

## Host plant protection by arboreal ants: looking for a pattern in locally induced responses

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### ABSTRACT

**Background:** Among arboreal ants, both territorially dominant species and plant-ants (e.g. species associated with myrmecophytes or plants housing them in hollow structures) protect their host trees against defoliators. Yet, locally induced responses, or the recruitment of nest-mates when a worker discovers a wound on its host-tree, were only noted in plant-ants. We wondered whether this might be due to the examination of the phenomenon being restricted to only six plant-ant species belonging to four genera. Based on the ant genus *Azteca*, a Neotropical group of arboreal species, we compared five species. The territorially dominant, carton-nester *A. chartifex*, three plant-ant species [*A. alfari* and *A. ovaticeps* associated with myrmecophytic *Cecropia* (Cecropiaceae), and *A. bequaerti* associated with *Tococa guianensis* (Melastomataceae)], and *A. schimperi* thought to be a temporary social parasite of true *Cecropia* ants.

**Methods:** We artificially inflicted wounds to the foliage of the host tree of the different ant species. We then compared the number of workers on wounded versus control leaves.

**Results:** We noted a locally induced response in the three plant-ant species as well as in the territorially dominant species, but very slightly so in *A. schimperi*.

*Keywords:* aggressiveness, ant–plant relationships, *Azteca*, biotic defence, induced responses.

### INTRODUCTION

To defend themselves against herbivorous arthropods, plants have evolved direct and indirect means of defence. A direct defence involves plant traits that directly deter herbivores and consequently reduce the damage they inflict. An indirect defence controls herbivores using the third trophic level, their predators or parasitoids (Heil, 2007, 2008).

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In both cases, the defence can be constitutive (present at all times) or induced in response to herbivory. Constitutive direct defences include permanent, physical barriers (e.g. trichomes, thorns, and leaf toughness) and the production of toxic, repellent or anti-feedent compounds. Constitutive indirect defences include the possibility of housing certain predators (mostly mites and ants) and/or attracting them (mostly ants) through food rewards in the form of extrafloral nectaries and food bodies. An induced direct defence occurs when a part of a plant attacked by a herbivore elicits increased resistance mechanisms that can be physical (e.g. longer trichomes) and/or chemical (e.g. toxic compounds produced locally or that are systemic). In induced indirect defence, the damage triggers the emission of volatiles that attract the natural enemies of herbivorous insects and mites (Tollrian and Harvell, 1999; Heil, 2007, 2008). Induced defences are particularly adaptive when defences are costly to implement, the presence of herbivores is spatially and/or temporally variable, and when different enemies co-occur because defence against one could increase susceptibility to another (Frost *et al.*, 2008).

The compounds emitted during attacks by herbivores and which act as induced indirect defence, herbivore-induced volatile organic compounds (HI-VOCs), are emitted immediately upon damage ( $C_6$  volatiles such as the isomers of hexenol, hexenal, hexenyl acetate known as green-leaf volatiles) or 24 h after attack (jasmonates, monoterpenes, sesquiterpenes) (Turlings and Wäckers, 2004; Pichersky *et al.*, 2006). Also, the secretion of extrafloral nectar can be involved in induced mechanisms, which is greater after leaf damage, increasing in turn the number of visiting ants that then reduce herbivory (see Heil, 2007, 2008).

Induced defence corresponds theoretically to the effect of HI-VOCs on plant fitness (Karban and Baldwin, 1997), something that is difficult to demonstrate. In plant defence by ants, a locally induced response has been seen in several myrmecophytes (or plants housing ants in hollow structures called domatia). Their guest plant-ants recognize and recruit nest-mates to the artificially wounded leaves of their host plant, which mimics an attack by herbivores; recruited workers patrol the wounded and even neighbouring leaves (Fiala *et al.*, 1989; Agrawal, 1998; Agrawal and Dubin-Thaler, 1999; Christianini and Machado, 2004; Romero and Izzo, 2004; see also Brouat *et al.*, 2000). This locally induced response is not specific, as *Allomerus* workers recruit nest-mates after discovering extracts not only from their host myrmecophyte, but also from another plant species if to a lesser extent (Romero and Izzo, 2004). This shows that the workers' response does not correspond only to specific plant HI-VOCs, but also to volatiles present in different plant species. In fact, they probably respond to what they perceive as threats to their nest-site and/or territory.

It was with this idea in mind that, using the Neotropical and strictly arboreal genus *Azteca* (subfamily Dolichoderinae), we wished to determine if locally induced responses exist in new plant-ants as well as in a territorially dominant arboreal species and a species suspected to be a temporary social parasite.

## MATERIALS AND METHODS

### Model system

Polydomous colonies of the territorially dominant species *A. chartifex* (*trigona* group) occur in clusters of several large (up to 2 m in height), pendant carton nests. They are frequent in wet forests (Longino, 2007). *Azteca alfari* and *A. ovaticeps* (*alfari* group) are both obligate *Cecropia* ants that are often locally sympatric. *Cecropia obtusa*, abundant in French

Guiana, houses its guest *Azteca* colonies in hollow stems [primary domatia, consisting of natural cavities as opposed to leaf pouches or secondary domatia, which derive from a structure that has undergone major modifications (Benson, 1985)]. It provides ants with glycogen-rich Müllerian bodies produced by the *trichilia* situated at the base of each leaf petiole, and with lipid- and amino acid-rich pearl bodies produced on the abaxial leaf surfaces (Heil and McKey, 2003; Davidson, 2005). *Azteca schimperi* (*aurita* group) construct ovoid, carton nests on the upper part of the trunk of *Cecropia* trees. Workers attend hemipterans on the vegetation surrounding their host tree, a trait that contrasts with typical *Cecropia* ants, and perform group ambush hunting, with individuals placing themselves side by side along the leaf margins (Dejean *et al.*, 2007). This species is probably a temporary social parasite of *Cecropia* ants, usurping an existing *Azteca* colony before establishing its own (Longino, 2007). In French Guiana *A. bequaerti* is mostly associated with the myrmecophyte *Tococa guianensis* (Melastomataceae) that forms shrubs up to 3 m in height along forest edges. The colonies shelter in leaf pouches situated at the base of each leaf lamina. Food-bodies are produced inside of the leaf pouches. Workers of this species react to the vibrations transmitted by the lamina when an alien insect lands on a leaf, making patrolling unnecessary. In contrast, *A. chartifex*, *A. alfari*, and *A. ovaticeps* workers discover alien insects by contact while patrolling the foliage of host plants, and then spread-eagle these insects (Dejean *et al.*, 2008).

### Study site

This study and the preliminary surveys that permitted us to develop the experimental protocols were conducted between 2004 and 2007 along forest edges in zones situated around the field station at Petit Saut, Sinnamary, French Guiana (5°03'39"N; 53°02'36"W). The surveys were conducted during the hours of maximum activity for four species (i.e. between 13:00 and 18:00 h for *A. chartifex*, and 17:00 and 19:00 h for *A. alfari*, *A. ovaticeps*, and *A. bequaerti*). Because *A. schimperi* workers ambush in a group along leaf margins mostly at dusk, experiments were conducted between 09:00 and 10:00 h when there were numerous workers on the leaves. Because our experimental methods placed the ants 'on alert', we conducted only one test per day and per tree, each time using a different leaf.

### Recruitment after a leaf wound is discovered

To determine whether discovering a leaf wound can induce the recruitment of workers, we counted the workers on experimental (i.e. artificially damaged) leaves and control leaves, using a well-known paired-leaf design (see Agrawal, 1998; Lapola *et al.*, 2003; Romero and Izzo, 2004). For each *Azteca* species tested, the experiment was repeated 30 times on 15 colonies. Each time we chose control and experimental leaves of similar size (opposite leaves or leaves next to each other), randomly assigning one as the experimental leaf and the other as the control. Because *Cecropia* leaves are large and multi-lobed, we counted only the ants on one lobe of each leaf. But in the case of *A. chartifex*, we counted the ants at the ends of the twigs on the five leaves situated around the damaged leaf, or its equivalent for the control condition. Before each treatment, we counted the number of ants patrolling the leaves or the targeted lobes. Then, we simulated herbivory by cutting out a triangular piece (~1 cm<sup>2</sup>) of the lamina (experimental treatment), while at the same time shaking the control leaf or lobe

slightly to counterbalance the disturbance caused by the treatment. We immediately began to count the ants present on both leaves (or lobes), and again every 30 s. While doing so, we noted each time when the first worker entered into physical contact with the wound. This contact was recorded as the point after which recruitment can occur – and, indeed, it did occur in the first 30 s in many cases.

We compared the transformed number of ants [ $y = \ln(x + 1)$ ] between the two treatments using SPSS version 11.5 software to run a repeated-measures analysis of variance (in which time was the repeated factor). To account for violations of the assumption of sphericity of variances, the Greenhouse-Geisser correction was applied to the degrees of freedom. For clarity of presentation, all graphs were generated using non-transformed data.

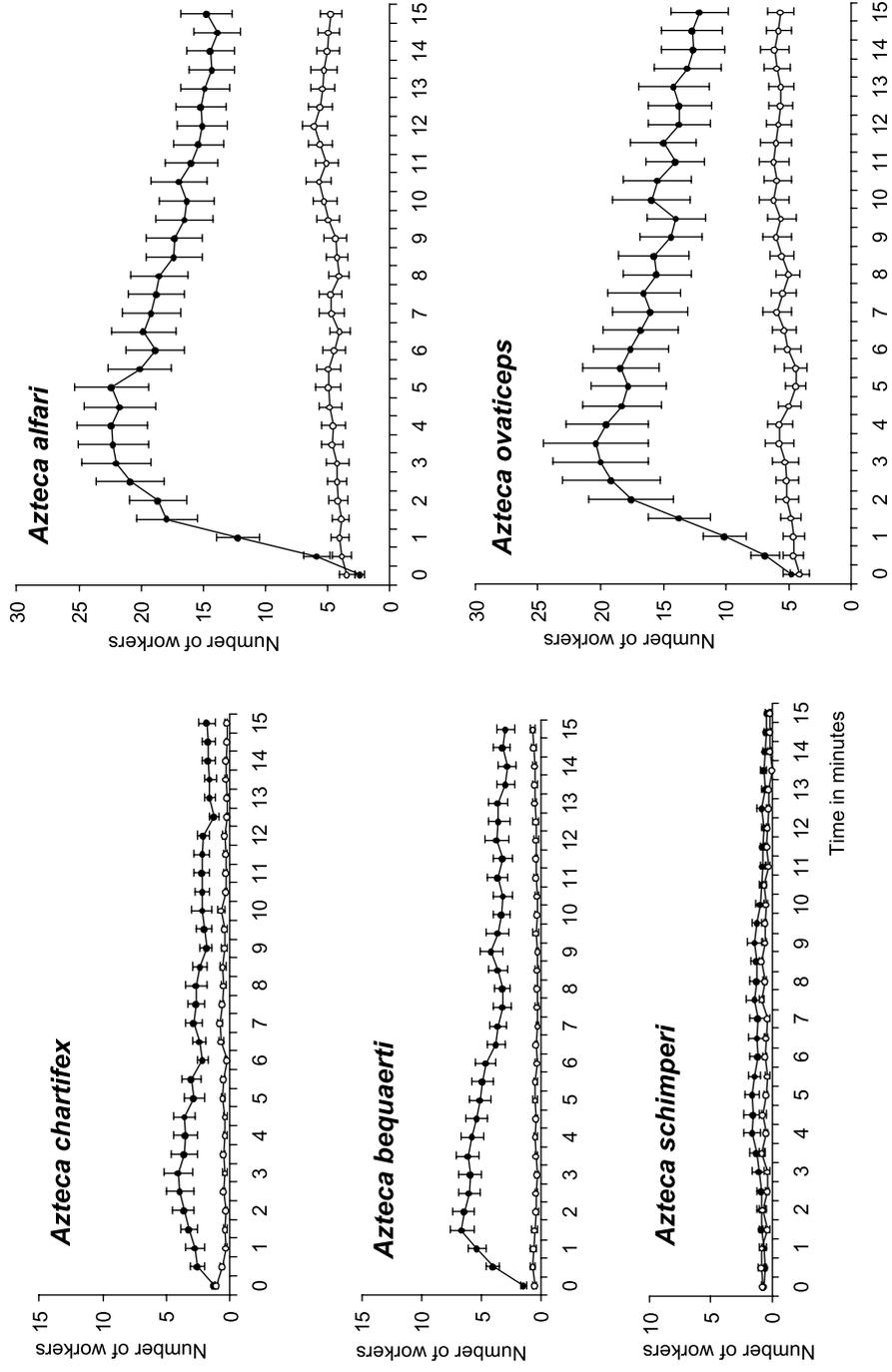
## RESULTS

Except for *A. schimperi*, the discovering workers were apparently attracted to the wounds because they antennated the edges of the wounds for a few seconds. One or two other ants patrolling on the same leaf occasionally did the same. Then, the discovering workers recruited nest-mates at either short (directly through the emission of an alarm pheromone) or long range (by returning to the host plant domatia) and some recruited workers amplified this action by recruiting in turn. As a result, the number of workers increased over time for the damaged leaves but not for the control leaves with the differences being significant in four cases (Table 1; Fig. 1). For *A. schimperi*, the main effect of treatment just reached significance, but the interaction of time  $\times$  treatment did not; overall, there was only a slight tendency towards recruitment.

## DISCUSSION

To date, locally induced responses through the recruitment of nest-mates have been demonstrated several times, but only in the associations between myrmecophytes and plant-ants (Agrawal and Dubin-Thaler, 1999; Lapola *et al.*, 2003; Christianini and Machado, 2004; Romero and Izzo, 2004). Plant-ant species can be generalist, for example not specialized in only one or a reduced number of myrmecophytes (Lapola *et al.*, 2003). During this study, we found new cases, including in *Cecropia* ants from the *alfari* group, boosting the data already obtained on the *muelleri* group by Agrawal (1998), and the association between *T. guianensis* and *A. bequaerti*. In contrast, *A. schimperi* workers reacted only very weakly to leaf damage, illustrating a divergence from all other *Azteca* species studied for which an induced response has been recorded. One interpretation might be that *A. schimperi* lost the capacity to respond in this way. Longino (2007) suggested that *A. schimperi* is probably a temporary social parasite of *Cecropia* ants, the ant parasitizing the *Azteca*–*Cecropia* association, something that has been noted in other myrmecophytes (Heil and McKey, 2003). This can also be due to the predatory behaviour of the species, with the workers placing themselves side by side along the leaf margin almost daily at dusk (Dejean *et al.*, 2007), making a locally induced response unnecessary.

The originality of our results resides in the fact that we noted induced responses even in a carton-nesting, territorially dominant arboreal species. In other words, induced responses are not limited to plant-ant species as previously believed, so that it is likely that their origin is not related to the rise of plant-ants. We can therefore conclude that induced responses are probably more general than previously thought, but there are exceptions.



**Fig. 1.** Number of *Azteca* spp. workers (means  $\pm$  standard error) patrolling experimental (a wound was inflicted to the leaf; solid circles) and control leaves (open circles) recorded every 30 s for 15 min after the start of the experiment (see statistical comparisons in Table 1).

**Table 1.** Results of repeated-measures ANOVA of the effect of the experimental treatment (artificially inflicted wound) versus control leaves on ant recruitment (increase in the number of workers)

	d.f.	MS	<i>F</i>	<i>P</i>	G-G
<i>Azteca chartifex</i>					
Treatment	1	174.71	26.03	<0.001	
Error	58	6.711			
Time	30	0.853	4.25	<0.001	<0.001
Time × treatment	30	0.539	2.68	<0.001	0.003
Error	1740	0.201			
<i>Azteca alfari</i>					
Treatment	1	560.9	25.6	<0.001	
Error	58	21.9			
Time	30	2.148	12.93	<0.001	<0.001
Time × treatment	30	1.774	10.67	<0.001	<0.001
Error	1740	0.166			
<i>Azteca ovaticeps</i>					
Treatment	1	358.73	15.35	<0.001	
Error	58	23.38			
Time	30	1.004	5.85	<0.001	<0.001
Time × treatment	30	0.893	5.20	<0.001	<0.001
Error	1740	0.172			
<i>Azteca bequaerti</i>					
Treatment	1	504.22	66.58	<0.001	
Error	58	7.573			
Time	30	1.089	6.1	<0.001	<0.001
Time × treatment	30	1.162	6.51	<0.001	<0.001
Error	1740	0.179			
<i>Azteca schimperi</i>					
Treatment	1	11.89	4.28	0.043	
Error	58	2.78			
Time	30	0.655	3.83	<0.001	<0.001
Time × treatment	30	0.268	1.57	0.026	0.127
Error	1740	0.171			

Note: MS = mean square; G-G = Greenhouse-Geisser correction applied to the degrees of freedom.

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