Oecologia (2004) 138: 558–565
DOI 10.1007/s00442-003-1455-5

PLANT ANIMAL INTERACTIONS

**Emilio M. Bruna · David M. Lapola ·
Heraldo L. Vasconcelos**

# Interspecific variation in the defensive responses of obligate plant-ants: experimental tests and consequences for herbivory

Received: 24 July 2003 / Accepted: 6 November 2003

**Abstract** The aggressive behavior of ants that protect plants from herbivores in exchange for rewards such as shelter or food is thought to be an important form of biotic defense against herbivory, particularly in tropical systems. To date, however, no one has compared the defensive responses of different ant taxa associated with the same plant species, and attempted to relate these differences to longer-term efficacy of ant defense. We used experimental cues associated with herbivory—physical damage and extracts of chemical volatiles from leaf tissue—to compare the aggressive responses of two ant species obligately associated with the Amazonian myrmecophyte *Tococa bullifera* (Melastomataceae). We also conducted a colony removal experiment to quantify the level of resistance from herbivores provided to plants by each ant species. Our experiments demonstrate that some cues eliciting a strong response from one ant species elicited no response by the other. For cues that do elicit responses, the magnitude of these responses can vary interspecifically. These patterns were consistent with the level of resistance provided from herbivores to plants. The colony removal experiment showed that both ant species defend plants from herbivores: however, herbivory was higher on plants colonized by the less aggressive ant species. Our results add to the growing body of literature indicating defensive ant responses are stimulated by cues associated with herbivory. However, they also suggest the local and regional variation in the composition of potential partner taxa could influence the ecology and evolution of defensive mutualisms in ways that have previously remained unexplored.

**Keywords** *Azteca* · *Crematogaster laevis* · Myrmecophytes · Mutualism · *Tococa bullifera*

## Introduction

Ants that indirectly defend plants from herbivores in exchange for rewards such as food or shelter are one of the defining characteristics of tropical forests, and there are over 200 species of myrmecophytic plants in Amazonia alone (Benson 1985). These ants are often obligately associated with their host plants, only establishing colonies in swollen thorns, leaf pouches, hollow stems, or other domatia on the plant (Benson 1985; Hölldobler and Wilson 1990). Following the pioneering work of Janzen (1966, 1967), a number of studies have experimentally confirmed that ants can reduce rates of herbivory on host plants (reviewed in Beattie 1985; Davidson and McKey 1993; Bronstein 1998). Since then, considerable research has attempted to identify the proximate cues associated with herbivory that can elicit ant defensive responses (reviewed in Agrawal and Rutter 1998).

Studies quantifying the defensive capability of ant residents typically focus on a single ant species (Schupp 1986; Fiala et al. 1989; Vasconcelos 1991; Dyer and Letourneau 1999; but see McKey 1984; de la Fuente and Marquis 1999). Ant-plant relationships in which a plant hosts a single species of ant are rare, however, and most

E. M. Bruna (✉)
Department of Wildlife Ecology and Conservation, University of Florida,
Gainesville, FL 32611-0430, USA
e-mail: BrunaE@wec.ufl.edu
Tel.: +1-352-8460634
Fax: +1-352-3926984

E. M. Bruna
Tropical Conservation and Development Program, University of Florida,
Gainesville, FL 32611, USA

D. M. Lapola
Departamento de Ecologia, Universidade Estadual Paulista,
13506-900 Rio Claro, SP, Brazil

H. L. Vasconcelos
Instituto de Biologia, Universidade Federal de Uberlândia,
38400-902 Uberlândia, MG, Brazil

E. M. Bruna · D. M. Lapola · H. L. Vasconcelos
Biological Dynamics of Forest Fragments Project, INPA-PDBFF,
CP 478, 69011-970 Manaus, AM, Brazil

myrmecophytic plants host multiple ant taxa (Fonseca and Ganade 1996; Bronstein 1998). These ants often vary with respect to traits that could influence their response to stimuli eliciting defensive behavior, such as aggressiveness, colony structure, foraging behavior, and evolutionary history (Davidson and McKey 1993). If there is interspecific variation in response to cues associated with herbivory, it could help explain why some ant species are better defenders of host plants than others (Bronstein 1998). To date, however, studies comparing the short-term defensive responses of different ant species associated with the same plant species are rare (e.g., Gaume and McKey 1999; Lapola et al. 2003), and no studies have related these differences to longer-term efficacy of ant defense.

*Tococa bullifera* (Melastomataceae) is an Amazonian myrmecophyte associated with *Crematogaster laevis* and an undescribed species of *Azteca* (Fowler 1993; Fonseca and Ganade 1996; Vasconcelos and Davidson 2000). In this study, we compare the responses of these resident ant species to physical and chemical cues resulting from simulated herbivory. We then attempt to link these responses to each species' defensive capability using a colony removal experiment. We hypothesize that *T. bullifera* colonized by the ant species with the superior defensive response will experience lower rates of herbivory over time.

## Materials and methods

All fieldwork was conducted from April to September 2002 in Reserve No. 1501 of the Biological Dynamics of Forest Fragments Project (BDFFP; Bierregaard et al. 2002). This is a large continuous forest site located 70 km north of Manaus, Brazil (2°30′S, 60°W). The habitat in the reserve is non-flooded lowland rain forest, with a 30- to 35-m-tall canopy and an understory dominated by stemless palms (Rankin-de Mérona et al. 1992). Soils in the sites are highly acidic and nutrient poor xanthic farralsols with poor water retention capacity (Fearnside and Leal Filho 2002). Annual rainfall ranges from 1,900 to 3,500 mm per year, and there is a pronounced dry season from June to October (BDFFP records).

*T. bullifera* (Melastomataceae) is an understory shrub that grows to a maximum height of 3 m. It has two pouches at the base of each leaf in which ant queens establish colonies (Michelangeli 2000; Vasconcelos and Davidson 2000). Although up to six putative species of ants have been found inhabiting these domatia (Fonseca and Ganade 1996), most *T. bullifera* are colonized by either *C. laevis* or an undescribed species of *Azteca* (Vasconcelos and Davidson 2000). These ants forage for insects on the host plant's leaves and may also tend scale insects for honeydew (Vasconcelos and Davidson 2000). At any given time, only one species of ant inhabits a plant; both ant species also colonize an additional 2–3 plant species (Fonseca and Ganade 1996).

### Patrolling behavior

To evaluate the baseline number of *Azteca* sp. and *C. laevis* workers patrolling *T. bullifera* leaves, we conducted instantaneous counts of worker numbers. For these counts we used one randomly selected leaf that had recently expanded (hereafter 'new leaf') and one randomly selected older leaf from the base of the plant (hereafter 'mature leaf') on 16 plants inhabited by each species. The numbers of ants patrolling the new and mature leaves (expressed per $\mathrm{cm}^2$ of leaf surface to correct for different leaf sizes) were compared using Wilcoxon signed rank tests. Plants inhabited by *Azteca* sp. and *C. laevis* were analyzed separately; all surveys were conducted between 8:00 and 11:00 a.m. because preliminary surveys indicated ant activity was independent of time of day (results not shown).

### Response to cues associated with herbivory

To quantify the defensive responses of ants, we used two classes of experimental cues that have been shown to elicit responses in tropical ant-plant systems: leaf damage and extracts of volatile compounds from leaf tissue (Agrawal 1998; Agrawal and Dubin-Thaler 1999). The responses of ants to these cues were assessed using a paired-leaf design, in which each plant has both a control and experimental leaf. For each of the trials described below, we selected ten *T. bullifera* with active colonies of each species. On each plant, we selected two similarly sized new leaves with comparable levels of herbivory. One leaf was randomly assigned to the 'control' treatment, while the other was assigned to the 'experimental induction' treatment. We counted the number of ants patrolling leaves immediately prior to applying the treatment (time=0), then 2, 4, 6, 8, 10, 15, 30, 60, 120, and 240 min after treatments were applied. A final census was conducted 24 h after induction.

The two types of leaf damage we applied—pin wounds and punch holes—both mimic naturally occurring herbivore damage to *T. bullifera* (caused by coleopterans and orthopterans, respectively). Pin damage was applied using a total of 90 steel pins passed through a styrofoam block: the leaf assigned to the 'induction treatment' was damaged by pressing the pins completely through the leaf while the control leaf had the styrofoam end pressed against it several times without damaging the leaves. For punch hole damage, the 'control' leaf was tapped five times with a hole punch while the leaf assigned to the 'induction treatment' had five holes punched along the leaf margins. Pins and punch holes removed approximately the same amount of leaf tissue (~190 $\mathrm{mm}^2$), thereby controlling for potential variation in responses resulting from differences in the absolute amount of leaf tissue removed.

Leaf volatiles were extracted by soaking approximately 5 g fresh *T. bullifera* tissue in 60 ml of methanol for 24 h, after which we removed the leaf tissue with a strainer (Agrawal and Dubin-Thaler 1999). Five drops of the extract were applied to the leaf assigned to the induction treatment, while the control leaf received five drops of methanol.

The effect of all cues on ln(ant abundance) over 24 h was analyzed using repeated measures analysis of variance. Each plant had both treatments, with no replication within plants. Treatment (i.e., the form of induction) was considered a fixed effect, with each plant considered a random effect. In these types of tests the between-subjects mean squared and degrees of freedom are used in the denominator for the calculation of the *F*-statistic (Zar 1999). Counts of patrolling ants were made immediately prior to initiating the experimental treatments to confirm there was no significant difference in the number of patrolling ants on control and experimental leaves (results not shown). Systat v.8.0 was used for this analysis (SSI 2001).

### Protection of host plants against herbivory

To determine the efficacy of defense against herbivores provided to *T. bullifera* by resident ants we performed a colony-exclusion experiment. We began by selecting 40 *T. bullifera* growing along the trails in Reserve No. 1501, half of which were inhabited by *C. laevis* and half of which were inhabited by *Azteca* sp. Ten of the plants inhabited by each species were randomly assigned to have their colonies removed using an organophosphate contact insecticide (Dimmy, Serv-San, Cajamar, SP, Brazil), while the other ten were controls in which colonies were left undisturbed. Previous studies have shown ants may differentially protect leaves of different ages (e.g., McKey 1984; Izzo and Vasconcelos 2002), therefore we randomly selected three mature and three recently expanded leaves on each plant on which to track changes in herbivory following colony removal.

Immediately prior to colony removal we quantified leaf area for all six leaves using the regression equation $\sqrt{\text{Leaf area (cm}^2)} = 0.473 \times \text{Leaf length (cm)}$ ($P<0.0001$, $F_{1,228}=53{,}821.06$, $R^2=0.996$, $n=229$ leaves), from which we subtracted the area that had previously been removed by herbivores (based on leaf tracings made on millimeter-squared graph paper). These measurements were repeated 6 weeks after colonies had been removed: periodic surveys revealed no plants were recolonized during this time period.

We compared the initial and final percentage of leaf tissue removed by herbivores with a repeated-measures split-plot analysis of variance. Ant species (*C. laevis* or *Azteca* sp.) and Treatment (colony removal or control) were main effects, with each plant considered a separate plot and leaf age as the within-plot factor. There were three replicates nested in each within-plot factor, i.e., herbivory was measured on three leaves per plant in each of the leaf age categories. We rank-transformed the initial and final values of herbivory because the residuals for the raw data were significantly non-normal (Conover and Iman 1981): however, throughout the manuscript we present back-transformed values. This analysis was conducted using S-Plus 2000 (Mathsoft 1999).

Of particular interest in this analysis are not the main effects, but rather how (1) the Species×Treatment interaction and (2) the Species×Leaf Age×Treatment interaction change during the 6 weeks of the experiment. A significant interaction of these terms with Time indicates the patterns of herbivory in these treatment combinations changed significantly between the first and final measurement.

## Results

### Patrolling behavior

Overall, the mean number of *Azteca* sp. workers patrolling *T. bullifera* leaves was 2.4-fold greater than the number of *C. laevis* workers (1.88±0.26 SE vs 0.78±0.18 SE). However, the median number of ants per cm$^2$ of leaf tissue was not significantly different between new and mature leaves for either *Azteca* sp. (median$_{\text{new}}$=0.021, mean$_{\text{new}}$=0.021±0.003 SE; median$_{\text{mature}}$=0.014, mean$_{\text{mature}}$=0.021±0.005 SE; $Z=-0.115$, $P=0.88$), or *C. laevis* (median$_{\text{new}}$=0.009, mean$_{\text{new}}$=0.011±0.003 SE; median$_{\text{mature}}$=0.000, mean$_{\text{mature}}$=0.008±0.005 SE; $Z=-0.764$, $P=0.44$).

### Response to cues associated with herbivory

All three cues significantly increased the number of *Azteca* sp. workers patrolling experimental leaves relative to control leaves (Table 1; Fig. 1A–C). The main effect of time was also significant in all cases, with worker numbers increasing rapidly followed by a decrease 15 min after induction cues were applied (Fig. 1A–C). For *C. laevis*, only leaf damage elicited a significantly increase in ant numbers (Table 1; Fig. 1D–F). Although we did not compare the intensity of the responses by *C. laevis* and *Azteca* sp. statistically, visual inspection indicates the intensity of the response elicited was much lower in plants inhabited by *C. laevis* (Fig. 1). For both ant species, the

**Table 1** Repeated measures ANOVA for the effect cues on ant recruitment

| | *Azteca* sp. | | | | *Crematogaster laevis* | | | |
|---|---|---|---|---|---|---|---|---|
| Experiment | Source | *df* | *MS* | *F* | Source | *df* | *MS* | *F* |
| Punched leaves | Treatment | 1 | 35.705 | 18.013** | Treatment | 1 | 16.817 | 17.720** |
| | Plant | 9 | 6.583 | 3.321* | Plant | 9 | 2.433 | 2.564 |
| | Error | 9 | 1.982 | | Error | 9 | 0.949 | |
| | Time | 9 | 4.367 | 27.619**** | Time | 9 | 1.527 | 6.160**** |
| | Time×Treatment | 9 | 0.384 | 2.431* | Time×Treatment | 9 | 0.150 | 0.604 |
| | Time×Plant | 81 | 0.280 | 1.77** | Time×Plant | 81 | 0.242 | 0.976 |
| | Error | 81 | 0.0158 | | Error | 81 | 0.248 | |
| Pin wounds | Treatment | 1 | 14.894 | 8.092* | Treatment | 1 | 17.566 | 16.632** |
| | Plant | 9 | 4.095 | 2.225 | Plant | 9 | 11.301 | 10.7** |
| | Error | 9 | 1.841 | | Error | 9 | 1.056 | |
| | Time | 9 | 9.338 | 42.269**** | Time | 9 | 1.467 | 6.063**** |
| | Time×Treatment | 9 | 0.404 | 1.828 | Time×Treatment | 9 | 0.125 | 0.515 |
| | Time×Plant | 81 | 0.481 | 2.179*** | Time×Plant | 81 | 0.282 | 1.167 |
| | Error | 81 | | | Error | 81 | 0.242 | |
| Extract | Treatment | 1 | 27.520 | 10.037* | Treatment | 1 | 3.182 | 1.754 |
| | Plant | 9 | 8.816 | 3.215 | Plant | 9 | 2.445 | 1.348 |
| | Error | 9 | 2.742 | | Error | 9 | 1.814 | |
| | Time | 9 | 5.128 | 25.606**** | Time | 9 | 0.729 | 2.993** |
| | Time×Treatment | 9 | 1.622 | 8.098**** | Time×Treatment | 9 | 0.133 | 0.545 |
| | Time×Plant | 81 | 0.379 | 1.891** | Time×Plant | 81 | 0.222 | 0.911 |
| | Error | 81 | 0.200 | | Error | 81 | 0.243 | |

Significance values are denoted with asterisks: *$P<0.05$, **$P<0.01$, ***$P<0.001$, ****$P<0.0001$

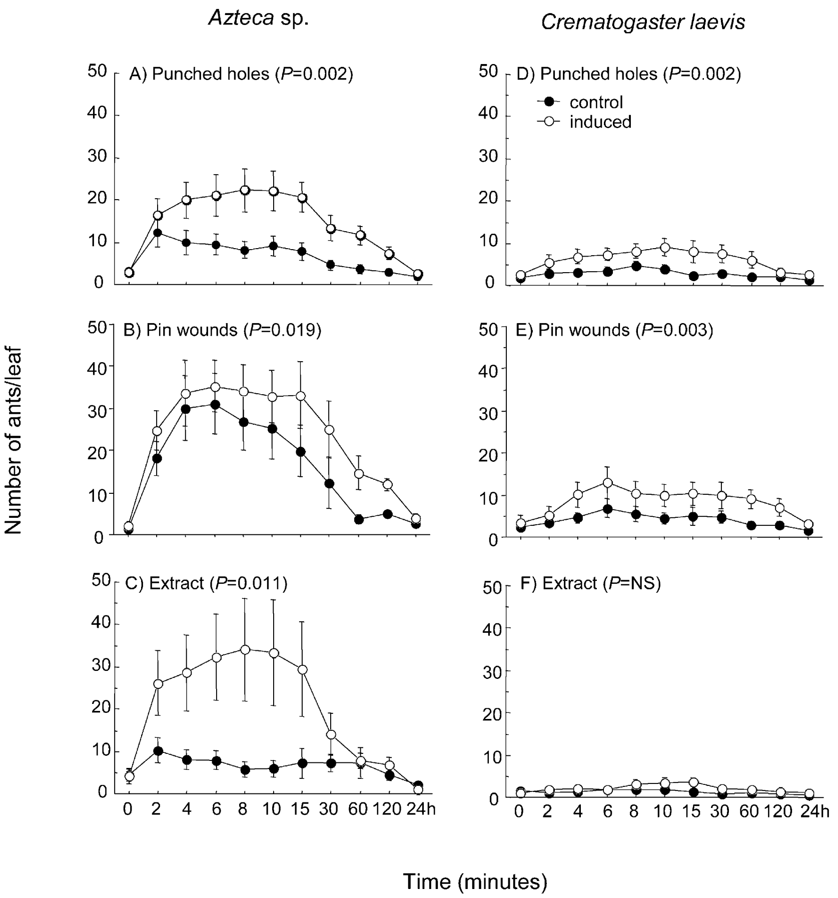

**Fig. 1A–F** Number of *Crematogaster laevis* and *Azteca* sp. workers patrolling *Tococa bullifera* leaves 2 h after experimental induction cues were applied. The cues were either damage to leaves [punched holes (**A**, **D**) or pin wounds (**B**, **E**)] or the application of leaf tissue extract (**C**, **F**). *Open circles* represent the leaves receiving the induction cue; *filled circles* are the control leaves. The *P* values indicate significant main effects of the experimental treatment (i.e., herbivory cue vs no cue; Table 1)

**Table 2** Repeated-measures split-plot ANOVA for the effect of ant species[a], leaf age[b], replicate nested within leaf age[c] and time[d] and on the amount of herbivory on plants. Significant results are in *bold*[e]

| Source | *df* | *MS* | *F* | *P* |
|---|---|---|---|---|
| Between subject | | | | |
| Species | 1 | 162,251.3 | 22.7 | **<0.0001** |
| Treatment | 1 | 39,985.8 | 5.6 | **0.02** |
| Replicate (leaf age) | 1 | 106,632.8 | 14.95 | **0.0005** |
| Species×Treatment | 1 | 1,904.0 | 0.3 | 0.61 |
| Species×Replicate (leaf age) | 1 | 26,477.9 | 3.7 | 0.6 |
| Treatment×Replicate (leaf age) | 1 | 26.2 | 0.003 | 0.95 |
| Species×Treatment×Replicate (leaf age) | | 6,403.4 | 0.90 | 0.35 |
| Error | 32 | 228,223.1 | | |
| Within subject | | | | |
| Time | 1 | 0.0 | 0.0 | 1.0 |
| Age | 1 | 154,047 | 67.89 | **<0.0001** |
| Replicate (leaf age) | 2 | 68,953.7 | 30.39 | **<0.0001** |
| Time×Species | 1 | 79,644.8 | 35.10 | **<0.0001** |
| Time×Treatment | 1 | 49,633.7 | 21.87 | **<0.0001** |
| Time×Leaf age | 1 | 137,329.5 | 60.52 | **<0.0001** |
| Time×Replicate (leaf age) | 2 | 63,098.4 | 27.81 | **<0.0001** |
| Time×Species×Treatment | 1 | 15,142.5 | 6.67 | **0.01** |
| Time×Species×Leaf age | 1 | 9,666.9 | 4.26 | **0.04** |
| Time×Treatment×Leaf age | 1 | 1,809.0 | 0.80 | 0.37 |
| Time×Species×Replicate (leaf age) | 2 | 2,449.2 | 1.08 | 0.34 |
| Time×Treatment×Replicate (leaf age) | 2 | 199.9 | 0.09 | 0.92 |
| Time×Species×Treatment×leaf age | 1 | 9,370.9 | 4.13 | **0.04** |
| Time×Species×Treatment×leaf number (leaf age) | 2 | 167.6 | 0.07 | 0.93 |
| Error | 412 | 934,818 | | |

[a] *C. laevis* or *Azteca* sp.

[b] Mature or new

[c] Leaf 1–3

[d] Measurement date 1 or 2

[e] The average of the ranked values must be identical in the two time intervals, therefore the effect of time cannot be significant

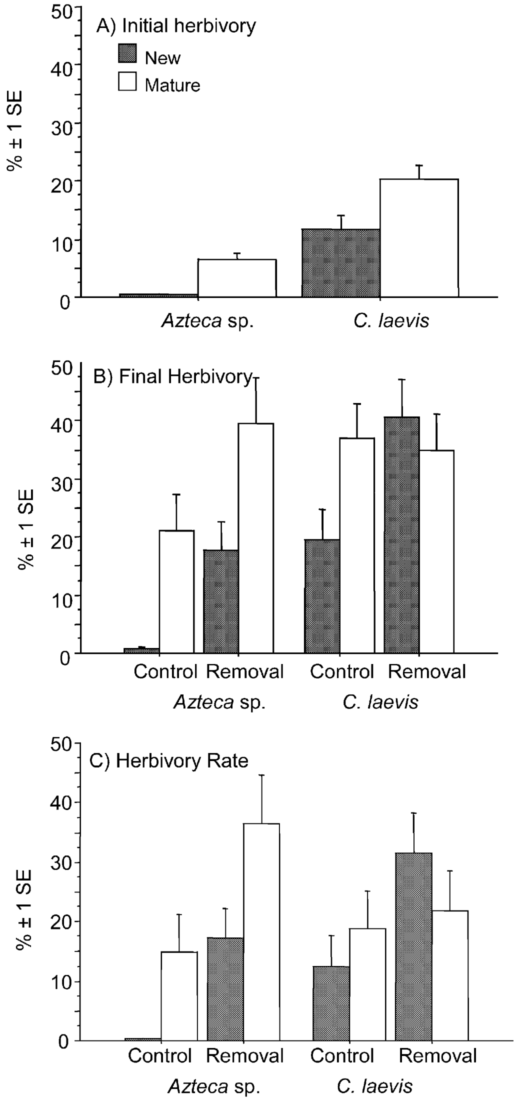

**Fig. 2** **A** Initial levels of herbivory (% leaf tissue missing ± 1 SE) on *T. bullifera* leaves from which *Azteca* sp. and *C. laevis* colonies were to be experimentally removed. **B** Levels of herbivory after 6 weeks (% leaf tissue missing ± 1 SE) on leaves from which colonies were removed or left undisturbed. **C** Herbivory rate over the course of the experiment (% change in leaf area due to herbivory ± 1 SE) on leaves from which colonies were removed or left undisturbed. On all panels the *shaded bars* represent new leaves, while *open bars* represent mature leaves

number of patrolling workers 24 h after cues were applied was identical on control and experimental leaves.

### Protection of host plants against herbivory

Leaves defended by *C. laevis* had 4.5× greater herbivory at the start of the experiment than leaves defended by *Azteca* sp. (16.0%±1.7 SE vs 3.5%±0.6 SE, respectively; mean initial rank$_{\textit{C. laevis}}$=126.0 vs mean initial rank$_{\textit{Azteca}\ \text{sp.}}$=89.2; Fig. 2A). Mature leaves also had double the initial levels of damage that new leaves did (13.4%±1.4 SE vs 6.1% ±1.3 SE; mean initial rank$_{\text{mature}}$=155.3±4.69 SE vs mean initial rank$_{\text{new}}$=85.7±6.15 SE; Fig. 2A). Furthermore, the pattern of initial herbivory on leaves of different ages was different for plants inhabited by each ant species. While plants defended by *Azteca* sp. had 13× greater herbivory on mature leaves than new ones (6.5%±1.2 SE vs 0.5% ±0.1 SE, respectively), mature leaves on plants defended by *C. laevis* had only 1.7-fold more herbivory than new leaves (20.3%±2.3 SE vs 11.7%±2.4 SE, respectively; Fig. 2A).

Experimental colony removal significantly increased the rate of herbivory on leaves, as indicated by the significant Time×Treatment interaction ($MS$=49,633.7, $F_{1,412}$=21.87, $P$<0.0001; Table 2; Fig. 2B–C). Furthermore, the significant Time×Treatment×Species interaction indicates the two ant species did not defend plants equally over the course of the experiment ($MS$=15,142.5, $F_{1,412}$=6.67, $P$=0.01; Fig. 2B). Overall, the rate of herbivory was almost two-fold higher on control plants defended by *C. laevis* than on control plants defended by *Azteca* sp. (15.6%±4.0 SE vs 7.6%±3.2 SE, respectively), while the rate of herbivory on plants from which colonies had been removed was similar for plants defended by the different species (*Azteca* sp.: 26.9%±4.9 SE; *C. laevis*: 26.7±4.8 SE). The significant Time×Species×Treatment ×Leaf age interaction indicates that, within plants, the herbivory rate suffered by leaves of different ages differed in each species×treatment combination ($MS$=9,370.9, $F_{1,412}$=4.13, $P$=0.04; Table 2; Fig. 2C). Whereas new leaves inhabited by *Azteca* sp. colonies had a rate of herbivory of only 0.3%±0.1 SE, the rate of herbivory on new leaves defended by *C. laevis* was 12.5%±5.1 SE (Fig. 2C). Mature leaves are less well defended than new ones by both species, although those defended by *Azteca* sp. still had lower rates of leaf damage over the course of 6 weeks than those defended by *C. laevis* (14.9%±6.3 SE vs 18.8%±6.3 SE, respectively; Fig. 2C).

## Discussion

This study is the first to explicitly compare the response of different predatory ants obligately associated with a plant species to multiple cues associated with herbivory, and the first to link levels of herbivore damage with variation in ant responses. Our experiments demonstrate that (1) cues eliciting a strong response by one ant species may induce no response by the other; and (2) for cues that do elicit responses, the magnitude of responses can vary interspecifically. Theoretical and empirical work on biotic plant defenses has focused almost exclusively on dichotomy between obligate and facultative ant associates (e.g., Agrawal and Rutter 1998; Heil et al. 2001a), with obligate ant partners presumed to be more responsive than facultative ones due to the tight link between plant condition and colony fitness. The results of our study suggest there can also be substantial variation in the capacity for eliciting responses from obligate associates, and that short-term variation is consistent with longer-term patterns of protection from herbivores.

## Interspecific variation in response to herbivory

Our results are similar to those of previous studies demonstrating that ant species can respond strongly to physical damage and host plant volatiles (Fiala and Maschwitz 1990; Agrawal 1998; Agrawal and Dubin-Thaler 1999; Brouat et al. 2000). However, the lack of a response to chemical cues by *C. laevis* is somewhat surprising, because such responses have previously been observed in *Crematogaster* species inhabiting *Macaranga*, *Acacia*, and other tropical ant-plants (e.g., Wheeler et al. 1975; Itioka et al. 2000; Inui et al. 2001). Interestingly, *C. laevis*' reduced sensitivity to volatiles does not appear restricted to those emitted by *T. bullifera*. We have found that *C. laevis* inhabiting the ant-plant *Maieta guianensis* (Melastomataceae) also responded strongly to physical damage but not to leaf-tissue extracts (Lapola et al. 2003). Benson (1985) suggested that *C. laevis* is an evolutionary newcomer to ant-plants and has played only a limited role in the evolution of their myrmecophytic traits. If so, it may be that the lack of sensitivity to plant volatiles is the result of limited coevolutionary history. *C. laevis* is broadly distributed throughout Amazonia (Benson 1985) and inhabits the domatia of several other myrmecophytic genera in the family Melastomataceae (e.g., *Maieta*, *Clidemia*, and *Myrmidone*): experiments conducted with these taxa, as well as phylogenetic analysis of both the plants and ants, would be necessary to test this hypothesis.

For cues that did elicit a response from both species, there was substantial interspecific variation in the intensity of the responses. For instance, 8 min after the cues were applied, the number of *Azteca* sp. workers patrolling experimental leaves was up to 11-fold greater than the number of patrolling *C. laevis* workers (Fig. 1). *Azteca* sp. workers also responded to cues more rapidly. Worker numbers reached 89.7–95.5% of their peak abundance just four minutes after leaves were damaged, whereas after four minutes *C. laevis* had reached only 75.8–78.3% of its maximum response (Fig. 1). These differences in the intensity of responses are consistent with differences in the baseline number of workers patrolling leaves.

Larger colonies may have more workers available both to patrol leaves and to respond to herbivory (Rocha and Bergallo 1992; Agrawal 1998). Therefore, it may be that *Azteca* sp.'s greater numerical response to physical damage is due in part to having larger colonies than *C. laevis*. While we do not yet have robust estimates of colony size for either ant species, we believe this is probably not the mechanism responsible for the observed differences in response intensity. In a preliminary study, we found no difference between the two ant species in the median number of workers per domatium (Mann-Whitney *U* test: $U'=64.5$, $P=0.27$, $n=10$ domatia for each ant species). In fact, the mean number of workers per domatium was actually higher for *C. laevis* than for *Azteca* sp. (mean±SE for *C. laevis* 50.2±12.38, range 11–138; mean±SE for *Azteca* sp. 37.9±12.6, range 3–126). These numbers are well in excess of the number of *C. laevis* workers counted on leaves at peak response to experimental damage.

Instead, the variation in response intensity is probably due to behavioral differences in how *Azteca* sp. and *C. laevis* recruit conspecifics to sites of damage. Both genera have a well-developed and complex repertoire of behaviors and alarm signals used to stimulate aggressive responses by nestmates (reviewed in Hölldobler and Wilson 1990). Despite this, only *Azteca* sp. appears to actively recruit nestmates to sites of damage using such stimuli. We observed *Azteca* sp. workers lifting their gasters after locating damage or dragging them along leaf surfaces. These behaviors are consistent with the release of the highly volatile ketone-based alarm pheromones common to this genus (Wheeler et al. 1975; Do Nascimento et al. 1998), as well as the laying down of scent trails for nestmates to follow (Hölldobler and Wilson 1990). We also observed workers returning to domatia, which was followed by the rapid emergence of large numbers of workers. In contrast, *C. laevis* workers usually remained in the vicinity of damage, with increases in worker number over time resulting from the subsequent discovery of damage by other patrolling ants. While some species of myrmecophytic *Crematogaster* do produce volatile alarm pheromones (Crewe et al. 1972; Wood et al. 2002), the limited long-distance recruitment of nestmates may mean *C. laevis* does not. Instead, it is possible that *C. laevis* individuals remain in the vicinity of damage in an attempt to find and attack herbivores with the contact toxins produced by their Dufour and poison glands (Laurent et al. 2003).

Finally, it is worth noting that the within-plant spatial scale at which the two ant species recruited workers differed as well. *C. laevis*' response was limited to workers from the domatium at the base of the damaged leaf, while *Azteca* sp. workers were frequently recruited to damage from the domatia of adjacent leaves (Bruna et al., personal observation). It is unclear if these individuals were recruiting in response to alarm pheromones released by the ants or the volatiles emitted following experimental damage. However, these observations do suggest the within-plant spatial scale at which biotic defenses operate may not solely depend on plant characteristics, and that it may also vary as a function of partner identity.

## Consequences for plants of variation in ant responses

The results of the colony removal experiments indicate that both ant species provide some protection from herbivores to *T. bullifera*. However, by the end of our experiment the amount of herbivory on plants inhabited by *C. laevis* colonies was three times that of plants inhabited by *Azteca* sp. (28% vs 11%, respectively). In part, this is because herbivory on *C. laevis* plants was initially higher (Fig. 2A). However, the rate of herbivory during our experiment was twice as high on plants defended by *C. laevis* as on those inhabited by *Azteca* sp. (15.7% vs 7.6%; Fig. 2C). While we do not yet know if the observed differences in herbivory between plants inhabited by *C. laevis* and *Azteca* sp. will lead to differences in individual fitness, high herbivory has been shown to dramatically lower fruit production in other myrmecophytic Melastomataceae (Vasconcelos 1991). Therefore, we suggest that both the sensitivity to cues associated with herbivory and the intensity of subsequent short-term responses will be important correlates of an ant species' effectiveness as a biotic defense.

Differences in protection at the whole-plant level also mask considerable within-plant variation in protection. At the end of our experiment, total herbivory on new leaves with intact *Azteca* sp. colonies was 20-fold lower than on leaves from which colonies had been removed. In contrast, herbivory on new leaves without *C. laevis* colonies was twice that of control leaves (Fig. 2B). The differences in protection provided to mature leaves was also notable. Removing *Azteca* sp. from mature leaves resulted in a rate of herbivory that was twice as high as that on control leaves, while the rate of herbivory on mature leaves inhabited by *C. laevis* was almost identical in the control and experimental treatments (18.8% vs 21.8%, respectively). Since new leaves frequently have higher concentrations of defensive chemicals than mature ones (Coley and Barone 1996), the asymmetry in defense provided to leaves of different ages observed in this and other studies (McKey 1984; Fiala et al. 1994; Heil et al. 2001a) may reflect, in part, asymmetry in the distribution of chemicals capable of inducing ant responses. In future studies we will evaluate this possibility by conducting induction trials with extracts of leaves of different ages on both new and mature leaves.

### Are plant-ants 'induced defenses'?

Plant defenses against herbivory, including biological ones, can either be expressed constitutively or induced by herbivores (Karban et al. 1997; Heil 2002). It has been proposed that plant-ants are an important type of induced biological defense (Agrawal 1998; Agrawal and Rutter 1998) since they meet many of the criteria of induced defenses, including mobility, a rapid response time, and the ability to be redeployed as necessary (Agrawal and Rutter 1998). However, the characterization of ants, parasitoid wasps, or other carnivorous insects as inducible biological defenses requires (1) that a plant trait, such as extra-floral nectar or food body production, change following herbivory, and (2) that this change cue the response of the putative defender (e.g., Thaler 1999; Heil et al. 2001b; Ness 2003). We have no evidence for such a trait in *T. bullifera*; furthermore, there are persistent costs incurred by plants maintaining active ant colonies (e.g., herbivory by coccids, domatia production). We therefore suggest *Azteca* sp., *C. laevis*, and other plant-ants should be considered 'constitutive' rather than 'induced' defenses until a specific induction cue has been identified.

### Conclusion

Spatio-temporal geographical variation in the ant inhabitants of myrmecophytic plants is common (Longino 1989; Alonso 1998; Rico-Gray et al. 1998): furthermore, plants frequently have multiple species of ant inhabitants over the course of their lifetimes (Palmer et al. 2000; Vasconcelos and Davidson 2000; Stanton et al. 2002). Our results add to the growing body of literature indicating that the aggressive defensive responses of these ants can be related to cues associated with herbivory. However, the strength of these responses can vary interspecifically in ways that have previously remained unexplored. We also found evidence that the intensity of a species' response to cues is correlated with one measure of ant defensive capability—rates of herbivory on leaves. Taken together, the results of this study therefore suggest new ways that variation in composition of potential partner taxa, both locally and across a species' geographic range, could influence the ecology and evolution of defensive mutualisms (Bronstein 1998; Thompson 1999).

**Acknowledgements** We would like to thank N. Underwood, P. Ward, D. Davidson, J. Rudgers, and two anonymous reviewers for helpful discussions and comments on the manuscript and J.M.S. Vilhena for help with ant identifications. The Biological Dynamics of Forest Fragments Project provided logistical support; permission to conduct the research was provided by the Manaus Free Trade Zone Authority (SUFRAMA). Financial support was provided by an NSF Minority Postdoctoral Fellowship, the NSF AMERICAS program, the University of Florida's Institute for Food and Agricultural Sciences (E.M.B.), the SUNY-BDFFP Internship Program (D.M.L.), and the Conselho Nacional de Desenvolvimento Científico e Tecnológico (H.L.V.). This is publication number 414 in the BDFFP Technical Series and FAES Publication number R-09881.

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