

THE EFFECT OF LARVAL DIET AND SEX ON NECTAR NICOTINE FEEDING PREFERENCES IN *MANDUCA SEXTA* (LEPIDOPTERA: SPHINGIDAE)

DAVID N. SHARP¹, AMANDA J. LENTZ-RONNING¹, JOHANNA BARRON¹ AND LYNN S. ADLER²

¹Department of Biology, Virginia Polytechnic Institute and State University, Blacksburg VA

²Department of Plant, Soil, and Insect Science, University of Massachusetts, Amherst MA

Many Lepidoptera interact with the same plant species as both herbivorous larvae and nectar-feeding adults (Adler & Bronstein 2004), providing the potential for plant secondary compounds to influence both pollination and herbivory through expression in floral and foliar tissue. Secondary compounds in nectar may be costly to plants if they deter pollinators, but beneficial if they deter oviposition of herbivorous offspring. Several studies have examined how pollinators respond to secondary compounds in nectar (Detzel & Wink 1993; Adler 2000; Liu et al. 2004; Tadmor-Melamed et al. 2004; Adler & Irwin 2005; Singaravelan et al. 2005; Kessler & Baldwin 2007). However, foraging responses can vary between individuals due to several factors, including sex and previous experience. For example, female insects often require amino acids for egg maturation (Jervis et al. 2005), so sexes may differ in foraging criteria. Larval experience could also influence adult oviposition (e.g., Hopkin's host selection principle, Barron 2001).

We asked how sex and larval exposure to nicotine affected adult feeding and oviposition in response to nicotine in nectar. *Manduca sexta* L. (Lepidoptera: Sphingidae) is a larval specialist herbivore on solanaceous plants including *Nicotiana* species (e.g., Madden & Chamberlin 1945; Lou & Baldwin 2003), and non-nicotine species such as tomato (Chen et al. 2005). Thus, some larvae consume nicotine while others do not. Several *Nicotiana* species have nicotine in nectar (Detzel & Wink 1993; Raguso et al. 2003; Adler et al. 2006; Kessler & Baldwin 2007).

First instars of *M. sexta* (North Carolina State University Insectary) were placed in individual 170-g cups with plastic lids and randomly assigned to no-nicotine or nicotine treatments ($n = 177$ and 173) with synthetic diet (F9783B, Bioserv, Inc. Frenchtown, NJ, USA) with or without 2% incorporated nicotine by wet weight ((-) nicotine, Sigma-Aldrich, Inc. N3876), well within the range in tobacco plants (Sisson & Severson 1990). Larvae were provided fresh diet *ad libitum* and allowed to pupate. We recorded mortality, time to pupation, pupal weight, and sex for each individual. Pupae were then divided into 4 greenhouse (9.5:14.5 L:D, temperature 25-26°C) cages (55-61 cm³) by larval diet treatment and sex. After emergence, moths were marked on their forewings to indicate larval diet treatment and sex, and transferred to a holding cage for mating and feeding.

We measured moth feeding preferences with artificial flowers (Kinko's gray fleck paper, Goyret & Raguso 2006) with 3 nectar compositions: high (0.0005% nicotine; 5 ppm), low (0.0001% nicotine; 1 ppm), and no nicotine. Flowers were placed into 1.5-mL microcentrifuge vials containing 0.5-mL of synthetic nectar without touching the nectar. Nectar was made from a 14% sugar solution (2:1:1 ratio of sucrose: glucose: fructose) to match the sucrose equivalents of *N. tabacum* nectar (Adler, unpublished data) and the ratio of sugars in other *Nicotiana* species (Kaczorowski et al. 2005). Nicotine concentrations were based on levels in *N. tabacum* (low nicotine) and *N. quadrivalis* nectar (high nicotine) (Adler et al., unpublished data), which *M. sexta* moths encounter in the wild (Madden & Chamberlin 1945; Lou & Baldwin 2003).

Moths were placed in trial cages for individual testing. Cages contained 6 artificial flowers (3 nectar treatments x 2 replicates) in stands wiped with 50% ethanol each night to remove pheromones. Trials lasted 24 h, after which moths and vials were weighed and we counted eggs on flowers and vials. Vial weights were compared with starting weights to calculate nectar consumption. A control experiment found no effect of nicotine concentration on evaporation ($F_{2, 24} = 1.92$, $P > 0.15$).

Analyses were conducted in SAS v9.1 (SAS-Institute 2004). Nectar consumption was analyzed by ANCOVA, with moth weight and age as covariates, and cage and date as blocks. Larval diet, sex, and nectar treatment were main fixed effects and all interactions between main effects were included. Responses were averaged across the 2 vials per treatment per cage each night, and log-transformed. Number of eggs was analyzed with the same model (without sex) for female moths only.

Nicotine increased larval mortality and time to pupation and reduced pupal weight (data not shown); negative effects of nicotine on larval performance are consistent with previous studies (Bentz & Barbosa 1990; Appel & Martin 1992; Glendinning 2002; Kester et al. 2002).

Adult nectar consumption was reduced in moths that consumed nicotine as larvae ($F_{1, 372} = 13.75$, $P = 0.0002$), and males drank more nectar than females ($F_{1, 372} = 23.80$, $P < 0.0001$). These differences are not simply due to weight variation because moth weight was included as a covariate. Nectar consumption also varied with cage and date

($P < 0.002$ for both). There was a significant interaction between sex and nectar nicotine concentration on consumption ($F_{1,372} = 3.6$, $P = 0.028$), with females deterred by nicotine in nectar more than males (Fig. 1). Oviposition was not affected by treatments or interactions ($F < 0.6$, $P > 0.4$ for all).

Manduca sexta adults responded in a sex-specific manner to nicotine in nectar. Female aversion to secondary compounds in nectar may be adaptive if such compounds interfere with survival or egg development, or if nicotine in nectar provides information about host quality for offspring. Concentrations of nicotine in leaves and nectar were phenotypically correlated within *N. tabacum* (Adler et al. 2006) and across *Nicotiana* species (Adler et al., unpublished data), but not within *N. quadrivalvis* (S. Halpern, L. S. Adler & M. Wink, unpublished data). Thus, in some but not all cases, nicotine in nectar could provide information to female moths about foliar nicotine levels that may be experienced by offspring. Nicotine in nectar could be a novel pro-active defense against oviposition by deterring female moths without influencing visits by males, which pollinate without laying eggs, but our results on oviposition preference do not support this hypothesis. Further tests with real flowers with manipulated nectar may provide greater insights into moth behavior. Alternatively, nicotine in nectar could be costly to plants by deterring potential pollinators (Adler & Irwin 2005). In fact, some wild tobacco species have decreased floral nicotine concentrations at dusk and dawn, when most visits by *M. sexta* occur (Euler & Baldwin 1996; Raguso et al. 2003). Nicotine did not influence male nectar consumption; although adult *M. sexta* do not sequester alkaloids, perhaps these specialized herbivores are more tolerant of nicotine as adults than other pollinators that do not encounter nicotine outside of nectar feeding. Future research should examine sex-specific differences in the physiology of tolerance and detoxification to shed light on the distinct responses of male and female moths to nectar alkaloids.

We thank B. Reynolds, G. Evanylo, K. Haering, and D. Wiley for assistance; K. Kester, L. Phelan, E. Preisser, R. A. Raguso and 2 anonymous re-

viewers for manuscript comments; and the Virginia Tech Department of Biology and National Science Foundation Grant No. DEB-0211480 for funding.

SUMMARY

Secondary compounds in nectar could be costly to plants by deterring pollinators, but beneficial if they reduce oviposition by nectar-feeding adults with herbivorous larvae. We found nectar consumption in *Manduca sexta* varied by sex in response to nicotine in nectar, with greater aversion in females than males. Thus feeding responses to nectar secondary compounds may be sex-specific, warranting consideration of both sexes when studying nectar-mediated interactions.

REFERENCES CITED

- ADLER, L. S. 2000. The ecological significance of toxic nectar. *Oikos* 91: 409-420.
- ADLER, L. S., AND BRONSTEIN, J. L. 2004. Attracting antagonists: Does floral nectar increase leaf herbivory? *Ecology* 85: 1519-1526.
- ADLER, L. S., AND IRWIN, R. E. 2005. Ecological costs and benefits of defenses in nectar. *Ecology* 86:2968-2978.
- ADLER, L. S., WINK, M., DISTL, M., AND LENTZ, A. J. 2006. Leaf herbivory and nutrients increase nectar alkaloids. *Ecology Letters* 9: 960-967.
- APPEL, H. M., AND MARTIN, M. M. 1992. Significance of metabolic load in the evolution of host specificity of *Manduca sexta*. *Ecology* 73:216-228.
- BARRON, A. B. 2001. The life and death of Hopkins' host-selection principle. *Journal of Insect Behavior* 14: 725-737.
- BENTZ, J. A., AND BARBOSA, P. 1990. Effects of dietary nicotine (0.1%) and parasitism by *Cotesia congregata* on the growth and food consumption and utilization of the tobacco hornworm, *Manduca sexta*. *Entomol. Exp. Appl.* 57: 1-8.
- CHEN, H., WILKERSON, C. G., KUCHAR, J. A., PHINNEY, B. S., AND HOWE, G. A. 2005. Jasmonate-inducible plant enzymes degrade essential amino acids in the herbivore midgut. *Proc. Natl. Acad. Sci., USA* 102: 19237-19242.
- DETZEL, A., AND WINK, M. 1993. Attraction, deterrence or intoxication of bees (*Apis mellifera*) by plant allelochemicals. *Chemoecol.* 4: 8-18.
- EULER, M., AND BALDWIN, I. T. 1996. The chemistry of defense and apparency in the corollas of *Nicotiana attenuata*. *Oecologia* 107: 102-112.
- GLENDINNING, J. I. 2002. How do herbivorous insects cope with noxious secondary plant compounds in their diet? *Entomol. Exp. Appl.* 104: 15-25.
- GOYRET, J., AND RAGUSO, R. A. 2006. The role of mechanosensory input in flower handling efficiency and learning by *Manduca sexta*. *J. Exp. Biol.* 209: 1585-1593.
- JERVIS, M. A., BOGGS, C. L., AND FERNS, P. N. 2005. Egg maturation strategy and its associated trade-offs: a synthesis focusing on Lepidoptera. *Ecol. Entomol.* 30: 359-375.
- KACZOROWSKI, R. L., GARDENER, M. C., AND HOLTSFORD, T. P. 2005. Nectar traits in *Nicotiana* section

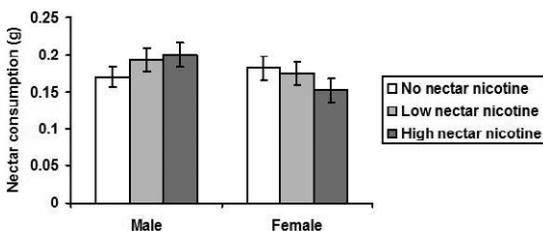


Fig. 1. Interaction between moth sex and nectar nicotine concentrations on adult nectar feeding in *Manduca sexta*. Error bars represent ± 1 SE.

- Alatae (Solanaceae) in relation to floral traits, pollinators, and mating system. *American J. Bot.* 92: 1270-1283.
- KESSLER, D., AND BALDWIN, I. T. 2007. Making sense of nectar scents: the effects of nectar secondary metabolites on floral visitors of *Nicotiana attenuata*. *Plant J.* 49: 840-854.
- KESTER, K. M., PETERSON, S. C., HANSON, F., JACKSON, D. M., AND SEVERSON, R. F. 2002. The roles of nicotine and natural enemies in determining larval feeding site distributions of *Manduca sexta* L. and *Manduca quinquemaculata* (Haworth) on tobacco. *Chemoecol.* 12: 1-10.
- LIU, F. L., FU, W. J., YANG, D. R., PENG, Y. Q., ZHANG, X. W., AND HE, J. Z. 2004. Reinforcement of bee-plant interaction by phenolics in food. *J. Apicul. Res.* 43: 155-157.
- LOU, Y., AND BALDWIN, I. T. 2003. *Manduca sexta* recognition and resistance among allopolyploid *Nicotiana* host plants. *Proc. Natl. Acad. Sci., USA* 100: 14581-14586.
- MADDEN, A. H., AND CHAMBERLIN, F. S. 1945. Biology of the tobacco hornworm in the southern cigar tobacco district. *U.S.D.A. Tech. Bull.* 896: 1-51.
- RAGUSO, R. A., LEVIN, R. A., FOOSE, S. E., HOLMBERG, M. W., AND MCDADE, L. A. 2003. Fragrance chemistry, nocturnal rhythms and pollination "syndromes" in *Nicotiana*. *Phytochem.* 63: 265-284.
- SAS INSTITUTE. 2004. *Base SAS 9.1 Procedures Guide*, Vol 1-4. SAS Publishing, Cary, NC.
- SINGARAVELAN, N., NEE'MAN, G., INBAR, M., AND IZHAKI, I. 2005. Feeding responses of free-flying honeybees to secondary compounds mimicking floral nectars. *J. Chem. Ecol.* 31: 2791-2804.
- SISSON, V. A., AND SEVERSON, R. F. 1990. Alkaloid composition of *Nicotiana* species. *Beitrag Tabakforschung Intl.* 14: 327-339.
- TADMOR-MELAMED, H., MARKMAN, S., ARIELI, A., DISTL, M., WINK, M., AND IZHAKI, I. 2004. Limited ability of Palestine sunbirds (*Nectarinia osea*) to cope with pyridine alkaloids in nectar of tree tobacco, *Nicotiana glauca*. *Functional Ecol.* 18: 844-850.