

Carrying and Effect of Granulated Baits Formulated with Entomopathogenic Fungi among *Atta sexdens rubropilosa* Colonies (Hymenoptera: Formicidae)

by

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ABSTRACT

The present study aimed to evaluate the carrying and effect of (dry) granulated baits containing conidia of entomopathogenic fungi among colonies of the leaf-cutting ant *Atta sexdens rubropilosa* in the laboratory. This bait type was chosen to facilitate its eventual commercial use. Two applications were performed: in the first, baits with 1×10^8 conidia/g were utilized while in the second employed concentrations 5 to 8.6 times greater. The baits were formulated with a citric pulp base, with 2 isolates of *Beauveria bassiana*, 1 of *Paecilomyces lilacinus* and 1 of *Isaria fumosorosea*. The following controls were utilized: (I) baits with sulfuramid insecticide, (II) without active ingredient, and (III) *Acalypha* spp. leaf discs. It was verified that the baits containing fungal conidia were rapidly carried to the nest interior in both applications and were rejected minimally. Thus, the (dry) granulated bait formulation appears to be an adequate vehicle for entomopathogenic fungi. At the doses and concentrations utilized, the fungi provoked only limited worker mortality, not killing the colonies. Given the rapid carrying and low rejection, a higher conidial dose per colony can, perhaps, kill them. Thus, it is inferred that all the isolates tested present potential as an agent to control colonies of leaf-cutting ants.

Keywords: *Atta sexdens rubropilosa*, *Beauveria*, *Paecilomyces*, *Isaria*, bait, leaf-cutting ant.

INTRODUCTION

The leaf-cutting ants, genera *Atta* and *Acromyrmex* (Hymenoptera: Formicidae: Attini), utilize exotic and native plants as substrate for cultivating

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symbiotic fungus (a Basidiomycete, and therefore a macrofungus) (Hölldobler & Wilson 1990), and are considered pests that have a great impact on agriculture, ranching and silviculture (Boaretto & Forti 1997).

To control these pests, in general, a chemical approach must be employed, principally via granulated toxic baits (dry), with a citric pulp base, given their practicality and effectiveness. Relatively persistent synthetic insecticides are always utilized as the active ingredient (AI) in commercial granulated baits, in such a manner that most of them have been gradually prohibited or discontinued (Boaretto & Forti 1997; Grosman *et al.* 2002; Nagamoto *et al.* 2004; Gunasekara *et al.* 2007).

This context has prompted a search to develop alternatives including microbial control with entomopathogenic fungi (Kermarrec *et al.* 1986; Machado *et al.* 1988; Lopez & Orduz 2003). Although the literature has already demonstrated the pathogenicity of these fungi to leaf-cutting ants, they remain far from being usable in the field on a large scale. Furthermore, to the best of our knowledge, such fungi have never before been tested in a dry granulated formulation based on citric pulp, despite high attractiveness of this (Nagamoto *et al.* 2004, 2011).

In general, fungi are considered important agents for controlling insects (Alves 1998); however, leaf-cutting ants possess a diverse array of defenses against these microorganisms: the use of specific and generic antibiotics, grooming, and removal of contaminated material (Kermarrec *et al.* 1986; Hölldobler & Wilson 1990; Bot *et al.* 2001; Currie & Stuart 2001; Poulsen *et al.* 2002; Caldera *et al.* 2009), represent a great challenge in the development of efficient products that are commercially viable.

Thus, the present study aimed to evaluate the carrying and the effect of (dry) granulated baits containing conidia of entomopathogenic fungi among colonies of *Atta sexdens rubropilosa* Forel, under laboratory conditions.

MATERIAL AND METHODS

The present work was carried out in the Laboratory of Social-Pest Insects (LISP), FCA, UNESP, Botucatu, in 2010. The baits utilized were in the form of (dry) granulated pellets and had been manufactured artisanally with an attractive citric-pulp substrate and AIs comprised of the following fungi isolates for testing: BBOT11 and BBOT12 (*Beauveria bassiana*), PBOT33

(*Paecilomyces lilacinus*) and IBOT25 (*Isaria fumosorosea*), which had been previously isolated from queens of *Atta* spp. (Cardoso 2010).

The formulation process consisted of mixing the dried ground citric pulp, 5% CMC (carboxymethylcellulose) agglomerating agent, conidial suspension (1.0 mL of suspension/gram of final intended bait weight), refined soy oil at 5% w/w and distilled water, similar to the description of Nagamoto *et al.* (2011), but utilizing some different ingredients and sterile conditions. On account of dehydration, and considering the conidial weight to be negligible, the final concentration was 1×10^8 conidia/gram of bait. The conidial suspensions were prepared by scraping mycelium from colonies of fungi (cultured in a appropriate culture medium, PDA), adding distilled water, homogenizing in an orbital vortex shaker, and filtering the result through a double layer of cheesecloth; then, the conidia were quantified under a microscope utilizing a Neubauer chamber. The bait fillets were produced according to the method of Nagamoto *et al.* (2011), added onto Petri dishes and dehydrated in a BOD chamber at 5 °C for 48 hours; next, they were placed into a plastic bag and manually broken into pellets about ~0.5 cm in length, similarly to commercial baits (Lima *et al.* 2003; Nagamoto *et al.* 2011). Finally, the pellets were conserved at 5 °C for subsequent use.

The study consisted of two applications. At the first moment, the formulated baits were utilized with 1×10^8 conidia/gram of bait. Due to the low mortality of worker ants after 144 hours, a second application was performed at the same dose, but utilizing higher concentrations (with the totality of conidia available): 6.0×10^8 , 8.6×10^8 , 5.0×10^8 and 5.0×10^8 (conidia/g), respectively, for BBOT11, BBOT12, PBOT33 and IBOT25.

The control baits TC1 (with standard AI, sulfluramid, from Dinagro Agropecuária Ltd., at 0.3% w/w), and TC2 (neutral, without AI), were also manufactured under sterile conditions. To control against a possible effect of the bait components, a third control treatment (TC3) was utilized: fresh 8.0 mm (Ø) leaf discs of *Acalypha* spp, cut with the aid of a hole puncher.

Colonies of *Atta sexdens rubropilosa* with about 500 ml of fungal sponge were used, maintained at 25°C. In each colony 1 g of bait or leaf discs was applied in the foraging arena.

The delineation utilized in the first application was completely randomized, with 7 treatments and 4 repetitions. In the second application, each colony

received the same type of product, except the colonies treated with sulfluramid, which had died and were thus excluded from this new application. Prior to the applications, the colonies stayed 24 hours without being supplied leaves and only 48 hours after the application this procedure was resumed.

After application, the total carrying time was evaluated and, at 6 and 24 hours after application, the percentages of pellets and discs carried were determined. It was observed whether this material was incorporated into the fungus culture or rejected (foraging arena or waste chamber). The effect of baits was evaluated through the number of dead workers. For this, the numbers of dead ants in the foraging arena and waste chamber were counted, cumulatively, every 24 hours.

The carrying time was assessed through descriptive analysis (mean, standard deviation), besides maximum and minimum carrying time observed by treatment. The percentage of pellets carried and the mortality data were submitted to ANOVA and the means compared by the Tukey test at 5% probability

RESULTS AND DISCUSSION

In both applications, baits formulated with fungus were carried and incorporated into symbiotic culture (fungal sponge), with the isolates BBOT12 (*B. bassiana*) and PBOT33 (*P. lilacinus*) presenting the shortest mean carrying times, 0.52 ± 0.37 h and 0.75 ± 0.26 h, respectively in the first application, and 0.56 ± 0.30 h and 0.91 ± 0.53 h, in the second one (Fig. 1). In the control treatments, at 6 and 24 hours after application, there were still pellets in the foraging chamber in the majority of colonies, while colonies treated with fungal baits presented carrying near or equal to 100% (Fig. 2). The carrying percentage among treatments was not significant at 24h ($p = 0.6996$ and 0.2234 , respectively, for applications I and II).

Thus, the baits containing conidia of entomopathogenic fungi were carried rapidly, evidencing the favorability of these baits, since in field conditions, more rapid carrying of baits implies their lesser exposure to adverse climatic conditions, which may increase the efficiency of control (Boaretto & Forti 1997; Lima *et al.* 2003).

The rejection behavior was verified only in colonies under sulfluramid treatment and in one colony treated with BBOT12 (*B. bassiana*), corresponding to the paucity of pellets found in the waste chamber.

As to the effect of the baits on the ant population, it was verified, starting at 96h of application, that treatment TC1 (sulfuramid) provoked a greater number of ant deaths than all the others (Table 1). In the second application, the worker mortality provoked by PBOT33 (*P. lilacinus*) was greater than in the other treatments in all evaluation periods (Table 2), although significant differences appeared only in the final evaluations (Table 2). None of the colonies treated with fungal isolates died, but the dead ants found in the waste chamber presented extrusion of the pathogen applied (data not shown).

The fact that the fungus-treated colonies presented almost no rejection of baits, and incorporated them into the fungus garden appears to indicate that the formulation utilized, dry granulated bait, can also be a good vehicle for entomopathogenic fungi, like the moist bait of Lopez & Orduz (2003), formulated with orange juice (50%), wheat bran, and spores of *Metarhizium anisopliae* and/or *Trichoderma viride*. The advantage of the formulation utilized in the present work is its practicality of application and eventual commercialization without requiring adaptation of the bait formulation for large-scale production, storage and distribution. The presence of dead ants

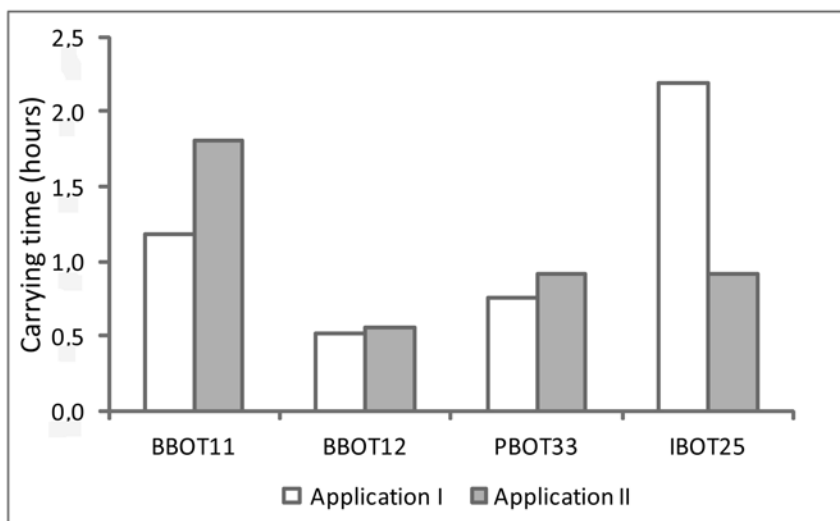


Fig. 1. Mean bait carrying time (hours) by *Atta sexdens rubropilosa* workers after application of baits with fungal isolates: BBOT11 and BBOT12 (*Beauveria bassiana*), PBOT33 (*Paecilomyces lilacinus*) and IBOT25 (*Isaria fumosorosea*), after first and second application.

parasitized by the applied fungus and the increase in waste volume produced in the waste chamber, findings also observed by Lopez & Orduz (2003), indicate its deleterious effect on the colonies, and thus the possibility that fungal isolates would kill the colonies at higher concentrations or doses.

There are still many important gaps in the research of leaf-cutting ants (Nagamoto *et al.* 2004), including lack of knowledge about the manner and extent to which some microfungi such as *Trichoderma*, in the form of spores, can sometimes be tolerated or not perceived (Lopez & Orduz 2003), and at

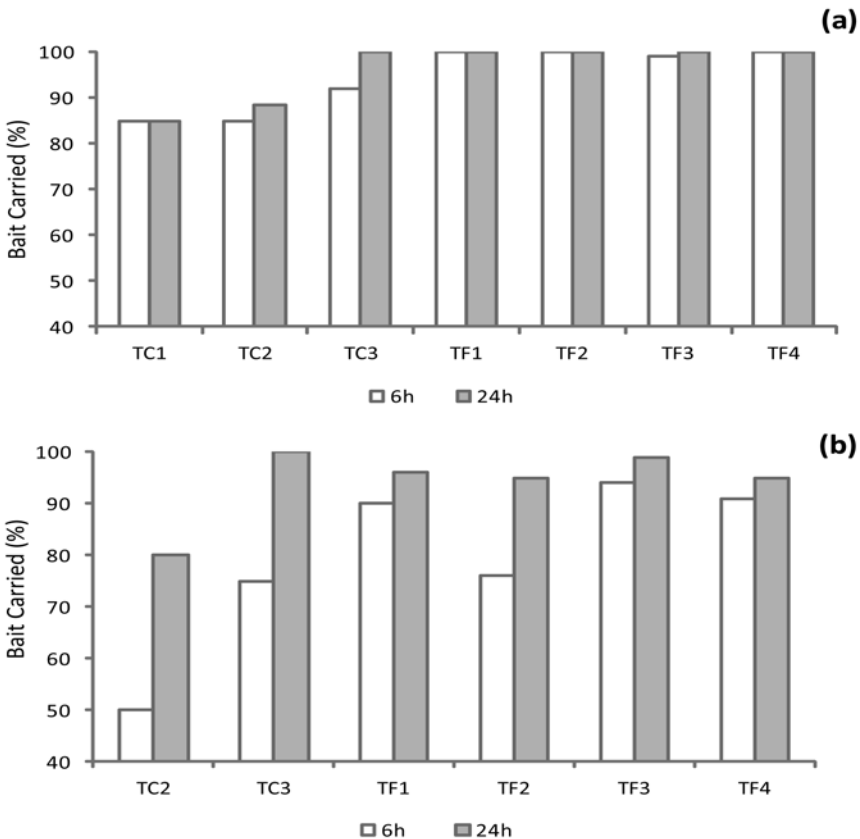


Fig. 2. Percentage (%) of baits carried by *Atta sexdens rubropilosa* workers, by treatment, at 6 and 24 hours after application of baits with fungal isolates: TF1 (isolate BBOT11 of *Beauveria bassiana*), TF2 (isolate BBOT12 of *Beauveria bassiana*), TF3 (isolate PBOT33 of *Paecilomyces lilacinus*), TF4 (isolate IBOT25 of *Isaria fumosorosea*), bait with the active ingredient sulfluramid (TC1), bait without active ingredient (TC2), and *Acalypha* leaf discs (TC3), in (a) application I and (b) application II.

Table 1. Number of *Atta sexdens rubropilosa* workers found dead after being supplied baits with conidia of *Beauveria bassiana*, *Paecilomyces lilacinus* and *Isaria fumosorosea* at the concentration 1×10^8 conidia/g – application I.

Treatment*	Hours after application**					
	24	48	72	96	120	144
Bait with AI	11.50 a	48.50 a	144.00 a	212.00 a	270.50 a	270.50 a
Neutral bait	0.50 a	0.00 b	1.00 b	1.00 b	1.00 b	1.00 b
Leaves	0.50 a	0.50 b	0.50 b	1.5 b	1.50 b	6.50 b
BBOT11	0.00 a	0.00 b	0.00 b	0.00 b	0.50 b	3.00 b
BBOT12	3.00 a	4.00 a	5.50 ab	10.50 b	6.50 b	14.00 b
PBOT33	3.00 a	1.00 ab	1.50 ab	2.50 b	1.50 b	5.00 b
IBOT25	0.00 a	1.00 ab	1.00 b	1.00 b	0.50 b	3.00 b
p	0.061	0.033	0.035	0.029	0.035	0.033

* Bait with AI: with AI sulfluramid (synthetic insecticide); neutral bait: without AI; leaves: leaf discs of *Acalypha*; baits with fungal isolates: BBOT11 (*B. bassiana*), BBOT12 (*B. bassiana*), PBOT33 (*P. lilacinus*), and IBOT25 (*I. lilacinus*).

** Means followed vertically by the same letter do not differ among the treatments by Tukey's test at 5% probability.

Table 2. Number of *Atta sexdens rubropilosa* workers found dead after being supplied baits with conidia of *Beauveria bassiana*, *Paecilomyces lilacinus* and *Isaria fumosorosea* at the concentration 5×10^8 to 8×10^8 conidia/g – application II.

Treatment*	Hours after application**								
	24	48	72	96	120	144	168	336	672
Neutral bait	5.25 a	5.25 a	6.50 a	8.25 a	12.50 a	14.50 a	16.00 a	18.00 ab	71.00 ab
Leaves	2.50 a	3.25 a	4.75 a	6.75 a	8.75 a	9.75 a	10.00 a	15.00 b	24.50 b
BBOT11	1.50 a	4.50 a	3.25 a	5.75 a	7.25 a	9.25 a	10.00 a	14.50 b	45.50 ab
BBOT12	4.47 a	6.75 a	8.25 a	13.25 a	13.50 a	26.50 a	26.75 a	38.50 ab	125.25 ab
PBOT33	13.00 a	21.00 a	30.50 a	36.00 a	46.25 a	55.00 a	62.50 a	97.00 a	133.25 a
IBOT25	4.00 a	5.75 a	9.50 a	14.00 a	16.25 a	19.00 a	22.25 a	23.50 ab	103.25 ab
p	0.3297	0.2982	0.1777	0.1691	0.2111	0.1774	0.1321	0.0281	0.0214

* Neutral bait: without AI; leaves: leaf discs of *Acalypha*; baits with fungal isolates: BBOT11 (*B. bassiana*), BBOT12 (*B. bassiana*), PBOT33 (*P. lilacinus*), and IBOT25 (*I. lilacinus*).

** Means followed vertically by the same letter do not differ among the treatments by Tukey's test at 5% probability.

other times, intensely excluded (Currie & Stuart 2001). In fact, the most likely expectation is that the workers seek to evade and eliminate microfungi and other undesirable microorganisms (Bot *et al.* 2001; Currie & Stuart 2001); however, the reality is that this does not always occur (Lopez & Orduz 2003,

present study). A surprising case has already been observed in which most baits with abundant growth of some microfungi, including *Paecilomyces* sp., were carried extensively (Carlos *et al.* 2009).

It is concluded that, although the (dry) granulated baits with fungi of the present study failed to overcome the defenses of the colonies (Kermarrec *et al.* 1986; Machado *et al.* 1988; Hölldobler & Wilson 1990; Bot *et al.* 2001; Currie & Stuart 2001; Poulsen *et al.* 2002; Caldera *et al.* 2009), these isolates show potential as a control agent by virtue of all having presented optimum carrying and provoked deleterious effects. Thus, new studies can be performed on these fungi, in this formulation, especially with an increase in conidial concentration in the baits, in order to better evaluate the control potential for leaf-cutting ants.

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