

## TERPENOIDS AND THE LEAF-CUTTING ANT-FUNGUS MUTUALISM

### TERPENOIDES Y EL MUTUALISMO HONGO-BACHACO

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#### ABSTRACT

*Pinus caribaea* needles contain  $\alpha$ - and  $\beta$ -pinenes which are toxic to insects. However, *Atta laevigata* workers prepare a substrate with these needles for their mutualistic fungus: the ant larvae's main food source. Three-one fungus garden nests were excavated and the fungal substrate separated as processed or fresh fractions. In each fraction, immature and adult forms were divided and counted. Also, a two-fungus garden nest was dug out, each garden ant population counted, and sorted into adults and immatures. Chloroformic extracts of pine needles and fungus fractions were analyzed by gas chromatography. It was found that immatures concentrate in the processed substrate. Workers were found anywhere.  $\alpha$ -pinene was found present in pine needles but not in fungal fractions.  $\beta$ -pinene was found in needles as well as in the two-garden nest fresh fungal substrate. All this suggest that  $\beta$ -pinene does not harm workers, but immatures might need to remain isolated until the toxic effect disappears. One could speculate that in this mutualistic association, adults carry out the work involved in fungal substrate preparation; immatures benefit from both protection from allelopathic effects as well as from available fungal food. In turn, the fungus performs the chemical work involving plant allelochemical and cellulose degradation.

KEY WORDS: Leaf-cutting ant, Fungus, Mutualism, *Atta laevigata*, *Pinus caribaea*, Terpenoids,  $\alpha$ -pinene,  $\beta$ -pinene, Allelochemicals.

#### RESUMEN

Los pinenos  $\alpha$ -y  $\beta$ - de las acículas de *Pinus caribaea* son tóxicos para los insectos. Pero las obreras de *Atta laevigata* preparan un sustrato de acículas para su hongo mutualista del cual se alimentan las larvas. Se excavaron tres nidos de una honguera y el sustrato fungal se separó en fracciones procesadas o frescas. En cada fracción se contaron las formas inmaduras y adultas. También se excavó un nido de dos hongueras, se contaron las adultas e inmaduras de cada honguera. Se analizaron por cromatografía de gases extractos en cloroformo de acículas y de fracciones fungales. Las inmaduras se concentran en el sustrato procesado. Las obreras están en cualquier parte. Se encontró  $\alpha$ -pineno en las acículas pero no en las fracciones fungales.  $\beta$ -pineno estuvo presente en acículas y en el sustrato fungal fresco del nido de dos hongueras. Todo sugiere que  $\beta$ -pineno no

perjudica a las adultas, pero estas podrían aislar a las inmaduras hasta que desaparezca el efecto tóxico. Podría especularse que en este mutualismo, las adultas efectúan el trabajo de preparar el sustrato fungal; las inmaduras se benefician por la protección de los efectos alelopáticos y por el alimento fungal disponible. A su vez, el hongo realiza el trabajo de degradar celulosa y sustancias aleloquímicas vegetales.

PALABRAS CLAVE: Bachaco, Hongo, Mutualismo, *Atta laevigata*, *Pinus caribaea*, Terpenoides,  $\alpha$ -pineno,  $\beta$ -pineno, Sustancias Aleloquímicas.

## INTRODUCTION

There is no direct herbivory in the leaf-cutting ant-plant interaction because it is mediated by a fungus that maintains an obligatory mutualism with the ant. In this way, the ant prepares a plant substrate for the fungus which, in turn, produces food bodies for the ant.

According to Martin and Weber (1969), mutualism is the means by which the ant gains access to the vast food reserves available in plants since the fungus is able to degrade cellulose and transform it into assimilable material. On the other hand, Cherrett (1980) considers that mutualism is possible only as an alliance to break down plant defenses: the ant removes barriers to fungal penetration by selecting new leaves which have low levels of allelochemicals and by removing the wax on the leaf surface (Quinlan and Cherrett, 1977); in turn, the fungus transforms plant allelochemicals into assimilable substances for the ant. There are no direct evidences for fungus activity, although Little et al (1977) have shown that a juvenile hormone analogue present in some plants does not affect the ant larvae when it is used as substrate for the fungus.

Plants produce compounds that act

as a chemical defense against insects (Feeny 1976, Levin 1976, Rhoades and Cates 1976, Hubbell et al 1983, Howard 1987). Terpenoids and tannins are examples of these compounds. In spite of this, many plants containing this kind of allelochemicals, e.g., pines, eucalyptuses, mangoes, are attacked by leaf-cutting ants.

In selecting plant food, the leaf-cutting ant *Atta laevigata* is a generalist if several plant species are available, but it is also successful on monocultures. It has reached enormous population growth in the pine plantation of Estado Monagas (Venezuela), where *Pinus caribaea* is being planted in extensive monocultures (Cedeño-León 1984).

*P. caribaea* produces terpenoids  $\alpha$ - and  $\beta$ -pinenes (Green et al. 1974). These terpenoids have antiinsect properties (Mabry and Gill 1979, Harborne 1977).

The ways in which terpenoids affect insects vary greatly (Slàma 1979, Williams 1970). Some terpenoids mimic the insect juvenile hormone, while some are strong toxic substances, and others have ovicide or sterilizing effects. Howard (1987) points out that the non-polar nature of the terpenoid molecules

may make them suited for penetrating the insect cuticle.

The ant's biting, during the process of pine needle fragmentation, in the preparation of fungus substrate, should expose the terpenoids present in the pine needles. If they have potentially insecticidal properties, why is the ant not affected?

Based on Cherret's (1980) hypothesis, this paper intends to illustrate the mechanism of mutualism by using both *Atta laevigata* and *Pinus caribaea* terpenoids. I postulate that terpenoids affect immature forms but not adult ants. Therefore, immature ants should be somehow protected from contact with pine allelochemicals.

### METHODS

Three one-fungus garden nests approximately 8 months old were excavated (Fig. 1). Fungus gardens containing their respective ant populations, were dug out and separated

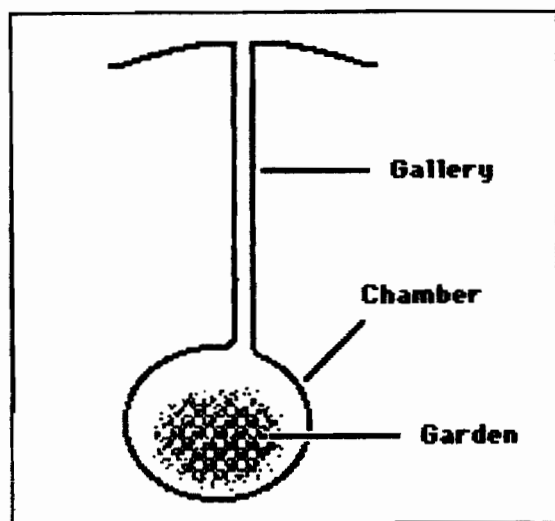


FIGURE 1. One Chamber nest (schematic).

into processed and fresh substrate fractions. Ants in each fraction were counted and classified as immature (eggs, larvae, pupae) or adult forms.

Also, a two-fungus garden nest was excavated (Fig. 2), each garden of the ant population counted and sorted into adults and immatures.

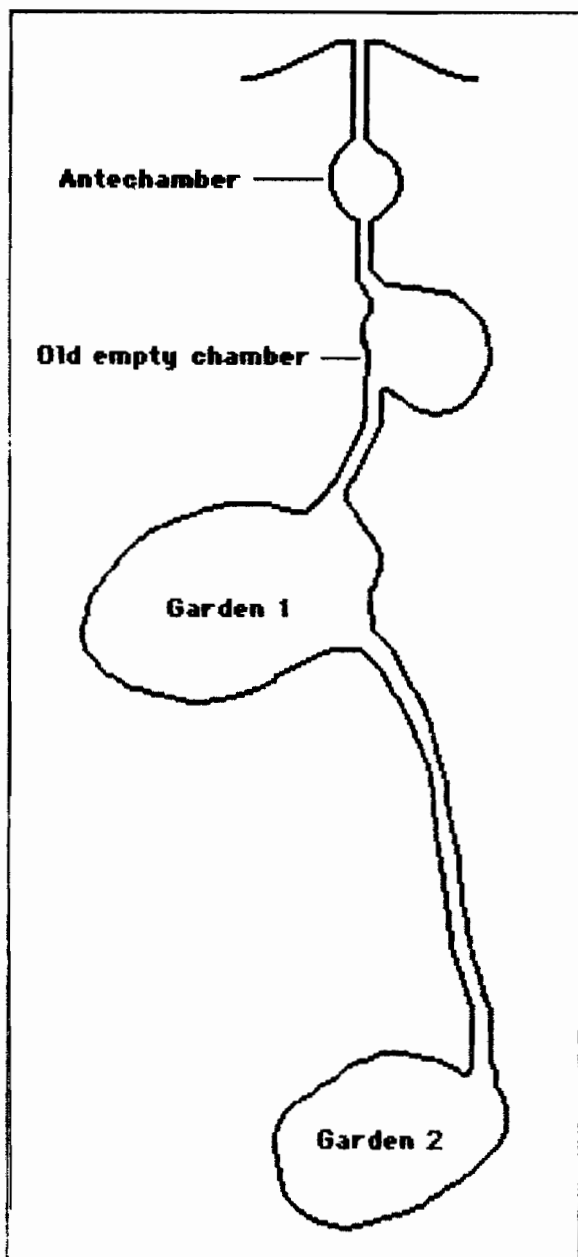


FIGURE 2. Two-fungus garden nest (schematic)

Terpenoids were determined by qualitative chromatographic analysis. For this purpose, nest fractions were fixed first in 70% ethanol, the ant population separated, and the fungal portion fixed in chloroform.

Chloroformic extracts of pine needles and fungal fractions were analyzed in a HP-5710A gas chromatographer with a HP-3390A integrator. The glass column was 36 cm long, 2.5 mm internal diameter and 6.0 mm external diameter in a 3.8% UCW-98/chromosorb WAW-DMCS (80-100) mesh. Both injection and detection temperatures were 300° C.

## RESULTS

### Population composition

Table 1 shows the population distribution in three one-fungus garden nests. Immature forms concentrated in the processed substrate of the garden. Adults were anywhere.

Table 2 shows the population distribution in the two-fungus garden

**TABLE 1.** Immature and adult numbers in one-fungus garden nests.

Nest	Type of substrate	No. Immatures	No. Adults
1	Processed	157	27
	Fresh	7	123
2	Processed	160	61
	Fresh	0	126
3	Processed	58	134
	Fresh	48	15

nest, the superficial garden (Garden 1, see Fig. 2) being the more recent one, as the presence of green material indicated. Although Garden 1 was the largest, it had only 3 immature forms compared to 413 adults and the foundress queen.

**TABLE 2.** Immature and adult numbers found in a two-fungus garden nest.

Location	Immatures	Adults
Fungal garden 1 (younger)	3	413
Fungal garden 2 (older)	282	266

The deeper garden (Garden 2), where fresh substrate was absent, had a total number of 282 immatures and 266 adults.

### Chromatographic analysis of terpenoids

Chloroformic extracts of one-fungus garden nests do not present any pinene.

Table 3 shows that  $\alpha$ -pinene is found in pine needles but not in fungal fractions.  $\beta$ -pinene is present in needles and in Garden 1 but not in Garden 2.

In general, fungal chromatograms are more complex than those of needles.

**TABLE 3.** Chromatographic analysis of terpenoids in pine needles and the two fungus garden nest.

Extract	$\alpha$ -pinene	$\beta$ -pinene
Pine needles	+	+
Fungus garden 1	-	+
Fungus garden 2	-	-

## DISCUSSION

The fungus garden is initiated by the *Atta laevigata* queen as a white sheet inside the chamber she previously excavates (Cedeño and León 1989). Eggs are laid on the fungal sheet and the newly-hatched immatures live embedded in the fungus. Thereafter adult workers begin to forage, incorporating processed plant material into the fungal surface. The garden grows centrifugally, and eventually adopts a globular shape.

It was found that immature forms are located in the central part of the garden in nests up to 5 months old; after this age, the tendency may change (Cedeño and León, in preparation).

If the garden's center is the oldest portion, one may assume that it is free of allelochemicals, but these must be present in fresh substrates. No pinene was found in any one-garden nest. The low rate of green material incorporation may explain the absence of terpenoids in these gardens.

As nests grow older, more chambers are excavated and new fungal gardens

established. The two-fungus garden nest we unearthed shows the probable trend of nest development. Cedeño and León (1989) have shown that after a second chamber has been excavated, the fungus and the immatures are moved into the deeper chamber. Afterwards, the superficial chamber is enlarged, a new fungus garden is cultivated there, and fresh green material is massively incorporated to it. This new situation exempts immatures from the action of plant allelochemicals since only few leaves are incorporated into the deeper fungus garden (Cherrett, personal communication). As Table 2 shows, the bulk of the forage of the workers processing was carried out in Garden 1, while the immature ant population concentrated in Garden 2. Worker ants were present anywhere in the gardens.

$\alpha$ -pinene was not found in the fungal fractions by the same method used to detect it in pine needles. It is possible that  $\alpha$ -pinene evaporates during the process of pine needle fragmentation by the ant, or else, it decays or is rapidly transformed by the fungus. It can be inferred that  $\beta$ -pinene is transformed more slowly because it is present in some fractions of the new garden (Garden 1), but not at all in the old one. Since the queen was found in the new garden, one may infer that  $\beta$ -pinene is neither harmful to her nor to adult workers. However, it could be dangerous to immature forms which should be safeguarded until the potential toxic effects disappear.

The modes of action of  $\alpha$ - and  $\beta$ -pinenes are unknown. One may suppose

that they can mimic the juvenile hormone (JH) (Slàma 1979, Williams 1970) or penetrate the soft cuticle of immatures (Howard 1987). Either adults are not affected by JH analogues or their cuticle is hard enough to hinder terpenoid penetration.

In order to have a clearer picture, it would be necessary to test the biological effects of  $\alpha$ - and  $\beta$ -pinenes on immatures as well as adults.

Plant species containing terpenoids should be selected by ants only if their fungus is able to degrade them. *Atta cephalotes* discriminates against plant species containing terpenoids that are harmful to its fungus (Hubbell et al. 1983, Howard 1987).

Some substances potentially harmful to insects seem to affect larval growth. Leaf-cutting ants defeat a plant's chemical defense strategy when adults process fresh material containing allelochemicals. However, the fungus should be able to degrade a wide range of antilarval substances (Cherret 1980, Jones 1984). This work lends support to that claim, since fungal chromatograms are more complex than those of pine needles.

I speculate on the roles played by the pair involved in this mutualistic association. Adult workers bear the weight of the interaction since they carry out all the work involved in the preparation of fungal substrate. Immatures benefit from both protection against allelopathic effects as well as from available fungal food. In turn, the fungus performs the chemical work

required for plant allelochemical and cellulose degradation.

This work supports Cherrett's hypothesis of mutualistic breakdown of plant species.

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