

Hiding From Defenders: Localized Chemical Modification on the Leaves of an Amazonian Ant-Plant Induced by a Gall-Making Insect (Diptera: Cecidomyiidae)

by

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ABSTRACT

Defense against herbivores is considered the main factor influencing the evolution of specialized ant-plant associations. Early studies have demonstrated that ants chemically recognize and recruit on host plant leaves due to herbivory simulation. Gall-forming insects are herbivores that are able to manipulate chemical plant properties and thus could change ant responses. The goal of this study was to evaluate recruitment responses of *Allomerus octoarticulatus*, an ant species associated with the myrmecophyte *Hirtella myrmecophila*, which is attacked by a gall-forming insect (Cecidomyiidae). We conducted two different experiments to assess whether *A. octoarticulatus* is able to recognize chemical compounds from galled tissues and whether galled and ungalled healthy leaves represent different chemical stimuli to worker ants. We did not observe differences on recruitment between galled and ungalled leaves. However, gall tissue extracts did not induce ant recruitment on *H. myrmecophila*, while galled leaves extracts resulted in a stimulus to ant recruitment. These results indicate that insect gall tissues on this myrmecophyte probably do not possess compounds which can be detected by the ants. A local change seems to be very helpful to gall-forming insects because they are protected from, and by, ants which deter herbivores.

Keywords: myrmecophytes, insect galls, ants, induced defenses, recruitment, herbivory, Cecidomyiidae.

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INTRODUCTION

Myrmecophytes are obligatory ant-associated plants which provide nest sites – domatia - and in some cases, food resources for ant colonies (Davidson & McKey 1993). For their part, ants inhabiting myrmecophytes generally offer benefits to the host plant by acting as allelopathic agents (Davidson & McKey 1993, Federle *et al.* 1998, Suarez *et al.* 1998), by providing nutrient accumulation that can be absorbed by the plant (Janzen 1974, Treseder *et al.* 1995), and by defending plants against herbivores (e.g., Janzen 1966, Fonseca 1994, Heil *et al.* 2001).

Defense against herbivores has been considered the main factor influencing the evolution of myrmecophily (Fonseca 1994). Ants associated with these plants are able to recognize chemical compounds which are released when the host plants suffer injury from insect herbivores (Agrawal & Rutter 1998, Brouat *et al.* 2000, Romero & Izzo 2004). Upon recognition of these compounds, volatiles or not, ants quickly recruit other workers to find and capture the responsible agent, diminishing the permanence time and damage extension caused by the herbivore (Agrawal & Rutter 1998, Brouat *et al.* 2000, Bruna *et al.* 2004).

Gall-forming insects represent an herbivore guild that possesses harmful relationships with the host plant (Fernandes 1987). The gall structure provides shelter and food to the insect larvae (Price *et al.* 1987), which is the responsible agent for genetic and chemical manipulation, and subsequent alterations on the plant tissues (Hartley 1998, Sopow *et al.* 2003). Insect galls act as natural sinks, changing nutrient allocation in the host plant (Kirst 1974, Kirst & Rapp 1974, Larson & Whitham 1991, Hartley 1998). Higher concentrations of phenolic compounds and tannins in galls compared to ungalled leaf tissues have been reported as an anti-enemy defense mechanism (Fernandes & Price 1991, see also Larew 1982). These chemical modifications on galling tissues may have two consequences: (i) make the nutrients more easily available to the gall-making larvae (Shorthouse 1986); and (ii) turn the gall tissues unpalatable to other herbivores (Mani 1964, Fernandes *et al.* 2005).

Despite the large myrmecophyte diversity in the Neotropics, no study evaluated the occurrence of gall-forming insects on these plants. However, of the 16 myrmecophyte species recorded by Fonseca & Ganade (1996) in

central Amazonia, insect galls were observed only on 4 species: *Cordia nodosa*, *Maieta guianensis*, *Hirtella physophora*, and *H. myrmecophila* (T. Izzo pers. obs). The gall formation on this particular group of plants can be affected by ants. In three species of the south Asian Macaranga myrmecophytes (Euphorbiaceae), *Crematogaster* and *Camponotus* ants were very efficient in reducing the incidence of gall-forming insects (Itino & Itioka 2001). Mutualistic ants may be able to recognize and prevent gall-forming insect oviposition, by detecting chemical and/or tactile perturbations on the leaf surface, and then killing the adult female insect. However, ants may also influence the galling success later or during the gall life cycle. Once that ants can recognize chemical compounds released when their hosts are damaged, at the stage of gall-making adult emergence, ants may be able to capture the gall-making insect during gall tissue rupture. Moreover, the newly emerged adult gall-making insect cannot fly immediately and has to spend time to expand its wing, therefore being vulnerable to ant attack. Nevertheless, a mechanism to prevent the ants from finding emerging gall-making adults may be present in insects which induce galls on myrmecophytes such as *H. myrmecophila*. We hypothesize that the gall-making insect is able to shift or exclude the chemical compounds that would induce the recruitment of *Allomerus octoarticulatus* (Romero & Izzo 2004). The chemical manipulation by the gall-making insect should be extremely localized to maintain the ant protection on the leaf, which would prevent leaf loss to other herbivores.

The present study aims to answer the following questions: (1) Does *A. octoarticulatus* recognize the chemical compounds from insect gall tissues? (2) Do galled and ungalled healthy leaves represent different chemical stimuli to worker ants?

MATERIAL AND METHODS

Study Area

This study was conducted out in a 10,000-ha forest reserve, located at km 41 of the ZF3 road, c.a. 70 km north of the city of Manaus, Amazonas, Brazil (54050'00" W & 02025'00" S). This reserve harbours 800-ha of undisturbed upland forest (Fonseca & Ganade 1996) and is managed by the Biological Dynamics of Forest Fragments, a project of National Institute for Amazonian Research (INPA) with collaboration with the Smithsonian Institution.

Study species

Hirtella myrmecophila is a small (<8 m) understory tree commonly found in non-flooded forests of central Amazonia, and produces leaf pouches as domatia. *Allomerus octoarticulatus* is a tiny ant (<2 mm), a relative of the fire ants, that lives exclusively in myrmecophytes and locally have a strict relationship with *H. myrmecophyla* (Izzo & Vasconcelos 2002). This ant species is extremely efficient to prevent herbivory on *H. myrmecophila*'s leaves (Izzo & Vasconcelos 2002). It's presence on plants promotes decreases in the richness and abundance of arthropods that have a sedentary relationship with the plant, such as herbivores and spiders (Izzo & Vasconcelos 2005). *A. octoarticulatus*, is also capable of recognizing, at short distance, chemical compounds released by it's host plant at the moment of tissue rupture. Then the ant recruits other ants to find and capture the possible herbivore responsible of this rupture (Romero & Izzo 2004). Galls in *H. myrmecophila* are a natural rare phenomenon. Of 227 plants surveyed for galls, just 14 were found in the study area. However, gall formation on Central Amazonian understory trees is not frequent and it is locally more common on the canopy (G.R. Julião, unpublished data). Just one cecidomyiid gall species was found in *H. myrmecophila*. The gall is closed, unilocular, and covered by a high density of plant trichomes.

Experiment 1: leaf extract vs. gall extract

To assess whether *A. octoarticulatus* is able to recognize chemical compounds from galled tissues, an experiment with paired blocks in each plant was employed. Galled leaves were sampled from nine *H. myrmecophila* individuals to produce aqueous extracts of both gall tissues and leaves that present galls (excluding gall tissue in this case). Gall extracts were obtained by macerating 1 g of gall tissues (Cecidomyiidae larvae was excluded) in 10 ml of water, resulting in an extract with 0.1 g/ml concentration. The extract production was done twice, using galls of different plants. As we didn't find statistical differences between both groups of gall extracts, its effect was not considered on the statistical model. The same procedure was adopted to obtain the galled leaves extract, using mixed tissues of all sampled leaves (excluding the insect gall). Both substances were applied separately on two randomly selected young leaves of 18 *H. myrmecophila* plants using syringes. All experimental plants hosted *A. octoarticulatus* ants and were 50 m far from each other. The

number of ants were counted just before the application of the extracts (time 0) and at 1, 3, 5, 7 and 9 minutes after the treatments on both leaves. This method was employed successfully by Romero & Izzo (2004) in this same host plant species to observe ant recruitment responses to leaf extracts and on damaged leaves.

Experiment 2: galled leaf extracts vs. ungalled leaf extracts

To evaluate whether ants present a differential response to chemical compounds from galled and ungalled leaves, leaves were collected in other nine randomly ungalled plants to obtain aqueous extracts with the same concentration, using the procedures described above. Other ten plants in the study area were randomly chosen to develop the experiment, employing the same design described above. In both experiments, all sampled leaves used to obtain the extracts were mature and totally expanded. Even after an extensive survey, we could not find galls on new expanded leaves.

Statistical Analysis

For the experiment 1, analysis of variance (ANOVA) with repeated measures on random blocks by plant was used to determine possible differences of ants responses to galled leaves and gall extracts (each experimental plant was considered as a block and response time as a repetition factor). For the experiment 2, comparisons were done among different plants. Thus, ANOVA with repeated measures was used to test possible differences between ant response to galled leaves and ungalled leaves extracts. In both cases the probabilities were corrected with the Greenhouse-Geisser (G-G) approximation procedure to avoid sphericity (Zar 1996).

RESULTS AND DISCUSSION

Gall tissue extracts did not induce ant recruitment on *H. myrmecophila*, while galled leaves extracts resulted in a stimulus to recruitment of *A. octoarticulatus* (Table. 1; Fig. 1a). These results indicate that insect gall tissues on this myrmecophyte probably do not possess compounds which can be detected by the ants. Alternatively, ants could not recognize the trace concentrations of chemical compounds on the gall tissues by the presence of other masking substances. Some gall-forming insects has the ability to manipulate the chemical pathways of its host plant, as resource allocation to gall tissues

Table 1. Repeated measures ANOVA examining the recruitment of ants overtime (number/leaf) on 1) gall tissue vs. galled leaves and 2) Galled leaves vs. ungalled leaves. The factor Time was considered as the factor of repetition and, in the first test, plants as experimental blocks.

| Source of Variation | df | F | P | G-G |
|--|-----|--------|-------|--------------|
| 1-Galls vs. galled leaves | | | | |
| Plant (Block) | 15 | 1.264 | 0.328 | |
| Treatment | 1 | 2.869 | 0.100 | |
| Error | 15 | | | |
| Time | 5 | 6.191 | 0.000 | 0.002 |
| Time x Plant | 75 | 0.865 | 0.734 | 0.675 |
| Time x Treatment | 5 | 8.06 | 0.000 | 0.000 |
| Error | 75 | | | |
| 2-Galled leaves vs. not galled leaves | | | | |
| Treatment | 1 | 3.311 | 0.082 | |
| Error | 24 | | | |
| Time | 5 | 13.316 | 0.000 | 0.000 |
| Time x Treatment | 5 | 1.157 | 0.334 | 0.323 |
| Error | 120 | | | |

(Kirst 1974, Kirst & Rapp 1974, Larson & Whitham 1991), reduction of phenolics content at gall interior (Larew 1982, Nyman & Julkunen-Tiitto 2000) and elevated content of phenolics in the galled tissues compared to host plant ungalled tissues (Hartley 1998). In *H. myrmecophila*, the chemical manipulation by gall-forming insects in the gall tissue should benefit the adult insect at the emergence stage, since tissue rupture could be chemically masked. In this stage, cecidomyiid flies are vulnerable to predators because adult emergence and further wing hardening are relatively slow processes. Gall-inducing sawflies are able to control concentration of phenolic compounds on their host *Salix* spp. leaves and gall interiors presented fewer different low molecular-weight phenolics than leaves, and total concentration of these compounds was reduced (Nyman & Julkunen-Tiitto 2000).

Chemical changes driven by gall-making insects seem to be a punctual phenomenon, occurring only in gall tissues, since galled (but excluding the insect gall) and ungalled leaves extracts showed the same results on ant recruitment (Fig. 1b, Table 1). A local change seems to be very helpful to gall-forming insects because they are free of toxic compounds, and, in the studied system, protected of and by ants which avoid herbivores. A similar pattern occurs in *Quercus turbinella* (Fernandes *et al.* 1999). In this host plant, the *Disholcaspis*

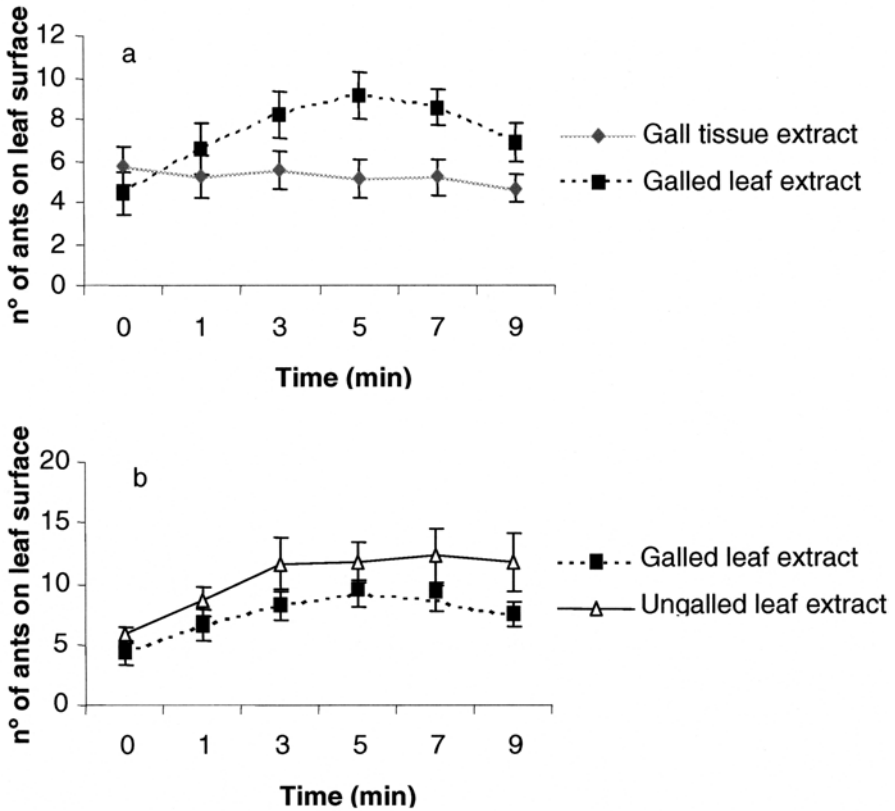


Fig. 1. Mean number (± 1 SE) of *A. octoarticulatus* on young leaves of *H. myrmecophila* overtime (in minutes) that received (a) galled tissue and galled leaves extracts and (b) extracts of galled leaves (excluding galls) and ungalled leaves.

edura (Cynipidae) galls secrete an attractive substance to ants whose presence reduces gall-forming insect mortality due to parasitoid attack. Even without direct observations, absence of chemical recognition of galls by *Allomerus octoarticulatus* is strong evidence that ants should be inefficient in gall forming insect capture at post-emergence stage. If a protective effect of ant's presence on the host plant exists, it would be during the oviposition stage, where ants may recognize and eliminate females. Other post-oviposition mechanisms can also explain the low incidence of galls on *H. myrmecophila*. For instance, some host plants submitted to high infestation can abscise galled leaves, such as in *Neea madeirana* (Nyctaginaceae) (G.W. Fernandes, *pers. obs.*), elicit induced defenses as hypersensitivity reactions (Fernandes 1998, Fernandes

& Negreiros 2001), or even abscise other plant parts, such as branches, as a response to stress conditions (Espírito-Santo & Fernandes 2002). These mechanisms may regulate gall-forming insect populations independently of ant presence. Further studies are required to obtain more details about interactions among mymecophytes, gall-making insects and ants, a poor surveyed system, which combines new approaches of chemical ecology and insect-plant interactions.

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