

Assessment of Microfungi in Fungus Gardens Free of the Leaf-Cutting Ant *Atta sexdens rubropilosa* (Hymenoptera: Formicidae)

by

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ABSTRACT

The purpose of this study was to determine the microfungi present in young nests of *Atta sexdens rubropilosa* when the fungus gardens were deprived of worker ants. The results were compared with another study in which worker ants had been killed by using toxic baits, and some species such as *Acremonium kiliense*, *Escovopsis weberi*, *Moniliella suaveolens* and *Trichoderma* sp. were confirmed among the most common inhabitants of this microenvironment, but differences in composition and proportion of species were observed. The importance and the role of these and other species of fungi within the symbiosis are discussed.

Keywords: symbiosis, leaf-cutting ant, filamentous fungi.

INTRODUCTION

Distributed only in the New World, leaf-cutting ants (Hymenoptera: Formicidae: Attini) are considered to be the major herbivores in the tropics (Hölldobler & Wilson 1990), causing various losses in plant production. It is well known that they cut fresh plant material and use it as a resource of nutrient for a symbiotic fungus, *Leucoagaricus gongylophorus* (Agaricales: Basidiomycota). This fungus is used mainly for nourishment of broods (Silva 2003); and is transferred to offspring colonies by the ant queens when founding a new nest after the nuptial flight (Weber 1972). The ants improve the growth of its partner by planting large amounts of fungal mycelium on the surface of the fresh plant fragments added to the garden (Weber 1979).

The aim of this strategy, combined with other hygienic behaviors, including the secretion of antiseptic substances, is to avoid the contamination of fungus gardens by alien microorganisms (Currie &

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Stuart 2001; Bot *et al.* 2002). However, an additional microbiota constituted of bacteria, yeasts and filamentous fungi can be found not only in the fungus gardens but also in other parts of the nests (Bacci *et al.* 1995; Fisher *et al.* 1996; Pagnocca *et al.* 1996; Carreiro *et al.* 1997). Apparently, this microbiota is under the control of the ants and little is known about its role within the symbiosis. Recently, an association between Attini ants and an antibiotic-producing bacterium was described (Currie *et al.* 1999a). This group of bacteria formerly identified as *Streptomyces* sp. and now recognized as a member of *Pseudonocardiace* (Currie *et al.* 2003) can suppress the growth of a fungus found only in the fungus growing ants' nests (Currie *et al.* 1999a,b). The harmful effect of this fast-growing fungus (*Escovopsis* sp.) over the nests is dramatic (Currie 2001a; Reynolds & Currie 2004).

In a previous work Rodrigues *et al.* (2005) described a number of microfungi species isolated from both field and lab nests of *Atta sexdens rubropilosa*, under stress conditions, which were induced by toxic baits. In order to know whether the toxic treatment could have affected the proportion and diversity of these microfungi, nests of the same ant species were collected at the same site, their workers were manually removed and many of the opportunistic fungi were identified

MATERIALS AND METHODS

Ant collection and bioassay

In April 2003, twelve young nests of *Atta sexdens rubropilosa* (with only one chamber of fungus garden) occurring in a citrus orchard near Corumbatai, S.P., Brazil (S 22°17'22"; W 47°39'23") were carefully dug out. The fungus gardens together with the ants tending it were aseptically collected and transported to the lab within sterile plastic Petri dishes.

Then, 60 pieces (ca 0.3 g five from of each fungus garden) were taken and the ants were removed using a sterile forceps. These parts of the fungus garden free of ants were maintained in a wet chamber (20 x 150 mm) at 25° C for twelve days in the dark and monitored daily for fungal growth. Simultaneously, another similar set of 10 pieces of the fungus garden with the workers still present were randomly taken from two among the twelve nests and maintained in the same conditions as control. During the period of the trial only sterile water (1 mL, daily) was available to the workers.

Isolation and maintenance of the fungi

Fungus garden fragments were observed under a stereomicroscope (Zeiss: Stemi SV6) and pieces of mycelium and/or spores of any non-

symbiotic fungi were plated into malt agar 2% (Bio Bras, Inc.) supplemented with chloramphenicol (150µg. L⁻¹, Sigma) and incubated at 25 °C in the dark. All of the isolates were identified based on morphological and cultural characteristics (Domsch *et al.* 1980; Samson *et al.* 1995). During the experimental period all isolates were maintained in agar slants at 5° C in the same culture medium.

RESULTS AND DISCUSSION

In the ant free fungus gardens (experimental) a total of 8 genera and two sterile mycelia of filamentous fungi were found just three days after the beginning of the assay and in most cases more than one species were isolated from the same colony (Table 1). Most of the fungi were anamorphic ascomycetes with exception of *Rhizopus stolonifer* var. *stolonifer*, a Zygomycete. *Acremonium kiliense*, *Escovopsis weberi*, *Moniliella suaveolens* and *Trichoderma* sp. were the most common microfungi isolated (Table 1). No fungal contamination was observed in the control at the same time.

When comparing the results with those obtained by using toxic baits (Mirex-S[®], commercial bait), the following fungal species were common to both approaches: *A. kiliense*, *Acremonium strictum*, *Cladosporium cladosporioides*, *E. weberi*, *Trichoderma harzianum* and *M. suaveolens* (Rodrigues *et al.* 2005). However, some differences in the profile and prevalence of species were observed. Thus, *M. suaveolens* which had been isolated in less than 10 % of the nests treated with baits (Rodrigues *et al.* 2004) was the prevalent species (50%) in the fungus garden manually deprived of workers (Table 1).

Table 1. Filamentous fungi isolated from ant free fungus-gardens of twelve *Atta sexdens rubropilosa* colonies, incubated in wet chambers at 25 °C for 12 days.

Fungal species	Ant Colony												(%)
	1	2	3	4	5	6	7	8	9	10	11	12	
<i>Acremonium kiliense</i>			+	+		+		+				+	42.0
<i>Acremonium strictum</i>					+								8.0
<i>Chrysosporium sulphureum</i>								+					8.0
<i>Cladosporium cladosporioides</i>									+				8.0
<i>Clonostachys rosea</i>												+	8.0
<i>Escovopsis weberi</i>	+	+		+				+				+	42.0
<i>Moniliella suaveolens</i>	+			+			+	+	+			+	50.0
<i>Rhizopus stolonifer</i> var. <i>stolonifer</i>			+										8.0
<i>Trichoderma harzianum</i>			+			+							17.0
<i>Trichoderma</i> sp.	+	+	+		+				+	+			50.0
Sterile mycelium							+		+				17.0

These fungus gardens contain high amounts of simple sugars as glucose (Silva *et al.* 2003) and the presence of *M. suaveolens* in this microenvironment is unusual because it has been often found in osmophilic and lipophilic environments, such as honey and dairy products (De Hoog 1979; Samson *et al.* 1995). It is interesting to note that Möller (1893) provided detailed descriptions of a fungus similar to *M. suaveolens* and erroneously associated it with a “morphological state” of the symbiotic fungus, otherwise a frequent contaminant as shown by the present work.

A similar result was achieved with *Trichoderma* sp. and *A. kiliense*. Both were found in less than 10 % in colonies treated with toxic baits but they were isolated in more than 40 % of the samples in nests manually deprived of workers. Currie (2001a) pointed out that *Trichoderma* sp. is a prevalent fungus in leaf-cutting ant nests and it could act as an antagonist to the symbiotic fungus, according to the *in-vitro* experiments of Ortiz & Orduz (2000).

Despite of their low frequency (Table 1) *Chrysosporium sulfureum*, *Clonostachys rosea* and *R. stolonifer* var. *stolonifer* were only found in fungus gardens manually deprived of workers. Probably, they are members of an ephemeral and transient microbiota that comes into the nests by forager ants and there is no evidence that they can threaten the symbiosis. Their absence in the bait-treated nests may indicate that they are probably more sensitive to the toxic chemicals present in the baits than other fungi. Yet, there is evidence that the toxic baits affected even some of the prevalent species. For instance, *E. weberi*, which had been found in 21 % and 15 % in lab and field colonies both treated with Mirex-S® (Rodrigues *et al.* 2005), respectively, was recovered in 42 % of the colonies in the present study.

According to Currie (2001a), *Escovopsis* sp. is present in a higher rate in older colonies (1-2 years-old) than in young ones (*ca* 6 months). Our data are quite different, since the rate of 42 % found in this study was higher when compared with only 6.6 % of young colonies of *Atta colombica* infected with *Escovopsis* sp. in the Panama Canal region (Currie *et al.* 1999b). It could be explained by species level variation, geographic distribution or even at random, but the rate of transmission of this parasite can be higher than previously assumed.

Therefore, it is important to rely on different approaches when studying the diversity of microfungi in leaf-cutting ant nests because according to the method used to eliminate the ants, variation in the community of fungi can occur. The reasons why these differences were observed are not clear yet but it is possible that some of the bait chemicals had a selective toxic effect to some species of fungi, as

indicated in the case of *Chrysosporium sulfureum*, *Clonostachys rosea* and *R. stolonifer* var. *stolonifer* A. *kiliense*, *E. weberi*, *M. suaveolens*, and *Trichoderma* sp.

Currie (2001b) considers that microorganisms, associated with fungus gardens of leaf-cutting ants, are, in fact, “invaders” or “weeds” for the cultivated fungus. Many of the fungi isolated in this research are consistently present in this microenvironment, suggesting that a process of specialization may be in course for them. The fact that they can act as opportunists when colonies undergo in an unbalanced state (for instance, major losses of ant population) makes them a matter of interest to further insights in possible interactions with the organisms involved in the symbiosis. To find out a suitable way to control these insects, a combination of biological and chemical methods should be tested using some of these fungi instead of the classical entomopathogenic fungi.

ACKNOWLEDGMENTS

This research was supported by CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) and FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo). The authors would like to thank Edson Luis Rezende, Mirian Salgado and Ricardo T.G. Pereira from Universidade Federal de Lavras for assistance during the identification of fungi.

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