

## Contrasting Responses to Induction Cues by Ants Inhabiting *Maieta guianensis* (Melastomataceae)<sup>1</sup>

### ABSTRACT

The two most common ant associates of the understory myrmecophyte *Maieta guianensis* (Melastomataceae) respond in different ways to experimental cues associated with herbivory. While *Pheidole minutula* is induced by both physical damage and extracts of leaf tissue, *Crematogaster laevis* is induced by leaf damage only. We suggest such interspecific variation in induced responses is common and could influence the quality of defense against herbivores provided to host-plants by ants.

### RESUMO

As duas formigas que mais comumente se associam com a mirmecófito de sub-bosque *Maieta guianensis* (Melastomataceae) respondem de maneiras diferentes a simulações experimentais de herbivoria. Enquanto *Pheidole minutula* é induzida tanto por danos físicos e extratos de tecido foliar, *Crematogaster laevis* é induzida apenas pelos danos físicos. Sugerimos que tal variação interespecífica em respostas induzidas é comum e pode influenciar a qualidade das defesas contra herbívoros que as formigas oferecem às plantas que elas ocupam.

*Key words:* Amazonia; ants; biotic defense; induced defense; mutualism; myrmecophytes.

PLANTS EMPLOY CHEMICAL, PHYSICAL, AND BIOLOGICAL DEFENSES AGAINST HERBIVORES (Coley & Barone 1996). Many of these defenses are expressed constitutively; however, the prevalence and importance of defenses that are induced by herbivory is now widely recognized (Karban & Baldwin 1997, Agrawal *et al.* 1999). Examples of induced defenses include the increased concentration of secondary defensive compounds in leaves after herbivory, the production of spines by trees following browsing, and the release of volatiles that attract the enemies of herbivores (reviewed in Karban & Baldwin 1997).

Recently, several authors have suggested that ants having mutualistic associations with tropical plants constitute an important category of induced defense (Agrawal 1998, Agrawal & Rutter 1998, Di Giusto *et al.* 2001). This is thought to be particularly true of ants inhabiting plants known as myrmecophytes, which have leaf pouches, hollow stems, and other domatia in which the ants establish colonies. In the Neotropics, more than 230 species of plants in 17 families have putatively mutualistic associations with the ants of 5 subfamilies (Benson 1985, Hölldobler & Wilson 1990). The aggressive behavior of these ants toward herbivores has long been recognized (Belt 1874) and explored experimentally (Janzen 1966, Fiala *et al.* 1989, de la Fuente & Marquis 1999). Ecologists, however, have only recently begun to investigate the proximate cues that induce aggressive ant responses, as well as quantify their dynamic nature (Agrawal 1998, Agrawal & Dubin-Thaler 1999). For example, recent studies have demonstrated that *Azteca* ants residing in Central American *Cecropia* trees rapidly and aggressively recruit in response to plant sap, leaf damage, and chemical volatiles (Agrawal 1998, Agrawal & Dubin-Thaler 1999) and that the numbers of workers patrolling leaves can remain elevated up to 24 hours after induction. Similar results have been found in both the Southeast Asian *Macaranga*–*Crematogaster borneensis* association (Fiala & Maschwitz 1990) and that of African *Leonardoxa* trees inhabited by *Petalomyrmex* ants (Brouat *et al.* 2000). Based on results such as these, it has been argued that induced ant responses to herbivory are likely to be common among ants inhabiting myrmecophytic plants and that they may be an important component of a plant's defenses against herbivory (Agrawal 1998).

One limitation of previous studies investigating induced ant responses is that they have focused on a single species of ant mutualist (*cf.* Gaume & McKey 1999 and Ness 2003b for comparisons of mutualist ants with parasitic or invasive species). Obligate ant–plant associations are rare, however, and most species of myrmecophytic plants serve as hosts to multiple ant species (Longino 1989, Fonseca & Ganade 1996).

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These ant species often differ in colony structure, behavior, and evolutionary history (Hölldobler & Wilson 1990), all of which can influence their responses to induction cues (Agrawal & Rutter 1998). If induced responses are indeed an important component of the defense provided by plant-ants, then interspecific variation in the intensity of these responses could alter the quality of the defense provided by each ant species. There could therefore be substantial costs and benefits to plants of associating with different ant taxa (Heil 2002). Here, we experimentally compared the induced responses of two ant species associated with a common Amazonian myrmecophyte to cues associated with herbivory and demonstrated that there is significant interspecific variation in ant responses to different types of induction cues.

This study was conducted in Reserve 1501 of the Biological Dynamics of Forest Fragments Project (Bierregaard *et al.* 2002), which is administered cooperatively by Brazil's Instituto Nacional de Pesquisas da Amazônia and the Smithsonian Tropical Research Institute. The 800 ha reserve is ca 70 km north of Manaus (2°25'S, 59°48'W) and is embedded in over 10,000 ha of continuous rain forest. The habitat at the site is nonflooded lowland tropical forest with annual precipitation ranging from 1900 to 3500 mm. A detailed description of the study site can be found in Bierregaard *et al.* (2002).

*Maieta guianensis* (Melastomataceae) is an understory shrub that grows to a height of 1.5 to 2.0 m (Vasconcelos 1993, Vasconcelos & Davidson 2000). It has highly dimorphic paired leaves, with a pair of foliar pouches at the base of the largest leaf in which ants nest. At our study sites, two ant species were associated with *M. guianensis*; most plants contained colonies of *Pheidole minutula* (86.2%), with the remainder occupied by *Crematogaster laevis* (Vasconcelos & Davidson 2000). In addition to scavenging for insects on the leaf surface, these ants tend coccids for honeydew inside domatia and may also collect food bodies from glandular trichomes located inside domatia (Vasconcelos 1991).

To quantify the induced responses of ants, we used two classes of experimental cues that have previously been shown to induce tropical plant-ants: leaf damage and extracts of compounds from leaf tissue (Agrawal 1998, Agrawal & Dubin-Thaler 1999). The responses to these cues were assessed using a paired-leaf design in which each plant had both a control and experimental leaf. For each of the trials described below, we selected ten *M. guianensis* with active colonies of each species. On each plant, we selected two new, similarly sized 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 minutes after treatments were applied (Agrawal 1998).

Leaves were damaged with a hole puncher, which mimics natural damage by orthopterans (Lapola *et al.*, pers. obs.). The leaf assigned to the induction treatment had five holes punched in (removing ca 190 mm<sup>2</sup> of leaf tissue, or <3% of total leaf area), while the control leaf was tapped with the hole punch five times. Chemical compounds in leaf tissue were extracted by soaking ca 0.5 grams of fresh *M. guianensis* tissue in 60 ml of methanol for 24 hours, after which the leaf tissue was removed with a strainer (Agrawal & Dubin-Thaler 1999). Five drops of the extract were applied to the leaf in the induction treatment, while the control leaf received 5 drops of methanol. Ten plants were used for each cue for each ant species (total  $N = 40$ ).

The effect of all cues on the abundance of patrolling workers 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 square and degrees of freedom are used in the denominator for the calculation of the  $F$ -statistic (Sokal & Rohlf 1995). Counts of patrolling ants were made immediately prior to initiating the experimental treatments to confirm that there was no significant difference in the number of patrolling ants on control and experimental leaves (results not shown). In addition, we compared the baseline number of *C. laevis* and *P. minutula* workers patrolling leaves prior to induction using a Mann-Whitney  $U$ -test.

The number of *P. minutula* workers patrolling leaves that were receiving damage and chemical cues was significantly greater than on control leaves (Fig. 1; damage treatment:  $MS = 13.96$ ,  $F_{1, 72} = 8.18$ ,  $P = 0.008$ ; extract treatment:  $MS = 24.72$ ,  $F_{1, 72} = 17.16$ ,  $P = 0.003$ ). In contrast, only leaf damage caused a significant increase in the number of patrolling *C. laevis* workers (leaf damage:  $MS = 21.89$ ,  $F_{1, 72} = 6.08$ ,  $P = 0.006$ ; leaf extract:  $MS = 4.31$ ,  $F_{1, 72} = 2.654$ ,  $P = 0.14$ ; Table 1). Prior to induction, the number of *P. minutula* workers patrolling leaves was two-fold greater than the number of

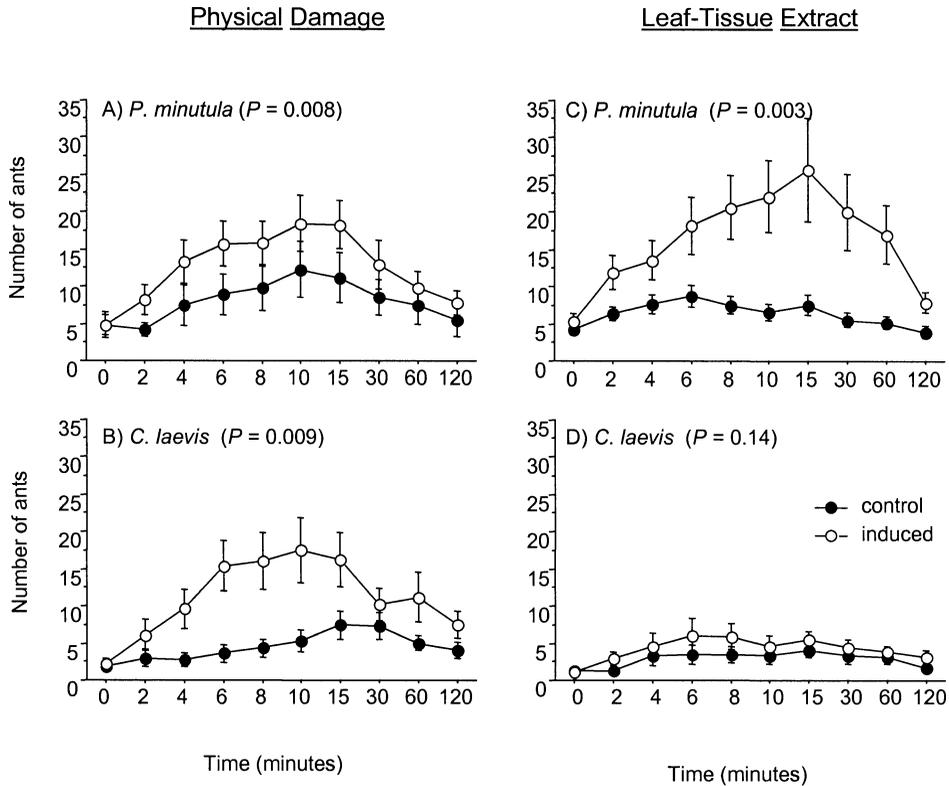


FIGURE 1. Number of *Crematogaster laevis* and *Pheidole minutula* workers patrolling *Maieta guianensis* leaves two hours after experimental induction cues were applied. The cues were either damage to leaves or the application of leaf tissue extract. Open circles represent leaves receiving the induction cue; filled circles are the control leaves.

patrolling *C. laevis* workers (*P. minutula*: median = 4,  $\bar{x} \pm 1 \text{ SE} = 4.52 \pm 0.89$ ; *C. laevis*: median = 2,  $\bar{x} \pm 1 \text{ SE} = 1.6 \pm 0.29$ ;  $U = 319.5$ ,  $P = 0.0038$ ). Fifteen minutes after the cues were applied, the number of patrolling workers was 1.6- to 3.4-fold greater on experimental leaves than control leaves, with values after two hours slightly higher than when the experiment began (Fig. 1).

Our results were consistent with those of previous studies showing that ants inhabiting myrmecophy-

TABLE 1. Repeated measures ANOVA for the effect of induction treatments on ant recruitment.

Experiment	<i>Pheidole minutula</i>				<i>Crematogaster laevis</i>			
	Source	DF	MS	F	Source	DF	MS	F
Physical damage	Treatment	1	13.96	8.181**	Treatment	1	21.89	6.08**
	Error	9	1.71		Error	9	2.00	
	Time	8	1.32	6.754***	Time	8	1.47	7.733***
	Time $\times$ Treatment	8	0.10	0.492	Time $\times$ Treatment	8	0.44	2.309*
	Error	72	0.20		Error	72	0.19	
Extract	Treatment	1	24.72	17.159**	Treatment	1	4.31	2.654
	Error	9	1.44		Error	9	1.62	
	Time	8	0.99	8.951***	Time	8	0.77	3.578**
	Time $\times$ Treatment	8	0.31	2.832**	Time $\times$ Treatment	8	0.06	0.276
	Error	72			Error	72	0.22	

\*  $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$ .

tic plants are induced by cues associated with herbivory (Agrawal 1998, Agrawal & Dubin-Thaler 1999). Not unexpectedly, however, comparisons with other studies revealed that the magnitude of the response can vary substantially between ant-plant systems. For example, while Agrawal (1998) found 3- to 4-fold differences in recruitment following induction, using similar methods we found that the number of ants patrolling *M. guianensis* increased a maximum of 1.5- to 3.4-fold (Fig. 1). In addition, the length of time that ant numbers remained elevated also varied. Previous studies have found that ant numbers can remain significantly elevated for one to five days (Agrawal 1998, Ness 2003a); however, we found that *C. laevis* and *P. minutula* abundances on control and experimental leaves were similar within 15 minutes of induction (Fig. 1).

The results of our experiments also suggest that not all cues will induce responses by the different ant species associated with a single plant. Although sample sizes were relatively low ( $N = 10$  plants in each species  $\times$  induction treatment combination), we nonetheless found that *P. minutula* recruitment was strongly induced by both physical damage to *M. guianensis* and leaf extracts. In contrast, numbers of patrolling *C. laevis* workers only increased significantly after physical damage to the host plant's leaves occurred (Fig. 1). If sensitivity to induction cues is indeed an important component of the quality of defense provided to plants by ants, then these differences could have important ecological consequences for plants inhabited by less responsive ants. Given the substantial variation in the ecology and behavior of ant-plant associates (Benson 1985, Hölldobler & Wilson 1990), we suggest that interspecific variation in the induced responses to different induction cues may be a common feature of ant-plant systems.

What factors are responsible for the variation in response to induction cues exhibited by *C. laevis* and *P. minutula*? Previous studies have found that the intensity of induced responses can be positively correlated to preinduction levels of ant patrolling (Agrawal 1998), which may in turn be the result of differences in colony size (Rocha & Bergallo 1992). We found that the baseline number of *C. laevis* workers patrolling leaves was significantly lower than the number of patrolling *P. minutula*. While that may explain a delayed or less intense response by *C. laevis* to induction cues, it does not explain why that species did not respond to the leaf tissue extracts with significantly greater numbers of patrolling ants once cues were discovered.

Instead, it may be that *C. laevis* has yet to evolve a sensitivity to the volatiles emitted as a consequence of herbivory. Although *C. laevis* is widely distributed throughout Amazonia and inhabits the domatia of several myrmecophytic genera (e.g., *Tococa*, *Clidemia*, and *Myrmidone*), it has been suggested *C. laevis* is an evolutionary newcomer to these taxa and has played a limited role in the evolution of their myrmecophytic traits (Benson 1985). There is some ecological evidence consistent with this hypothesis: *C. laevis* occasionally nests in twigs at the base of host plants, and the openings of domatia are frequently too small for *C. laevis* queens to enter without enlarging them (Vasconcelos & Davidson 2000). In addition, *C. laevis*' limited sensitivity to host plant chemistry may not be limited to that of *M. guianensis*. We found that *C. laevis* inhabiting *Tococa bullifera* (Melastomataceae), another myrmecophyte with which it is obligately associated, also responded strongly to physical damage but not to leaf tissue extracts (Bruna *et al.*, pers.obs.). Additional studies with other *C. laevis* host plants, as well as phylogenetic analyses of both plant and ant partners, are needed to test the hypothesis that *C. laevis*' limited sensitivity to host plant chemistry is the result of limited coevolutionary history.

Colony fitness is thought to be directly related to host plant vigor in obligate ant-plant systems (Agrawal & Rutter 1998). Therefore, it is predicted that ants will exhibit strong induced responses to herbivory, even in systems without extrafloral nectaries or other direct rewards (Agrawal & Rutter 1998). The results of our study have demonstrated that even among obligately associated ants, there can be significant variation in response to induction cues, and that the efficacy of induced biotic defenses may vary with mutualist partner identity in ways that have previously remained unexplored.

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