

Optimal defence theory predicts investment in extrafloral nectar resources in an ant–plant mutualism

J. Nathaniel Holland,* Scott A. Chamberlain and Katherine C. Horn

Department of Ecology and Evolutionary Biology, Rice University, Houston, TX 77005, USA

Summary

1. Nearly all mutualisms entail the production of resources by one species that attract and reward the species with which it interacts. As such resource production could otherwise be allocated to growth or reproduction, mutualists are predicted to minimize these investment costs. Here, we employ optimal defence theory for plant secondary compounds to evaluate plant production of extrafloral nectar (EFN) resources to attract and reward ants for resistance against herbivores.

2. Through ant exclusion and artificial herbivory experiments, we examined investment in EFN by both buds and fruits of *Pachycereus schottii* (senita cacti) in the Sonoran Desert of North America. We tested predictions of optimal defence theory that plants invest more in high value parts (fruits) through constitutive EFN, less in low value parts (buds) through induced EFN, and that for a given plant part (buds or fruits), constitutive and induced EFN are negatively correlated.

3. Constitutive levels of EFN were greater in fruits than in buds. Only buds showed induced EFN production following artificial herbivory. Constitutive EFN scaled positively with fruit size but not bud size. Induced EFN was negatively correlated with constitutive EFN for fruits (but not buds), suggesting a potential trade-off in these two herbivore resistance traits.

4. These results indicate that *P. schottii* minimizes its costs by investing in induced EFN for buds and constitutive EFN for fruits. Moreover, by shifting from induced to constitutive EFN with the morphogenesis of buds to fruits, our results show that investment in EFN can change as a particular plant tissue increases from a lower to a higher value.

5. *Synthesis.* Indirect defence through EFN production is in accord with that of direct defence through plant secondary compounds, thereby supporting optimal defence theory as a general framework with which to evaluate the costs of producing resources (i.e. the defence) by plants, lycaenids and homopterans to attract and reward ants for their resistance against natural enemies in protection mutualisms.

Key-words: ant–plant interaction, consumer–resource interaction, constitutive, costs, distraction, extrafloral nectar, induced, mutualism, optimal defence theory, *Pachycereus schottii*, senita

Introduction

Mutualisms are increasingly recognized to be mediated through the production and consumption of resources among interacting species (Holland *et al.* 2005). For instance, plants produce nectar and fruit resources that attract and reward pollinators and seed dispersers; mycorrhizae supply nutrients to plants and obtain carbohydrates in return; and larvae of lycaenid butterflies produce food secretions for ant protection from natural enemies. The production of such resources (and other secondary traits, products or structures)

by mutualistic species to attract and reward another species are generally considered the costs of mutualism (Bronstein 2001a,b; Holland *et al.* 2005). While costs of mutualism are increasingly recognized for their role in the ecology and evolution of mutualistic interactions, they remain less well understood than the benefits of mutualism (Bronstein 2001a,b). When and how much do mutualists invest in their provision of resources to attract and reward the species with which they interact? Here, we employ optimal defence theory (McKey 1979; Rhoades 1979) to evaluate plant production of extrafloral nectar (EFN) resources that attract and reward ants for their resistance against natural enemies in protection mutualisms.

*Correspondence author. E-mail: jholland@rice.edu

Central to optimal defence theory for plant–herbivore interactions is the underlying cost of producing secondary compounds (and mechanical structures) for herbivore resistance, which may otherwise be allocated to reproduction or growth (McKey 1979; Rhoades 1979; Karban & Baldwin 1997; Strauss *et al.* 2002). To minimize costs of producing defences and maximize herbivore resistance, optimal defence theory predicts that defences be deployed among plant parts in direct proportion to their value and likelihood of attack (McKey 1979; Rhoades 1979). Optimal defence theory further predicts that plants invest in ever-present constitutive defences for plant parts of high value and likelihood of attack, and induced defences (after initial herbivore attack) for plant parts of lower value and/or likelihood of attack (Karbon & Myers 1989; Zangerl & Rutledge 1996). Moreover, as investment in constitutive defence of a plant part increases, induced defence is predicted to decrease. This leads to the much discussed trade-off between constitutive and induced production of plant defences (Karbon & Myers 1989; Zangerl & Rutledge 1996; Morris *et al.* 2006).

Unlike plant secondary compounds and other direct defences, resource production by plants, homopterans and lycaenids (e.g. domatia, food bodies, EFN, honeydew and food secretions) to attract and reward mutualistic ants is an indirect defence, as natural enemy resistance occurs through the defensive behaviour of ants that exploit the resource (Agrawal & Rutter 1998; Bronstein 1998; Agrawal & Fordyce 2000; Heil & McKey 2003; Heil & Kost 2006). Optimal defence theory does nevertheless predict that plants and insects employing indirect defences minimize their investment costs of producing resources to attract and reward ants for their resistance against natural enemies. Particularly common in nature are ant–plant mutualisms entailing EFN resources, with at least 330 plant genera among 93 families known to produce EFN (Koptur 1992). Despite being so widespread, we are only beginning to understand the investment costs of EFN as a constitutive and induced defence, including how common inducible EFN and nectaries are among plants (e.g. Dyer *et al.* 2001; Heil *et al.* 2001a,b, 2004; Mondor & Addicott 2003; Ness 2003; Huntzinger *et al.* 2004; Rutter & Rausher 2004). Recently, however, studies have begun to provide some support for optimal defence theory as a means for evaluating investment in indirect defences (Wäckers & Bonifay 2004; Radhika *et al.* 2008; Rostás & Eggert 2008). For example, predictions of optimal defence theory have been explicitly tested and confirmed for EFN as an indirect defence of agricultural cotton, lima bean and castor oil plants (Wäckers & Bonifay 2004; Radhika *et al.* 2008).

In this study, we employed optimal defence theory to examine the production of EFN resources by buds and fruits of *Pachycereus schottii* Engelmann (senita cacti) in the Sonoran Desert of North America. We do not examine the effects of EFN on ant deterrence of herbivory (see Chamberlain & Holland 2008), nor do we attempt to explicitly quantify the exact costs of EFN as an indirect defence in this long-lived plant. Rather, we examine constitutive and induced EFN in the context of predictions of optimal defence theory, assum-

ing that EFN is a cost to plants. We use ant exclusion and artificial herbivory experiments to examine EFN production by both buds and fruits. Buds are of a relatively lower value than fruits, as they entail fewer invested resources, lower survival and a lower fitness value than fruits with developing seeds. We tested multiple predictions of optimal defence theory, including whether plants invest more in high value parts (fruits) through constitutive EFN and less in low value parts (buds) through induced EFN, and whether or not constitutive and induced EFN are negatively correlated for buds and fruits. While studies have demonstrated that investment in constitutive or induced defences can differ among plant parts (e.g. Zangerl & Rutledge 1996; Wäckers & Bonifay 2004), this study is one of the first to examine whether plant investment can shift from induced to constitutive defence during the morphogenesis from a less to more valuable plant part (i.e. buds to fruit).

Methods

STUDY SITE AND STUDY SPECIES

We studied *Pachycereus schottii* (Cactaceae) near Bahia de Kino, Sonora, Mexico (28°53'N, 111°57'W) during the flowering season from April through July of 2007. *Pachycereus schottii* is a multi-stemmed columnar cactus attaining heights of 2–4 m and living for > 75 years. Plants can produce thousands of buds from their areoles (spine-bearing pads) over this time period, many of which do not survive the 9–12 days of development prior to anthesis (J.N. Holland, unpubl. data). Flowers are hermaphroditic, with an inferior ovary containing many ovules, hundreds of anthers and one pistil. Flowers resulting from the surviving buds open at sunset and typically close prior to sunrise, thereby excluding diurnal pollinators. *Pachycereus schottii* relies on a seed-eating moth, *Upiga virescens* Hulst, for pollination, as plants are self incompatible and co-pollinating bees are rarely important (Holland & Fleming 2002). During flower visitation, female moths pollinate stigmas, after which they usually lay a single egg on the open corolla. After active pollination, moths collect pollen by rubbing their abdomen among anthers. Only approximately 50% of flowers initiate fruit due to limited resources, such that low fruit-to-flower ratios and fruit set (fraction of flowers initiating fruit maturation) arise from trade-offs in resource (water) allocation between flower production and fruit set, but pollen limitation can occur (Holland 2002, Holland *et al.* 2004, Holland & Chamberlain 2007). Fruit abortion occurs within 6 days after anthesis and fruit mature within 30 days. All immature fruit do not survive the 20–25 days of development (Holland 2002; Holland *et al.* 2004), as some (15–29%) are lost to moth larvae and others to herbivores. Mature fruits are berry-like, with approximately 180 seeds per fruit.

Cactus reproductive tissues are especially vulnerable to herbivores, as they largely lack alkaloid and silica compounds common in stem tissue (Gibson & Nobel 1986). Buds, flowers and immature fruits of *P. schottii* are all susceptible to herbivory from a diverse range of chewing and sucking insects, some of which include larvae of a pyralid moth (*Cactobrosis fernaldialis*), longhorn beetles (e.g. *Moneilema gigas*), leaf-footed bugs, lace wings, aphids and among others, mirid bugs. Developing buds, flowers and immature fruits produce extrafloral nectar (EFN) from the tips of tepals, photographs of which can be found in Chamberlain & Holland (2008). In addition to reproductive tissues, *P. schottii* has extrafloral nectaries just below

their areoles (sub-areole nectaries), but secretion from them has been largely observed after flowering seasons on new stem growth (S.A. Chamberlain & J.N. Holland, pers. obs.). Flies, parasitoids and beetles have been observed to exploit EFN of *P. schottii*, but ants are the primary consumer, including 14 species among four subfamilies: *Crematogaster depilis*, *Monomorium* n. sp. 'desert', *Pheidole obtusospinosa*, *Pheidole vistana*, *Solenopsis xyloni*, *Tetramorium hispidum* and *Cephalotes rowheri* (Myrmicinae); *Camponotus fragilis*, *Camponotus atriceps/sayi* and *Myrmecocystus mimicus* (Formicinae); *Pseudomyrmex pallidus*, *Pseudomyrmex gracilis* (Pseudomyrmecinae); *Dorymyrmex bicolor* and *Forelius mccooki/pruinosis* (Dolichoderinae).

EXPERIMENTAL DESIGN

We marked and measured EFN on one bud and one immature fruit on each of three randomly chosen stems for each of 31 randomly chosen plants. Each stem per plant was assigned to one of three treatments: control with ants, ant exclusion and ant-exclusion plus artificial herbivory. All three treatments were included within each replicate plant to facilitate larger samples and the control of individual plant effects. We did not attempt to exclude herbivores from the 93 stems. The ant-exclusion treatment was established by applying Tanglefoot (Tanglefoot, Grand Rapids, MI) to the base of stems, as in prior study of *P. schottii* (Chamberlain & Holland 2008). The ant-exclusion plus artificial herbivory treatment was set up by applying Tanglefoot and mechanically damaging with scissors approximately 10% of haphazardly chosen buds and fruits of the stem (not including marked buds and fruits for measuring EFN). EFN secretion has been shown to be similar for natural herbivory, artificial damage and jasmonic acid treatments, indicating the same biochemical pathway for plant responses among such treatments (Heil *et al.* 2001b). Prior to implementing artificial herbivory, we measured EFN production by the marked buds and fruits on all three stems per plant in 8-h time increments over 48 h. We then implemented artificial herbivory at approximately 2000 h and measured EFN of marked buds and fruits 8 h later (04.00 h). EFN volumes were measured using 2 μ L micropipettes. EFN volume was calculated as the length of the pipette filled with EFN, divided by the pipette length multiplied by 2 μ L. At the end of the experiment we measured bud and fruit mass (0.01 g), bud length, and fruit diameter of each marked bud and fruit for which EFN was measured.

Including each treatment within each replicate plant is reasonable for *P. schottii*, as stems behave largely independent of one another in the short term. For example, *P. schottii* does not appear to re-allocate resources among reproductive units (Holland & Fleming 2002; Holland & Chamberlain 2007). Our experimental design provided us with the versatility and statistical power to address multiple questions of constitutive and induced EFN production. The design also allowed us to use different metrics (e.g. pre- and post-manipulation of artificial herbivory) that have been advocated for testing constitutive and induced defences (Morris *et al.* 2006). Sample sizes, with plant as the unit of replication, vary among statistical analyses because during the study some buds matured into flowers, some immature fruit matured into ripe fruit, and some buds and fruits were lost to abortion and other natural events. All analyses were performed using nonparametric statistics, as normality could not be met even after transformation due to the many zeros in the data set. Removing such zeros from the data set can lead to erroneous results for constitutive and induced levels of EFN. Below, we describe statistical analyses for each of the questions we addressed with the above-described experimental design.

SCALING OF EFN PRODUCTION WITH BUD AND FRUIT SIZE

Because buds and immature fruits grow and develop, changing in size and age with time (*cf.* leaf age, Heil *et al.* 2000), we examined whether EFN production for buds and fruits scales with their respective sizes. Using Spearman's correlation, we tested for relationships between EFN and bud and fruit size using data for the two ant-exclusion stems, each stem tested separately, with plants as replicates. We tested for relationships between EFN production (total μ L over 24 h) and bud mass, fruit mass, bud length, and fruit diameter. We only present analyses for size measurements based on mass, as bud and fruit mass were highly correlated with bud length ($r_s = 0.98$, $P < 0.0001$, $n = 19$ plants) and fruit diameter ($r_s = 0.97$, $P < 0.0001$, $n = 30$ plants), respectively.

INDUCED AND CONSTITUTIVE EFN PRODUCTION

Using Wilcoxon paired difference tests, we compared levels of constitutive and induced EFN for buds and fruits using ant-exclusion (control) and ant-exclusion plus artificial herbivory treatment stems. As constitutive and induced plant defences may differ in pre-herbivory levels of EFN, our analyses incorporated levels of EFN production pre- and post-artificial herbivory (Morris *et al.* 2006). Pre-artificial herbivory levels of EFN were based on the 8 h nocturnal time period from the night prior to implementing the treatment, whereas post-artificial herbivory levels of EFN were based on the 8 h nocturnal time period following the treatment. We conducted an additional analysis of induced EFN by standardizing post-artificial herbivory EFN relative to pre-artificial herbivory EFN values (post-divided by pre-artificial herbivory EFN). Finally, we examined whether the relative frequency of buds or fruits producing EFN differed between ant-exclusion and ant-exclusion plus artificial herbivory treatments using Fisher's exact test.

CORRELATION BETWEEN INDUCED AND CONSTITUTIVE EFN PRODUCTION

We tested whether a negative correlation occurred between constitutive and induced EFN for both buds and fruits. While multiple metrics have been used to test for such a relationship, the preferable metric is one that compares absolute measures of each defence between control and herbivory treatments (Morris *et al.* 2006). Constitutive defence (C) is the level of defence on controls, whereas induced defence (I) is the level of defence on the herbivore damaged treatment (D) minus the level of constitutive defence (C) on the control (i.e. $I = D - C$). Using Spearman's correlation analysis, we tested for the predicted negative correlation between constitutive (C) EFN produced on ant-exclusion (control) stems and induced (I) EFN produced on ant-exclusion plus artificial herbivory stems, with plant as the unit of replication. This is reasonable, as each stem within a plant behaves largely independent of the other, while a plant genotype's relative investment in constitutive and induced EFN should remain unchanged. In this way, we were able to examine for a negative correlation among plants (but see Discussion). We conducted two separate analyses of (I) vs. (C). First, we tested for a negative correlation using the preferred metric between (I) and (C) (Morris *et al.* 2006). For comparison with prior work, we also used a standardized measure of (I) relative to pre-artificial herbivory levels of EFN, that is $(D_t/D_0 - C_t/C_0)$ vs. (C), where t is the post- and 0 is the pre-artificial herbivory level of EFN. We added 0.5 to each parameter of $(D_t/D_0 - C_t/C_0)$ due to the many zeros in the denominator of the ratios.

Results

SCALING OF EFN PRODUCTION WITH BUD AND FRUIT SIZE

EFN production was not correlated with bud mass for either of the two ant-exclusion stems ($r_s = 0.17$, $P = 0.441$, $n = 23$ plants; $r_s = -0.05$, $P = 0.805$, $n = 23$ plants), but did correlate positively with fruit mass on one stem ($r_s = 0.47$, $P = 0.011$, $n = 28$ plants) but not the other ($r_s = 0.22$, $P = 0.248$, $n = 30$ plants).

INDUCED AND CONSTITUTIVE EFN PRODUCTION

Both buds and fruits produced some amount of constitutive EFN, but only buds had inducible EFN following artificial herbivory (Fig. 1). No difference in pre-artificial herbivory EFN occurred between ant-exclusion and ant-exclusion plus artificial herbivory treatments for buds (Fig. 1a; Wilcoxon paired difference, $Z = 24$, $n = 20$, $P = 0.110$) or fruits (Fig. 1b; $Z = -18$, $n = 29$, $P = 0.375$). Post-artificial herbivory EFN of buds on ant-exclusion plus artificial herbivory stems was > 90% higher than that of the ant-exclusion stems (Fig. 1a; $Z = 11$, $n = 12$, $P = 0.031$); fruits showed no significant change in post-artificial herbivory EFN (Fig. 1b; $Z = 32$, $n = 28$, $P = 0.245$). When post-artificial herbivory EFN is standardized relative to pre-treatment levels, EFN production by buds

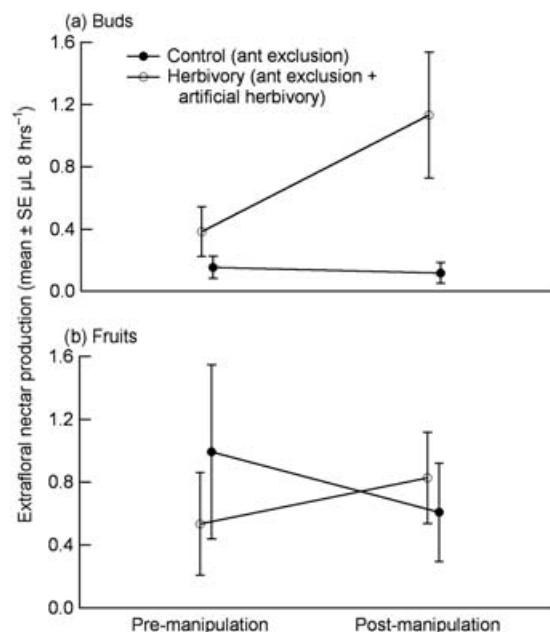


Fig. 1. Experimental test of inducible extrafloral nectar (EFN) production by buds (a) and fruits (b) of *P. schottii*. Pre-manipulation EFN is that level of nocturnal EFN on the night prior to implementing the artificial herbivory treatment, while post-manipulation EFN is the level of nocturnal EFN produced the night following artificial herbivory. Sample sizes [number of plants]: buds [ants excluded, 31; ants excluded + damage, 27] and fruits [ants excluded, 30; ants excluded + damage, 29]. Symbols are slightly offset to show standard error bars.

remained higher for ant-exclusion plus artificial herbivory stems than ant-exclusion stems ($Z = 15$, $n = 12$, $P = 0.039$) and insignificant for fruits ($Z = 33$, $n = 28$, $P = 0.393$). In keeping with inducible EFN by buds and not fruits, the relative frequency of buds producing EFN was greater for ant-exclusion plus artificial herbivory (0.63) than ant-exclusion alone (0.32) (Fisher's exact test, $P = 0.034$). Likewise, the relative frequency of fruits producing EFN did not differ between ant-exclusion plus artificial herbivory (0.45) and ant-exclusion (0.37) treatments (Fisher's exact test, $P > 0.05$).

CORRELATION BETWEEN INDUCED AND CONSTITUTIVE EFN PRODUCTION

A significant correlation did not occur between induced and constitutive EFN for buds for either metric used to estimate induced EFN (Fig. 2a,c). However, a significant negative correlation did occur between induced and constitutive EFN for fruits for both metrics (Fig. 2b,d). Because buds (but not fruits) showed induced EFN (Fig. 1) and fruits (but not buds) showed a negative correlation between induced and constitutive EFN (Fig. 2), buds were predicted to have lower constitutive EFN than fruits. In accord, pre-artificial herbivory EFN on ant-exclusion stems was lower for buds than fruits (Fig. 1; Wilcoxon sign-rank test, one-tailed; $Z = 21.5$, $n = 23$, $P = 0.097$), but no such pre-artificial herbivory difference in EFN of buds and fruits occurred for ant-exclusion plus artificial herbivory stems (Fig. 1; $Z = -7.0$, $n = 24$, $P = 0.640$). Collectively, these results are consistent with buds, but not fruits, showing induced EFN.

Discussion

In this study, we employed optimal defence theory to evaluate investment in the production of EFN resources by both buds and fruits of *P. schottii* (senita) to attract and reward mutualistic ants as a defence against herbivores. To reduce costs of EFN production as an indirect defence and increase herbivore resistance, optimal defence theory predicts that EFN be deployed among plant parts in proportion to their value and likelihood of attack. This prediction rests on the underlying assumption that plant defences represent a cost which could otherwise be allocated to growth or reproduction. Although few studies have explicitly examined costs of EFN to plant fitness, empirical work continues to support the validity of the assumption. For instance, EFN and nectaries can be subject to selection (Rudgers & Strauss 2004; Rutter & Rausher 2004; Wooley *et al.* 2007); can be relaxed in the absence of herbivores (Huntzinger *et al.* 2004); and can be inducible (e.g. Mondor & Addicott 2003), which itself is an indication of a costly defence (Simms 1992). In our study, we too did not directly quantify costs of EFN for plant fitness, as *P. schottii* is a long-lived cactus with many reproductive episodes. Nevertheless, our results (Table 1) and those of other studies (Wäckers & Bonifay 2004; Radhika *et al.* 2008) are consistent with and support the application of optimal defence theory to the production of EFN resources as an indirect defence.

Fig. 2. Correlation between constitutive (C) and induced (I) extrafloral nectar (EFN) production by buds (a, c) and fruits (b, d) of *P. schottii*. Constitutive defence is the level of EFN on ant-exclusion (controls) stems, whereas induced defence is the level of EFN on ant-exclusion plus artificial herbivory stems (D) minus constitutive EFN produced on the control stem (i.e. $I = D - C$). A significant negative correlation, as measured by Spearman's correlation (r_s , $\alpha = 0.10$), is predicted to occur between (I) and (C) if a trade-off occurs between induced and constitutive EFN (Morris *et al.* 2006). We present two analyses of (I) vs. (C). The first is an analysis of (D - C) vs. (C), that is (I) vs. (C) (panels a, b). The second is a standardized measure of (I) relative to pre-artificial herbivory EFN, that is ($D_t/D_0 - C_t/C_0$) vs. C (panels c, d), where t is post- and 0 is pre-artificial herbivory EFN. The ordinate in panels (c) and (d) is unit-less due to division by pre-artificial herbivory EFN.

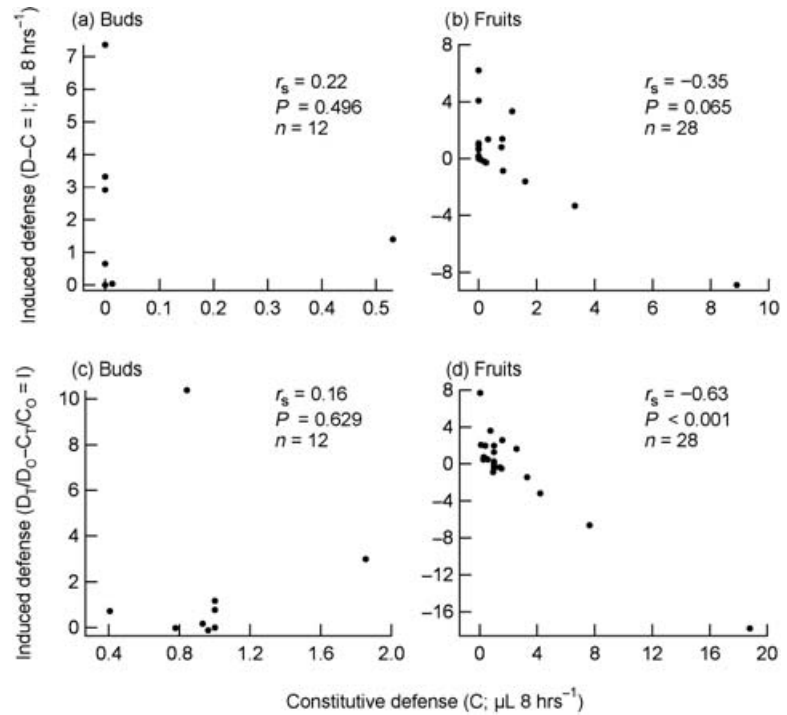


Table 1. Summary of extrafloral nectar (EFN) production by buds and fruits of *P. schottii* in relation to predictions of optimal defence theory. Value represents the relative cost of the plant parts to plant reproduction if lost to herbivores. Size scaling is whether EFN production varied with bud or fruit size. Constitutive refers to the level of constitutive EFN, while induced refers to whether EFN was inducible following artificial herbivory. C-I correlation refers to whether a negative correlation occurred between constitutive (C) and induced (I) EFN. This qualitative summary is largely consistent with optimal defence theory, predicting in particular that plants invest more in higher value parts through constitutive EFN, less in lower value parts through induced EFN, and constitutive and induced EFN are negatively correlated

| | Extrafloral nectar production | | | | |
|--------|-------------------------------|--------------|--------------|---------|-----------------|
| | Value | Size scaling | Constitutive | Induced | C-I correlation |
| Buds | Low | No | Low | Yes | No |
| Fruits | High | Yes | High | No | Negative |

Before discussing predictions of optimal defence theory for constitutive and induced EFN of *P. schottii*, we present some potential limitations of our experimental design. Using multiple treatment stems within replicate plants may be confounded by possible within-plant effects. If constitutive and induced EFN responses on different treatment stems within a plant were not independent, then this could lead to falsely accepting a null hypothesis. This is not likely the case for *P. schottii*, as stems within plants do appear to behave independently of one another on short time scales. For example, plants do not readily re-allocate resources among reproductive units given differences in pollination (Holland &

Fleming 2002; Holland & Chamberlain 2007). For this reason, we did not expect artificial herbivory on one stem to alter short-term EFN of another stem within a plant. Nevertheless, treatments within replicate plants may be confounded by plant volatiles released in response to herbivory, which may induce EFN and attract ants (Choh *et al.* 2006; Edwards *et al.* 2006; Kost & Heil 2006; Heil & Silva Bueno 2007). If we had found no significant effects, then we would not be able to confidently 'fail to reject' the null hypothesis of no differences in constitutive and induced EFN due to a possible lack of independence associated with plant volatiles. We did find significant differences, however, indicating that such a caveat is not likely to alter the qualitative results of the experiments. In addition, our results, like any such study of EFN, may be unavoidably confounded by artificial effects that sampling EFN may have on later EFN production (Heil *et al.* 2000), though any such sampling effects would likely be similar among study plants and treatment stems. Despite such caveats, as we describe below our results do nevertheless indicate that plants may minimize their investment costs in accord with optimal defence theory.

Though a relatively unexplored aspect of optimal defence theory in general, and of EFN production in particular, plants may minimize their investment costs in a given plant part as it grows and develops by scaling their defences with the value of the plant part as it changes in size and age. Indeed, EFN often occurs on foliar or reproductive tissues that grow and change in size and age (*cf.* leaf age, Heil *et al.* 2000), such that EFN may scale with their size- or age-dependent values. In our study, EFN of *P. schottii* did not scale with bud mass in either of the two stems analyzed, but it did increase with fruit mass for one of the two stems analyzed. These results are consistent with minimal constitutive and mostly induced

EFN for buds, and constitutive (and not inducible) EFN for fruits. Moreover, these results suggest the need for more studies that test whether plants can minimize costs and maximize herbivore resistance by scaling constitutive EFN with the size and age of plant parts, as ant defence may in turn scale with EFN resources (Willmer & Stone 1997; Oliveira *et al.* 1999; Chamberlain & Holland 2008). Such speculation is indeed tentative, as further studies are needed of size-dependent scaling of plant defences.

Central to optimal defence theory is the prediction of constitutive defence for plant parts of high value, and inducible defence for plant parts of low value (McKey 1979; Rhoades 1979; Karban & Myers 1989; Zangerl & Rutledge 1996). We examined buds and immature fruits in this context, though in actuality they represent two different stages during the morphogenesis of a single type of plant tissue. Nevertheless, buds are of a relatively lower value than fruits, as they have fewer invested resources and are of lower fitness value than fruits with developing seeds. For both buds and fruits, constitutive EFN (C) is that level produced for ant-exclusion controls, whereas induced EFN (I) is that level produced for ant-exclusion plus artificial herbivory stems (D) minus the level of constitutive EFN on ant-exclusion controls (i.e. $I = D - C$). Consistent with optimal defence theory, constitutive EFN occurred for high valued immature fruits and induced EFN for lower valued buds (Table 1), corresponding with empirical results of some other studies (e.g. Stephenson 1982; Swift & Lanza 1993; Young *et al.* 1997; Wäckers & Bonifay 2004). Not only does this study document induced EFN production in another plant species, but by shifting from induced to constitutive EFN with the morphogenesis of buds to fruits, our results show that investment in EFN can change as a given plant tissue increases from a low to higher value. To our knowledge, such a shift from induced to constitutive defence during the morphogenesis of a single plant tissue is a new dimension of optimal defence theory, especially as applied to EFN. To this end, EFN secretion is now recognized to be induced by jasmonic acid, the same hormone which often increases (and is routinely expressed) during fruit development (Creelman & Mullet 1997). Taken together, these observations suggest an attractive plant physiological mechanism responsible for the change from induced (buds) to constitutive (fruits) EFN production.

Optimal defence theory also predicts that as investment in constitutive defence of a plant part increases, investment in induced defence decreases, leading to the hypothesized trade-off between constitutive and induced defences (Karbon & Myers 1989; Zangerl & Rutledge 1996; Morris *et al.* 2006). In our study, costs of EFN may be reduced by employing induced EFN after initial attack, rather than maintaining constitutive EFN. As constitutive EFN increases, inducible EFN is predicted to decrease, leading to a negative correlation between I and C. We were able to test for a negative correlation between I and C with our experimental design, as treatment stems within replicate plants likely behave independently in the short term (see above), while their genotype and thus relative investment in C and I remained unchanged. Including

each treatment within replicate plants has the added advantage of controlling for environmental effects on plant levels of C and I (rather than using familial level analyses to account for genotypic trade-offs). Our results are consistent with optimal defence theory in that, for both metrics analyzed (Fig. 2), we found significant negative correlations between I and C for fruits, but not for buds (Table 1, Fig. 2). Some plants exhibited induced susceptibility, that is negative values of I, indicating high constitutive EFN before herbivore attack and lower EFN production (and resistance) after herbivore attack (Fig. 2; Morris *et al.* 2006). Though we incorporated principles of some recently recommended protocols for testing for negative correlations (Morris *et al.* 2006), results of which suggest a possible trade-off between I and C for *P. schottii*, our analyses do still entail some of the general shortcomings associated with evaluating trade-offs. For this reason, caution is warranted in interpreting any such negative correlation as a trade-off, even for well-recognized trade-offs, such as that between plant reproduction and growth (Knops *et al.* 2007). While some studies have begun to examine relationships between direct and indirect defence (e.g. Dyer *et al.* 2001; Agrawal *et al.* 2002), more are needed that examine relationships between induced and constitutive EFN as an indirect defence.

We have thus far presented EFN as an indirect defence to attract and reward mutualistic ants for herbivore resistance. However, EFN may serve an additional or alternative function as a direct defence against florivorous ants. Ants do indeed exploit floral resources, which can result in antagonistic interactions with plants by disrupting plant reproduction through florivory, pollen consumption, pollinator interference, reduced pollen viability and depletion of floral nectar (Galen 2005; Junker *et al.* 2006; Nicklen & Wagner 2006; Ness 2006; Chamberlain & Holland 2008). By distracting ants from floral resources (Wagner & Kay 2002), EFN may be a direct defence against antagonistic ants, rather than an indirect defence against herbivores mediated through ants. In the senita ant–plant–pollinator system, EFN and ant abundance on plants are greatest at night, the only time at which flowers are open and visited by their obligate pollinating moth (Holland & Fleming 1999; Chamberlain & Holland 2008). Prior study has shown that EFN is as likely to distract ants from flowers as it is to reward them as mutualists (Chamberlain & Holland 2008). Ants were largely commensalistic, possessing the potential both to protect buds and fruits from herbivores and to disrupt pollination and plant reproduction. Likewise, Wäckers & Bonifay (2004) found that in addition to attracting ants for protection against herbivores, EFN affects pollination processes by deterring ants from flowers. It remains unresolved why EFN resources are maintained by plants when benefits of ant protection cannot accrue in the absence of herbivores (Bronstein *et al.* 2006). As pairwise ant–plant interactions are studied more often in a community context of multi-species interactions, greater recognition is being made of the need to integrate the many facets of EFN production into understanding its causes and consequences.

Regardless of whether EFN is an indirect defence against herbivores mediated through ant protection or a direct defence

for distracting florivorous ants, optimal defence theory has provided a predictive means with which to understand investment in EFN by *P. schottii*. Specifically, plants can minimize their investment costs through the production of constitutive and induced EFN consistent with predictions of optimal defence theory (Table 1). As indirect defence through EFN, for example, is in accord with direct defence through plant secondary compounds, optimal defence theory may provide a general framework with which to evaluate the resources produced by plants, lycaenids and homopterans (e.g. domatia, food bodies, EFN, honeydew and food secretions) to attract and reward mutualistic ants for natural enemy resistance. More generally, as the study of such resources supplied by one mutualistic species to another grows, so will our understanding of the implications of mutualism for pairwise and multi-species interactions.

Acknowledgements

We thank C. Fresquez and J. Carillo for field assistance and P. Ward for assisting in ant IDs. We are grateful for comments on an earlier version of this paper by J. Rudgers, F. Massey and three reviewers. We used the SDC approach for author ordering (Tscharrntke *et al.* 2007).

References

- Agrawal, A.A. & Fordyce, J.A. (2000) Induced indirect defence in a lycaenid-ant association: the regulation of a resource in a mutualism. *Proceedings of the Royal Society of London B*, **267**, 1857–1861.
- Agrawal, A.A. & Rutter, M.T. (1998) Dynamic anti-herbivore defense in ant-plants: the role of induced responses. *Oikos*, **83**, 227–236.
- Agrawal, A.A., Janssen, A., Bruin, J., Posthumus, M.A. & Sabelis, M.W. (2002) An ecological cost of plant defence: attractiveness of bitter cucumber plants to natural enemies of herbivores. *Ecology Letters*, **5**, 377–385.
- Bronstein, J.L. (1998) The contribution of ant-plant protection studies to our understanding of mutualism. *Biotropica*, **30**, 150–161.
- Bronstein, J.L. (2001a) The costs of mutualism. *American Zoologist*, **41**, 825–839.
- Bronstein, J.L. (2001b) Mutualisms. *Evolutionary Ecology* (eds C. Fox, D. Fairbairn & D. Roff), pp. 315–330. Oxford University Press, New York.
- Bronstein, J.L., Alarcon, R. & Geber, M. (2006) The evolution of plant-insect interactions. *New Phytologist*, **172**, 412–428.
- Chamberlain, S.A. & Holland, J.N. (2008) Density-mediated, context-dependent consumer-resource interactions between ants and extrafloral nectar plants. *Ecology*, **89**, 1364–1374.
- Choh, Y., Kugimiya, S. & Takabayashi, J. (2006) Induced production of extrafloral nectar in intact lima bean plants in response to volatiles from spider mite-infested conspecific plants as a possible indirect defense against spider mites. *Oecologia*, **147**, 455–460.
- Creelman, R.A. & Mullet J.E. (1997) Biosynthesis and action of jasmonates in plants. *Annual Review of Plant Physiology and Plant Molecular Biology*, **48**, 355–381.
- Dyer, L.A., Dodson, C.D., Beihoffer, J. & Letourneau, D.K. (2001) Trade-offs in antiherbivore defenses in *Piper cenocladum*: ant mutualists versus plant secondary metabolites. *Journal of Chemical Ecology*, **27**, 581–592.
- Edwards, D.P., Hassall, M., Sutherland, W.J. & Yu, D.W. (2006) Assembling a mutualism: ant symbionts locate their host plants by detecting volatile chemicals. *Insectes Sociaux*, **53**, 172–176.
- Galen, C. (2005) Catching ants with honey: an experimental test of distraction and satiation as alternative modes of escape from flower-damaging ants. *Oecologia*, **144**, 80–87.
- Gibson, A.C. & Nobel, P.S. (1986) *The Cactus Primer*. Harvard University Press, Cambridge, MA.
- Heil, M. & Kost, C. (2006) Priming of indirect defences. *Ecology Letters*, **9**, 813–817.
- Heil, M. & McKey, D. (2003) Protective ant-plant interactions as model systems in ecological and evolutionary research. *Annual Review of Ecology, Evolution, and Systematics*, **34**, 425–453.
- Heil, M. & Silva Bueno, J.C. (2007) Within-plant signaling by volatiles leads to induction and priming of an indirect plant defense in nature. *Proceedings of the National Academy of Sciences of the United States of America*, **104**, 5467–5472.
- Heil, M., Fiala, B., Baumann, B. & Linsenmair, K.E. (2000) Temporal, spatial and biotic variations in extrafloral nectar secretion by *Macaranga tanarius*. *Functional Ecology*, **14**, 749–757.
- Heil, M., Greiner, S., Meimberg, H., Kruger, R., Noyer, J.L., Heubl, G., Linsenmair, K.E. & Boland, W. (2004) Evolutionary change from induced to constitutive expression of an indirect plant resistance. *Nature*, **430**, 205–208.
- Heil, M., Hilpert, A., Fiala, B. & Linsenmair, K.E. (2001a) Nutrient availability and indirect (biotic) defence in a Malaysian ant-plant. *Oecologia*, **126**, 404–408.
- Heil, M., Koch, T., Hilpert, A., Fiala, B., Boland, W. & Linsenmair, K.E. (2001b) Extrafloral nectar production of the ant-associated plant, *Macaranga tanarius*, is an induced, indirect, defensive response elicited by jasmonic acid. *Proceedings of the National Academy of Sciences of the United States of America*, **98**, 1083–1088.
- Holland, J.N. (2002) Benefits and costs of mutualism: demographic consequences in a pollinating seed-consumer interaction. *Proceedings of the Royal Society of London B*, **269**, 1405–1412.
- Holland, J.N. & Chamberlain, S.A. (2007) Ecological and evolutionary mechanisms for low seed: ovule ratios: Need for a pluralistic approach? *Ecology*, **88**, 706–715.
- Holland, J.N. & Fleming, T.H. (1999) Mutualistic interactions between *Upiga virescens* (Pyralidae), a pollinating seed-consumer, and *Lophocereus schottii* (Cactaceae). *Ecology*, **80**, 2074–2084.
- Holland, J.N. & Fleming, T.H. (2002) Co-pollinators and specialization in the pollinating seed-consumer mutualism between senita cacti and senita moths. *Oecologia*, **133**, 534–540.
- Holland, J.N., Bronstein, J.L. & DeAngelis, D.L. (2004) Testing hypotheses for excess flower production and low fruit-to-flower ratios in a pollinating seed-consuming mutualism. *Oikos*, **105**, 633–640.
- Holland, J.N., Ness, J.H., Boyle, A.L. & Bronstein, J.L. (2005) Mutualisms as consumer-resource interactions. *Ecology of Predator-Prey Interactions* (eds P. Barbosa & I. Castellanos), pp. 17–33. Oxford University Press, New York.
- Huntzinger, M., Karban, R., Young, T.P. & Palmer, T.M. (2004) Relaxation of induced indirect defenses of acacias following exclusion of mammalian herbivores. *Ecology*, **85**, 609–614.
- Junker, R., Chung, A.Y.C. & Blüthgen, N. (2006) Interactions between flowers, ants and pollinators: additional evidence for floral repellence against ants. *Ecological Research*, **22**, 665–670.
- Karban, R. & Baldwin, I.T. (1997) *Induced Responses to Herbivory*. University of Chicago Press, Chicago and London.
- Karban, R. & Myers, J.H. (1989) Induced plant responses to herbivory. *Annual Review of Ecology and Systematics*, **20**, 331–348.
- Knops, J.M.H., Koenig, W.D. & Carmen, W.J. (2007) Negative correlation does not imply a tradeoff between growth and reproduction in California oaks. *Proceedings of the National Academy of Sciences of the United States of America*, **104**, 15982–15985.
- Koptur, S. (1992) Extrafloral nectary-mediated interactions between insects and plants. *Insect-Plant Interactions* (ed. E. Bernays), pp. 81–129. CRC Press, Boca Raton, FL.
- Kost, C. & Heil, M. (2006) Herbivore-induced plant volatiles induce an indirect defence in neighbouring plants. *Journal of Ecology*, **94**, 619–628.
- McKey, D. (1979) The distribution of secondary compounds within plants. *Herbivores: Their Interaction with Secondary Plant Metabolites* (eds G.A. Rosenthal & D.H. Janzen), pp. 56–134. Academic Press, Orlando, FL.
- Mondor, E.B. & Addicott, J.F. (2003) Conspicuous extra-floral nectaries are inducible in *Vicia faba*. *Ecology Letters*, **6**, 495–497.
- Morris, W.F., Traw, M.B. & Bergelson, J. (2006) On testing for a tradeoff between constitutive and induced resistance. *Oikos*, **112**, 102–110.
- Ness, J.H. (2003) *Catalpa bignonioides* alters extrafloral nectar production after herbivory and attracts ant bodyguards. *Oecologia*, **134**, 210–218.
- Ness, J.H. (2006) A mutualism's indirect costs: the most aggressive plant bodyguards also deter pollinators. *Oikos*, **113**, 506–514.
- Nicklen, E.F. & Wagner, D. (2006) Conflict resolution in an ant-plant interaction: *Acacia constricta* traits reduce ant costs to reproduction. *Oecologia*, **148**, 81–87.
- Oliveira, P.S., Rico-Gray, V., Diaz-Castelazo, C. & Castillo-Guevara, C. (1999) Interaction between ants, extrafloral nectaries and insect herbivores in Neotropical coastal sand dunes: herbivore deterrence by visiting ants increases fruit set in *Opuntia stricta* (Cactaceae). *Functional Ecology*, **13**, 623–631.

- Radhika, V., Kost, C., Bartram, S., Heil, M. & Boland, W. (2008) Testing the optimal defence hypothesis for two indirect defences: extrafloral nectar and volatile organic compounds. *Planta*, **228**, 449–457.
- Rhoades, D.F. (1979) Evolution of plant chemical defense against herbivores. *Herbivores: Their Interaction with Secondary Plant Metabolites* (eds G.A. Rosenthal & D.H. Janzen), pp. 4–55. Academic Press, Orlando, FL.
- Rostás, M. & Eggert, K. (2008) Ontogenetic and spatio-temporal patterns of induced volatiles in *Glycine max* in the light of the optimal defence hypothesis. *Chemoecology*, **18**, 29–38.
- Rudgers, J.A. & Strauss, S.Y. (2004) A selection mosaic in the facultative mutualism between ants and wild cotton. *Proceedings of the Royal Society of London B*, **271**, 2481–2488.
- Rutter, M.T. & Rausher, M.D. (2004) Natural selection on extrafloral nectar production in *Chamaecrista fasciculata*: the costs and benefits of a mutualism trait. *Evolution*, **58**, 2657–2668.
- Simms, E.L. (1992) Costs of plant resistance to herbivores. *Plant Resistance to Herbivores and Pathogens. Ecology, Evolution, and Genetics* (eds R.S. Fritz & E.L. Simms), pp. 392–425. University of Chicago Press, Chicago, IL.
- Stephenson, A.G. (1982) The role of the extrafloral nectaries of *Catalpa speciosa* in limiting herbivory and increasing fruit production. *Ecology*, **63**, 663–669.
- Strauss, S.Y., Rudgers, J.A., Lau, J.A. & Irwin, R.E. (2002) Direct and ecological costs of resistance to herbivory. *Trends in Ecology and Evolution*, **17**, 278–285.
- Swift, S. & Lanza, J. (1993) How do *Passiflora* vines produce more extrafloral nectar after simulated herbivory? *Bulletin of the Ecological Society of America*, **74**, 451.
- Tscharntke, T., Hochberg, M.E., Rand, T.A., Resh, V.H. & Krauss, J. (2007) Author sequence and credit for contributions in multiauthored publications. *PLoS Biology*, **5**, e18.
- Wäckers, F.L. & Bonifay, C. (2004) How to be sweet? Extrafloral nectar allocation by *Gossypium hirsutum* fits optimal defense theory predictions. *Ecology*, **85**, 1512–1518.
- Wagner, D. & Kay, A. (2002) Do extrafloral nectaries distract ants from visiting flowers? An experimental test of an overlooked hypothesis. *Evolutionary Ecology Research*, **4**, 293–305.
- Willmer, P.G. & Stone, G.N. (1997) How aggressive ant-guards assist seed-set in *Acacia* flowers. *Nature*, **388**, 165–167.
- Wooley, S.C., Donaldson, J.R., Gusse, A.C., Lindroth, R.L. & Stevens, M.T. (2007) Extrafloral nectaries in aspen (*Populus tremuloides*): heritable genetic variation and herbivore-induced expression. *Annals of Botany*, **100**, 1337–1346.
- Young, T.P., Stubblefield, C.H. & Isbell, L.A. (1997) Ants on swollen thorn acacias: species coexistence in a simple system. *Oecologia*, **109**, 98–107.
- Zangerl, A.R. & Rutledge, C.E. (1996) The probability of attack and patterns of constitutive and induced defense: a test of optimal defense theory. *American Naturalist*, **147**, 599–604.

Received 15 February 2008; accepted 12 September 2008

Handling Editor: Fergus Massey