

# Is dump material an effective small-scale deterrent to herbivory by leaf-cutting ants?<sup>1</sup>

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**Abstract:** It has been suggested that refuse dumps from leaf-cutting ant nests work as a herbivory deterrent for leaf-cutting ants because dumps harbour micro-organisms that are dangerous to ants and their symbiotic fungus. However, this deterrent effect was tested for the narrowly distributed *Atta colombica* only during a five-day period. We experimentally tested the refuse-deterrent effect for a longer period using the broadly distributed *Acromyrmex lobicornis*. Although refuse mounds around plants significantly delayed the initiation of attack by *A. lobicornis*, almost all plants (~90%), surrounded or not by refuse, were killed by leaf cutters in a few weeks. Therefore, the refuse-deterrent technique may not be useful as a widespread method of biological control for leaf-cutting ants. The refuse showed an improved deterrent effect when both the dump material and the leaf-cutting ant came from the same colony, suggesting that the pathogens for both ant and fungus found in refuse dumps might be nest-specific.

**Keywords:** *Acromyrmex lobicornis*, biological control, dump material, herbivory, leaf-cutting ants, Patagonia.

**Résumé :** Les amas de déchets des nids de fourmis coupeuses de feuilles exerceraient un effet dissuasif contre les autres fourmis coupeuses de feuilles en raison de la présence dans les amas de dangereux micro-organismes et de champignons. Cet effet dissuasif n'a toutefois été testé que pendant cinq jours et chez une fourmi peu répandue, *Atta colombica*. Nous avons vérifié pendant une plus longue période l'effet dissuasif de ces amas de déchets chez une fourmi très répandue, *Acromyrmex lobicornis*. Bien que des monticules de déchets placés autour de plantes aient retardé, de façon significative, les attaques effectuées par *A. lobicornis*, presque toutes les plantes (~90 %) ont été tuées par les fourmis en quelques semaines, qu'elles aient été entourées ou non de déchets. Par conséquent, les amas de déchets ne seraient pas très utiles comme méthode de contrôle biologique des fourmis coupeuses de feuilles. Les détritux ont un meilleur effet dissuasif lorsque l'amas de déchets et les fourmis proviennent de la même colonie, ce qui suggère que les pathogènes sont spécifiques au nid à la fois pour les fourmis et les champignons.

**Mots-clés :** *Acromyrmex lobicornis*, contrôle biologique, déchets, herbivore, fourmis coupeuses de feuilles, Patagonie.

**Nomenclature:** Holldöbler & Wilson, 1990.

## Introduction

Several forest insects considered pests are important limiting factors in forestry production and in native forest management and conservation (Carroll *et al.*, 1990). Leaf-cutting ants (*Atta* and *Acromyrmex*) are one of the most serious insect pests of many crops and plantations in South and Central America (Blanton & Ewel, 1985; Cherrett, 1986a,b; Fowler *et al.*, 1986). Fungi cultivated by leaf cutters can grow on a wide range of plant substrates; therefore, these ants are highly polyphagous and cause extensive damage to native forests as well as agricultural crops (Vilela, 1986; Vasconcelos & Cherrett, 1997). For example, a single colony of leaf-cutting ants caused 48% conifer seedling mortality and reduced growth of 40% of the surviving fraction in a 2-ha area in Venezuela (Jaffe, 1986). Similar reports are common in several American countries (Blanton & Ewel, 1985; Vilela, 1986) where forestry establishment depends on a reasonable degree of ant control (Cherrett, 1986a).

A wide range of control methods for leaf-cutting ants, including poison baits, thermal fogging, and the use of parasites and pathogens, have been used for the last 50

years (Cherrett, 1986b; Kermarrec *et al.*, 1986). However, these techniques do not generally keep leaf-cutting ant populations below economic thresholds, and they have adverse effects on the environment and human health (Cherrett, 1986b; Vilela, 1986).

In a recent study, Zeh *et al.* (1999) reported a new approach to protecting plants from leaf-cutting ants that eliminates the risks associated with poison techniques and the introduction of exotic species. This method involves the use of refuse dumps from leaf-cutting ant nests. Plant parts harvested by leaf-cutting ants are transported to underground fungus gardens. Organic materials from the fungus culture and associated debris are known to harbour microorganisms harmful to both the ants and their symbiotic fungus (Bot *et al.*, 2001; Hart *et al.*, 2001). These materials are removed from the fungus gardens to subterranean chambers or external disposal areas known as refuse dumps (Farji-Brener & Medina, 2000). Zeh *et al.* (1999) suggested that external refuse dumps-from *Atta colombica* colonies can be used as an effective control method to protect plants from leaf-cutting ant defoliation because ants avoided contact with the refuse. While leaf cutters removed all leaves from control plants, plants surrounded with a mound of ant refuse showed no damage during five days.

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However, the following facts suggest that the available evidence in that study is not conclusive. As the leaf-cutting ant species used (*Atta colombica*) has a narrow geographic distribution (Holl Dobler & Wilson, 1990), the useful range of the refuse technique is restricted. In addition, ant nests were not used as replicates in the experimental design, and thus there was no information about variation of the refuse-deterrent effect between ant nests. Finally, and possibly the most important fact, there was no report about what happened after five days. It is clear that any biological control method to protect plants from defoliation by leaf-cutting ants requires a longer sampling period to prove whether it is effective or not. Therefore, we believe that the usefulness of refuse dumps as a widespread method for leaf-cutter control needs to be reassessed.

We used the broadly distributed *Acromyrmex lobicornis* ant species to examine whether refuse material can be used as an effective natural deterrent to protect plants from leaf-cutting ant attack. We also checked the refuse-deterrent effect using ant nests as replicates and extended our study period for more than five days. Finally, we investigated whether the success of the refuse-deterrent method varied if dump material was from the same nest or from another conspecific nest (nest-dependent effect). In addition to its biological relevance, this point is important if we want to test the practicality of the refuse-deterrent method.

## Methods

### LEAF-CUTTING ANT SPECIES AND STUDY SITE

*Acromyrmex lobicornis* is one of the leaf-cutting ant species with 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 (Boneto, 1959; Coll, 1997; Pillati *et al.*, 1997). *Acromyrmex lobicornis* waste, such as organic material from the fungus culture, dead ants and debris are transferred to the soil surface, forming conspicuous external refuse dumps (Farji-Brener, 2000).

The study was carried out during November and December 2001 (summer season) along the eastern border of the Nahuel Huapi National Park, Argentina (41° s, 71° w). The mean annual temperature is 8°C, and the mean annual precipitation 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 (Farji-Brener, 2000).

### METHODOLOGY

We employed seedlings (15 cm height) of *Goedertia* spp. (F<sub>1</sub> hybrid), a common ornamental plant highly palatable for leaf-cutting ants. Seedlings rather than adult plants were used because they are preferred by leaf cutters (Vasconcelos & Cherrett, 1997) and they facilitate a randomized, replicated experimental design.

We selected 10 *A. lobicornis* adult nests (mounds 1 m in diameter) separated by approximately 20 m. Around each nest (1-2 m away from the mound) and near active foraging trails (15 cm), we planted 4 randomly selected

seedlings. One of the following four treatments was randomly assigned to each seedling: (1) seedling surrounded by a refuse mound from the sampled nest (own refuse), (2) seedling surrounded by a refuse mound from another randomly selected nest (foreign refuse), (3) seedling surrounded by a mound of soil to control for mound effects (mound control), and (4) seedling with no mound around it (control). Following Zeh *et al.* (1999), mounds in treatments 1-3 were *ca* 7 cm high and had a diameter of 20 cm. We counted the leaves on each seedling before planting. Each replication in this experiment consisted of an adult nest of *A. lobicornis* ( $n=10$ ) with four treatment conditions. The mean number of leaves ( $\pm$  SE) at the beginning of the experiment ( $51 \pm 2$ ) did not differ significantly among the four treatment conditions ( $F_{3,36}=0.84$ ,  $P=0.48$ ). We watered the seedlings daily for the first 15 days and then every other day until the 28<sup>th</sup> day (end of the experiment). At the same time, we checked each seedling for the presence of *A. lobicornis* damage and counted the number of leaves.

Variation in defoliation levels was examined using analysis of variance. Prior to analysis we tested dependent variables for normality, and when necessary we used arcsine transformations. We examined differences in defoliation level and timing using a one-way, repeated measures randomized block design (Zar, 1999). Each ant nest was considered as a block because the foraging activity of each colony may influence defoliation levels. By the fourth day, leaf cutters had harvested all the leaves of treatments 3 and 4, so we employed the above-described analysis twice: first, to detect differences in defoliation levels between all the treatments on day four and, second, to detect differences in defoliation levels between the remaining treatments (1 and 2) until the end of the study period.

## Results and discussion

Considering the first four days of sampling, there was a highly significant effect on the seedlings' defoliation level due to time ( $F_{3,27}=9.7$ ) and treatment ( $F_{3,27}=34.5$ ; both  $P<0.001$ ). The percentage of leaves remaining decreased during the sampling period, and seedlings surrounded by refuse suffered much less leaf-cutter attack than control and mound control seedlings (Figure 1). This effect was marginally influenced by ant nests ( $F_{9,27}=2.0$ ,  $P=0.07$ ). On the fourth day of sampling, all leaves from seedlings without refuse were gone: seedlings with own and foreign refuse had  $68 \pm 3.5\%$  and  $46 \pm 4.3\%$  of their leaves intact, respectively (mean  $\pm$  SE, Figure 1).

Between days 5 and 28 there was a marginally significant difference between own and foreign refuse defoliation level ( $F_{1,9}=3.2$ ,  $P=0.10$ ) and a significant difference between nests ( $F_{9,9}=3.6$ ,  $P=0.03$ ). At the end of the experiment (day 28), the percentage of leaves that were intact on own refuse seedlings was slightly higher than that on foreign refuse seedlings ( $30 \pm 5\%$  versus  $10 \pm 2\%$  respectively, mean  $\pm$  SE, Figure 1, paired *t*-test,  $t=1.75$ ,  $P=0.057$ ).

Our experiment demonstrates that mounds of refuse material cannot be considered as a highly effective biological control method to protect plants from defoliation by *A. lobicornis*. Although refuse mounds significantly

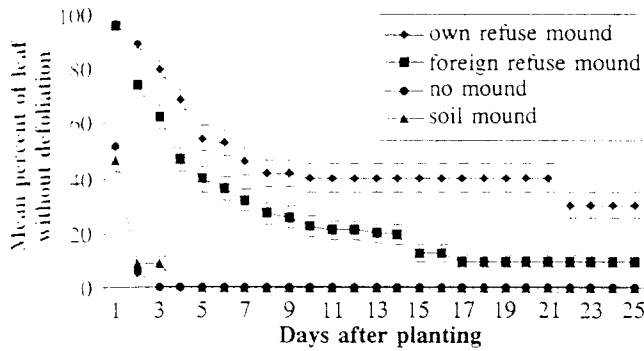


FIGURE 1. Mean percent ( $\pm$  SE) of remaining leaves in seedlings surrounded by own refuse mound, foreign refuse mound, soil mound, and no mound. Ten leaf-cutting ant nests and one seedling for each treatment condition per nest were employed (total=40 seedlings). In own refuse mounds, seedlings were surrounded by refuse from the same ant colony being sampled. In foreign refuse mounds, the refuse used came from another, randomly selected conspecific nest.

delayed the attack, almost all plants of all treatments were eventually attacked and killed by leaf-cutting ants (Figure 1). Seedlings surrounded by refuse dumps suffered significantly lower defoliation during the first days, but a few weeks later only 10-30% of the leaves were not removed and only 3 of 20 seedlings were not attacked. Additionally, not all the nests responded equally to the deterrent effect of the refuse; therefore, wide application of this control method would be problematic. Despite the potential positive aspects of this technique and its relatively good results in the earliest days of use, it does not seem likely that a control method that is effective for only a few days would be a useful technique for protecting plants from leaf-cutting ants.

Why did the refuse have only a short-term deterrent effect? It is known that refuse dumps harbour microorganisms that are dangerous to both ants and their symbiotic fungus (Bot *et al.*, 2001; Hart *et al.*, 2001). It is possible that many of these microorganisms die or lose their hazardous effect through time in external refuse dumps. This, in fact, could be the explanation for why some ant species construct external refuse dumps. It is also possible that the abiotic condition of the environment influences the loss of refuse-deterrent potency. In the dry and cold conditions of our study area, the refuse dumps lost 50% of their deterrent effect in the first 5 days and ~80% twenty days later. Unfortunately, the study of Zeh *et al.* (1999), which was carried out in a warm and humid environment, was performed only for 5 days. Therefore, we do not know whether the loss of potency of the refuse-deterrent effect showed in this study is a general phenomenon or it depends on both the leaf-cutting ant species and the abiotic habitat condition. Both of these aspects are worthy of further work. However, if different leaf-cutting ant species do not respond consistently to the refuse-deterrent method, and the refuse has only a short-term deterrent effect (as we confirmed), this technique will be ineffective as an extensive method of leaf-cutting ant control. It is possible that repeating the dump treatment every two weeks would help to prevent leaf-cutting ant attack for a longer period. However, this practice might be too laborious, and its effectiveness deserves further study.

On the other hand, the results of our experiment show the possibility of studying a novel aspect of the refuse-dump biology of leaf-cutting ants. Surprisingly, the refuse had an enhanced deterrent effect when both the dump material and the attacking ant came from the same colony (Figure 1). This suggests that many of the pathogens and competitors for both ant and fungus found in refuse dumps may be nest specific.

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