Ecological Entomology (2008), DOI: 10.1111/j.1365-2311.2007.00974.x

The nectar alkaloid, gelsemine, does not affect offspring performance of a native solitary bee, *Osmia lignaria* (Megachilidae)

SUSAN E. ELLIOTT[1], REBECCA E. IRWIN[1], LYNN S. ADLER[2] and NEAL M. WILLIAMS[3] [1]Department of Biological Sciences, Dartmouth College, Hanover, New Hampshire, U.S.A., [2]Department of Plant, Soil, and Insect Sciences and Graduate Program in Organismic and Evolutionary Biology, University of Massachusetts, Amherst, Massachusetts, U.S.A. and [3]Department of Biology, Bryn Mawr College, Bryn Mawr, Pennsylvania, U.S.A.

Abstract. 1. The ecology and evolution of foliar-feeding insects are thought to be closely tied to plant secondary compounds. Although secondary compounds are also abundant in floral nectar, their role in mediating pollinator preference and performance remains relatively unexplored.

2. This study tested the effects of an alkaloid, gelsemine, found in the nectar of Carolina jessamine (*Gelsemium sempervirens* L., Loganiaceae), on the performance of a native solitary bee (*Osmia lignaria lignaria* Say, Megachilidae). Nectar gelsemine reduces visits from pollinators, including *O. lignaria lignaria*, and gelsemine is toxic to vertebrates and possibly non-native honey bees (*Apis mellifera* L., Apidae). To test the hypothesis that the deterrent effects of nectar gelsemine reflect negative consequences for pollinator performance, *O. lignaria lignaria* offspring provisions were supplemented with nectar containing different gelsemine concentrations. Effects on larval development time, prepupa cocoon mass, adult emergence, and adult mass were measured.

3. Nectar gelsemine had no effect on any measure of offspring performance. Thus, although gelsemine deters foraging by adult bees, this behaviour did not optimize offspring performance under the experimental conditions of this study. In contrast, sugar added to nectar treatments increased offspring mass.

4. While adult pollinators may avoid nectar with secondary compounds, this could hinder offspring performance by reducing sugar in provisions if nectar is limiting in the environment. Preference-performance trade-offs, which are studied extensively with foliar herbivores, have seldom been tested for pollinating plant consumers. Future studies of nectar secondary compounds and insect pollinator preference and performance may help to integrate studies of foliage-consuming insect herbivores with nectar-consuming insect pollinators.

Key words. Gelsemine, *Gelsemium sempervirens*, insect foraging, *Osmia lignaria*, pollinator performance, secondary compounds, toxic nectar.

Introduction

Herbivorous insects often control their offspring's fate through oviposition and provisioning choices. These choices may be influenced by a diversity of plant secondary compounds that could affect both adult and offspring performance (Mayhew, 2001). Plant secondary compounds are often thought to increase plant fitness in the presence of herbivores by reducing herbivore feeding rates (Fritz & Simms, 1992). However, not all secondary compounds deter insects and some even increase herbivore survival and reproduction (Vrieling *et al*., 1991; Fritz & Simms, 1992; Cole, 1997; Awmack & Leather, 2002), especially for phytophagous insects that specialize on plants with particular secondary compounds (Ehrlich & Raven, 1964). Theoretically, adult herbivore preference should evolve to optimize offspring performance, especially when there is no parental care and low

Correspondence: Susan E. Elliott, Department of Biological Sciences, Gilman Lab, Dartmouth College, Hanover, NH 03755, U.S.A. E-mail: susan.e.elliott@dartmouth.edu

offspring mobility (Mayhew, 1997). However, while some studies support these predicted positive preference-performance correlations (Craig *et al*., 1989; Barker & Maczka, 1996; Craig & Ohgushi, 2002; Groenteman *et al*., 2006), others do not (Thompson, 1988; Scheirs *et al*., 2000; Mayhew, 2001; Holland *et al*., 2004).

While secondary compounds are frequently found in leaves, where their effects on foliar herbivores have been studied extensively, they are also found in floral tissues (Euler & Baldwin, 1996; Irwin & Adler, 2006; McCall & Karban, 2006), including nectar (Baker, 1977; Adler, 2000; Adler & Irwin, 2005). For example, in a study of flowering species from diverse habitats in Costa Rica, California, and Colorado (26–275 species per habitat), alkaloids and phenolics were present in the nectar of 0–12% and 19–51%, respectively, of the species examined (Baker, 1977). For most species, it is not known how or why secondary compounds occur in nectar (e.g. independent physiological or genetic control of nectar compounds vs pleiotropic constraints with defence compounds in other plant tissues; Adler, 2000). Along with our limited understanding of the physiological mechanisms underlying nectar secondary compounds, very little is known about their effects on pollinating insect preference and performance (Adler, 2000). While secondary compounds in nectar might benefit plants by encouraging specialist pollinators (e.g. Johnson *et al.*, 2006) or deterring nectar robbers, they could also have indirect negative effects on plants by deterring or even poisoning their legitimate pollinators (Adler & Irwin, 2005).

Studies that have addressed the effects of secondary compounds on insect pollinator behaviour demonstrate that the effects are contingent on many factors, including the source plant species (Hagler & Buchmann, 1993), the consumer species (Stephenson, 1981, 1982; Adler & Irwin, 2005; Johnson *et al*., 2006), alternative nectar sources (Gegear *et al*., 2007), and the secondary compound concentration (Adler & Irwin, 2005; Singaravelan *et al*., 2005, 2006). In a survey of 64 allelochemicals, 39 reduced, three increased, and 22 had no significant effect on honey bee feeding rates (Detzel & Wink, 1993). In addition, compounds that are deterrent to honey bees are not necessarily toxic (London-Shafir *et al*., 2003; Kevan & Ebert, 2005), and others that are thought to be toxic can stimulate feeding (Liu & Fu, 2004; Liu *et al*., 2005). Native solitary bees may respond differently to nectar secondary compounds than social and largely introduced honey bees because native solitary bees generally do not store nectar and pollen resources and they may have had more time to adapt to plants with secondary compounds in their native range. In this study, the effects of a nectar alkaloid were tested on the performance of the offspring of a native solitary bee.

Alkaloids, found in 20% of flowering plant species, are the most diverse group of secondary metabolites (Hartmann, 1991), with equally diverse effects on animal physiologies (Macel *et al*., 2002, 2005). Alkaloids are known for their deterrent properties, harmful effects on the nervous system (Hartmann, 1991; Wink, 2000), and toxicity to honey bees at high concentrations (Detzel & Wink, 1993). However, the performance of some specialized insects is not affected by alkaloids, and some insects even sequester alkaloids for their own protection (Hartmann & Ober, 2000). In this study, it was hypothesized that the deterrent effects of a nectar alkaloid on adult foraging may reflect negative consequences of the alkaloid for pollinator offspring performance. To test this hypothesis, different concentrations of the alkaloid were added to native solitary bee pollinator offspring provisions, and the effects on larval development time, prepupa cocoon mass, adult emergence, and adult mass were measured. In addition, the effect of sugar addition on offspring performance was measured.

Materials and methods

Study system

Gelsemium sempervirens (hereafter, *Gelsemium*) is a perennial vine that is native to the southeastern United States (Ornduff, 1970). It blooms from early March to late April, producing several hundred yellow tubular flowers per plant. The flowers are self-incompatible and distylous, requiring animal visitors for effective pollination (Ornduff, 1970). *Gelsemium* nectar contains an alkaloid, gelsemine, which ranges in concentration from 5.8–246.1 ppm (mean = 48 ppm) (Adler & Irwin, 2005) and is also found in *Gelsemium* leaves and corollas (Irwin & Adler, 2006). Gelsemine in leaves and corollas is highly toxic to mammals (Kingsbury, 1964) and *Gelsemium* nectar may deter and possibly poison some bees (Eckert, 1946; Hardin & Arena, 1969). For example, Burnside and Vansell (1936) reported that honey bee larvae developed abnormally extended abdomens and eventually died after feeding on a light-coloured honey that was presumed to contain *Gelsemium* nectar.

At the study sites near Athens, Georgia (U.S.A.), experimentally augmented gelsemine concentrations in *Gelsemium* nectar generally reduced visitation by five pollinating bee species, including *Osmia lignaria lignaria* Say (Megachilidae), hereafter referred to as *Osmia* (Adler & Irwin, 2005). For example, in 2002, high levels of nectar gelsemine (3259 ppm) reduced the time *Osmia* spent per flower by 57% relative to plants with low levels of nectar gelsemine (approximately 12 ppm), and in 2004, naturally high levels (174.6 ppm) reduced the per cent of flowers probed per plant by *Osmia* by 40% (Adler & Irwin, 2005).

In nature, *Osmia* females provision several offspring individually in wood tunnels, using pollen and nectar from a diversity of plant species (Krombein, 1967). Each provision consists of approximately 50% pollen and 50% nectar by mass (Williams, 2003) and contains one egg. In our study area, female *Osmia* provision 8.3 ± 0.3 (mean ± 1 SE) offspring per 15-cm tunnel ($n = 54$ artificial tunnels). Over approximately three weeks, offspring consume their provision, pass through five larval stages, and spin a cocoon (Torchio, 1989). Offspring then pupate, over-winter as adults, and the following spring these adults emerge, mate, and establish new nests (Torchio, 1989).

Experimental nectar treatments

To test the effects of nectar gelsemine on *Osmia* offspring performance, gelsemine was added to field-collected provisions and offspring were reared under controlled conditions in the laboratory. A total of 431 *Osmia* offspring were collected from artificial nesting blocks (blocks of wood with holes containing paper straws) between late March and early April, 2004. In the study area, *Osmia* were observed foraging on *Gelsemium*, and 5% of their provisions contained *Gelsemium* pollen ($n = 136$ provisions) (S. E. Elliott, unpubl. data). Offspring were collected while *Gelsemium* was in bloom so that bees used in the experiment would be those that could normally encounter *Gelsemium*. However, bees were not collected from areas where *Gelsemium* was abundant, so that the control treatment (described below) would have little to no natural *Gelsemium* nectar or pollen in the provision. Pollen from a sub-sample of 136 provisions was identified to verify that there was no systematic bias in pollen species among treatments. Once nests were completed, they were moved to the lab and each was opened to expose provisions. Because adults anchor each egg into its provision, the provisions can be removed with the egg safely attached. Each provision (with egg attached) was transferred into a small plastic cup (cryogenic tube 10 mm wide cut to a height of 12 mm; Perfector Scientific Cryo-Store®, Atascadero, CA) and weighed to the nearest 0.01 mg. All provisions were treated prior to larvae reaching the second instar, when feeding begins (Torchio, 1989). Larvae reached feeding stages within 1–5 days after the nectar additions.

Individual provisions were treated with one of five nectar solutions: (i) water only, (ii) honey-water only, (iii) low (250 ppm) gelsemine in honey-water, (iv) high (5000 ppm) gelsemine in honey-water, or (v) natural nectar collected from *Gelsemium* flowers ($n = 35$–49 bees/sex/treatment; Table 1). After mixing a 30-µl dose of the randomly-assigned treatment into each provision (weighing approximately 200 mg), the low and high gelsemine concentrations in the entire provision would be approximately 37.5 ppm and 750 ppm, respectively. Thus, gelsemine concentrations in the provisions were just under average and three-times greater than the maximum gelsemine concentration that adult bees encounter in natural *Gelsemium* nectar in the wild (Adler & Irwin, 2005). The low gelsemine concentration was used to test whether offspring would suffer if adult females used solely gelsemine nectar in their provisions. The high gelsemine concentration was used as an exploratory measure to test whether high concentrations *could* affect *Osmia* offspring performance. Therefore, this treatment was not used to create inferences about toxicity of natural alkaloid levels but rather to explore *Osmia* offspring response to an extreme gelsemine concentration (as in nutrient enrichment and species composition manipulations Chapin *et al*., 1986; Power *et al*., 1998). Natural *Gelsemine* nectar was used to test how offspring performance would be affected if adults added a small amount of *Gelsemium* nectar, including all other *Gelsemium* nectar compounds, to the provision.

Table 1. Least squared means (±1 SE) and sample sizes (*n*) for female and male *Osmia* offspring in each nectar treatment and for each performance variable.

	Females		Males	
Treatment	LSMean (±1 SE)	*n*	LSMean (±1 SE)	*n*
Development time (days)				
Honey	15.7 (±0.3)	40	15.2 (±0.3)	46
Low	15.5 (±0.3)	39	15.2 (±0.3)	45
High	15.8 (±0.3)	41	15.3 (±0.3)	46
Natural	15.2 (±0.3)	37	15.1 (±0.3)	46
Water	16.0 (±0.4)	41	15.5 (±0.3)	49
Cocoon mass (mg)				
Honey	160.1 (±3.0)	40	103.3 (±2.1)	46
Low	161.7 (±2.9)	39	104.2 (±2.1)	46
High	160.7 (±2.9)	40	103.0 (±2.1)	45
Natural	159.6 (±3.0)	37	103.6 (±2.0)	46
Water	152.1 (±2.0)	41	89.8 (±2.6)	49
Adult mass (mg)				
Honey	102.8 (±1.2)	37	60.8 (±0.6)	42
Low	101.1 (±1.2)	36	61.4 (±0.6)	42
High	101.1 (±1.2)	35	60.8 (±0.6)	39
Natural	101.4 (±1.2)	36	61.1 (±0.6)	44
Water	94.4 (±0.9)	36	56.7 (±0.6)	46

The nectar treatments were made as follows. For artificial gelsemine treatments, gelsemine hydrochloride (Indofine Chemical Company, Hillsborough, New Jersey, U.S.A.) was mixed into a 60% honey-water solution, which matches the natural sugar concentration of *Gelsemium* nectar found in 2004 (S. E. Elliott, unpubl. data). A local organic wildflower honey was used so that natural nectar constituents that might have synergistic effects with the alkaloid would be included to better simulate natural nectar additions. If nectar harvested by bees for entire provisions averaged 40% sugar, and given that provisions are roughly 50% nectar (Williams, 2003), then the additional honey-water represented a 45% sugar increase from the original untreated provisions. The water-only treatment was included as a control to determine if the amount of sugar added affected offspring performance. For the natural *Gelsemium* nectar treatment, nectar was collected from *Gelsemium* flowers growing in Athens, GA. Prior to use, the artificial and natural nectar solutions were stored at −4 °C.

To ensure that offspring sex was not confounded with nectar treatment and to test for sex-specific effects, offspring sex was estimated before assignment to nectar treatments using nest position and provision mass, which are indicators of gender for this species (Torchio, 1989). Females generally have larger provisions than males, and females are generally provisioned at the back of the straw (Torchio, 1989). At the end of the experiment, sex was confirmed under a dissecting microscope at 4×. Because the same adult female likely provisioned all offspring within a straw, treatments were divided within straws to control for maternal effects. However, there were not enough offspring per straw of each sex to block treatments by straw.

Bee performance

Offspring performance was measured as larval development time, cocoon mass, probability of adult emergence, and adult mass. Offspring were kept in a dark 25°C chamber and monitored daily as they passed through each of the five larval instars.

Larval development time was measured as the number of days between the first and fifth larval instars. The fifth larval instar, the prepupa, was defined as the point at which larvae initiated cocoon spinning. Prepupae in their cocoons were weighed to the nearest 0.01 mg as soon as spinning was completed and faeces were removed. Body mass can be an important indicator of future fecundity and survival of megachilid bees (Tepedino & Torchio, 1982; Kim, 1997; Steffan-Dewenter & Schiele, 2004). After cocoons were spun, the bees were transferred into a dark container and kept at 21 °C until early October to simulate normal dormancy periods (Bosch & Kemp, 2000). In October, cocoons were transferred to a 4°C room to simulate over-wintering conditions. In March 2005, each cocoon was moved into a greenhouse, transferred into a 1.5-ml micro-centrifuge tube with an air hole in the top, allowed to emerge in the tubes, and frozen within 1 h of emergence. The frozen adult bees were weighed to the nearest 0.01 mg and their sexes confirmed.

Statistical analyses

ANCOVAs were used to test the effects of nectar treatment and provision mass on development time, prepupa cocoon mass, and adult mass. Analyses were separated by sex because the range of the covariate (provision mass) for males was smaller than females (male provision mass: 134–254 mg; female provision mass: 107–469 mg). In addition, development time increased more steeply with provision mass for males than females (ANCOVA sex × provision interaction: $F_{1,1} = 10.6, P = 0.0013$), suggesting that males and females had qualitatively different physiological responses to provisions and should be analysed separately. All provision mass × nectar treatment interactions were non-significant ($P \geq 0.28$) except for male development time ($F_{3,175} = 4.0$, $P = 0.008$) and female adult mass ($F_{3,136} = 2.9$, $P = 0.04$). However, homogeneity of slopes tests are sensitive to finding different slopes (Sokal & Rohlf, 1995), especially with large sample sizes and numerous treatment levels (Quinn & Keough, 2002). Thus, because scatter plots of the data showed no detectable differences in slopes by eye, the recommendations of Quinn and Keough (2002) were followed, and ANCOVAs were used for all response variables.

Indicator-variable multiple logistic regressions were used to test the effects of nectar treatment and provision mass on the probability of survival from egg to adult for both sexes. Before emergence in the greenhouse, seven bees were lost when they were displaced from their cups, so they were omitted from adult mass and survival analyses. One additional bee escaped from its tube during emergence, so it was included in analyses for survival, but not adult mass.

To test for gelsemine effects on offspring performance, the analyses included four nectar treatments: honey only, low gelsemine, high gelsemine, and natural nectar. If gelsemine influenced any aspect of offspring performance, then offspring performance should vary among gelsemine levels or between gelsemine and honey-only treatments. To test for sugar effects on offspring performance, the analysis compared honey-only and water-only treatments. The four metrics of offspring performance (larval development time, cocoon mass, adult mass, and adult survival) were analysed separately because mortality during development eliminated individuals at later stages (Table 1). Also, for one bee, the date of spinning initiation was not recorded so that bee was not included in the development time analysis. Type III sums of squares and least squared means were used to account for unbalanced sample sizes among treatments. All statistical analyses were performed using SAS Version 8.2; PROC GLM was used for the ANCOVAs, and PROC GENMOD was used for the logistic regressions (SAS Institute, 2001).

Results

Gelsemine addition

Gelsemine addition to larval provisions did not affect any aspect of offspring performance (Tables 1, 2). On average, female provisions were 51% heavier than male provisions, leading to females that were 67% heavier as adults. Average development time increased with provision mass (Table 2). Offspring survival was high overall (394 out of the original 424 bees survived). Survival was highest in the natural nectar treatment (96.4%), followed by high gelsemine (94.0%), honey-only (91.9%), and low gelsemine (86.0%) treatments. Neither nectar treatments nor provision mass had a statistically significant effect on the probability of survival from egg to adult (nectar treatment: $\chi^2 = 6.6$, $P = 0.09$; provision mass: $\chi^2 = 2.7$, $P = 0.10$).

Table 2. Results of ANCOVAs, Type III SS, testing the effects of gelsemine nectar treatment (honey-water, low artificial, high artificial, and natural nectar) and provision mass on offspring development time, cocoon mass, and adult mass.

		Development time			Cocoon mass			Adult mass		
Source	d.f.	SS	*F*	*P*	SS	*F*	*P*	SS	*F*	*P*
Female										
Nectar treatment	3	8	1.0	0.40	120	0.1	0.95	73	0.5	0.68
Provision mass	1	194	71.1	**<0.0001**	72 412	213.4	**<0.0001**	29 097	593.6	**<0.0001**
Male										
Nectar treatment	3	1	0.1	0.95	32	0.1	0.98	9	1.9	0.13
Provision mass	1	130	53.7	**<0.0001**	8095	42.1	**<0.0001**	3095	209.0	**<0.0001**

Effects in bold are statistically significant at $P < 0.05$.

Sugar addition

The additional sugar in the honey-only treatment affected offspring mass but not development time (Table 3). Compared with the water-only treatment, cocoon mass in the honey-water treatment was 5% greater for females and 15% greater for males, and adult mass was 9% greater for females and 7% greater for males. On average, female and male bees with the honey-water addition developed 0.3 days sooner, but this effect was not statistically significant ($P \geq 0.08$; Table 3). Finally, as in the gelsemine treatments, provision mass affected development time and offspring mass (Table 3), but there were no significant effects of the sugar addition in the honey-water treatment or of provision mass on survival (sugar addition: $\chi^2 = 2.3$, $P = 0.13$; provision mass: $\chi^2 = 2.1$, $P = 0.15$).

Discussion

Insect pollinators face a diversity of plant species on which to forage. Nectar and pollen provide the sole energy and protein source for most bee pollinators but may contain secondary compounds, which could affect adult foragers and the offspring they provision. In contrast to the well-studied roles of plant secondary compounds on foliar herbivores, the effects of nectar secondary compounds on bee pollinator foraging and offspring performance remain relatively unexplored. In this study, natural and experimental concentrations of a nectar alkaloid, gelsemine, had no effect on the performance of *Osmia* offspring under the experimental conditions studied. Although the alkaloid is toxic to vertebrates (Kingsbury, 1964), potentially toxic to non-native honey bees (Burnside & Vansell, 1936), and deterrent to native *Osmia* adults (Adler & Irwin, 2005), offspring of *Osmia* were not affected by even unnaturally high concentrations of the alkaloid.

At least two non-mutually exclusive hypotheses may explain why secondary compounds found in nectar deter *Osmia* adults but have no effect on *Osmia* offspring performance. First, *Osmia* offspring may not be harmed by the alkaloid if they can physicologically detoxify or sequester the compound. While the ability of *Osmia* larvae to detoxify or sequester secondary compounds is unknown, honey bees can detoxify some secondary compounds (Yu *et al*., 1984), and their ability to detoxify can depend on their developmental stage (Smirle & Winston, 1988). If *Osmia* larvae had the ability to sequester secondary compounds to make themselves less palatable, this might benefit them as they are susceptible to numerous predators, parasites, and pathogens (Bosch & Kemp, 2001). Second, although larvae fed on the provisions within 1–5 days of alkaloid addition, it is possible that the compound broke down into a non-toxic form by the time of consumption (Liu *et al*., 2005). Similarly, other natural nectar constituents (e.g. Manson *et al*., 2007) in the provision or honey may have counteracted the gelsemine.

Harvesting or avoiding alkaloid-rich nectar may have different effects on adult and larval energetics, depending on the ecological context. Both adult and larval bees consume nectar to fuel their energetic requirements. Each 200 mg larval provision contains approximately 40 mg of sugar (given 40% nectar sugar concentration); this amount of sugar would supply adults with energy for over 10 h in flight (Heinrich, 1979). If alkaloids negatively affect adult performance (e.g. vigor, fecundity, survival), then avoiding alkaloid-rich nectar may benefit adults when alternative nectar sources are abundant. However, if alternative nectar resources are scarce, avoiding *Gelsemium* may come at the cost of reduced sugar intake for adults, as well as reduced rate of offspring provisioning. Therefore, adults may have greater tolerance for harvesting alkaloid-containing nectars if nectar resources are limited (London-Shafir *et al*., 2003). For example, bumble bees (*Bombus impatiens* Cresson Apidae) only preferred artificial flowers with gelsemine when non-gelsemine flowers had low sugar concentrations relative to the flowers with gelsemine (Gegear *et al*., 2007). Accordingly, the current study supports the prediction that the benefit of increased sugar content in provisions outweighs any costs of even excessively high gelsemine concentrations.

Herbivore foraging theories suggest a variety of mechanisms to explain mismatches in preference-performance relationships, including physical, physiological, and genetic constraints (Bernays, 1991, 1999; Futuyma *et al*., 1995), host variability (Thompson, 1988), and trade-offs between adult and offspring performance (Scheirs *et al*., 2000). Given that alkaloids and sugar in nectar additions had neutral and positive effects, respectively, on pollinator offspring performance, and that past studies demonstrated negative effects of alkaloids on adult preference (Adler & Irwin, 2005), these results suggest potential energetic trade-offs at the larval versus adult stage. The benefit of avoiding versus harvesting, consuming, and

Table 3. Results of ANCOVAs, Type III SS, testing the effects of sugar addition (honey-water vs. water-only) and provision mass on offspring development time, cocoon mass, and adult mass.

		Development time			Cocoon mass			Adult mass		
Source	d.f.	SS	*F*	*P*	SS	*F*	*P*	SS	*F*	*P*
Female										
Sugar addition	3	1	0.3	0.62	1429	8.4	**0.005**	1375	44.9	**<0.0001**
Provision mass	1	86	25.8	**<0.0001**	40 038	235.9	**<0.0001**	16 151	527.5	**<0.0001**
Male										
Sugar addition	3	7	3.1	0.08	3856	12.0	**0.0008**	322	23.1	**<0.0001**
Provision mass	1	20	9.1	**0.0033**	4695	14.7	**0.0002**	1863	133.6	**<0.0001**

Effects in bold are statistically significant at $P < 0.05$.

provisioning offspring with alkaloid-rich nectar may depend on the degree to which nectar availability in the environment is limiting to adult and/or offspring performance, as well as physiological constraints and alternative resources for adults and offspring.

Our current knowledge of insect pollinator preference and performance relates largely to variation in floral morphologies and nutritional floral rewards (Chittka & Thomson, 2001). This study confirms that bee pollinator behaviours that increase nutritional rewards, such as sugar, will benefit offspring performance (Schmidt *et al*., 1987; Roulston & Cane, 2002). However, adult behavioural responses to non-nutritional quality components (e.g. avoidance of nectar secondary compounds) may not link directly to pollinator offspring performance.

Acknowledgements

We thank J. Barron, G. Crutsinger, B. DeGasperis, A. Lentz, and H. Norden for collecting *Gelsemium* nectar and L. Burkle for providing comments on the manuscript. This research was supported by a grant from the National Science Foundation (NSF DEB-0211480 to L. S. Adler and R. E. Irwin).

References

Adler, L.S. (2000) The ecological significance of toxic nectar. *Oikos*, **91**, 409–420.

Adler, L.S. & Irwin, R.E. (2005) Ecological costs and benefits of defenses in nectar. *Ecology*, **86**, 2968–2978.

Awmack, C.S. & Leather, S.R. (2002) Host plant quality and fecundity in herbivorous insects. *Annual Review of Entomology*, **47**, 817–844.

Baker, H.G. (1977) Non-sugar chemical constituents of nectar. *Apidologie*, **8**, 349–356.

Barker, A.M. & Maczka, C.J.M. (1996) The relationships between host selection and subsequent larval performance in three free-living graminivorous sawflies. *Ecological Entomology*, **21**, 317–327.

Bernays, E.A. (1991) Evolution of insect morphology in relation to plants. *Philosophical Transactions of the Royal Society of London Series B Biological Sciences*, **333**, 257–264.

Bernays, E.A. (1999) Plasticity and the problem of choice in food selection. *Annals of the Entomological Society of America*, **92**, 944–951.

Bosch, J. & Kemp, W.P. (2000) Development and emergence of the orchard pollinator *Osmia lignaria* (Hymenoptera: Megachilidae). *Environmental Entomology*, **29**, 8–13.

Bosch, J. & Kemp, W.P. (2001) *How to Manage the Blue Orchard Bee*. Sustainable Agriculture Network, Beltsville, Maryland.

Burnside, C.E. & Vansell, G.H. (1936). *Plant Poisoning of Bees*, Report No. E-398. USDA, Bureau of Entomology and Plant Quarantine, Washington, District of Columbia.

Chapin, F.S., Vitousek, P.M. & Cleve, K.V. (1986) The nature of nutrient limitation in plant communities. *American Naturalist*, **127**, 48–58.

Chittka, L. & Thomson, J.D. (2001) *Cognitive Ecology of Pollination: Animal Behavior and Floral Evolution*. University Press, Cambridge, U.K.

Cole, R.A. (1997) The relative importance of glucosinolates and amino acids to the development of two aphid pests *Brevicoryne brassicae* and *Myzus persicae* on wild and cultivated brassica species. *Entomologia Experimentalis et Applicata*, **85**, 121–133.

Craig, T.P., Itami, J.K. & Price, P.W. (1989) A strong relationship between oviposition preference and larval Performance in a shoot-galling sawfly. *Ecology*, **70**, 1691–1699.

Craig, T.P. & Ohgushi, T. (2002) Preference and performance are correlated in the spittlebug *Aphrophora pectoralis* on four species of willow. *Ecological Entomology*, **27**, 529–540.

Detzel, A. & Wink, M. (1993) Attraction, deterrence or intoxication of bees (*Apis mellifera*) by plant allelochemicals. *Chemoecology*, **4**, 8–18.

Eckert, J.E. (1946). Injury to bees by poisoning. *The Hive and the Honey Bee* (ed. by R. A. Grout), pp. 570–582. Dadant and Sons, Hamilton, Illinois.

Ehrlich, P.R. & Raven, P.H. (1964) Butterflies and plants–a study in coevolution. *Evolution*, **18**, 586–608.

Euler, M. & Baldwin, I.T. (1996) The chemistry of defense and apparency in the corollas of *Nicotiana attenuata*. *Oecologia*, **107**, 102–112.

Fritz, R.S. & Simms, E.L. (ed.) (1992) *Plant Resistance to Herbivores and Pathogens: Ecology, Evolution, and Genetics*. University of Chicago Press, Chicago, Illinois.

Futuyma, D.J., Keese, M.C. & Funk, D.J. (1995) Genetic constraints on macroevolution–the evolution of host affiliation in the leaf beetle genus *Ophraella*. *Evolution*, **49**, 797–809.

Gegear, R.J., Manson, J.S. & Thomson, J.D. (2007) Ecological context influences pollinator deterrence by alkaloids in floral nectar. *Ecology Letters*, **10**, 375–382.

Groenteman, R., Guershon, M. & Coll, M. (2006) Effects of leaf nitrogen content on oviposition site selection, offspring performance, and intraspecific interactions in an omnivorous bug. *Ecological Entomology*, **31**, 155–161.

Hagler, J.R. & Buchmann, S.L. (1993) Honey-bee (Hymenoptera, Apidae) foraging responses to phenolic-rich nectars. *Journal of the Kansas Entomological Society*, **66**, 223–230.

Hardin, J.W. & Arena, J.M. (1969) *Human Poisoning from Native and Cultivated Plants*. Duke University Press, Durham, North Carolina.

Hartmann, T. (1991) Alkaloids. *Herbivores: Their Interactions with Secondary Metabolites* (ed. by G. Rosenthal and M. Berenbaum), pp. 79–116. Academic Press, San Diego, California.

Hartmann, T. & Ober, D. (2000) Biosynthesis and metabolism of pyrrolizidine alkaloids in plants and specialized insect herbivores. *Topics in Current Chemistry*, **Vol 209**, 207–243.

Heinrich, B. (1979) *Bumblebee Economics Harvard*. University Press, Cambridge, Massachusetts.

Holland, J.N., Buchanan, A.L. & Loubeau, R. (2004) Oviposition choice and larval survival of an obligately pollinating granivorous moth. *Evolutionary Ecology Research*, **6**, 607–618.

Irwin, R.E. & Adler, L.S. (2006) Correlations among traits associated with herbivore resistance and pollination: implications for pollination and nectar robbing in a distylous plant. *American Journal of Botany*, **93,** 64–72.

Johnson, S.D., Hargreaves, A.L. & Brown, M. (2006) Dark, bitter-tasting nectar functions as a filter of flower visitors in a bird-pollinated plant. *Ecology*, **87**, 2709–2716.

Kevan, P.G. & Ebert, T. (2005) Can almond nectar & pollen poison honey bees? *American Bee Journal*, **145**, 507–509.

Kim, J.Y. (1997) Female size and fitness in the leaf-cutter bee *Megachile apicalis*. *Ecological Entomology*, **22**, 275–282.

Kingsbury, J.M. (1964) *Poisonous Plants of the United States and Canada*. Prentice-Hall, Englewood Cliffs, New Jersey.

Krombein, K.V. (1967) *Trap-Nesting Wasps and Bees: Life Histories, Nests and Associates*. Smithsonian Press, Washington, District of Columbia.

Liu, F.L. & Fu, W.J. (2004) Plant with toxic nectar kills native honey bees in SW China. *American Bee Journal*, **144**, 707–708.

Liu, F.L., He, J.Z. & Fu, W.J. (2005) Highly controlled nest homeostasis of honey bees helps deactivate phenolics in nectar. *Naturwissenschaften*, **92**, 297–299.

London-Shafir, I., Shafir, S. & Eisikowitch, D. (2003) Amygdalin in almond nectar and pollen–facts and possible roles. *Plant Systematics and Evolution*, **238**, 87–95.

Macel, M., Bruinsma, M., Dijkstra, S.M., Ooijendijk, T., Niemeyer, H. M. & Klinkhamer, P.G.L. (2005) Differences in effects of pyrrolizidine alkaloids on five generalist insect herbivore species. *Journal of Chemical Ecology*, **31**, 1493–1508.

Macel, M., Klinkhamer, P.G.L., Vrieling, K. & van der Meijden, E. (2002) Diversity of pyrrolizidine alkaloids in *Senecio* species does not affect the specialist herbivore Tyria jacobaeae. *Oecologia*, **133**, 541–550.

Manson, J.S., Lachance, M.A. & Thomson, J.D. (2007) *Candida gelsemii* sp nov., a yeast of the Metschnikowiaceae clade isolated from nectar of the poisonous Carolina jessamine. Antonie Van Leeuwenhoek. *International Journal of General and Molecular Microbiology*, **92**, 37–42.

Mayhew, P.J. (1997) Adaptive patterns of host-plant selection by phytophagous insects. *Oikos*, **79**, 417–428.

Mayhew, P.J. (2001) Herbivore host choice and optimal bad motherhood. *Trends in Ecology & Evolution*, **16**, 165–167.

McCall, A.C. & Karban, R. (2006) Induced defense in *Nicotiana attenuata* (Solanaceae) fruit and flowers. *Oecologia*, **146**, 566–571.

Ornduff, R. (1970) Systematics and breeding system of *Gelsemium* (Loganiaceae). *Journal of the Arnold Arboretum*, **51**, 1–17.

Power, M.E., Dietrich, W.E. & Sullivan, K.O. (1998). Experimentation, observation, and inference in river and watershed investigations. *Experimental Ecology Issues and Perspectives* (ed. by W. J. J. Resetarits), pp. 113–132. Oxford University Press, New York.

Quinn, G. & Keough, M. (2002) *Experimental Design and Data Analysis for Biologists*. Cambridge University Press, New York.

Roulston, T.H. & Cane, J.H. (2002) The effect of pollen protein concentration on body size in the sweat bee *Lasioglossum zephyrum* (Hymenoptera: Apiformes). *Evolutionary Ecology*, **16**, 49–65.

SAS Institute (2001) JMP 4.04, SAS Institute, Carey, North Carolina.

Scheirs, J., De Bruyn, L. & Verhagen, R. (2000) Optimization of adult performance determines host choice in a grass miner. *Proceedings of the Royal Society of London Series B Biological Sciences*, **267**, 2065–2069.

Schmidt, J.O., Thoenes, S.C. & Levin, M.D. (1987) Survival of honeybees, *Apis mellifera* (Hymenoptera, Apidae), fed various pollen sources. *Annals of the Entomological Society of America*, **80**, 176–183.

Singaravelan, N., Inbar, M., Ne'eman, G., Distl, M., Wink, M. & Izhaki, I. (2006) The effects of nectar-nicotine on colony fitness of caged honeybees. *Journal of Chemical Ecology*, **32**, 49–58.

Singaravelan, N., Nee'man, G., Inbar, M. & Izhaki, I. (2005) Feeding responses of free-flying honeybees to secondary compounds mimicking floral nectars. *Journal of Chemical Ecology*, **31**, 2791–2804.

Smirle, M.J. & Winston, M.L. (1988) Detoxifying enzyme-activity in worker honey bees–an adaptation for foraging in contaminated ecosystems. *Canadian Journal of Zoology-Revue Canadienne De Zoologie*, **66**, 1938–1942.

Sokal, R. & Rohlf, F. (1995) *Biometry: The Principles and Practice of Statistics in Biological Research*, 3rd edn. W.H. Freeman and Company, New York.

Steffan-Dewenter, I. & Schiele, S. (2004) Nest-site fidelity, body weight and population size of the red mason bee, *Osmia rufa* (Hymenoptera: Megachilidae), evaluated by Mark-Recapture experiments. *Entomologia Generalis*, **27**, 123–132.

Stephenson, A.G. (1981) Toxic nectar deters nectar thieves of *Catalpa speciosa*. *American Midland Naturalist*, **105**, 381–383.

Stephenson, A.G. (1982) Iridoid glycosides in the nectar of *Catalpa speciosa* are unpalatable to nectar thieves. *Journal of Chemical Ecology*, **8**, 1025–1034.

Tepedino, V.J. & Torchio, P.F. (1982) Phenotypic variability in nesting success among *Osmia lignaria propinqua* females in a glasshouse environment (Hymenoptera, Megachilidae). *Ecological Entomology*, **7**, 453–462.

Thompson, J.N. (1988) Evolutionary ecology of the relationship between oviposition preference and performance of offspring in phytophagous insects. *Entomologia Experimentalis et Applicata*, **47**, 3–14.

Torchio, P.F. (1989) In-nest biologies and development of immature stages of 3 *Osmia* species (Hymenoptera, Megachilidae). *Annals of the Entomological Society of America*, **82**, 599–615.

Vrieling, K., Smit, W. & Vandermeijden, E. (1991) Tritrophic Interactions between aphids (*Aphis jacobaeae* Schrank), ant species, *Tyria jacobaeae* L, and *Senecio jacobaea* L lead to maintenance of genetic-variation in pyrrolizidine alkaloid concentration. *Oecologia*, **86**, 177–182.

Williams, N.M. (2003) Use of novel pollen species by specialist and generalist solitary bees (Hymenoptera: Megachilidae). *Oecologia*, **134**, 228–237.

Wink, M. (2000). Interference of alkaloids with neuroreceptors and ion channels. *Bioactive Natural Products* (ed. by Atta-Ur-Rahman), Vol 11, pp. 3–129. Elsevier, Amsterdam, the Netherlands.

Yu, S.J., Robinson, F.A. & Nation, J.L. (1984) Detoxication capacity in the honey bee, *Apis mellifera* L. *Pesticide Biochemistry and Physiology*, **22**, 360–368.

Accepted 12 November 2007