Trichome Removal by Hitchhikers in Two Leaf-cutting Ant Species (Hymenoptera: Formicidae)

by

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ABSTRACT

Despite the known evidence that hitchhiker ants protect workers against attack by phorid parasitoids, several alternative hypotheses are suggested for the occurrence of hitchhikers on leaf-cutting ants. One hypothesis suggests that hitchhikers clean leaf fragments and remove pathogens. We hypothesized that hitchhikers can act in the removal of leaf trichomes. Activities of hitchhikers (HH) are reported based on three laboratory and eight field colonies of leaf-cutting ants (*Atta* spp.). We evaluated whether the presence of trichomes increases the frequency of HH in leaf fragments transported to the colony. Furthermore, we evaluated if fragment size and the time that the fragment remains in the foraging arena could influence HH frequency. The removal of trichomes by HH of laboratory colonies was recorded on video. Hitchhikers were more frequent in fragments with trichomes in both laboratory and field colonies. In the field, the distance from the foraging site did not influence the amount of HH. The proportion of HH in laboratory, however, was most frequent only during the first hour of foraging. The presence of HH is correlated to the size of fragments. We also observed removal of trichomes as an additional role of hitchhikers.

Key words: foraging behavior, *Atta sexdens*, *Atta laevigata*, social insects, trunk trails.

RESUMO

Apesar das evidências que reforçam o papel de formigas caroneiras (hitchhikers) na proteção das operárias forrageadoras contra o ataque de forídeos,

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hipóteses alternativas tentam explicar a ocorrência desse comportamento em formigas cortadeiras. Uma das hipóteses sugere que hitchhikers atuam limpando fragmentos de folhas contra patógenos. Nossa hipótese é que hitchhikers podem atuar na remoção de tricomas foliares. Atividades de caroneiras são relatadas com base em três colônias de *Atta* spp. mantidas em laboratório e oito colônias de campo. Nós avaliamos se a presença de tricomas aumenta a frequência de caroneiras em fragmentos de folhas transportados para a colônia. Além disso, foi avaliado se o tamanho do fragmento e o tempo em que o fragmento permanece na arena de forrageamento poderiam influenciar na frequência de hitchhikers. A remoção de tricomas por caroneiras em laboratório foi gravado em vídeo. Caroneiras foram mais frequentes em fragmentos com tricomas tanto em colônias de laboratório como nas de campo. No campo, a distância do local de coleta não influenciou a quantidade de caroneiras. A proporção de caroneiras em laboratório, no entanto, foi mais frequente apenas durante a primeira hora de forrageamento. A presença de caroneiras foi correlacionada com o tamanho do fragmento. A remoção de tricomas caracteriza-se como um papel adicional de caroneiras.

Palavras-chave: caroneiras, forrageamento, mata atlântica, trilhas

INTRODUCTION

During foraging, the smallest workers ("minims") of some leaf-cutting ant species are commonly seen "hitchhiking" on leaf fragments transported to the colony by larger foragers and the likely explanations for this behavior has been discussed in previous studies. Several papers report the activity of hitchhikers (HH) in seven leaf-cutting ant species, most of which relates to forager defense against phorid parasitoids as the main function of these hitchhikers (Lutz 1929; Stahel 1943; Eibl-Eibesfeldt & Eibl-Eibesfeldt 1967; Feener & Moss 1990; Erthal &Tonhasca 2000; Linksvayer *et al.* 2002; Vieira-Neto *et al.* 2006).

Linksvayer *et al.* (2002) reported hitchhiking behavior of *Atta cephalotes* as a means of forager protection and, more recently, Vieira-Neto *et al.* (2006) tested, in addition to the aforementioned function, two other hypotheses concerning to hitchhiking behavior in *A. sexdens* and *A. laevigata*: defense against fungal contamination and leaf sap acquisition. These authors suggested that the hitchhiking behavior probably has multiple functions.

For laboratory colonies, a previous study (Kitayama *et al.* 2010) had shown that foragers of *A. sexdens* remove leaf trichomes prior to transport of leaf fragments to the colony. In field colonies, however, workers are seen carrying leaf fragments with trichomes (author's personal observation). This suggests that trichome removal is performed during the journey to the colony or in the subterranean chambers of the nest.

In this study, we test the hypothesis that there is a positive relationship between the presence of leaf trichomes and the number of hitchhikers, thus suggesting that hitchhikers may act in the removal of trichomes of the leaf fragments during the transport of them, to increase the efficiency of cleaning the substrate, an activity that has not been yet reported for this behavior. In addition, we tested whether larger foraging trails or large fragments have a higher proportion of hitchhikers and if this activity decreases with processing time of the plant material in the foraging area.

MATERIAL AND METHODS

Laboratory colonies and experimental design

Experiments were carried out using three colonies of *Atta sexdens rubropilosa* Forel, 1908. The colonies were maintained in laboratory conditions at 25 ± 2 °C under a 12 h photophase. Colonies were daily supplied with leaves without trichomes of *Bauhinia variegata* L. (Fabaceae) and leaves with trichomes of *Ochroma piramidalis* (Bombacaceae). These plant species were chosen because they are commonly seen being cut by leaf-cutting ants in the field.

The colonies (five years-old) were maintained in artificial nests, consisting of two interconnected plastic chambers with fungus and brood (volume: 40,000 cm$_3$) linked to a foraging arena (70 cm in length × 40 cm in width × 30 cm in height) by a transparent plastic tube (2.5 cm in diameter; 16 m in length).

Presence/absence of leaf trichomes and proportion of hitchhikers

For each leaf type, small leaf discs (0.5 – 1.0 cm^2) and large discs (1.1 – 1.8 cm^2) were offered to ant colonies for 6 hours. The proportion of hitchhikers in the leaf discs was assessed by collecting all the workers carrying leaf fragments back to the colony during 30 minute intervals in two phases: (1) thirty minutes after the start of the experiment and (2) six hours after the first sampling.

The size of leaf discs collected as well as the head capsule of all collected ants (workers and hitchhikers) was measured using a digital caliper.

Trichome removal by hitchhikers

The removal of trichomes by hitchhikers on the way back to the fungus garden from the foraging tray was videotaped inside the plastic tube and also in a transparent box of 20×10×5 cm in length, height and width connected to the plastic tubes.

Field colonies and sampling

Field study was conducted in an Atlantic forest reserve of the Parque Estadual do Rio Doce (PERD), state of Minas Gerais (19°30'21"S and 42°41'19" W). The park is considered the largest reserve of Atlantic Forest of Minas Gerais state with total area covering approximately 36,000 ha. Colonies of leaf-cutters *Atta sexdens rubropilosa* and *A. laevigata* are common on the edge of roads that cross the park. For this study we selected eight adult colonies (five *A. sexdens rubropilosa* and three *A. laevigata*) with nest area from 64 to 120 m^2 and at least 100m apart from each other. The observations were made in three days (15-17) of July 2011, at the peak of foraging activity (18-23h). Each colony had two to four well developed foraging trails, with an activity of approximately 68 ± 11 ants/minute. For each colony, a foraging trail was chosen arbitrarily for sampling. The total length of the trail (from the nest entrance to foraging site) was accessed and a fixed point was established 1 m away from the nest entrance. During the observation period (18 - 23h) we established a sampling period of 5 minutes at 60 minute intervals, totaling 5 samples per colony in total (25 minutes). In each sample we collected all loaded ants crossing a fixed point in the foraging trail and we determined the proportion of fragments with hitchhikers. Also, the collected fragments were separated in relation to the presence/absence of trichomes. Leaf fragments without trichomes were identified as being from trees of *Mabea fistulifera* Mart., while the plant species of fragments with trichomes could not be identified.

Data analysis

To test whether the size of leaf discs may affect the proportion of hitchhikers, a Wilcoxon rank test was performed. For laboratory colonies, a generalized linear model (GLM; Mccullagh & Nelder 1989) with quasibinomial error distribution was performed to test the effects of presence of trichomes and foraging time (independent variables) on the proportion of hitchhikers in leaf fragments (dependent variable). For field colonies, the GLM model was performed to test if the proportion of HH changes with leaf substrate (with or without trichomes) and length of the foraging trail.

RESULTS

Laboratory colonies

Major leaf discs (1.55 ±0.76 cm^2) had consistently higher number of hitchhikers than small leaf discs (1.02 ± 0.37 cm^2) (W=0.85; p=0.04). As HH may climb up or down the leaf at anytime, it is likely that larger loads are more easily climbed, and this may explain the higher occurrence of HH in larger fragments. Also, the presence of trichomes significantly affected the presence of HH. Leaf fragments of *Ochroma* sp. had more HH than leaf fragments of *Bauhinia* sp. (F =38.61; df =1 $p < 0.001$) in the first hour of foraging (Fig. 1). After a six hour interval, however, there was no significant difference in the proportion of leaf fragments with HH between leaf type (F = 1.87; $p > 0.05$). The decrease in the proportion of leaves with HH in *Ochroma* treatment after six hours of exposure to leaves in the foraging arena may be due to the reduction of trichomes (Kitayama *et al.* 2010).

Field colonies

As in the laboratory, the field colonies showed a similar foraging behavior, with a higher proportion of HH on leaves with trichomes. In total, 3300 leaf fragments were collected, but only 132 (4%) had trichomes. The proportion of HH in these fragments, however, was three times higher than in fragments without trichomes (mean of 60% of leaves with HH versus 23% in fragments without trichomes) (F =

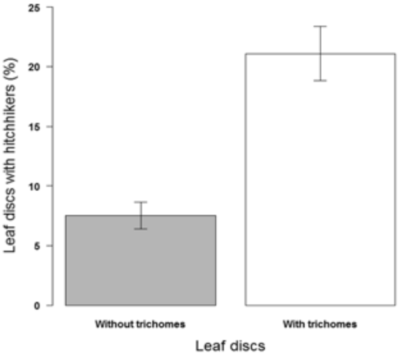

Fig. 1: Hitchhiker presence (%) in response to trichomes in leaf discs in three laboratory colonies (mean ± SE).

Table 1: GLM Analysis showing the effects of leaf type and distance of foraging site to the colony on proportion of hitchhikers of *Atta sexdens rubropilosa* and *A. laevigata* colonies.

Response Variable	Error distribution	d.f.	*F-value*	*p*
Leaf type	Quasibinomial	2	32.98	0.001
Distance to the nest	Quasibinomial	1	1.58	ns
Atta species	Quasibinomial	2	1.79	ns

32.98 p < 0.001). The distance from the foraging site to nest entrance did not significantly influence the proportion of HH in the fragments (F = 1.58 p = 0.24) (Table 1, Fig. 2).

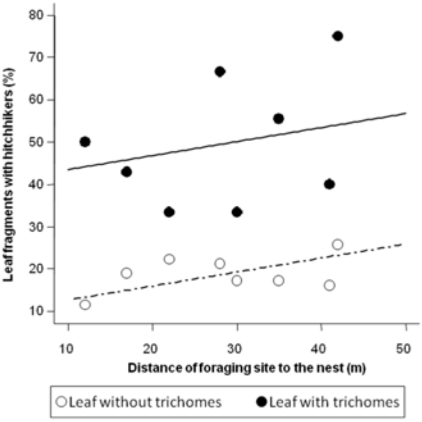

Fig. 2: Proportion of Hitchhikers in response to distance of foraging site to the nest and trichomes in leaf fragments in eight field colonies (curves: $Y_{\text{White circles}} = 0.0936+(0.0033^*x)$ and $Y_{\text{black circles}} = (0.401+(0.0033^*x))$.

DISCUSSION

Ants may hitch onto loads at anytime, anywhere along way back to the fungus garden (colony), for several different hypothetical reasons: (1) protection of leaf carriers from attack by phorid (Diptera: Phoridae) parasitoids; (2) to save energy of walking themselves to the nest; (3) to feed on leaf sap from cut leaves; and (4) prior preparation of leaf fragments, removing microbial contaminants (Eibl-Eibesfeldt & Eibl-Eibesfeldt 1967; Linksvayer *et al.* 2002; Vieira-Neto *et al.* 2006; Gerstner *et al.* 2011).

Our results support the previous processing hypothesis since not only were there more frequent hitchhikers in leaf with trichomes but also because their frequencies were reduced after six hours of processing the leaf discs. In the absence of ant-parasitizing phorids, pathogens or trichomes, the function of hitchhikers is probably not much required. In addition, the energy saving hypothesis (Feener & Moss 1990) was not supported, because the ratio of HH was not affected by the distance of the foraging site to the colony (Fig. 2).

As the presence of phorid parasitoids may reduce foraging activity (Bragança *et al.* 1998) parasitoid defence could be the primer function of hitchhiking. This fact is reinforced by the study of Roces & Holldobler (1995) that showed increased hitch behavior followed by sound signals caused by stridulatory vibrations during the cutting of leaf fragments before being loaded into the colony. So hitchhikers seem "to know" the right time to climb on the leaf fragments through this short-range recruitment signal. The occurrence of hitchhikers in periods that phorid parasitoids do not occur (night hours, for example), however, has raised several questions about alternative functions of hitchhikers on the trails of foraging.

After observation of 14 field colonies of *A. cephalotes*, Linksvayer *et al.* (2002) concluded that the prior preparation of leaves before entering the nest may be the most likely possibility for the occurrence of hitchhikers, as these authors found no evidence that hitchhikers were feeding on sap. This study reinforces the hypothesis that HH have multiple functions because HH were observed at night, with the highest proportion found in leaves with trichomes. Moreover, as this behavior was observed in two different species in the field, cleaning leaves of trichomes can be performed by all species of leaf-cutting ants. Similar functions performed by HH of different ant species were observed (Vieira-Neto *et al.* 2006).

Leaves without trichomes are preferred for cutting when there is a choice for ants (Howard 1988; Kitayama *et al.* 2010). In the present study, only 4% of the harvested leaves had trichomes. In fact, *Mabea fistulifera* is one of the most abundant tree species of the study site (Drumond & Neto 1999; personal observation), being possibly the main plant resource cut by *Atta* colonies throughout the year. Other plant species, however, may be more suitable for workers and this may explain the harvesting of leaves with trichomes. It is possible that such leaves with trichomes were chosen by some either nutritional or stress factor of the host plant, since plants under stress (i.e. drought-sensitive individuals) suffer greater attacks on the edge of forest environments than healthy plants (Meyer *et al.* 2006). In several Brazilian ecosystems, however, climate seasonality leads to many plants developing defence mechanisms against desiccation and herbivores (Saraiva *et al.* 1996; Marques *et al.* 2000). Thus, for several months, plants with leaves with abundant pubescence or trichomes are probably more resistant to drought and an important resource to herbivores (Woodman 1991). For ants in the cerrado and semi-arid habitats, leaves with trichomes are an important resource and the role of hitchhikers in these environments is crucial for the best utilization of this substrate for the colony.

Concerning the size of HH, we found that, according to our classification, 95.2% of ants are between 0.4 and 1.9 mm in head width and only 4.7% were larger than 2mm in head width. As nestmate (or load) defence is the main role of hitchhikers and small workers are more aggressive than larger ones, this size difference appears to correspond to this function.

As workers smaller than 2mm in head width are more likely to remove trichomes (Kitayama *et al.* 2010) and because HHs were also smaller than 2mm, a new function of HH was hypothesized and observed, that is, they could remove trichomes while moving back to the fungus garden. This new function was videotaped and is available for viewing at http://www.entomoufs.com.br/Hitchhikers2012.wmv.

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