

# Induced biotic responses to herbivory and associated cues in the Amazonian ant-plant *Maieta poeppigii*

Alexander V. Christianini<sup>1\*</sup> & Glauco Machado<sup>2</sup>

<sup>1</sup>Departamento de Zoologia and <sup>2</sup>Museu de História Natural, Instituto de Biologia, CP 6109, Universidade Estadual de Campinas, 13083-970 Campinas SP, Brazil

Accepted: 28 April 2004

**Key words:** biotic defense, domatia, mutualism, myrmecophyte, *Maieta poeppigii*, Melastomataceae, *Pheidole minutula*, Hymenoptera, Formicidae

## Abstract

Ants inhabiting ant-plants can respond to cues of herbivory, such as the presence of herbivores, leaf damage, and plant sap, but experimental attempts to quantify the dynamic nature of biotic defenses have been restricted to a few associations between plants and ants. We studied the relationship between certain features of the ant-shrub *Maieta poeppigii* Cogn. (Melastomataceae) and the presence or absence of ant patrolling on the leaf surface in plants occupied by the ant *Pheidole minutula* Mayr (Hymenoptera: Formicidae). We also carried out field experiments to examine ant behavior following plant damage, and the potential cues that induce ant recruitment. These experiments included clipping of the leaf apex, as well as the presentation of a potential herbivore (live termite worker) and a foliar extract from *Maieta* on treatment leaves. The presence of ants patrolling the leaves of *M. poeppigii* is influenced by the number of domatia on the plant. Ant patrolling on the leaves of *M. poeppigii* was constant throughout a 24 h cycle, but the mean number of patrolling ants decreased from young to mature leaves, and from leaves with domatia to those without domatia. There was an overall increase in the number of ants on experimental leaves following all treatments, compared to control leaves. Visual and chemical cues associated with herbivory are involved in the induction of ant recruitment in the *Maieta*–*Pheidole* system. The continuous patrolling behavior of ants, associated with their ability to respond rapidly to foliar damage, may result in the detection and repellence/capture of most insect herbivores before they can inflict significant damage to the leaves.

## Introduction

Plants have evolved various strategies to deal with herbivores and the risk of herbivory, including the development of constitutive (e.g. tannins and other phenolic compounds) and/or inducible defenses (Coley, 1986; Schultz, 1988). Inducible responses to herbivory are related to traits that can be adjusted in response to herbivory or the risk of herbivory (Karban et al., 1999). For example, in the wild radish *Raphanus sativus* (Brassicaceae) exposure to chewing herbivores induces an increase in the concentration of chemical defense substances in plant tissues and setose trichomes on the surface of young leaves. After several weeks of exposure to natural conditions, induced wild radish

plants show lower levels of herbivory, fewer herbivores per plant, and increased female fitness compared to the controls (Agrawal, 1998a).

In addition to physical and chemical changes in plant traits in response to damage by herbivores, some studies have focused on the inducible nature of biotic defenses. Volatile chemicals released by damaged plant tissues can attract predators or parasites of herbivores (Dicke et al., 1990; Turlings et al., 1990). Thus, ants inhabiting ant-plants can respond to cues associated with herbivory, such as the presence of herbivores, leaf damage, and plant sap (Fiala & Maschwitz, 1990; Agrawal, 1998b; Agrawal & Dubin-Thaler, 1999; Brouat et al., 2000). Numerous studies have shown ants to be very effective defenders against herbivores (Agrawal & Rutter, 1998, and references therein), but the dynamic nature of anti-herbivore defense by ants has often been neglected (but see Agrawal, 1998b; Agrawal & Dubin-Thaler, 1999). Moreover, the ability of ants inhabiting plants to recognize the signals associated with herbivory and the

\*Correspondence: Alexander V. Christianini, Departamento de Zoologia, Instituto de Biologia, CP 6109, Universidade Estadual de Campinas (Unicamp), 13083-970 Campinas, SP, Brazil. E-mail: avc@unicamp.br

risk of herbivory is only now becoming clear (reviewed in Agrawal & Rutter, 1998).

Ant patrolling behavior among and within ant-plants can be influenced by various plant traits. Several indicators of the efficacy of ant patrolling, such as the number of ants visiting sites of plant damage, the time until discovery of herbivores, the residence time of herbivores, and the overall plant damage, are linked to the number of ants patrolling the leaves before damage, and to the size of resident ant colonies (Vasconcelos, 1991; Rocha & Bergallo, 1992; Agrawal, 1998b). Ant colonies allocate more workers to defend young leaves than older leaves, probably because young leaves have a greater contribution to plant productivity, which may influence the production of ant rewards (Agrawal & Rutter, 1998, and references therein). Some studies have shown that ants act as an inducible biotic defense by recognizing and defending specific sites of recent damage (Agrawal, 1998b; Agrawal & Dubin-Thaler, 1999; Brouat et al., 2000). Although Agrawal & Rutter (1998) have proposed the possible evolution of similar mechanisms of inducible biotic defenses in other obligate ant-plant systems, there is still little information available from manipulative experiments in systems other than *Cecropia-Azteca* (Agrawal, 1998b; Agrawal & Dubin-Thaler, 1999) and *Leonardoxa-Petalomyrmex* (Brouat et al., 2000).

Myrmecophytes are widespread through the tropics, and occur in at least 141 plant genera belonging to 47 families (Davidson & McKey, 1993). In the central Amazon, myrmecophytes can be conspicuous forest elements, where they may reach a density of 380 individuals, ha<sup>-1</sup> (Fonseca & Ganade, 1996). In this region, the local ant-myrmecophyte community consists of at least 25 ant species and 16 plant species (Fonseca & Ganade, 1996), some of which have been intensively studied (Vasconcelos, 1991, 1993; Fonseca, 1993, 1994; Izzo & Vasconcelos, 2002). In this study, we used field experiments and observations to examine the dynamic nature of biotic defenses in the ant-plant *Maieta poeppigii* (Melastomataceae). We were especially interested in five questions: (1) What are the plant traits influencing ant patrolling on the leaves? (2) Are ants efficient biotic defenses against herbivores? (3) Do foliar damage and/or its associated cues induce ant recruitment? (4) What is the pattern of ant responses to inducing cues? (5) Do ants behave as inducible defenses?

## Materials and methods

### Study area and organisms

This study was done in August and September of 2001 in the Reserva Florestal do Km 41 (2°24'S, 59°44'W), a continuous 'terra firme' (upland) forest that is one of the control plots of the Biological Dynamics of Forest Fragments

Project (BDFFP) in the central Amazon, about 100 km north of the city of Manaus, northern Brazil. Its altitude ranges between 100 and 150 m, and rainfall varies from 1900 to 2500 mm annually, with a dry season from June to October. A detailed description of the area can be found in Lovejoy & Bierregaard (1990).

*Maieta poeppigii* is a shrub that occurs in clumps or individually in the understory of terra firme forest, along forest streams (Ribeiro et al., 1999). The leaves are pubescent, opposite, and dimorphic. Only one leaf of each pair bears ant pouches located at the base of its petiole. Each pouch consists of two chambers that have a small opening on the lower leaf surface, between the junction of the central and lateral veins (Benson, 1985). The ants *Pheidole minutula* Mayr and one unidentified species of *Crematogaster* (both Formicidae: Myrmicinae) colonize the domatia of *M. poeppigii* (H.L. Vasconcelos, pers. comm.), with the former species occurring in nearly 70% of ant-bearing plants (Fonseca & Ganade, 1996).

*Pheidole minutula* is an obligate plant-ant with dimorphic workers (major and minor individuals) that tend coccids within domatia (Vasconcelos, 1991; Fonseca, 1999). No experimental studies with *M. poeppigii* have been reported. However, the removal of *P. minutula* from the congeneric *M. guianensis*, which is syntopic and locally more abundant, markedly decreased the reproductive success of the plants (Vasconcelos, 1991). *Pheidole minutula* occurs on more than 95% of individuals of *M. guianensis* and only survives in myrmecophytic plants, which suggests an plant-ant co-dependency (Vasconcelos, 1991). Moreover, individuals of *M. guianensis* colonized by *C. laevis* grow more slowly and may have a lower life expectancy than plants colonized by *P. minutula* (Vasconcelos & Davidson, 2000). Indeed, *Crematogaster* is considered a relatively recent colonist of Amazonian melastome ant-plants and is believed to have played a lesser role in the evolution of myrmecophytic traits in these plants (Benson, 1985). For these reasons, in our observations and experiments we only used plants colonized by *P. minutula*.

### Biotic determinants of ant patrolling

To investigate the plant traits influencing ant patrolling on leaves, we examined the effect of some features of individual plants on the presence or absence of ant patrolling on the leaf surface. On each of 50 different plants (1–2 plants per plant cluster), we measured the height of the plants (from the ground surface to the top of the apical bud), the total number of leaves, and the number of leaves bearing domatia. We recorded the presence or absence of ant patrolling on the leaf surface by carefully scanning the upper surfaces of all the leaves of a given plant. We were aware that some plants could already be colonized by a founding queen but

workers had not yet been produced, or had not begun to patrol. Our protocol did not take into account whether the plant was occupied or not, but focused on the activity of foraging ants on the leaf surface, a relevant behavior from the plant's point of view.

We also followed the time schedule of ant patrolling on the leaf surface using visual inspections of marked leaves at 2 h intervals for 24 h. The leaves were marked with a permanent ink marker that had no detectable effect on ant patrolling behavior (A.V. Christianini, pers. obs.). We checked the number of ants patrolling marked leaves on each of 11 plants in a total of 10 plant clusters. A single, fully expanded leaf in one of the following categories was marked per plant: young leaf bearing domatium, young leaf without domatium, mature leaf bearing domatium, and mature leaf without domatium. The young leaves were randomly assigned to one of the two higher nodes in a given plant, bearing expanded leaves, whereas older leaves were randomly marked in the lower nodes. Leaf age was controlled between categories in a paired design, using a leaf without domatium in the same node as a leaf with domatium.

#### Experimental design

The experiments were always done on paired leaves of individual shrubs (30–100 cm tall) to eliminate inter-plant differences, such as in ant colony size or the location of the plant, which could affect the results. The leaves in a given pair were of similar size and both bore domatia. Treatments were randomly assigned on two leaves bearing domatia. These leaves were at adjacent nodes localized near the top of the plant (young leaves, see above).

#### Ant recruitment to herbivores

To test whether ant recruitment was stimulated by the presence of a potential herbivore, a live termite worker of *Neocapritermes* spec. (Termitidae: Capritermitinae), a non-chemically defended species, was placed on the surface on one of a pair of leaves on each of 10 plants with active ant colonies (cf. Oliveira et al., 1987). At the same time, a small stick the size of a termite was placed on the other leaf (control). The time required for the first ant to establish physical contact (using its antennae or legs) with the termite or the stick was recorded. We also counted the ants on the leaf surface at 2, 4, 6, 8, and 10 min after contact.

#### Ant responses to foliar damage

To examine whether ants recognized damaged tissues and were recruited to leaves with signs of recent damage following visual and/or chemical cues, one leaf on each of 10 shrubs occupied by ants was chosen at random and damaged, while an undamaged leaf served as the control.

Damage was caused by clipping the distal third of the leaf. Concomitantly, both surfaces of the control leaf were pressed with forceps at a similar position as the cut leaf, but with no external sign of foliar damage. Both treatments were applied to the same amount of surface area. We recorded the time required for the first ant to establish physical contact with the treated area, as well as the number of ants on the leaf surface at 2, 4, 6, 8, and 10 min after the initial contact.

#### Ant responses to plant chemical cues

To test whether ants recognized plant chemical cues associated with herbivory, we quantified ant recruitment following elicitation using plant sap. In this test, no visual or tactile cues were present that could affect ant recruitment to sites of recent damage. A rectangular portion (2.5 × 1.5 cm) of the leaf clipped in the previous experiment was macerated with 2 ml of water in a small jar. After allowing the mixture to stand for ca. 15 min, a 0.2 ml droplet was placed near the apex of a treated leaf and 0.2 ml of distilled water was applied to the control leaf. The time until the ants came into contact with these liquids was recorded, as was the number of ants on the leaf surface at 2, 4, 6, 8, and 10 min after contact with the treated area.

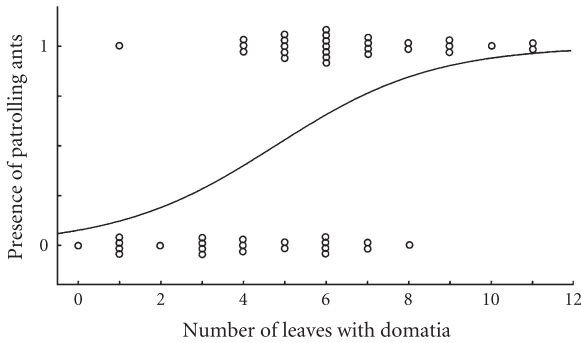
#### Statistical analysis

Logistic regressions were used to investigate the influence of plant traits on the presence or absence of ant patrolling. Temporal patterns of ant patrolling were compared using the Kruskal–Wallis test applied to the number of ants patrolling all leaves at a given time. The relationship between ant activity and leaf age was compared using Friedman analysis of variance. Planned comparisons with a Wilcoxon paired-test and critical values corrected for multiple comparisons (Zar, 1999) were used to investigate the effect of leaf age and the presence or absence of domatia. All experiments were done in a paired design, in which each shrub received both treatments, without replication within a plant. Repeated measures analysis of variance was used to test for treatment effects in all experiments. The data were transformed (square root of the number of ants plus one) in order to correct for normality and homocedasticity. A random sample of the number of ants patrolling young leaves was used as a baseline level for ant activity on the leaf surface before applying treatments. Treatments were considered fixed effects.

## Results

#### Biotic determinants of ant patrolling

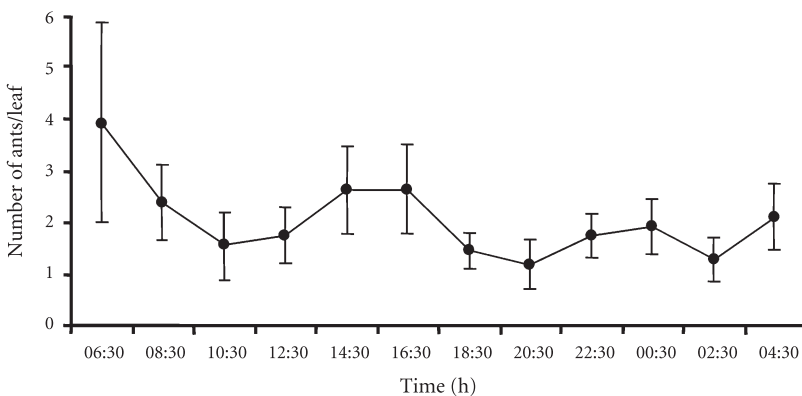
The presence of patrolling ants on the leaves of *M. poeppigii* was strongly influenced by the number of domatia on each



**Figure 1** Influence of the number of leaves with domatia on the presence of patrolling ants on the leaf surface in the Amazonian ant-plant *Maieta poeppigii* (odds ratio = 8.67). The presence and absence of patrolling ants are indicated as 1 and 0, respectively, and each dot represents one plant. Dots were displaced vertically in order to avoid overlapping. Equation of the curve:  $y = \exp(-2.503 + 0.524x) / (1 + \exp(-2.503 + 0.524x))$ .

plant (logistic regression;  $\chi^2 = 14.52$ , d.f. = 1,  $P < 0.001$ ), which explained most of the variance associated with the presence of ants (Figure 1). In general, small plants with few domatia had no patrolling ants on their leaves, whereas the probability of finding patrolling ants on plants bearing nine domatia was nearly 90% (Figure 1). Moreover, the number of domatia in a given plant was influenced by the number of leaves ( $n = 50$ ;  $r^2 = 0.84$ ,  $F = 248.6$ ,  $P < 0.001$ ) and plant size ( $n = 50$ ;  $r^2 = 0.68$ ,  $F = 105.8$ ,  $P < 0.001$ ), but these two variables explained a lower proportion of the variation in the presence of patrolling ants when considered alone in a logistic model or with all variables combined.

Ants patrol the leaves of *M. poeppigii* throughout the 24-h cycle (Figure 2). Major workers were seen in only one event above the leaves of *M. poeppigii*, when an unidentified katydid (Tettigoniidae) was captured. The number of patrolling ants was constant throughout the day ( $H = 4.976$ , d.f. = 11,  $P > 0.90$ ), considering the number of ants on young leaves with domatia used in the experiments.



**Figure 2** Daily cycle of patrolling activity by the ant *Pheidole minutula* on domatia-bearing young leaves of the host plant *Maieta poeppigii*. The points are the mean  $\pm$  SE of the number of ants observed on leaf surface at a given time.

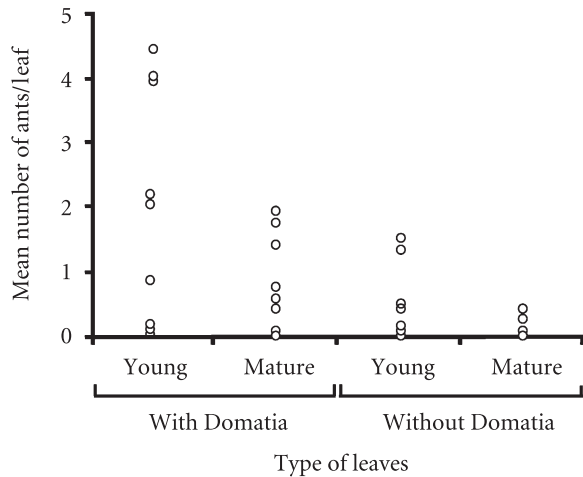
However, ant activity was not independent of leaf age or of the presence/absence of leaf domatia (Friedman ANOVA:  $\chi^2 = 11.67$ , d.f. = 3,  $P < 0.01$ ). Thus, the number of patrolling ants decreased in the absence of domatia and with increasing leaf age (Figure 3). Leaves with domatia had a median of 1.13 patrolling ants at a given time, compared to 0.08 patrolling ants on leaves without domatia ( $n = 18$ ;  $z = 3.74$ ,  $P = 0.0002$ ), a 14-fold difference. Young leaves had a median of 0.46 patrolling ants at a given time, whereas mature leaves had 0.33 ants ( $n = 18$ ;  $z = 2.82$ ,  $P = 0.005$ ).

#### Ant recruitment experiments

There was no difference in the time to contact between control (sticks) and treated (termites) leaves (Wilcoxon paired-test;  $n = 10$ ;  $z = 1.48$ ,  $P = 0.14$ ). Similarly, there was also no difference in the time to contact between the two groups in the experiments involving leaf damage ( $n = 10$ ;  $z = 1.36$ ,  $P = 0.17$ ) and chemical cues ( $n = 10$ ;  $z = -0.059$ ,  $P = 0.95$ ). The time until first contact by an ant was not significantly different among treatments (mean  $\pm$  SD in seconds): herbivores,  $85.3 \pm 126.5$ ; foliar damage,  $45.5 \pm 57.0$ ; chemical cues,  $89.7 \pm 175.2$  (Kruskal-Wallis:  $H = 0.53$ , d.f. = 2,  $P = 0.77$ ).

However, there was an overall increase in the number of ants on experimental leaves following treatments (Table 1, Figure 4). Ant recruitment to leaves with a potential herbivore was much greater than for control leaves. Ant recruitment was rapid and localized, with up to 43 ants attending the leaf with a termite at a given time, compared to a maximum of seven ants on control leaves. The peak of ant recruitment occurred 8–10 min after the first contact (Figure 4A). Similar results were obtained for ant responses to leaf damage and chemical cues, but the number of ants attending treated leaves was lower, peaking at 4 min after contact (Figures 4B,C). Ant activity on control leaves was unchanged following manipulation (Figure 4).

Ants were visibly excited on all treated leaves, with fast movements and the recruitment of major workers, which



**Figure 3** Mean number of ants on the leaves of *Maieta poeppigii* throughout a 24-h cycle. The number of ants determined by visual scans at 2 h intervals is shown according to type of leaves. Each dot represents the mean for one plant, with  $n = 11$  for all types of leaves, except for mature leaves without domatia ( $n = 7$ ). Not all points are seen because of overlap.

did not occur during regular patrolling activities. In several cases, the attending ants came from other domatia within the plant. The first ant that detected the termite either returned to the domatium, followed by an influx of ants to the leaf, or attacked the termite, biting a leg or antenna to hinder termite movements. A swarm of ants recruited to this leaf within a few minutes, immobilized the intruder and started to cut the prey into smaller pieces. Attending

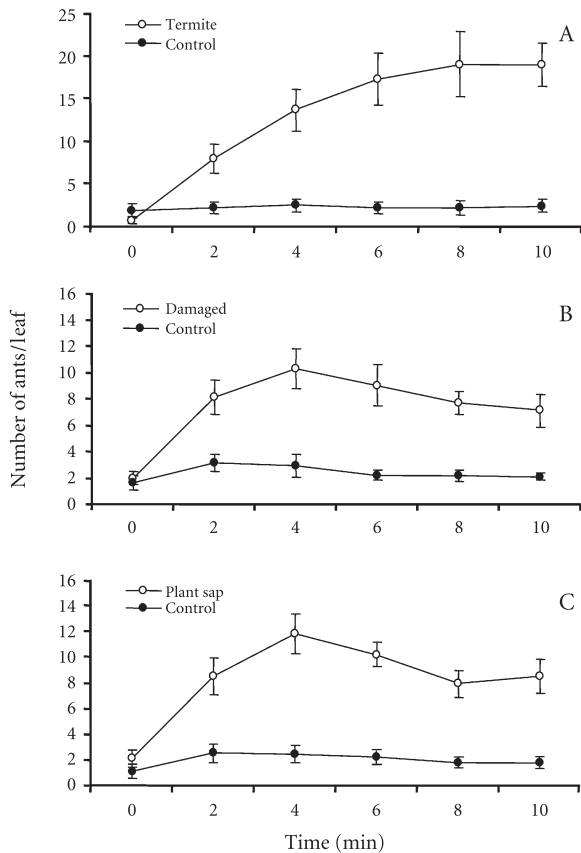
ants transported the remains of the termite into the domatium on the same leaf. Ants spent ca. 40 min cutting and transporting the termite, after which time the activity of the ants on the leaf surface returned to control levels. In experiments using leaf damage and plant sap, patrolling ants always returned to the domatium after detecting the treatments and showed excitement characterized by bursts of running, vigorous vibrations of the antennae, and curling of the gaster.

## Discussion

Nesting space rather than food or territory is the main limiting factor of ant colony size in species living exclusively on myrmecophytes (Fonseca, 1993, 1999, but see Itino et al., 2001). If the domatia are restricted to the leaves, as in *Maieta*, the greater the number of leaves, the larger the ant colony (Fonseca, 1999). The production of young leaves and, consequently, young domatia, is controlled by the plant. However, ants can accelerate the growth of their partners by making some crucial nutrients available to the plant (e.g., Beattie, 1989) and/or by efficiently defending the photosynthetic tissues against herbivores (e.g., Vasconcelos & Davidson, 2000). The speed and intensity of ant responses to herbivory vary depending on colony size (Rocha & Bergallo, 1992), the number of ants patrolling the leaves before damage (Agrawal, 1998b), and the recognition of cues indicative of herbivory (Fiala & Maschwitz, 1990; Agrawal, 1998b; Agrawal & Dubin-Thaler, 1999; Brouat et al., 2000). Our results showed that the number of

**Table 1** Repeated-measures analysis of variance for the effects of induction treatments on the recruitment of *Pheidole minutula* ants by *Maieta poeppigii* shrubs. The probabilities corrected for sphericity were obtained using the Greenhouse–Geisser (G-G) and Huyn–Feldt (H-F) corrections

Experiment	Source	d.f.	MS	F	P	G-G	H-F
Herbivore (termite)	Treatment	1	89.130	27.637	<0.001		
	Error	18	3.225				
	Time	5	7.639	26.288	<0.001	<0.001	<0.001
	Time × Treatment	5	6.557	22.564	<0.001	<0.001	<0.001
	Error	90	0.291				
Foliar damage	Treatment	1	29.698	38.540	<0.001		
	Error	18	0.771				
	Time	5	2.392	10.486	<0.001	<0.001	<0.001
	Time × Treatment	5	1.067	4.677	<0.001	0.006	0.003
	Error	90	0.228				
Chemical cues	Treatment	1	46.812	44.625	<0.001		
	Error	18	1.049				
	Time	5	3.176	14.401	<0.001	<0.001	<0.001
	Time × Treatment	5	1.315	5.960	<0.001	0.001	<0.001
	Error	90	0.221				



**Figure 4** Recruitment of ants to leaves that received a termite (A), foliar damage (B), and plant sap (C). The controls were a termite-sized stick, foliar pressure, and water, respectively. The points are the mean  $\pm$  SE of the number of ants observed on the leaf surface at a given time.

domatia in *M. poeppigii* was an important predictor of *P. minutula* activity on the leaves. Larger plants have colonies with more individuals and, consequently, more workers available for patrolling the leaves, thereby creating a positive feedback between the ants and their plant partner.

As in other ant-myrmecophyte systems (e.g., Downhower, 1975; McKey, 1984; Madden & Young, 1992; Moog et al., 1998, but see Vasconcelos, 1991), the ant colonies on *M. poeppigii* concentrate their efforts in patrolling young leaves bearing domatia. This pattern could reflect the proximity of the ant colony to domatia bearing leaves, although older leaves with domatia had fewer patrolling ants. Another possibility is that young leaves attract more herbivores (Fonseca, 1994; Heil et al., 2001a), and because *P. minutula* forage on these, they were simply working in more profitable areas (see also Brouat et al., 2000). Furthermore, leaves of different ages can have different values for the plant, since photosynthetic rates and the efficiency of nitrogen use decline with leaf age (Kitajima et al., 2002). If

the fitness of an ant colony is directly linked with the quality of its host (Heil et al., 1997, 2001b), it is expected that the ant colony will use more resources in patrolling those leaves with a greater value for the plant.

Ants patrolled the leaves of *M. poeppigii* throughout the 24-h cycle, with similar levels of activity during the day. During this time, they inspected almost every portion of the leaf surface and provided an effective barrier against potential herbivores (see also Vasconcelos, 1991). The continuous patrolling behavior of ant colonies may result in the detection and repellence/capture of most insect herbivores before they can cause significant damage to the leaves. The lack of difference in the time of detection between control and treated groups provides additional support for this idea. Although the detection of herbivores and leaf damage is likely to be controlled primarily by ant patrolling, the presence of ants on the leaf surface per se can be enough to prevent the action of herbivores. For example, females of the non-myrmecophilous butterfly *Eunica bechina* avoid oviposition on leaves of their host plant occupied by artificial rubber ants (Freitas & Oliveira, 1996). The presence of ants on the leaf surface can also elicit an avoidance response in other herbivorous insects, especially those that use visual cues to evaluate plant quality prior to oviposition or feeding.

The response of the ant colonies on *M. poeppigii* to herbivores or cues associated with herbivory may be considered an induced biotic defense, since the number of ants on the leaves increased rapidly and locally after damage. Ants also recruit in response to the aqueous extract of leaves from *M. poeppigii*, suggesting that such induction is chemically mediated, as previously recorded in other ant-plant systems (reviewed in Agrawal & Rutter, 1998). However, it is important to stress that the leaves used in the maceration were previously in contact with ants from another colony and it is therefore difficult to discriminate if the recruiting ants were responding to either plant chemicals or ant pheromones deposited on leaf surface. This methodological problem was not mentioned in any previous study on ant induced defenses (e.g., Agrawal, 1998b; Lapola et al., 2003), but the curves of ant recruitment produced by leaf damage and plant sap were always quite similar. Although we believe that ant recruitment was driven mainly by chemical cues from the plant, future experimental manipulations should isolate the putative noise represented by ant pheromones on the macerated leaf.

We found no evidence of a direct reward (e.g., nectar: Heil et al., 2001c) produced by the plant immediately after the damage used to induce ant recruitment. The behavior of the ants attending sites of recent damage or sites treated with plant sap suggested that the ants could be releasing alarm substances or pheromones (Agrawal & Dubin-Thaler,

1999), in order to communicate information about leaf damage to the rest of the ant colony. In such cases, the ants could be 'inducing themselves', as was proposed for obligate ant-plant mutualistic associations (Agrawal & Rutter, 1998). In obligate ant-myrmecophyte systems, ant colony fitness is so tightly linked with plant vigor that selection has favored the evolution of ant responses to damage and associated cues, regardless of any reward from the plant itself. Previous evidence indicated a strong ant-plant codependency in the system *P. minutula* – *Maieta* (Vasconcelos, 1991; Vasconcelos & Davidson, 2000), and our results corroborate the prediction regarding the self-induction of *P. minutula* after leaf damage in *M. poeppigii*.

The rapid induction of ant recruitment should be particularly effective against unpredictable and mobile herbivores. Moreover, in *Maieta*, the combination of physical (trichomes), biotic (ants), and possibly chemical defenses may provide an unpredictable environment for herbivores, thereby constraining the benefits to herbivores and the evolution of counter defenses (see Karban et al., 1997). Only a few specialized herbivores feed on individuals of *M. poeppigii* occupied by ants, and the damage caused by herbivores to young leaves is generally very low (pers. obs.). When there is a successful attempt by herbivores to feed, ant recruitment should provide a fast response to minimize the subsequent damage suffered by the plant. The mean time required for the ants to detect a potential herbivore or experimental leaf damage in *M. poeppigii* ranged from 45 to 90 s. Ant recruitment peaked 8–10 min after detection, at which time up to 43 workers (minors and majors) could be found on the leaf. This fast response reinforces the importance of ant self-induction as a barrier against widespread damage.

Because obligate plant-ants are very dependent on their hosts for food and shelter, ant populations are directly limited by their host plant populations (Fonseca, 1999). This association generates strong intra- and interspecific competition among ants, and may be driving the specialization of ants towards plant partners that provide most of their requirements (Fonseca & Ganade, 1996, and references therein). The result could be the highly species-specific interactions seen in many studies (e.g., Benson, 1985; Fiala & Maschwitz, 1990; Fonseca & Ganade, 1996). During the colonization of myrmecophyte seedlings, the recognition of the appropriate partner plant by foundress queens is a crucial phase. Foundress queens recognize their own specific partner-plant species by the chemical characteristics of the seedling surface (Inui et al., 2001). It is possible that the workers use these same chemical characteristics of the host plant to respond to the compounds released by damaged tissues, but such a hypothesis remains to be experimentally tested.

## Acknowledgements

This work was done during the 9th edition of the course 'Ecologia da Floresta Amazônica' supported by the Instituto Nacional de Pesquisas da Amazônia (INPA), the Biological Dynamics of Forest Fragments Project (BDFFP), and the Smithsonian Institution. The authors thank the coordinators of the course, Eduardo M. Venticinque and Jansen Zuanon, and all participants for their helpful suggestions and encouragement during the fieldwork. We also thank Drs Heraldo Vasconcelos, Carlos R. Fonseca, Gustavo Q. Romero, and an anonymous reviewer for their comments on the manuscript. A.V.C. (grant 02/12895-8) and G.M. (grant 02/00381-0) are supported by fellowships from Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP).

## References

- Agrawal AA (1998a) Induced responses to herbivory and increased plant performance. *Science* 279: 1201–1202.
- Agrawal AA (1998b) Leaf damage and associated cues induce aggressive ant recruitment in a Neotropical ant-plant. *Ecology* 79: 2100–2112.
- Agrawal AA & Dubin-Thaler BJ (1999) Induced responses to herbivory in the Neotropical ant-plant association between *Azteca* ants and *Cecropia* trees: response of ants to potential inducing cues. *Behavioral Ecology and Sociobiology* 45: 47–54.
- Agrawal AA & Rutter MT (1998) Dynamic anti-herbivore defense in ant-plants: the role of induced responses. *Oikos* 83: 227–236.
- Beattie A (1989) Myrmecotrophy: plants fed by ants. *Trends in Ecology and Evolution* 4: 172–176.
- Benson WW (1985) Amazon ant-plants. Amazonia (ed. by G Prance & T Lovejoy), pp. 239–266. Pergamon Press, New York.
- Brouat C, McKey D, Bessière J-M, Pascal L & Hossaert-McKey M (2000) Leaf volatile compounds and the distribution of ant patrolling in an ant-plant protection mutualism: Preliminary results on *Leonardoxa* (Fabaceae: Caesalpinioideae) and *Petalomyrmex* (Formicidae: Formicinae). *Acta Oecologica* 21: 349–357.
- Coley PD (1986) Costs and benefits of defense by tannins in a neotropical tree. *Oecologia* 70: 238–241.
- Davidson DW & McKey D (1993) The evolutionary ecology of symbiotic ant-plant relationships. *Journal of Hymenoptera Research* 2: 13–83.
- Dicke M, van Beek TA, Posthumus MA, Ben Dom N, van Bokhoven H & de Groot AE (1990) Isolation and identification of volatile kairomone that affects acarine predator-prey interactions: involvement of host plant in its production. *Journal of Chemical Ecology* 16: 381–396.
- Downhower JF (1975) The distribution of ants on *Cecropia* leaves. *Biotropica* 7: 59–62.
- Fiala B & Maschwitz U (1990) Studies of the South East Asian ant-plant association *Crematogaster borneensis*/Macaranga: adaptations of the ant partner. *Insectes Sociaux* 37: 212–231.
- Fonseca CR (1993) Nesting space limits colony size of the plant-ant *Pseudomyrmex concolor*. *Oikos* 67: 473–482.

- Fonseca CR (1994) Herbivory and the long-lived leaves of an Amazonian ant-tree. *Journal of Ecology* 82: 833–842.
- Fonseca CR (1999) Amazonian ant–plant interactions and the nesting space limitation hypothesis. *Journal of Tropical Ecology* 15: 807–825.
- Fonseca CR & Ganade G (1996) Asymmetries, compartments and null interactions in an Amazonian ant-plant community. *Journal of Animal Ecology* 65: 339–347.
- Freitas AVL & Oliveira PS (1996) Ants as selective agents on herbivore biology: effects on the behaviour of a non-myrmecophilous butterfly. *Journal of Animal Ecology* 65: 205–210.
- Heil M, Fiala B, Linsenmair KE, Zotz G, Menke P & Maschwitz U (1997) Food body production in *Macaranga triloba* (Euphorbiaceae): a plant investment in anti-herbivore defence via symbiotic ant partners. *Journal of Ecology* 85: 847–861.
- Heil M, Fiala B, Maschwitz U & Linsenmair KE (2001a) On benefits of indirect defence: short- and long-term studies of antiherbivore protection via mutualistic ants. *Oecologia* 126: 395–403.
- Heil M, Hilpert A, Fiala B & Linsenmair KE (2001b) 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 KE (2001c) 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 USA* 98: 1083–1088.
- Inui Y, Itioka T, Murase K, Yamaoka R & Itino T (2001) Chemical recognition of partner plant species by foundress ant queens in *Macaranga-Crematogaster* myrmecophytism. *Journal of Chemical Ecology* 27: 2029–2040.
- Itino T, Itioka T, Hatada A & Hamid AA (2001) Effects of food rewards offered by ant-plant *Macaranga* on the colony size of ants. *Ecological Research* 16: 775–786.
- Izzo TJ & Vasconcelos HL (2002) Cheating the cheater: domatia loss minimizes the effects of ant castration in an Amazonian ant-plant. *Oecologia* 133: 200–205.
- Karban R, Agrawal AA & Mangel M (1997) The benefits of induced defenses against herbivores. *Ecology* 78: 1351–1355.
- Karban R, Agrawal AA, Thaler JS & Adler LS (1999) Induced plant responses and information content about risk of herbivory. *Trends in Ecology and Evolution* 14: 443–447.
- Kitajima K, Mulkey SS, Samaniego M & Wright SJ (2002) Decline of photosynthetic capacity with leaf age and position in two tropical pioneer tree species. *American Journal of Botany* 89: 1925–1932.
- Lapola DM, Bruna EM & Vasconcelos HL (2003) Contrasting responses to induction cues by ants inhabiting *Maieta guianensis* (Melastomataceae). *Biotropica* 35: 295–300.
- Lovejoy TE & Bierregaard RO Jr (1990) Central Amazonian forests and the Minimum Critical Size of Ecosystem Project. Four Neotropical Rainforests (ed. by AH Gentry), pp. 60–71. Yale University Press, New Haven, USA.
- Madden D & Young TP (1992) Symbiotic ants as an alternative defense against giraffe herbivory in spinescent *Acacia drepanolobium*. *Oecologia* 91: 235–238.
- McKey D (1984) Interaction of the ant-plant *Leonardoxa africana* (Caesalpiniaceae) with its obligate inhabitants in a rainforest in Cameroon. *Biotropica* 16: 81–99.
- Moog J, Drude T & Maschwitz U (1998) Protective function of the plant-ant *Cladomyrma maschwitzi* to its host, *Crypteronia griffithii*, and the dissolution of the mutualism (Hymenoptera: Formicidae). *Sociobiology* 31: 105–129.
- Oliveira PS, Oliveira-Filho AT & Cintra R (1987) Ant foraging on ant-inhabited *Triplaris* (Polygonaceae) in western Brazil: a field experiment using live termite-baits. *Journal of Tropical Ecology* 3: 193–200.
- Ribeiro JELS, Hopkins MJG, Vicentini A, Sothers CA, da Costa MAS, Brito JM, Souza MAD, Martins LHP, Lohmann LG, Assunção PACL, Pereira EC, Silva CF, Mesquita MR & Procópio LC (1999) Flora da Reserva Ducke: Guia de Identificação das Plantas Vasculares de uma Floresta de Terra-Firme na Amazônia Central. INPA-DFID, Manaus, Brazil.
- Rocha CFD & Bergallo HG (1992) Bigger ant colonies reduce herbivory and herbivore residence time on leaves of an ant-plant: *Azteca muelleri* vs. *Coelomera ruficornis* on *Cecropia pachtachia*. *Oecologia* 91: 249–252.
- Schultz JC (1988) Plant responses induced by herbivores. *Trends in Ecology and Evolution* 3: 45–49.
- Turlings TCJ, Tumlinson JH & Lewis WJ (1990) Exploitation of herbivore-induced plant odors by host-seeking parasitic wasps. *Science* 250: 1251–1253.
- Vasconcelos HL (1991) Mutualism between *Maieta guianensis* Aubl., a myrmecophytic melastome, and one of its ant inhabitants: ant protection against insect herbivores. *Oecologia* 87: 295–298.
- Vasconcelos HL (1993) Ant colonization of *Maieta guianensis* seedlings, an Amazon ant-plant. *Oecologia* 95: 439–443.
- Vasconcelos HL & Davidson DW (2000) Relationship between plant size and ant associates in two Amazonian ant-plants. *Biotropica* 32: 100–111.
- Zar JH (1999) *Biostatistical Analysis*, 4th edn. Prentice Hall, New Jersey.