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LETTER

Leaf herbivory and nutrients increase nectar alkaloids

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Abstract

Correlations between traits may constrain ecological and evolutionary responses to multispecies interactions. Many plants produce defensive compounds in nectar and leaves that could influence interactions with pollinators and herbivores, but the relationship between nectar and leaf defences is entirely unexplored. Correlations between leaf and nectar traits may be mediated by resources and prior damage. We determined the effect of nutrients and leaf herbivory by *Manduca sexta* on *Nicotiana tabacum* nectar and leaf alkaloids, floral traits and moth oviposition. We found a positive phenotypic correlation between nectar and leaf alkaloids. Herbivory induced alkaloids in nectar but not in leaves, while nutrients increased alkaloids in both tissues. Moths laid the most eggs on damaged, fertilized plants, suggesting a preference for high alkaloids. Induced nectar alkaloids via leaf herbivory indicate that species interactions involving leaf and floral tissues are linked and should not be treated as independent phenomena in plant ecology or evolution.

Keywords

Anabasine, herbivory, induced defences, *Manduca sexta*, *Nicotiana tabacum*, nicotine, optimal defence theory, phenotypic correlation, pollination, toxic nectar.

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INTRODUCTION

Most organisms interact with a range of other species, and the outcome of these interactions is mediated by numerous traits. Pleiotropy or correlations between traits involved in multispecies interactions may constrain or shape trait evolution. For example, the evolution of beak morphology in response to food availability may constrain vocal evolution in Darwin's finches (Grant & Grant 1995; Podos 2001), garter snakes that have evolved resistance to newt prey toxins are slower and may be less able to avoid their own predators (Brodie 1999), and wild radish plants with flower colour morphs preferred by pollinators have leaves that are preferred by herbivores (Irwin *et al.* 2003). Recent theoretical models demonstrate that genetic correlations between traits may qualitatively change the dynamics and outcome of three-species interactions, especially when one of the interactions is antagonistic (Nuismer & Doebeli 2004). Thus, understanding correlations between traits that mediate multispecies interactions is fundamental to predicting how such traits may evolve.

Although plant chemical defences are typically studied in leaves, these compounds are often present in floral tissue (reviewed in Strauss *et al.* 2004) and in nectar (reviewed in Wink 1992; Adler 2000). While defensive compounds in nectar may benefit plants if they deter antagonists such as nectar robbers, such compounds may also have ecological costs if they are deterrent to pollinators (e.g. Adler & Irwin 2005). However, no study has examined the extent or sources of intraspecific variation in nectar defensive compounds. Leaf herbivory induces changes in leaf defensive compounds that can reduce subsequent damage (Karban & Baldwin 1997). Only a handful of studies have examined whether leaf damage induces changes in floral defensive chemistry (Euler & Baldwin 1996; Ohnmeiss & Baldwin 2000; Strauss *et al.* 2004), and no study has explicitly considered nectar, the resource used by many pollinators. Floral induction might be adaptive if leaf herbivory provides the plant with a reliable cue predicting future floral herbivory (Karban *et al.* 1999), but costly if induced defences are deterrent to pollinators (e.g. Strauss *et al.* 1999).

Attractive and defensive traits can be correlated via shared physiology, linkage and/or pleiotropy. Thus, selection on resistance by herbivores may drive the evolution of floral traits, and vice versa. If defence concentrations in nectar and leaves are correlated, plants may be unable to evolve optimal solutions in response to selection by both herbivores and pollinators. However, the extent to which defence

concentrations are correlated across different tissues within plants is largely unknown. Furthermore, the abiotic environment may alter the expression of traits that influence biotic interactions. Nutrient availability has played a central role in theories of defence allocation (e.g. Coley *et al.* 1985), and spatial heterogeneity in nutrients may alter the expression of defence traits across tissues and thus mediate plant–animal interactions at the level of both leaves and flowers.

Plant traits in leaves and flowers may not be independent of each other, and neither are leaf herbivores and pollinators. Several insect taxa include species that are pollinators as adults and herbivores as larvae (Adler & Bronstein 2004). Thus, adults might use nectar traits to evaluate plant quality and make oviposition decisions if nectar is an honest signal reflecting plant quality. For example, soil nutrients alter the composition and concentration of nectar amino acids (Gardener & Gillman 2001), which may provide an indicator of plant nutritional status to nectar-feeding adults (Gardener & Gillman 2002). Similarly, biotic and abiotic factors could affect chemical defences in leaves and nectar, providing information to nectar-feeding insects about host quality for offspring.

Nicotiana tabacum L. (domestic tobacco) and *Manduca sexta* L. (hawkmoth; tobacco hornworm) provide a model system to examine how biotic and abiotic factors influence nectar defensive compounds, leaf defensive compounds and moth oviposition. *Nicotiana tabacum* produces alkaloids in tissues including nectar (Detzel & Wink 1993). *Manduca sexta* larvae are specialist herbivores on *Nicotiana* and related species (del Campo *et al.* 2001; Wink & Theile 2002), and adults are dusk-flying hawkmoths that forage on night-blooming flowers including *Nicotiana attenuata* (Euler & Baldwin 1996) and *N. tabacum* (L.S. Adler, personal observation). Male and female moths can both pollinate flowers, but only females will lay eggs. *Nicotiana tabacum* flowers are also visited by bumble bees, honey bees, and hummingbirds (L.S. Adler, personal observation), indicating that *M. sexta* is not the sole agent for outcrossing in *N. tabacum*. We used this system to ask the following questions:

(1) How do nutrients and herbivory affect leaf alkaloid levels, nectar alkaloid levels and other floral traits?
(2) Are nectar and leaf alkaloid concentrations correlated between plants?
(3) Do nutrients and herbivory influence moth behaviour and oviposition?

MATERIALS AND METHODS

Experimental design

One hundred and forty *N. tabacum* seedlings (Richer's Herbs, Ontario, Canada) were transplanted into 3.78 L pots with 50 : 50 sand and Metromix 360 soil (Scotts-Sierra Horticultural Products Company, Marysville, OH, USA) on 14 December 2002. Typical dry weight of soil and sand per pot was 1570 g. Supplemental greenhouse light from 1700 to 2100 was provided with alternating 1000 W sodium and metal halide lights. Plants were randomly assigned to nutrient (low or high) and leaf herbivory (herbivory or control) treatments in a factorial design. Plants from each treatment combination were randomly assigned to either morphology/chemistry measurements or to moth behaviour/oviposition measurements (see below) to avoid impacts of handling and nectar collection on moth behaviour. Plants were randomly arranged together until measurements began.

Nutrient treatments were applied on 18 December 2002. Plants in the high nutrient treatment received 2.68 g of fertilizer (equivalent to 0.2856 g N, 0.1247 g P and 0.2371 g K; Osmocote 14-14-14, 4-month slow release; Scotts-Sierra) and low nutrient treatment plants received 0.67 g. With a pot radius of 85 mm, the 'high' treatment resulted in an application of 12.58 g m^{-2} N, 5.49 g m^{-2} P and 10.44 g m^{-2} K and the 'low' application had 25% of these amounts. Leaves were damaged when flower buds first appeared by placing one fifth-instar *M. sexta* (North Carolina State University Insectary, USA) on each of the three youngest expanded leaves within mesh bags; control plants received bags without larvae. Larvae and bags were removed when at least 50% of leaf material was consumed, and on many plants leaves were entirely consumed. This damage level (up to three leaves per plant) is well within the range of natural herbivory (e.g. van Dam *et al.* 2001). Herbivory treatments typically took 1–3 days and were complete before plants flowered. Damaged leaves were removed from each plant 4–5 days after treatment completion using a clean razor to avoid biasing moth behaviour and to maintain uniformity across studies. Leaves were removed from control plants simultaneous with removal from damaged plants. The next youngest leaf was also collected at the same time from each plant for alkaloid analysis. Leaves were stored individually in plastic bags at −20 °C until analysis. Plants were divided into four blocks based on flowering phenology; thus, these represent blocks in time rather than space. Plants in blocks 1–4 were damaged on 17 January, 29 January, 11 February and 20 February respectively. Plants in block 4 were used for moth behaviour only.

Effect of nutrients and herbivory on floral morphology and chemistry

Half the plants from each treatment–block combination were randomly assigned to floral morphology and chemistry measurements. Floral morphology, nectar volume and nectar sugar concentration were measured for every flower

on its first day of maturity (mean ± SE: 19.7 ± 1.0 flowers per plant; range 5–37). Flowers were considered 'mature' when corollas were open and pink, anthers were dehiscing and the stigma appeared wet. Measurements were initiated every day at 1600. We measured corolla length, corolla tube width at base, corolla tube width at top opening, and corolla display (from tip of one petal to opposite indent) to the nearest 0.01 mm. We measured nectar volume with a 25 μL microcapillary tube inserted near the corolla base, and nectar sugar concentration with a pocket refractometer. Additional nectar was collected from all open flowers every day, pooled within plants and stored at −20 °C for chemical analysis. Care was taken to avoid floral damage from microcapillary tubes that could contaminate nectar with petal alkaloids.

Alkaloid analysis

Leaves

Leaves were freeze dried, heated at 70 °C, and ground. Alkaloids were extracted with 5% acetic acid and quantified as mg g^{-1} with a colorimetric determination on a Technicron Auto-Analyzer following methods of Davis (1976).

Nectar

Nectar samples were pooled within plants and stored in 1 mL of 95% ethanol. We analysed the nicotine and anabasine concentrations by high-performance liquid chromatography (HPLC). Nectar samples were dried by a speedvac (VR-Maxi; Heto, Allerod, Denmark) and then kept at −20 °C. Methanol (150 μL) was added to each sample, and after vortexing the samples were centrifuged at 13 000 rpm (10 000 × ***g***) for 5 min. Fifty microlitres of the supernatant was derivatized, and the following solutions were sequentially added: 25 μL of 4 M acetate buffer (pH 4.7); 10 μL of 1.5 M potassium cyanide in water; 10 μL of 0.4 M chloramine-T in water; and 50 μL of 50 mM thiobarbituric acid in water–acetone (50 : 50 v/v). The contents were mixed and incubated for 5 min; the reaction was stopped by the addition of 10 μL of 0.1 M sodium metabisulphite in water. HPLC analysis was performed exactly 3 min after the reaction had been stopped. The HPLC configuration (HPLC, System Gold Nouveau, Beckmann, Fullerton, CA, USA) for determination of anabasine and nicotine consisted of a HPLC pump (Beckmann 125P) connected to a photodiode array detector (Beckmann 168; wavelength: 505 nm). The mobile phase–linear gradient was water–acetonitrile from 0% to 100% acetonitrile in 15 min. The column used was Merck LiChroCART RP-18 (250 × 4 mm ID, 5 μm particle size) (Merck, Darmstadt, Germany). Injection volume was 20 μL and the flow-rate was 1 mL min^{-1}. Before the next injection, the column was equilibrated for 3 min. Concentrations of nicotine and anabasine were determined by calibration curves using standards at concentrations between 0.3 and 50 ng μL^{-1}. The primary alkaloid we detected in nectar was anabasine rather than nicotine; only 15 of 140 samples had detectable nicotine while all but three samples contained anabasine. As nicotine is quite volatile as a free base when compared with anabasine, nicotine could have been lost during sample processing and so was excluded from nectar analysis. Across *Nicotiana* species, nectar nicotine and anabasine concentrations are marginally positively correlated ($n = 22$, $r = 0.39$, $P = 0.07$; M. Gittinger, L.S. Adler, G. Morse and M. Wink, unpublished data), but no data are available regarding correlations within species. Nectar alkaloid concentration was calculated in μg anabasine per mL nectar.

Effect of nutrients and herbivory on moth behaviour and oviposition

The other half of the plants from each treatment–block combination was used in moth foraging behaviour and oviposition measurements. Plants were placed upon flowering in a large mesh enclosure 2.7 × 4.2 × 3.3 m stocked with adult *M. sexta*. Plant positions were randomized on two benches with supplemental high-pressure sodium light from 1700 to 2200. Eggs were counted and removed every day until plants ceased flowering. Total eggs were summed within plants.

Moths were marked with paint pen (uni®Paint Medium line; Mitsubishi Pencil Co. for Sanford Corporation, Bellwood, IL, USA) on their thorax to indicate sex. Behaviour was observed on 6, 11, 13, 24 and 25 February between 18:00 and 19:00 hours, when moths were most active. Individual moths were followed and the plants visited, flowers probed and time per flower were recorded. Hereafter, a 'visit' refers to each time a moth initiates foraging on a new plant, and a 'probe' refers to each time a moth begins feeding at a new flower.

Statistical analysis

Leaf alkaloid concentrations were measured and analysed for every plant. Other responses were measured on separate plants allocated to either morphology/chemistry or to moth behaviour/oviposition studies; these responses were analysed separately. We determined the effect of herbivory, nutrient treatment, their interactions, and block on leaf and nectar alkaloid concentration, male and female moth foraging behaviour, and on the total eggs per plant using ANOVAs, and on floral display and rewards (number of flowers, floral morphology, nectar production and nectar sugar) using MANOVA. Plant was the unit of replication in all analyses. All responses were normal without transformation except for leaf alkaloid levels and moth behaviour, which were log(x) or

log(x + 1) transformed. One outlying value for corolla length and one for nectar alkaloids was deleted; each value was more than three standard deviations from the mean and retaining these values did not qualitatively change the results. We calculated the phenotypic correlation between nectar and leaf alkaloid concentrations using Pearson correlation coefficients. Correlations were calculated pooled across treatments and also separately within each treatment group. Because some plants did not flower, 112 were used for leaf alkaloids, 57 in morphology/chemistry analyses, 67 for oviposition and 58 for foraging behaviour (block 4 was not observed). Flowers probed and time per flower were only analysed for plants that were visited; thus 38 plants were included for analysis of female moths and 14 plants for male analysis.

RESULTS

Effect of nutrients and herbivory on morphology and chemistry

Leaf alkaloid levels ranged from 0 to 6 mg g^{-1} (mean ± SE: 1.55 ± 0.105) and nectar alkaloid levels ranged from 0 to 1.04 μg mL^{-1} (mean ± SE: 0.33 ± 0.031). Leaf herbivory induced 33% higher nectar alkaloid concentrations compared with undamaged plants ($F_{1,51} = 4.39$, $P = 0.04$; Fig. 1a) but did not increase leaf alkaloids ($F_{1,105} = 3.09$, $P = 0.08$; Fig. 1b). High nutrients increased nectar alkaloid concentrations by nearly 80% ($F_{1,51} = 6.31$, $P = 0.015$; Fig. 1a) and more than doubled leaf alkaloid concentrations compared with unfertilized plants ($F_{1,105} = 47.67$, $P < 0.0001$; Fig. 1b). Herbivory and nutrients had additive effects on nectar and leaf alkaloids (interaction terms: $F < 0.6$, $P > 0.4$ for both). Nectar and leaf alkaloid concentrations also varied between blocks ($F > 4.65$, $P < 0.005$ for both).

Nectar and leaf alkaloids were significantly positively correlated across plants ($n = 44$, $r = 0.4$, $P = 0.008$; Fig. 2). Adding a quadratic term to a regression model did not explain significant variation in nectar alkaloids (leaf alkaloids squared: $t_{1,41} = -1.42$, $P > 0.15$), suggesting the relationship is linear. The correlation between leaf and nectar alkaloids was also positive but non-significant within each treatment group where sample sizes were much smaller (r between 0.23 and 0.41, n = 8–13 per group, $P > 0.2$ for all).

Nutrients influenced floral traits, but herbivory and the interaction term did not (MANOVA, nutrients: Wilks' $\lambda = 0.66$, $F_{7,45} = 3.23$, $P = 0.007$; herbivory and interaction: Wilks' $\lambda > 0.81$, $F_{7,45} < 1.45$, $P > 0.2$). High nutrients increased total flowers and decreased nectar volume per flower (flowers, mean ± SE: high nutrients 23.5 ± 1.6, low nutrients 18.5 ± 1.4; $F_{1,51} = 12.07$, $P = 0.001$; nectar volume: high nutrients 30.2 ± 0.6 μL, low nutrients 32.8 ± 1.3 μL; $F_{1,51} = 6.19$, $P = 0.016$), but did not affect floral morphology or nectar sugar ($F_{1,51} < 1.5$, $P > 0.2$ for all). Block also significantly influenced floral traits in MANOVA and univariate analyses (Wilks' $\lambda = 0.13$, $F_{14,90} = 11.22$, $P < 0.0001$; univariate analyses: $F_{2,51} > 8.81$, $P < 0.0005$ for all but nectar volume, where $F_{2,51} = 2.3$, $P > 0.1$).

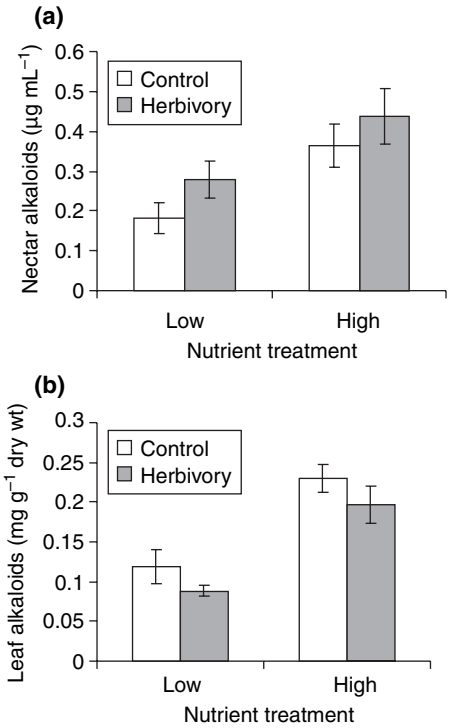

Figure 1 Effect of nutrient treatment and herbivory on alkaloid concentration in (a) nectar and (b) leaves. Note different units for nectar and leaf concentrations. Error bars represent standard error.

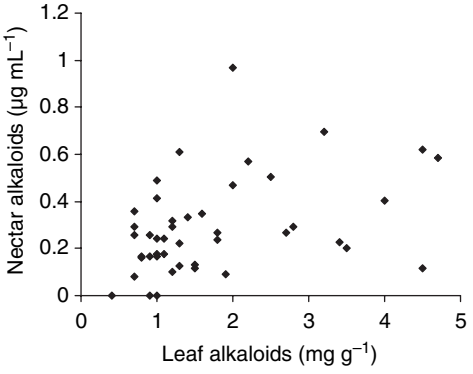

Figure 2 Phenotypic correlation between leaf and nectar alkaloid concentrations. Note different units for leaf and nectar concentrations. Each point represents one plant and plants from all treatments in the 'floral morphology and chemistry' study are included.

Table 1 Effect of herbivory, nutrient level and their interactions on the total eggs oviposited on each plant

Source	d.f.	SS	*F*	*P*-value
Nutrient	1	9429.3	6.8	0.012
Herbivory	1	6539.1	4.7	0.034
Nutrient × herbivory	1	6220.2	4.5	0.039
Block	3	64101.1	15.4	0.0001
Position	1	7501.9	5.3	0.025
Error	59	82109.8		

'Position' is a covariate representing spatial arrangement in the arena and plants were grouped into four blocks in time based on phenology.

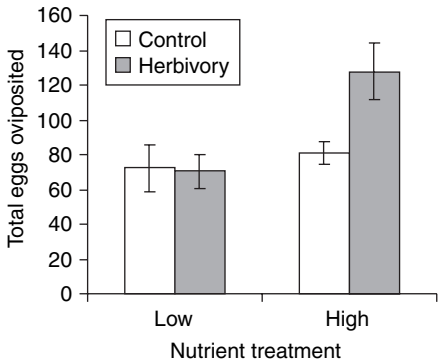

Figure 3 Combined effect of herbivory and nutrient treatment on oviposition by *Manduca sexta*. Error bars represent standard error.

Effect of nutrients and herbivory on moth behaviour and oviposition

Plants with high nutrients received more visits by female but not male moths (female: high nutrients 2.2 ± 0.4, low nutrients 1.0 ± 0.3; $F_{1,53} = 6.07$, $P = 0.017$; male: high nutrients 0.82 ± 0.3, low nutrients 0.6 ± 0.3; $F_{1,53} = 0.09$, $P > 0.7$). Nutrients, herbivory and their interactions did not affect any other measure of male or female moth foraging behaviour.

Herbivory and high nutrients both increased the total eggs per plant, and affected oviposition non-additively (Table 1). Moths laid more eggs on damaged compared with control plants when nutrients were high, but not when nutrients were low (Table 1; Fig. 3).

DISCUSSION

Here, we provide the first data documenting intraspecific variation in nectar secondary compounds (Fig. 2), and demonstrating that both leaf herbivory and higher resources increase nectar alkaloids. Defensive compounds in nectar may influence interactions with a wide range of floral visitors, including pollinators (e.g. Stephenson 1981, 1982; Detzel & Wink 1993; Hagler & Buchmann 1993; Adler & Irwin 2005), nectar robbers (Adler & Irwin 2005), nectar thieves (Guerrant & Fiedler 1981; Stephenson 1981, 1982), microbes (Thornburg *et al.* 2003), parasitoids (Wackers 2001) and leaf herbivores (Adler & Bronstein 2004; Romeis *et al.* 2005). Secondary compounds have been documented in nectar from numerous locations and plant families (Baker 1977, 1978; Adler 2000), suggesting their importance in many systems. We found a positive phenotypic correlation between leaf and nectar alkaloid concentrations, suggesting that expression of traits in flowers and leaves is not independent. Because domestic tobacco is highly selfing, there is little or no genetic variation within commercial tobacco varieties. Thus, this positive correlation is likely driven by the positive effect of nutrients on both leaf and nectar alkaloids, although we also found positive but non-significant correlations between these traits within each treatment group. If such correlations have a genetic basis in wild plants, they provide a mechanism by which pollinator selection against nectar defensive compounds could drive indirect selection to decrease leaf defences or herbivore selection for increased leaf defences could drive increased defensive compounds in nectar. Although our correlation is relatively low ($r = 0.4$), this number reflects a correlation between leaf alkaloid levels at one point in time and nectar alkaloid concentrations pooled over several weeks of collection per plant. Thus, nectar alkaloid levels may represent a remarkably robust indicator over time of leaf defence. Our results suggest that models describing the ecology and evolution of floral or leaf traits need to consider correlated plant traits and interactions between community members occurring across multiple tissues.

Leaf herbivory can reduce floral display and deter pollinators (e.g. Lehtila & Strauss 1997; Mothershead & Marquis 2000), but the actual mechanisms of deterrence are generally unclear. While herbivory induces extrafloral nectar production as an indirect defence in several systems (e.g. Agrawal & Rutter 1998), the role of leaf or floral herbivory on floral nectar production or composition has rarely been explored (but see for example, Krupnick *et al.* 1999; Lehtila & Strauss 1999). Induced nectar defensive compounds may provide a mechanism by which leaf herbivory reduces pollinator preference. Such an ecological cost of defence has been demonstrated in *Brassica rapa* lines selected for high or low expression of myrosinase, an enzyme involved in herbivore resistance. Pollinators spent significantly less time on high-resistance plants in the absence of herbivory, suggesting that either myrosinase itself or allocation costs of expression made flowers less palatable to pollinators (Strauss *et al.* 1999). Here, we demonstrate a direct link between leaf damage and nectar defensive compounds, indicating that herbivory may

rapidly induce floral changes that could decrease pollinator preference.

Leaf herbivory in budding plants induced nectar alkaloids more strongly than leaf alkaloids. Greater amounts of leaf herbivory on bolting plants also induced higher floral but not leaf alkaloids in *Nicotiana sylvestris* (Ohnmeiss & Baldwin 2000), induced higher corolla nicotine pools in *N. attenuata* (Euler & Baldwin 1996) and reduced floral and fruit damage in *N. attenuata*, suggesting that induced floral defenses may be adaptive (McCall & Karban 2006). The timing of damage may be critical; leaf damage to early rosettes induced higher leaf alkaloids in *N. sylvestris* while leaf damage to bolting plants induced floral but not leaf alkaloids, consistent with predictions of optimal defence theory (Ohnmeiss & Baldwin 2000). However, protecting floral tissue in response to leaf herbivory is only adaptive if leaf damage provides a reliable cue for incipient floral damage (Karban *et al.* 1999), and if the benefits of induction outweigh potential costs in terms of deterring mutualists such as pollinators (Strauss *et al.* 2002). At dusk when moth pollinators are most active, corolla nicotine pools decrease and the attractive volatile benzyl acetone increases in *N. attenuata* (Euler & Baldwin 1996), suggesting that plants may be able to control diurnal variation to reduce costs of deterring pollinators. Thus, plants may be able to allocate defences to reproductive tissues as predicted by optimal defence theory and avoid deterring pollinators if pollinators and herbivores forage at different times of day (N. Theis, R.A. Raguso and M. Lerdau, unpublished data).

The positive correlation between nectar and leaf alkaloids suggests that moths could use nectar taste as an indicator of leaf defence for oviposition decisions. This hypothesis assumes that moths are capable of tasting alkaloids in nectar and that the correlation is strong enough to provide reliable information. Moth oviposition was highest on plants that had the highest levels of both nectar and leaf alkaloids; i.e. plants that experienced both herbivory and high nutrients. Moth oviposition preference for high-alkaloid plants may seem surprising as nicotine decreased larval performance in several laboratory studies with colony *M. sexta* (Barbosa *et al.* 1991; Appel & Martin 1992; Voelckel *et al.* 2001; Kester *et al.* 2002). However, the impact of a third trophic level in the field may decrease the costs of consuming secondary compounds, particularly for a specialist herbivore like *M. sexta* that is relatively nicotine tolerant (Glendinning 2002; Wink & Theile 2002). For example, nicotine had stronger effects on the parasitoid *Cotesia congregata*, which can parasitize up to 100% of hornworms in the eastern USA (Thorpe & Barbosa 1986), than on *M. sexta* (Barbosa *et al.* 1991). Nicotine ingestion also reduced ant predation in choice tests (Cornelius & Bernays 1995). Balances between nicotine consumption and parasitism were thought to determine oviposition choices in *M. sexta* on *N. tabacum* (Kester *et al.* 2002), and high predation risk from *Geocoris* bugs correlated with *Manduca quinquemaculata*'s preference for younger *N. attenuata* leaves despite 2.1-fold higher nicotine concentrations (Kessler & Baldwin 2002). Thus, the benefits of consuming nicotine in terms of reduced predation or parasitism may outweigh the physiological costs in field settings, and explain moth preferences to oviposit on highly defended plants.

Alternatively, hawkmoths may choose oviposition sites based on factors other than secondary compounds. High nutrients may increase nectar amino acids (Gardener & Gillman 2001), which increase *M. sexta* oviposition on *N. tabacum* (A.J. Lentz and L.S. Adler, unpublished data), and which increase female but not male preference in other Lepidoptera (Rusterholz & Erhardt 2000). The lack of treatment effect on refractometer readings, which represent amino acid as well as sugar concentration (Inouye *et al.* 1980), suggest that amino acid concentrations did not differ strongly between treatments. However, a more sensitive analysis is necessary to determine conclusively whether nectar amino acids varied between treatments and alter moth behaviour. The non-additive effect of herbivory and nutrients on moth oviposition was not reflected by any similar change in floral morphology, nectar alkaloids or flower visitation by female moths, suggesting that as yet unmeasured traits, such as volatiles (e.g. De Moraes *et al.* 2001) or leaf contact stimulants (e.g. Severson *et al.* 1991), may be responsible for oviposition decisions. Herbivory and nutrients can alter many aspects of plant chemistry and growth (e.g. Gershenzon 1984; Karban & Baldwin 1997; Galen 1999); additional studies manipulating single factors are necessary to determine which mechanisms are responsible for the non-additive effect of herbivory and nutrients on moth oviposition.

Female moths were more likely to forage on plants with high nutrients, which also had high alkaloid levels in nectar and leaves. Thus, our results are not consistent with the hypothesis that nectar alkaloids deter moths as pollinators. The impacts of nectar alkaloids on preference or performance of other pollinators have only been examined for a few systems. Palestine sunbirds, which pollinate non-native *Nicotiana glauca* in Israel, are deterred by nectar anabasine at naturally occurring concentrations of 5 p.p.m. (Tadmor-Melamed *et al.* 2004). Honey bees are deterred by a variety of alkaloids including nicotine in sugar solutions, although the lowest concentration tested was 10 p.p.m. (Detzel & Wink 1993). Multiple pollinating bees (*Bombus bimaculatus*, *Osmia lignaria*, *Habropoda laboriosa* and *Apis mellifera*) as well as the nectar-robbing *Xylocopa virginica* are all deterred by the alkaloid gelsemine that occurs in *Gelsemium sempervirens* nectar (Adler & Irwin 2005). Adult *M. sexta* appear relatively tolerant to alkaloids compared with other studies, and are the only pollinator examined whose larvae are specialist

herbivores that regularly encounter the same alkaloids in their diet. Data from more systems are needed to evaluate the hypothesis that insects that encounter secondary compounds as larvae are better able to tolerate such compounds as pollinating, nectar-feeding adults.

Our work suggests that leaf herbivory may impact pollination and subsequent herbivory via induced nectar defensive compounds. We found a positive phenotypic correlation between nectar and leaf alkaloids, suggesting that interactions occurring at multiple tissue levels may be linked via correlated trait expression. While effects of leaf herbivory on pollination have been demonstrated, the induction of nectar defensive compounds provides a direct mechanism by which herbivores could deter pollinators. Furthermore, the correlation between nectar and leaf alkaloids may provide a mechanism for nectar-feeding adult insects to assess host quality for offspring. We hope this study will inspire future research in wild systems to determine whether correlations between nectar and leaf defensive compounds have a genetic basis, to manipulate nectar alkaloids independent of other traits and measure their impact on pollinator preference, and to examine the fitness costs and benefits of induced nectar defensive compounds in systems where pollinators are and are not herbivores. Addressing these questions is necessary to quantify the extent to which the evolution of plant defence is determined by interactions with pollinators as well as leaf herbivores.

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