

**REPLY TO
BALDWIN****A reply to Baldwin: critique does not weaken major conclusions**

Lynn S. Adler,^{1*} Michael Wink,²
Melanie Distl² and Amanda J.
Lentz³

Abstract

Baldwin's critique raises some valid points. However, none invalidates our main findings of correlations between leaf and floral defences, and induction of nectar alkaloids. We believe our study successfully demonstrated linkages between leaf and floral traits, and we hope it inspires further research in multiple systems and settings.

Keywords

Alkaloids, induced defences, *Manduca sexta*, nectar, *Nicotiana tabacum*, phenotypic correlations.

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The goal of our research was to demonstrate the potential for interactions between leaf herbivores and floral visitors using *Nicotiana tabacum*. We exposed greenhouse plants to fertilizer and leaf damage treatments, and subsequently measured leaf and nectar alkaloids. Leaf and nectar alkaloids were correlated, and leaf damage induced higher nectar alkaloids (Adler *et al.* 2006). This is the first research to examine intraspecific variation in nectar secondary compounds and to demonstrate links between leaf interactions and nectar defensive chemistry. While we agree with some of the general concerns raised by Baldwin, these concerns do not negate our findings.

Dr Baldwin's primary concern was that leaf induction is constrained in pot-bound *Nicotiana sylvestris*, and therefore possibly in *N. tabacum* (Baldwin 1988). We were surprised by the emphasis on this point for three reasons. First, our main goals were to correlate leaf and nectar alkaloids, and to show that leaf damage could induce nectar defences. If pot effects existed, they likely reduced rather than increased the correlation between leaf and nectar alkaloids because damage increased the nectar but not leaf alkaloids. Indeed, rather loose correlation between leaf and nectar alkaloids may have been tighter without potentially constrained leaf induction. Second, our results are very similar to a study by Baldwin and colleagues, in which leaf damage induced floral but not leaf alkaloids in reproductive *N. sylvestris* (Ohnmeiss

& Baldwin 2000). While pot effects cannot be ruled out of our study, similar results have been found in a closely related species where pot effects presumably did not occur. Third, all experiments involve potential artefacts. We chose to conduct this research in the greenhouse rather than the field for several reasons. Primarily, we were concerned that uncontrolled herbivore damage on multiple tissues in the field would reduce our ability to interpret any correlations, or lack thereof, between leaf and nectar alkaloids, or any effects of treatments on plant chemistry. Attempts to control herbivory with pesticides or cages produce other potential artefacts (e.g. Stamp & Bowers 1994; Molina *et al.* 1998). Thus, we chose the more controlled environment of the greenhouse.

Dr Baldwin's concern about loss of nectar nicotine is a valid point, and represents a current gap in our knowledge about *Nicotiana* nectar chemistry. Although we do not know how much nicotine is in *N. tabacum* nectar, the high proportion of nicotine in other tissues suggests that nectar nicotine is likely. However, we were frank about the possible loss of nectar nicotine in our methods, and our addition of HCl to the nectar before extraction should have converted free nicotine to non-volatile nicotine salts, reducing the probability of loss. The correlation between leaf and nectar alkaloids was most likely driven by the positive effect of nutrients on alkaloid concentration in both tissues. Given

¹Department of Plant, Soil and Insect Sciences and Graduate Program in Organismic and Evolutionary Biology, University of Massachusetts, Amherst, MA, USA

²Institut für Pharmazie und Molekulare Biotechnologie, Universität Heidelberg, Heidelberg, Germany

³Department of Biology, Virginia Polytechnic Institute and State University, Blacksburg, VA, USA

*Correspondence: E-mail: lsadler@ent.umass.edu

that nutrients increased alkaloids in other Solanaceous species (e.g. Al-Humaid 2004; Armer *et al.* 2004) and nicotine yield in *N. tabacum* (Gondola 1994, 2002), the simplest expectation is that nectar nicotine would have a similar pattern and also correlate with leaf alkaloids. Furthermore, our results regarding anabasine, which we found consistently in nectar, are likely to be ecologically significant regardless of nicotine patterns. Anabasine has physiological effects on animals at concentrations well below the maximum we found (Tadmor-Melamed *et al.* 2004), is more deterrent in nectar than nicotine to honey bees (Singaravelan *et al.* 2005), and is considerably more toxic than nicotine to aphids (Richardson *et al.* 1936; Bowen & Weigel 1948) which often infest tobacco flowers. Although we would have preferred to correlate total alkaloids in leaves with total alkaloids (rather than anabasine only) in nectar, the possible loss of nectar nicotine does not negate the correlation between leaf alkaloids and nectar anabasine, nor the result that leaf damage increased nectar anabasine.

Finally, Baldwin contends that artificial selection for high leaf nicotine and large leaves in *N. tabacum*, a domesticated species, could drive the phenotypic correlation between leaf and nectar alkaloids. Although *N. tabacum* is not a wild-growing species, it has been a model system for research on plant physiology and genetics. Nicotine is synthesized in roots; thus selection on leaf size would seem unrelated to alkaloid production. We fail to understand how artificial selection for large leaves with high nicotine would drive a correlation with nectar alkaloids that would be different from that found in a wild species.

In conclusion, while Baldwin's critique has valid points, most of the issues he raised would make it less, rather than more, likely that we would find a correlation between leaf and nectar alkaloids. None invalidates our finding of induced nectar anabasine in response to leaf damage. We believe our research has successfully achieved the goal of demonstrating linkages between leaf and floral traits and interactions, and we hope it inspires further work on this topic in both the greenhouse and the field, on domesticated and wild species.

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