Extrafloral nectary phenotypic plasticity is damage- and resource-dependent in *Vicia faba*

Edward B. Mondor*, Michelle N. Tremblay and Russell H. Messing

Kaua'i Agricultural Research Center, University of Hawai'i at Manoa, 7370 Kuamo'o Road, Kapa'a, Hawai'i 96746, USA

*Author and address for correspondence: Department of Biology, Georgia Southern University, Statesboro, GA 30460, USA (emondor@georgiasouthern.edu).

Phenotypic plasticity enables many damaged plants to increase nectar secretion rates from extrafloral nectaries (EFNs), or in the case of broad bean, *Vicia faba* L., to produce additional EFNs, to attract natural enemies of herbivores. While plants benefit greatly from these defensive mutualisms, the costs of producing EFNs are largely unknown. We hypothesized that if EFN production is costly, then damaged plants with high resource levels would be able to produce more EFNs than plants that are resource-limited. Here, we show that this indirect inducible defence does follow this general pattern. *Vicia faba* enriched with 6 or 12 g of 14:14:14 NPK fertilizer increased EFN numbers after leaf damage by 46 and 60%, respectively, compared with nutrient-poor plants. Thus, EFN production is both damage- and resource-dependent. Analogous to direct defences, production of EFNs may limit the overall loss of leaf tissue when risk of herbivory increases.

Keywords: ant–plant interaction; herbivory; inducible defence; mutualism; nectaries; polyphenism

1. INTRODUCTION

Phenotypic plasticity enables organisms to produce rewards, so that benefits are maximized and production costs are minimized (Moran 1992). In response to herbivory, many plants produce defensive compounds or structures to reduce leaf damage (Karban & Baldwin 1997; Tollrian & Harvell 1999). If such defences are costly, individuals expressing these traits in the absence of herbivory will have lower fitness than individuals not expressing similar traits (Bentley 1977; Baldwin 1998).

In ant–plant defensive mutualisms, plants produce food rewards from extrafloral nectaries (EFNs) to attract ants (Beattie 1985; Huxley & Cutler 1991). Consequently, ants often increase plant survival and fitness by deterring herbivore damage (Heil *et al.* 2001a). Plants often alter the volume and (or) composition of nectar to increase the chances of attracting mutualists (Stephenson 1982). However, some plants, such as broad bean *Vicia faba* L., produce additional EFNs in response to leaf damage (Mondor & Addicott 2003). As *V. faba*'s EFNs are visually conspicuous, additional nectaries may present

a greater visual stimulus for ant attraction (Mondor & Addicott 2003).

While plants benefit greatly from EFN-mediated defences, neither the absolute nor the relative costs of producing these structures have been addressed. Nectar may be costly to produce (Southwick 1984; Pyke 1991) and plants may lessen these costs by reducing (Heil *et al.* 2000) or reabsorbing (Bentley 1977) unused nectar. If nectar production is costly, then the production of additional EFNs is likely to be as, if not more, energetically expensive. A better understanding of nectary production costs would provide great insights into the ecology and evolution of EFNs (Rosenzweig 2002).

If EFN production is costly, then this response may also be resource-dependent; though opinions do vary widely (Herms & Mattson 1992; Stamp 2003). Thus, we hypothesized that damaged broad bean plants with high resource levels would have the ability to produce more EFNs than plants that are resource-limited. To test this hypothesis, we assessed whether plants alter EFN numbers in response to leaf damage and/or increasing nutrient levels.

2. MATERIAL AND METHODS

Individual broad bean, *V. faba* cv. 'Broad Windsor', seeds were planted in 4.5 in. pots containing Sun Gro Sunshine Mix no. 4 (Sun Gro Horticulture Canada Ltd, Seba Beach, AB). Pots were top-dressed with 0, 6 or 12 g ($n=30$ of each level) of Scotts Osmocote 14:14:14 NPK controlled-release fertilizer (Scotts-Sierra Horticulture Products Company, Marysville, OH). These levels were chosen to achieve 0, 100 and 200% of the optimal nutrient levels for greenhouse plants (i.e. on the basis of manufacturer's recommendations). Plants were grown in a greenhouse at 21–35°C, 38–96% relative humidity and a 13L:11D photoperiod.

Once the plants reached ca 10 cm and the 2–3 leaf stage, plant height, number of expanded leaves, number of immature leaves and number of EFNs (using a magnifying glass) on each plant were recorded. Broad bean plants generally produce either zero or two EFNs on the stipules at the base of each bean leaf. After recording plant traits, they were subjected to one of the following treatments: (i) control, no leaf damage ($n=15$ of each nutrient level); and (ii) damage, the distal third of the topmost leaf was excised with scissors ($n=15$ of each nutrient level). Nutrient and leaf damage treatments were randomly determined using JMP IN v. 5.1 (SAS Institute 2005).

Mechanical damage allowed us to carefully control the amount of tissue removed, compared to real herbivory (Tiffin & Inouye 2000). Plants frequently increase nectar secretions in response to both natural and mechanical damages, indicating that a herbivore-specific elicitor is not required for this response (Wackers & Wunderlin 1999; Heil *et al.* 2000). Furthermore, *V. faba* has previously been shown to increase EFN numbers in response to mechanical leaf damage alone (Mondor & Addicott 2003).

After 7 days, the same plant traits were assessed. Pre-treatment values were then subtracted from post-treatment values to quantify the degree of change in each character. Immediately after trait measurements, plant shoots were cut at soil level and roots washed. Roots and shoots were dried at 55±5°C for two weeks and then weighed to the nearest 0.01 g (Ohaus GT4100 balance; Ohaus Corporation, Pine Brook, NJ).

3. STATISTICAL ANALYSES

To determine if EFN production was damage- and/or resource-dependent, data were analysed with a two-way ANCOVA. Main factors in the analysis were leaf damage (no versus yes) and nutrient level (0 versus 6 versus 12 g of 14:14:14 NPK). The following covariates were included simultaneously: change in plant height, change in number of expanded leaves, change in number of immature leaves, dry root weight, and dry shoot weight. However, owing to the strong correlation between dry root weight and dry

Table 1. Effects of leaf damage and/or nutrient levels on EFN production in broad bean, *Vicia faba*. (Δ indicates the degree of change in the trait over 7 days, following leaf damage.)

variable	parameter estimate	F	p
leaf damage _(d.f. = 1,74)		25.94	<0.0001
nutrient level _(d.f. = 2,74)		7.42	0.0012
leaf damage $\times$ nutrient level _(d.f. = 2,74)		9.93	0.0002
Δ in plant height _(d.f. = 1,74)	0.03	3.94	0.051
Δ in expanded leaves _(d.f. = 1,74)	-0.42	41.86	<0.0001
Δ in immature leaves _(d.f. = 1,74)	0.19	4.97	0.029
shoot weight _(d.f. = 1,74)	1.39	16.32	0.0001

shoot weight, dry root weight was removed prior to running the final analysis. The dependent variable was the change in the number of EFNs per expanded leaf for each plant. We believe that this dependent variable accurately represents differences in relative EFN production among treatments. This variable is also evolutionarily relevant, as it directly assesses the trade-off between the plant's physiological investment (nectary production) and the area to be defended (number of expanded leaves).

Six seeds did not germinate and were not included in the analyses. For all analyses, calculations were conducted using JMP IN v. 5.1 (SAS Institute 2005).

4. RESULTS

Production of EFNs was found to be both damage- and resource-dependent (table 1). When subjected to mechanical leaf damage, plants increased overall nectary numbers by 33% (figure 1). Plants also increased nectary production in response to augmented resource levels. Overall, plants fertilized with 6 or 12 g of 14:14:14 NPK increased nectary numbers by 26 and 42%, respectively, compared to those with limited resources (figure 1). Most interestingly, however, there was a significant interaction between leaf damage and resource abundance. Nutrient-poor plants did not increase EFN numbers, while plants with 6 or 12 g of 14:14:14 NPK increased EFN production by 46 and 60%, respectively, in response to leaf damage (figure 1).

Three covariates were found to significantly influence EFN numbers. Nectary production was negatively associated with expanded leaf numbers, but positively associated with immature leaf numbers and shoot weights (table 1). There was also a strong trend towards greater numbers of EFNs on taller plants (table 1).

5. DISCUSSION

As suggested by Heil & McKey (2003), EFNs are plant defences 'worn on the outside', and, thus, analogous to chemical defences, indirect defences are also the products of selection (McKey 1974; Rhoades 1979; Heil & McKey 2003). If costly, such defences

Figure 1. Extrafloral nectaries produced by broad bean plants over 7 days, following mechanical leaf damage, at different nutrient levels. Nutrient levels of 0, 100 and 200% represent 0, 6 and 12 g of 14:14:14 NPK per pot. Columns with different letters are significantly different, $p < 0.05$, Tukey's test.

should be preferentially expressed only when risk of herbivory increases (Bentley 1977; Baldwin 1998) so as to optimize resource allocation. However, as nutrient levels increase, additional resources may sometimes be allocated to defence (Herms & Mattson 1992; Stamp 2003).

When subjected to mechanical leaf damage, broad bean plants produced additional EFNs only when nutrient levels increased. It is important to note that plants never produced more than two EFNs per leaf, but rather produced multiple EFN-bearing stipules on the apical meristem prior to the leaves unfolding. This altered developmental trajectory not only results in higher EFN: expanded leaf ratios, but also focuses defensive efforts around the most actively growing part of the plant. This induction response corresponds well with the ant-rewarding, food body production in *Macaranga bancana* (Heil *et al.* 2001b), which is also nutrient-dependent. While research regarding the inducibility of EFNs is still in its infancy, both these results would suggest that the induction of indirect defences is indeed physiologically costly.

Though variability in EFN production may occur in response to soil nutrients, it is uncertain whether nitrogen, phosphorous or potassium is most important for EFN production. Once the key nutrient is identified, one could predict how other mutualistic associations, such as plant-*Rhizobium* (Denison 2000; Simms & Taylor 2002) and plant-mycorrhizal fungi (Johnson *et al.* 1997; Klironomos 2003), might alter this defence. For example, if nitrogen is the limiting resource, plants with *Rhizobium*-containing, nitrogen-fixing root nodules (Graham 1998) would be anticipated to have greater EFN production. Conversely, if phosphorous is the key nutrient, mycorrhizae may take on an increased importance (Koide 1991).

Many questions remain to be addressed regarding EFN production as an ecological and evolutionary strategy. As many species increase nectar production in response to leaf damage (Stephenson 1982), it is unclear why others increase EFN numbers. As plants

have finite resources available for defence (McKey 1974), a trade-off may ensue between different forms of defence. These two forms of defence would also operate on very different temporal scales, with nectar induction being relatively rapid compared to nectary induction. Furthermore, it is unclear how this inducible defence is manifested under field conditions, i.e. in response to variable light levels, moisture levels and types of herbivory. Further experimentation is required to understand the conditions necessary for the evolution of EFN phenotypic plasticity.

Secondary plant compounds frequently respond to changes in resource availability (Hermes & Mattson 1992). Indirect, inducible plant defences, such as the production of EFNs in broad bean plants, appear to respond similarly. Direct and indirect plant defences may function comparably, as they both evolve in response to herbivory (McKey 1974; Rhoades 1979; Heil & McKey 2003). A better understanding of the costs, as well as the benefits, of indirect defences will provide greater insight into the evolution of inducible defences in plants.

We thank J. Gordines and the KARC staff for research support. We also thank Martin Heil and two anonymous reviewers for their constructive comments on this manuscript. This work was supported by the USDA-CSREES-T-STAR (Tropical and Sub-Tropical Agricultural Research) program, grant no. 2002-34135-12762.

- Baldwin, I. T. 1998 Jasmonate-induced responses are costly but benefit plants under attack in native populations. *Proc. Natl Acad. Sci.* **95**, 8113–8118. (doi:10.1073/pnas.95.14.8113)
- Beattie, A. J. 1985 *The evolutionary ecology of ant-plant mutualisms*. Cambridge, UK: Cambridge University Press.
- Bentley, B. L. 1977 Extrafloral nectaries and protection by pugnacious bodyguards. *Annu. Rev. Ecol. Syst.* **8**, 407–427. (doi:10.1146/annurev.es.08.110177.002203)
- Denison, R. F. 2000 Legume sanctions and the evolution of symbiotic cooperation by rhizobia. *Am. Nat.* **156**, 567–576. (doi:10.1086/316994)
- Graham, P. H. 1998 Symbiotic nitrogen fixation. In *Principles and applications of soil microbiology* (ed. D. M. Sylvia, J. J. Fuhrmann, P. G. Hartel & D. A. Zuberer), pp. 325–347. Englewood Cliffs, NJ: Prentice Hall.
- Heil, M. & McKey, D. 2003 Protective ant-plant interactions as model systems in ecological and evolutionary research. *Annu. Rev. Ecol. Syst.* **34**, 425–453. (doi:10.1146/annurev.ecolsys.34.011802.132410)
- Heil, M., Fiala, B., Baumann, B. & Linsenmair, K. E. 2000 Temporal, spatial and biotic variations in extrafloral nectar secretion by *Macaranga tanarius*. *Funct. Ecol.* **14**, 749–757. (doi:10.1046/j.1365-2435.2000.00480.x)
- Heil, M., Fiala, B., Maschwitz, U. & Linsenmair, K. E. 2001a On benefits of indirect defence: short- and long-term studies of antiherbivore protection via mutualistic ants. *Oecologia* **126**, 395–403. (doi:10.1007/s004420000532)
- Heil, M., Hilpert, A., Fiala, B. & Linsenmair, K. E. 2001b Nutrient availability and indirect (biotic) defense in a Malaysian ant-plant. *Oecologia* **126**, 404–408. (doi:10.1007/s004420000534)
- Hermes, D. A. & Mattson, W. J. 1992 The dilemma of plants: to grow or defend. *Quart. Rev. Biol.* **67**, 283–335. (doi:10.1086/417659)
- Huxley, C. R. & Cutler, D. F. 1991 *Ant-plant interactions*. Oxford, UK: Oxford University Press.
- Johnson, N. C., Graham, J. H. & Smith, F. A. 1997 Functioning of mycorrhizal associations along the mutualism–parasitism continuum. *New Phytol.* **135**, 575–586. (doi:10.1046/j.1469-8137.1997.00729.x)
- Karban, R. & Baldwin, I. T. 1997 *Induced responses to herbivory*. Chicago, IL: University of Chicago Press.
- Klironomos, J. N. 2003 Variation in plant response to native and exotic arbuscular mycorrhizal fungi. *Ecology* **84**, 2292–2301.
- Koide, R. T. 1991 Nutrient supply, nutrient demand and plant response to mycorrhizal infection. *New Phytol.* **117**, 365–386. (doi:10.1111/j.1469-8137.1991.tb00001.x)
- McKey, D. 1974 Adaptive patterns in alkaloid physiology. *Am. Nat.* **108**, 305–320. (doi:10.1086/282909)
- Mondor, E. B. & Addicott, J. F. 2003 Conspicuous extrafloral nectaries are inducible in *Vicia faba*. *Ecol. Lett.* **6**, 495–497. (doi:10.1046/j.1461-0248.2003.00457.x)
- Moran, N. A. 1992 The evolutionary maintenance of alternative phenotypes. *Am. Nat.* **139**, 971–989. (doi:10.1086/285369)
- Pyke, G. H. 1991 What does it cost a plant to produce floral nectar? *Nature* **350**, 58–59. (doi:10.1038/350058a0)
- Rhoades, D. F. 1979 Evolution of plant chemical defense against herbivores. In *Herbivores: their interaction with secondary plant metabolites* (ed. G. A. Risenenthal & D. H. Janzen), pp. 4–53. New York, NY: Academic Press.
- Rosenzweig, M. L. 2002 The distraction hypothesis depends on relatively cheap extrafloral nectaries. *Evol. Ecol. Res.* **4**, 307–311.
- SAS Institute, Inc. 2005 *JMP start statistics*, 3rd edn. Belmont, CA: Brooks/Cole-Thomson.
- Simms, E. L. & Taylor, D. L. 2002 Partner choice in nitrogen-fixation mutualisms of legumes and rhizobia. *Integr. Comp. Biol.* **42**, 369–380. (doi:10.1093/icb/42.2.369)
- Southwick, E. E. 1984 Photosynthate allocation to floral nectar a neglected energy investment. *Ecology* **65**, 1775–1779. (doi:10.2307/1937773)
- Stamp, N. 2003 Out of the quagmire of plant defense hypotheses. *Quart. Rev. Biol.* **78**, 23–55. (doi:10.1086/367580)
- Stephenson, A. G. 1982 The role of the extrafloral nectaries of *Catalpa speciosa* in limiting herbivory and increasing fruit production. *Ecology* **63**, 663–669. (doi:10.2307/1936786)
- Tiffin, P. & Inouye, B. 2000 Measuring tolerance to herbivory: accuracy and precision of estimates made using natural versus imposed damage. *Evolution* **54**, 1024–1029. (doi:10.1554/0014-3820(2000)054[1024:MTTHAA]2.3.CO;2)
- Tollrian, R. & Harvell, C. D. 1999 *The ecology and evolution of inducible defenses*. Princeton, NJ: Princeton University Press.
- Wackers, F. L. & Wunderlin, R. 1999 Induction of cotton extrafloral nectar production in response to herbivory does not require a herbivore-specific elicitor. *Entomol. Exp. Appl.* **91**, 149–154. (doi:10.1023/A:1003618617497)