

IMPLICATIONS OF ENEMY ESCAPE ON CHEMICALLY MEDIATED INTERACTIONS WITH MUTUALISTS: WILD PARSNIP POLLINATION IN TWO HEMISPHERES

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Abstract—When plant species invade new areas, they can escape from specialist enemies and thereby reduce investment in chemical defense. Enemy release may have other impacts on plant chemistry; in the absence of specialists, plants may be able to increase production of volatiles that enhance attractiveness to pollinators. In the United States (US), the introduced Eurasian wild parsnip, *Pastinaca sativa*, has long been subject to attack by an introduced coevolved florivore, *Depressaria pastinacella*, the parsnip webworm. In 2004, webworms were found for the first time attacking parsnips in New Zealand (NZ). Relative to US *P. sativa*, NZ *P. sativa* produces lower levels of defenses but higher levels of certain floral volatiles, suggesting that escape from its specialist florivore may have resulted in changes in volatile profiles to increase pollinator attraction.

In this study, we examined the influence of wild parsnip floral volatiles on pollinator attraction and seed production in NZ and the US. While many insect groups were observed on parsnip umbels, the percentage of flowers that set seed could significantly be predicted by the occurrence of large calyprate flies and small syrphids in both localities. In the US, β -pinene, γ -terpinene, hexyl butyrate, octyl butyrate, germacrene D and an unknown monoterpene were all positively correlated with visitation by large calyprates and small syrphids. In NZ, transocimene, carene and octyl butyrate were positively correlated with visitation. Remarkably, most compounds positively associated with visitation are produced in significantly higher proportions in NZ flowers, suggesting that NZ flowers, in the absence of specialized florivores, may be chemically better constituted to attract pollinators.

Keywords: *Coevolution, floral volatiles, florivory, plant-pollinator interactions, invasive species, pollination*

INTRODUCTION

When plant species invade new areas, they often escape from coevolved specialist enemies; escape in certain circumstances can be accompanied with reduced investment in chemical defense. This relationship in fact forms the basis of multiple hypotheses aimed at predicting the success of invasive plants (Maron & Vila 2001; Keane & Crawley 2002; Joshi & Vrieling 2005; Liu & Stiling 2006). Escape from coevolved specialists, however, may have impacts on the chemistry of plants other than reducing investment in defense. Some plants avoid herbivores by reducing the production of attractants used by adapted specialists to find host plants (Feeny 1977). Colonizing new habitats where coevolved enemies are absent may result in altered selection on volatile attractants, particularly if these chemicals mediate interactions with mutualists such as pollinators. While floral volatiles render plants attractive to beneficial pollinators, they may also make them conspicuous to detrimental specialist herbivores that have evolved the ability to

recognize host-specific volatiles (Theis 2006, Theis et al. 2007, Raguso 2008, Kessler & Baldwin 2011, Kessler et al. 2012, Theis & Adler 2012).

In the presence of coevolved specialist herbivores, plant species may experience considerable selection pressure to alter their floral profiles to reduce their apparency (Baldwin et al. 1997). A potential cost of this alteration is a reduction in pollinator attraction, especially if the same components of the floral blend are attractive to both the herbivores and pollinators. For example, although benzyl acetone is a pollinator attractant for the flowers of *Nicotiana attenuata* Torr., it is emitted at low levels because plants with elevated production are subject to greater herbivory (Kessler et al. 2008). As well, benzaldehyde and phenylacetaldehyde emitted by flowers of *Cirsium arvense* (L.) Scop. are attractive to both florivores and pollinators (Theis, 2006). Increased scent production in *Curcubita pepo* results in greater herbivore damage and reduced fitness (Theis and Adler 2012). Understanding how volatiles mediate both antagonistic and mutualistic interactions is thus a prerequisite for predicting the phytochemical and ecological consequences of enemy release.

A system in which the chemical consequences of herbivore escape for pollinator attractiveness may be

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examined is the Eurasian weed *Pastinaca sativa* L., wild parsnip, and its European florivorous specialist, *Depressaria pastinacella* Duponchel, the parsnip webworm. The defensive chemistry of this interaction has been well characterized both in its native Europe and in the introduced range in North America, where the wild parsnip has been part of the flora for over 300 years and where it has been reassociated with the parsnip webworm for more than 150 years (Bethune 1869; Riley 1888).

In 2004, webworms were discovered on wild parsnips in New Zealand (NZ), where the plants had grown free from significant herbivory since the introduction and establishment of the weed in the mid-nineteenth century (Zangerl et al. 2008). This accidental introduction provided a unique opportunity for a real-time analysis of evolution in this interaction. Extracts of parsnip buds and flowers in NZ contained lower amounts of certain chemicals, including linear furanocoumarins, octyl butyrate, and myristicin, which function in defense against webworms in the US (Berenbaum & Zangerl 1996; Carroll & Berenbaum 2002; Mao et al. 2008). NZ flower and fruit parsnip extracts also contained higher amounts of octyl acetate, an olfactory attractant for larval webworms (Carroll & Berenbaum 2002). Reduced levels of costly defensive compounds, such as the furanocoumarins, are a predictable consequence of enemy escape, but increased production of other compounds is consistent with a response to other agents of selection, such as pollinators.

Throughout its native and invasive range, *P. sativa* is thought to be "promiscuously pollinated" by a wide range of insects (Bell 1971), including, in central Illinois, dipterans in nine families (Lohman et al. 1996). Nectar is secreted by a stylopodium that is easily accessed by generalist visitors, particularly flies. Many of the volatile constituents of parsnip flowers, including octyl esters and sesquiterpenes (Borg-Karlsen et al. 1993), are attractive to saprophilic pollinating flies (Jürgens et al. 2006). Visitation by a wide variety of generalist pollinators has been suggested to contribute to the ability of parsnips and other apiaceous species to establish a broad geographic distribution and to succeed in new habitats after introduction (Bell 1971). Yet, despite an abundance of potential pollinator species, pollination success is rarely 100% in US populations. In many populations, a substantial proportion of ovules go unfertilized, as evidenced by a high frequency of parthenocarpy (normal-sized seeds lacking endosperm or embryo) (Zangerl et al. 1991). Parsnips prevent selfing by protandry, so seed set is heavily limited by visitation. Thus, pollinator visitation can impose strong selection on reproductive fitness; release from its specialist enemy in a newly invaded area may free *P. sativa* from constraints on production of volatile attractants and enhance its ability to attract pollinators.

The invasion of webworms in NZ allows us to compare floral chemical traits of wild parsnips in habitats with and without a coevolved specialist. Tradeoffs between conspicuousness to antagonists and to mutualists should be particularly striking in interactions between florivores and their hostplants. Although the interaction between parsnips and webworms has been studied in detail for over 30 years,

little information is available on the interaction between parsnips and their pollinators and how this interaction might shape floral traits. We hypothesized that volatiles are important in mediating interactions between parsnips and their pollinators and that changes in volatile chemistry accompanying range expansions may reflect differences in the composition of the pollinator community. Thus, the primary goals of this study were to: 1) evaluate how variation in floral volatiles influences pollinator visitation and seed production; and 2) determine if differences in parsnip floral chemistry associated with introduction into NZ are consistent with a role in increasing pollinator attraction. In this study, we examined the floral bouquet of wild parsnips in NZ and US and its influence on pollinator attraction and pollination success. Volatiles and floral visitors were collected, identified, and quantified from parsnips in the US and NZ, and we utilized the natural variation in the volatile profiles to determine which chemical differences among individual plants explained differences in pollination success and consequently in fitness gained through seed production.

MATERIALS AND METHODS

Experiments were conducted in June 2009 in the "Overpass" parsnip population (40.121173, -88.143400) in Champaign County, Illinois, USA, which has long hosted webworm populations (Zangerl & Berenbaum 2009) and in December 2009 in the "Cottage" population in Seacliff, Otago, New Zealand (45.678541, 170.625247), known to be infested by webworms only since 2004 (Zangerl et al. 2008). Identical methods were used in both localities to measure insect visitation, volatile production, and pollination success.

Insect visitation and volatile collection

Flowering in *P. sativa* commences with the maturation of the primary apical umbel followed by flowering in the secondary umbels, which branch out laterally from the main stalk. Insect visitation and volatile production were monitored on separate secondary umbels on the same plant. *P. sativa* is protandrous and all measurements were made when the flowers were receptive to pollen (i.e., when most of the inflorescence had matured into the female stage). For all plants in this study, we removed primary umbels to ensure that sufficient resources were allocated to the full development of secondary umbels and that fruit development was not resource-limited.

P. sativa flowers transition from male to female within the span of a few days. We secured fine nylon mesh bags over secondary umbels with male flowers that were beginning to lose their anthers and develop stigmal structures but were still unreceptive to pollen. We identified 4 to 5 plants per day that were intermediate in size and uninfested by webworms and bagged two secondary umbels per plant to prevent insect visitation. Bagged plants were checked daily for the appearance of mature female flowers (denoted by the presence of bifurcated stigmas). On maturation, bags were removed from both umbels. A camera was positioned above one of the umbels to photograph visitors and volatiles were collected from the other umbel. Photographs and volatiles

were collected from a total of 52 plants in Illinois and 32 plants in NZ.

The camera and the volatile collection apparatus were affixed to a customized aluminum pole that was inserted into the ground next to the plant. Four adjustable arms that extended from the pole held the camera, the volatile collection apparatus and two umbels in place securely. The camera (Canon Powershot S31S, 6 megapixels) was placed directly over one of the umbels and programmed to take high-resolution photographs every 5 minutes for 5 hours (100 pictures per plant). All experiments were conducted over a standardized time period within a day, 9 am to 2 pm for the US component and 10 am to 3 pm for the NZ component. A small picture-card containing printed text was placed on the surface of the umbel and used to focus manually on the flowers to obtain sufficient image resolution of floral visitors for identification. The card was removed prior to the experiment. The height of the camera over the umbel was adjusted to obtain the best resolution of the picture-card.

Volatiles were collected from the second umbel on the same plant over the same time period by dynamic headspace sampling. An inverted Erlenmeyer flask was secured around the umbel. Air passing through the flask entered via a small tube filled with activated charcoal for filtration. Air from the flask was drawn out at the rate of 0.5L/min through an adsorbent Porapak Q cartridge with the aid of a vacuum pump (Airchek XR5000, Eighty four, PA). The cartridge consisted of 150 mg of Porapak Q packed inside stainless steel tubing, 5 cm in length and 2.5 mm in diameter. Both the charcoal filter and the Porapak Q were held in place by insertion into a foam stopper that plugged the opening of the Erlenmeyer flask. The flask was covered with an aluminum sun-shield (Zangerl & Berenbaum, 2009) to keep temperature inside the flask close to ambient temperature. At the end of the 5-hour period, the umbels were re-bagged to prevent further pollination and remained enclosed for 2-3 weeks until fruits completed development.

All photographs were scored for the presence and identity of visitors. The number of visits per morphospecies was generally very low so we combined visitor groups based on taxonomy and size (Table 1). The detail in the photographs allowed us to identify certain insects to species (*Apis mellifera*, *Chauliognathus pennsylvanicus*), whereas others were identifiable to family (Diptera: Syrphidae, Bibionidae, Stratiomyidae; Hymenoptera: Vespidae, Formicidae; Coleoptera: Coccinellidae, Lampyridae, Cerambycidae), groups of families (calyptrate Diptera, acalyptrate Diptera), or order (Thysanoptera). In addition, we used the photographs to count number of flowers as an estimate of umbel size and the amount of nectar available per umbel. A glossy sheen, generally clearly visible in the photographs, indicates the presence of nectar on the flower; the last photograph for each plant (of the 100 taken) was scored for the proportion of flowers bearing nectar. *P. sativa* flowers do not age synchronously within an umbel but age centripetally. Owing to the non-synchronous maturation of flowers in an umbel, we counted the number of receptive stigmas from the photograph to measure pollination success,

the percentage of receptive flowers that had set seed. The number of receptive stigmas was obtained from the final photograph for each umbel following the protocols in Zangerl & Berenbaum (2009).

Porapak Q cartridges from the volatile apparatus were rinsed with 0.5 ml of hexane containing a 0.0001 % tridecane internal standard and the contents were eluted into 2 ml GC-vials. They were then cleaned with pentane and dried with nitrogen gas. Eluents were analyzed by gas chromatography with flame ionization detection and subjected to gas chromatography-mass spectrometry (GC-MS) to identify the volatile compounds. US samples were analyzed by GC-MS on the date of collection and NZ samples were stored in a -20° C freezer and shipped to the US for analysis. A small number of the NZ samples unavoidably volatilized in transit, leaving a total of 19 NZ samples to be analyzed for floral volatiles.

One μ L per sample was injected in the GC-MS (Shimadzu QP2010 Plus, SHRXI-5MS capillary column, 30m \times 2.5mm \times 0.25 μ) in splitless mode for 1.5 minutes with an inlet temperature of 250° C and helium as the carrier. The initial oven temperature was set at 60° C, held for 30 seconds, increased at a rate of 10° C/min until 80° C, at a rate of 30 C/min until 120° C, followed by 3° C/min until 150° C and finally at a rate of 30° C/min for 1 minute. The spectra were recorded in Single Ion Mode (SIM) and the ions detected were m/z 93, 56, 71, 161, 192. Volatile peaks from *P. sativa* flowers were identified in this study from a small sample of flowers after completing a full-scan of total ions; these have also been identified in a prior study (Zangerl and Berenbaum, 2009). We used SIM to increase the sensitivity of peak detection. We selected five ions because of their relative abundance. Peaks were identified by comparison to the NIST08 library, to Kovat indexes obtained from an alkane ladder, and to standards when available. Amounts were quantified based on peak area integrations of total ion chromatograms. Areas were adjusted with the peak area of the internal standard (tridecane). The absolute areas of each compound were converted to a proportion of the total emission. Using proportions, we were able to standardize floral emissions and compare the composition of floral volatiles between US and NZ populations.

Bagged umbels with fully developed fruit were clipped and brought to the laboratory, where all fruits were evaluated for the presence of endosperm. The parsnip fruit is a schizocarp; each of the two mericarps of an individual fruit was placed over a light source to determine whether it was "empty" or filled with endosperm (Zangerl et al. 1991). The presence of an endosperm on each side of the mericarp fruit indicates a successful pollination of the corresponding stigma on the flower.

Pollen analysis

On days when experiments were in progress, insect visitors were collected from female secondary umbels that were not photographed in this study. Insect were collected with an aspirator and placed in a 2-ml tube with 70% ethanol. Pollen counts for all captured insects were estimated

following the protocols in Zangerl & Berenbaum (2009). Vials with insects were sonicated for two minutes, insects were removed, and the remaining ethanol was centrifuged at 15,000X for 30 seconds. Five μ L of fuschin dye solution (in glycerol) were mixed with the pellet and pipetted onto a glass slide, which was then covered with a cover slip. Parsnip pollen grains were counted in three microscope fields (109 objective, field width = 1.9 mm) of an Olympus CH-2 compound microscope. Pollen counts determined if an insect visitor was carrying parsnip pollen when captured and accordingly had the potential to be a successful pollinator.

Statistical analysis

Statistical analyses were conducted in R version 2.13.0 (R Development Core Team 2011) and PASW Statistics version 18. Data sets from the US and NZ study were analyzed separately using Generalized Linear Models. Pollination success was analyzed as a binomial variable with successes measured as the number of fertilized fruit and failures as the number of receptive flowers that were not fertilized (total number of receptive flowers minus the number of fertilized fruit). Pollination success was fitted using a General Linear Model with a quasi-binomial error structure, with visitor pollinator potential (frequency of visitation*average pollen load) as the explanatory variable. We included only frequency of visitation for NZ thrips and acalyprate flies as we did not have pollen load data for these

visitor groups. Visitors categorized as “very small insects” were frequently observed but their very small size precluded both taxonomic identification and quantification of pollen load.

We used the “drop1” function in R to determine the significance of each explanatory variable in the model. The drop1 function drops each explanatory variable in turn and each time compares the difference between the partial model deviance and the full model to a Chi-square distribution (Zuur *et al.*, 2009). Based on the results, we could identify visitor groups that are most likely to be important in predicting pollination success. Insect groups with very rare visits (less than an average of 5 visits per umbel per 5 hours) were considered unlikely pollinators and thus were not included in the analysis. After calculating correlations and VIF values for all explanatory variables, we found that two groups, large flies and small syrphids, were highly correlated in the US data set (VIF: Large flies = 6.26, small syrphids = 4.68, $r = 0.83$). Because of the difficulty in separating out the individual effects of each group, we ran the model once with large flies and once with small syrphids.

Absolute peak areas for each volatile were converted to a relative proportion of the total and proportions were compared between countries using multivariate analysis of variance (MANOVA). We used a multivariate distance-

TABLE 1: Frequency of insect visitation on *Pastinaca sativa* umbels. Observations were made from 100 photographs (5 hours) for each umbel and the frequencies were averaged over all umbels. Visitation frequencies were calculated as the average number of times the insect was observed in the span of 5 hours (in 100 photographs). Pollen counts were divided by the weight of the insect to obtain a pollen count per mg measure, which was averaged over all specimens collected.

	Pollinators	Frequency of visitation/5 hours		Average pollen count per mg (US/NZ)
		US	NZ	
COLEOPTERA	<i>Chauliognathus pennsylvanicus</i>	8.94		107.0
	Cerambycidae		0.03	
	Coccinellidae	0.96	0.07	39.3/50.3
	Lampyridae	2.86		51.4
	Other Coleoptera		4.87	
DIPTERA	Acalyprate flies		36.47	
	Anthomyiidae	7.98		
	Bibionidae		465.97	96.1
	Large calyprate Diptera (Calliphoridae, Muscidae, Tachinidae, Sarcophagidae)	14.39	58.27	64.7/69.8
	Large Syrphidae	1.14	2.80	679.3/20.2
	Small Syrphidae	44.67	9.83	64.6/136.1
	Stratiomyidae		0.97	206.1
HYMENOPTERA	<i>Apis mellifera</i>	0.08		75.4
	Large Formicidae	13.69		55.2
	Small Formicidae	10.63		84.5
	Small Hymenoptera	0.41	2.03	
	Vespidae	0.18		2.9
LEPIDOPTERA	Lepidoptera		0.10	
THYSANOPTERA	Thysanoptera		671.47	
< Imm, unidentified		63.84		

based (square root transformed, Bray-Curtis distances) approach DISTLM in Permanova+ for Primer 6.1 to identify the relative contribution of individual volatiles to pollinator visitation. Absolute values, adjusted with the internal standard, were used in the Permanova+ analysis. A stepwise selection procedure based on AIC measures of fit was used to determine which volatiles in the bouquet could best predict large fly and small syrphid visitation. In US plants, cis-ocimene and trans-ocimene were highly correlated and we excluded trans-ocimene from the stepwise selection procedure. In the NZ data, isomers of alpha-farnesene were higher correlated and only (E,E) alpha-farnesene was included in the model. Individual volatiles deemed important from the DISTLM analysis were plotted against total visitation to discern the overall direction of the relationship.

We also fitted a GLM model with a negative binomial error structure to determine if visitation counts of insect groups were dependent on nectar (proportion of flowers bearing nectar) and umbel size (total number of flowers per umbel).

RESULTS

Visitation and pollination success

Pollen loads were correlated with insect size (by weight) in both the U.S. and New Zealand (US $r^2 = 0.45$, NZ $r^2 = 0.22$), but certain groups of large insects, such as honey bees and large syrphid flies, were observed too infrequently to

have contributed to pollination success in this study irrespective of pollen load size (Table 1). In both the US and NZ, wild parsnip flowers were pollinated primarily by large calyptrate and syrphid flies, but the identity of dipteran visitors varied by country (Table 1). In the US, although small syrphids were the most frequent identifiable visitors, they carried relatively small pollen loads; large calyptrate flies (Calliphoridae, Muscidae, Sarcophagidae, Tachinidae) were likely the most important pollinators as they carried relatively large pollen loads and were also frequent visitors (Table 1). In contrast with the US, wild parsnips received proportionately fewer visits from syrphids in NZ, where large calyptrate flies carried large amounts of pollen and were also frequent visitors. Despite striking geographic differences in visitation frequency, in both the US and NZ the percentage of flowers that set seed could significantly be predicted by the occurrence of large calyptrate flies and small syrphids (Table 2).

Insects with high pollen loads did not necessarily predict pollination success. In the US, soldier beetles (*C. pennsylvanicus*) carried large amounts of pollen and were also frequent visitors but their visitation was not significantly associated with pollination success (Table 2). Soldier beetles appeared to utilize the large, flat umbels of *P. sativa* as a mating site and movements between umbels were not observed. Similarly, NZ umbels were visited primarily by bibionid flies, which carried relatively large pollen loads but were not predictive of pollination success (Table 1, 2). Like

TABLE 2. Deviance (Chi-sq) tests for GLMs (quasi-binomial model) predicting pollination success by insect visitation frequency x average pollen load in US and NZ parsnips. Significant differences at $P < 0.001$ are indicated by *** and $P < 0.05$ by *

	Df	Deviance	Scaled deviance	p-value	
US: Pollination success: with large calyptrate Diptera					
Full model		4,765.60			
Anthomyiidae	1	4,786.40	0.20	0.65	
Large calyptrate Diptera	1	7,442.40	26.13	0.00	***
Small Formicidae	1	4,811.70	0.45	0.50	
Large Formicidae	1	5,071.40	2.99	0.08	.
<i>Chauliognathus pennsylvanicus</i>	1	4,852.20	0.85	0.36	
US: Pollination success: with small Syrphidae					
Full model		4,798.40			
Anthomyiidae	1	4,993.60	1.84	0.17	
Small Syrphidae	1	7,442.40	24.93	0.00	***
Small Formicidae	1	4,817.60	0.18	0.67	
Large Formicidae	1	4,982.20	1.73	0.19	
<i>Chauliognathus pennsylvanicus</i>	1	4,817.50	0.18	0.67	
NZ: Pollination success					
Full model		1561.30			
Large calyptrate Diptera	1	1957.00	5.10	0.02	*
Small Syrphidae	1	2260.30	9.02	0.00	**
Bibionidae	1	1763.90	2.61	0.10	
Thysanoptera (frequency only)	1	1664.90	1.34	0.24	
Acalyptate flies (frequency only)	1	1632.50	0.91	0.33	

C. pennsylvanicus in the US, they aggregate and mate in large numbers on the umbels without moving often from flower to flower.

We did not directly compare pollinator visitation rates between the US and NZ because of local differences in insect diversity and because visitation rate is not necessarily correlated with effective pollination. Although pollination success in NZ appeared to be higher (32.1% of plants experienced >50% fertilization) than in the U.S. (20% of plants experienced >50% fertilization), this difference was only marginally significant ($P=0.056$, as determined by a binomial GLM model).

Volatile chemistry

The floral bouquet of US parsnips comprised 16 distinct compounds, 14 of which could be identified; two monoterpenes could not be definitively characterized. The most abundant constituents were *cis*- (30.81%) and *trans*-ocimenes (23.69%) (Figure 1). As in the US, the floral bouquet of *P. sativa* in NZ was dominated by *cis*- (32.27%) and *trans*- (49.12%) ocimenes and octyl butyrate. NZ umbels emitted low quantities (<1%) of five compounds

absent in the US floral bouquet: linalool, carene, allo-ocimene and two isomers of α -farnesene (Figure 1). We found a total of 20 identifiable constituents but we were unable to identify two compounds present in low concentrations. A comparison of US and NZ floral volatiles revealed significant differences in the proportional representation of seven compounds (Wilks λ $F_{13,57} = 680.64$, $P < 0.01$); NZ flowers emit a greater proportion of beta-pinene, trans-ocimene, gamma-terpinene, octyl butyrate and germacrene whereas US flowers emit a greater proportion of octanol and octyl acetate.

Association between pollination and volatile chemistry

In the US, volatiles that best explain the variation in the pollinator matrix were β -pinene, γ -terpinene, hexyl butyrate, octyl butyrate, germacrene D and an unknown monoterpene. This was the model with the lowest AICc score (AICc = 336.23, $R^2 = 0.34$). These compounds were all positively associated with pollinator visitation (large calyprate flies and small syrphids, Figure 2). In NZ, volatiles that best explain

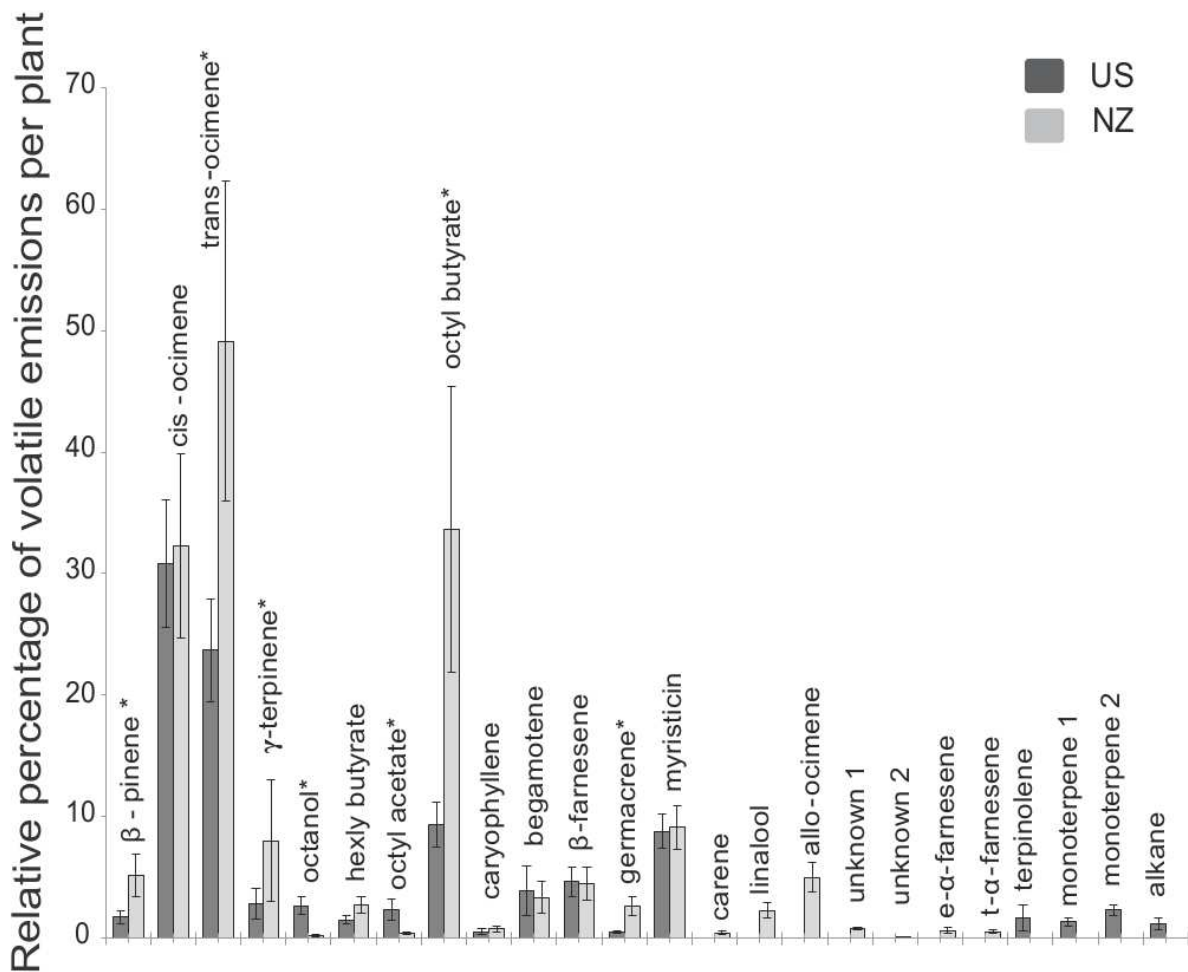


FIGURE 1. A comparison of the composition of volatile emitted from US and NZ flowers. Error bars represent standard error. Significant differences (MANOVA) indicated by *

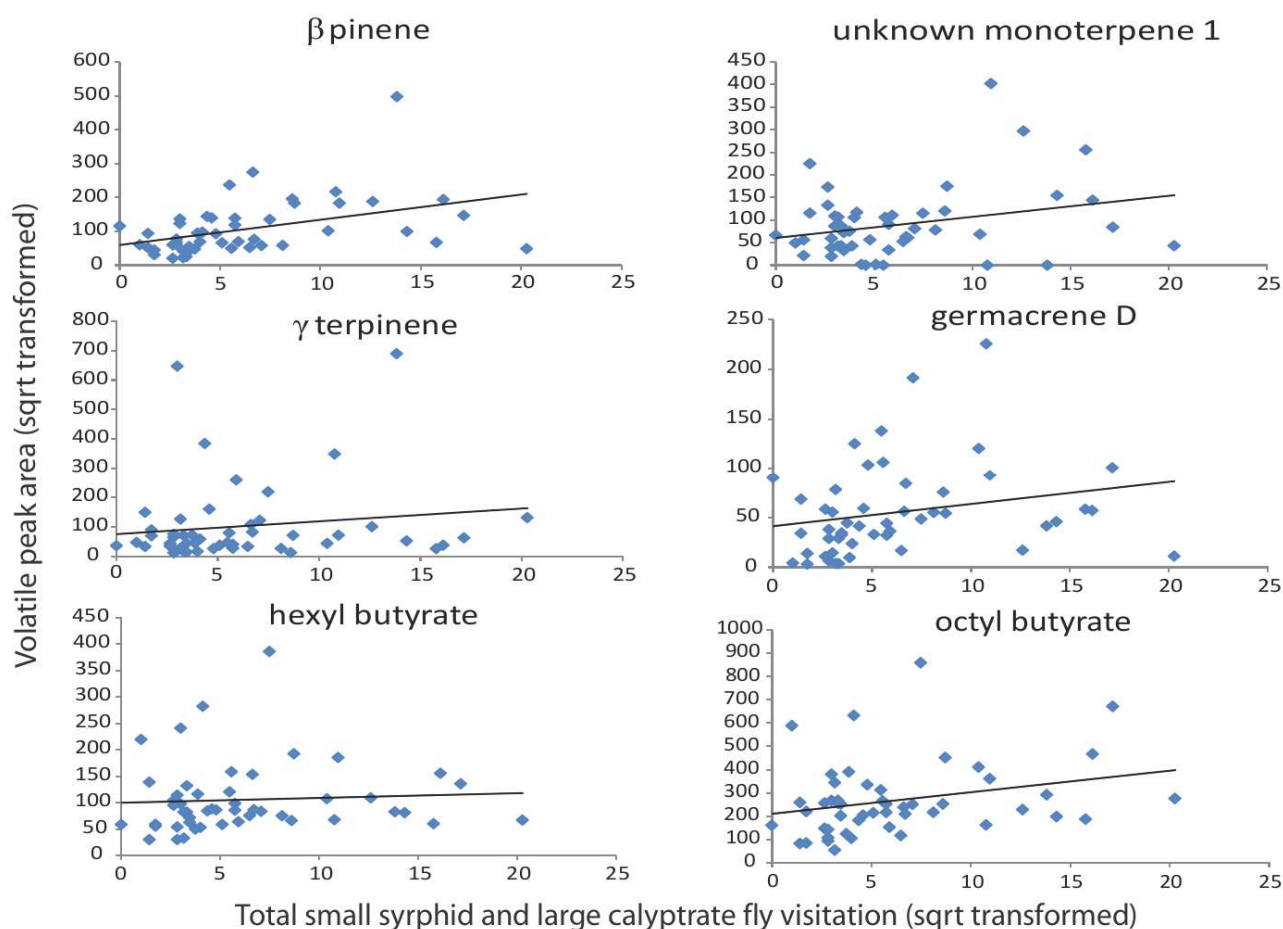


FIGURE 2. Compounds that best explain variation in the US pollinator matrix (DISTLM analysis: $AIC_c = 336.23$, $R^2 = 0.34$). Graphs represent the direction of relationship between pollinator visitation (counts of large calyprate flies and small syrphids) and individual volatile compounds in US parsnip flowers. The Kovat index for the unknown monoterpene (monoterpene 1) calculated from C9 and C10 alkanes at 80°C is 916 and m/z ion fragments in order of relative abundance are 93, 56, 71, 192, 161

the variation in the pollinator matrix were trans-ocimene, carene, octyl butyrate and octyl acetate. This model had the lowest AIC_c score ($AIC_c = 103.95$, $R^2 = 0.69$). Trans-ocimene, carene and octyl butyrate were positively associated with pollinator visitation while octyl acetate was negatively associated with visitation (large calyprate flies and small syrphids) (Figure 3). Most compounds positively associated with visitation (β -pinene, γ -terpinene, octyl butyrate, germacrene D, trans-ocimene and carene) were emitted in higher proportions in the NZ plants, whereas octyl acetate, which is negatively associated with visitation, is emitted in lower proportions in NZ plants.

Association between pollination and nectar availability

Large calyprate and small syrphid visitation was significantly associated with nectar availability in the US but not in NZ (Tables 3a and b, Figures 4a and b). The number of visitors was positively associated with umbel size for large calyprate and anthomyiid flies in the US and large calyprate, small syrphid and bibionid flies in NZ (Tables 3a and 3b).

DISCUSSION

Wild parsnips depend heavily on insect visitation for reproduction, indicating that seed production in this monocarpic biennial is highly contingent on the plant's ability to attract effective pollinators. Easily accessible nectar and large conspicuous inflorescences render wild parsnips a target for visitation by a wide range of insects. Many (possibly most) frequent visitors, however, do not appear to be effective pollinators. Frequency of visitation and pollen loads did not prove to be a reliable indicator of pollinator efficacy in this study; *C. pennsylvanicus* in the US and bibionid flies in NZ, for example, were extremely abundant and carried substantial amounts of pollen but were poor predictors of pollination success, possibly because of their tendency to aggregate and remain on the inflorescence for long periods (personal observations). Increased duration of visits per flower likely reduces the frequency with which other flowers are visited, an important determinant of pollinator effectiveness (Madjidian et al. 2008). Similarly, large ants, although common on flowers, do not appear to contribute proportionately to pollination success.

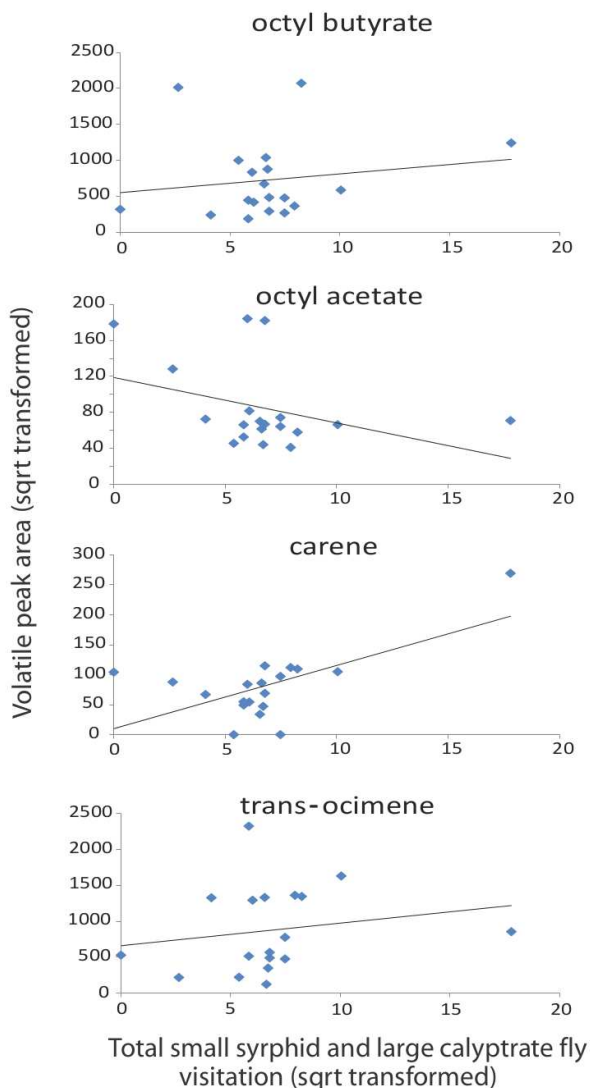


FIGURE 3. Compounds that best explain variation in the NZ pollinator matrix (DISTLM analysis: AICc = 103.95, $R^2 = 0.69$). Graphs represent the direction of relationship between pollinator visitation (counts of large calyptrate flies and small syrphids) and individual volatile compounds in NZ parsnip flowers.

Despite an apparent “promiscuous” strategy (*sensu* Bell 1971), in both the US and NZ the insects chiefly responsible for parsnip pollination in this study were species in the order Diptera. In fact, this so-called “promiscuously pollinated” plant may be somewhat specialized for pollination by flies. Visits by large calyptrate flies and small syrphids were significantly predictive of seed set in both the US and NZ. Moreover, the composition of floral volatiles of *P. sativa* is suggestive of some degree of specialization for dipteran pollinators. Although containing representatives from phytochemical classes widespread among angiosperms, the floral volatiles of *P. sativa* and closely related species in the genus *Heracleum* are essentially unique in containing octyl esters (Borg-Karlson et al. 1993, Knudsen et al. 2006). Evidence suggests that some dipterans may rely on aliphatic esters as cues for locating floral hosts (Knudsen & Stahl, 1994). Similarly, attractiveness to flies among some plant

species has been linked to production of trans-ocimene (e.g., *Prestoea schultzeana*, Knudsen et al. 2001; *Silene otitis*, Dotterl et al. 2012). Indeed, we found that many of these floral volatiles have a significant role in attracting dipteran pollinators to *P. sativa*. Pollinators appear to respond mainly to monoterpenes, sesquiterpenes and esters in the floral bouquet. Trans-ocimene and octyl butyrate dominate the floral bouquets of both NZ and US flowers (Figure 1) and are positively associated with visitation. In addition to floral volatiles, pollinator visitation is also significantly driven by nectar rewards (Figure 4a) and the conspicuousness of the umbels (umbel size, Tables 3a and 3b).

Remarkably, most compounds positively associated with visitation are produced in significantly higher proportions in NZ flowers. In the absence of herbivory, NZ plants might be under selection to increase fitness by producing more pollinator attractants. However, in this study, the tradeoff between compounds that mediate pollinator and herbivore attraction/deterrence may be constrained or otherwise influenced by biosynthetic considerations. Octyl butyrate is a known webworm deterrent but also a pollinator attractant, whereas octyl acetate is a webworm attractant and a pollinator deterrent, suggesting that selection should favor plants with high octyl butyrate and low octyl acetate. However, octyl acetate and octyl butyrate are extremely polymorphic in *P. sativa* and their production might be genetically constrained via pleiotropic associations with other chemical pathways. In a heritability study conducted on North American wild parsnip mature fruit, Carroll et al. (2000) determined that amounts of each ester are independently phenotypically correlated with linear furanocoumarins; if these phenotypic correlations reflect underlying genetic correlations, each ester may thus respond differently to selection on furanocoumarins or on other defense compounds. Despite absence of direct evidence of conflicting selection on floral chemistry in wild parsnips, the floral bouquet of wild parsnips in NZ reflects overall a profile of volatiles that is more attractive to pollinators.

A previous examination of wild parsnip floral chemistry in the US and NZ compared the composition of ethyl acetate extracts of buds and flowers (Zangerl et al. 2008). However, our focus in this study was attraction of pollinators and we compared headspace volatiles (Knudsen et al. 1993) as more ecologically relevant. Accordingly, the content and composition of floral volatiles differ between studies, with greater proportional representation of monoterpenes and lower representation, or absence, of compounds of higher molecular weight (e.g., palmitolactone) in this study.

The presence of herbivores can also have a direct impact on pollination. In *P. sativa*, florivory by webworms can greatly reduce the number of floral visitors to *P. sativa* (Lohman et al. 1996). Even a single webworm can reduce visitation; webworms not only web together and consume flowers, reducing the size of the floral display and restricting access of pollinators to flowers, they also deposit frass in their webs, altering the composition of the floral bouquet. Zangerl et al. (2009) demonstrated that webworm frass contributes octanol to the floral bouquet, through webworm

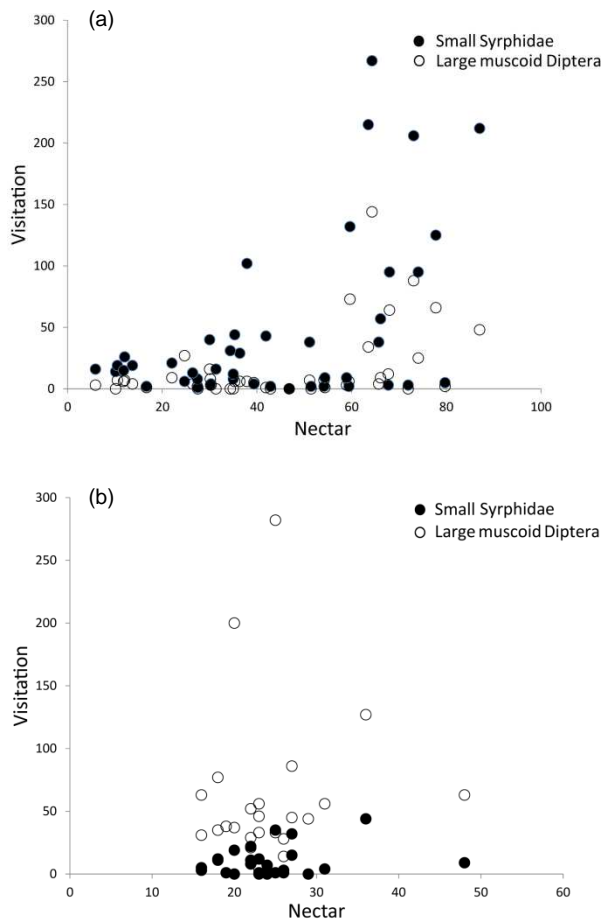


FIGURE 4. Relationship between the number of visits and nectar availability (percentage of flowers per umbel bearing nectar on the day's last photograph) on a) US parsnips and b) NZ parsnips for large calytrate flies (US: $P < 0.001^*$, NZ: $P = 0.75$, GLM negative binomial) and small syrphids (US: $P = 0.01^*$, NZ: $P = 0.93$ GLM negative binomial)

detoxification of the esters octyl acetate and octyl butyrate. Conversion of octyl esters to octanol may thus contribute to reductions in pollinator visitation via reducing the abundance of a potential pollinator attractant and increasing the abundance of an otherwise rare volatile component.

In undertaking this study, we found evidence consistent with the hypothesis that floral chemical traits in wild parsnips free from webworm florivory will reflect a volatile profile that maximizes fitness via the attraction of pollinators. Our findings suggest that future studies of phytochemical impacts of enemy release should address interactions with mutualists as well as interactions with enemies, in order to obtain a more comprehensive understanding of both the ecology and evolution of plant-insect interactions in non-indigenous environments.

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TABLE 3. Log-likelihood ratio (Chi-sq) tests for GLMs (negative binomial model) predicting large fly and small syrphid visitation by nectar availability in US and NZ parsnips. Significant differences at $P < 0.001$ are indicated by *** and $P < 0.05$ by *

a) US						
	Df	Deviance	AIC	LRT	P-value	
Large calyptrate Diptera						
Full Model		52.43	294.42			
Nectar	1	63.29	303.22	10.802	0.00	***
Umbel size	1	69.40	309.33	16.911	0.00	***
Small Syrphidae						
Full Model		51.95	409.18			
Nectar	1	62.89	418.12	10.943	0.00	***
Umbel size	1	55.48	410.71	3.5304	0.06	
Anthomyiidae						
Full Model		49.80	266.16			
Nectar	1	56.03	269.84	5.678	0.01	*
Umbel size	1	55.48	270.38	6.226	0.01	*
<i>Chauliognathus pennsylvanicus</i>						
Full Model		50.07	240.93			
Nectar	1	50.91	270.49	0.84	0.35	
Umbel size	1	50.74	270.31	0.67	0.41	
Small Formicidae						
Full Model		43.90	240.93			
Nectar	1	44.93	239.96	1.02	0.311	
Umbel size	1	46.37	241.40	2.46	0.11	
Large Formicidae						
Full Model		43.49	228.94			
Nectar	1	46.10	262.06	2.60	0.10	
Umbel size	1	43.52	259.48	0.03	0.86	
b) NZ						
	Df	Deviance	AIC	LRT	P-value	
Large calyptrate Diptera						
Full Model		33.09	270.80			
Nectar	1.00	33.19	268.89	0.09	0.75	
Umbel size	1.00	39.23	274.94	6.14	0.01	*
Small Syrphidae						
Full Model		31.48	176.88			
Nectar	1.00	31.49	174.89	0.06	0.93	
Umbel size	1.00	39.76	183.18	8.27	0.00	*
Bibionidae						
Full Model		31.01	385.77			
Nectar	1.00	31.66	384.41	0.64	0.42	
Umbel size	1.00	35.36	388.12	4.34	0.03	*
Acalyptrate flies						
Full Model		31.60	252.96			
Nectar						
Umbel size	1.00	32.57	251.94	0.97	0.32	
Thysanoptera						
Full Model		32.06	415.06			
Nectar	1.00	31.65	251.01	0.04	0.82	
Umbel size	1.00	32.78	413.78	0.72	0.39	

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