likely being produced by *D. formosa*. All the terpenoids we IDed through comparison with authentic standards have been reported elsewhere in *D. formosa* leaves (Lucero et al. 2005). It seems unlikely that both host and parasite are synthesizing the exact same suite of terpenoids. Moreover, the lack of any terpenoid peaks detected in the whole flower extracts from *P. thurberi* strongly suggests they are not originating from the *P. thurberi* flowers. These compounds could have emitted from the host plant stem, which of necessity was bagged along with the *P. thurberi* flowers during headspace collection. Alternatively, these could be secondary compounds taken up from the host through the parasite's haustorial connection and then emitted by the flowers. Regardless of their origin, they may influence the pollination relationships of *P. thurberi*. Host chemistry has been shown to influence a parasitic plant's interactions with both herbivores (e.g. Marvier 1996) and pollinators (e.g. Troncoso et al. 2010). Troncoso et al (2010) showed that the mistletoe *Tristerix verticillatus* emitted a different suite of volatiles depending upon which of three host plants it parasitized, and that these odor differences affected visitation rates by insects.

We do not know the degree to which *P. thurberi*'s floral scent is different from other plants in its community, nor do we know how it compares to its congeners with different pollination systems. For example, *P. aethiopica* is pollinated by blowflies (Calliphoridae) (Bellot & Renner 2013). Blowfly pollinators typically visit flowers with scents similar to rotting meat or dung, and evolutionary shifts to and from fly pollination have been found to be accompanied by the evolution/loss of floral scents with sulphurous compounds (Shuttleworth & Johnson 2010). How such transitions might play out in a parasitic plant, whose chemical ecology likely involves complex interactions among the parasites, host plants, pollinators, and herbivores, remains a topic for future research.

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