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Leaf-Cutter Ants and Microbial Control

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Abstract

The attini tribe comprises fungusgrowing ants, such as the basal *Apterostigma* and other more specialized genera, including the higher attine and the ones that cut the fresh plant tissue (*Atta* and *Acromyrmex*), maintaining an obligatory mutualistic relation with the fungus *Leucoagaricus gongylophorus*, which serves as a food source for the ants. Leaf-cutter ants are considered agriculture pests and populate the soil, a rich environment, especially due to the presence of several microorganisms. Some of these microorganisms are natural enemies that may cause epizootics (quickly spreading opportunistic diseases). Such defence strategies include polyethism, that is, division of labor among the individuals. The older ants take on the responsibility of foraging, as their integument is harder and heavily sclerotized, serving as a protective barrier against pathogens (including bacteria and antagonistic fungi). The younger ants, whose metapleural glands synthesize important secretions to eliminate and control microorganisms that could attack the colony fungus garden and the immature (larvae and pupae), remain inside the colony cultivating symbiont fungi. The sum of the survival strategies of ants in general, including social immunity and nest-cleaning behavior, represents a barrier for the application of biological control programs, mainly microbial ones.

Keywords: epizootics, entomopathogenic, fungi

1. Origin

Leaf-cutter ants, classified into more than 250 species within 17 genera, are found exclusively in the American continent [1, 2]. The basal genera *Cyphomyrmex*, *Mycetophylax*, *Mycocepurus*,

Myrmicocrypta, *Apterostigma*, *Mycetosoritis* and *Mycetarotes* use dead vegetable matter and insect feces and carcasses as substrate for the fungi. The genus *Pseudoatta* comprises parasite species and do not have a worker caste. The genera *Trachymyrmex*, *Sericomyrmex*, *Attaichnus*, *Kalathomyrmex*, *Paramyrcetophylax*, *Acromyrmex* (quenquens) and *Atta* (sauvas) belong to the superior attini group.

Based on the nidification habits of these species, the lowland arid environments with open vegetation in South America are suggested to be the centers of diversification [3, 4]. However, some studies have reported that this diversification occurred in the wet environment of the Amazon Basin [5, 6]. Recent molecular studies on attini ants point to dry environments as a decisive factor in the symbiont fungus domestication [7].

2. Mutualistic interactions

Attini ants have maintained a mutualistic relationship with basidiomycete fungi, in an obligatory association for around 50 million years [8]. The interaction with the fungus is not restricted to feeding, once symbiotic associations between larvae and fungi in basal attini have been reported in the literature [9]. Based on morphometric analyses, *Sericomyrmex* and *Trachymyrmex* together form a distinct group from the other genera. The transition from the ancestral agricultural system toward the derived leaf-cutting habit is followed by remarkable changes in nest size and architecture, colony size and worker size and polymorphism. Considering *Sericomyrmex* and *Trachymyrmex* as possessing transitional habits, differently from species that cultivate fungus by using mostly non-plant items (insect feces and carcasses) as well as from typical leaf-cutters *Atta* and *Acromyrmex*. Studies detect correlations of nest traits with worker number and size of the fungus garden in the less conspicuous attini [10].

3. Agronomic importance

The genera *Atta* and *Acromyrmex* play an important role in protecting the soil structure by recycling organic matter; however, these ants have been reported to damage the aerial parts of cropped plants in a short period of time [11]. Several strategies have been developed to control these insects [12]. In general, to be efficient against leaf-cutter ants, toxic baits must contain active ingredients with retarded action [13]. In addition, the insecticide must present specific characteristics, such as being lethal in low concentrations, not killing the ants immediately when applied in high concentrations, being absorbed through ingestion and having slow action, being easily distributed throughout the colony, being highly degradable to avoid environmental impact and not having a repellent or deterrent action on the ants [14]. Thus, the search for new alternatives to control leaf-cutter ants is ongoing, and the use of plant extracts and microbial control has been proven to be promising strategies.

4. Biology and behavior

The colonies of *Atta* ants are founded by the queens, that visit the colony fungus gardens before the nuptial flight and place a small piece of fungal mycelium (*Leucocoprinus*) in their infrabuccal cavities [15]. After this, the queen burrows into the ground [16], lays the eggs and takes care of the brood until the first workers eclose, take over the fungus culture activities and help in the construction and expansion of the chambers, more specifically the garbage and symbiont ones [11].

Ants are holometabolous insects, that is, their life cycle includes three stages: larva, pupa and adult. They are organized into castes, which perform different functions, including the maintenance of the colony and brood care and feeding. The males and queens are part of the reproductive castes, ensuring the survival of the species.

Leaf-cutting ants are prevalent herbivores and dominant invertebrates in tropical forests: the volume of the soil occupied by a single 6-year-old nest of *Atta sexdens* weighs approximately 40,000 kg, and a colony with such dimensions can collect more than 5892 kg of leaves [17]. Therefore, *Atta* ants are considered important ecosystem engineers once they modify the soil composition and create regions with high concentrations of organic matter, causing the development of more demanding vegetal species in terms of nutrition and altering the flora composition [18]. These effects may remain up to 15 years after the death of the colony of nest abandonment [19].

The colonies present a broad behavioral repertoire, varying according to the morphology (polymorphism) and age (polyethism) of the workers [20–22]. The foraging behavior consists of cutting and transporting the vegetal substrate to the interior of the colony, where it is processed and incorporated in the symbiont fungus garden [5, 17]. It is the most intense and energy-demanding activity performed in the colony, requiring approximately 90% of the workers [23].

5. Microbial control

5.1. Entomopathogenic fungi

Entomopathogenic fungi (EF) have been proven a promising strategy to control leaf-cutter ants through microorganisms. These fungi present two phases in their biological cycle: anamorphic (vegetative) and reproductive, when they produce conidia, and, according to Sung et al. [24], they belong to the phylum Ascomicota and class Hypocreales (**Table 1**).

Considered one of the most efficient pathogens in microbial control, entomopathogenic fungi need a host to spread in the environment, and the literature has reported that they attach to the cuticle of pest insects through physical and chemical processes, producing chitinolytic enzymes and developing a penetration clamp from the apical region of the hyphae [25, 26]. When the host insect is debilitated (low immunity level), some opportunistic fungi can act as saprophytes, accelerating the insect's death process [27]. Most fungi penetrate the host through the integument [28, 29]. According to Quiroz [30], who

	Family	
	Clavicipitaceae	Cordycipitaceae
Teleomorphs	<i>Hypocrella</i> , <i>Metacordyceps</i> <i>Regiocrella</i> , <i>Torrubiella</i>	<i>Cordyceps</i> s.str., <i>Torrubiella</i>
Anamorphs	<i>Aschersoni</i> , <i>Metarhizium</i> ,	<i>Beauveria</i> , <i>Isaria</i> , <i>Lecanicillium</i>

Table 1. Some fungi.

studied *A. mexicana* ants, entomopathogenic fungi are one of the most important mortality agents for the queens. The author identified the following species: *Aspergillus parasiticus*, *Paecilomyces farinosus*, *Beauveria bassiana* and *Metarhizium anisopliae*. In Brazil, most studies have used *B. bassiana* and *M. anisopliae*. Alves and Sosa-Gomez [31] reported these fungi virulence in worker and reproductive castes of *Atta sexdens rubropilosa*. In forests of *Eucalyptus grandis*, promising results were obtained using *B. bassiana*, in baits to control *Acromyrmex* spp [32].

Studies by Kermarrec et al. [33], which lasted approximately 10 years, used several entomopathogenic fungi, applied on *Acromyrmex octospinosus*, popularly known as ‘quenquem’, and they showed that this insect is able to identify and isolate the pathogen, leading the authors to the conclusion that the social surveillance/immunity and altruism of the workers would be essential for microbial control. Fungi as *B. bassiana*, *M. anisopliae* and *Trichoderma viridae* were proven inefficient when used in the control of *Acromyrmex heyeri* ants [34].

Machado et al. [35] reported that ants of the genus *Acromyrmex* would present systems, probably olfactive, with the function of recognizing entomopathogenic fungi. Such functions would be the result of selective pressure that occurred during the evolution of this social insect, which nests on pathogen-abundant soils. This would be accompanied by behaviors related to defense strategies aimed at reducing the dissemination and the effects of entomopathogenic fungi on the colony [36].

In this sense, it is known that the ant antennae “analyze” different types of material, allowing the recognition of pathogenic elements that could harm the colony [37]. Kermarrec et al. [33] observed that the sensitivity of the antennae can be demonstrated by the fact that attractive baits containing entomopathogenic fungi and spores are not cut but placed away from the colony area by *Ac. octospinosus*. Studies using electroantennography demonstrated that the olfactive function of *Ac. octospinosus* would be dependent on neuroreceptors located in the antennae [36]. Morphological analyses using scanning electron microscopy showed differentiation in these sensory structures in *Atta robusta* [38]. In a recent study, whose results serve as a model for ants in general, Slone et al. [39] analyzed *Harpegnathos saltator* and identified several subfamilies of olfactory receptors, demonstrating the complexity and sophistication of this sensory organ.

6. Other entomopathogenic microorganisms

In addition to entomopathogenic fungi, other organisms have been tested to control pest insects. Entomopathogenic nematodes (*Steinernema* and *Heterorhabditis*) were proven inefficient against urban ants [40] or caused only partial mortality to a more susceptible leaf-cutter species. These nematodes associate with entomopathogenic bacteria (*Xenorhabdus* and *Photorhabdus*) found in their digestive tract. Isolated and multiplied in aqueous media, *Acromyrmex subterraneus* were reported susceptible to the entomopathogenic bacterium *Photorhabdus temperate* K122, highly virulent when inoculated in worker abdomens [41]. Fungicide metabolites produced in the cultivation media (supernatant) have been used to control phytopathogenic fungi in field applications [42]; therefore, leaf-cutter ant control can be performed by targeting the symbiont fungus. The use of endophytic bacteria to overcome the morphological, mechanical and biochemical defenses of leaf-cutter ants aiming to contaminate the symbiont fungus [43], as well as the use of endophytic fungi [44], has been regarded as promising research lines; however, the assumptions are still hypothetical.

It is important to emphasize that the associations of the bacteria *Pseudonocardia*, *Streptomyces* and *Burkholderia* with the integument of leaf-cutter ants would represent a barrier for the microbial control success, once these bacteria secrete compounds to defend the host, occupying the niche where the pathogenic agent would settle [45, 46].

7. Use of synthetic chemicals

Once toxic substances are capable of overcoming insect immunity barriers (individually and collectively) [47, 48], the development research on strategies to impair the colony organization through immunosuppression, weakening the humoral system of the ants, is fundamental. Such strategies often include the use of chemical substances [49]. In this sense, subdoses of insecticides would aid the biological control; however, this would lead us to the use of granular baits containing a low concentration of the active ingredient. Nevertheless, the bait itself is highly specific to the target insect and, consequently, environmentally friendly, in contrast with other insecticides available in the market, and the demand for efficient and sustainable products is on the increase, considering the stricter requirements of regulatory agencies [50].

8. Capability to neutralize pathogens through gland secretions

Leaf-cutter microbial control has been subject to the same criticism directed toward plant extracts, that is, considering that the ants evolve in an environment where toxic plants and pathogenic microorganisms abound, probably the concentration is not the only issue to be taken into consideration for the success of these control methods [51–53]. The virulence

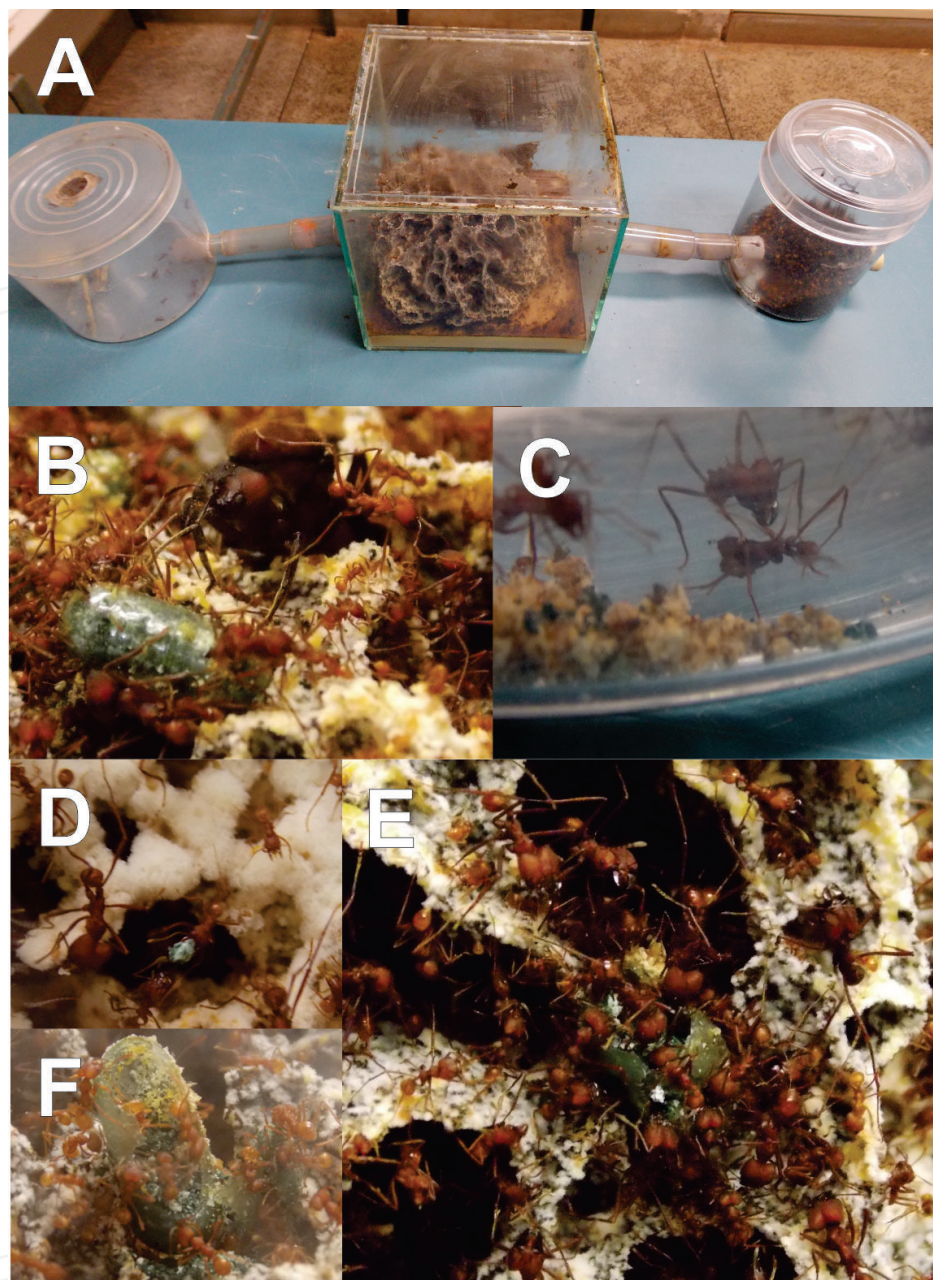


Figure 1. Previews with parted gelatin capsules containing *Trichoderma asperellus*: (A) colony kept in laboratory, (B) queen next to the capsule containing *T. asperellus*, (C) worker disposing the antagonist fungus in the waste chamber, (D) workers manipulating the fungus *T. asperellus*, (E and F) workers incorporating the capsule and content. (Source: Author).

of entomopathogenic fungi has been intensely investigated, especially the length of time needed to cause the pest population mortality; however, further investigation is needed on the forms of application and compatibility with adjuvants. Moreover, microbial control faces several natural barriers, including the capability of the ants to inhibit the germination of the conidia through secretions produced by their salivary glands (4-methyl 3-heptanone) and through their fecal fluid (chitinolytic enzymes) as well [54]. According to Pagnocca [55], in order to prevent the contamination of the symbiont fungus garden,

the ants lick the foraged substrate surface, keeping microorganisms in the infrabuccal cavity to dispose them in the waste chamber. Thus, the infrabuccal cavity would function as a filter, preventing the entrance of solid particles into the worker body and in the nest as well [56, 57].

The metapleural glands represent another defense mechanism, secreting several substances, such as phenylacetic acid; 3-hydroxydecanoic acid; indoleacetic acid; and skatole [58], which produce acid secretion that inhibits the germination of some entomopathogenic fungi, protecting the cuticle against microbial infections [57]. Fernandez-Marin et al. [59] identified a higher level of cleanliness by the gland secretion in workers inoculated with *Paecilomyces lilacinus* conidia. This chemical strategy developed by the ants to protect the fungi present in the environment is maximized by the grooming habit, either individual or collective, definitely removing the pathogenic microorganism from the host's body, preventing adhesion and, consequently, the infection onset [60].

However, some authors reckon that the pathogen is transmitted to part of the population during grooming, possibly contaminating larvae (immature) and ultimately the queen [61, 62] (Figure 1).

9. Perspectives

Pest control must be thought of from a preventive point of view and, in the case of leaf-cutter ants, in the initial phase of colony establishment. The scientific scenario today allows a more thorough and accurate research [34].

- The use of advanced analysis tools, such as scanning, light and confocal microscopes to investigate what happens in the moment when the insect is infected by the fungus [63].
- Cutting-edge technology software to monitor the agronomic environment and the emergence of entomological radars [64] can be incorporated as control strategies, indicating the best moment for the technique application (nuptial flight or "revoada").
- Drones have been used in some sampling techniques for leaf-cutter ants [65].
- The use of microbial control agents in different formulations (conidia, blastospores or microscleroids) synergically applied along with adjuvants is a tendency in microbial control [66].

10. Final consideration

Overall, microbial control has been proven efficient to control some pest insects (*Cosmopolite sordidus*, *Mahanarva fimbriolata*, *Diaphorina citri*, among others), except the ones presenting social behavior [67]. Leaf-cutter ants are endowed with an accurate system to identify and fight microorganisms (antennae) and secrete several antimicrobial compounds through

different glands located throughout their bodies [68, 69]. In addition, the grooming behavior constantly performed by the workers makes microbial control almost impossible with the use of the currently available tools.

Various isolates of *Escovopsis* sp. were analyzed in order to understand whether the antagonism of these fungi involves natural products, as suggested by the genome filled with genes encoding mycotoxins and fungal cell wall degrading-enzymes [71], the greater knowledge of pathogens such as *Escovopsis* sp. and *Trichoderma* sp. can be a way to found potential control agent for the control of leafcutting ants [72], reported in experiments, including those of systematic fungal analyzes of the symbiotic fungal garden where antagonistic fungi manifest aggressive development in the presence of stresses in the colonies, even those maintained for a long period in the laboratory [73]. In the case of the antagonistic fungi is the possibility of a joint action between microorganisms for control. However, although there is potential in the laboratory, it will not necessarily be promising in the field, due to the necessity of successive applications and the longer time in relation to the chemical, which is already long (30 days), to obtain colony death [70].

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