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Refuse dumps of leaf-cutting ants as a deterrent for ant herbivory: does refuse age matter?

S. A. Ballari & A. G. Farji-Brener*

Laboratorio Ecotono, Departamento de Ecología, CRUB, Universidad Nacional del Comahue, Quintral 1250, (8400) Bariloche, Argentina

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Abstract

Leaf-cutting ants often avoid contact with their waste because it harbors microorganisms that are dangerous to the ants and their symbiotic fungus. Therefore, the use of ant waste (i.e., refuse dumps) has been proposed as a deterrent method against leafcutter attack. We tested experimentally whether the age of the refuse dump (fresh vs. old) affects the herbivory-deterrent effect against the leaf-cutting ant *Acromyrmex lobicornis* Emery (Hymenoptera: Formicidae). Refuse placed around seedlings significantly delayed the initiation attacks of leaf-cutting ants, and this deterrent effect decreased gradually over a period of 30 days. The initial strength of this decrease was the same for newly-discarded ('new') refuse and refuse from the bottom of the ants' waste pile ('old' refuse). However, the loss of deterrent effect over time was more rapid for new than old refuse. A further experimental manipulation, replacement of refuse every 3 days, had no effect on the deterrent effect for old refuse, but increased this effect for new refuse, although the amount of this increase gradually weakened over the course of the 30-day experiment. We speculate on the possible causes of these effects, their consequences for the hygienic behavior of leaf-cutting ants, and on the use of ant debris as short-term control method against leaf-cutting ants.

Introduction

Leaf-cutting ants (genera *Atta* and *Acromyrmex*) (Hymenoptera: Formicidae) are considered as one of the keystone herbivore species in natural habitats of America (Cherrett, 1989), and the most serious insect pests in agricultural and forest systems (Cherrett, 1986a,b). For example, a single colony of leaf-cutting ants caused 48% conifer seedling mortality and reduced growth in 40% of the surviving fraction in a 2-ha area in a Venezuelan forest plantation (Jaffé, 1986). Similar damage is common in several American countries (Blanton & Ewel, 1985; Fowler et al., 1986; Vilela, 1986), and forestry establishment often depends on a reasonable degree of leaf-cutting ant control (Cherrett, 1986b).

A wide range of control methods for leaf-cutting ants, including poison baits, parasites, and pathogens, has been used (see Cherrett, 1986b; Kermarrec et al., 1986). However, these techniques do not generally keep leaf-cutting ant populations below economic thresholds, and often have adverse effects on the environment and on human health (Cherrett, 1986b; Vilela, 1986). Recent studies have suggested that the refuse dumps of leafcutters could provide an alternative control method, eliminating the risks associated with poison techniques and the introduction of exotic species (Zeh et al., 1999; Farji-Brener & Sasal, 2003).

Leaf-cutting ants selectively collect large quantities of fresh vegetation from a large area and carry it to their nest chambers, where the plant material is degraded by a mutualistic fungus. The waste material from the fungal decomposition, dead ants, soil particles, and debris are removed from the fungus gardens to specific external or internal disposal areas (hereafter called refuse dumps). This refuse harbors microorganisms harmful to the ants and their symbiotic fungus (Fisher et al., 1996; Bot et al., 2001; Hart & Ratnieks, 2001, 2002). For example, fungi of the genus *Escovopsis*, which can infect the fungus culture of the leafcutters, causing the death of the colony, is common in refuse dumps (Currie et al., 1999, 2003). Therefore, leaf-cutting ants avoid contact with their own refuse. This natural avoidance was tested in a preliminary manner as a control method against leafcutter attacks. Zeh et al. (1999) showed that seedlings surrounded by refuse dumps were not harvested by *Atta cephalotes* ants during 5 days.

*Correspondence: E-mail: alefarji@crub.uncoma.edu.ar; alefarji@yahoo.com

Nevertheless, the deterrent effect of refuse dumps drops after 1 week, and almost all seedlings surrounded by refuse dumps are finally attacked by *Acromyrmex lobicornis* Emery (Hymenoptera: Formicidae) ants after 30 days (Farji-Brener & Sasal, 2003). These results suggest that refuse dumps lose toxicity under field conditions, thus reducing their potential value as an effective control method against leafcutters. However, the factors affecting this reduction have not been explored. An understanding of why refuse dumps lose their deterrent effect may help to understand the ant management of waste, and improve their value as an effective control method.

Two factors that may affect the deterrent level of refuse dumps over time are their age and the location of the waste inside the refuse mound. When ants deposit waste on a mound, the fresh waste is located in the superficial layer while older wastes are in lower strata. Therefore, ant waste suffers two different biotic conditions as it ages. First, when freshly deposited, it is directly exposed to the external climatic conditions (e.g., daily variation in temperature, humidity, etc.). Later, when it is older and covered with other waste, microclimatic conditions change: inside the mound, both humidity and temperature are higher and less variable (AG Farji-Brener, unpubl.). These different abiotic conditions may affect the microorganisms that inhabit the refuse dumps.

We experimentally tested whether refuse age affects the antiherbivore properties of refuse. In addition, we also tested the deterrent effect of both types of refuse by replacing fresh and older refuse every 3 days. To do this, we employed the same protocol as Zeh et al. (1999) and Farji-Brener & Sasal (2003), monitoring the ant attack on seedlings that were surrounded by different types of refuse vs. controls.

Materials and methods

Study site and leaf-cutting ant species

The study was carried out between November and December 2003 (summer) on the eastern border of the Nahuel Huapi National Park, Argentina (41°S, 71°W). In general, weather in the region is dry and cold. The mean annual temperature is 8 °C but soil temperatures range between −15 °C in winter and 70 °C in summer (AG Farji-Brener, unpubl.). The mean annual rainfall is about 600 mm. The experiment was conducted in an area covered by herbaceous/shrub steppe vegetation, where the density of *A. lobicornis* is very high (up to 43 nests per ha; Farji-Brener, 2000). The dominant plant species include shrubs from the genera *Baccharis*, *Fabiana*, *Adesmia*, *Mulinum*, and *Senecio*, as well as herbaceous species from the genera *Poa, Stipa*, and *Festuca.*

The subject of the study, *A. lobicornis*, is the leaf-cutting ant species occurring in Argentina that has the widest latitudinal range, reaching from subtropical areas in southern Brazil and Bolivia (23°S) to Patagonia (44°S) (Farji-Brener & Ruggiero, 1994). *Acromyrmex lobicornis* occurs in a broad range of plant communities and is a major pest of agricultural and forestry areas (Bonetto, 1959; Pilati et al., 1997; Coll, 2004). *Acromyrmex lobicornis* refuse dumps are located on the soil surface, forming conspicuous waste mounds (Farji-Brener, 2000).

Methodology

We followed the same basic protocol of Zeh et al. (1999) and Farji-Brener & Sasal (2003): planted seedlings were surrounded by various types of refuse (treatments), and ant attack was monitored until ca. 80% of seedlings were harvested. We used seedlings (15 cm height) of *Godetia* spec. (F_1 hybrid), a common ornamental plant highly palatable to leaf-cutting ants (Farji-Brener & Sasal, 2003). Seedlings rather than adult plants were used because they are preferred by leafcutters (Vasconcelos & Cherrett, 1997), and they facilitate a randomized, replicated experimental design. We used seven *A. lobicornis* adult nests (mounds at least 1 m in diameter) separated by ca. 20 m. Around each nest (1–2 m away from the mound) and 15 cm from the two to three main active foraging trails, we planted a total of six randomly selected seedlings. One of the following four treatments was randomly assigned to each seedling: (1) seedling surrounded by fresh refuse (waste from the superficial layer of the mound), (2) seedling surrounded by old refuse (waste from inside the mound), (3) seedling surrounded by a mound of soil as control for mound effects (‘mound control’), and (4) seedling with no mound around it (control). Treatments (5) and (6) were similar to (1) and (2), but in these we replaced the refuse around the seedling every 3 days, removing the refuse previously placed around the seedlings and replacing it with the same type of refuse (i.e., fresh for fresh, old for old). The refuse dump used as a source was of the same colony, as a colony’s own waste has a stronger deterrent effect than foreign debris (Farji-Brener & Sasal, 2003). The mounds of the treatments were ca. 7 cm high and 20 cm in diameter. The mean number of leaves (± SE) at the beginning of the experiments (80 ± 10) did not differ significantly among the plants in the six treatment conditions ($F_{1,36} = 0.64$, $P = 0.48$). Each replication in this experiment consisted of an adult nest of *A. lobicornis* (n = 7) with six treatment conditions. We watered the seedlings daily for the first 15 days, and then every other day until the end of the experiment on the 30th day. We checked each seedling for the presence of *A. lobicornis* ants and counted the number of leaves approximately every 2 days (18 times over a period of 30 days). Because of the leaf-cutting behavior and the leaf size, leaves of this plant species were often entirely cut (i.e., partially damaged leaves were not found, Farji-Brener & Sasal, 2003).

Variation in defoliation was expressed as the number of remaining leaves (in percentage), and differences were tested using ANOVA. Prior to analysis, we tested response variables for normality, and when necessary, used arcsine transformations. We examined differences in defoliation level among treatments (fixed factor) and time (repeated measure) using a one-way, repeated measures randomized block design. Each ant nest was considered as a block because the foraging activity of each colony probably influences defoliation levels. At the end of the experiment, the final level of defoliation between treatments was analyzed using a one-way, randomized block design ANOVA. Duncan post hoc comparisons of means were employed when ANOVA results were statistically significant ($P<0.05$).

Results

Refuse dumps showed a high initial deterrent effect against leaf-cutting ant attack (i.e., ants avoided refuse dumps often after contacting with the waste), but this effect decreased gradually over a period of 30 days ($F_{17,612} = 121.6$, $P<0.0001$). Despite some variability between ant nests ($F_{6,612} = 2.2$, $P = 0.07$), the loss of the deterrent effect over time depended on the type of treatment (treatment*time interaction: $F_{85,612} = 10.6$, $P<0.0001$). Control seedlings and seedlings surrounded with soil mound were defoliated in the first day, while those surrounded by refuse were attacked 2 weeks later (Figure 1). However, the loss of the deterrent effect over time depended on the refuse age. New refuse showed the highest decrease rate in its deterrent effect; seedlings surrounded with this treatment lost 40% of their leaves in only 1 week. However, their deterrent properties were maintained longer when the refuse was replaced every 3 days. On the other hand, the loss of the deterrent effect over time of old refuse was similar with and without replacement until the last week of the experiment (Figure 1). At the end of the experiment, the level of defoliation was significantly different between treatments ($F_{5,36} = 6.8$, $P = 0.0001$). Refuse that was replaced every 3 days showed a higher deterrent effect against leafcutters than the non-replaced ones (Figure 1). Control seedlings and seedlings surrounded by soil mound were the most defoliated ($0 \pm 7\%$ of remaining leaves), followed by those surrounded by fresh and old refuse without replacement ($8.1 \pm 6\%$ and $23.0 \pm 11\%$ of remaining leaves, respectively), and by fresh and old refuse with replacement each 3 days ($34.5 \pm 10\%$ and $45.5 \pm 8\%$ of remaining leaves, respectively; mean ± SE, all $P<0.05$, post hoc Duncan test).

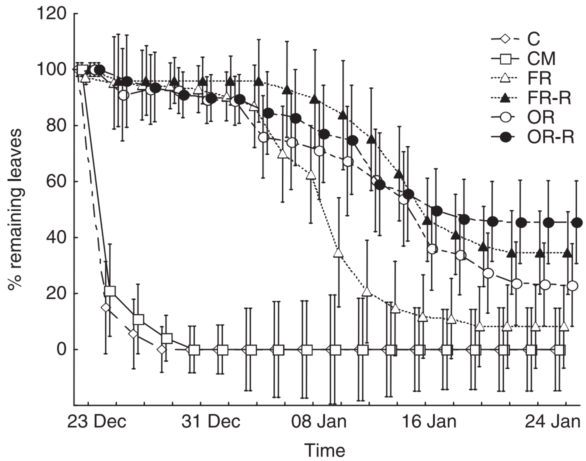

Figure 1 Remaining leaves (in percentage, mean ± 1 SD) due to *Acromyrmex lobicornis* attack on *Godetia* spec. seedlings with different treatments during the 30 days of the experiment. FR: seedlings surrounded by fresh refuse, FR-R: as FR, but replacing the refuse every 3 days, OR: seedlings surrounded by old refuse, OR-R: as OR, but replacing refuse every 3 days, CM: seedlings surrounded by soil to control mound effect, and C: seedlings without anything around (controls). The form of the symbols represent similar treatments (e.g., circles, old refuse; triangles, fresh refuse), and their color if refuse was replaced every 3 days or not (black, with replacement).

Discussion

Ant debris showed a good initial deterrent effect against leaf-cutting ant attack, but this effect decreased rapidly over a month. The initial deterrent effect and its subsequent decrease were not a result of the mound per se (e.g., mounds around seedlings may hinder their localization or harvest); seedlings surrounded by soil mounds were discovered and attacked at the beginning of the experiment. The deterrent effect and its decrease over time apparently depend on the refuse characteristics. Our results suggest that refuse dumps have, at least, two different types of toxicity for leafcutters. Recently dumped waste showed a high deterrent effect, but a faster loss of deterrence over time in field conditions. However, its deterrence was maintained when it was renewed every few days. These results strongly suggest that the climatic conditions of the study area (e.g., extreme aridity, high temperature variations, etc.) are an important source of mortality for the microorganisms that inhabit the refuse dump and are responsible for the deterrent effect (Powell & Stradling, 1986; Roces & Kleineidam, 2000). On the other hand, old refuse showed a similar deterrent effect as fresh refuse, but a lower decrease of deterrence over time, independently of its replacement (Figure 1). These results suggest that the microclimatic conditions inside the mounds may favor the regrowth of the pathogens that have either survived or colonized from the upper layers of the mound of debris. This would explain why older refuse presents similar deterrence to that of new refuse. However, old refuse showed a slower decrease in deterrence over time. The different decrease rate between fresh and old refuse in their deterrent effect is difficult to explain. Probably old refuse harbor and/or stimulate the growth of microorganisms relatively more resistant to external climatic conditions. However, this hypothesis deserves more study. Our findings that fresh and old refuse have different deterrent effects can be considered as the first step to fully understand the mechanisms involved in the toxicity of refuse dumps. To extend this idea it is necessary to know the identity of the microorganisms from fresh and old refuse, and to analyze their tolerance to different abiotic conditions.

This work offers some information that can be useful to understand both the hygienic behavior of leaf-cutting ants, and how to control this agricultural and forest pest. First, our results suggest that the behavior of *A. lobicornis* of locating their refuse dumps in mounds on the soil surface is doubly hygienic: external climatic conditions rapidly decrease the toxicity of fresh waste, diminishing the risk of colony contamination; and when the waste recovers some level of toxicity it is inside the mound, reducing the possibility of ant contact and/or an eventual dispersion to the nest. It would be interesting to study whether this behavior of locating waste outside the nest is generally performed by leaf-cutting ants that inhabit environments that are harsh for the microorganisms responsible for the deterrent effect (e.g., deserts). On the other hand, because the deterrent effect of ant wastes decreases within a few weeks, its use appears to be ineffective as a long-term, extensive method of leaf-cutting ant control. However, it may be useful as a short-term control method, especially if ant refuse is renewed every 3 days. This methodology can effectively protect 90% of seedlings from ant attack for almost 15 days and 50% for 1 month (Figure 1). Because the earlier stage of seedlings is the most vulnerable plant stage to the leaf-cutting ant attack (Vasconcelos & Cherret, 1997), the use of this cheap and simple control method may be important in the first phase of some agricultural or forest plantation.

Leaf-cutting ants are among the most serious pests in America, therefore identifying and isolating the microorganisms responsible for the deterrent effect, and determining the abiotic conditions favorable for their growth may have important consequences. Our hope is that the results of this work will stimulate development of a short-term pest control technique that is cheap and healthy for humans and ecosystems.

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