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## Patterns of *Azteca* ants' defence of *Cecropia* trees in a tropical rainforest: support for optimal defence theory

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**Abstract** Optimal defence theory (ODT) predicts that, whereas high risk of herbivory should select for high constitutive levels of defence, induced defences should be more advantageous in environments with a low probability of herbivory. In the present field study, conducted on the *Azteca*–*Cecropia* ant–plant system in a Neotropical rainforest, we evaluated whether the constitutive and induced ant defence of leaves are directly and inversely related to an estimate of herbivory risk, respectively. To assess the constitutive level of *Azteca* defence in *Cecropia obtusifolia* trees, we recorded the number of ants patrolling undamaged leaves. To evaluate the induced level of *Azteca* defence, the same leaves were subjected to simulated herbivory by punching circular holes in them. We recorded the maximum number of ants patrolling the damaged leaves from 2 to 15 min after damage. Past herbivory (% defoliation of old leaves) was assumed to indicate a risk of herbivory.

Regression analyses showed that, whereas the constitutive level of ant patrolling was positively associated with the magnitude of herbivory on old leaves, there was a negative association between the magnitude of induced ant defence and past herbivory. These preliminary results lend support to ODT.

**Keywords** Ant–plant interactions · *Azteca* · Biotic defences · *Cecropia* · Indirect defences · Optimal defence

### Introduction

Optimal defence theory (ODT; McKey 1979; Rhoades 1979) predicts that plants should allocate anti-herbivore defences in such a way that plant fitness is maximised, taking the cost of defences into account, i.e. that allocation to defences divert resources otherwise devoted to plant growth or reproduction. Consequently, defence investments should be directly associated with the value of plant tissue and the probability of herbivore attack. Although originally formulated for constitutive defences, ODT can incorporate induced defences (Gianoli and Niemeyer 1997; Zangerl and Rutledge 1996; Karban and Baldwin 1997; Koricheva et al. 2004). In this regard, Zangerl and Bazzaz (1992) stated that the relative expression of constitutive and induced defences should be related to herbivory risk. Thus, whereas high herbivory pressure should select for high constitutive levels of defence, induced defences should be more advantageous in environments with a low probability of herbivory. Empirical tests of this hypothesis are few (Zangerl and Rutledge 1996; Karban and Nagasaka 2004).

Plant protection by ants has been considered as a biotic defence, analogous to chemical defence (Janzen 1966), and induced ant recruitment following experimental damage has been reported in several myrmecophilous tree species (Agrawal and Rutter 1998). Ant–plant mutualisms have been used as model systems to test general theories on anti-herbivore plant defence (Heil and McKey

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2003). Working with *Crematogaster* ants and a *Maca-ranga* tree species, Heil et al. (2004) showed that ants preferentially defended young leaves and remained active for a longer time when the stimulus mimicked long-term stress. These patterns are consistent with the predictions of ODT. In the present field study, conducted in the *Azteca-Cecropia* ant-plant system, we tested for a direct relationship between constitutive biotic defence (basal levels of ant patrolling) and herbivory risk, and an inverse relationship between induced biotic defence (ant recruitment to a damaged leaf) and herbivory risk, as predicted by ODT.

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## Materials and methods

The study was carried out in February 2004 at La Selva Biological Station (Organization for Tropical Studies), situated in the Caribbean lowlands of northern Costa Rica (10°26'N, 83°59'W). *Cecropia* trees (Urticaceae), typical of disturbed lands in wet Neotropical regions, are commonly inhabited by *Azteca* ants (Dolichoderinae), whose colonies occupy the hollow internodes of the tree's main stem (Janzen 1969; Longino 1989). The primary food source of the ants are the glycogen-rich food bodies that the plant continuously produces at the base of leaf petioles (Longino 1989). Ants defend the plant against herbivores, thereby, increasing the fitness of occupied plants (Janzen 1969; Schupp 1986; Rocha and Bergallo 1992). At La Selva, two closely related and very similar species of *Azteca*, *A. xanthocroa* and *A. constructor*, inhabit young *C. obtusifolia* trees and show very aggressive behaviour (Agrawal 1998).

We evaluated the baseline level of ant patrolling and the induction of ant recruitment after simulated herbivory on the leaves of young *C. obtusifolia* trees. Procedures to evaluate constitutive (basal) and induced defences by ants were modified from two early studies on the same system conducted at La Selva (Agrawal 1998; Agrawal and Dubin-Thaler 1999). To assess the constitutive level of *Azteca* defence in the plant, we recorded the number of ants patrolling a leaf during 2 min. This was done on two fully expanded leaves per plant. The leaves were located at approximately the same height (mid-crown) and had minimal or no herbivory. To evaluate the induced level of *Azteca* defence, the same leaves were then subjected to a simulated herbivory treatment by punching five circular holes (6 mm in diameter) in the margin of each of five lobes of the leaf. A typical leaf of *C. obtusifolia* had 10–12 lobes of area approximately 200 cm<sup>2</sup> each. We counted the number of ants patrolling the damaged leaves 2, 4, 6, 8, 10, 12 and 15 min after punching the holes. The damage created by the simulated herbivory treatment is very similar to that caused by natural herbivores (Agrawal 1998; E. Gianoli, personal observation). Although manipulation of the leaf without making any damage does not induce ant recruitment in this system (Agrawal 1998; E. Gianoli,

personal observation), we inflicted the damage carefully in order to minimise physical disturbance.

Herbivory on old leaves was assumed to indicate the risk of herbivory. Although within-year variation in herbivory pressure should be expected, in the particular case of pioneer species of lowland tropical forests such as *Cecropia*, there are no major differences in the rate of herbivory among seasons (Coley 1983). We assessed past herbivory pressure on trees by quantifying damage in the two oldest leaves of each tree. Foliar damage was estimated in the field by visually assigning a percentage of leaf area lost to herbivory. These old leaves had started to wilt. We found ant colonies in 14 out of 22 *Cecropia* trees evaluated, hence, the final sample size was  $N=14$ . The trees were between 2 and 4 m tall, had approximately 12–20 leaves and occurred along the Tres Ríos and Arroyo-Zompopa trails in an abandoned plantation, young secondary forests and path edges.

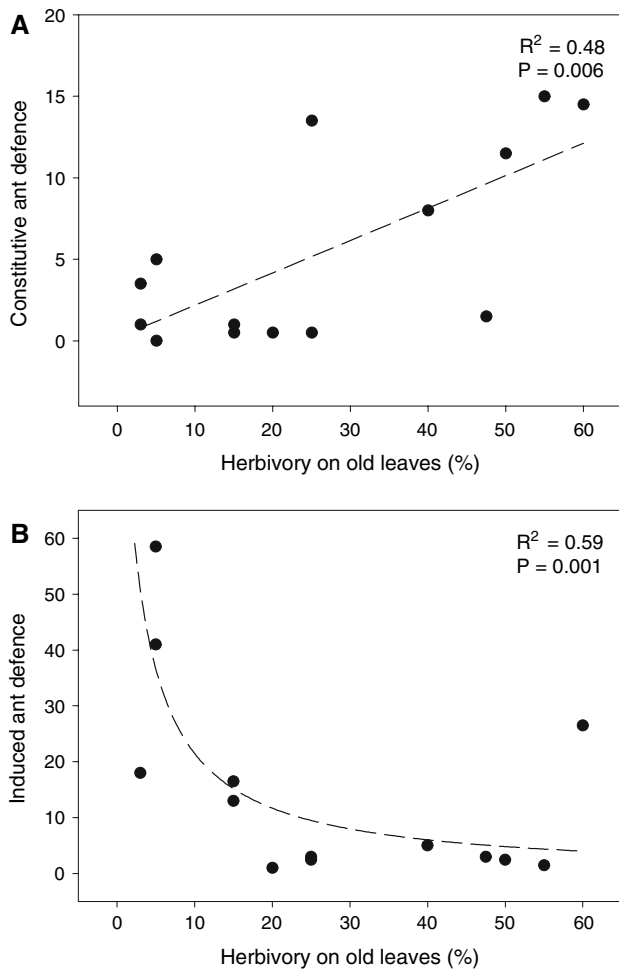
Data from the two leaves evaluated for each tree were averaged to give the value of the individual tree for each of the measured variables: constitutive and induced *Azteca* defence, and herbivory. The induced level of ant defence was defined as the maximum number of ants recorded during the observation of ant recruitment. We chose this criterion in order to standardise possible differences among trees due to variable distances from the colony to the focal leaf. To evaluate the relationship between either type of ant defence and past herbivory in *Cecropia* trees, we used regression analysis ( $N=14$ ).

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## Results and discussion

We found that the relationships between both constitutive and damage-induced levels of ant defence and herbivory risk in the *Azteca-Cecropia* system were consistent with the predictions of ODT. Whereas the constitutive level of ant patrolling was positively associated with the magnitude of herbivory on old leaves ( $R^2=0.48$ ,  $F_{1, 12}=11.27$ ,  $P=0.006$ ; Fig. 1a), there was a negative association between the magnitude of induced ant defence and past herbivory ( $R^2=0.59$ ,  $F_{1, 12}=17.06$ ,  $P=0.001$ ; Fig. 1b). In the case of constitutive ant defence versus past herbivory, the data showed good fit to a linear regression (Fig. 1a). For the relationship between induced ant defence and herbivory, a non-linear regression (hyperbolic function:  $y=ab/b+x$ ) explained a greater percentage of variance (Fig. 1b).

These results add to the body of literature showing that the predictions of ODT are met in ant-plant systems: higher defence with greater magnitude of damage (Agrawal 1998; Agrawal and Dubin-Thaler 1999) and preferential protection of young, more valuable leaves (Gaume and McKey 1999; Heil et al. 2001, 2004). Interestingly, we also found that, while ant patrolling peaked 10–15 min after damage in *Cecropia* trees with high herbivory on old leaves (>25% damage), it showed a peak between 4 and 8 min after damage for trees with



**Fig. 1a, b** The relationship between herbivory on old leaves (% leaf damage) in *Cecropia* trees and (a) constitutive ant defence: number of *Azteca* ants patrolling undamaged leaves; and (b) induced ant defence: number of *Azteca* ants recruited to experimentally damaged leaves. See text for details on the regression analyses

low past herbivory (0–25% damage) (data not shown). Thus, the greater damage-induced ant recruitment, in terms of ant number, observed for *Cecropia* trees with low past herbivory was accompanied by a more rapid response.

Although the aim of our study was to detect patterns and not to elucidate mechanisms, the rapid ant response observed suggests that a volatile cue released by wounded leaves could elicit ant recruitment. A systemic response is less likely to be involved because such plant responses commonly have a time lag of hours (Karban and Baldwin 1997). There is some evidence that defensive ants respond to plant volatile cues (Agrawal 1998; Brouat et al. 2000), but several other factors may account for the location and behaviour of patrolling ants (Agrawal and Rutter 1998). Interestingly, the hyperbolic function that describes the relationship between induced ant defence and herbivory (Fig. 1b) might suggest the existence of a damage threshold that, once

surpassed, “switches off” induced ant recruitment, given the increased basal ant patrolling (Fig. 1a).

Our results indicate a negative relationship between constitutive and induced levels of ant defence at the leaf scale. Interestingly, previous work on the same ant–plant system and site reported a positive association between the peak number of recruited ants following leaf damage and the basal number of ants patrolling leaves before damage (Agrawal 1998). However, this study and the present research are not entirely comparable. The study by Agrawal (1998) was conducted on smaller trees (1.5–2.5 m tall), did not include past herbivory as a factor and spanned 2 years with contrasting rainfall (it pooled data from 1995—a “dry” year for La Selva Biological Station: 2,892 mm—and 1996—an “average” year in terms of rainfall: 4,323 mm; data from La Selva Organization for Tropical Studies (OTS) Meteorological Station). There is evidence that plant ontogeny (Bonato et al. 2003; Del Val and Dirzo 2003) and environmental conditions (Yu and Davidson 1997; Trimble and Sagers 2004) may influence the *Azteca*–*Cecropia* association. The present preliminary study, despite its small sample size and short-term nature, strongly suggests that there is a relationship between herbivory pressure and the patterns of constitutive and damage-induced activity of *Azteca* ants in *Cecropia* trees. Further research should address the fitness consequences for the plant of this apparent optimal defence pattern and the mechanism responsible for the rapid recruitment of ants observed.

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