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Use of alternatives to PFOS, its salts and PFOSF for the control of leaf-cutting ants *Atta* and *Acromyrmex*

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ABSTRACT

Several biological, chemical, cultural and mechanical methods have been studied for the control of leaf-cutting ants due to their economic importance in forestry, agriculture and pastures. Applied biological methods such as manipulating predators, parasitoids and microorganisms; conservative control, non-preferred plants (resistents), extracts of toxic plants or active ingredients of botanical origin and cultural methods; have been unsatisfactory with inconsistent results. With the development of synthetic insecticides, chemical methods have been effectively used to control these ants. Currently, the use of toxic bait with active ingredient with delayed action on a wide range of concentrations, is being employed and it's sufficient, viable and efficient. However,it is extremely time consuming and difficult to find new active ingredients that are viable and efficient because of the great limitations associated with finding the essential features desired of the active ingredient (the action by ingestion, odourlessness and non-repellant, delayed toxic action, lethality at low concentrations and paralyses of plant cutting activities in the first days after application). Chemical control with toxic baits is still the only method that is technologically available to control leaf-cutting ants with technical, economic and operational viability. Beyond efficiency, chemical control has great advantages over other methods such as low cost, high performance and low hazard to humans and the environment. Sulfluramid is among the active ingredients currently registered in Brazil; the only one that has all the characteristics necessary for proper functioning of toxic bait. Therefore, maintaining this active ingredient is essential; at the risk of a dangerous set back in the control of leaf-cutting ants such as pest population growth and huge losses to the Brazilian agribusiness, if sulfluramid production is discontinued. In the light of current knowledge, it is believed that the future in the control of leaf-cutting ants remains exclusively chemical and the commercial formulation is toxic bait, because of the limitations of other formulations.

INTRODUCTION

The leaf-cutting ants belong to the family Formicidae, subfamily Myrmicinae, tribe Attini known as *saúvas* (genus *Atta*) and "*quenquéns"* (genus *Acromyrmex*). They are cited as the main pest of forest plantations, agriculture and livestock, due to the damage they cause, as well as their wide occurrence in those areas. Concerns about damage by leaf-cutting ants have been around since the discovery of Brazil and in some States legal acts were created for the management of these pests (Mariconi, 1970). Since then, there have been various attempts to estimate the damage posed by the pests in various agro-ecosystems.

The damage caused by the *Atta sexdens rubropilosa* activity (Forel, 1908) at a density of 4 colonies/ha was estimated at 14% for eucalyptus plantations and 14.5% for pine production (Amante, 1967c).

Eucalyptus trees die after 3 consecutive defoliations caused by *saúvas* and the loss of Eucalyptus stumps may reach up to 30% in areas with 200 colonies of *quenquéns* per hectare on average (Mendes Filho, 1979).

Defoliation caused by leaf-cutting ants in *Pinus taeda* has shown that this species seedlings with 100% of defoliation at 30 days of age suffered losses of 13.3% in height and 20% in diameter in comparison to plants which were not attacked, one year after the defoliation (Reis Filho et al., 2011). Moreover, the complete defoliation of *Eucalyptus grandis* and *P. taeda* at one month of age caused mortality from 10 to 25% for each species, respectively. The attack of the *Acromyrmex* genus ants in *P. taeda* plants reduced the increase in diameter and height during the first two years of planting (Cantarelli et al., 2008).

Amante (1967a, b), using unknown methods, concluded that *Atta capiguara* consumes a tremendous amount of 255-639 kg of dry matter/colony/year of Gramineae, which can be converted into losses of 512 to 870 thousand head of cattle per year. These data were reanalyzed by Fowler et al. (1990), whose losses can be estimated between 30 and 150 kg of dry matter/colony/year. For *Atta vollenweideri,* which is also a grass-cutting ant, it consumes 90 to 250 kg/colony/year (Fowler et al., 1986). In general, *A. capiguara* does not reduce the number of cattle by more than 30% (Amante, 1967a, b).

The economic losses caused by grass-cutting ants beyond go beyond the grass consumption, and such as: loss of productive pasture surface, accidents with animals and farm machinery, weed proliferation, loss of soil fertility and land value, etc. (Fowler et al., 1986). An adult *Atta* nest causes a drop in productivity of 3.2 t/ha of sugar cane, corresponding to 5.3% of the productivity (Precetti et al., 1988).

In the agricultural commodity chains such as grains, fruits and others, big losses in agricultural productivity are added, since in these cultures the occurrence of leaf-cutting ants is very expressive. For all these cultures, it can be said that when the plants are young, losses can reach 100% (Forti and Boaretto, 1997).

The management of leaf-cutting ants is essential for Brazilian agribusiness, as these can cause great damage to agricultural and forest cultivations, bringing huge losses to the country.

Problems with leaf-cutting ants have led to the development of mechanical, cultural, biological and chemical control methods for the integrated management of this pest since the 50s. The previous control prevents damage to crops, reduces the amount of applied insecticide during the forthcoming years, can prevent the occurrence and re-infestation, reducing damage, and the environmental impact of a later combat.

Cultural control with "resistant plants" and applied biological, manipulating predators, parasitoids, and microorganisms have produced poor and inconsistent results without indication of technical, economic, and operational feasibility. There are basic studies under development at research centers and universities assessing biological products such as entomopathogenic fungi, as well as natural products such as plant extracts, for the control of leaf-cutting ants. The results, however, have been inconsistent, showing technical, economic and operational infeasibility (Forti and Boarettto, 1997; Moreira et al., 2004a).

With the development of synthetic insecticides, chemical methods have been effectively used to control *Atta* and *Acromyrmex* (Mariconi, 1970; Cherrett, 1986a; Camargo, 2007; Nagamoto et al., 2007). Chemical control is the only one which presents the available technology for practical use with leaf-cutting ants control. Toxic bait represent the only method that offers technical, economic and operational feasibility with the control of leaf-cutting ants, and consists of a mixture of attractive (usually orange pulp and vegetable oil) and an active ingredient (insecticide), in pellet form. They are highly efficient, presenting huge advantages over other methods, low cost, high performance, and low hazard to humans and the environment. The ants carry the bait to their nests (Lima et al., 2003; Teixeira and Santos, 2008). Baits can be used in nests of any size (Zanetti et al., 2002). Toxic baits of leaf-cutting ants slowly paralyze the cutting activity in a few days (Zanetti et al., 2004).

Sulfluramid, generally used as the active ingredient of toxic baits, presents full efficiency in the control of all species of leaf-cutting ants and grass-cutting ants, presenting technical feasibility, excellent cost/effectiveness, availability, and affordability, matching the

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Toxicological Class IV - lightly toxic. The forestry industry has been a pioneer in the replacement of dodecachlor-based baits by the sulfluramid-based ones (Zanuncio et al., 1992, 1993).

In order to meet the request made by Decision SC-6/7, item 5, letter C of UNEP/POPS/COP.6/33, adopted at the Conference of the Parties (COP. 6) held in May 2013, the authors designed this pilot project.

The project consubstantially searched available literature on all documents (scientific and technical publications) and information, in order to survey the possible alternatives to perfluoro octane sulfonyl (PFOS), its salts and perfluoro octane sulfonyl fluoride (PFOSF) for leaf-cutting ants control and carefully analyzed the feasibility of the presented alternatives, commenting on and discussing aspects, considering especially the criteria established in the Stockholm Convention, defined in the document General Guidance on Considerations Related to Alternatives and Substitutes for Listed Persistent Organic Pollutants and Candidate Chemicals-UNEP/POPS/ POPRC.5/10/Add.1, from the perspective of Integrated Pest Management (IPM).

To promote discussion by reviewing in pairs, the feasibility of using PFOS, its salts and PFOSF alternatives, within an approach of integrated pest management (IPM), for the submittance to the Secretariat of the Convention, considering aspects and criteria established in the Stockholm Convention, which are technical feasibility, effects to man and environment, cost/benefit, efficiency, availability and viability and defined in the document General Guidance on Considerations Related to Alternatives and Substitutes for Listed Persistent Organic Pollutants and Candidate Chemicals - UNEP/POPS/POPRC.5/10/Add.1 as well as in the Brazilian Pesticides Legislation.

NATURAL HISTORY OF LEAF-CUTTING ANTS

Currently, it is estimated that there are over 24,000 species of ants belong to 23 subfamilies (Bolton et al., 2005), with 13,954 already described (Agosti and Johnson, 2005). These insects constitute a monophyletic group whose main synapomorphies are:

- the presence of petiole and post-petiole,
- metapleural gland and;
- antennas divided into layers (Fernández and Palacio, 2003), which enables distinguishing them from any other hymenopteran (Fernandez, 2003).

They appeared in the Cretaceous period in between 115 and 135 million years ago (Brady and Schultz, 2006) and are distributed across the planet, except the poles (Hölldobler and Wilson, 1990; Ward et al., 2015).

Among the ants, there are the fungi-growing ants (Formicidae: Myrmicinae) which belong to the Attini tribe, which gathers ants with the ability to cultivate fungi and use them as food for their larvae (Silva et al., 2003a). It is estimated that the origin of the habit of cultivating fungi occurred about 50 million years ago, period during which the ancestor of the tribe changed from a hunting behavior to fungal cultivator (Mueller et al., 2001; Schultz and Brady, 2008).

Currently, the tribe Attini comprises 13 genera with about 230 described species (Brandão and Mayhé-Nunes, 2001; Schultz and Brady, 2008). The tribe is found in the Americas only, but covering a wide geographical area, from Northern Argentina to Southern United States. Within this range, the Attini ants occur in most diverse biomes, for example, the Amazon rainforest (Solomon et al., 2008) and in extreme environments such as the desert (Weber, 1972).

Among the Attini *Atta* and *Acromyrmex* stand out, which are the leaf-cutting ants. The leaf-cutting ants use as a substrate for cultivating fungi, which the feed on, as already mentioned: living tissues, often by cutting the most diverse plant species (Belt, 1874; Souza, 1965; Cherrett, 1968; Mariconi, 1970). Occasionally, Attini species of the genera *Sericomyrmex* and *Trachyrmymex*, may also use plant live tissue (Weber, 1972; Fernández-Marín et al., 2003). The leaf-cutting ants of genus the *Atta* are popularly known as *saúvas* and ones from the genus *Acromyrmex* are called *quenquéns*. In Brazil, they are renowned agricultural pests of planted forests and pasture (Cherrett, 1986a, b, c). The success of this group of insects is related both to social organization and the ant - plant - fungus interaction. They cut about 29 to 77% of the plants in natural environments (Cherrett, 1968; Forti, 1985a; Dejean et al., 1992; Garcia et al., 2003). They can cut parts of the plants, such as flowers and leaves. They are known by the complexity of their preferences, dependent in part, on the plant's physical characteristics (Reis Filho et al., 2007). Chemical and physical features of the plants influence the acceptance by the ants (Fowler and Stiles, 1980). Generally, leaf-cutting ants prefer the plant's tender parts (Reis Filho et al., 2007) and due to this, leaf-cutting ants oppose themselves against human activity such as large scale agriculture.

Atta and *Acromyrmex* genera are considered pests in several regions of Brazil and America (Cherrett, 1986 a, b, c; Robinson and Fowler, 1982; Hernandez et al., 1999; Varon et al., 2007). The economic losses caused by these ants reaches millions of dollars per year in Texas (Cameron and Riggs, 1985) and about US$ 130 million per year in the State of São Paulo, Brazil (Fowler et al., 1986). Although they are considered pests, ants of the genus *Atta* are important to the ecosystems in which they occur and play an important role in nutrient cycling by bringing organic matter to their nests (Hölldobler and Wilson, 1990; Moutinho et al., 2003; Silva et al., 2003b; Wirth et al., 2003; Sternberg et al., 2007; Sternberg et al., 2005).

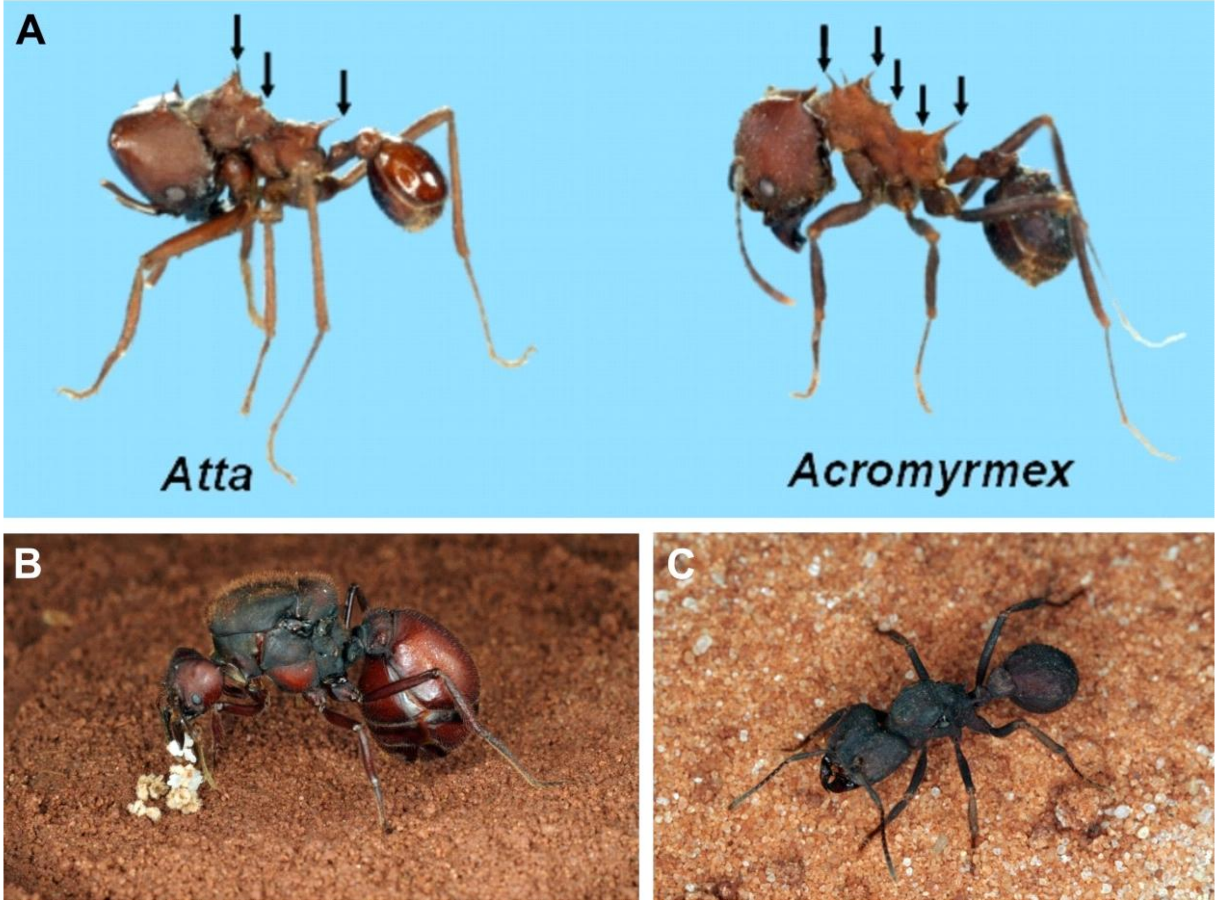

Figure 1. Differences between *Atta* and *Acromyrmex*: **A,** The largest *Atta* workers have three pairs of spines over the thorax while the *Acromyrmex ones* can have 4-5 pairs; **B,** *Atta* queen with initial fungal culture and eggs; **C,** *Acromyrmex* queen is much smaller in size than the *Atta* queen (Forti and Boaretto, 1997).

Geographical distribution of leaf - cutting ants

Leaf-cutting ants from *Atta* and *Acromyrmex* (Figure 1) are widely distributed. They occur throughout the American continent, from the Southern United States (Gonçalves, 1961; Weber, 1970) going through Central America (except for some islands of the Antilles) (Mariconi, 1970; Weber, 1972; Della Lucia et al., 1993) and proceeding through all countries of South America (except Chile) to the center of Argentina (Gonçalves, 1961; Weber, 1970; Farji-Brener and Ruggiero, 1994). The leaf-cutting ants species, distribution and relative pest status, are shown in Table 1, according to the literature (Fowler et al., 1990).

In Brazil, they are distributed throughout the national territory, with presence of nine species of *Atta* (Table 2) (Delabie, 1998) and 21 species of the genus *Acromyrmex* and 9 taxonomical subspecies. Some of these species are mentioned in Table 3 (Gonçalves, 1961, 1967; Fowler et al., 1986; Fowler, 1988; Della Lucia et al., 1993).

The five most important species of *Atta* (*saúvas*) in Brazil are: *Atta sexdens*, *Atta laevigata*, *Atta bisphaerica*, *Atta capiguarae,* and *Atta cephalotes* (Figure 2). Species of *A. sexdens* and *A. laevigata*, have wide distribution across the country (Forti and Boaretto, 1997). Other species occur in restrict regions, like *A. cephalotes*, with a distribution among the Amazon region and the Southern Bahia Atlantic Forest, *Atta opaciceps*, with occurrence in North-eastern Brazil, *A. bisphaerica* in São Paulo, Minas Gerais and Rio de Janeiro states, *A. goiana* in a narrow portion of Mato Grosso and Goiás, *Atta robusta* only in the State of Rio de Janeiro, and *Atta vollenweideri* in small portions of the States of Rio Grande do Sul and Mato Grosso do Sul (Forti and Boaretto, 1997). *A. capiguara,* in particular, increased its species distribution towards the State of Paraná, and a few areas in Mato Grosso, Mato Grosso do Sul, Goiás and Minas Gerais (Forti and Boaretto, 1997).

A. laevigata (*saúva-de-cabeça-de-vidro*) and *A. capiguara* (*saúva-parda*) are expanding species. *A.*

Table 1. Species of leaf-cutting ants, their distribution, and their relative pest status, as assessed by citations (Modified from Fowler et al., 1990).

Leaf-cutting species monocots (m)/dicots (d)	Plant cut (amplitude)	Distribution (*Brazil)	Relative pest status §
***Acromyrmex*(*Acromyrmex*)**			
ambiguous	d	*Pandemic	Strong
aspersus	d	*Pandemic	Dubious
coronatus	d	*Pandemic	Weak
crassispinus	d	*Pandemic	Strong
diasi	d	Endemic	Dubious
disciger	d	*Pandemic	Weak
*gallardoi**	d?	*Endemic	Dubious
hispidus	d	*Pandemic	Weak
hystrix	d	*Pandemic	Weak
laticeps	d	*Pandemic	Weak
lobicornis	d/m	*Pandemic	Strong
lundii	d	*Pandemic	Weak
niger	d	*Pandemic	Weak
nobilis	d	*Endemic	Dubious
octospinosus	d	*Pandemic	Strong
rugosus	d	*Pandemic	Strong
subterraneus	d	*Pandemic	Strong
***Acromyrmex* (*Moellerius*)**			
balzani	m	*Pandemic	Strong
fracticornis	m	*Pandemic	Strong
heyery	m	*Pandemic	Strong
landolti	m	*Pandemic	Strong
mesopotamicus	m?	Endemic	Dubious
pulvereus	m?	Endemic	Dubious
silvestrii	m	Pandemic?	Weak
striatus	m	*Pandemic	Strong
versicolor	d/m?	Endemic	Dubious
Atta			
bisphaerica	m	*Pandemic	Strong
capiguara	m	*Pandemic	Strong
cephalotes	d	*Pandemic	Strong
colombica	d	Pandemic	Weak
goiana	m	*Endemic	Dubious
insularis	d	Endemic	Weak
laevigata	d/m	*Pandemic	Strong
mexicana	d/m	Pandemic	Weak
opaciceps	d	*Endemic	Weak
robusta	d	*Endemic	Dubious
saltensis	d	Pandemic	Weak
sexdens	d	*Pandemic	Strong
texana	d	Pandemic	Moderate
vollenweideri	m	*Pandemic	Weak

laevigata occurs at the margins of the newly-open roads in the States of Rondônia, Acre and Amapá. Agricultural activity can also contribute to the species expansion. For instance, *A. capiguara* had no occurrence in Northwestern Paraná before 1975 and today they occur in areas close to the municipality of Paranavaí, PR. Until

Table 2. Species of the genus *Atta* in Brazil.

Scientific name	Vulgar name	Federative unit
Atta bisphaerica (Forel, 1908)	*Saúva-mata-pasto*	SP, MG, RJ, MT
Atta capiguara(Gonçalves, 1944)	*Saúva-parda*	PR, SP, MT, MG, GO,MS
Atta cephalotes (L., 1758)	*Saúva-da-mata*	AM, RO, RR, PA, AP, MA, PE, BA, AC, MT
Atta goiana(Gonçalves, 1942)	*Saúva*	GO, MT
Atta laevigata (F. Smith, 1858)	*Saúva-de-vidro*	RO, SP, AM, RR, PA, MA, CE, AC,PE, AL, BA, MG, RJ, MT, GO
Atta opaciceps (Borgmeier, 1939)	*Saúva-do-sertão-do-nordeste*	PI, CE, RN, PB, PE, SE, BA,
Atta robusta (Borgmeier, 1939)	*Saúva-preta*	RJ
Atta sexdens piriventris (Santschi, 1919)	*Saúva-limão-sulina*	SP, PR, SC, RS
Atta sexdens rubropilosa (Forel, 1908)	*Saúva-limão*	SP, MG, ES, RJ, MT, GO, PR
Atta sexdens sexdens (L., 1758)	*Formiga-da-mandioca*	AM, AC, RO, RR, PA, AP, MT, GO, MA, PI, CE, RN, PB, PE, AL, SE, BA, MG
Atta silvai (Gonçalves, 1982)	*Saúva*	BA
Atta vollenweideri (Forel, 1939)	*Saúva*	RS, MT

Table 3. Some species of the genus *Acromyrmex* in Brazil.

Scientific name	Vulgar name	Federative unit
Acromyrmex ambiguus Emery, 1887	*Quenquém-preto-brilhante*	SP, BA, RS
Acromyrmex aspersus (F. Smith, 1858)	*Quenquém-rajada*	SC, RS, MG, SP, BA, ES, RJ, MT, PR,
Acromyrmex coronatus (Fabricius, 1804)	*Quenquém-de-árvore*	SP, PA, CE, BA, ES, MG, RJ, MT, GO, SC, MS
Acromyrmex crassispinus Forel, 1909	*Quenquém-de-cisco*	SP, RJ, PR, SC, RS, MG, DF
Acromyrmex diasi Gonçalves, 1983	*Quenquém*	DF, SP
Acromyrmex disciger Mayr, 1887	*Quenquém-mirim e formiga-carregadeira*	SP, RJ, MG, PR, SC
Acromyrmex heyeri Forel, 1899	*Formiga-de-monte-vermelha*	PR, SC, RS, SP
Acromyrmex hispidus fallax Santschi, 1925	*Formiga-mineira*	PR, SC, SP, RS
Acromyrmex landolti balzani Emery, 1890	*Formiga-rapa*	SP, MG, SC, GO, MS
Acromyrmex rugosus rugosus(F. Smith, 1858)	*Formiga-mulatinha*	MS, RS, SP, PA, MA, PI, CE, RN, PB, PE, SE, BA, MG, MT, GO
Acromyrmex subterraneus subterraneus Forel, 1893	*Caiapó*	SP, AM, CE, RN, MG, RJ, MT, PR, SC, RS

1975, in Northwestern Paraná, there was a coffee cultivation, and from this date the coffee fields were replaced by pasture (Forti and Boaretto, 1997).

Hence, there are species which cut preferably dicotyledonous plants with high pest status, such as *A. sexdens, A. cephalotes*, while others, for instance the *A. capiguara* and *A. bisphaerica* prefer grasses (Mariconi, 1970; Forti, 1985a; Fowler et al., 1986; Nagamoto et al., 2009), causing significant losses in pastures (Amante, 1967a, b, c) and in corn and sugarcane cultivations (Amante, 1972; Precetti et al., 1988). The *A. laevigata* species may cut both dicotyledonous plants and grass, and it is quite interesting because of that biological feature, and constitutes a species of great economical importance due to this biological plasticity (Forti and Boaretto, 1997). In addition to the *Atta silvai,* which was also cited as a leaf-cutting ant of dicotyledonous and grass, this species was defined by Delabie (1998) as being a synonym for *A. laevigata.*

The species from the *Acromyrmex* genus occupy an important position in pasture and reforesting areas, as they can, sometimes, overcome *saúvas* in abundance (Forti and Boaretto, 1997; Forti, 1988; Forti et al., 1987). The occurrence of those ants (*quenquéns*) goes from California (USA) to Patagonia. Usually, we find individual variations in the head and thorax spine proportion in species within the same colony. The taxonomical characterization is carried out based on the proportion

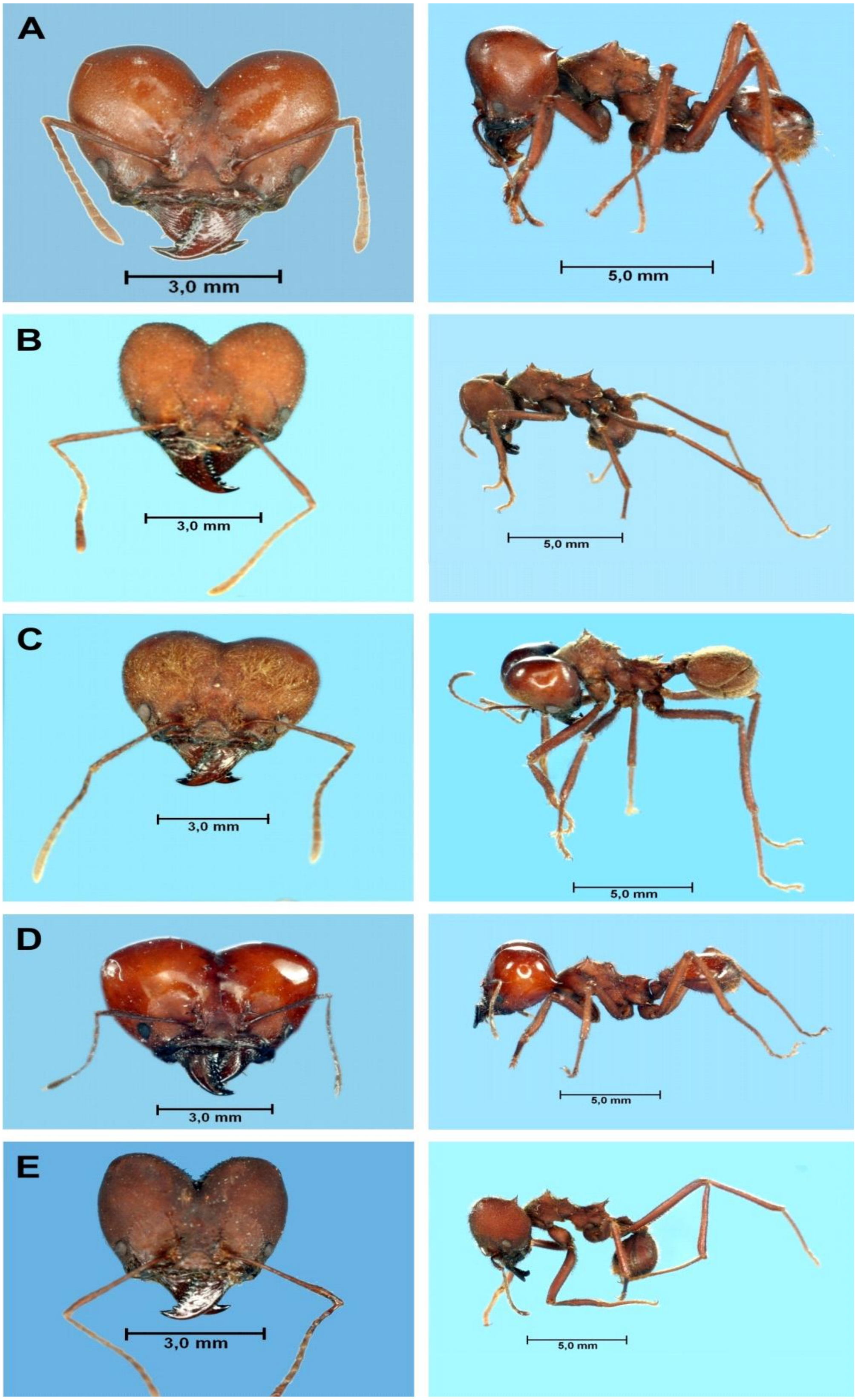

Figure 2. The five most important species of *Atta* (*saúvas*) in Brazil: **A,** *A. bisphaerica*; **B,** *A. sexdens*; **C,** *A. cephalotes*; **D,** *A. laevigata;* **E,** *A. capiguara*.

and shape of the thorax and spines, type of sculpturing, and the arrangement of tubers in the gaster.

Generally, it can be said that Brazil's Southern States gather the greater number of *Acromyrmex* (*quenquéns*) species (Table 3). A few species of *quenquéns*, like the grass-cutting ants occur in small portions of the Brazilian territory, for example, the *Acromyrmex heyri, Acromyrmex lobicornis and Acromyrmex striatus,* which occur only in the Southern States of the Country. The *Acromyrmex landolti,* also a grass-cutting ants, occurs in the States of Amapá, Roraima, Pará and Amazonas (Table 3). Only in the State of São Paulo there occurs 11 species of *Acromyrmex,* and in Paraná, 9 species (Forti and Boaretto, 1997). Some species present wide occurrence in large land areas, like the *Acromyrmex balzani, Acromyrmex rugosus, Acromyrmex subterraneus* and *Acromyrmex coronatus* (Table 3) (Forti and Boaretto, 1997).

The *Acromyrmex* genus distribution and systems still have innumerous issues to be clarified, needing urgent update. About the geographical distribution, we can notice cases as the *A. lobicornis,* which occurs in Rio Grande do Sul and Bahia, and, inexplicably, does not occur in Santa Catarina, Paraná, São Paulo, Minas Gerais, Rio de Janeiro, and Espírito Santo. One can formulate two hypotheses to explain that “vacuum” in distribution: either there was no sufficient survey to realize the presence of these species in the States where it does not occur, or the species was incorrectly identified. Regarding the systems, it is possible to state that there are generalizes mistakes in the group (Forti and Boaretto, 1997).

In the *A. subterraneus* case: That species has 3 subspecies, which can hardly be distinguished using identification keys, without taking into consideration the morphological variations within the same subspecies (Forti and Boaretto, 1997).

The survey for the species occurrence update in different regions is extremely important. A survey was carried out in the State of São Paulo, elevating the number of species from 8 to 11 (Forti and Boaretto, 1997).

However, the Southern States of our Country really present the greatest number of *quenquéns* species in comparison with other States. In São Paulo, there occur 11 species, in Paraná 9, in Santa Catarina and Rio Grande do Sul together there are 10 (Table 3) (Forti and Boaretto, 1997).

Among the *Acromyrmex* there are also species, which are preferably grass-cutting ants and other which are leaf-cutting ants, as well as the ones which cut both plant groups (Table 1). Depending on the region of the country and on the cultivated plant, some species of *Acromyrmex* can cause severe damage to the plant cultivation, and can even limit its cultivation. As an example, *Acromyrmex crassipinus*, *A. subterraneus*, *A. octopinonis*, *A. rugosus,* and *A. lobicornis* can be found in large areas planted with *Eucalyptus* and constitute severe pests, limiting the production. Among the grass-cutting ants, *A. landolti*, *A. striatus, A. labicornis,* and *A. heyry* are the pests of greater importance. As mentioned, the *Acromyrmex* can cause such severe economic damages just as the *Atta*, but there are only few studies about the *Acromyrmex.* Also, they are hardly mentioned in literature (Forti and Boaretto, 1997).

Biology of leaf-cutting ants

Nest foundation

Adult colonies of *Atta* and *Acromyrmex* produce winged individuals, male and female, which depart from their original colony to found new colonies, perpetuating the species. The dispersion of the sexual individuals occurs shortly after the beginning of the rainy and hot season in different regions of the country. During the months from September to December in Central Brazil, from September to April in the North, from December to April in the North-east, and from June to December in the South (Forti and Boaretto, 1997). The winged females of *saúvas* are called popularly by “*içás”* or “*tanajuras”* and males are called by “*bitus”* (Forti and Boaretto, 1997).

The mating flight occurs when winged females and males come in flight and copulate in the air. Later, the male dies and the female, after being fertilized by 3-8 males, lands and sheds her wings, starting to excavate the new nest, which lasts 6-10 h (Autuori, 1942), with the construction of a tunnel with a depth ranging from 8 to 25 cm and an hemispherical initial chamber (Camargo et al., 2011; Autuori, 1945).

After the excavation, the channel is sealed with soil excavated by the queen herself. Before the nuptial flight, the queen spit out a small portion of the original colony fungus garden, and lodges it inside her infrabucal cavity. After the nest construction, the queen encloses herself. After 48 h, it regurgitates the fungus pellet, which, in turn, is cultivated with secretions and feces until the first workers appear, in a period from 80 to 100 days (Figure 3). Soon thereafter, the workers remove the soil that is sealing off the narrow tunnel and goes outside to cut the plants beginning to bring leaves into the nest for the symbiotic fungus (Forti and Boaretto, 1997).

During this time, the queen lays feeding eggs (called trophic eggs) which will serve for nutrition of larvae and small eggs that originate the workers, both gardeners and foragers. These workers tend the fungus and larvae, promote both mutual and queen grooming, host the larvae and the queen, then carry their brood inside the small chamber (Forti and Boaretto, 1997).

After 5 days of founding the initial nest by the queen, the first eggs appear, after 25 days the first larvae.

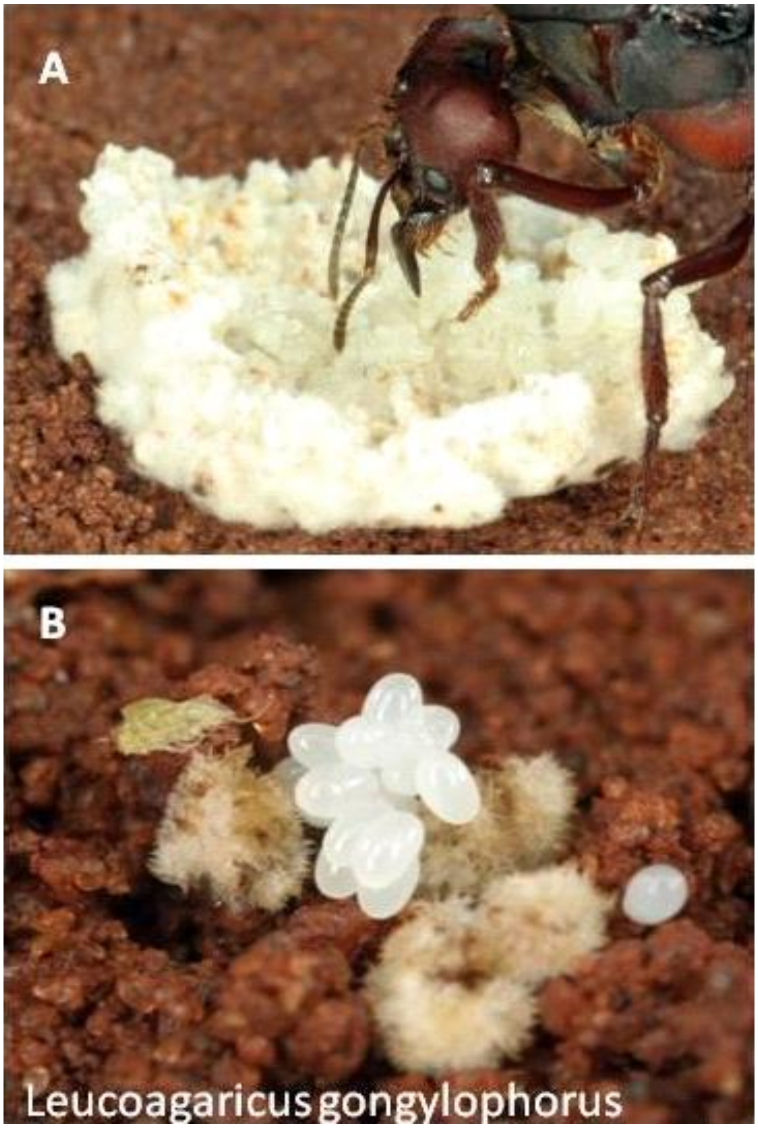

Figure 3. A, *A. laevigata* queen care for the offspring in fungus garden; **B,** Symbiotic fungus with the queen's first oviposition.
Photo: Luiz C. Forti.

Subsequently, after 22 days the first pupae appear (Figure 4), and then the first adults (10 days), totaling from 62 to 75 days (Autuori, 1942; Camargo et al., 2011) (Figure 4). In *A. capiguara*, in the sampled periods, the eggs were most prevalent at 1 to 18 days, larvae at 21–38 days, pupae at 39–55 and adults at 58–67 days (Pereira-Da-Silva, 1979).

As the colony grows, the queen lays the eggs that are going to originate workers of all sizes. The second entrance hole of the nest is opened after about 421 days. It is observed that after the opening of the second, the colony expands rapidly (Forti and Boaretto, 1997).

Sometimes, the mortality rate in colonies mortality reaches 100%, but most times that do not happens. For the *A. sexdens rubropilosa*, mortality reaches 99.5%. Hence, 0.05% of *içás* produced by an *A. sexdens rubropilosa* colony remain alive, originating into adult colonies (Autuori, 1942).

Social organization

In *Atta* spp. nests, the colonies reach maturity in about 3 years (Autuori, 1942). In this phase, the nest size, population, and fungal biomass grow. This growth is the result of the increased number of workers with greater

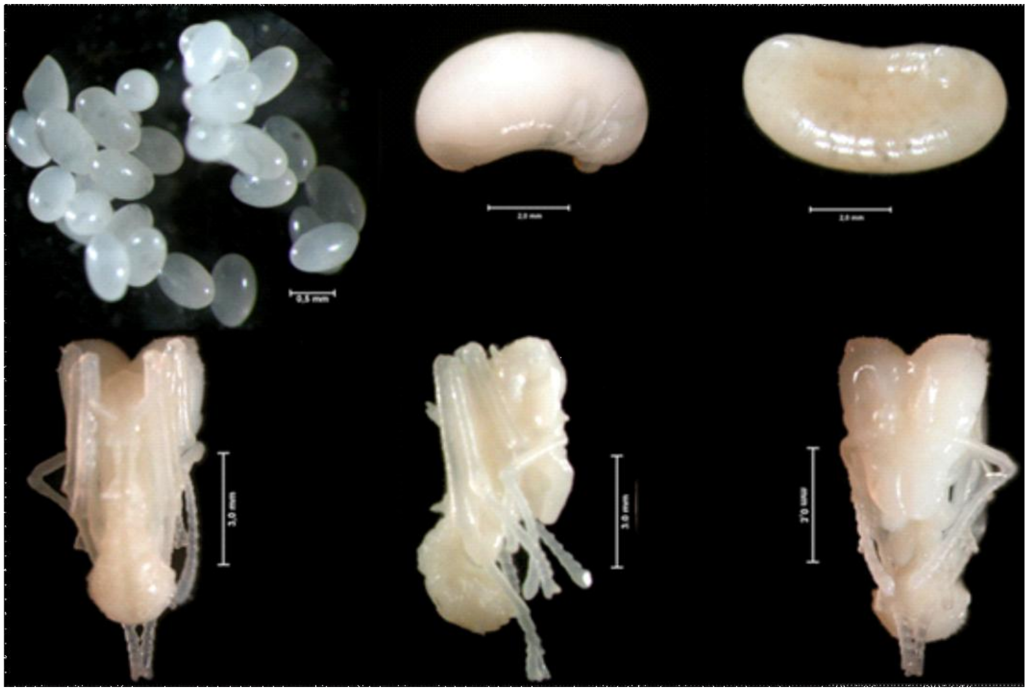

Figure 4. Immatures of *A. sexdens rubropilosa*: egg, larva and pupa, accounted for during the initial phase of colony development.
Photo: Ricardo T. Fujihara.

diversification of their size classes, as well as specialty and distribution of their tasks inside the nest. The polyethism can be divided by age, when the individual present different behaviors during their live, and by caste, when they present morphological and physiological differences (Hölldobler and Wilson, 1990). Polyethism allowed for the differentiation of roles in society, through the specialization of individuals, promo-ting an optimal mix of specialists who perform tasks more efficiently than equal groups made up of generalists (Wilson, 1968).

Leaf-cutting ants are polymorphic. *Atta* (Fabricius, 1804) and *Acromyrmex* (Mayr, 1865), in which the larger workers are specialized for the colony defense (called soldier), average workers forage the vegetable species (called forager)and minor workers are specialized to work with the fungus garden (called generalist and gardener) (Wilson, 1980a,b). The aloethism intensifies when workers are involved in plant foraging and processing to obtain and generate food resources. Such process involves a number of specific tasks performed according to the body size of the workers (Wilson, 1980a,b, 1983). A colony of leaf-cutting ants has reproducers and workers castes. Some individuals are permanent in the colony, as the queen who founded it, and the workers, which are sterile females. The colony's temporary individuals are the winged sexual forms as the females (*içás, tanajuras*) and males (*bitús*) (Forti and Boaretto, 1997).

The queen (*içá* or *tanajura*) is the female who produces eggs in the colony. Throughout her life, the queenwill lay eggs for the production of sterile workers (gardeners, scouts and/or foragers and soldiers) and only during a time of the year, will lay eggs for the production of new queens and males, which at the beginning of the rainy warm season of each year will leave to found new colonies. The queens are fewer than males (Forti and Boaretto, 1997).

The workers constitute the largest population of the leaf-cutting ants nests, and they are responsible for feeding the colony, they are wingless and sterile. According to their size, we can divide the workers into 4 categories: soldiers, foragers, generalists and gardeners.

Large workers are called "soldiers" and are related to the defense of the colony. Average workers, called "foragers", as their name suggests, they cut and carry the leaf fragments to the colony. Smaller workers, called "generalists and gardeners", cut the leaf fragments in pieces and placed them the fungal culture ("fungus sponge") and, inoculate the fungi mycelia that they culture in those small pieces of leaves (Forti and Boaretto, 1997).

In *A. sexdens rubropilosa,* after the leaf foraging, the generalist workers (headwidth capsule between 1.3 to 1.6 mm) lick and tear leaves into pieces 1-2 mm in diameter. Then, smaller workers (head width from 0.8 to 1.2 mm) chew along the edges until the reduction of leaves into a wet pulp, and then they insert these fragments in the fungus garden (Andrade et al., 2002). Finally, hyphae tufts are transplanted to the fragments surfaces (Weber, 1956).

The genus *Acromyrmex* presents a moderate polymorphism, with a distinct division of the tasks between the physical and age castes (Camargo, 2007). The cultivation of the symbiotic fungi occurs similarly to the *A. sexdens rubropilosa*, according to ethological sequence of Figures 5 and 6.

Atta and Acromyrmex nesting

The characterization of these ants' nests is also a difficulty in using new control techniques. These underground nests present many interconnected chambers, and connected with the surface through long galleries, which may impair the wide dissemination of toxic products inside the colonies, as well as determining the proper dosages (Marinho et al., 2006).

Most ant nests are located underground, however a few species build them in rotten logs, parts of plants and under leaves (Weber, 1945, 1972; Pereira-Da-Silva et al., 1981; Mayhé-Nunes, 1995; Hölldobler and Wilson, 2008).

The external architecture of the Attini nests has similarities and differences between genera (Weber, 1972) (Figures 7 and 8). The external appearance is made up by the soil removed from the excavation of the chambers and tunnels, carried out by the workers during the nest construction, by plant residues such as straw, leaves and/or twigs and in some more primitive genera only the nest entrance hole is found. Then the nest plays an important role not only for the ants, but also for other social insects, to protect their brood and queen of natural enemies and other hazards, as well as to control the food supply distribution among the descendants and microclimatic control (Amante, 1960; Sudd, 1970, 1982; Brian, 1983). The microclimatic variables regulated by the colonies are: temperature, humidity and gas concentration (Seeley and Heinrich, 1981; Hölldobler and Wilson, 1990; Bollazzi and Roces, 2002; Bollazzi et al., 2012; Roces and Kleineidam, 2000).

Forti et al. (2011) reported that an *Atta* genus nest consists of an externally visible part, the mound or hill of loose dirt, which is characterized by its large amount of holes that lead to the inside, which in turn is formed of tunnels of various diameters and shapes that allow the transit of the ants and interconnect the holes with the chambers.

The loose soil mound of the *Atta* nests constitutes one of the most important aspects for the control, because it is used as the basis for computing the chemical dosage (Forti and Boaretto, 1997; Forti et al., 2011).

The shape of the mound is one of the features originally observed in the field for species identification, but there may be small or may have large variations, depending on the species. In spite of the peculiarities of each species with respect to the loose soil mound shape and to the location of nests, it has been observed, in practice, that there is great variation in that deposition of soil by the ants and in the choice of location for nesting, according to different regions of the country, and can incur serious errors when only this factor is observed to identify species in the field (Forti and Boaretto, 1997; Forti et al., 2011).

Nests of *A. laevigata* are built in either sunny or shady places (Mariconi, 1970; Pereira-Da-Silva, 1975; Moreira et al., 2004a). Instead, the nests of *A. sexdens* are built only in shady locations (Jacoby, 1935; Stahel and Geijskes, 1939; Mariconi, 1970; Pretto, 1996). *A. bisphaerica* and *A. capiguara* build their nests in areas with high insolation (Mariconi, 1970; Moreira et al., 2004b; Andrade et al., 2005). *A. opaciceps* can build their nests in both open and shaded areas, this is species is the most tolerant to low humidity and high temperatures, in a way they can they build their nests even in the regions of Caatingabiome. The deposition of soil over the nests is quite irregular, similar to the *A. sexdens',* but can sometimes be regular, similar to *A. laevigata* (Silva, 1981). *A. cephalotes*, in turn, usually build their nests in humid and shady locations of woods and forests and these are not very deep (Stahel and Geijskes, 1939; Weber, 1966). The *A. robusta* species build their nests in shady areas in regions of sandbanks and due to this nesting in this type of habitat, their nests are not very deep (Table 1). The external appearance of the nest is similar to the *A. sexdens* one, that is, loose soil deposition in a very irregular shape (Gonçalves, 1945).

The nests of several species of leaf-cutting ants, as the *A. cephalotes*, *A. vollenweideri*, *A. sexdens, A. laevigata* and *A. bisphaerica* (Stahel and Geijskes, 1939; Jonkman, 1980; Pretto, 1996; Moreira et al., 2004a, b), are characterized for having the chambers with fungus and wasteunderground, in the projection of a single mount of loose soil, which is considered the main mound. Moreover, the nests of *A. capiguara,* besides having a great mount of loose soil, have other adjacent smaller mounds of soil, and the chambers containing fungi are located outside the larger loose soil mount projection, but inside up to a 10 m radius around the small mounts, being scattered and difficult to locate (Amante, 1967a; Mariconi, 1970; Forti, 1985a; Forti andBoaretto, 1997; Andrade et al., 2005; Forti et al., 2011). Under the highest mount, the chambers with waste can be found. Moreover, the peculiar form of deposition of the soil removed from the excavation, for the various species of the *Atta* genus, has also important role in regulating the temperature inside the nest, especially in species that construct the fungus chambers near the soil surface (Forti et al., 2011).

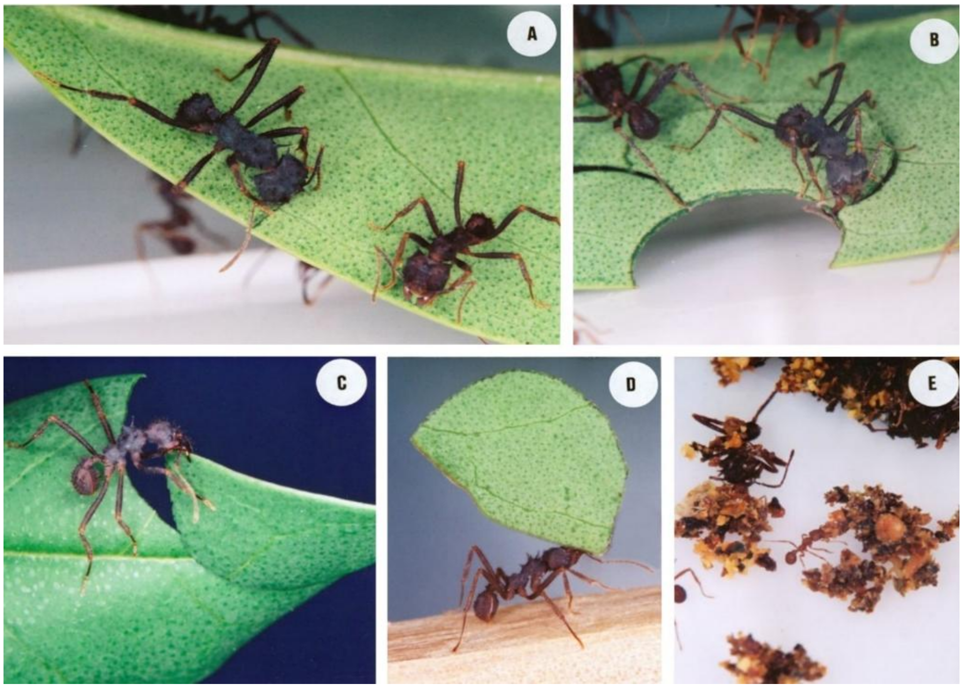

Figure 5. Behavioral acts of *Acromyrmex subterraneus brunneus* workers, in laboratory colonies. Acts outside of the nest: Leaf-cutting (A and B), Leaf fragments carrying (C and D) and tasks inside the trash chamber (E).
Photo: Roberto S. Camargo.

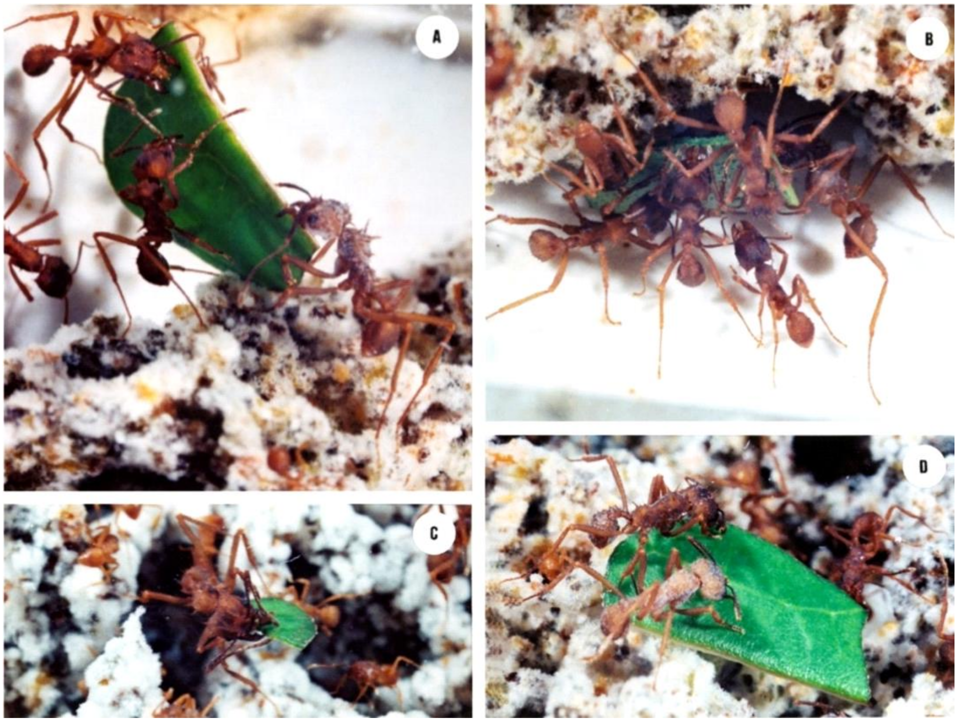

Figure 6. Behavioral acts of *A. subterraneus brunneus* workers, in laboratory colonies. Culture of the Fungus Garden: Licking the leaf fragment (A, B and D), Holding foliar fragment on fungal surface (A), and Tearing the leaf fragment (C).
Photo: Roberto S. Camargo

Figure 7. External architecture of an *Atta laevigata* nest.
Photo: Luiz C. Forti

Figure 8. External architecture of an *A. balzani* nest (Caldato, 2010).

A feature of the area surrounding the nests of leaf-cutting ants is the presence of well-defined paths, known as foraging trails, vegetation and obstacle-free, leading the supplying holes to the harnessed plants (Cedeño-Leon, 1984). According to Weber (1972), the physical and chemical trails are used for worker recruitment from the same nest, to harness the plant species.

The foraging trails can also serve as territory markers, providing a means of massive recruiting of workers to protect the integrity of resources of the colony against competitors (Fowler and Stiles, 1980). However, it should be considered that the positions are provisional and they are reallocated from time to time, as observed by *A. capiguara* (Forti, 1985a,b). In those territories, colonies harness available resources without invading the territory of others, foraging in places unexplored by other colonies. Forti (1985a,b) observed that the maximum foraging distance of an *A. capiguara* colony is closely related with the distance from the nearest colony, in the direction in which the foraging trail is oriented.

Regarding the internal architecture, leaf-cutting ants show a great expertise in nest building, which are of greater structural complexity in Atta genus species, among the Attini (Figures 9 and 10). A leaf-cutting ant nest is constituted by chambers with different functions and tunnels (channels) excavated in the ground by the workers. Inside the chambers, ants culture the symbiotic fungus that in addition to serving as food, also house the eggs, larvae, pupae, workers and the queen, and, when the colony becomes adult, the fungus can also house the winged individuals in a certain time (Mariconi, 1970). There are also chambers with the function of storing the waste (plant material under decomposition, dead ants and exhausted fungus) and chambers with soil or empty, resulting from excavation aiming nest growth (Forti et al., 2011).

Nests of several species of ants are characterized by having underground fungus chambers, under the loose soil (mound), which is considered the main mound of nest (Amante, 1967a). The distinguished architecture of *Atta* nests is an important aspect to be considered in adopting control techniques (Forti et al., 2011).

Regarding the depth, the *A. laevigata* nests are the deepest, reaching 7 m (Moreira et al., 2004b), while in *A. bisphaerica* nests depth donot exceed 2.5 m, presenting only a certain lateral growth (Moreira et al., 2004a). For the *A. vollenweideri*, Bonetto (1959) and Jonkman (1980) found chambers 3m deep under the soil surface. For the *A. capiguara,* chambers up to 4.5 m deep were found (Andrade et al., 2005) and for the *A. sexdens rubropilosa* Pretto (1996) found chambers down to 5 m. The variations in the depth of the nest of different species may be related to microclimatic requirement, since the deepest chambers suffer less from temperature and humidity variation than the more superficial ones. On the other hand, the groundwater level can also affect the depth (Geijskes and Stahel, 1939).

Studies with *A. vollenweideri* performed by Bonetto (1959), Carvalho (1976) and Jonkman (1980) observed that fungus chambers had oval shape with a flat bottom and waste chambers were conical shaped. For the *A. sexdens rubropilosa,* Jacoby (1950) concluded that the chamber structure is simple, varying from spherical to ellipsoid while to Gonçalves (1964) they are spherical and flat at the base and, according to Pretto (1996), live chambers showed semi-ellipsoid shape, as well as the waste chambers, but the latter had greater dimensions, displaying cylindrical extensions, similar to arms. In *A. laevigata* nests, Pereira-Da-Silva (1975) observed oval chambers, ellipsoid and spherical, a fact also observed by Moreira et al. (2004b), with the spherical model being the closest to the real shape of the chamber. The chambers found in *A. bisphaerica* nests were also spherical (Moreira et al., 2004a). However, in the excavated nest of *A. laevigata* and *A. bisphaerica* there were no specialized chambers containing waste (Forti et al., 2011).

For the *A. capiguara*, the form of their fungus chambers was ellipsoid and the waste chambers were conical (Forti, 1985a; Andrade et al., 2005). This species build extremely large waste chambers, and Andrade et al. (2005) estimated that the average volume of chambers with fungus was 173 L, however, the estimated total volume to 11 chambers was 166.5 L, according Forti (1985a).

In *A. cephalotes'* nest Stahel and Geijskes (1939) found a total of 373 chambers. In *A. laevigata's* nests, Pereira-Da-Silva (1975) found a total of 7164 chambers and Moreira et al. (2004b) 7864 chambers distributed in different depths, most of them located between 1 and 3 m, with an average volume of 124 L. In *A. bisphaerica,* 285 chambers were found with an average volume of 7 L (Moreira et al., 2004a). The number of chambers found in *A. capiguara* nests is much smaller. Andrade et al. (2005) found 72 chambers containing fungus, distributed from 0.6 to 4.5 m deep and 10 waste chambers from 0.43 to 1.6 m deep, with respect to ground level. The average volume of fungus chambers was 5.5 L. It can be assumed that for species which dig fewer chambers, the volume of such chambers is greater.

Tunnels going to the nest loose soil surface, open through holes that are the exits of the landfill tunnels, where ants bring the soil removed from excavations (Figure 9). These tunnels of various diameters and shapes, besides allowing the flow of workers from the outside into the nest and vice versa, are also interconnected with the chambers. The foraging tunnels, where the ants carry cut leaves, open to the outside of the nest in holes (Gonçalves, 1964) located generally beyond the limit of the loose soil area, within variable distances from it.

In studies of foraging tunnels in *A. sexdens rubropilosa*,

Figure 9. Internal architecture of an *A. bisphaerica's* nest.
Photo: Luiz C. Forti

Figure 10. Internal architecture of an *A. laevigata* nest.
Photo: Luiz C. Forti.

Pretto (1996) concluded that they are shown in a complex shape, being interconnected, in a similar way as a mesh, joining a main tunnel leading to the nest. These tunnels have elliptical cross-section, with an average width of 4 cm and a height of 3 cm, similar to those observed by Moreira et al. (2004b) for *A. laevigata*, which have an average width of 16 and 4 cm in height. In *A. bisphaerica*, the tunnels have an average width of 7 cm and height of 1.8 cm and reached the greatest chamber concentration area generally in radial ways (Moreira et al., 2004a), similarly to the *A. capiguara's* tunnels (Forti, 1985a).

Andrade and Forti (unpublished) used the cement molding technique in *A. capiguara's* nests and found the existence of a main tunnel, which was connected directly to the outside (rosettes), of rectangular cross-section, with an average width of 21 cm and 3.6 cm high, which was attached to the chambers through peduncles. This tunnel, called the main one, was continuous, forming an outer ring, communicating virtually with the entire nest. Branched laterally to the main tunnel were found the so called branched tunnels, which more chambers with fungus were found, connected to these through peduncles. The chambers connected to the main tunnel were shallow (54 cm from ground level), and the ones connected to the branched were deeper (up to 4.5 m). The branched tunnels were not as wide (11 cm) as the main tunnel, and they were also flattened and presented an almost rectangular cross-section (Forti et al., 2011).

The communication between the region where the fungus and waste chambers were found occurred through a single tunnel, similarly to those previously described (11 cm wide). The little contact between the two parts of the nest (alive and dead zones) is interesting and advantageous to the species, because this fact reduces or prevents contact with harmful microorganisms to the symbiotic fungus, present in the waste disposal area (Forti et al., 2011). Foraging tunnels had and almost sectorial arrangement, that is, the region of the nest where they concentrated had no fungus chambers. These tunnels had elliptical cross-section with an average width of 8 and 1.3 cm thickness, similarly to the pattern found in other species (Forti et al., 2011).

The nests of the species and subspecies of *Acromyrmex* vary both in structure and shape, They are smaller than the *Atta* nests and some species make their superficial nests covered by straw or plant residues, while others build them underground and may be covered with loose soil or sometimes one does not even notice the excavated soil (Gonçalves, 1961, 1964; Weber, 1972; Lewis, 1975; Pereira-Da-Silva et al., 1978; 1981; Diehl-Fleig and Droste, 1992; Della Lucia and Moreira, 1993; Wetterer, 1993; Wetterer et al., 1998; Andrade, 2002; Verza et al., 2007). Frequently, the nests are inconspicuous, hindering its location and control and this fact contributes to a greater density of them.

Despite quenquéns presenting variations in nest building, the external form of their nests may sometimes contribute to the identification of the species, but this feature is not safe, as there are those who build similar nests (Gonçalves, 1967; Pacheco and Berti-Filho, 1987).

Acromyrmex ants exhibit interspecific differences in relation to the depth of their nests (Fowler and Claver, 1991). Some species build superficial nests, with the fungus garden located above ground level covered by a mount made of straw or plant fragments. In contrast, other species build nests composed of multiple excavated chambers at depths of up to 3 m (Bonetto, 1959; Fowler, 1985; Gonçalves, 1961; Lapointe et al., 1998; Andrade, 2002; Verza et al., 2007). Even though, nests built by this genus are smaller and less complex compared to the *Atta*'s nests (Forti et al., 2011).

Acromyrmex species inhabiting shallow nests are distributed through colder areas of South America, suggesting that nests composed of straw or wood fragments have the same properties of the mound nests built by *Formica* spp., in the Northern Hemisphere (Seeley and Heinrich, 1981), that is, straw or plant fragments help the colony achieve higher and more stable temperatures than the surrounding environment (Farji-Brener, 2000; Pirk and Farji-Brener, 2013). There is a predominance of occurrence of superficial nests with increasing latitudes for *Acromyrmex* and other ants (Seeley and Heinrich, 1981), related with the concomitant decrease in the average soil temperature (Hillel, 1998; Rosenberg et al., 1983).

Bollazzi et al. (2008) made a survey of the nesting habits of *Acromyrmex* species that take place in South America and found that there is a correlation between temperature and the nest construction form. The highest number of species inhabiting underground nests is in the warmer soil regions of the continent and those that inhabit shallow nests are in colder soils. Experiments have shown that the workers of *Acromyrmex lundii* prefer digging with soil temperature between 20 to 30.6°C (Bollazzi et al., 2008). This species shows great plasticity in nest building, due to having wide distribution over the continent, being able to inhabit both underground nests in regions of warmer soil (Fowler, 1985), and surface mound nests composed of straw, often on trees in soils of flood zones (Bonetto, 1959).

The *A. balzani* build their nests underground always up to 2 m deep and, according to Forti et al. (2006) with a maximum of 5 chambers. However, in a work performed in Bahia by Silva et al. (2003b), up to 14 chambers were found. Sometimes there is a small woven straw tube into the nest entrance. *A. landolti* also nests in open areas, their nests exceeding 4 m deep with up to 10 overlapping chambers connected by a vertical gallery (Weber, 1972), and they also builds small woven straw tube that, according to Espina and Timaure (1977) and Navarro and Jaffe (1985), protects against rain and flooding.

Generally, *Acromyrmex aspersus* and *A. rugosus rochai* build underground nests (Gonçalves, 1961, 1967; Forti et al., 2006), although Silva (1964) have found that *A. aspersus* can also nest on trees or in the hollow portion of felled trees and covered by a layer of straw (Gonçalves, 1967). Nests of *A. ambiguus* are usually described in the literature as underground or partially underground (Luederwaldt, 1926; Silva, 1964; Fowler, 1979). However, Forti et al. (2006) only found underground nests of this species in a survey on *Acromyrmex* species present in the State of São Paulo. Probably *A. aspersus* and *A. ambiguus* present plasticity with respect to the construction site of their nests, as verified by Bollazzi et al. (2008) in *A. lundii.*

Nests of *A. coronatus* can be found in the most diverse places, such as on trees, on ground surface, inside hollow wood, and on rural and urban buildings. The nests of this species consist of a small amount of straw or dried plant fragments that protect a single fungus chamber (Forti et al., 2011). This species has even been found on the soil surface (Gonçalves, 1967; Forti et al., 2006), but it is very common to find it on trees (Silva, 1964; Gonçalves, 1967; Pereira-Da-Silva et al., 1981; Forti et al., 2006). However, Fowler (1985) found underground nests and only in a few there were an underground portion covered by dry vegetation.

The species *A. disciger, Acromyrmex laticeps nigrosetosus* and *Acromyrmex crassispinus* build underground or partially underground nests covered by a mound of straw or plant fragments, housing one single fungus chamber (Gonçalves, 1961; Forti et al., 2006; Marsaro Júnior et al., 2007). Gonçalves and Nunes (1984) and Fowler (1985) observed almost superficial nests of *A. disciger* externally covered by straw and excavated dirt. Gonçalves (1967) observed underground nests of *A. laticeps nigrosetosus*, not very deep and covered by a prominent mound of soil. However, Forti et al. (2006) also observed nests externally composed of straw and loose soil. Gonçalves (1961) and Fowler (1985) in addition to observing superficial mound nests in *A. crassispinus,* observed totally underground nests.

The three subspecies of *A. subterraneus* most times build underground nests, or partly underground, next to the root system of plants (Bondar, 1923; Gonçalves, 1961; Andrade, 1991; Della Lucia and Moreira, 1993; Andrade, 2002; Bondar, 1938). Nests of *A. subterraneus brunneus* can have up to 5 m² of loose soil (Andrade and Forti, 1999). They build foraging tunnels of up to 10 m in length, as noted by Ihering (1928). These nests may have a single chamber of up to 40 cm in diameter, located below the ground surface covered by sticks, straw, pieces of dried leaves and other debris (Silva, 1964; Gonçalves, 1961; 1967; Delabie, 1989). Instead, Andrade and Forti (1999) found a variable number of chambers which could contain fungus chambers up to 24 cm deep.

Nests of the *A. subterraneus subterraneus* studied by Andrade (2002) had up to 3.96 m² of loose soil area, although there is in the literature a quote of nests with up to 20 m² (Della Lucia and Moreira, 1993), but they were never observed by Gonçalves (1961), Andrade (1991, 2002). This subspecies can build nests with up to three irregularly shaped fungus chambers reaching a depth of 90 cm from ground level (Andrade, 2002).

Nests of *A. subterraneus molestans* are predominantly externally covered by straw or plant debris and loose soil and are partially underground, almost superficial, composed internally by a single fungus chamber (Della Lucia and Moreira, 1993; Andrade, 2002).

The *Acromyrmex diasi* species build surface nests covered by grass fragments, protecting the fungus garden, up to 0.50 m of high (Gonçalves, 1982).

Verza et al. (2007) were the first to use cement molding to study the internal architecture in *Acromyrmex*. The authors chose *A. rugosus rugosus*, which is the subspecies that has the greatest distribution in Brazil (Rando and Forti, 2005).

The nest of *A. rugosus rugosus* is externally composed of one or more mounds of excavated earth (Gonçalves, 1961; Forti et al., 2006; Verza et al., 2007). The mounds have irregular shape with the soil generally arranged in a Crescent pattern, with the hole in the center (Verza et al., 2007). The nest coverage area from 0.01 to 9.89 m². The nests can be composed of 1 to 26 chambers which may contain both fungi, the excavated soil, waste disposal or even be empty. The chambers have an irregular shape and distribution and, in most cases, are arranged vertically along a single tunnel. The chambers were found from surface (6 cm) to 3.75 m below ground level, usually below the pile of loose soil. The greater the depth, the smaller the number of chambers found. The fungus chambers were located in shallower depths and the waste chambers were deeper (Verza et al., 2007).

Lapointe et al. (1998) found exhausted substrate from the inside of *A. landolti* nests, but in most cases this material was deposited outside the nests. Until then, it was known that most species of *Acromyrmex* deposited rejected substrates and exhausted plant material (waste) externally, on the mound or next to it, as in the species *A. balzani, A. coronatus, A. fracticornis, A.hispidus, A. landolti, A. lobicornis, A. lundii pubences, A. striatus* and *A. subterraneus* (Bonetto, 1959; Gonçalves, 1961; Zolessi and Abenante, 1973; Zolessi and González, 1974; Fowler, 1979, 1985; Pereira-Da-Silva et al., 1981; Navarro and Jaffe, 1985; Mayhé-Nunes, 1991; Della Lucia and Moreira, 1993; Farji-Brener, 2000; Andrade, 2002; Forti et al., 2006). Verza et al. (2007) demonstrated for the first time that in *Acromyrmex* there may occur in different chambers for waste disposal. The construction of internal chambers for waste can be a strategy to reduce the exposure of workers to predators and parasites (Wetterer, 1995).

The excavation of a nest has a considerable energy cost to the colony and the ant need to coordinate their work in nest building. Even though, the energy invested has significant benefits reverted to the own colony (Forti et al., 2011).

Among the behavioral factors that may constitute control obstacles, the chemical communication, olfactory sensitivity, learning capacity, selectivity and production of antibiotic substances stand out (Marinho et al., 2006). Pheromones are widely used by them in communication and may be for alarm, individual recognition, track and recruitment, territorial, and leaf demarcation. The alarm pheromones trigger defense and are produced in the mandibular glands. When an ant is alarmed, it releases a small amount of this pheromone, which spreads rapidly and alarms other ants, leaving them ready to attack the enemy (Della Lucia and Vilela, 1993). The ability of forragers to recognize their nest mates is also an important feature, as the presence of an intruder in the colony would be quickly transmitted to other partners, which increases the defense potential of the colony (Della Lucia et al., 2001).

These ants use a wide variety of plants as a substrate for their fungus, but have certain preferences that make them choose a few plants from this variety or parts of them over others. This selective capacity with respect to the plant material to be cut is also further evidence of this insect's behavioral complexity, which would also be a contributor to its success. This plant selection may occur due to chemical defense, such as toxic substances to the symbiotic fungus, to workers or both (Hubbell et al., 1983). An interesting type of interaction between organisms, according to Diehl-Fleig (1995), is one where in the one hand are the ants, whose evolutionary success is based on its high social organization, and on the other hand, their enemies, which can be predators, parasites or pathogens. So it is assumed that, in the evolutionary process the ants developed defense systems, not just structural, morphological and physiological, but also behavioral.

Conclusion of natural history

Leaf-cutting ants have a wide geographical distribution, from northern Argentina to the southern United States, in the most diverse biomes, for example, the Amazon rainforest and extreme environments such as the desert (Haines, 1981). The great adaptive potential of this group resulted in an intriguing natural history, which motivates researchers from around the world to research basic knowledge about mutualism with the fungus symbiotic and the ants, about the reproduction and division of labor between the workers, about temperature and humidity regulation mechanisms of the colony combined with the knowledge of nest architecture, foraging system and preferred plant species, among numerous researches carried out until the present time.

However, as this is diverse group, leaf-cutting ants are considered pests in several regions of Brazil and America in agricultural, forestry and urban systems. Despite being considered pests, ants of *Atta* genus are important to the ecosystems in which they occur and play an important role in nutrient cycling by bringing organic matter to the nests, thus enriching the soil where they live (Sousa-Souto et al., 2007).

All the basic knowledge generated about the natural history of leaf-cutting ants helps us better understand how the control tactics can be effective or not. Several examples can be listed:

- Knowledge of the workers' behavior in the symbiotic fungus culture fosters subsidies to interpret how formicide baits are processed inside the nest, and how the workers' contamination occurs,
- External and internal architecture of the nest subsidizes the understanding of the amount of the insecticide active ingredient to be used in the control, as well as understanding their distribution inside the nest, which may connected to a formicide bait,
- Understanding the workers' foraging system, allows for finding a way to make the bait more attractive, with fast carrying to the interior of the colony, as well as understanding why some plant species are rejected by the foragers.

A broader understanding of the role of leaf-cutting ants in ecosystems also provides subsidies for more efficient management of these organisms, aimed at environment and human safety.

INJURIES AND DAMAGES CAUSED BY LEAF-CUTTING ANTS

Injuries caused by leaf-cutting ants to plants, historically, are acknowledged since 1560 and these injuries extend beyond agriculture, forestry and livestock, and being also caused in buildings, dams, highways and railways (Mariconi, 1970). In Brazil, the concern to control leaf-cutting ants came to involve 1.100 km², only in the city of Rio de Janeiro (Former Federal District). In 1929, about 12 teams with 10 men each were necessary to kill colonies of leaf-cutting ants, using arsenic and sulfur (Oliveira Filho, 1934).

Thus, the concern to control leaf-cutting ants is very old due to the economic importance of species of the genera *Atta* and *Acromyrmex* and the losses caused because of they being insects of importance in the Neotropical agriculture (Cramer, 1967). Despite the great economic importance of these insects, little is known about how data on losses were obtained (Fowler et al., 1990).

Several methods have been proposed to estimate losses caused by leaf-cutting ants (Fowler et al., 1990) and the first time it was held was by Amante (1967a) for the *A. sexdens rubropilosa* and *A. laevigata* species in *Eucalyptus alba* and *Pinus* sp.plantations. It is concluded that wood losses are between 14 and 14.5%, considering an infestation of 4 colonies per hectare. Despite the criticism of the method of obtaining these data (Fowler et al., 1990), we cannot yet say whether they were under or overestimated.

The greater number of studies have been performed on trees of economic importance used in planted forests, mainly in Brazil and, to a lesser extent, in other Latin American countries, being studies of artificial plant defoliation (Oda and Berti Filho, 1978; Anjos et al., 1987; Freitas and Berti Filho, 1994; Oliveira, 1996) and, of course, plants defoliated by leaf-cutting ants (Hernández and Jaffé, 1995; Cantarelli et al., 2008; Nickele et al., 2012).

Plants that were completely defoliated showed wood losses between 13 and 50% (Hernández and Jaffé, 1995, Oliveira, 1996). Plants, when young, may die after defoliation (Mendes Filho, 1979). When *Pinus* sp. individuals do not die because of the successive attacks of leaf-cutting ants, they show a reduction in diametral growth (Cantarelli et al., 2008) and in other cases losses can also occur in a decrease in plant height (Nickele et al., 2012).

More realistic data on the loss of *Eucalyptus* wood production caused by *Atta* spp. in different sites were obtained by Zanetti et al. (2000, 2003b) and Souza et al. (2011).

These studies showed that colonies of *Atta* decrease the production of wood, through defoliation of *Eucalyptus* areas. Densities greater than 80 nests (loose soil area of 2.76 m²) per hectare can reduce more than 50% of Eucalyptus wood production (Zanetti et al., 2003b; Oliveira et al., 2011).

Leaf-cutting ants are nonspecific pests of cultivated plants in such a way that cause loss of productivity in various crops and the defoliation is the main cause, decreasing the plant's area responsible for the photosynthesis. Crops which are important to the country are attacked by leaf-cutting ants. Besides those already mentioned, crops of grain (rice, corn, sorghum, wheat), oilseeds (soybeans, beans, coffee, cassava), fruit (citrus), vegetables, ornamentals, etc.

Cassava plants, very important crop, are severely attacked by leaf-cutting ants and losses can reach 55% (Bertorelli et al., 2006). Coffee plants (*Coffe arabica*) may also suffer defoliation by leaf-cutting ants, including injuries in branches and fruits (Barreto et al., 1998).

Several species of cutting ants also attack cocoa plants in Brazil and in Trinidad and Tobago. Defoliation and destruction of flower buds limit plant growth and cause production losses (Delabie, 1990; Montoya-Lerma et al., 2012).

The species of *Atta* cause significant losses in forestry, agricultural and pasture production, but species of the genus *Acromyrmex* are also economically important (Montoya-Lerma et al., 2012; Boulogne et al., 2015). In Brazil, the *A. crassispinus*, a common species (Rando and Forti, 2005) cause significant forest loss of *P. taeda* (Lopes and Camargo, 2004; Nickeleet al., 2009, 2012). Severe losses from 20-30% of the production were documented in Trinidad and in Guadalupe, Central America, for fruit, cocoa and other plants' crops (Boulogne et al., 2015). In Argentina, Cantarelli (2005) reported that 20.8% of *P. taeda* seedlings were attacked by the *Acromyrmex* species. This attack can cause total defoliation of small plants and more significant losses (15% mortality) occur when the ants also cut the apical meristem (Nickele et al., 2012).

Grass-cutting ants also cause considerable losses in pastures and sugarcane crops (Fowler et al., 1986). Amante (1967a, b) estimated that *Atta capiguara* can consume 255-639 kg of dry matter per colony per year, which equals losses 512-870 thousand head-of-cattle per year in the State of São Paulo. Even if these data are overestimated (Fowler et al., 1990), they still indicate significant losses to livestock. Robinson and Fowler (1982) showed that actually, *Atta capiguara* competes directly with cattle for grass. These estimates are considered potential losses, thus *Atta vollenweideri* and *A. landolti* can also cause significant losses (Fowler et al., 1986).

The leaf-cutting ants *A. capiguara*, *A. bisphaerica* and *A. laevigata* are very important for the culture of sugarcane. The *A. capiguara* and *A. bisphaerica* species account for average losses in productivity of sugarcane ranging from 1.7 to 3.2 tons of sugarcane per colony, each crop cycle. Consequently there is a 30% reduction in sugarcane sucrose content (Precetti et al., 1988; Stingel, 2007). The reduction of the quantity of leaves in sugarcane caused by the attack of grass-cutting ants can cause losses of 3.26 kg of sugar cane plants per m² (Albuquerque, 1997).

CHEMICAL CONTROL OF LEAF-CUTTING ANTS HISTORY AND ACTUALITIES

Formicides in dry powder formulation

Formicides in dry powder formulation began to be used in the later 50s to control leaf-cutting ants. It is basically an active ingredient which acts by contact, added to ground powder as inert and application vehicle. It is applied via a manual pump, coupled to a conical container where the product is placed (Mariconi, 1970; Forti and Boaretto, 1997).

The first formulations had as active ingredient insecticides belonging to the organochlorinated group, with long residual power in the soil, which is about 12 years. The use of these products, which was carried out on a large scale has been decreased with the introduction of new chemical methods, such as thermal fogging and the toxic bait. With the discovery that the insecticides of this group are carcinogenic and accumulate in the fatty tissue, began the use of insecticides of the pyrethroid group and others with contact action (Mariconi, 1970; Forti and Boaretto, 1997).

The organochlorinated ones, more frequently used in the initial studies aimed at determining the lethal dose, were Aldrin and Heptachlor, being Aldrin indicated as effective at a dose of 30 g/m² of loose soil. The efficiency this commercial product had was questionable because it showed contradictions when the results obtained under different experimental conditions were confronted, reaching less than 12.5% of control over *A. sexdens rubropilosa,* being considered as unsatisfactory (Mariconi and Castro, 1960), or moderately satisfactory with 40% of control of *A. laevigata* (Zanuncio et al., 1980) and 44.7% in *Atta* (Mariconi and Castro, 1960; Ribeiro and Woessner, 1979), or even highly satisfactory with control from 65 to 91.6% (Amante, 1963a,b; Amante, 1962, 1967c; Gonçalves, 1956).

This contradiction in efficiency is difficult to analyze, since, in general, the authors have not detailed the methodology used in the study. Another aspect not considered by researchers is the individual result of the efficiency experiments such as the study by Amante (1962), in which nests with 73 and 60 m² of loose soil remained alive after the Aldrin application, while others with 72 and 64 m² were killed or even nests with 37m² remained alive and nests with 210 m² died with the same dosage of Aldrin. In addition, there are conflicting results, where a higher dosage resulted in lower efficiency as compared to a lower dose as shown in the works of Gonçalves (1956) and Mariconi and Castro (1960). This way, it was evident that there are methodological problems in these studies. Besides Aldrin, there were tests with mixtures of this active ingredient with Esther - 2 - chlorophenol of sultovidila acid, whose technical name is F214, which presented more effective control than Aldrin (5%) (Amante, 1963a,b).

Another product tested was the Heptachlor (5%), which at a dose of 30 g/m² presented control around 90% in *A. sexdens rubropilosa* (Mariconi, 1963a; Amante, 1967c), 88% for *A. laevigata* (Amante, 1967c) and 70% for *A. sexdens* (Amante, 1968a,b), although other studies have indicated a 50% efficiency at a dose of 10 g/m² for *A. laevigata* (Zanuncio et al., 1980). In addition to those, the efficiency of powder-formulated phosphine was studied in controlling *A. capiguara,* having zero formicide response (Mariconi, 1974a,b). Malatol (5%) and deltamethrin were also tested in *Atta* sp. control using a dosage of 15 g/m² of loose soil, providing an efficiency of 77.78 and 50%, respectively (Mendonça et al., 1987).

The concern in the control of leaf-cutting ants of the *Acromyrmex* genus guided a few works also with powder-formulated insecticides, including the malathion (4%), parathion (1.5%), fenthion (5%), trichlorfon (2.5%) and bendiocarb (1.0%). According Juruena (1984), these products were effective in control, but the methodology used by the author and the control percentage achieved were unclear.

Recently, some products in this formulation were researched for controlling *Atta* and *Acromyrmex.* Among them is “K-Othrine 2P” with 100% efficiency (Bendeck et al., 1995a, b) and Birlane, with 25% efficiency for *A. sexdens rubropilosa* and 50% for *Acromyrmex crassispinus* (Bendeck et al., 1995a, b). It is noteworthy that in other fieldwork using the product Birlane in the *A. sexdens* rubropilosa and *A. crassispinus* control, the efficiency was poor, being below 34% (Bendeck and Nakano, 1998).

Generally, studies performed show methodological errors, which are readily apparent when we compare the results obtained by different researchers, in different regions of the country, in addition to the inexistence of a detailed interpretation of the results, such as the fact that nests with similar size behave differently when exposed to the same product. Currently, the products “Landrin Pó” (Chlorpyrifos 50 g kg^{-1}) and “K-Othrine 2P”(deltamethrin 2 g kg^{-1}) are registered in MAPA-Ministry of Agriculture, Livestock and Supply, Brazil (AGROFIT, 2015) for the control of leaf-cutting ants. However, among these only the “K-Othrine 2P”(deltamethrin) is now being marketed.

The active ingredient deltamethrin is used exclusively in dust formulation and there are several reasons that limit their application, for example, this insecticide is used only for young colonies of *Atta* and *Acromyrmex* colonies. Colonies with more than one year old have many chamber between 58 to 7,864, reaching as deep as 7 meters underground (Moreira et al., 2004a, b). The chamber volume ranged from 0.03 to 51 L. The foraging tunnels extended as far as 70m from the loose soil region (Moreira et al., 2004b). As we can observer, dust formulation (for example, the active ingredient deltamethrin) has great difficulty in achieving the ants in their fungus gardens. The difficulties of application increase during the rainy season because the soil becomes moist and dust sticks to the walls of the tunnels and do not reach the target. The dust product insecticides are formulated into solid vehicles (for example, talc powder) and are applied with hand-held equipment called dusters. Therefore, it is recommended using it only when the soil is dry. Furthermore, there is the need to remove loose soil before application of the product, which makes the technique impractical operatively. Another limitation is the risk of contamination to the environment and the operator (Forti and Boaretto, 1997).

The insecticides in dry powder formulation are recommended for complementary use in very specific situations, for example, to control some species of *Acromyrmex* and initial nests of *Atta* spp. However, it is clear that the powder formulations cannot be recommended as the main one and not even as the sole method of control, especially in large areas, because they present low efficiency. Thus the powder insecticides cannot be considered as an alternative to the use of toxic baits.

Fumigants

Pioneering products

Fumigant products were pioneers in control of leaf-cutting ants, being widely used. Some of the first fumigants such as hydrocyanic acid, sulphurous anhydride, sulfur dioxide and sulfo-arsenious oxide were applied with the aid of special equipment. The first, because of being very dangerous and not having proper equipment to apply it, was abandoned. The sulphurous anhydride, although being not flammable and heavier than air had the inconvenience of blocking the thermal fogger pipes it went through, due to freezing (Mariconi, 1970).

By the combustion of sulfur, one can obtain sulfur dioxide, but to apply it there was the need to dig trenches approximately 1m in diameter and 2m deep, filling it with wood, making a fire and throwing on this a lot of powdered sulfur, subsequently the hole was covered with an iron plate of which had a hole in the center. Then, the tip of the bellows was inserted in the plate orifice and inoculated the gas in the nest channels existing in the trench. The sulfo-arsenious oxide was produced by burning arsenic and sulfur powder, two gases were produced this way, sulfur dioxide and arsenious oxide which had better efficiency when compared to the isolated chemicals. For the application of those gases is a simple device called bellows was used (Townsend, 1921). The carbon disulfide was widely used in the control of leaf-cutting ants and when used in the gaseous form had the need to use special devices, gasifiers (Mariconi, 1970). This device consisted of a container, in which burning coal was placed for heating and volatilizing the chemical (sulfur and arsenicals), which was blown into the nest through the natural tunnels via a manual blow. The bellows also helped the application of insecticides that emitted gases when in contact with air, such as the carbon disulfide (Mariconi, 1970). Initially, the fumigant was applied into the natural orifices of the nest. However, to improve the control system probes or drills were developed, called "JP" probes (Autuori, 1940) and "Favorite” drill (Borgmeier, 1948), which were used to open artificial channels, allowing the application of the product. To make this system of control feasible, all the loose soil was removed from the mound (mount of loose soil), and later drilling was initiated with the probe, processes which required plenty of water and a lot of physical effort. When the drilling was over, the product was applied, closing the holes so that they remained inside the nest.

Fumigants products applied with the bellows and in need of heating were sulfur (which by burning released SO_2) and arsenicals. The methyl bromide and carbon disulphide were other fumigants used as formicides, differing from the others by not requiring heating to volatilize, needing only contact with air, and the use of bellows with coal for heating was not necessary, but required the using a suitable applicator. The application of sulfur as sulfur anhydride, was among the first to be used for controlling leaf-cutting ants, already being tested in 1921 (Townsend, 1921) and later by Walter et al. (1938). However, their effectiveness has not been proven (Autuori, 1942). Indeed, it seems that the oldest formicide in the control of *Atta* was carbon disulfide used with privileges by Dr. Guilherme S. de Capanema for 18 years (1873-1891) (Dafert and Rininius, 1896; Mariconi, 1970).

The carbon disulfide was widely used in the control of leaf-cutting ants (Autuori, 1940; Durval, 1949; Morais and Pinheiro, 1951; Amante, 1962, 1963a,b), however, it was highly dangerous to humans. It is interesting to note the recommendation to users of carbon disulfide, that as soon as the gas began to spread in the channels and chambers, it was necessary to set it on fire to cause major explosions. But by the time this need was doubted because the insecticide itself was already highly toxic. Other products such as hydrocyanic gas, emitted from sodium cyanide or potassium cyanide, were applied both in solution and dry form, without the need for equipment, with great killing power. The chlorine gas was produced from the mixture of lime dichloride and sulfuric acid for the treatment of *Atta* nests, applied without machine aid (Tonwsend, 1921). No compound having chlorine as active ingredient has reached commercialization, however, those containing white phosphorus and mercury were marketed (Mariconi, 1970). The first equipment and control methods employed against leaf-cutting ants were very well described with many details by various authors (Townsend, 1921; Oliveira Filho, 1934; Carvalho, 1935; Torres et al., 1936; Rego and Brandão Filho, 1945; Borgmeier, 1948; Mariconi, 1970).

In 1936, in order to provide a new stimulus to the Campaign against *Atta*, the Ministry of Agriculture appointed a Commission to judge and report on the best control methods. The Commission concluded that the best insecticides were carbon disulfide and white arsenic, with the blowers and carbonated devices being handled by skilled workers (Torres et al., 1936; Borgmeier, 1948; Mariconi, 1970).

The second generation

A fumigant widely used until recently was methyl bromide, which over time has undergone a few changes, acquiring new brands, but always with a very good control efficiency, between 88 to 100%, however, it is highly toxic to the applicator (Amante, 1962; Morais and Pinheiro, 1951; Mariconi and Castro, 1960; Costa, 1949; Durval, 1949; Autuori, 1950; Autuori and Pinheiro, 1950; Vilela, 1986; Amante, 1963a,b). According to the Montreal Protocol, it is no longer environmentally acceptable, as it is a ozone layer destructive gas, and thus its use should be gradually discontinued (Silva, 2009). In Brazil, its use in agriculture it is no longer allowed. Now, the "IVI.M.33" was used on a small scale, presenting very variable results, with efficiency between 65 to 100% in the control of *Atta* spp. and *A. capiguara*. This product is no longer used due to its high dangerousness to the applicator (Morais and Pinheiro, 1951; Amante, 1963a,b, 1950).

Most recently, a single product was developed: the Bunema 330 CS (Sodium-N-methyldithiocarbamate) which present fumigants characteristics. It was also tested for efficiency in the control of *Atta* and *Acromyrmex* by Nakano et al. (1993), presenting 100% control at a dose of 60 ml/m². However, as this study is only a symposium summary, it is difficult to assess whether the methodology used was adequate. One should also consider that this product is highly toxic to humans and can cause irritation to the lung tissue due to its corrosive characteristics, in addition to attacking especially the skin, liver and bladder.

Currently, among the fumigant products, only the CS 330 Bunema (Metam-sodium [methyl isothiocyanate]) at 383 g L^{-1} is registered in MAPA (AGROFIT, 2015), and it was never commercialized.

Thus, the fumigants cannot be recommended for the control of leaf-cutting ants, as they are extremely toxic, not available and present difficulties in technical and operational feasibility. They cannot be considered as alternatives.

Liquid formulation formicides

For the use of liquid formicides, the recommended procedure was as follows:

The day before the application of the formicide, the pile of loose earth is measured and removed to locate the active holes. The next day, the product is placed in the active holes or in perforations made with "JP" probe, with a funnel coupled to a hose of 30 cm length (Autuori, 1940, 1947).

Aldrin liquid was the most studied product, and provides variable results in efficiency in the control of *Atta* sp., with a range of 30 to 60% (Mariconi and Castro, 1960, 1961; 1963a,b; Ribeiro and Woessner, 1979) and 80% (Gonçalves, 1959). Another formicide tested was Heptachlor, which showed satisfactory results, achieving 80% efficiency in the control of *A. capiguara* (Mariconi, 1967). However, in another experiment, it controlled only 20% of the colonies of the same species (Amante, 1968a,b). Thus, it is concluded that this product has not been fairly studied, not allowing for a more careful analysis of such efficiency. Similarly, chlordane was also little studied, with unsatisfactory results, with only 50% of control (Mariconi and Castro, 1960). The control method with liquid insecticides efficiency is highly questionable, due to the large variations that occur in the results obtained by different researchers, considering the same product and dosage, in addition to events unexplained by the authors as the fact that a product as Aldrin, for example, being more efficient at lower dosages compared to larger. Certainly there are explanations for these events and they reside in the methodological procedure of the evaluations and interpretations of the researchers.

Currently, registered in MAPA-Ministry of Agriculture, Livestock and Supply, there is only Fipronil in the WG (water dispersible granule) formulation, specifically for *A. capiguara* in crops of sugarcane and corn, for an application of 50 mL insecticide mixture/nest opening, using a backpack sprayer, aiming for the center of the opening, the physical path where the ants walk (0.5 m), the individuals there and also the ground where there are circulating (AGROFIT, 2015). There is no data available in the literature about the effectiveness of this product.

The volume of water required to use over large areas or in remote locations is a serious problem for the use this type of product (Della Lucia and Vilela, 1993; Forti and Boaretto, 1997). Additionally, using this type of product, the risk of contaminating the groundwater table is greater than in the other formulations. The products have inconsistent experimental results and are also extremely persistent in the environment, as they contaminate soil (Gunasekara et al., 2007).

Fipronil is highly toxic to bees, aquatic organisms, fish, birds and mammals (Gunasekara et al., 2007; Kitulagodage et al., 2011; Beggel et al., 2012). Fipronil is highly toxic to Melipona scutellaris (Hymenoptera: Apidae), confirming the toxicity of fipronil to native bees in Brazil (Lourenço et al., 2012). In China, it was also proven the high toxicity of fipronil to non-target organisms, mainly *Apis mellifera* (Hymenoptera: Apidae) (Li et al., 2010). Therefore, the application for full coverage can cause a major environmental disaster.

The ecological impacts of fipronil are extremely worrying because as new studies are completed and been published more problems than benefits are increasing from their use, both in terrestrial communities, as aquatic communities (Gunasekara et al., 2007; Hayasaka et al., 2012).

Are very strong the evidence that fipronil, even utilized in low concentrations can cause problems at non-target organisms, since has already been detected in drainage water in agricultural and urban areas (Stratman et al., 2013).

In such a way, there is no perspective that this type of product may, in the future, be recommended as an alternative to leaf-cutting ants control, which even the development of efficient products to control *Acromyrmex* spp. or small *Atta* spp. colonies is no guarantee. So theuse of this type of product does not serve even as a complementary method.

Fogging

Thermal fogging

Thermal fogging is the production of deadly smoke through heating and volatilization of liquid insecticide, acting by contact, conveyed together with the products of combustion carried by a motor. This application is made directly into the holes on the mount of loose soil, placing the exhaust hose and waiting for the smoke reflux. When this occurs, the application hole is changed. The formicide should have a formulation appropriate to withstand high temperatures without active ingredients (AI) degradation (Nakano et al., 1978; Forti et al., 1999; Zanetti et al., 2002).

The first liquid insecticides for use in thermal foggers were heptachlor and aldrin, presenting high efficiency, with control between 80 and 100% of the ant nest (Nogueira et al., 1981). However, with the ban of this insecticide group, others were tested, including carbamates which did not present satisfactory effect on the control (Forti and Boaretto, 1997), contradicting the results presented by Mendonça et al. (1987), who claimed a 80% efficiency for this product. The same researchers mentioned efficiency of 50% for deltamethrin, 78.95% for Malafog (15%) and 48 to 90% for deltamethrin when the concentration changed from 2.5 to 6.0 g/l. However we must emphasize that the information obtained by these researchers are contradictory, as, among the used insecticides, it was found that in larger doses the efficiency of the products were lower (Horton et al., 1982). Chlorpyrifos, in turn, showed high efficiency, with 100% control action in *Atta* spp. (Mendonça and Gomes, 1989; Gomes et al., 1989; Gomes and Gomes, 1993).

The formicides suitable for this method are restricted to those discussed above, however, regarding the equipment, one can use a two-stroke motor with adjustments, or an application system coupled to motorcycles (Vilela, 1986) or even in tractors or chainsaws, facilitating the application and making the insecticide distribution in nest more efficient.

As advantages, for some cultures, such as sugarcane, usually subjected to intensive cultivation during the crop cycle, thermal fogging can sometimes be used as an alternative to the use of granulated baits, especially during rainy periods (Cruz et al., 1984). Thermal fogging can present some efficiency for large ant nests, especially in initial combat operations during the implementation of cultivated forests (Zanetti et al., 2002). In addition, this control method can be used any time of year in soggy or dry land and requires no preparation and nest measurement (Anjos et al., 1998).

However, this method of application presents operational and economic disadvantages and brings risks to the health of workers and the environment (Cherrett, 1986d; Della Lucia and Araujo, 2000; Moreira et al., 2004b). The equipment is costly and requires constant maintenance, some of these being the main disadvantages against their viability. In cases of large estates, it may be feasible to use, but for the vast majority of small Brazilian producers this would be a totally impractical methodology, mainly due to the high equipment cost. Even in large properties, these issues are very important in cultures with lower density of cultivation, such as the reforestation of *Eucalyptus* and *Pinus*, where the thermal fogging is not a cost-effective alternative, being used only sporadically as a complementary method. In these cultures, the cost of control using granulated baits already is very high in proportion to gross income(so it is worst with thermal fogging). In addition, there is a risk to cause forest fire, due to the emission of sparks during use and flames when shutting down the thermal fogger.

A serious problem and that is independent of culture, is the great exposure of workers to the insecticide, which can be easily inhaled during application.

Another item that should be considered, which has only recently been lifted, is soil contamination by these products, according to the study by Bollazzi et al. (2014). Due to their formulation at high active ingredient concentrations, which after application remain attached to the ground, it is evident that these products can bring great impact to the soil macrofauna and microfauna, and possibly contaminate the groundwater table.

The products Lakree Fogging [chlorpyrifos (organophosphate) 720 g kg^{-1}], Sumifog 70 [fenitrothion (organophosphate) 70 g kg^{-1}] and Gemini [permethrin (pyrethroid) 100 g kg^{-1}] are registered in MAPA (AGROFIT, 2015), but only the latter is commercially available.

In commercial plantations of *Eucalyptus* and *Pinus* in Brazil and in other crops (sugarcane, citrus, pasture) this control method has been put in the background, being used in specific situations when there is a high rate of infestation and initial preparation of land for cultivation. And only the larger nests end up being controlled by this method. In the case of early nests of *Atta* and *Acromyrmex* becomes unviable the control effort with these products. Taking into account that in a farming area there are nests of various sizes, in most cases it is more appropriate to use toxic baits for succeeding in the control. The use of thermal fogging does not prevent the use of toxic baits, since small and unknown nests must be controlled with toxic baits.

Some factors may explain the low interest in the use of the thermal fogging when compared to the use of baits, for example: high cost of equipment, equipment operational problems and maintenance, increased exposure of equipment operators and their colleagues to the insecticides, contamination of soil and water, some equipment sparkle in beginning of the process and when shutting down there is the possibility of flames via the injection pipe of the device, which can cause fires in forests or grasslands, accidents with operator burns, increased use of workers, since an operator and at least two helpers are necessary, for cleaning the opening for the application and the channels cloggings through which the “smoke” is spreading. For these reasons large companies in Brazil have dropped by 100% the use of thermal fogging and keeping the control in their fields made with toxic baits.

This method of control cannot be considered a substitute or alternative to the use of ant toxic baits but only as a complement to the use of baits and in some specific situations.

Cold fogging

The development of an equipment called Aero System was an attempt to technically enabling the cold fogging method using the Bistar product (bifenthrin), a specific formulation. The equipment consisted of a cylindrical steel tank coupled to a hose, rod and nozzle, suitable for placement of the product inside the nest through the hills. The formulation consists of the active ingredient bifenthrin diluted in solvent and mixed with butane and propane gases. The higher density of the gas mixture with respect to the air, on average 1.77 times, allows the downward movement of the product inside the nests (Forti and Boaretto, 1997; Raetano and Wilcken, 1998).

Some authors obtained high levels of efficiency (80-90%) in their studies when using this application system, in colonies of *A. capiguara*, *A. laevigata* and *A. sexdens rubropilosa* (Nakano and Nogueira, 1993). Other studies have shown lower efficiency rates (around 60%) for *A. sexdens rubropilosa* and maximum control rates (100%) for *Acromyrmex* (Alves et al., 1995).

However, according to Raetano and Wilcken (1998), this equipment applying the Bistar product showed only good performance for new nests (<1m²) of *Atta* and *Acromyrmex*, whereas larger nests presented disorders, population decline and inactivity for long periods.

It appears that the control results are closely linked to the size and therefore the age of the colonies, considering that with age there is a large increase in their architectural complexity. Large nests of *A. laevigata*, for example, have from 6 to 7 thousand chambers and reach depths of up to 6m (Pereira-Da-Silva, 1975; Moreira et al., 1995; Moreira et al., 2004b). In addition, knowledge about the diffusion and gas flow in most *Atta* and *Acromyrmex* colonies is still incipient, which hinders the development of products which depend on this parameter. Therefore, it is doubtful the efficiency of recommending this method as unique as a function of the architecture of *Atta* spp. colonies, Combined with random distribution of nests of different sizes in the field. Facts widely discussed are the practicality and cost of application, time saving and applicator safety.

As for operational efficiency, studies by Branco et al. (1995) showed that the effectiveness of bifenthrin (Aero System) was 21.1% lower than the use of systematic bait-holder (BH), and 17.7% lower than the application of bulk baits. According to the authors, the system has shown potential to control leaf-cutting ants, being able to reduce these differences in operating efficiency with more intensive training. As system benefits, Raetano and Wilcken (1998), cited its use under unfavorable conditions to the control with bait and the use of returnable packaging. The limitations cited by the authors describe the application bar freezing, causing flow obstruction, as well as the possibility of leaks in valves. These operational issues that would require a well-trained professional for the use of the equipment in field. This method has never been used commercially, it was actually a development attempt that did not work due to technical problems with equipment, low efficiency, and added costs to use.

Currently, Bistar products [bifenthrin (pyrethroid) 5.28 g kg^{-1}] and Bistar UBV [bifenthrin (pyrethroid) 5.28 g kg^{-1}] are registered in MAPA (AGROFIT, 2015), but the products are not being marketed. This type of product is not available, does not have technical and operational viability, and therefore, it clearly does not have the necessary features to be recommended as an alternative control method.

Toxic bait

The product

Toxic baits are made up of attractive and insecticide. In the case of leaf-utting ants, it must be made of a material that can be foraged and used with and the mutualistic fungal culture of the ants and an insecticide as an active ingredient.

By 1957 the first toxic baits for leaf-cutting ants appeared, which were made with Aldrin (as AI, active ingredient) and with cassava, corn and wheat bran used as substrate (Gonçalves, 1960a,b; Mariconi and Castro, 1962). Research on new products continued as aldrin was a contact action insecticide. Therefore, Amante (1968b) started the first works in Brazil with dechlorane ($C_{10} Cl_{12}$) in 1964 in the State of São Paulo, obtaining promising results. At the same time, in other countries, many researchers came to the same conclusion as Amante, such as Zarate (1964) in Peru, Lopes (1965) in Mexico and Echols (1965,1966a,b) in the United States of America.

In Brazil, research on dechlorane (dodecachlor) continued with Freire and Vanetti (1968) in Minas Gerais and with Kober and Juruena (1968) in Rio Grande do Sul, always with high control efficiency. In 1968, the Department of Agriculture of the São Paulo State authorized the Campanha Agrícola Imobiliaria e Colonizadora (CAIC) company to import bait formulated with dechlorane from the United States of America. Thereafter, many National Capital companies began formulating baits with dechlorane and continued producing them until the end of 1993, when the permission ended. The official ban on bait manufacturing was enacted in January 1992 and sales on April 30 of that year, but there was an extension. The manufacture of baits with dechlorane was prohibited only because there was already a replacement substance called sulfluramid, which was registered at the Ministry of Agriculture under the trade name of Mirex-S, from the prohibition of dechlorane. Currently, the toxic bait is a pelletized formicide, formulated with attractive substrate (citrus pulp and soybean oil), with several trademarks. It is an active ingredient (AI) with suitable characteristics for use with toxic baits, including especially the delayed action in a good range of concentrations (Nagamoto and Forti, 1997; Nagamoto et al., 2004, 2007).

After all, only a few years later more detailed studies on the action of toxic baits for leaf-cutting ants began to be conducted, for example the work on dispersion of insecticide by Pretto et al. (1995) and the and the work on formicide action in workers, by Nagamoto and Forti (1997). This way, to better understand the efficiency mechanisms, we will discuss, in the following topics, some aspects of the mode of action, dispersion and characteristics of the insecticide, as well as the acceptance and distribution of the substrate by the colonies.

Advantages of bait use

The use of granulated bait has significant advantages over other methods of controlling leaf-cutting ants, as it does not require hand labor or specialized equipment, it is practical and economical. It is a safe method to the operator (Forti and Boaretto, 1997; Zanetti et al., 2014). The efficiency of this method is very high, provided it properly meets the basic requirements: Using proper AI and an attractive substrate in its formulation, and right application (Forti et al., 1998; Nagamoto et al., 2004, 2007). Considering the architecture of the colonies (Moreira et al., 2004a, b), the toxic bait strategy is what makes the most sense because the pest-ants themselves take the product inside the nests and distribute it evenly in the colony, even if eventually the nest is constituted of thousands of chambers, promoting widespread poisoning in the colony (Forti and Boaretto, 1997; Moreira et al., 2004a, b). This is the inverse of the other formulations, whose application is forced to the inside of the nests and the resulting distribution is often not wide, causing inefficiency (Forti and Boaretto, 1997).

Features and requirements of the substrate

In the colony, the workers prepare the leaf fragments by licking them intensely, to later incorporate them to the fungus (Peregrine and Cherrett, 1974). During this process of licking the leaves, they remove the wax layer and inhibit the action of microorganisms which are competitors to cultivated fungus. The time taken to lick the leaf is influenced by the thickness of the wax layer, by chemical attraction and by the contamination of the plant species used (Quinlan and Cherrett, 1977; Fowler et al., 1991).

Thus, it is easy to understand how the *Atta* workers poisoning occurs. Initially the workers take the bait pellets to the inside of the fungus chambers, licking them intensely, as they usually do in any usable substrate for fungus growth, ingesting the insecticide as the pellets are hydrated, and then they incorporate the substrate to the fungus culture in small pieces. This occurs relatively quickly, beginning approximately 6 h after the supply of bait and ending about 18 h later.

Another important aspect to be addressed is regarding the distribution of the substrate in the colony, which behaves in a highly advantageous manner in this control method, as it occurs uniformly, reaching virtually every fungus chamber, regardless of its depth and the supply hole through which the substrate enters (Mariconi et al., 1981a,b; Forti and Silveira Neto, 1989; Forti et al., 1995; Moreira et al., 1995; Pretto, 1996).

The vehicle most commonly used in the manufacture of insecticide bait for controlling leaf-cutting ants is citrus pulp, although it may be used alone or in a mixture with other ingredients such as ground corn, wheat bran, *Eucalyptus* foliage and other ingredients such as cassava flour, soybean bran, wheat flour, grass leaves, jatobá fruit pulp and sugarcane bagasse (Gonçalves, 1960a,b; Cherrett and Merrett, 1969; Robinson et al., 1980; Lima et al., 2003; Teixeira and Santos, 2008). It seems that the discovery of using citrus pulp and other ingredients used as the toxic bait vehicles come from the observation that, in nature, *Atta* ants carry the most different materials to the inside of its colonies, such as cow and bird feces, pieces of paper, food (Mariconi, 1970), plastics (Garcia and Forti, 1993; Fowler et al., 1993).

To improve the attractiveness of granulated bait, it has been thought about adding vegetable oils (Echols, 1966a,b; Cherrett et al., 1973), *Eucalyptus* leaves, grass leaves, trail pheromone (Robinson et al., 1982) and, in some cases, sugarcane molasses. It is known that the leaf-cutting ant workers are attracted by numerous odoriferous substances, but this attraction is always within short distance, as the scout workers are the ones that find the ideal material to be brought to the colony, and only find them because they move thought the forage territory looking for some source of cellulose. This behavior explains why certain substances known to be attractive, have not been significantly important to improve the attractiveness of toxic bait, as the ones mentioned above.

Bait application

The localized application of bulk bait, with dosage calculation for each colony, is the most recommended way to implement the bait, because then you can better dose the quantity of product per colony, ensuring efficiency and minimizing product waste, although there is a need for well trained manpower and is not always easy to find small colonies (Forti and Boaretto, 1997; Zanetti et al., 2003a,b, 2014). This form is used even for well-controlled experiments, aimed at making reports with the purpose of bait registration in the MAPA (Brazil, 2011).

The traditional method of applying baits, which consists of the distribution of bulk baits in ant colonies, present a few drawbacks, such as the inability to work every day of the year due to rain and accumulated moisture, complicating the operations planning and other interdependent activities, generating extra spending on labor and product loss by exposure to moisture, the substantial costs of implementing the bait is due to the high use of manpower. However, all these drawbacks can be overcome with the use of the bait holder and the micro-bait holder. A bait holder is an apparatus, made from different materials such as metal, waterproof paper, plastics, polyethylene films, which contain a certain amount of baits, suitably packaged, in order to protect them from moisture, heat and non-target animals (Forti and Boaretto, 1997; Zanetti et al., 2003a,b).

Active ingredients (AI) features and requirements

To control leaf-cutting ants, the insecticide formulated in toxic bait should act by ingestion and basically have the same characteristics of insecticides that act primarily on workers of *Solenopsis* spp., according to early studies of Lofgren et al. (1962), followed by the proposal of Stringer et al. (1964), and supplemented by Lofgren et al. (1967) and Vander Meer et al. (1985), which are:

- Toxic action with mortality less than 15% after the first day and greater than 89% by the end of the experiment (the fourteenth or twenty-first day),
- Lethal at low concentrations,
- Be readily spread in the colony and kill the receiving individuals,
- 4) Do not damage the environment.

Thus, pesticides were divided into different classes to serve as a “screening” of formicides (Stringer et al., 1964; Lofgren et al., 1967; Vander Meer et al., 1985), being:

- Class I: compounds cause less than 90% mortality at the end of the test period.
- Class II: compounds kill rapidly in high concentrations (mortality over 15% after 24 h and greater than 90% at the end of the test period), and causes total mortality below 90% at low concentrations.
- Class III: compounds exhibit delayed action: mortality lower than 15% after 24 h but greater than 90% at the end of the test period, over a range of concentrations from 1 to 9 times.
- Class IV: similar to class III, differing from those for having delayed action over a range of concentrations of 10 to 99 times.
- Class V: rare compounds, with delayed action over a range of concentrations over 100 times.

Based on these studies performed on *Solenopsis* spp., a methodology for testing AIs directly in leaf-cutting ants was developed, as substantial differences may exist between different ants (Nagamoto et al., 2004, 2007). Therefore, the chosen species was *A. sexdens rubropilosa*. This classification is very similar to the one used for *Solenopsis* spp. in terms of mortality and classes, but it differs greatly in the feeding of ants separated from the colony and in the formulation of AIs used in the tests.

The results of Nagamoto et al. (2004, 2007), in general, were comparable with those obtained for *Solenopsis* spp., although with some differences, particularly, the result for fipronil was less positive: it was a little less delayed for *A. sexdens rubropilosa* (Nagamoto et al., 2004) than for *Solenopsis* (Collins and Callcott, 1998; Nagamoto and Forti, 1999a; Nagamoto, 2010). In Nagamoto et al. (2004), fipronil is clearly framed in class III, with apparent indication of small amplitude concentrations, while sulfluramid presented class III with an apparent indication of wider range of concentrations, approaching class IV (Nagamoto et al., 2004).

It is noteworthy that the spread of AI in *Solenopsis* and many other ants occurs by trophallaxis, exchange of fluids between the workers because it is inherent to their anatomy and behavior to make trophallaxis with their food. However, morphological and behavioral studies have shown that the same is not true in large proportions with leaf-cutting ants. Thus, it is concluded that the spread of AI in the colonies mostly occurs in other ways, such as direct contact of the workers with the bait (Nagamoto et al., 2004, and included references).

Thus, sulfluramid falls within the class III (with proximity to the class IV) of Vander Meer et al. (1985) and Nagamoto et al. (2004). To discover an insecticide with these characteristics is very difficult. Just to illustrate, in the period 1958-1981, the USDA Department studied 5,730 products, of which only one had the aforementioned characteristics (Williams, 1983). According Vander Meer et al. (1985), for a period of 10 years less than 1% of a total of 7000 insecticide compounds were of delayed action large range of concentrations. The importance of these AI features has been highlighted by studies on their dispersion in the colony (Echols, 1966a,b; Peregrine et al., 1972; Peregrine and Cherrett, 1976; Pretto, 1996; Forti et al., 2007). The dispersion of the insecticide in the study of Echols (1966a,b) in *Atta texana*, indicated that 120 h after the bait was offered, with marked insecticide, over 90% of the individuals had a dyed digestive tract. In Peregrine and Cherrett (1976), made using *A. cephalotes*, this behavior was similar, further demonstrating that the workers of average size are the ones working with the substrate. A similar study carried out in the laboratory with *A. sexdens rubropilosa* using dye as insecticide marker, demonstrated that 24 h after offered dyed bait, about 70% of workers were marked, thus demonstrating good colony contamination (Forti et al., 2007; Pretto, 1996).

In colonies of *A. sexdens rubropilosa*, under field conditions, this dispersion is different, contaminating 40.86 and 49.15% of the population, within 24 and 48 h after the bait is offered, respectively (Pretto, 1996).

Beyond the suggestion of the dispersion through trophallaxis (Echols, 1966a,b), an ingestion of insecticide contaminated fungus could also occur (Peregrine and Cherrett, 1976). However, it is generally believed the workers do not have enough time to ingest the insecticide contaminated fungus (Moreira et al., 1999) because most ants are contaminated 24 h after being in contact with the insecticide bait (Forti et al., 2007; Pretto, 1996), even because the period of time for the fungus to grow on the substrate with insecticide is quite long, and in this interval the ants are already dead. Thus, the workers are contaminated primarily by contact with the bait during the preparation and the incorporation of bait in the fungal culture. There is evidence of the existence of limited trophallaxis in leaf-cutting ants (Quinlan and Cherrett, 1979; Peregrine and Cherrett, 1976; Forti et al., 1993; Moreira et al., 2006; Da-Silva et al., 2009). Even if there is trophallaxis, it does not necessarily mean that this mechanism has a significant role in the dispersion of toxic insecticide baits in colonies (Nagamoto et al., 2004). It is noteworthy that this behavior is not frequent to the extent of sometimes not even being detected (Andrade et al., 2002), and the volume appearing to be very small (Moreira et al., 2006), which is consistent with the relatively low capacity of leaf-cutting ants to ingest and transport liquids, if compared with other species of ants (Paul and Roces, 2003).

Another important feature that should be stressed is that the effective insecticide not being noticeable to the colony's workers, otherwise it would not present the expected lethal effect (Forti et al., 1998).

Moreover, when exposed to environmental heat and light, it must remain stable long enough for it to be carried into the colonies. For example, if the AI is easily photodegraded, efficiency can be compromised (Brennan, 1990), and it will not be able to volatilize or be inactivated when more than 8 h under direct sunlight, since the application of the baits, many times, is done in the morning, on sunny days in warm periods, in which case the foraging of leaf-cutting ants are nocturnal.

It can be said that an AI suitable for use in toxic bait of leaf-cutting ants must necessarily possess all of the following characteristics: Have delayed action on adult workers (Nagamoto, 1998, 2003; Nagamoto et al., 2004, 2007), not be repellent (Forti et al., 1998; Nagamoto, 2003), be well dispersed inside colonies (Pretto and Forti, 1993, 1995; Forti et al., 2007), have sufficient stability when exposed to the environment (Cameron, 1990), and not be overly toxic to humans or other bodies.

Active ingredients (AI) currently used

Sulfluramid: Fluoroaliphatic sulfones, which belongs to the compounds “PFOS, its salts, and PFOSF” (Starkov and Wallace, 2002), is a chemical group in which several compounds show formicide activity (Vander Meer et al., 1985). Studying certain components of the group of the fluoroaliphatic sulfones for *Solenopsis* spp., Vander Meer et al. (1985) reported that they present a general structure R_fSO_2A, where R_f is a fluoroaliphatic radical and A is theoretically just a compatible chemical structure. However, the formula of the most studied component was the fluoroaliphatic sulfonamide, $R_fSO_2NR_1R_2$ where R_1 and R_2 are compatible chemical structures. Among the components studied by these researchers, the most abundant analogous presented the R_f radical equal to C_8F_2-, which is of fundamental importance to put it in the formicide category, and the sulfluramid best suited to control *Solenopsis invicta*. This product has obtained registration in the US to control household pests such as ants and cockroaches, with certain compounds of this new insecticide class having excellent action against hous insecticide on leaf-cutting ants has been widely studied, house flies and mosquitoes larvae. The effect of this both in Brazil and other countries, demonstrating its lethal delayed action, as observed in the *Camponotus pennsylvanicus* ant (Reid and Klotz, 1992).

The bait with sulfluramid has been well accepted by the *Atta* spp. ants, that cut dicotyledonous and grasses, for example, *A. sexdens rubropilosa*, *A. laevigata* and *A. cephalotes*, *A. capiguara* and *A. bisphaerica,* for which it has been reported control from 90 to 100% of the analyzed colonies (Alves et al., 1995; Forti et al., 1993, 2003; Langer Jr et al., 1993a, b; Laranjeiro and Zanuncio, 1995; Nakano et al., 1993; Pinhão et al., 1993; Zanuncio et al., 1992, 1993a, b, c, 1996; Laranjeiro and Alves, 1987; Laranjeiro, 1994). In the case of leaf-cutting ants of the genus *Acromyrmex*, published results showed high efficiency of control at a dose of 10 g of the commercial product (Mirex-S) per colony (Caetano et al., 1993; Pacheco et al., 1993; Zanuncio et al., 1993a, b).

Molecular characterization and mode of action of sulfluramid: The sulfluramid, when present in the body, gets broken, becoming a main component called perfluorooctane sulfonamide (DESFA), which operates in oxidative phosphorylation process (aerobic respiration), interrupting the production of ATP in mitochondria (Schnellman and Manning, 1990) differing from dechlorane that acts in the nervous system. Both sulfluramid as DESFA act in the mitochondria increasing the 4th breathing stage on the succinate. DESFA does not allow the protons coupling in the process of oxidative phosphorylation, blocking his path. That is not the case with the sulfluramid (Schnellmann and Manning, 1990). Thus, there is an interruption in the ATP production, becoming lethal to the insect. The characteristic symptoms of intoxication by sulfluramid in *Atta* spp. workers, manifested by slow movements and a great decrease in aggressiveness due to the energy reduction of their organism. The energy tends to decrease until the metabolism is interrupted, causing the death of the worker.

Mode of action of sulfluramid containing bait in colony: Sulfluramid mode of action in colonies is almost identical to the dechlorane, with the only differences lying in the fact of dechlorane killing the gardener workers more slowly and in the intoxication symptoms, which are predominantly lethargic (sulfluramid). The action of bait with sulfluramid in a colony of leaf-cutting ant in laboratory can be described as follows:

Step 1: Foraging workers carry the bait inside the colony and then the pellets are distributed over the fungus garden;
Step 2: One or more workers hold the bait pellets hanging them in the fungus garden, being licked by many other workers until hydrated, as they do with leaf fragments (Fowler et al., 1991);
Step 3: After the pellets start to become hydrated, they are deposited on the fungus garden and then the workers start to remove small pieces and incorporate them into the fungus garden. This process of incorporation may be started after 6 h after the bait is offered and extends up to 18 h later (Forti et al., 1993). During this process 70% of workers are contaminated with the insecticide as data obtained by Pretto and Forti (1993, 1995);
Step 4: After 3 or 4 days of the bait application, the workers no longer cut leaves, since the colony disorder is very intense (Forti et al., 1993) and one can observe high mortality of gardener workers (2 mm body length). The remaining workers who were not contaminated begin to die by starvation. The ants show the low mobility or even complete immobility as a symptom of intoxication;
Step 5: From day 4 onwards, occurs disorganisation in the fungal culture, with large mycelial growth of micro-fungi, with no possibility of recovery of the mutualistic fungal culture;
Step 6: From the 13th day, only the queen is still alive and she can live up to 40 days, however, in most colonies, the death of all individuals is given between 16-22 days (Forti et al., 1993). Under field conditions living ants have been observed in colonies within 60 days of application (Forti, personal observation).

Use of sulfluramid for the control of leaf-cutting ants: The control of leaf-cutting ants in Brazil, until 1993, was made with the use of organochlorinated insecticides, especially dechlorane. Organochlorines were banned in Brazil in 1985, and the dechlorane was kept as an exception for the control of leaf-cutting ants until 1993, for not having an effective substitute. In 1993 the dechlorane was banned in the country, being then replaced by sulfluramid, after proof of its efficiency (Forti et al., 2007; Nagamoto et al., 2007).

The active ingredient sulfluramid is used in Brazil as an active ingredient in the manufacture of insecticide bait to control of leaf-cutting ants of the genus *Atta* (*saúvas*) and *Acromyrmex* (*quenquéns*), which are the insects that cause greater damage to the national agriculture. The use of toxic baits based on sulfluramid, it is now the main method of control of these pests.

In addition to presenting excellent control, toxic baits represent the only method that offers technical, economic, and operational feasibility in the control of leaf-cutting ants. It presents great advantage over other products and current methods. It features low cost, high performance and low acute danger to workers because its formulation is developed with very low concentration of the active ingredient and its application is localized, dispensing application equipment (Cherrett, 1986d; Cameron, 1990; Moreira et al., 2004b).

The sulfluramid has full control efficiency of leaf-cutting ants with efficiency between 90 and 100% at a dosage of 8 to 10 g/m² (Zanuncio et al., 1992, 1993a, b, c; Forti et al., 1993, Langer Jr et al., 1993 a, b, Nakano et al., 1993; Pinhão et al., 1993; Alves et al., 1995). Sulfluramid is, among the active ingredients registered in Brazil in the form of toxic bait, the only one which has all the necessary characteristics for a safe operation and, as not having a replacement with the same characteristics places, it is the only effective option to control cutting ants (Cameron, 1990; Forti et al., 2007; Nagamoto et al., 2004, 2007).

The toxic baits with sulfluramid are produced in Brazil by national companies that have been operating in this market for several decades representing more than 95% of the formicide bait market. In addition, these baits are exported to several countries in Latin America. This whole problem of finding an effective product is recurrent in Latin America and in some of these countries, sulfluramid baits imported from Brazil are important to facilitate the fight against these pests (Cassanello, 1998; Coll, 1997; Forti et al., 1998, Rivera-Heredia, 2015).

Fipronil (phenyl pyrazoles): Among the new chemical groups used in the control of leaf-cutting ants, the insecticide fipronil, from the chemical group of phenyl pyrazoles, acts on the central nervous system, specifically in the gamma-aminobutyric acid (GABA) system (Tomlin, 2000). This compound acts by contact and ingestion (Tomlin, 2000), which is not ideal for use in toxic baits.

This compound presented high efficiency in most early studies in laboratory colonies and in field for leaf-cutting ants (Forti et al., 1997; White, 1998). It has delayed action in *S. invicta* workers (Collins and Callcott, 1998). However, even with these favorable initial results, it was subsequently found that in *A. sexdens*, delayed action exists only in a narrow range of concentrations (Nagamoto et al., 2004; Nagamoto, 2003), and a large bait rejection can occur with this AI (Forti et al., 2003).

Many insects (beneficial and pests) are highly sensitive to fipronil in low doses, both in young and adult forms (Gunasekara et al., 2007). This AI has soil mobility (EPA, 1998), and the own ingredient fipronil as their degradation products are persistent and toxic (Gunasekara et al., 2007). Fipronil and its metabolites were also detected in water bodies, with profound alteration the structure of invertebrate communities (Mize et al., 2008; Stevens et al., 2011).

The high toxicity of fipronil has been demonstrated for all organisms, including humans. There is a report of people who ingested insecticide baits containing fipronil at 0.01% and showed various symptoms such as vomiting, agitation and seizures (Gunasekara et al., 2007).

The bait based on fipronil has low efficiency for grass-cutting ants (Nagamoto et al., 1999; Zanuncio et al., 1999; Forti et al., 2003). In the study of Buczkowski et al. (2001), in cockroaches, it presented quick action, so it is not ideal to control this semi-social insect. In addition, Ríos de Saluso (2010) found that for *A. lundii*, the fipronil efficiency was only 71%. Thus, since even under well controlled experimental conditions there are inconsistencies, it is evident that the efficiency of AI is not always good in operational conditions of use.

In Brazil, fipronil is registered only for dicotyledonous leaf-cutting ants (*A. sexdens* and *A. laevigata*) and is not registered for grass-cutting ants. Thus, both by issues of toxicity to non-target organisms and by inconsistencies in efficiency under some conditions, and the occurrence of colonies of various sizes and species in the same area in Brazil, the AI is not an alternative that can replace sulfluramid. Therefore, fipronil is not an alternative insecticide for the control of leaf-cutting ants, because its use may affect health and the environment (UNEP/POPS/POPRC.8/10, item b-SC6-5), and because it has low efficiency in this control.

Researches on other active ingredients in toxic bait

Organochlorinated chemical group: The AIs in this group were the first ones formulated in toxic bait as they were available on the market and some of they were efficient. Among the products used, there was the heptachlor, hexachlor, monachlor, pentachlor, aldrin and dechlorane (Gonçalves, 1960a,b; Mariconiand Castro, 1962, Echols and Biesterfeldt,1966; Echols, 1966a,b; Amante, 1968b; Freire and Vanetti, 1968; Juruena, 1984; Kober and Juruena, 1968); Lofgren et al., 1964; Ribeiro and Woessner, 1979). However, most of them caused low mortality to *Atta* colonies, both for its rapid lethal action and the perception of its odor. Among these products, aldrin and dechlorane stood out, due to the high mortality rate caused in the nest, controlling around 95% (Gonçalves, 1960a,b; Mariconi and Castro, 1960; Lofgren et al., 1964; Echols, 1966a,b; Echols and Biesterfeldt, 1966; Amante, 1968a, b; Freire and Vanetti, 1968; Kober and Juruena 1968; Juruena, 1973; Ribeiro and Woessner, 1979; Costa et al., 1997).

Aldrin was established in the control of leaf-cutting ants at a concentration of 2%, however it present-ed different characteristics compared to dechlorane (0.45%) regarding the dispersion and incorporation into the fungal culture, with the former contaminating more gardeners, while the latter acted in a large number of workers of all castes, besides being better incorporated into the fungus. Pelleted baits are broken into smaller particles by the worker when using dechlorane instead of aldrin

(Peregrine and Cherrett, 1974).

Thus, the dechlorane presented several appropriate insecticide characteristics for proper ant control, however, its use is not acceptable in commercial products as it is carcinogenic to humans and accumulate in their fatty tissue, besides the high residual period in the environment, which led its registration being canceled on 1977 in the United States of America (Vander Meer et al., 1985) and on 1992 in Brazil (Forti et al., 1998). Therefore, chlorinated insecticides have been banned and are no longer marketed.

Chlorpyrifos and fenitrothion (organophosphates):

i. Chlorpyrifos: is banned in Brazil by ANVISA for domestic use since 2004 and so in other countries like USA and Argentina (Resolution MSN456/09), as the intoxication can cause brain disorders in children development. Chlorpyrifos is an insecticide that was widely used in domestic environments and its active ingredient is part of the chemical group of organophosphates, with high health risk, leading to problems in the nervous system and deficits in cognitive function (ANVISA, 2004). Plus, it does not present good characteristics for use as formicide bait and is no longer used in Brazil for the control of leaf-cutting ants. It has been shown that the active ingredient kills the ants quickly, has no delayed action at any concentration (Nagamoto, 2003). Thus, the chlorpyrifos is typically a fast acting insecticide on leaf-cutting ants, that is, belongs to Class II, in the insecticide action rating by Nagamoto et al. (2004). Due to this characteristic, the control effectiveness in field conditions is generally low or null (Forti et al., 1998, 2003; Nagamoto et al., 1999), with only a few reports of substantial efficiency (Linket al., 1997; Zanuncio et al., 1999b).

It is noteworthy that bait with this active ingredient was not efficient for grass-cutting ants as *A. capiguara* and *A. bisphaerica* (Forti et al., 1998, 2003; Nagamoto et al., 1999). For other leaf-cutting ants, the efficiency was very variable: (i) low efficiency, from 0 to 40% (Forti et al., 1998, 2003; Cruz et al., 1996; Link et al., 1995, 1999a, b) (ii) high, > 80% (Link et al., 1997; Zanuncio et al., 1999b; Link et al., 2000), or (iii) intermediate (Zanuncio et al., 1999a). So it was indicated, when it presents a great level, an inconsistency and variability in efficiency, a fact that would not be acceptable for a product to be recommended for the control. In fact, in the light of many field-control studies ever made, it can be affirmed that baits containing this AI are inefficient in controlling leaf-cutting ants.

ii. Fenitrothion: by being an organophosphorus and having high vapor pressure (157 mPa) (Tomlin, 2000), this insecticide is also not suitable for use in the formulation of toxic bait. In addition, this insecticide presents contact and stomach action. Its application is made exclusively in thermal fogging solutions (Isenring and Neumeister, 2010).

Cypermethrin, deltamethrin and silafluofen (pyrethroids):

i. Cypermethrin and deltamethrin: these pesticides are not suitable for use in toxic bait formulation. Both have strong contact action and high "knockdown" (Tomlin, 2000; Ware and Whitacre, 2004).

Cypermethrin is highly repellent to many insects, and their use as barrier treatment for "pharaoh” ants have been suggested (Buczkowski et al., 2005). Therefore, this active ingredient does not have the least chance to be used for leaf-cutting ants in toxic bait formulation. Deltamethrin 0.2% is used exclusively in powder formulation (Isenring and Neumeister, 2010).

ii. Silafluofen (Silaneophan, Silaneofane): is not a typical pyrethroid, because it acts mainly by ingestion, although it also has action by contact (Katsuda et al., 2011; Macbean, 2012). On the other hand, pyrethroids are very fast acting. This AI is used in chemical barrier to termites (Katsuda et al., 2011; Macbean, 2012) and insecticides of this type are not typically good AIs for toxic baits.

In a screening test in *A. sexdens rubropilosa* colonies, the AI did not show good results, because the mortality was negligible and there was a quick colony recovery (Forti et al., 1998). If the action is too fast, the ants simply do not carry a lethal amount of bait, leading to total inefficiency (Forti et al., 1998). Thus, this AI is not even a bit promising for use with cutting ant toxic baits.

Hydramethylnon (amidinohydrazones):

The anhydrous amidinohydrazone, containing 0.8% of active ingredient AC 217 300 whose chemical name is Tetrahydro- 5, 5- dimethyl- 2(1H)- pirimidinone, 3- 4-tritluoromethyl) feni1-1-2-4 (trifluoromethyl) phenyl-2-ethenyl propenilidena hydrazone), was registered in the US on 1980 for the control of *Solenopsis* spp. ants under the name Amdro® (Lofgren, 1986). This AI compound belongs to the chemical group of amidinohydrazones, an active ingredient that inhibits the mitochondrial electron transport, with delayed action mode (Hollingshaus, 1987). The amidinohydrazones were investigated by Williams et al. (1980) in laboratory studies for the control of fire ants in the USA and only the chemical AC 217 300 showed promising results, although a new class of toxic substances have been discovered. This active ingredient is quite toxic to *S. invicta* workers and queens, even at low concentrations, but has delayed action, an important feature for the control of ants. This AI has been used to control *Pheidole megacephala* in pineapple plantations in Hawaii (Su et al., 1980; Reimer and Beardsley, 1990) and coconut in Zanzibar (Zerhusen and Rashid, 1992) as well as in citrus ants in South Africa (Samways and Tate, 1985), with relative success.

The biggest disadvantage of amidinohydrazone AC 217 300 is their rapid degradation in the environment by light, probably by the action of UV rays (Vander Meer et al., 1982; Cameron, 1990; CYANAMID, 1999) forcing users to apply the baits for many ant species at times of low lighting (Cameron, 1990; Zerhusen and Rashid, 1992). Hydramethylnon residues were undetectable in pastures where baits were applied for *Solenopsis* spp., within 24 h of application, indicating its instability (Apperson et al., 1984). This is a feature considered undesirable for the production of formicide baits in tropical and subtropical conditions (Forti et al., 1998).

Its use for leaf-cutting ants (*A. texana*) was investigated by Cameron (1990). This author concluded that hydramethylnon (Hydramethylnon) managed to control 80-100% of the colonies but has found a lot of control variability between colonies. Its rapid degradation by sunlight is the likely explanation for the mixed results obtained by Cameron (1990), although Mendonça et al. (1987) and Nogueira et al. (1981) have found promising results for the use of this AI compound.

Other studies were performed to evaluate the potential of this active ingredient in the control of leaf-cutting ants of the genus *Atta*. The results were even more inconsistent, despite the use of high concentrations of the AI in the formulation and higher dosages (Mendonça et al., 1987; Papa et al., 1997; Wilcken et al., 1998).

In the US, there is the Amdro®Ant Block product (AI hydramethylnon), registered for leaf-cutting ant, *A. texana*, for which it has been shown ineffective (Grosman, 2015). In accordance with the Texas Forest Service, Amdro®Ant Block, AI hydramethylnon, shows only 30% of efficiency and there is the need for more than one application, especially for large colonies (Grosman, 2015). Another disadvantage is that this bait cannot be stored for long periods of time due to its relatively short useful life (Grosman, 2015).

Although proven to have delayed action for workers of fire ants *S. invicta* (Williams et al., 1980) and for the leaf-cutting ant *A. sexdens rubropilosa* (Nagamoto, 1998), hydramethylnon was also not efficient to control other ants: this AI was used to *Wasmannia auropunctata*, and the results were not satisfactory (Roque-Albelo et al., 2000; Wetterer and Porter, 2003). Even though it is a delayed action insecticide, this is not enough for many situations, and assigned, again, its inefficiency due to its rapid degradation by ultraviolet and quick worker mortality (Krushelnycky and Reiner, 1998; Harris, 2002).

Despite being a known insecticide for more than 30 years, it has not been registered or used in Brazil for leaf-cutting ants, certainly because they do not have proven effective for them, due to numerous drawbacks related to the AI, reported in this document. Among these drawbacks, the rapid sunlight degradation may be the limiting factor for the use of these substances as an active ingredient in baits for leaf-cutting ants.

***Ethiprole and acetoprole (phenyl pyrazoles)*:**

Ethiprole ($C_{13}H_9Cl_2F_3N_4OS$ – development code: RPA 107382) and acetoprole ($C_{13}H_{10}Cl_2F_3N_3O_2S$– development code: RPA 115782) are the other phenyl pyrazoles which are being evaluated for use with cutting ants (Link et al., 1999a,b; Nagamoto, 2003; Macbean, 2012; Bernardini et al., 2014a, b).

As well as fipronil, ethiprole and acetoprole have contact action and are potent insecticides, with very low DL_{50} and CL_{50} for insects (Nagamoto, 2003; Macbean, 2012a). However, they are not as broad action spectrum as fipronil (Macbean, 2012). Fipronil broad spectrum correlates with its limited effectiveness, with important restrictions to social and semi-social insects. Conversely, ethiprole and acetoprole have a narrower action spectrum insecticide, that is, they are effective in fewer species and groups of pest insects.

Bernardini et al. (2014a, b), considered that bait with this AI (3g/kg, 10 g p.c./m²) is promising based on the results obtained by them with *A. laevigata* and *A. sexdens rubropilosa* with efficiency from 90 to 100%. However, Link et al. (1999a,b) found no promising results, because unlike fipronil, standard of the conducted experiment, ethiprole did not reach 80% of *A. striatus* control and had shocking action. Further studies, more detailed, and independent, should be carried out with ethiprole, before it can be recommended or discarded.

Anyway, it seems that ethiprole and acetoprole do not have potential for future use as effective alternatives for the control of leaf-cutting ants. They are not currently available and the efficiency is doubtful. They are not promising.

***Imidacloprid and thiamethoxam (neonicotinoids)*:**

The imidacloprid and thiamethoxam are neonicotinoids insecticides, which despite having ingestion and systemic action, they also have great contact action (Forti et al., 1998; Tomlin, 2000), and therefore its pointed out that they do not have the desirable characteristics for being used in toxic bait formulation (Forti et al., 1998; Nagamoto, 1998, 2003).

Imidacloprid with entomopathogenic fungus:

Imidacloprid, specifically, when at very low concentrations and associated to the entomopathogenic fungus *Beauveria bassiana* or *Metarhizium anisopliae*, can change the ant behavior by slightly increasing their susceptibility to this fungus in workers isolated from colonies (Santos et al., 2007; Galvanho et al., 2013; Oi et al., 1994). However, this combination is far from feasible for control of leaf-cutting ants, as they only subtly increases the efficiency of products with little or no effectiveness on killing colonies.

Moreover, among these attempts to use imidacloprid in toxic baits, virtually all of them were limited to very different conditions for operational use in the field, and failed to proceed because of the strong contact action that this insecticide has. These recent researches on association with entomopathogenic fungi are limited to behavioral studies and are far from any feasibility of practical application (Santos et al., 2007; Galvanho et al., 2013; Forti et al., 1998).

Such studies, in general, aim for the enhancement of the active ingredients, that is, a synergistic effect: effect greater than the sum of the two isolated assets (Tammes, 1964). In this particular case, there is an attempt of turning these two products which are inefficient when alone into efficient when together, but this did not occur since the effect is only additive (less than the sum of the actions of each active alone).

Moreover, it has not been evaluated in colonies. If it is, quite simply, the act of isolated workers kill the colonies, as in these studies by Santos et al. (2007), Galvanho et al. (2013), little is added, as it is infinitely easier to kill isolated workers than killing small laboratory colonies, and killing small laboratory colonies is much easier than killing adult colonies in the field (Forti et al., 1998; Nagamoto, 2003). Therefore, at least so far, these attempts to combine entomopathogenic with imidacloprid are: (i) very incipient, and, additionally (ii) the results were not promising.

Formicide activity as a function of time

As already pointed out in previous sections regarding the use of toxic baits, it is useful to evaluate the ant activity versus time for workers isolated from the colonies, and this was done for imidacloprid and thiamethoxam. The first presented a substantial, quick, but little ant activity. But the thiamethoxam is a powerful AI, but with very fast action (Nagamoto, 1998, 2003). Additionally, imidacloprid and thiamethoxam have strong contact action (Forti et al., 1998; Tomlin, 2000), therefore, they do not meet the characteristics required for use with toxic baits. They are not promising.

Avermectins (macrocyclic lactones):

The avermectins are macrocyclic lactones with nematicide, insecticide and acaricide properties, obtained from the filamentous soil bacteria *Streptomyces avermitilis* (actinomycetes). The avermectins were identified in essays with natural products with anthelmintic activity for animal health in 1979 (Lasota and Dybas, 1991).

The mode of action of avermectin in vertebrates is in the channel of gamma-aminobutyric acid (GABA) chloride, and, in invertebrates, the exact mechanism by which the chloride channels are activated are unknown (Lasota and Dybas, 1991). In arthropods (Fritz et al., 1979; Albrecht and Sherman, 1987; Mellin et al., 1983) the avermectins increased the permeability of the muscle to chloride ions, thereby reducing the excitatory potential and pulse input resistance, believing this is caused by the reduction in permeability of the cell membrane. Lofgren and Williams (1982) found that avermectin B_1a showed to be a potent inhibitor of the reproduction of the queen of fire ants *S. invicta* Buren at low concentration (0.0025%), in soybean oil. This insecticide also caused low workers mortality in concentrations greater than 0.025% and showed good results in field tests.

Subsequently, Glancey et al. (1982) described the histological effects of avermectin on the reproductive system of *S. invicta* queens. They observed irreversible cells and tissue damage in the queen's ovaries. The damage included hypertrophy of squamous epileptic and porous of the nursing cell's nucleus. No reduction in ovary size or elimination of nursing cells was observed.

Avermectins have shown high activity against fire ants and according to Lasota and Dybas (1991) they have potential for use as an ingestion toxic, resulting in sterilization of queens and the gradual destruction of worker population.

Although these authors believe these facts, we prefer to say that the avermectin can reduce the population of workers in fire ant colonies due to delayed insecticide action response, although field trials with avermectin 0.07% in soybean oil reduced the population by 87% (Lofgren and Williams, 1982; Williams, 1985). Even in laboratory studies the mortality and the decrease in the number of workers was quite variable, with 71 to 100% mortality after 112 days of treatment (Williams, 1983, 1985; Williams, 1986).

Although numerous authors assigned to avermectin a delayed mode of action, there is evidence that avermectins act much faster than dechlorane and sulfluramida to *C. pennsylvanicus,* and avermectin does not operate in a wide concentration range (Reid and Klotz, 1992) and has contact action to *Atta* (Nogueira et al., 1995).

Avermectins are environmentally acceptable because they are used at low concentrations and quickly are lost in the environment, not accumulating in tropic chains (Lasota and Dybas, 1991). It is known that the avermectins B_1a and B_2a degrade rapidly when applied in a very thin layer on surfaces, regardless of the presence of light. However, the presence of light accelerates degradation, resulting in a half life of 4 to 6 h (Macconnell et al., 1989). Avermectins degrade in the presence of ultra-violet (UV) light, in water exposed to sunlight and when dissolved in organic solvents (Lasota and Dybas, 1991; Wislocki et al., 1989; Mrozik et al., 1988). In soil, the product decomposes in at least 13 other products, with a half-life ranging from 20 to 47 days (Bull et al., 1984).

Taking into account the physical and chemical characteristics of avermectins, the commercial product most likely will not have sufficient stability to be commercialized as its rapid environmental photodegradation could compromise the bait efficiency.

Perhaps, because of the features described previusly, Cameron (1985, 1990), has not been successful when tried baits with avermectin in *A. texana,* neither has Vilela (1986) for colonies of *Atta* and *Acromyrmex* in Brazil. In workers of *A. sexdens rubropilosa*, this insecticide caused a mortality greater than the control treatment in three tested concentrations, with mortality concentrated in the early days, but without reaching 90% (Nagamoto, 2003), being therefore included in formicide action class I (Nagamoto et al., 2004, 2007). Similar results were obtained for *S. invicta* (Lofgren and Williams, 1982; Lofgren et al., 1989). This AI did not kill laboratory colonies. The detoxification process is also observable, that is, leaf-cutting ants can metabolize this pesticide which gradually ceases to kill the workers, which reinforces its low efficiency (Nagamoto, 2003).

The abamectin also acts on the leaf-cutting ant queen fertility if direct application is made (Antunes et al., 2000), but direct contact with bait did not appear to be substantial with the queens in colonies, probably due to the lack of trophallaxis between workers and queens and because queens feed basically only on grown fungus. Unlike other species of pest-ants, an AI will only be effective in leaf-cutting ants if it present delayed action on adult workers (Cherrett et al., 1986a; Forti et al., 1998; Nagamoto, 1998, 2003; Nagamoto et al., 2004, 2007).

The work done with avermectin to control leaf-cutting ants does not clarify which key or similar components the authors experimented with (for example, B_1a, A_1a or A_2a etc.). This observation is extremely important, as each one present different insecticide features and avermectin B_1a was developed for plant protection; even though we know that for certain insects the avermectin B_2a has greater activity (Lasota and Dybas, 1991). In addition, according to the information previously exposed, avermectins have not shown good results for the control of leaf-cutting ants, in a way a lot of basic research needs to be done before the product is tested in the field. Hence the urgent need to research avermectins with more criteria for leaf-cutting ants. With the research results that we have today with leaf-cutting ants, we can say that the future of avermectin does not look promising for control.

Spinosad (macrocyclic lactones):

The use of spinosad, a new insecticide class considered low risk, activates the enzyme acetylcholinesterase and prolongs the acetylcholine responses, stimulating the insect's nervous system (California Department of Pesticide Regulation, 2002). *S. invicta* colonies were not killed when treated with Spinosad-based baits in laboratory, while in field they showed 17% efficiency (Oi and Oi, 2006). As the distribution of Spinosad by ants is done by trophallaxis (Barr, 1997), the size of the colony and the foraging rate can influence the effectiveness of the product (Oi and Oi, 2006). Nests of leaf-cutting ants, especially of the *Atta* genus, have large dimensions, and control is not effective when killing only some soldiers and workers of the colony, and so even small colonies can survive (Forti and Nagamoto, personal observations). While the queen stays alive after the colony goes through a period of decline, it returns to normal activity in a process similar to what happens in the application of formulation in chemical powder (Mariconi et al., 1981a,b). Furthermore, the application of Spinosad may reduce the frequency density of other ant groups (non-leaf-cutting ants) in corn crops (Pereira et al., 2010).

Copper oxychloride and copper chloride (inorganic):

The idea that copper oxychloride could be used for controlling leaf-cutting ants was disclosed by Loeck and Nakano (1987), Nakano et al.(1987) and Nakano et al. (1987). At the end of 1987 and early 1988, these substances were tested in field conditions, in the granulated bait formulation for control of *A. sexdens rubropilosa* and *A. laevigata*, verifying that the bait with this active ingredient showed no efficiency in control (Guassu and Crocomo, 1989). In addition, bioassays were developed in laboratory with numerous other possible active ingredients, such as boric acid, borax, potassium chloride, potassium sulfate and copper oxychloride, with the result being highly disturbing, since the colonies which had been used as control and had received baits without active ingredient, also died (Forti et al., 1993).

An investigation started on the reason why the colonies that received baits without active ingredient died. Sample baits were subjected to residue testing and the outcome was that there were very small quantities of dechlorane both in the bait with no active ingredient, as well as in those containing boric acid, borax, potassium chloride and sulfate and copper oxychloride (Forti et al., 1993). It was obvious that ants have died due to contamination by dechlorane because the baits were made of industrial equipment that was formulating the commercial dechlorane bait.

Further evidence of contamination with dechlorane were symptoms of poisoning and death of the workers. Furthermore, analyzing the reports of Nakano et al. (1987) about the stoppage of the cutting activity on colonies treated with copper oxychloride, we have the distinct impression of a contamination by dechlorane.

These authors mention that the colonies treated with copper oxychloride stopped cutting and transporting leaves between 18 and 38 days, while those with dechlorane stopped in about 8 days (Loeck, 2003). From that moment, there was the development of a project with copper oxychloride to investigate their insecticide action advocated by Loeck and Nakano (1982).

Initially, there was a failed attempt to prepare a lethal dose (DL_{50}) curve with copper chloride applied to the oral cavity of ants, as mortality was very low (Forti et al., 1993). Concomitantly, were made in laboratory bioassays with bait formulated in pilot equipment using copper oxychloride in concentrations from 0.2 to 0.8, and no colonies died. Incidentally, copper oxychloride baits formulated in industrial equipment had varying amounts of dechlorane, but in very low concentration. Therefore, we believe that the widely varied results of copper treated colony mortality were obtained due to the fact of baits containing different concentrations of dechlorane.

It proved the inefficiency of copper in the oxychloride and chloride forms to kill leaf-cutting ants (Forti et al., 1993, 1998).

***Boric acid (inorganic)*:**

Boric acid causes mortality of workers of *A. sexdens rubropilosa*, but does not control the colony (Takashi-Del-Bianco, 2002; Forti and Nagamoto, unpublished data). Sumida et al. (2010) also found lower survival of workers of this species, but only *in vitro* essays of isolated colony workers, subjected to treatments with boric acid with different concentrations. Its efficiency in the control of colonies was zero (Forti and Nagamoto, unpublished data), so there is no indication that this AI can be effective for the control of leaf-cutting ants.

***IGRs (including chitin synthesis inhibitors)*:**

Several other chemicals for the control of leaf-cutting ants have been or are being researched. In this context, we found laboratory and field tests within insect growth regulators (not strict sense, including chitin synthesis inhibitors) in different genus of ants. The use of insect growth regulators as an efficient insect control is in the fact that researchers consider that these products act competing in finals enzymes, which are synthesizing chitin (Ker, 1978), so the effect would act to reduce this by providing malformation of workers and even sterilizing the queens.

The IGRs tested for leaf-cutting ants such as fenoxycarb, pyriproxyfen, diflubenzuron, teflubenzuron, tidiazuron, tefluron, prodrone, methoprene has not cause mortality in colonies of leaf-cutting ants and results has not differed from the control treatment (Forti et al., 1998; Nagamoto et al., 2004, 2007).

***Diflubenzuron* (*chitin synthesis inhibitors*):**

As for the mode of action, the most commonly widespread biochemical interference is that the action of diflubenzuron acts directly in the biosynthetic chitin chain (Post and Mulder, 1974; Marks and Sowa, 1976; Verloop and Ferrel, 1977; Hajjar and Casida, 1978, 1979; van Eck, 1979, 1980; Mitsui et al., 1981). It appears that the effect of diflubenzuron happens particularly on the enzyme chitin synthase, and this fact is only explained by the fact that accumulation of uridine diphosphate-N-acetylglucosamine (UDPAGA) (Deul et al., 1978).

Another modification in the body of insects assigned to diflubenzuron is the change in permeability of the cell membrane which may explain the poor cuticle deposition. This fact helped to understand the mode of action of the product PH6040 (diflubenzuron) which prevents the incorporation of thymidine into DNA (Deloach et al., 1981). On the other hand, the enzyme chitin synthase of *Saccharomyces cerevisiae* fungal mycelium is indifferent to the action of diflubenzuron.

Researchers have shown that the diflubenzuron have interference on the composition of the hemolymph (Baronio and Pasqualini, 1984). We cannot, with the research done to date, draw a definite conclusion about the action of diflubenzuron on insects and even we can even believe that its effect cannot be attributed only on chitin biosynthesis, as many researchers claim today. Therefore, we cannot classify the diflubenzuron as an insect growth regulator. The effect of substances such as the growth regulators and diflubenzuron was on the larval development and on fire ant castes, not being toxic to adults (Vinson and Robeau, 1974; Banks, 1990; Banks and Lofgren, 1991; Banks et al., 1977). Moreover, Kruger and Schumann (1993) reported high mortalities of workers in *Leptothorax acervorum* colonies treated with diflubenzuron. However, not enough assessments were made to test the effect of diflubenzuron on fire ants (Lofgren, 1986), perhaps because it is an extremely insoluble substance.

Nagamoto (personal communication) evaluated diflubenzuron in various concentrations about *A. sexdens rubropilosa* adult workers and did not verified significative mortality. In other observations, the effect of high concentrations of diflubenzuron on *Atta* colonies did not changed the population of larvae and pupae. Diflubenzuron was also tested in colonies of leaf-cutting ants of *Atta* genus, under laboratory conditions (Frank and Haden, 1991; Crispolin et al., 1989; Link and Costa, 1993) presenting good efficiency. Other authors (Pacheco et al., 1989; Löeck et al., 1993) also tested diflubenzuron and reported the good efficiency of this AI. Moreover, this efficiency has been challenged (Costa et al., 1997; Pacheco, 1987). This contradiction, regarding the formicide efficiency of diflubenzuron, is more accentuated knowing that this AI, when offered directly to the workers of *A. sexdens piriventris* increases their longevity (Brancher et al., 1991). As discussed initially, insect growth regulators are considered active in the process of chitin formation, though some researchers consider it as lethal to the growth of fungus cultivated by ants (Frank and Haden, 1991; Löeck et al., 1993). Subsequently, the efficiency tests conducted with various insect growth regulators in different concentrations on *A. sexdens rubropilosa* in laboratory also have not detected positive effect in the control (Forti et al., 1998; Forti et al., unpublished data). Therefore, although this substance is considered a formicide and a fungicide for control of leaf-cutting ants, it is believed that these claims lack sufficient scientific basis to support the condition of diflubenzuron as being a good formicide to control *Atta* and *Acromyrmex* (Busoli, 1993).

In colonies of *A. sexdens rubropilosa*, under laboratory conditions, bait containing diflubenzuron to 40, 200 and 1200 ppm was offered. All colonies received 0.5 g of bait, surviving 120 days and no change was observed in the population. In another experiment, diflubenzuron was offered at 400 ppm to other *A. sexdens rubropilosa* colonies, and also they survived without any change in structure of the population (Forti and Nagamoto, unpublished). These facts were also observed for *Solenopsis* sp. colonies that received insect growth regulator substances, which can survive for a year or more, although the population kept decreasing, while in field there are reports of colonies that survived from four to six months (Banks, 1990). Additionally, Nagamoto et al. (2007) did not find any insecticide activity on adult workers. Although the mode of action is still not known enough, we can consider that its supposed effect on chitin should change the young population of the leaf-cutting ants' colony, however, this change was not found. Likewise, if its action happens on the permeability of the cell membrane, making the deposition of cuticle difficult, which should change the young population of the colony, this was not observed. Conversely, if the product acts in the composition of hemolymph, it is believed that it can take action on young and adult ants, however, there is no experimental evidence demonstrating such action mode.

The effects of diflubenzuron in the colonies of leaf-cutting ants are not very encouraging. Initially, we must consider that the best toxics discovered to kill leaf-cutting ants, as sulfluramid, have lethal effect on adults in a few days, and that was not the case with diflubenzuron. In addition, we can see that the diflubenzuron apparently has no lethal effect on the population of ants, neither young nor adult, and neither on the fungus they cultivate.

***Fenoxycarb (juvenile hormone mimics)*:**

Fenoxycarb is a substance from the chemical group of carbamates, but has the property of being aninsect growth regulator, unlike other carbamates which inhibit the action of cholinesterase. The fenoxycarb mode of action in fire ants is unknown (Glancey and Banks, 1988). This carbamate in *Solenopsis* spp. and *P. megacephala* intoxicates larvae and pupae, reduces or stops the production of eggs by the queen and can still change the caste differentiation (Banks et al., 1988; Banks,1990; Reimer et al., 1991).

This pesticide was registered in 1985 in the United States of America under the trade name of Logic for controlling *Solenopsis invicta* and *Solenopsis richteri* (Glancey and Banks, 1988). Because it is an environmentally safe product, it was used in the USA in large-scale applications for *S. invicta* (Banks, 1986), in just a single application (Phillips Jr and Thorvilson, 1989). *Solenopsis* colonies treated with fenoxycarb may have active ants up to 5 weeks or more after the treatment.

Fenoxycarb (0.1, 0.5 and 1.0%) tested in colonies of *A. sexdens rubropilosa* in three consecutive experiments, proved to be inefficient in their control, and no colonies died within 120 days of testing in laboratory conditions (Forti and Nagamoto, unpublished). Cameron (1990) also experienced fenoxycarb (1%) for *A. texana* and was it not promising in control, showing low colony mortality. Interestingly, the fenoxycarb formulated in baits for leaf-cutting ants did not cause any rejection by the workers, being very well accepted, although Glancey et al. (1990) have noted repellency to *P. megacephala*.

More recently Forti and Nagamoto (unpublished) tested fenoxycarb 2% in powder formulation for *A. sexdens rubropilosa* in laboratory, and not even that way have the colonies shown any change in growth, surviving for more than 12 months. Fenoxycarb applied in bait of fire ants altered the tissue of queens' ovaries, causing retro-regression (Glancey et al., 1990; Lemke and Kissam, 1987). However, this parameter has not been studied in *Atta* sp. and *Acromymex* spp. queens.

According to Banks (1990) many insect growth regulators were tested for controlling *Solenopsis* spp., but only a few showed effective action, and lately only fenoxycarb and pyriproxyfen had been proven their effectiveness for *Solenopsis*.

We do not know exactly what happens with insect growth regulators and, neither the reason of its inefficiency in the control of leaf-cutting ants. However, we can hypothesize that perhaps the fungus of leaf-cutting ants degrades these active compounds, and that they are not toxic to adults, neither being able to contaminate larvae by trophallaxis, because this was not found in larvae (Forti et al., 1993).

Pyriproxyfen (*juvenile hormone mimics*):

Recent studies using pyriproxyfen have shown that this substance does not cause mortality of leaf-cutting ants, and does not affect the reproduction of the queen to the extent that changing the position and feasibility of the eggs has been considered (Forti, 2001).

There is evidence that the substances that act as reproduction inhibitors, for example, avermectins and growth regulators, such as methoprene, hydroprene, fenoxycarb, pyriproxyfen, diflubenzuron and others are not efficient for leaf-cutting ants, because it was suggested that the fungus garden acts as a “filter” performing the detoxification of substances. Such evidence was observed by Little et al. (1977). When the juvenile hormone analogue (altozar) was tested against *A. sexdens* in petri plates without fungus garden, there was mortality of adults and larvae, and the pupae development was quite affected (Little et al., 1977). However, when the toxic was introduced in intact colonies, there was little effect detected. These researchers suggested that the presence of the fungus garden “protects” the younger forms possibly via detoxication, once it is worthy to remind that both larvae and adults ingest hyphae of the fungus cultivated *Leucoagaricus gongylophorus*.

Other IGRs:

Products with insect growth regulation properties have shown contradictory results regarding its effectiveness in the control of leaf-cutting ants and other ants. Most studies have been conducted in *Solenopsis* sp., demonstrating quantitative interference in offspring population leading to colony death (Banks et al., 1978, 1983; Phillips et al., 1985; Banks, 1986; Kruger and Schumann, 1993; Etheridge and Phillips, 1976). They can still cause morphological changes on the wings of mating males and females, as well as modify their proportion in the colony (Banks et al., 1978). These substances can cause morphologic alterations in males and females, but they do not sterilize the queen (Banks et al., 1983; Glancey et al., 1990).

In the research by Banks et al. (1978), among the 26 insect growth regulators (IGRs) administered via bait to *S. invicta* colonies, 14 negatively affected the younger forms, 8 killed one or more colonies, and 4 of the best AI compounds killed about 75%: AI3-36206, AI3-36206, AI3-36093, and AI3-35477.The AI3-36206 was acquired by the company Stauffer, coded as MV-678 and the bait with this AI was registered in United States Environmental Protection Agency (EPA) in 1983 with the brand name Prodone®, however, this product stopped being marketed few years later (Womack, 2006).

Despite the promising results for *Solenopsis* sp., the effectiveness of IGRs in the control of *A. sexdens* is unsatisfactory, especially on the offspring, so that its use in the field has questionable efficiency (Little et al., 1977).

The same was observed for *A. capiguara*, with unsatisfactory results in their control (Nakano et al., 1987).

The efficiency of some insect growth regulators and substances inhibiting the chitin synthesis is contradictory in the literature, especially for leaf-cutting ants (Forti et al., 1998), but also for other ants (for example, *Solenopsis* spp.) (Banks, 1986).

The IGRs tested for leaf-cutting ants as fenoxycarb, pyriproxyfen, diflubenzuron, teflubenzuron, thidiazuron, teflurom, prodrone, methoprene did not cause mortality in leaf-cutting ant colonies (Forti et al., 1998; Nagamoto et al., 2004, 2007). Pyriproxyfen studies have shown that this substance does not cause the mortality of leaf-cutting ants, and does not affect the queen reproduction, changing the posture and the viability of eggs (Forti et al., 2003). For leaf-cutting ants, diflubenzuron have not caused any formicide effect whatsoever in laboratory colonies (Forti et al., 1998; Nagamoto et al., 2007). Thus, in the case of leaf-cutting ants, one can conclude that in fact there is no contradiction, and these products are actually ineffective.

There is evidence that these substances that act as reproduction inhibitors are not effective for leaf-cutting ants, since the fungus garden acts as a “filter” doing the detoxification of these substances. This evidence was observed when the juvenile hormone analogue (altozar) was tested in *Atta sexdens* in Petri dishes without the fungus garden, occurring mortality of adults and larvae, as well as a greatly affected pupal development (Little et al., 1977). However, when the toxicant was introduced in intact colonies it was inefficient. The presence of the fungus garden "protects" the young forms possibly via detoxification, as both larvae and adults eat the cultivated fungus.

Other active ingredients (AIs):

Several other AIs are being, or have been, assessed in workers of *A. sexdens rubropilosa* in the Laboratory of Social-Pest Insects (LISP), FCA, UNESP, Botucatu:

closantel, potassium chloride, potassium sulfate, diafenthiuron, endosulfan, cryolite, thiodicarb, propoxur, indoxacarb, emamectin benzoate, cyhexatin, hexythiazox, acetamiprid, lufenuron, cartap, pyridaben, fenpyroximate, metan, white-nitnipyram, yellow-nitnipyram, bifenazate, tetradifon, pymetrozine, azocyclotin, fenbutatin, diafentiuron, phyraphentition, tetrachlorvinphos, dinotefuran, amitraz, chlorfenapyr, cyantraniliprole, flufenoxuron, rynaxypyr, metan + hexythiazox, pyridaben + dinotefuran, phyrphention + imidacloprid, sesamin, buprofezin, etoxazole, fenpyroximate, flufenoxuron, metan, pyridaben (LC Forti, personal communication). Generally, these AIs had no potential for use in toxic baits for leaf-cutting ants.

Chemical control conclusion

While it is desirable that other methods and products are developed and used in the chemical control of leaf-cutting ants, currently, the use of toxic bait with delayed action AIs on a wide range of concentrations is sufficient, viable, and effective. It is desirable develop new AIs making them available for replacing sulfluramid, the most used AI. However, it is extremely difficult and time consuming to develop new AIs or products that are highly viable and efficient in the control of leaf-cutting ants, to the extent of being possible to replace the sulfluramid.

This situation is similar to what happened in the past for other ants. However, it is noteworthy that it is much more difficult to develop AIs or products to control leaf-cutting ants than any other species of pest ant, because neither queens sterilizers, nor insect growth regulators, nor chitin synthesis inhibitors works. The causes of this great difficulty are still far from being well understood, but they are probably related to the symbiotic association of the ants with their mutualistic fungi and other microorganisms. Many and more in-depth basic studies that support applied research should be conducted so that these issues are better understood, allowing, perhaps, the use IGRs in control of leaf-cutting ants. It is noteworthy that, currently:

- A good efficiency is at least 80% of colony control under field conditions, where adult colonies occur,
- The successful preliminary tests in small laboratory colonies are often not repeated in the field, especially if the trial is not made in a single application, and
- The mortality parameter of workers isolated from the colony is an extremely incipient result, because it is very rarely a product that can cause 100% mortality in workers isolated from colonies ends up killing colonies under field conditions.

Since the successful replacement of dechlorane by sulfluramid in the early 1990s, the search for new AIs had no effect so far, despite the many efforts. To develop products that are highly efficient and viable is really an extreme technical and scientific challenge.

Chemical control with toxic bait is still the only one that shows available technology to control leaf-cutting ants *Atta* and *Acromyrmex* with technical, economic and operational viability. Toxic baits employ active ingredients in very low concentration (for example, sulfluramid at 0.3%) in the form of pellets. In addition to their high efficiency, this formulation shows great advantage over other methods such as low cost, high performance and low hazard to humans and the environment. The sulfluramid is, among the active ingredients, the only one who has all the necessary characteristics for a proper function as toxic bait, which places it as the only effective option for the control of leaf-cutting ants (Cameron, 1990; Forti et al., 2007; Nagamoto et al., 2007; Della Lucia et al., 2014). Therefore, its maintenance is very important, and if the production of sulfluramid is discontinued, it could lead to a dangerous setback in leaf-cutting ant control, such as increased pest population and therefore a great loss to the Brazilian agribusiness.

ORGANIC BAITS

Rice-based organic baits

In some small farms in Argentina, it is a common practice to use grains of rice for the control of *A. lundii* (Saluso and Anglada, 2010). Preliminary studies in the field have indicated that rice-based baits are highly attractive to ants of this species, however, they have not shown effectiveness in their control (Anglada et al., 2010). Later, it was decided to continue the studies using broken rice, broken rice + orange juice and broken rice used in increasing doses. In these studies, the treatments based on broken rice had zero efficiency, very different from bait with standard chemical insecticide (Saluso and Anglada, 2010; Anglada et al., 2013).

Carrere (2006) also concluded that rice only prevented the ants from continuing cutting the plants, and had no effect on killing the fungus garden.

Thus, the use of rice grains in baits for control of leaf-cutting ants showed no practical result, as research indicated that they do not have efficiency to control them.

Organic baits based on beer yeast

Organic baits based on beer yeast were not the best treatment for *A. lundii*, as we can see in the text by Borgetto (2009, 2010). It concludes that sulfluramid-based baits were the most effective treatment. These results were also confirmed by Coll (2003) and Anglada et al. (2010). Saluso and Anglada (2010) reported that the treatment with standard insecticide bait showed better results and lower ant activity. These surveys with organic bait based on beer yeast are incipient research and do not detail how the experiments were conducted. The lack of details of the research together with the lack of references in the literature of organic baits based on yeast for leaf-cutting ants hampers a more complete analysis on these baits.

Bait registered for organic agriculture with secondary plant compounds

This product is a granulated bait formulated from the aerial part of the plant *Tephrosia candida* (AGROFIT, 2015; (Kole et al., 1992). This commercial bait is registered in MAPA for *A. laevigata* and *A. sexdens rubropilosa* control, for use in organic agriculture (AGROFIT, 2015). Products for organic farming do not require agronomic efficiency of proof to be registered in MAPA. It should be noted that the same product registered in MAPA for use in conventional agriculture, had his registration revoked for failure in submitting reports proving the agronomic efficiency of this formicide bait. Furthermore, the product recommendation application mentions that if verified the green mass transport by the ants after 3 days, the application should be repeated in a number from 3 to 5 applications. Thus, the product is applied several times until the nest saturation. This practice is not feasible in operational scale.

The total control of the nest may last 60 days (AGROFIT, 2015). This recommendation of reapplication after the 3rd day does not exist for other control methods (AGROFIT, 2015). Normally, a single application of a product is good enough to handle the vast majority of colonies and only one possible maximum transfer is suggested. Therefore, even if that product is really efficient for organic farming, but this cannot be recommended for large areas. Thus, the organic baits based on *T. candida* can, at least, be recommended for very specific and limited cases, for instance, in organic farming, and yet, assuming its efficiency, aspect which should be further investigated, as scientific studies showing the effectiveness of this product for these ants are scarce in the literature.

A test run in colonies of leaf-cutting ants in laboratory (Forti, 1988; personal communication), resulted in no mortality of colonies treated with this ant bait.

A field experiment with colonies of *A. laevigata* held in Viçosa-MG (Zanuncio et al., 1999; personal communication), resulted in only 10% efficiency. Thus, it is necessary to carefully analyze the use of new products, in view of the many mistakes that happened in the past (Nagamoto et al., 2004, 2007; Schoereder et al., 2012).

OTHER PRODUCTS

Diatomaceous earth

Diatomaceous earth (DE) is formed by deposits of fossilized unicellular algae rich in silicon dioxide. It is presented in the form of a powder, fine grain, whose particles have epicuticular lipid absorption capacity in insects, also having abrasive action in the cuticle, thereby acting on the permeability of the cuticle thus causing water loss and consequent death by desiccation. The DE is an insecticide of low toxicity, commonly used to control pests in stored products and in homes and gardens (Quarles, 1992).

The diatomaceous earth association can increase the entomopathogenic or synthetic chemical insecticides action (Quarles, 1992; Brinkman and Gardner, 2001; Drees, 2006), but not enough for a control in field of any social insect. The mortality of *S. invicta* exposed to diatomaceous earth was only 29% after 10 days (Brinkman and Gardner, 2001). DE is typically of contact action and did not eliminate treated colonies of *Solenopsis* (Drees, 2006), which is not surprising since no solid or liquid (non-nebulized), contact action insecticide showed good efficiency in the control of cutting ants. In the case of leaf-cutter ants, Ríos De Saluzo (2010) and Ferreira-Filho et al. (2015) found that in doses from 1.0 to 50.0 g/m² of DE in field experiments, the efficiency was very low, similar to the control treatment, while in the standard treatment (ant bait), the efficiency was 94.54%.

Diatomaceous earth proved to be ineffective in controlling colonies of *A. sexdens rubropilosa* in operating conditions (Ferreira-Filho et al., 2015). Ríos De Saluzo (2010) also points out that the treatments with chemical insecticides were more effective than the treatment with diatomaceous earth when spraying nests of *A. lundii*.

Despite the efforts invested in research to develop new products to control leaf-cutting ants, studies with diatomaceous earth did not produce results that can be used in practice, in operational regime for the control of leaf-cutting ants.

Lime stone

Ravnborg et al. (2001) and Ravnborg and Westermann (2002) suggested to control *A. cephalotes*, in the Colombian *Andes*, by pumping lime inside the nests, replacing the use of insecticides. However, this proposal was made without any consistent basis.

As is apparent Schoereder et al. (2012), although Ravnborg et al. (2001) and Ravnborg and Westermann (2002) have reported the use of lime as an effective method for controlling leaf-cutting ants, they did neither study nor showed the action form of limestone inside the nests and it is unlikely that lime is, itself, a good insecticide. Even though, a possible mortality mechanism could be increased pH of the fungus garden, which would cause changes that could eventually kill the fungus and consequently the ants by starvation. The pH is an important factor for proteins activity in the fungus grown by leaf-cutting ants (Semenova et al., 2011), as well as limiting the growth of parasitic microorganisms which infect fungus gardens (Haeder et al., 2009). Furthermore, Van Gils et al. (2010) reported that *A. sexdens* live preferably in acidic soils, with approximate pH of 4.3.

From this information, Schoerederet al. (2012) studied, in details, the effect of lime to control the leaf-cutting ant *A. sexdens rubropilosa*, evaluating the effect on the fungus cultivated by the ants and their behavior. In the field, the lime has been applied by a pump inside nine colonies, which were compared with nine untreated nests. Nine laboratory colonies were used to evaluate the effect of lime on the fungus cultured by ants, both with and without ants. There was no significant difference in survival between nests treated with lime and untreated, both in the field and in the laboratory, although the behavior of harvester ants apparently changed in laboratory colonies. The application of lime does not control the ants, which was a contrary result to the reported results by other authors. The behavior of ants may have contributed to the inefficiency of the control method, because the ants actively removed the lime deposited on the fungus. Thus, Schoereder et al. (2012) concludes that the lime is inefficient and that alternative controlling methods must be rigorously tested before being recommended. Another point to note is that according to the report of the Stockholm Convention (UNEP/POPS/POPRC.5/10/Add.1) a product to be considered an alternative shall produce: technical feasibility, have no effect for humans and the environment, must have good cost-benefit relation, efficiency, availability and accessibility.

Chemical barrier

In the case of plants, newly planted trees are, due to its smaller dimensions, particularly susceptible to compromising damage by pests in general, and particularly by leaf-cutting ants of the *Acromyrmex* genus because the nests of this genus, depending on the species, are relatively difficult to find and then more efficiently controlled. The chemical barrier is a layer of a substrate (in this case, the ground), which is impregnated with insecticide of quick and/or repellent action.

Given this situation, Vitorino et al. (2015) evaluated the effectiveness of the imidacloprid IA in different dosages by soil conditioner gel in newly-planted *P. taeda* L. seedlings (Pinaceae). However, this procedure lasts only a few weeks or months, not protecting the plants through the following years and still the ants cut the treated plants. Thus, the chemical barrier for plants cannot be recommended as a cutting ant control technique in forestry. With respect to chemical barriers for seed treatment, in pasture there are fipronil-based products registered for *A. sexdens rubropilosa*, *A. capiguara* and *A. landolti*. These products probably protect plants during the initial period of culture development (AGROFIT, 2015). However, there are no scientific studies on this type of chemical barrier which have not been tested in practice. Also, they do not control the colonies of leaf-cutting ants and cannot be recommended as a control technique.

Nanostructured aluminum

Gorosito et al. (2013) evaluated the formicide activity of nano-structured aluminum (ANE) by contact, as well as the load of a (unspecified) food impregnated with the substance to verify the possibility of using it as a vehicle for fungicides or insecticides. These authors found a good load, and that there is formicide activity, killing, within 24 h, 100% of the workers in the largest concentration of ANE (1000 ppm). But it is pointed out that this is a study in early initial phase and that there was assessed only the mortality of *Acromyrmex* workers in laboratory (there was not verified the colony mortality).

In this publication, where authors evaluated the formicide activity of this substance, that cannot be measured much, because this study is only at its early stages, where there were not even data reported about the substance, as composition, formulation, etc. What is noticeable is that this is a substance in powder formulation and has also been tested to evaluate the loading for possible use as a vehicle for insecticides or fungicides. Therefore, there is no data in the literature for a more detailed analysis of nanostructured aluminum for leaf-cutting ants, since the researches are incipient.

Homemade preparations for the control of leaf-cutting ants

Regarding homemade preparations for leaf-cutting ants, there are many recommendations in the manual of ecological alternatives for the prevention and control of pests and diseases by Burg and Mayer (1999).

For the prevention of damage caused by ants, the authors report: a) vegetable seed treatment with diluted soap, b) physical barriers with inverted cones, c) repellent or toxic plants like, *Sesamum indicum*, *Ricinus communis*, rotenone, *Ipomoea batatas*, *Manihot sculenta,* among others.

For the control of the colonies after working with methods that prevent infestation at a higher level BurgandMayer (1999) recommend the following methods: physical methods, homemade chemical methods, biological methods, ant nests debris and toxic plants.

As physical methods, the authors mention the direct action on the ant nest (removing the fungus and the offspring, eggs and killing the queen), fire, running water, exhaust and combustion engine fumes (carbon dioxide). The authors recommended homemade chemical to use, as well as salty water, vinegar, cresol, burned oil, kerosene, gasoline, among others. They suggested the biological methods as fresh manure with molasses and, after the fermentation, putting it in the ant nest. Another suggestion is to use pieces of ant colonies with ground corn to be used in other nests.

In the case of homemade preparations mentioned by Burg andMayer (1999) for leaf-cutting ants, there is no scientific study proving that these preparations are effective to control the colonies of leaf-cutting ants.

Care must be taken with the information contained in this publication for the control of leaf-cutting ants, first because if we seek the information in literature on the biology of leaf-cutting ants we see that they are a very well organized social insects and, in the case of the genus *Atta,* these have large nests, which when adult may have thousands of fungus chambers and be in a soil depth of 8m with millions of individuals (Moreira et al., 2004b). In this case, the recommended products for the control of leaf-cutting ants should take these issues into account. Additionally, these recommendations are totally impractical when thinking about a commercial culture, and even in gardens they have no effective control.

These recommendations by Burg and Mayer (1999) are not scientific recommendations and have not been thoroughly tested in practice. Moreover, they are not feasible, unworkable, inefficient and without possibility of operational use.

Research with homeopathic preparations

Homeopathy has also been investigated through research as a possible control method for leaf-cutting ants. The research is incipient and existing results demonstrate inefficiency and impossibility of use in practice.

The effect of homeopathic preparations obtained the mother dye of crushed and macerated adult *Acromyrmex* spp. and symbiotic fungus *L. gongylophorus* were applied in colonies of these ants, reducing the foraging activity and the movement of the ants in these colonies (Giesel et al., 2012).

However, it did not control the colony and, after a period of 20 days, the activities were normalized. Homeopathic baits were also used in colonies of *A. bisphaerica* in field, but the loading of these baits was 30% lower than conventional, and the baits return was higher in the homeopathic treatment with a mortality rate up to 60% less than traditional baits (Ramos et al., 2013). There is, currently, no indication that homeopathy can be used in the control of leaf-cutting ants.

All new alternative control methods should be thoroughly tested before being recommended for the control of leaf-cutting ants (Schoereder et al., 2012). The efforts to develop alternatives to conventional insecticides seem misdirected, looking for substances that kill the workers and soldiers but not the nests. To develop efficient insecticides, research must turn towards substances that kill the ants that tend the fungus garden, provoking the killing of the colony.

Research on homeopathic preparations are incipient and, moreover, these homeopathic preparations tested so far did not show efficiency, are not available in the market, have no technical feasibility and cannot be used in practice.

RESEARCH WITH BIOLOGICAL CONTROL

The biological control has been defined by many authors, among them Debach (1968), for whom the biological control is the action of parasitoids, predators and pathogens in maintaining population density of other organisms, at a lower level than would normally occur in its absence. That is, biological control is the use or manipulation of natural enemies to control unwanted organisms (Debach, 1987). The biological pest control relies on the use of ecological principles of Applied Ecology, where not only the biological method applied originates control by using biotic agents to create a less suitable environment for the pest species, having as its greatest advantage is the characteristic of prolonging this situation, that is the biological control is essentially permanent and this is one of its most important aspects (Debach, 1987).

According van Den Bosch et al. (1982), biological control is the regulation of the number of plants and animals by natural enemies. Samways (1990), describes that an unwanted organism can be eliminated locally, or, more often, have its presence reduced to a level that no longer cause significant economic damage. Complete eradication is an ambitious goal and rarely reached. A natural enemy that completely eliminates their resources, when going without food or a host, is condemned to his own disappearance. In biological control, one should reduce the population of a pest to an acceptable level that does not cause damage to human health, economy, and environment, but enough to ensure the survival of the organism controller, creating a balance between the two populations.

Flint and Dreistadt (1998) describe that the biological control is any activity of a species that reduces the adverse effects of other species. Natural enemies occurring in the ecosystem are the biological control agents. The impact of a biological control agent results from interactions between the pest species populations and natural enemies. The population is the local individuals group of the same species that are born, feed, reproduce, and die.

Gullan and Cranston (2007) postulate that the regulation of the abundance and distribution of species are strongly influenced by the activities of naturally occurring enemies, particularly predators, parasites/ parasitoids/pathogens or competitors.

Facing all these definitions we can summarize the biological control as a natural phenomenon that consists of animal regulation by their natural enemies or mortality biotic agents (Parra et al., 2002).

Biological control can be natural or applied. The applied biological control can be divided into classical biological control, conservative and augmentative (Parra et al., 2002).

The natural biological control involves the combined actions (biotic and abiotic factors) of the whole environment in maintaining the characteristic densities of the population that is the natural balance. Many potential pest organisms can be maintained in densities far below the levels of damage by natural enemies that occur naturally in the field (Huffaker, 1971; Parra et al., 2002) estimated that 90% of all agricultural pests are kept under natural control.

The applied biological control involves the interference of man and functions in order to increase the antagonistic interactions occurring among living beings in nature. This type of control can be classic, conservative and augmentative. The Classical biological control involves the control agent importation from one country to another or from one region to another in order to establish a biological equilibrium to a given pest. In many cases, the complex of natural enemies associated with a pest-insect may be inadequate. This is especially evident when a pests-insect is accidentally introduced into a new geographic area without its natural enemies, which would involve then the search and the introduction of appropriate natural enemy to the pest or closely related species. A number of studies, however, must be previously held with these control agents so that there is certainty in terms of safety and effectiveness before the program implementation (Huffaker, 1971; Parra et al., 2002).

The conservative biological control involves measures that preserve natural enemies in an agro-ecosystem that is favorably manipulating their environment, like avoiding unsuitable cultural practices, preserving food sources or habitat, use of selective phytosanitaries. Conservation can result in greater diversity of beneficial species such as in a large population of each species, leading to better control of the pests (Huffaker, 1971; Parra et al., 2002).

In augmentative biological control, natural enemies are periodically introduced and released after the mass rearing in laboratory; this control is commercially applied in large areas in various cropping systems around the world. There are three forms of augmentative releases of natural enemies and they can be distinguished between: Inundative release – natural enemies are mass created in the laboratory, being periodically released in large numbers for an immediate pest control effect for one or two generations, that is, these organisms are used as "biological insecticides". It is used in annual crops or in crops where the level 7 damage is very low requiring a quick control of the pest in the early stages of infestation, or in cultures where only one generation of the pest occurs. Inoculative release – natural enemies are released in limited numbers, that is, only a small number is released, with the objective of long-term suppression of the pest population. It is used in perennial or semi-perennial crops and forests. It is typical of the classical biological control. Seasonal inoculative release, where natural enemies are released in greenhouses, with short duration crops, on the pest occurrence period. A large number of natural enemies is released both for immediate control and to enable the growth of the control agent population during the cultivation cycle (Parra et al., 2002).

Leaf-cutting ants natural biological control

The natural biological control of leaf-cutting ants occurs by various organisms that can be found in the nests of ants or near to them. Della Lucia et al. (1993, 2011) present a list of organisms associated with and/or found in the nests of the ants, whose occurrences were recorded in literature. Among these stands out the predators, parasitoids and pathogens a microorganism, among others (Della Lucia, 2011).

The natural biological control of leaf-cutting ants occurs from when the winged females and males (winged forms), leave the mother colony for the mating flight, during the excavation period of the original nest and extends until after the adult nest. According to Autuori (1949, 1950) in a study with *A. sexdens rubropilosa* it was found that the mortality rate of early nests up to 15 months of age is 99.95%. Possible causes of mortality in this phase are the diseases, low fertility of the queen, the decay of the symbiotic fungus and natural enemies as predators, parasitoids microorganisms, among others.

Autuori (1941) reports that there are four "critical periods" the winged females go through for a colony to become adult, able to produce new winged forms. The first critical period occurs when the winged forms (virgin females and males) leave for the mating flight. At this stage birds are usually the most effective predators ofwinged females, as they may attack winged females during flight and most often eat only part of the winged females abdomen.

The second period lasts from 6 to 8 h, and occurs during the incipient nest excavation work after the mating flight (Moser, 1967). This nest founding period represents one of the most critical moments for the survival and establishment of the colony (Wilson, 1971), requiring great effort by the founder. She, in addition to the chamber construction, use their body's reserves to maintain its activities and, until the emerging workers sprout and start foraging (Hölldobler and Wilson, 1990), keeps the parental care, symbiotic fungus cultivation and her own cleaning and the colony's (Autuori, 1942). The success in the foundation and establishment of the new colony, there-fore, depends on the queen survival (Autuori, 1941; Araújo et al., 2003), which most often is compromised because the chance of mortality of it high during the flight and the initial stage of foundation (Autuori, 1950). In the foundation of the nests, the birds still remain as important predators, followed by spiders, frogs, lizards, geckos and other predatory arthropods, such as the beetle *Canthon* spp.

The third hard critical period lasts for 80-100 days and is the stage where the queen is isolated inside the early nest and at this stage main reduction factors in the number of initial nests are the climatic conditions (for example, pouring rain, low humidity for the fungus development, etc.), armadillos and microorganisms associated with incipient nests of leaf-cutting ants. As well as the nutrient content in the soil can interfere in the early nests (Autuori, 1941).

The fourth critical period goes from the appearance of the first adult workers (1st ant hill) until the construction of the second chamber. This period can last up to 15 months. At this stage, in addition to the armadillos and weather conditions, other predatory ants also attack the nests and so does the anteaters (Autuori, 1941).

Leaf-cutting ants natural enemies

Predators: Among the predators of leaf-cutting ants stand out: birds (great kiskadees, hawks, sparrows, thrushes, anis, white-throated kingbirds, etc.), poultry, spiders, lizards, geckos, frogs and toads, anteaters and armadillos, predatory ants (*Nonamyrmex*, *Paratrechina*, *Solenopsis*, etc.), beetles of the *Canthon* spp. genus and mites (*Pyemotes tritici*) (Autuori, 1949; 1950; Almeida, 1979; 1982; Almeida et al., 1983; Mariconi, 1970; Della Lucia et al., 1993; Wilcken and Berti Filho, 1994; Araujo et al., 2011; Borgmeier, 1922). In this revision each group of predators was discussed, as follows.

Birds: In the case of birds, both wild birds as poultry are among the most effective predators of winged females during the mating flight (Borgmeier, 1948; Almeida, 1979; Almeida et al., 1983). Among wild birds that prey on the winged females in flight are the "bem-te-vi" (*Pitangus sulphuratus*), "nei-nei" (*Megarynchus pitanga*), "siriri" (*Tyranus albogularis*), "tesoura" (*Muscivora Tyranus*), "sábias" (*Turdus* spp.), the hawks "pinhé" (*Milvago chimachima*), "anu preto" (*Crotopahaga anu*), "anu branco" (*Guira guira*) and even the sparrow (*Passer domesticus*). On the floor the winged females are attacked by the quail (*Nothura maculosa*), "perdiz" (*Rhynchotus rufescens*) and the "inambus" (*Crypturellus* spp.) (Almeida, 1979). In another survey of birds in *Eucalyptus* spp., Almeida et al. (1983) identified 60 species of birds and correlated them with the number of nests of *Atta* in the area. The authors concluded that the presence of understory in *Eucalyptus* forests and the consequent bird populations *in situ* are factors that contribute in reducing the number of initial ant nests. However, it is noteworthy that the birds prey on the queens at the time of the mating flight, which takes place once a year, reducing the number of initial nests that would be founded. However, the birds are not effective to control nests already founded since these are underground. Several diurnal birds of prey were also found preying on flying individuals of leaf-cutting ants (Camacho et al., 2012). They are part of the natural biological control of those pests when they are establishing new colonies (Araujo et al., 2003; Costa et al., 2008).

Fierro-Calderon (2010) recorded the *Theristicus caudatus* (Boddaert), bird native of South-western Colombia, feeding on the queens of cutting ants. This species, along with the *Bubulcus ibis* (Linnaeus), *Crotophaga ani* (Linnaeus), and *Vanellus chilensis* (Molina) were identified as the main species of predatory birds of the flying ants during the mating flights of leaf-cutting ants (Molina et al., 2010). However, birds in general are simply natural predators of leaf-cutting ants and are effective only during the mating flight when they prey on winged females during flight, and during the foundation of the incipient nests. After the nest is founded birds are no longer effective. Furthermore, the only study carried out in Brazil with birds present in the understory of planted forests of *Eucalyptus* spp. only lasted 13 days in two surveys in the year (Almeida et al., 1983). Therefore, birds cannot be considered as biological control agents as they are not used in applied biological control programs.

Furthermore, it is not a control method and cannot be considered an alternative.

***Ants*:** Other species of ants are predators of *Atta* spp., for example, we have the species *Nomamyrmex esenbecki*, *Nomanyrmex hartigi*, *Paratrechina fulva* and *Solenopsis* spp. (WilckenandBerti Filho, 1994). Fire ants (*Solenopsis* spp.), attack both workers as eggs, larvae and pupae, not infrequently extinguishing initial nests (Lisboa, 1948).

According to Mariconi (1970), the *N. esenbecki* and *N.*

hartigi ants, both Ecitoninae, attack the leaf-cutting ants, but their potential as a control agent appears to be small.

In the case of *P. fulva*, the “crazy ant", which despite occasionally attacking the leaf-cutting ants of *Atta* genus, popular literature report the negative association with these ants.This ant has also been introduced in Colombia for the control of *Atta*, however, in addition to having no effect on the populations of *Atta,* it became an important coffee pest, protecting mealy-bugs (Zenner De Polania and Ruiz Bolaños, 1983; Fowler et al., 1990).

In addition to the existence of many questions about these studies with predatory ants, another point to be noted is that there is no possibility of these ants to be used to control leaf-cutting ants as they are generalist predators and not specific ones.

Beetles**:** In case of beetles, we highlight the *Canthon* genus (Scarabaeidae) which are important predators of winged females during the mating flights (Mariconi, 1970; Araujo et al., 2011). *Canthon virens* and *Canthon dives* beetle species, as they are specific predators of leaf-cutting ants queens, deserved attention. The species *C. virens* attacks queens right after the flight, before the foundation (Forti et al., 1992; Rinaldi et al., 1993; Forti et al., 2012). Soon after the capture of the queens, this predator buries itself in the soil leaving characteristic formations on the surface, indicating its presence. The queens are used to own feeding or the offspring’s. But the female reproductive potential is relatively low, with posture of no more than 3 eggs, the maximum larval period being 26 days and the pupal 23 days, occurring emergence of adults between 34 and 53 days (Rinaldi et al., 1993). Probably the creations of *Canthon* in Paraguay have not achieved success due to the reproductive potential of *Canthon* female being relatively low. Another factor associated with this low number of offspring per female, authors found that, in field conditions, most times, the number of predated queens increases non-linearly with predator density, so there might be isolated or combined factors acting as limiters in the number of explored prey (Fowler et al., 1986; Forti and Boaretto, 1997).

These insects also prey only the winged forms of leaf-cutting ants, which take place once a year when the winged females go out in mating flight, thus they are not effective to control the nests of leaf-cutting ants, which are deep underground nests.

Mites**:** Mites can be phoretic on workers and its winged forms, proliferating in several colonies (Waller and Moser, 1990). Some may live in the waste pots and debris of the colonies in high populations. The *P. tritici* species, obtained in laboratory, was introduced in the hills of an *A. sexdens rubropilosa* nest. Three days later, the nest activity ceased, but after a period of 16 days it returned to its normal activity, recovering fully (Fletchmann, 1981). Generally, mites, although they prey on the ant eggs, they cannot control the colonies by themselves, even as they proliferate in the nest chambers.

Parasitoids (Phorids): Among the natural enemies of leaf-cutting ants are the phorids, diptera from the Phoridae family, which consists of tiny parasitoid flies. Over 20 species of Phoridae are known, which are parasitic of *Atta* and *Acromyrmex* workers (Borgmeier, 1928, 1931; Feener and Moss, 1990; Bragança, 2007).

These phorids were studied by several authors, among them Waller and Moser (1990). The first record of parasites in leaf-cutting ants in Brazil was made by Borgemeie R. in 1922. After that the author published several works with phorids between the 1920s and 1970s. However, in these works there is information regarding the description of the species, with few biological and behavioral data (Bragança, 2011).

The phorids of leaf-cutting ants have several behavioral and biological characteristics that vary from species to species. Among those there is the preference for the place of oviposition and development of immature forms and emergence of adult flies. Some phorids can lay eggs in one or several species of ants. The oviposition site in the ant's body varies among the species of phorid and can occur in the gaster, in the back or in the right of the head or between the mandibles (Bragança, 2011). Their larvae develop in the head or torax of the parasitised ant. In these places, these immature forms feed from the internal content of the host, resulting in death of the ant (Erthal and Tonhasca, 2000; Bragança and Medeiros, 2006). The pupa can be formed inside the cephalic capsule, in the torax, between the mandibles or even on the ground. These are the places from which adult parasitoids may emerge (Bragança and Medeiros, 2006; Bragança, 2007). In the case of parasitoids phorid *Pseudacteon* Coquillett genus, the larvae migrate to the head and, because of this, the species are known as ant-decapitating flies (Porter et al., 1995; Porter, 1998).

Currently there are four known phorid genara that parasitize other leaf-cutting ants of the *Atta* (L.) genus: *Neodohrniphora* Malloch, *Myrmosicarius* Borgmeier, *Apocephalus* Coquillett and *Allochaeta* Borgmeier. According to a review study performed by Bragança (2007), seven species of *Atta* and five of *Acromyrmex* Mayr are parasitized by approximately 35 phorid species. However, the flies are not parasitoids uniquely of leaf-cutting ants, being able to use several species of other ants groups as hosts. An example is the phorids of the *Pseudacteon* Coquillett genus, which parasitize *Azteca instabilis* (F. Smith) (Philpott et al., 2004), *Linepithema humile* (Mayr) (Orr and Seike, 1998) and *S. invicta* (Almeida and Queiroz, 2009).

Flies use different location and recognition guides of the host, which may be, among others, chemical and visual stimuli, for example, movement, color, size or even different released chemicals (Orr, 1992; Moorehead and Feener, 2000; Wuellner et al., 2002).

Gazal et al. (2009) established that *Neodohrniphora elongata* Brown responds to the movements of its host, the *A. sexdens rubropilosa* Forel. But when the stimulus was only chemical, the parasitoid did not attack. In addition, visual stimuli associated with the pheromones of the leaf-cutting ants resulted in a longer inspection time by the phorid.

Environmental variables such as temperature, humidity and light, as well as biotic factors such as size of the head capsule and ants traffic seem to be limiting factors of the actuation of these parasitoids. Studies show that phorid species prefer to lay eggs on more favorable environmental conditions. Such conditions, biotic or abiotic, are responsible for the success of the actuation of the parasitoid and subsequent development of immature forms (Pesquero et al., 1996; Calcaterra et al., 2005; Bragança et al., 2008; Almeida and Queiroz, 2009; Pesquero et al., 2010). Lighting seems to be essential for enabling some species of phorid on recognizing their hosts. In a laboratory study, Bragança et al. (2008) investigated the influence of lighting on the activity of parasitoids phorid of *N. elongata* Brown and *Neodohrniphora tonhascai* Brown species on *A. sexdens rubropilosa.* Ants were subjected to three different brightness levels: high (simulating daytime light), medium (simulating dusk and dawn) and absence of light (simulating the night). There was only an attack of phorid parasitoids under the high brightness level, suggesting that these species of parasitoids phorid are only active during the day and not active during dawn, dusk or at night. The authors concluded that visual stimulation can be an essential factor in the location and recognition of the host for *Neodohrniphora* spp. In field studies conducted by Orr (1992), it was found that the phorids of the *Neodohrniphora curvinervis* Malloch species also attack the host, the ant *A. cephalotes* (Linnaeus), during the daytime. However, according to the authors, these phorid remained active under artificial light at night.

Also, there are known temporal and seasonal patterns of parasitism (inactive at night and in winter, in Texas, according Waller and Moser (1990), there is selectivity on the size (larger phorids pursuing soldiers of *A. cephalotes* in Trinidad (Weber, 1972), parasitism rates and defense by the ants. Different phorid species may have different preferences as to the location of attack in colonies, which may occur in ant hills, tracks or areas of foraging. Tonhasca et al. (2001) found *Myrmosicarius grandicornis* Borgmeier, most often within or near the ant hills, while the species *Neodohrniphora* sp. was found along the foraging trail of *A. sexdens* (L.) colonies. Also, *Apocephalus attophilus* attacks its host only in areas of foraging while they are cutting plant material. They take advantage of this fact to insert eggs between their mandibles. Bragança et al. (2003) found that *A. bisphaerica* Forel is attacked by phorid species *A. attophilus*, *M. grandicornis* and *Neodohrniphora bragancai* Brown. However, these species, although coexisting in the same place, attack the same host at different locations (foraging area and along the tracks).

In Mexico, there were identified *Megaselia scalaris* (Loew) and *Puliciphora* sp. attacking queens of *Atta mexicana* (Quiroz, 1996). Similarly, in Panama, Feener and Moss (1990) found parasitism of *A. attophilus* (Borgmeier) in foragers of *A. colombica*. More recently, in Argentina, Elizaldeand Folgarait (2010) reported 15 species of phorids associated with *Atta* and *Acromyrmex*. These phorid flies can be commonly found parasitizing forager ants (Elizalde and Folgarait, 2012). The richness of parasitoids may correlate positively with the richness and abundance of species of leaf-cutting ants (Elizalde and Folgarait, 2010).

Some studies show that in addition to the above abiotic factors, biotic factors such as the size of leaf-cutting ants workers, may also be responsible for modifying the behavior of the phorid attack, as verified by Silva et al. (2007). In this study, the largest workers, whose head capsules are also larger, were further attacked by parasitoids phorid than the younger workers. This selection will guarantee parasitoids greater reproductive success. Tonhasca (1996) found a positive relationship between the size of the phorids and the head capsule that originated them, concluding that certain species of female will search for the larger workers in the vicinity of the nests to lay eggs. In laboratory, Bragança and Medeiros (2006) studied the parasitism by the phorid species *A. attophilus* and *Neodorniphora erthali* Brown in *A. laevigata*. In this study, the authors found that both the greater parasitoids, such as *N. erthali*, and species which produce a greater number of larvae, such as *A. attophilus*, parasites larger workers.

Guillade and Folgarait (2010, 2011) provided information on duration of the lifecycles of *Apocephalus setitarsus* (Brown), *Myrmosicarius brandaoi* (Disney), *Myrmosicaus gonzalezae* (Disney), and *Eibes feldtphora trilobata* (Disney), parasitoids of *A. vollenweideri*. The natural parasitism varied with the seasons and seemed to be influenced by the extreme drought affecting the place of study. Since the first descriptions made by Borgmeier in the early 20th century, there were several advances in knowledge of the interactions of these insects and three species of leaf-cutting ants (*A. sexdens*, *A. laevigata* and *A. bisphaerica*), these studies suggest that these phorids can contribute to the integrated management of these ants. However, there is no result of practical use in the control of leaf-cutting ants.

Regarding the use of these parasitoids for biological control there is the need for further research both in biology, ecology and behavior of these parasitoids as well as further research aimed at obtaining the laboratory creation of phorid in Brazil (Bragança, 2011).

Efforts of the studies on the use of Phoridae for the biological control of *S. invicta* and *S. richteri*, which were accidentally introduced in the United States for more than 80 years, intensified this research line in Brazil. Currently, the parasitoids of the genus *Pseudacteon* imported from Brazil and Argentina, were successfully introduced in the United States, as populations of these parasitoids are established in Florida, including the success of artificial breeding in laboratory (Porter et al., 2004; Vazquez et al., 2006). In spite of all the studies of those parasitoids for the control of leaf-cutting ants by the current time, phorids cannot be considered as a control alternative.

***Fungi* (*Competing fungi and parasites of leaf-cutting ants mutualistic fungus*):** Many species of filamentous fungi coexist with mutualistic fungus (Weber, 1972; Fisher et al., 1996) of the leaf-cutting ants. The colonies of the leaf-cutting ants are composed of a mutualistic system comprised of ant-cultivated fungus-mutualistic bacteria and filamentous fungi (Currie, 2001a; Mueller et al., 2008; Caldera et al., 2009; Barke et al., 2011; Carlos et al., 2010). But in the fungus garden of the leaf-cutting ants lots of other species of bacteria and yeast can be found (Craven et al., 1970; Pagnocca et al., 1996a,b).

Invariably, leaf-cutting ants cultivate the mutualistic fungus a whitish colored structure called "fungus garden", which is composed of the collected substrate and finely divided by the workers, and by the mycelium of the mutualistic fungus (Rodrigues, 2009). It is known that in addition to this fungus, other microorganisms may be found in the nests of these insects, including bacteria, yeasts and other micro-fungi (filamentous fungi with microscopic structures) and thus could potentially participate in the symbiosis (Craven et al., 1970; Rodrigues, 2009).

It has been shown that ants import a diverse community of microfungi to their nests, probably from soil and from plant substrate that they use to culture their fungus (Rodrigues et al., 2008; Bittleston et al., 2011).

Considered specialized parasite symbiotic fungus, fungi of the *Escovopsis* genus (Ascomycota: Hypocreales) are anamorphic (that is, fungi the present only the asexual reproduction phase) found in almost all gardens of Attini ants (Currie et al., 1999). When present, these fungi can cause a decrease in the garden biomass accumulation, decreased production of pupae, larvae and workers, thus leading to a delay in the growth of the colony (Currie, 2001b). There is evidence that *Escovopsis* is a myco-parasitic fungus that feeds indirectly on hyphae of the symbiotic fungus (Reynolds and Currie, 2004). In addition to this parasite, other filamentous fungi are also frequent, as *Syncephalastrum racemosum*, *Trichoderma* and *Fusarium* (Rodrigues et al., 2005a, b, 2008).

Studies by Currie (2001) and Currie et al. (1999), carried out in Central America indicate that *Escovopsis* is the most prevalent microfunus in the gardens of leaf-cutting ants. However, studies carried out in South America (Nagamoto, 2003; Rodrigues et al., 2005a, b, 2008, 2014) indicates that some other fungi may be equally or more prevalent than *Escovopsis*. A hypothesis to attempt an explanation to this difference would be that there is geographic variation in the prevalence of micro-fungi in gardens (Rodrigues, personal communication).

Ants have defenses such as grooming (cleaning by licking) both of themselves but also of the fungal sponge, freeing them from most of the spores and other microbial structures. Moreover, the sponge is also protected by "weeding" which is removal of sponge fragments with mycelial growth of other fungi (Currie and Stuart, 2001). Metapleural gland secretions, salivary secretions and antimicrobial substances produced by the associated microbiotic also help keep unwanted organisms under control in the fungal sponge (Fernandez-Marin et al., 2013; Poulsen and Currie, 2006; Rodrigues et al., 2008; Aylward et al., 2012; Junior et al., 2001).

For these microorganisms associated to the fungal sponge of leaf-cutting ants, in order to better understand these relationships it is necessary that we know the microbial ecology of the colonies of leaf-cutting ants. Certainly these filamentous fungi, are important to the ants, however, more studies in this area are required to better understand the defensive strategies of leaf-cutting ants and thus develop methods to permeate this defense system. However, there is no result of practical use produced by science for control of ants with micro organisms associated to fungal sponge, so far.

***Entomopathogenic fungi*:** The entomopathogenic fungi are microorganisms that cause pathology to the insects. Various researches have been done with the intention of using entomopathogenic fungi for the control of leaf-cutting ants. However, so far, these studies did not produce any practical result, having technical feasibility, effectiveness, cost-benefit, is accessible and has availability, even for species of *Acromyrmex* that have smaller nests. Difficulties in obtaining the successful use of entomopathogenic fungi in field conditions is possibly related to morphological, mechanical and biochemical defense strategies of leaf-cutting ant colonies, along with their mutualistic fungus and the associated microbiotic, against parasites and pathogens (Forti and Boaretto, 1997). The extraordinary resistance of mutual fungi of leaf-cutting ants to epizootic and epiphytes diseases is due to many factors related to the nest internal hygiene (Kermarrec et al., 1986).

Several surveys were conducted in laboratory and in field with the fungi *B. bassiana* and *M. anisopliae* for *A. sexdens rubropilosa* (Alves and Soza-Gomez, 1983), *A. cephalotes* (López et al., 1999), *A. sexdens piriventris* and *Acromyrmex* sp. (Diehl-Fleig and Silva, 1986; Diehl-Fleig, 1987; Diehl-Fleig et al., 1988; Specht et al., 1994), but without conclusive results.

Silva and Diehl-Fleig (1985) tested different isolates of *B. bassiana* and *M. anisopliae* to control *A. sexdens piriventris*, species of large occurrence in Rio Grande do Sul. Through bioassays in laboratory and field tests, the pathogenicity of fungi to insects was proven. The authors obtained TL_{50} of 2.72 and 3.33 days for two isolates of *B. bassiana*. However, in this study there was no significant difference in the mortality of soldiers and workers. In the field, colonies inoculated with the fungi presented total reduction of external activity 60 days after the application.

The pathogenicity of the fungus *B. bassiana*, *M. anisopliae* and *Paecilomyces farinosus* was also tested on soldiers of *A. sexdens sexdens* in laboratory with 80% efficiency after four days of inoculation (Loureiro and Monteiro, 2005). Isolates ENA 04 of *M. anisopliae* were the most pathogenic for soldiers of *A. bisphaerica* with a TL_{50} of 1.15 days, over 80% mortality during the first three days of application, and increased production of spores in ant corpses (Castilho et al., 2010). These surveys isolated from entomopathogenic fungi are incipient and the efforts seem misdirected, since these surveys with virulence tests are conducted in the laboratory with ants isolated from colonies. There are no practical results for the field colonies.

Jaccoud et al. (1999) tested isolated dry spores of *M. anisopliae* on mini-nests (sub-colonies), of *A. sexdens rubropilosa* inlaboratory. There was a gradual decline in mini-nests up to about 26 days after application, until they began to deteriorate. The authors concluded that the decline of these mini nests after 26th day was neither due to the direct pathogenic action of *Metarhizium*, nor to the initial daily mortality it caused. Results suggest that social stress in these mini nests was sufficient to cause their death and final decline. Thus the symptoms that entomopathogenic fungi cause the colonies of leaf-cutting ants indirectly end up taking them indirectly to death. Thus, one cannot attribute the death of the mini-nests to the action of the entomopathogenic fungus. Ribeiro et al. (2012) tested isolates of *B. bassiana* and *Aspergillus ochraceus* in *A. bisphaerica* workers isolated from the colony. The results show that the mortality of ants was only 50% even when applied to isolated workers of the colonies which is not recommended, because the "social control" is nonexistent. The correct procedure would be applying the fungal spore in colonies at least two years old. For the conditions under which the experiment was conducted, it is expected is that the mortality reaches 100% of the ants.

In *Eucalyptus grandis* forests, promising results were obtained with the use of *B. bassiana* in baits for controlling *Acromyrmex* spp. (Diehl-Fleig et al., 1992), however, the results are inconclusive. Diehl-Fleig et al. (1993) tested isolates of *B. bassiana* and obtained 87.2% of mortality for *A. crassipinus* and *A. heyeri* after 35 days of application, in field colonies, 1.0 x 109 con/0.1g of rice sporulated with *B. bassiana*, *isolated Bsa*. Later Silva and Diehl-Fleig (1995) compared two application forms (direct and lures) from three lineages of *B. bassiana* (B_{SA}, LV and AC) and a *M. anisopliae* (E_{SA}) for the control of *Acromyrmex* sp. In direct application, rice containing conidia of the fungus was inoculated in the nests, while in the application in baits, five MIPIS were distributed containing 10 g of bait, in two applications in a 15 day interval. The results showed 50, 60 and 70% of mortality for the direct application with the BC and LV lineages, BSA and ESA, respectively. Mortality rates were lower when using baits, except for $B_{SA,}$ which for the two application forms showed 60% mortality. Similar results were found for *A. sexdens* as for the application of baits (Warumby et al., 1995).

Ortiz and Orduz (2000) found *in vitro* that some strains of *Trichoderma lignorum*, commonly found in soil, satisfactorily inhibit the mycelial growth of the *A. cephalotes* symbiote. In Cuba, Perez (2002) reports that in nests of *A. insularis*, there was a decrease of 90%, using the MB-1 strain of *B. bassiana* in baits. However, the author does not disclose research details and under what conditions the baits were applied, stating only that the highest efficiency occurs when the bio-preparation is placed directly in the cavities of the nests, where the foraging comes to cease. However, this process takes from 15 to 30 days. In this case, the research methodology reveals the conditions under which the research was conducted. There is the need for more details to understand the effect of *B. bassiana* on *A. insularis*.

Escobar et al. (2002a,b), in a similar study in the region of Chocó, found that the application of the fungi *B. bassiana*, *M. anisopliae* and *T. lignorum* in baits prepared with oatmeal flour and orange juice, decreased foraging activity of *A. colombica* and *A. cephalotes* from the first to the seventh week after the application, but later the activity of the colonies intensely resumed, suggesting a transient effect of the preparations applied as fungi-based baits, having no effect on the colonies of these ants.

Lopes and Orduz (2003) tested baits containing isolates of *M. anisopliae* and *Trichoderma viride* in field for *A. cephalotes*. Results were 100% for baits containing isolates of *M. anisopliae* and 80% for baits containing *T. viride*. However, the time required for the complete inactivity of the colony was 60 days. Also in these field experiments baits, standard insecticide baits were not used in comparison to the baits with isolates of entomopathogenic fungi, but powdered formicides, which is not a good method of control, and addition to the fact that methyl pirimiphos is an organophosphate insecticide with contact action and is not recommended for controlling leaf-cutting ants. In addition, there have been more than one application of baits with entomopathogenic fungi and the right would be applying a single dose. Another noteworthy point is the evaluation form of the experiments, in which the assessment parameter was the flow of foragers on the trails. The flow of the ants on the trails varies widely depending on weather conditions, daytime and the seasons (Nickele, 2013; Caldato, 2014), in which case it would not be the best parameter for the assessment, because depending on the environmental conditions the number of ants could be underestimated. The evaluation time of the experi-ments was also short, since they were evaluated for only 4 weeks (28 days). In field experiments with active ingredients, it is recommended that the assessment to be made after 150 days after the application of pesticides. After this period the nests are excavated (Mapa Normative Ruling No. 42, 2011). In addition to all the points raised in the present study, we stress that there are experimental products not commercially available for leaf-cutting ants.

Entomopathogenic fungi combined with lethal subdoses of insecticides**:** Another strategy which has been investigated was the combined use of pathogenic fungi with lethal subdoses of insecticides. The idea is to cause an initial stress by applying small dose of insecticide in the colonies, in order to make them more susceptible to the action of fungi, thus obtaining control success. Santos et al. (2007) tested isolates of *B. bassiana* and *M. anisopliae* on filter paper discs in Petri dishes, the results show that the mortality of the ants was below 10%. The same authors repeated the test using an isolate of *B. bassiana* combined with sub-lethal doses of the insecticide imidacloprid offered on filter paper discs in Petri dishes. The results show that the mortality of the foragers in these conditions was 64.3%. However, it is worth remembering that although the insecticide have increased the susceptibility of ants for this isolate of *B. bassiana,* this percentage is still low because the insecticides candidates for the control of leaf-cutting ants should present mortality lower than 15% in 24 h and greater than 90% at the 21st day after the application, so it can be considered a good insecticide (Nagamoto et al., 2004). It is worth remembering that these are preliminary laboratory tests, and have not been tested in the field. Even adding sub-lethal doses of insecticides to cause an initial stress in the leaf-cutting ant colony, the efficiency of the entomopathogenic fungi was low. Probably much of this percentage of dead ants (64.3%) is due to the action of the insecticide. Therefore, the use of entomopathogenic fungi in baits for leaf-cutting ants is still in the research phase and there is much to be further investigated in this area so that we can have better results. Even baits with *B. bassiana* and *M. anisopliae*, under field conditions, to nests of *Acromyrmex*, were not promising because the workers disinfect, prune and isolate the fungal culture and, in more extreme cases, the ants leave the nest, which does not happen when the colony gets a toxic bait with a suitable active ingredient. Another factor influencing the action of the fungus on the leaf-cutting ants is the biofilm of bacteria on the cuticle of these insects, which produce antibiotic substances that prevent the appearance of fungi antagonistic to the cultured fungus and provide protection against fungal pathogens such as *M. anisopliae*, to individuals themselves (Mattoso et al., 2012). Unlike other organiza-tions, ants are in advantage due to living socially, regarding the dynamics of diseases (Hughes et al., 2002a,b).

Despite some promising results regarding the microbial control of leaf-cutting ants, in many cases efficient pathogenicity is noted in laboratory, which is not repeated in field conditions, with the widely varying results of control efficiency.

Bacteria**:** The use of *Bacillus thuringiensis* (Bt) has been little explored for leaf-cutting ants. Pinto et al. (2003) isolated Bt from *A. crassispinus* and *A. lundii* and applied it on individuals of the second species in laboratory. The best results were with the isolates HA48 and HA58, with 100 and 80% mortality of ants after seven days, respectively, but without testing for colonies. Entomopathogenics seem to have their focus misdirected, for the control of individuals rather than the colony. In addition, the biofilm of bacteria that secrete antibiotic substances protect the cutting ants and symbiotic fungi from the action of other microorganisms, in addition to the metapleura glands that secrete antifungal substances of broad spectrum, protecting the ants and the entire colony (Estrada et al., 2013).

Regarding the bacterium *Photorhabdus temperata* K122, this was highly virulent for *A. subterraneus* with a mortality of 90% in 24 h, reaching 100% within 48 h (De Paula et al., 2006).

It is worth remembering that these studies were conducted with bacteria in laboratory conditions with workers isolated from the colonies. For this reason, studies are needed with colonies in field. Possibly, when these bacteria are field-tested in colonies the results will differ depending on the specific defense mechanisms used by leaf-cutting ants to defend their colonies. These ants have developed special behavior such as the disposal of the symbiotic fungus and ants infected in the waste chambers, and the morphological characteristics such as the cuticle sclerotization forming a protective barrier, and presence of hair to protect themselves against invading pathogens (Kermarrec et al., 1986; Shultz, 1999; Della Lucia et al., 2008).

Nematodes: The use of nematodes shows no possible use for the control of leaf-cutting ants. There are few possibilities for nematodes penetrate the ants, as they have the cuticle, important protective barrier, allied to the small size of the orifices, as oral opening, labial gland opening, the anus and the spiracles, potential points of entry into larvae. In the adult ants, certain morphological aspects, such as he infraoral filter and the hairiness of the anus, are morphological features that provide protection against intrusion (Kermarrec et al., 1986; Poinar, 1990).

In Brazil, Passos et al. (1995) tested, in laboratory, the pathogenicity of *Steinernema carpocapsae,* in Exhibit formulation in different castes of *A. sexdens piriventris* in bioassays with doses of 100 and 1000 infective larvae (L_3)/cm^2. The authors found that ants dispose the fungus (food) and sick ants in an attempt of isolating the nematodes in the trash, although there was mortality of individuals of the castes and reduced fungus garden volume. However, with all the difficulties for these nematodes to infect the leaf-cutting ants and with the fact that there is only a few studies on them, there is no possibility of using nematodes in control of leaf-cutting ants.

Conclusions on biologic control research

First, to better understand the biological control, it is necessary to know the microbial ecology of the leaf-cutting ant colonies.

Certainly, the filamentous fungi, mutualistic bacteria, other bacteria and yeasts are extremely important for the leaf-cutting ants, but so far there is no viable and feasible alternative in practice so we can use all this knowledge produced by science.

Many species of filamentous fungi coexist with the leaf-cutting ant mutualistic fungus (Weber, 1972; Fisher et al., 1996). With the work of Currie (2001a),, we learned more about a kind of fungus and its possible role within the colony, as well as a mutualistic bacteria. Therefore, colonies of leaf-cutting ants consistof a mutualistic system composed of ant-cultured fungus-mutualistic bacteria and filamentous microfungi. But in fact, in the fungus garden of the leaf-cutting ants there are loads of other species of bacteria and yeast (Pagnocca et al., 1996a,b; Rodrigues, 2009).

In the case of natural enemies, including predators (birds, beetles, ants), the parasitoids (Phoridae flies), nematodes and entomopathogenic fungi, in some cases they generally reduce the populations of leaf-cutting ants, especially during the founding of new nests such, as for the predators. However, the occurrence of these natural enemies is not considered a control method, because it does not have research on using them in applied biological control. The vast majority of researches reports the natural occurrence of these organisms, preying, parasitizing and infecting the ants. In other cases, the research is usually done only in laboratory conditions, having no continuity under field conditions. In the case of the *Canthon* beetle, there were attempts of raising them in laboratory for use in applied biological control of leaf-cutting ants in Paraguay, however, these raisings did not achieve success, probably due to the female reproductive potential of *Canthon* being relatively low. Another factor associated with this low number of offspring per female, authors found, in field conditions, most of the time, the number of predated queens increases non-linearly with predator density, there should be alone or combined factors acting as limiting the number of explored prey (Fowler et al., 1986; Rinaldi et al., 1993).

In the case of entomopathogenic fungi, these are microorganisms that cause insect pathology. Various researches have been done with the intention of using entomopathogenic fungi for the control of leaf-cutting ants. Some of this researches came to be conducted in field with entomopathogenic fungi-based bait (*B. bassiana* and *M. anisopliae*) with *Acromyrmex* nests. However, the results were not promising, because in that case the workers disinfect, prune and isolate the fungal culture and, in more extreme cases, ants leave the nest. Therefore, so far the results are not conclusive and this research produced no practical result having technical viability, the desired efficacy, cost-benefit, accessibility and availability, even for the *Acromyrmex* species that have smaller nests.

Difficulties in obtaining the successful use of entomopathogenic fungi in field conditions are related to morphological, mechanical and biochemical defense strategies of leaf-cutting ant colonies, along with their mutualistic fungus and the associated microbiotic, against parasites and pathogens (Forti and Boaretto, 1997). According Kermarrec et al. (1996), the extraordinary resistance of mutual fungi of leaf-cutting ants to epizootic and epiphytes diseases is due to many factors related to the nest internal hygiene.

The phorid flies (Diptera, Phoridae) despite parasitizing the workers of *Atta* spp. and *Acromyrmex* spp., present low levels of parasitism (5%) (Bragança, 2011), a fact that makes its use impossible nowadays.

The use of nematodes does not have any chance of use for the control of leaf-cutting ants. According Kermarrec et al. (1986), there are few possibilities for the nematodes to penetrate the ants: the cuticle, important protective barrier, allied to the small size of the orifices, as oral opening, labial gland opening, the anus and the spiracles, potential points of entry into larvae. In adult ants, certain morphological aspects, such as the infraoral filter and the hairiness of the anus, are morphological features that provide protection against intrusion. Another noteworthy expected is that there is little research on nematodes for leaf-cutting ants.

Thus, despite all this knowledge of the past few years produced by research with biological control of leaf-cutting ants, we still do not yet have a chance to use them in practice. It is certainly a field for a promising research, but currently the need for basic biological knowledge is clear, so that control strategies for leaf-cutting ants can actually be applied.

Although leaf-cutting ants possess "defenses that help them to counterbalance the effect of the control measures and allow them to inhabit the soil, in an environment that is heterogeneous and abundant in microorganisms', the mortality of the nests is fairly large in nature and the researches by (Autuori, 1942), found that the mortality from the foundation up to 3 years old reaches 99.95%. Perhaps the diseases can contribute to the number of mortality, but there are no studies that scientifically prove the importance of microorganisms. It is not known which key species cause mortality.

It is even more critical when considering symbiotic bacteria because these studies are new to science (Currie et al., 1999; Currie, 2001a).

Although several literature reviews address and discuss the biological control of leaf-cutting ants (Della Lucia et al., 2014; Montoya-Lerma et al., 2012; Zanetti et al., 2014; Zanetti, 1998), in practice we are far from using this science knowledge, although we have to, as scientists, always think about the development of research in the future applications of this control knowledge. In short, and facing the facts noted in the literature, the applied biological control of leaf-cutting ants is impraticable and unworkable today. There is a long way still to go to reach the proper techniques of biological control that can be used in practice in operating regime for the leaf-cutting ants.

So far there is no applied biological control program to reduce populations of leaf-cutting ants. Certainly, the biological control, also with entomopathogenic fungi is a promising research field, but, currently, the need for basic biological knowledge is clear so that control strategies can actually be applied. With the scientific results presented in this review, the applied biological control is still far from being used in the integrated management of leaf-cutting ants. Unfortunately, the biological control with micro-organisms, predators, and parasitoids, is not a tool that can be used in IPM.

PRODUCTS WITH BOTANICAL ORIGIN

Studies of plants with insecticidal and fungicidal properties

Some plant species of the Asteraceae (Howard et al., 1988; Giraldo-Echeverri, 2005; Castaño-Quintana et al., 2013), Apocynaceae (Boulogne et al., 2012), Euphorbiaceae (Bigi et al., 2004; Alonso and Santos, 2013), Myrtaceae (Marinho et al., 2005), Fabaceae (Aubad-Lopez, 2011; Valderrama-Eslava et al., 2009; Rodrigues et al., 2008; Castano, 2009), Calophyllaceae (Boulogne, et al., 2002), Caesalpiniaceae (Howard et al., 1988), Convolvulaceae (Hebling et al., 2000a,b), Clusiaceae (Howard et al., 1988), Curcubitaceae (Palacios and Gladstone, 2003), Meliaceae (Leite et al., 2005; Bueno et al., 2005), Myristicaceae (Pagnocca et al., 1996a,b), Rutaceae (Biavatti et al., 2002; Miyashira et al., 2012), Pedaliaceae (Bueno et al., 1995, 2004; Ribeiro et al., 1998), Piperaceae (Pagnocca et al., 2006), Phyllanthaceae (Escobar et al., 2002a,b), Simaroubaceae (Peñaflor et al., 2009) and Solanaceae (Boulogne et al., 2012) families may contain apparent toxicity to leaf-cutting ants.

Among those families are found the main species used in researches with leaf-cutting ants, with insecticidal and/or fungicidal activities. For an understanding on toxic plants, we list the species used in the research, as follows: *R. communis* (Hebling et al., 1996; Bigi et al., 1998; Kitamura et al., 1999; Bigi et al., 2004; Caffarini et al., 2008), *Sesamum* (Barbiellini, 1926; Gonçalves, 1944; Pagnocca et al., 1990; Betella, 1990; Hebling et al., 1991; Bueno et al., 1995; Ribeiro et al., 1998; Peres Filho and Dorval, 2003; Morini et al., 2005; Bueno et al., 2004; Peres Filho et al., 2002), *I. batatas* (Hebling et al., 2000), *Canavalia ensiformis* (Mullenax, 1979; Hebling et al., 2000a,b; Rodriguez et al., 2008; Valderrama-Eslava et al., 2009; Aubad-Lopez, 2011; Varon, 2006), *Raulinoa echinata* (Biavatti et al., 2005), *Simarouba versicolor* (Peñaflor et al.,2009), *Manihot esculenta* (Santos et al., 2013), *Melia* (Caffarini et al., 2008), *Trichilia* (Caffarini et al., 2008), *Hymenaea courbaril* (Howard et al., 1988), *Melampodium divaricatum* (Howard et al., 1988), *Vismia baccifera* (Howard et al., 1988), *Tithonia diversifolia* (Giraldo-Echeverri, 2005; Castano, 2009), *Cedrela fissilis* (Bueno et al., 2005), *Cipadessa fruticosa* (Leite et al., 2005), *Virola sebifera* (Pagnocca et al., 1996a,b), *Otaba parvifolia* (Pagnocca et al., 1996a,b), *Hellietta puberula* (Almeida et al., 2006), *Cucurbita máxima* (Palacios and Gladstone, 2003), *Azidarachta indica* (Bigi et al., 2004; Gruber and Valdix, 2003; Herrera, 2009), *Phyllanthus acuminatus* (Escobar et al., 2002a,b), *Clibadium asperum* (Escobar et al., 2002a,b), *Mammea americana* (Boulogne et al., 2012), *Nerium oleander* (Boulogne et al., 2012), *Nicotina tabacum* (Boulogne et al., 2012), *Gliricidia sepium* (Gruber and Valdix, 2003), *Havenia dulcis* (Specht et al.,1994), *Aleuritis fordii* (Specht et al., 1994) and*Hura crepitans* (Varon, 2006).

The active substances of plant extracts are responsible for deleterious effects, but little is known about its mode of action in these organisms. Below, we describe the most known substances with an adverse effect on workers and on the symbiotic fungus of leaf-cutting ants, which are: terpenoids (caryophyllene, caryophyllene epoxide, kolavenolandnerolidol) for *A. cephalotes* and *L. gongylophorus* (Howard et al., 1988), terpenoids and alkaloids for *A. cephalotes* and *L. gongylophorus* (Aubad-Lopez, 2011; Valderrama-Eslava et al., 2009; Rodrigues et al., 2008; Castano, 2009), demethylhomopterocarpin with fungicidal activity for *Atta* spp. (Mullenax, 1979), mexicanolidelimonoids for *A. sexdens rubropilosa* and *L. gongylophorus* (Leite et al., 2005), ricinine with synergistic activity of fatty acids for *A. sexdens* *rubropilosa* and *L. gongylophorus* (Bigi et al., 2004), sesamin, mix of fatty acids and lignan for *A. sexdens rubropilosa* and *L. gongylophorus* (Bueno et al., 2004; Ribeiro et al., 1998; Bueno et al., 1995), epigalgravin, lignans and sesamin for *A. sexdens rubropilosa* and *L. gongylophorus* (Pagnocca et al., 1996a,b), alkaloids, such as dictamnine, anthranilic acid and kokusaginine for *A. sexdens rubropilosa* and *L. gongylophorus* (Almeida et al., 2006), sesquiterpene farnesol as a temporary repellent for *A. mexicana* (Palacios and Gladstone, 2003) and cardenolides such as kaneroside, neridiginoside, neritaloside, neriumoside, nerizoside, odoroside, and finally nicotine (Boulogne et al., 2012). These active substances are present in plant extracts of different plant parts (leaves, flowers and seeds), however, we donot know the exact concentration of these actives, only the dosage of the extracts. It is noteworthy that the majority of tests with extracts were performed in a laboratory, with small colonies or groups of workers and symbiotic fungus, with an *in vitro* cultivation of symbiotic fungus and extract (Pagnocca et al., 1996a,b).

The mode of action of most of those compounds is still unknown, probably because of the difficulty in conducting physiological studies, common to a proper scientific accuracy. However, few researchers have been studying the mode of action of the compounds of toxic plants. For example, Bullatacin is a compound isolated from plants of the Annonaceae family, with a great potential as an insecticide. Such a compound causes a strong inhibition of cell respiration with an antagonistic effect on electron transport in the mitochondria, with specific action in the complex I (Ahammadsahib et al., 1993), however, up to now we still donot know about the progress of that research. Other compounds are being studied with a neurophysiological focus (approach), such as the silphinenes, a compound extracted from the leavesof the *Senecio palmensis*. This tricyclic sesquiterpene acts as antagonist of the ϒ-aminobutyricacid (GABA) system, more specifically chlorine chloride receptor channels (Bloomquis et al., 2008). Another active ingredient with neurotoxic activity is the monoterpenoid pulegone-1,2-epoxide isolated from the *Lippia steochadifolia* plant (GrundyandStill, 1985). The authors verified that this compound acts as carbamates with an irreversible inhibition of the acetylcholinesterase enzyme. However, a biochemically specific action, such as acetylcholine sterase inhibition, is related to the insect-plant co-evolution, that is, plants produce chemical compounds as a defense against herbivorous insects generally by producing substances of the terpenoids class (Ryan andByrne, 1988), but none of those active principles are marketed. They identified 6 terpenoids (pulegone, gossypol, citral, linalool, bornyl acetate and cineole) that inhibit the acetylcholinesterase enzyme, with effects of paralysis and insect death.

The substances with botanical origin also act antagonistically in biogenic amines, such as neurotransmitters, neuromodulators and neurohormones (Roeder, 1994).

The most studied are the octopamine (OA) and the tyramine (TA), both considered receptors and couplers of G-protein (GPCRs) (Vernier et al., 1995; Blenau and Baumann, 2001). The activation of GPCRs leads to change in intracellular concentrations of secondary messenger's cyclic adenosine 3',5'-monophosphate [cAMP] and [Ca^{2+}], whose function is to modulate information on the nervous and cardiovascular systems, cellular growth and differentiation, and general metabolism. In insects, the AO is synthesized from the TA through hydroxylation of the β-carbon, thus the TA is considered a direct precursor of the OA (Bischof and Enan, 2004). Plant essential oils (eugenol, α-terpineol, cinnamyl alcohol, thymol, carvacrol, L-carvone) are related to cellular changes, indicating that the toxicity of these chemicals is mediated by the TA receptor (Enan, 2001; Enan, 2005). But these substances are not marketed so far.

Meanwhile, researches goes on only in laboratory conditions, and do not advance, in most cases without the scientific continuity of discoveries or a researchline in particular, as in the case of Bullatacin, silphinenes, pulegone-1,2-epoxide, pulegone, gossypol, citral, linalool, bornyl acetate, cineole, as well as the antagonists of biogenic amines. Generally, researches include the offering of leaves, plant extracts and seeds to the colonies of leaf-cuttingr ants in laboratory, or in the use of plant extracts in the in vitro symbiotic fungus. Below, we describe the main research with leaves, plant extracts and seeds, as well as scientific discoveries.

Regarding the leaves directly offered to the colonies of leaf-cutting ants, Barreto (1930) was the first to observe the deleterious effect of sesame *S. indicum* to colonies of leaf-cutting ants, however, it was just a statement, without following any scientific method. Thus, the action of sesame *S. indicum* as a controller in nests has already been researched and reported for several decades (Barbielini, 1926; Borges, 1926), despite its insecticidal action being challenged in a laboratory experiment by Gonçalves (1944). Decades later, using the scientific method, Bueno et al. (1995, 2004) observed that initial nests maintained in the laboratory and treated exclusively with *S. indicum* (sesame) leaves were drastically affected and that toxic compounds for ants would be concentrated in the metabolic extract of this plant. An exclusive and continuous supply of *S. indicum* (sesame) leaves promotes a change in the respiration rate of ants, inducing a physiological response in the *A. sexdens rubropilosa* workers (Hebling et al., 1991). According to Hebling et al. (1996), laboratory nests of *A. sexdens rubropilosa* fed daily with *R. communis* (castor) leaves showed a gradual decrease in the fungus garden volume and an increase in the mortality of ants, with complete extinction of the nests after six weeks of treatment. Ribeiro et al. (1998) reported that sesame extracts added in a culture medium can cause up to 60% inhibition in the *in vitro* development of symbiotic fungus. Hebling et al. (2000a,b) exclusively and continuously fed colonies of leaf-cutting ants (*A. sexdens*) with *I. batatas* leaves for 5 weeks. Authors found a fungus garden reduction, as well as higher oxygen consumption by the ants, attributed to the *I. batatas* leaves, suggesting a physiological response to toxic effects. As per the experiment described above, Hebling et al. (2000a,b) offered *C. ensiformis* leaves for 11 weeks, achieving a high mortality of workers and reduction in the fungus garden volume. A similar fact was observed by Valderrama-Eslava et al. (2009), who during two months offered leaves of *C. ensiformis* and *T. diversifolia* to colonies of *A. cephalotes* under laboratory conditions. The authors observed a strong deleterious effect to the fungus garden, which consequently affected the survival of the workers.

Granulated baits containing leaves, branches and sesame seeds (*S. indicum*) showed lower results than toxic baits in field conditions (Peres Filho and Dorval, 2003). This is the only study found in the literature that was conducted under these conditions, and unfortunately it is not possible to draw conclusions because the results were not statistically analyzed, furthermore, the authors suggest that further studies should be conducted in order to improve baits containing leaves and seeds of *S. indicum*.These results show the inefficiency of these preparations compared to sulfluramid-based insecticide baits.

It can be concluded that continuous and exclusive offer of leaves to incipient colonies of leaf-cutting ants can mislead of the actual toxicological potential of plants. We can enumerate some methodology flaws:

- Studies only use incipient nests with hundreds of individuals, however, an adult nest of the studied species contains millions of individuals (Hölldobler and Wilson, 1990), therefore prevents a direct application of the leaves in the field;
- The effects are observed in the colony as a whole, which can lead to misinterpretation, is the ant or the symbiotic fungus under the action of the natural insecticide?
- The continuous and exclusive offer of leaves to colonies promoted a nutritional deficit to the symbiotic fungus, consequently, the workers, larvae and queen were also starving, simulating a possible toxic effect.

The last item can be proved by the study by Camargo et al. (2008) who continuously offered only plant species of high acceptability toincipient colonies of *A. sexdens rubropilosa*, showing that some plant species lead to a reduction of the fungus garden, less staphylae (gongylidia group, food), reduced brood, weight of the queen and oviposition rate (Camargo et al., 2013).

Other researches had been conducted under field conditions, but these were aimed at protecting plants against herbivory (Giraldo-Echeverri, 2005) by reduction of temporary activity of ants (Escobar et al., 2002a,b; Gruber and Valdix, 2003; Mullenax, 1979). None of these researches aimed to control colonies and they require other methods to be applied, as they alone will not solve the problem of losses caused by leaf-cutting ants, besides the fact that they are not available in the market, or are not economic (Gruber and Valdix, 2003) and therefore are technically unfeasible.

Regarding plant extracts taken from leaves, stems, fruits and seeds, these have much deeply studied, but all the effort has been focused on laboratory experiments and in most cases without a detailed continuity on the nature of the compounds, as well as the artificial synthesizing of the molecule. Howard et al. (1988) studied plants *H. courbaril*, *M. divaricatum* and *Vismea bacífera*, extracting the terpenoids: caryophyllene, caryophyllene epoxide, nerolidol and kolaenol. In the laboratory, on a liquid artificial diet for biotests on ants and culture medium with agar withthe symbiotic fungus, they found an insecticidal action and inhibition of fungal growth, except with caryophyllene who had no action in the mortality of ants.

In the same line of research, with an *in vitro* fungus culture, Pagnocca et al. (1990) verified that the extract of *S. indicum* promoted a decrease in the *L. gongylophorus* growth, however without identifying the compound of that extract. Later, the authors found the same result with lignans of *V. sebifera* and *O. parvifolia* (Pagnocca et al., 1996a,b). Lignans commonly occur in plants, with more than 500 compounds, with antitumor, antimitotic and antiviral activity (Macrae and Towers, 1984). Pagnocca et al. (1996a,b) have concluded that lignans were the most powerful compound for the symbiotic fungus, and because of that, ants do not cut the leaves of the *V. sebifera*. With a chromatographic analysis of the *S. indicum* extracts, Ribeiro et al. (1998) purified substances with fungicidal action, namely tetradecadonoic, hexadecanoic, 9-12-15 octadecatrienoic, octadecanoic, eicosanoic and docosanoic, but the attempt to isolate the substances was unsuccessful. A similar result was found with fatty acids and raw extract of *C. ensiformis* (Monteiro et al., 1998). With this knowledge, Castro Faria and Sousa (2000) used *S. indicum* seeds to the culture medium of the symbiotic fungus, and concluded that the seeds may affect its growth.

The citric seeds were also studied regarding its action on the symbiotic fungus (Fernandes et al., 2002). The authors extracted oils from *Citrus sinensis*, *Citrus limone* and *Citrus reticulata* seeds, adding them to the culture medium of the *L. gongylophorus*. Surprisingly, the results showed a low toxic effect, with only the sesamin in a high concentration (70 μ/Ml) showing a fungicidal effect.

These results differ from previous studies. Ribeiro et al. (1998) showed total inhibition of the fungus at low concentration (2.5 µ/Ml). Later, Bigi et al. (2004) stated that fatty acids are important compounds against the fungi of the leaf-cutting ants, when they studied the activity of the *R. communis* extracts, however, it is unknown which fatty acid was responsible for the inhibition of the fungus.

C. fruticosa and *C. fissilis* show extracts in the dichloromethane and hexane fractions with inhibitory effect in the symbiotic fungus (Bueno et al., 2005; Leite et al., 2005). Almeida et al. (2006) isolated six substances of *H. puberula*, among them anthranilic acid, kokusaginine, maculine and dictamnine, which show a strong effect in the inhibition of the symbiotic fungus, these alkaloids being a promising source of new substances for leaf-cutting ants control. Another alkaloid also showed similar results, the 4,5-dimetoxicantin-6-ona extracted from the *S. versicolor* (Peñaflor et al., 2009). Caffeine (1,3,7-trimethylxanthine) was also used to evaluate the *in vitro* growth of the symbiotic fungus of the *A. sexdens rubropilosa*, and concentrations of 0.1 and 0.50% caused a severe mortality of the fungus (Miyashira et al., 2011) in the laboratory.

The *Senna alata* plant also shows fungicidal activity, as its leaves contain compounds of alkaloids, phenolics and terpenoids for leaf-cutting ants, but when conducted in the laboratory (Boulogne et al., 2012). The authors also verified that extracts of *Allium cepa, Allium sativum, Lycopersicon esculentum* and *M. esculenta* showed a weak fungistatic or fungicidal effect, depending on the concentration used. All these experiments showed low potential of application to the control of leafcutter ants. Souza et al. (2012) studied the influence of the extract of 24 plant species added to the culture medium of the symbiotic fungus, concluding that some species show a deleterious effect, while others promote a better development of the fungus. Deleterious species are *Physocalymma scaberrimum*, *Amburana acreana*, *C. fissilis, Magonia pubescens*, *Leucaena leucocephala* and *Swietenia macrophylla*, while the beneficial species to the symbiotic fungus are *Genipa americana*, *Hevea brasiliensis*, *Inga edulis* and *Vochysia divergens*. Franco et al. (2013) following the same previous methodology, verified that *Zizyphus joazeiro, A. sativum* and *Croton urucurana* inhibited the growth of the fungus *L. gongylophorus*, but *Parapiptadenia rígida* was inefficient.

Regarding the insecticidal activity of toxic plants, we know that terpenoids, caryophyllene epoxide, nerolidol and kolaenol isolated from *H. courbaril*, *M. divaricatum* and *V. bacífera* showed insecticidal activity when fed to workers in an artificial diet (Howard et al., 1988). Leave extracts of *R. communis* in the hexane and dichloromethane fractions showed an insecticidal activity, in topical application and by ingestion (Bigi et al., 1998). Fatty acids and ricin were toxic to workers of *A. sexdens rubropilosa*, in the 0.2 and 0.4 mgmL^{-1} concentrations, in ingestion treatments (Bigi et al., 2004). However, the continuous and intentional ingestion of a diet with these extracts may cause toxicity to them, that is, a methodology error. The continuous application of the diet causes a cumulative toxicity to workers, which leads to error, that is, high mortality.

Oils extracted from *Citrus* seeds showed a strong insecticidal activity against workers of *A. sexdens* when diluted in ethyl acetate in topic application, but additional studies should be conducted, due to the high attractiveness of the leaf-cutting ants to the citric pulp (Fernandes et al., 2002; Fernandes et al., 1976). Bueno et al. (2005) showed a high mortality of workers of *A. sexdens* when they were fed continuously with a diet based on extracts of roots and leaves of *C. fissilis*, however, authors have not identified the compounds responsible for the mortality. Similar results were found by Leite et al. (2005), where hexane and dichloromethane fractions of the extract *Cipadessa fritucosa* affected the survival of workers in the laboratory, without identifying the compounds. On the other hand, Biavatti et al. (2005) conducted an X-ray diffraction analysis of compounds extracted from the stem of the *R. echinata*, where the limonoid limonexic acid showed high toxicity against *A. sexdens* workers in the concentration of 200 µg on each artificial diet. Morini et al. (2005) verified that the toxicity of the sesame seeds is mainly due to the mix of triglycerides, sesamolin and sesamin, which all act synergistically. Authors conducted topic application in workers in a diet with the concentration of 1 µL of sesame extract. The continuous application of that diet causes a cumulative toxicity to workers, which leads to misinterpretation that is high mortality.

The raw extract of *Azadirachta indica* seeds produced significant toxicity to workers of *A. sexdens* by ingestion and contact (Santos Oliveira et al., 2006). With the *Atta cephatoles,* extracts of *T. diversifolia* in the concentration of 1.5 ml/L was efficient in topic application and ingestion (Castano, 2009). The purified substances of *H. puberula* were toxic to workers of *A. sexdens*, being those anthranilic acid, kokusaginine and dictamnine, offered in artificial diets (Almeida et al., 2006). The alkaloid 5-metoxicantin-6-ona isolated from the plant *S. versicolor* was toxic to workers of *A. sexdens* (Peñaflor et al., 2009).

A study with plants from Amazonia Forest showed that six plants show insecticidal activity to *A. sexdens, A. laevigata* and *A. subterraneus molestans*, being those *Banara guianensis*, *Clavija weberbaueri*, *M. parvifolia*, *Ryania speciosa*, *Spilanthes oleraceae* and *Siparuna amazonica*, with topic application of the extracts in the concentration of 5 mg/mL (Gouvea et al., 2010). Boulogne et al. (2011) observed that extract of *M. americana* and *N. tabacum* was toxic to workers via topic application, and extracts of *N. oleander* showed delayed action, though many fractions were repellent to ants.

Extracts of ruta (*Ruta graveolens*), datura (*Datura stramonium*), erva balieira (*Cordia verbenacea*), menta (*Mentha piperita*) mentrasto (*Ageratum conyzoides*) were tested in workers of *A. laevigata* and *A. subterraneus subterraneus,* and the one with highest mortality was mentrasto, from which we obtain coumarin after fractioned, a potential insecticide for those species (Araujo et al., 2008), however, those extracts were tested only on workers of those species and not in nests. The essential oil of *Eugenia uniflora* and the extract of *Melia azedarach* may show insecticidal activity to the *A. laevigata* in laboratory conditions (Jung et al., 2013), in this case more field studies are needed to verify the insecticidal activity of such substances, however, there were no field tests conducted. Extracts of *V. sebifera* were toxic both to workers of *A. sexdens rubropilosa* and their symbiotic fungus in a laboratory (Bicalho et al., 2012), in this case, more field studies are required to verify the toxicity of the extracts in large colonies. Extracts from branches of *Spiranthera odoratissima* (Rutaceae) led to the isolation of insecticidal and/or fungicidal substances in colonies of *A. sexdens rubropilosa* (Terezan et al., 2010).

The use of cassava (*M. esculenta*) extracts, recommended by the FSC, also shows to be inefficient in field conditions. Santos et al. (2013) concluded that the methanolic extract of the leaf powder of *M. esculenta* containing gallic acid and catechins shows itself as a promising alternative for the control of *A. sexdens*, however, it was just a laboratory experiment. Cassava has its leaves cut by many species of leaf-cutting ants in Brazil, such as *A. sexdens*, *A. cephalotes*, *A. laevigata*, *A. rugosus* and *Acromyrmex octospinosus* (Belotti and Schoonhoven, 1978; Blanton and Ewel, 1985; Rando and Forti, 2005).

The oil of seeds of *R. communis* and *Jatropha curcas* showed a toxic action by ingestion in a concentration of 10 to 30 mg/ml and by topic application in a concentration of 0.1 to 0.2 mg/ml per ant (Alonso and Santos, 2013). The authors did not identify the substances responsible, only suggested the ricinoleic, oleic and linoleic acids as toxicity agents to workers, in the case of the *Ricinus* oil. Interestingly, caffeine (1,3,7-trimethylxanthine) did not cause mortality in workers of *A. sexdens* when they received a diet containing the substance, although the authors report that there could have been a methodology failure caused by the addition of glucose to the diet, leading to a higher survival by the workers (Miyashira et al., 2011).

While testing plant extracts of *Carapa guianensis*, *Elaeis guineenses*, *S. indicum*, *R. communis*, *Anacardium occidentale* and *A. indica*, toxicity was observed in workers of *A. sexdens rubropilosa*, but those extracts with fogging were not enough to control field nests (Oliveira, 2006).

As we mentioned above, studies with plant extracts were conducted *in vitro* in a laboratory, being far from an effective application in the field. Difficulties with the possible active ingredients of the plants are:

- Instability of the substances, which are complex and degraded by light, UV and heat,
- Lack of knowledge of the concentration to be used, as each extraction method shows different ways of isolating the chemical groups, besides the natural variability of the plants,
- Lack of knowledge of the mode of action of those compounds,
- Difficult synthesis of the active molecules,
- In practice, how to convey those substances in different formulas, baits, powder, fogging term, as most studies were conducted in a laboratory.

The β-eudesmol is a sesquiterpene derived from essential oils of *Eucalyptus maculata* that promotes an aggressive behavior among ants in the nest of the *Atta* genus (Marsaro Junior et al. (2004). The reason is the contact with that substance changes the cuticular composition of ants triggering the aggressiveness within the colony (Marinho et al., 2007). In ideal concentrations, the β-eudesmol breaks the cohesion of the colony, which may be another tool in the control of those pests (Marinho et al., 2005). However, this compound shows difficulties when used against leaf-cutting ants, as its formulation is solid and easily volatile when diluted in hexane (Marinho et al., 2007), and there's still no practical field application developed for this product. The application of β-eudesmol may be explored in the resistance of the plants against the attack of the leaf-cutting ants, because of the way of acts on its behavior.

Until now no publication has proved the effects of plant extracts in the control of nests of leaf-cutting ants in operational conditions. Most publications, among them (Pelicano et al., 2002; Caffarini et al., 2003, 2008; Silveira et al., 2003; Ácacio-Bigi, 1997) and many others, were only conducted in a laboratory. Caffarini et al. (2003) and Caffarini et al. (2008) have concluded that research should go on to prove the efficiency of plant extracts. Caffarini et al. (2008) describes that the field stages of their study were not conducted yet.

Conclusions on products of botanical origin

In general, we know that many plants may show substances that may cause changes in theinsect behavior, as well as their death. Meanwhile, thousands of years of coevolution went by acting on the interaction ant versus plant. Many times field observations demonstrate there's a change in the behavior of ants, but when isolated, some of those compounds don't show the same behavior on insects.

In an expressive majority of studies, research was stalled and restricted to laboratory conditions. The research that had some advances was also restricted to the laboratory in conditions completely different from the field reality. Laboratory studies include screenings, where a reduced number of workers is used isolated from the colony. It is clearly known that this is part of initial studies, however, those screenings should also be conducted in laboratory colonies, as the results of the substances evaluated may be different when in the presence of the associated interaction ant-symbiotic fungus-microbiota. On the next stage experiments should be conducted in field conditions and finally also on the field in operational situations. It is obvious we agree with the experiments in laboratory conditions, and it is not recommended and rash to experiment substances of botanical origin without having proven its effectiveness in the laboratory.

Laboratory researches with plants potentially toxic to ants or to the cultivated fungus converge, even when the purpose is not clear in terms of identifying, isolating or synthesizing new active ingredients of botanical origin, similar to what occurred with the pyrethroids and others (Rattan, 2010). Usually, active compounds of botanical origin tested and with biochemical modes of action unknown or not mentioned in the publications, make research progress difficult.

The most critical and recurring methodology problem is the daily offer of potential toxic compounds and substances extracted from the plants, added to artificial, liquid and more often solid diets, during the experimentation period that may last up to 25 days. This screening methodology procedure contradicts studies developed by Stringer et al. (1964) and Nagamoto et al. (2004). The correct procedure would be to offer compounds or substances at once as supported by research from Nagamoto et al. (2004).

Results shown on scientific publications regarding this matter, when they’re clear, they usually allow to conclude that substances or compounds of botanical origin are either toxic to workers of leaf-cutting ants or inhibit the growth of the symbiotic fungus.

They are results of initial research and that cannot be recommended for use in the field. Any substances, compounds or molecules that may come to be used for control of leaf-cutting ants should be thoroughly tested before being recommended for control (Schoereder et al., 2012).

Little is known about the active ingredients extracted from toxic plants researched for leaf-cutting ants. They are complex, unstable substances, difficult to synthesize in laboratory, without any technical feasibility in light of the current knowledge.

Experiments (98%) were conducted in laboratory and the little work conducted in field conditions does not allow drawing conclusions on their effectiveness for control of leaf-cutting ants. Even on laboratory studies, their effectiveness is uncertain, as numerous methodology errors were found in the scientific articles, compromising the actual effectiveness of any potential active ingredient.

Although the authors persistently state that these substances extracted from plants that are considered toxic, are innocuous to humans, animals and to the environment, no study has been conducted to investigate that question. The lack of standardization on purity and concentration of the substances may lead to intentional errors on their true toxicity.

Up to now there’s neither a market available substance nor a product. Probably, it will take decades until we can isolate and synthesize a molecule of any toxic plant to be marketed for leaf-cutting ants. Usually the development of a molecule is very costly.

All molecules of botanical origin that were tested until now in a laboratory and on the field with the purpose of controlling leaf-cutting ants did not show efficiency. This does not discredit the efforts to develop alternative products to conventional insecticides, but much basic research will still be required until we obtain a substitute for the products present today in the market.

PHEROMONES FOR LEAF-CUTTING ANTS

According to Hölldobler and Wilson (1990), communication between ants occurs more often through chemical communication, but may also occur through tactile and sound signals. The chemical communication is done through chemical substances that are named semiochemicals (Nordlund and Lewis, 1976). Semiochemicals may be classified as pheromones or allelochemicals, depending on the type of interaction in which they participate.

Pheromones are defined as a chemical substance of intraspecies action (provides information in an interaction between two individuals of the same species) and that are produced and stored in specific locations, most commonly exocrine glands (Nascimento and Sant’ana, 2001). Allelochemicals act in interspecies relationships and have varied origins and compositions, which may be allomones, kairomones or synomones (Della Lucia and Vilela, 2001). Pheromones may cause immediate changes in behavior, in which are called releasers (Shorey, 1973).

For leaf-cutting ants, main pheromones with releaser effect are pheromones for alarm, territorial behavior, trail marking and recognizing other individuals (Della Lucia and Vilela, 2001; Viana-Bailez et al., 2011).

By action of these substances, individuals of the colony are capable of performing several functions, such as setting foraging trails, recruiting workers and recognizing individuals of the same colony (Hölldobler and Wilson, 1990).

According to Vilela (1994), on leaf-cutting ants pheromones can be used in two ways: promoting the disorganization of the social system of the colony with its occasional weakening and death, or increasing the attractiveness of granulated baits by incorporating them into the bait's formulation, with the consequent increase of its transport to the interior of the nest.

Robinson and Cherrett (1978) in a laboratory increased the attractiveness of granulated baits formulated with citric pulp by adding the synthetic trail pheromone M4MP2C (methyl-4-methylpyrrole-2-carboxylate) for *A. cephalotes*, *A. sexdens* and *A. octospinosus.* The addition of that pheromone made it easier to find baits, but showed repellent action in high concentrations. The same attractive effect was observed when adding that pheromone to baits formulated with soya beans, by Robinson et al. (1982), but unsuccessful. Cross et al. (1979) verified that baits impregnated with a mix of pirazines 3E25DMP (3-ethyl-2-5-dimethylpirazine) and 2E25DMP (2-ethyl-2-5-dimethylpirazine) did not increase the bait's attractiveness in the field for *A. sexdens rubropilosa.* Vilela and Howse (1988) concluded that the 3E25DMP of the trail pheromone may be used as attraction in granulated baits for *A. sexdens rubropilosa.* Lima (1973) worked with the attractiveness of market baits impregnated with pheromone in the field, where approximately 50% of the baits were carried; however, market baits without the pheromone reached a transport rate higher than 80%.

Glancey et al. (1970) and Vilela and Howse (1988) impregnated corn grains with extract of larvae of *Solenopsis saevissima*, the fire ant, and observed that the grains were carried to the nest and treated as larvae by the ants for several hours. In the same way, Viana (1996) impregnated imitations of larvae made from filter paper with cuticular extract of larvae of *A. subterraneus subterraneus* (caiapo ant) and verified that imitations were carried and treated as larvae by the colony. Meanwhile, Robinson and Cherrett (1974) did not get positive responses while evaluating the larva pheromone as attraction in baits against *A. cephalotes*. On the other hand, Tatagiba-Araujo et al. (2012) verified that granulated baits impregnated with extract of poison gland (trail pheromone) were carried more often than baits formulated with extract of larvae cuticle, and in field tests the extract of the poison gland decreased the time for the bait's discovery and transport.

For *A. capiguara* and *A. bisphaerica* the compound of the alarm pheromone 4-methyl-3-heptanone increased significantly the bait's attractiveness, however, there was no difference in terms of carrying the baits, even when increasing the number of sachets or changing their position (Hughes et al., 2002a,b).

Research studies have been conducted aimed at using pheromones with this purpose, besides the search for knowledge on the different types of pheromones responsible for keeping the complex social organization of these insects, allowing for a great capacity of adaptation to environmental changes. Viana-Bailez et al. (2011) covers information on the different types of pheromones used in the communication chemical for leaf-cutting ants, such as the trail and recruitment pheromones, the territory pheromone, the pheromone recognition of the nest's mate and offspring, the alarm pheromone, the queen's pheromone and the pheromone for marking leaves.

The issue with using pheromones with leaf-cutting ants would be to find a single mix of pheromone components capable of acting against all the main species of leaf-cutting ants that exist, avoiding having to use different baits treated with a specific pheromone for each ant species, which would create some market difficulties (Vilela, 1994).

Research has been conducted with pheromones in order to increase the toxic bait's attractiveness, however, until now we did not get a practical and operational use of those pheromone compounds.

PREFERENCE AND NON-PREFERENCE OF PLANTS CUTTING BY LEAF-CUTTING ANTS

Food preferences and specialization

The foraging behavior of leaf-cutting ants has aroused interest among many researchers, as the understanding of this behavior and the selection of plants would imply a series of applications of generated knowledge in the advances of the technology of population management and control of these insects.

Many factors come into play in the choice of the vegetable substrate by leaf-cutting ants, so, for example, the anatomical, biochemical and physiological features of the plant species are related to its acceptance or rejection, and leaf-cutting ants show preferences for certain species (Cherrett, 1968; Rockwood, 1975; Littledyke and Cherrett, 1978; Pollard et al., 1983; Forti, 1985a,b; Vitório, 1996; Garcia, 2003; Neto et al., 2012). Although there are many works found in the literature, there's still no general theory on the exploration of plants by these ants.

Several evidences support the hypotheses about selection of plants, which can be attributed to chemicals defenses (Cherrett, 1968; Rockwood, 1975), physical (Stradling, 1978; Waller, 1982; Cherrett, 1972), water content (Rockwood, 1976; Bowers and Porter, 1981), nutritional value (Rockwood, 1976), secondary chemical substances (Cherrett, 1972; Howard, 1987, 1988), distribution of palatable resources (Fowler and Stiles, 1980; Forti, 1985a,b), among others.

Therefore, for a better understanding of plant-ant interaction, many studies have been conducted with the purpose of checking arresting (Cherrett and Seaforth, 1970; Littledyke and Cherrett, 1978), repellent (Hubert et al., 1987), or deterrent (Saladino et al., 1998) effects. Still a great variability is found in the preference shown by the leaf-cutting ants in terms of natural materials and their chemical fractions, contradicting much research conducted on the selection of plants. According to Cherrett (1972), this variability may be partially explained by the tendency of the colony to become saturated with the material offered every day, and so ants would tend to select new alternative sources of substrates available.

Another aspect would be related to the role of the fungus in the selection of plants when foraging, since the vegetable is used as a substrate for symbiotic fungus growth (Weber, 1972). The symbiotic fungus is a *L. gongylophorus* (Fisher et al., 1994), whose basic energetic reserve are the glycogen, in the first stages of formation of the gongylidia, and the polysaccharides, which are found in a form readily absorbable by the ants (Quinlan and Cherrett, 1979). Weber (1972) defines gongylidia as expansions in the central or end part of the hyphae and the staphylae as a set of gongylidia.

So, for example, the citric pulp is the most attractive substrate for several species of leaf-cutting ants (Mudd et al., 1978). However, Quinlan and Cherrett (1978) studied a selection of substrates for the cultivation of the symbiotic fungus in *A. octospinosus* and *A. cephalotes*, and verified that the workers of the *A. octospinosus* carried the substrates, barley, citric pulp, lentil and corn flakes, in this order of preference, while the *A. cephalotes* preferred the citric pulp. But when the growth rates of the fungus were observed in an artificial medium containing the same substrates, they verified that the growth was higher on those containing barley and lower on that with the citric pulp, for both species of ants studied. Therefore, one concludes that ants do not necessarily select materials that provide better fungus growth. Cherrett (1980) argues that the role of the fungus is to supplement with essential nutrients required by the insects, but are not synthesized by them, such as the sterols. Martin et al. (1969) showed that the fungus produces ergosterol, the same detected by the Peregrine et al. (1973) in the post-pharyngeal gland of the *A. octospinosus.*

In Brazil, much work has demonstrated that leaf-cutting ants select plants with attractive or stimulating compounds. However, the presence of repellent and toxic substances to the ants and/or fungus may be in disguise. Within this line of research of toxic plants for control work is found on sesame (*S. indicum*) (Hebling-Beraldo et al., 1986; Pagnocca et al., 1996a,b; Bueno et al., 1995; Sinhori et al., 1997; Costa et al., 1997), on castor (*R. communis*) (Fernandes et al., 1997), on *C. ensiformis* (Takashi-Del-Bianco et al., 1997), and on *Virola* (Pagnocca et al., 1996a,b), however, no study has been conclusive in understanding the toxicity relation ant versus symbiotic fungus.

With this perspective, evidence suggests that the selection factor may be more related with the preference of the leaf-cutting ants to the plant, and not with the substrate that promotes a higher growth rate for the symbiotic fungus.

Plant’s physical factors, such as hardness, resin or latex production also influence the selection of plants. According to Cherrett (1972), the *A. cephalotes* selects vegetables that are less hard, dense and vigorous, which may contain a higher amount of liquid, when new and old grapefruit (*Citrus paradisi*) leaves are offered. This preference for new leaves in detriment of the old from the same vegetable was also found by Cherrett and Seaforth (1970), Littledyke and Cherrett (1978), Waller (1982), Rockwood (1976), Nichols-Orians and Schultz (1989) and Barrer and Cherrett (1972).

Thus it is undeniable that physical factors directly influence the selection of plants, but there’s still no explanation as to how these occur individually and which ones have more influence.

Chemical composition and the physical aspects of the substrates do not make it possible to explain the mechanism of plant selection, since the selection is complex as it involves from the choice of material to be foraged down to its modification by the symbiotic fungus. The procedures involved in the preparation of the leaves as substrate for the fungus garden in colonies of *Acromyrmex*, as per Littledyke and Cherrett (1976), include the process of licking the surface of the leave and chewing its edges, with the ingestion of liquids. And during these processes, the composition of the vegetable material may be perceived by the workers, and in that second moment a second selection occurs (Camargo et al., 2006).

Due to that behavior in plant selection and the intricate relationship between ant and symbiotic fungus, it allowed the group to have a wide distribution in the Neotropics. These ants often attack plantations of coffee (*C. arabica*), cocoa (*Theobroma cacao*), citrus (*Citrus* spp.), cassava (*M. esculenta*), corn (*Zea mays*) and cotton (*Gossypium hirsutum*). However, native and introduced pastures, grass and plantations of forest and silvipastoral species are also affected (Cherrett, 1986d; Forti and Boaretto, 1997; Moulaert et al., 2002; Perez et al., 2011).

Studies on leaf-cutting ants have an uneven geographical distribution, and the number of published articles does not necessarily reflect either the damaged caused by them, neither the importance of each species. Most studies published several decades ago were conducted in Panama (Burd, 1996; Herz et al., 2007) and Costa Rica (Rockwood,1976; Burd, 2000) probably reflecting the availability of research financing and facilities.

In the latest years, research in Brazil, Argentina and other South American countries has become more and more important.

Plants preference and non-preference

The control of leaf-cutting ants in implanted forests, via preferred and non-preferred plants, has been investigated by many researchers, although little is known about the ability of these insects to select species and provenances of *Eucalyptus*. Anjos et al. (1993) evaluated the preference of the *A. sexdens rubropilosa* with 20 species of *Eucalyptus* in the laboratory, concluding that *E. maculata* and *E. deanei* were highly not preferred by the *Atta* for attack, the *E. dunnii, E. pilularis* and *E. propingua* species were considered moderately not preferred, while the other remaining 15 species were classified as preferred. The *Eucalyptus nesophila* showed to be not preferred by the *A. laevigata* and the *A. sexdens rubropilosa*, and the species *E. cloezina* was not preferred by the *A. laevigata* and preferred by the *A. sexdens rubropilosa* (Santana and Anjos, 1989). Regarding the non-preference of the ant *A. subterraneus* to cutting the *Eucalyptus*, Della Lucia et al. (1995) verified that *E. acmenioides* and *E. citriodora*, in this order, were the less carried species for the interior of the fungus garden, while *E. saligna*, *E. urophylla* and *E. torelliana* were well accepted by the foraging ants. Consistent results on *Eucalyptus* selectivity by the leaf-cutting ants could help in the population decrease of these pests, via planning in terms of composition of species and provenance of the forests. Some authors state the use of “resistant plants” of *Eucalyptus* species (Anjos et al., 1986; Santana and Anjos, 1989; Santana, 1988; Zanetti et al., 2014) due to the plant’s mechanical, physical and chemical features. In fact, these articles studied the preference and the non-preference of the leaf-cutting ants in relation to several species of *Eucalyptus* plants, so in a way, we cannot affirm that these plants are resistant or susceptible to leaf-cutting ants.

In laboratory, it was evaluated the preference of twenty species of *Eucalyptus* to the *A. sexdens rubropilosa* concluding that *E. mulata* and *E. deanei* were highly not preferred by the *Atta* for attack, while the species *E. dunnii*, *E. pilularis* and *E. propingua* were considered moderately not preferred, and the remaining ones were considered preferred (Anjos et al., 1996; Santana andAnjos, 1989). Under field conditions it was verified that the density of the colonies was higher in plantations of *E. grandis* (28.84 nests/ha) when compared to plantations of *E. pellita* and *E. tereticornis* (12.84 and 11.07 nests/ha) (Zanetti et al., 2000). Meanwhile, the *Eucalyptus grandis* was the less preferred between the species that were studied. It seems that while in the field leaf-cutting ants cut all species without distinction due to the high degree of polyphagia (Rockwood, 1976; Garcia et al., 2003). It is a consensus with Zanetti et al. (2000) that new studies are necessary to clarify this issue, as preference and non-preference studies conducted by Forti (1985a,b), Santana and Anjos (1989), Della Lucia et al. (1995) and Vendramim et al. (1995) did not show any unanimous non-preferred species. Little is known about the resistance mechanism of such species of *Eucalyptus*, if it is antixenosis or non-preference, antibiosis or tolerance, and as those species have adapted to other regions of the country, that’s a fact not studied by those authors.

Little attention has been given to study the causes of the selection by the leaf-cutting ants of plants with economical relevance. Ants may possibly stop cutting plants or avoid cutting and transporting leaves, due to the chemical substances undesirable to the fungus culture (Cherrett, 1972; Howards and Wiemer, 1986; Ridley et al., 1996; North et al., 1999). It seems that the best evidence to explain the inhibition of the culture of symbiotic fungus by the grass-cutting ants is the presence of chemical substances (Lapointe et al., 1996; Castellani, et al., 2009; Michels et al., 2001; Lopes et al., 2003). Bundle sheath on grass, due to the high starch content, may also explain the preference of some grass species by the *A. landolti fracticornis* (Fowler and Robinson, 1979; Fowler and Robinson, 1977; Robinson, 1979).

Some studies on plant preferences were conducted to find grass species or cultivars more attractive to grass-cutting ants aiming to include dry leaves bran from those grasses in the toxic baits, in order to increase the attractiveness to the grass-cutting ants (Castellani et al., 2009; Boaretto, 2000; Vitório et al., 2011).

However, the results obtained up to this point do not allow a practical application, including with *Citrus* plants studied by Pollard et al. (1983). Numerous factors affect the selection of the substrate by the leaf-cutting ants, and many conclusions have been drawn from tests with inappropriate methodologies, for example, tests without free choice. Could it be that, within the same genus of an exotic plant, there will be resistant species? It is known that, several species of leaf-cutting ants explores between 38 to 77% of the plants in natural forests (Rockwood, 1976; Garcia et al., 2003), making it clear that there is plants selection, but the range is too wide. Besides, there is evidence that the fungus cultivated by the leaf-cutting ants can metabolize toxic substances coming from the plants, making even more difficult the selection of plants that are resistant or have low preference (Little et al., 1977).

Conclusions on preference and non-preference of cutting plants by leaf-cutting ants

Regarding the so-called resistant plants little is known about the resistance mechanism, if there is any, of such of *Eucalyptus* species, whether it is antixenosis or non-preference, antibiosis or tolerance, and how these species will adapt in other regions of the country, a fact not studied by any of the above mentioned authors. Therefore, the results obtained up to this point do not allow a practical application. Numerous factors affect the selection of the substrate by the leaf-cutting ants, and many conclusions have been drawn from test with inappropriate methodologies, for example, tests without free choice. The effectiveness is uncertain, as numerous methodology errors were found in the scientific articles, besides, a recurring lack of replication of those cultivars in different regions of America. There's currently no cultivate, nor variety, nor a species in the market, and none of the studies indicate the availability of the species/cultivars in the agroforestry sector.

CULTURAL PRACTICES FOR LEAF-CUTTING ANTS

Studies on cultural practices

Cultural practices comprise a set of methods proposed to help in the control of leaf-cutting ants. The most quoted practices in the literature are: diversification of crop systems, trap crops, crop rotation, soil plowing and harrowing, the use of fertilizers, among others. These methods were quoted by numerous authors (Mariconi, 1970; Vilela, 1986; Montoya-Lerma et al., 2012; Della-Lucia et al., 2014; Zanetti et al., 2014). The consortium of plants such as sesame (*S. indicum*) (Vilela, 1986), sweet potato (*I. batatas*), castor (*R. communis*), and *Brachiaria* sp. with cultivation of *Eucalyptus* species in commercial crops was not successful (Forti and Boaretto, 1997; Oliveira et al., 2011; Zanetti et al., 2014). In Colombia, Montoya-Lerma et al. (2012) reported the plantation of *T. diversifolia* interspersed with the plantation of *Montana quadrangularis* for culture control of the *A. cephalotes*. This research based in an isolated case of herbivore study (Giraldo-Echecerri, 2005) may not be recommended for operational use without being more thoroughly tested in the practice.

Plantations of *Eucalyptus* with maintained understory have less colonization by leaf-cutting ants when compared with those without understory (Oliveira et al., 2011) and strips of native vegetation contribute to reduce the density of colonies in the areas planted with *Eucalyptus* next to those strips (Zanetti et al., 1999). The idea is that those strips provide shelter and refuge to the natural enemies of the queens of the *Atta* species and the birds are natural predators of the *Atta* species queens, but little research was made to progress on this topic that is so important to the natural biological control (Almeida et al., 1983; Montoya-Lerma et al., 2012).

However, it is believed that the maintenance of the understory vegetation and the diversification of crop systems quoted by Araújo et al. (2003), Oliveira et al. (2011), Montoya-Lerma et al. (2012), Della-Lucia et al. (2014), Zanetti et al. (2014) must yet be thoroughly tested in the practice (D'araújo et al., 1968).

The adoption of the practice of minimum tillage, which reduces soil preparation, may increase the number of nests of leaf-cutting ants (Zanetti el al., 2003a,b).

The soil's conventional preparation through plowing and harrowing may determine the mortality of new nests of *Atta* (up to four months old) (Lapointe et al., 1990; Della Lucia and Vilela, 1993; Forti and Boaretto, 1997).

However, with the practice of the minimum tillage or no-till farming, adopted in several crops and in reforestation, this control has basically ceased to exist. For adult nests of *Atta*, the effect may even be harmful, since soil mechanization may partially disrupt the nest, temporarily ceasing its activity, giving the wrong impression that it has been controlled and making its localization more difficult (disrupting the ant nest) (Della Lucia and Vilela, 1993; Forti and Boaretto, 1997).

Another cultural practice used in management leaf-cutting ants consists of digging the new nests and capturing the queens. This method is not recommended for colonies of *Atta* ants more than four months old, because after that period, the queen is found nested at a depth higher than 2 m, becoming not feasible due to the large effort required. In practice, digging is not feasible in areas of commercial crops, in reforestations, pasture systems and large cultivated areas, and it only recommended for small areas (Forti and Boaretto, 1997).

Crop rotation is impractical in the production of *Eucalyptus* and it is also not feasible to control *A. capiguara* in pastures, as recommended by Mariconi (1970).

The use of phosphate fertilizers was not operationally tested despite the evidences of experiments conducted in laboratory showing the decrease in damages to *Eucalyptus* species plants (Cabello and Robinson, 1975). The use of limestone was inefficient in the control of *A. sexdens rubropilosa* (Schoereder et al., 2012). Composting, a mix of organic (molasses, poultry manure, yeasts, leaf litter) and inorganic (agricultural lime and water) materials, used within the philosophy of sustainable and ecological agriculture for the control of *A. cephalotes*, as quoted by Montoya-Lerma et al. (2012), does not have a proven effectiveness nor technical feasibility.

The use of barriers is one of the oldest methods of control for leaf-cutting ants, but only in small orchards (MoressI et al., 2007). Plastic tape coated with grease, cylinders of plastic, and strips of aluminum, plastic or metal are attached around the trunks (Justi Júnior et al., 1996). These physical barriers around the plants are placed mainly in the young stage (seedlings) and are also recommended to prevent the ant's access, avoiding the cut. However, frequent inspections and repairs are necessary to protect the plants, and so they are not applicable to agricultural and forestry crops in larger scales (Della Lucia, 1993; Della Lucia and Fowler, 1993).

Conclusion on cultural practices used for leaf-cutting ants

The consortium of plants, the so-called trap plants, with plants of *Eucalyptus* species, and others, does not have any scientific proof, does not have practical application and was not thoroughly tested (Forti and Boaretto, 1997; Oliveira et al., 2011; Zanetti et al., 2014).

Crop rotation, plowing and harrowing, the use of fertilizers and limestone, the digging of nests and death of the queen, and the use of composting, do not have any practical feasibility, do not have proven efficiency and are not innovative, available nor accessible technologies (Forti and Boaretto, 1997; Oliveira et al., 2011; Zanetti et al., 2014).

The practice of minimum tillage, although providing numerous advantages, reducing soil preparation, may increase the number of nests of leaf-cutting ants (Zanetti et al., 2003a,b).The maintenance of the understory in the cultivation of the *Eucalyptus* with strips of native vegetation may promote a decrease in the number of nests of the *Atta* species (Zanetti et al., 1999; Oliveira et al., 2011), and that may be attributed to the increase of shelter and refuge locations for the natural enemies, such as birds (Almeida et al., 1983; Oliveira et al., 2011). Although these studies are very important, they were not conducted in many locations of Brazil and the study of birdlife (Almeida et al., 1983) was a short period of study, thus, to draw more general conclusions new research should be conducted.

INTEGRATED MANAGEMENT OF LEAF-CUTTING ANTS

Despite the idea of Integrated Pest Management (IPM) having emerged as a movement after the World War II and being now, approximately, 55 years old, with big stimuli in the 1960s, it still faces large barriers to its implementation, even though being aware of the advantages of its use for the environment and for the economy (Kogan and Bajwa, 1999; Ehler, 2006; Gullan and Cranston, 2005).

It is important to note that the management of leaf-cutting ants, based on monitoring, is uniquely to planted forests in order to provide raw materials for the pulp, wood, and energy industries (Della Lucia et al., 1993; Zanetti et al., 2014).

In its current, or successful, use a careful analysis of the integration of all the protection methods of plants available must be done in order to discourage or reduce the use of synthetic insecticides in levels that are economically and ecologically justified, reducing risks for the environment and human health (Ehler, 2006; Vasileiadis et al., 2011).

Historically the first ideas of IPM emerged from the realization of the indiscriminate use of synthetic organic insecticides soon after the end of World War II (Ehler, 2006). The term IPM with its modern concept, was printed for the first time in 1970 and included all pests. This concept is a solution mainly thanks to efforts of scientists from California who had the brilliant idea of including the word “integrated” in the context, creating what they called “Integrated Control”, which in its birth clearly combined and integrated chemical and biological control, with the introduction of economical concepts and injury damage levels. According to Ehler (2006), that was the first clear definition of “integration” in pest management.

The integrated control concept developed by entomologists combine the use of insecticides with the natural enemies to enable a more efficient control (Stern et al., 1959). This idea of integrated control expanded to the concept of IPM to include the management of all types of pests using a variety of compatible control tools (Flint andDreistadt, 1998).

The key concept in the IPM is the integration of the management methods. According to Ehler (2006), the absence of integration has been one of the larger obstacles to the implementation of the IPM. According to Kogan and Bajwa (1999), the IPM is a decision support system for the selection and use of pest control methods, isolated or harmoniously coordinated in a management strategy, based on a cost/benefit analysis that takes into account the interests of the producers and the impacts to society and the environment.

Prokopy (2003) defined IPM as a decision based on processes that involve the coordination of the use of multiple methods in order to perfect pest control (insects, pathogens, weeds, vertebrates) in an ecological and economically feasible way.

Despite the IPM’s frequent ecological appeal, from its beginning (Flint and van Den Bosch, 1981) to the most recent concepts (Kogan, 1998; Prokopy, 2003; Meissle et al., 2010; Vasileiadis et al., 2011), little attention has been giving to the practical application of ecology knowledge. IPM programs, including the more successful, have been implemented with little care for the processes of the ecosystems (Kogan, 1998) to the point where Geier and Clark (1978) suggest that IPM has been considerably successful, despite its weak theoretical support.

Since the start of the implementation of the concept of IPM until our days, the purpose is always to reduce the use of pesticides and their risks, using a combination of different control techniques that enable an efficient pest control (Meissle et al., 2010).

On the 2009/128/EC European Parliament directives covering the sustainable use of pesticides, IPM is defined as: a careful analysis of all plants protection methods available and the consequent integration of appropriate measures that discourage the development of populations of harmful organisms and keep the use of plant protection products and other forms of intervention in levels that are economically and ecologically justified, reducing or minimizing the risks to human health and to the environment (European Parliament, 2009; Vasileiadis et al., 2011).

Integration of compatible management tools

The IPM depends on a variety of control methods, for example, chemical, mechanical, cultural, and physical methods, and it depends on biological control agents (Flint and Dreistadt, 1998). The IPM programs often combine two or more compatible methods that when used together, produces excellent management (Kogan, 1998; Huffaker and Smith, 1980; Boller et al., 2004; Flint and Dreistadt, 1998; Meissle et al., 2010; Vasileiadis et al., 2011). In this context, the IPM promotes the use of different combined techniques to efficiently control pests (Meissle et al., 2010). After reviewing the literature, there is no doubt that the IPM's success depends on the integration of the compatible management methods and in all definitions of IPM integration is the key concept.

Critical analysis of current Integrated Management of leaf-cutting ants

Examining the articles in the literature that covers the IPM of leaf-cutting ants (Anjos et al., 1993; Della Lucia et al., 2014; Zanetti et al., 2014), the integrated management of leaf-cutting ants, from our point of view, may not be implemented. This impediment is not only due to a non-compliance with the principles of IPM, but due to the lack of integration of the control methods, and that integration does not exist because the control methods given as alternatives to chemical control are not efficient to the point of promoting an effective control in operational conditions. The alternative control methods to chemical control, in their current status, do not just require improvements to be integrated (Della Lucia et al., 2014) in the program of management of the leaf-cutting ants, they need to show proven efficiency obeying the assumptions of the Stockholm Convention. Adding to that, there is also insufficient data to determine the level of economical damage, despite big efforts from researchers in determining them (Zanetti et al., 2000, 2003; Souza et al., 2011).

In practice, what is done is to control the leaf-cutting ants using chemical control methods (Della Lucia et al., 2014), based or not in monitoring the colonies' population density. With not much data on its economical damage level (or threshold) and with the lack of biological, cultural, physical, mechanical methods, resistant plants, and of insecticides e fungicides of botanical origin, control gets based in unreliable and controversial information, except for the chemical method. So one can states that, what occurs is the management of the chemical control and not the integrated management of leaf-cutting ants, because only the first has enough data to show high efficiency of its control, mainly due to the use of the sulfluramid used in toxic baits both for the control of leaf-cutting ants of large leaves and grass-cutting ants, adding to that the high efficiency of the sulfluramid also for the species of *Acromyrmex*.

There is no doubt that the chemical control, with active ingredients of organic synthetic origin, is the only one available to obtain an efficient control.

Monitoring

In the case of planted forests in Brazil, the monitoring aims exclusively at evaluating the population of the nests of leaf-cutting ants in almost all the cases with different survey methods (Zanetti, 2011; Zanetti et al., 2014), and there are few cases where the injury is evaluated (Della Lucia et al., 2014). Obviously, such monitoring is practiced due to economical reasons, as according to Vilela (1986) timber producing companies spend more than 75% of their time with pest control and the cost involved is more than 75%. According to the currently implemented model of leaf-cutting ants control runs throughout the whole year and, thus, the areas are often monitored up to a certain age of the culture, although it varies between companies. The monitoring system of the population of nests of leaf-cutting ants is an adaptation of the monitoring programs used in annual and perennial agricultural crops. Although being an adaptation it works well for: (a) recognizing the species with damage potential (key pests), (b) estimate the density of nests or damage caused by the pests, (c) it follows up with a certain precariousness the plant's phenological development and its susceptibility to attack by the insects, (d) it follows up and checks the effects of the population decrease, in the case of chemical control in its different formula variants, but it lags behind on following up and verifying the effects of chemical control on non-target species, including their natural enemies. Naturally, the monitoring systems employed, being casual samplings (random sampling, transects or a worst focus technique) (Zanetti, 2011; Zanetti et al., 2014) were idealized to increase the efficiency of the chemical control and mainly reducing the costs with insecticides and hand labor, and do not have the purpose of the ecosystem evaluation, in the sense of measuring the impact on all non-target organisms, including predators, parasitoids, as advocated by the philosophy of IPM. Due to the absence of biological control applications in an operational scale, predators and parasitoids are not evaluated in these samplings, but there is also a lack of concern for evaluating the impact on other non-target species, although the formula in toxic bait has not revealed impact on non-target organisms (Santos, 2000).

Level of action or decision making of the control

Complete sampling methods and programs will be those that besides everything that we have discussed, enable to easily obtain data to correlate with the economical damage caused by the leaf-cutting ants (Crocomo, 1990; Zanetti et al., 2000, 2014), so that we can determine the level of economical damage and finally obtain the control action level, whenever possible supported by guidelines. The control action level and the guidelines do not show when one should adopt management actions to avoid losses caused by the leaf-cutting ants. The control action guidelines are not necessary for all pests, however, with the help of this tool we can often determine the need for the application of an insecticide and also report to the producer the proper moment to start other control methods.

Based on work developed by ZanettI et al. (2000, 2003a,b) and Souza et al. (2011), exclusively obtained for planted forests, it is possible, through monitoring or survey techniques for the population of the nest of leaf-cutting ants, obtain a control decision making or action level. It must be clear that this decision making is done exclusively to obtain a better cost-benefit relation for the chemical control, usually or exclusively done with toxic baits.

One fully agrees with Della Lucia et al. (2014) that the purpose is the control and not the integrated management of leaf-cutting ants. Although there are publications that reveal the economical threshold, usually the control decisions are still based in empirical observations (Della Lucia et al., 2014).

The efforts are undeniable in improving the sampling techniques for monitoring (Zanuncio et al., 2004; Sossai, 2005; Cantarelli et al., 2006; Reis et al., 2010) and estimating the economical damage in planted forests where there’s more studies (Hernández and Jaffé, 1995; Zanetti et al., 2000, 2003a,b; Souza et al., 2011; Zanuncio and Sossai, 1999a; Zanuncio et al., 1996).

As for the estimate of economical damage in other crops, results of studies are very few and limited to the consumption of vegetables by the colonies of grass-cutting ants (Amante, 1967a, b, c), or the damage caused by the leaf removal in the coffee plant, but without measuring the effect on the production (Barreto et al., 1998) or evaluating the losses caused by the leaf removal of cassava plants in areas treated and not treated with insecticides (Bertorelli et al., 2006).

The economical impact of the grass-cutting ants, which is not much studied, was reviewed by Fowler et al. (1986) and the estimation methods for the amount of vegetation consumed by the leaf-cutting ants were also reviewed by Fowler et al. (1990).

Conclusions on integrated management of leaf-cutting ants

The integrated management of leaf-cutting ants is not possible in our days as there is no effective method available in the market, except for chemical control, so it is not possible to use two or more control methods, such as advocated by the IPM. Reviews on control methods for leaf-cutting ants point that methods that could be alternatives to chemical control (biological control, use of extracts of plant-insecticides/fungicides of botanical origin, mechanical methods and cultural methods) are under study (Montoya-Lerma et al., 2012; Della Lucia et al., 2014; Zanetti et al., 2014), so one concludes that they are not available for use yet. Besides, effectiveness data of methods that can be alternatives to chemical control are scarce, variable and inconsistent (Della Lucia et al., 2014; Boaretto and Forti, 1997). On the other hand, chemical control with toxic baits with sulfluramid provides high effectiveness levels (Della Lucia et al., 2014) in all crops in different regions of Latin America, with different soil and weather conditions, as well as quite diverse social and economical conditions.

The estimate of the number of leaf-cutting ants nests through a monitoring program (Zanetti, 2011; Della Lucia et al., 2014) is an adaptation of the monitoring idea used in annual and perennial agricultural crops. That system works nicely in estimating the density of nests of leaf-cutting ants or their damage and verifies the effects of population decrease after the chemical control. The monitoring systems, as reviewed by Zanetti et al. (2014) and used exclusively in the cultivation of species of *Eucalyptus* was idealized to increase the efficiency of the chemical control, mainly with toxic baits in order to reduce the costs with hand labor and insecticides. The monitoring programs used for leaf-cutting ants do not intend to evaluate the ecosystem as advocated and proposed by the IPM.

Although there are publications on the cultivation of *Eucalyptus* species that demonstrate the economical threshold and control decision making, there is no such thing for all the other crops and the control decisions are based on empirical observations (Della Lucia et al., 2014).

Decision making in the cultivation of *Eucalyptus* species has the purpose of determining the moment of performing chemical control, therefore it is just the rational use of chemical control, very far from the ideal approach of IPM (Kogan, 1998).

If the key aspect is to reduce the use of insecticides in the cost/benefit relation, without examining other aspects (environmental, social and economical impacts), then good surveys are enough, taking into account that what is usually found in the literature is the integrated management of insecticides, the distinct use of insecticides. That is not new, and goes back to the 1940s, such as the supervised control described by Ehler (2006).

That is not necessarily bad and the careful use of insecticides should be encouraged. The biggest issue with this management approach or vision is that it perpetuates the idea of the “quick-fix mentality”, which targets the symptoms, but is not able to solve the deep causes of the control of leaf-cutting ants within the ideal vision of IPM (Smith andSmith, 1949; Kogan, 1998; Ehler, 2006).

The ideal situation is that in the future, the IPM can be implemented in the cultivation of species of *Eucalyptus* and in other crops, however, and especially for leaf-cutting ants, it is necessary that other technologies may be efficient and be available in the market to rural producers. The methods candidate to alternatives to chemical control, namely: (a) insecticides of botanical origin and/or from toxic plants, (b) “resistant plants”, (c) biological agents, (d) cultural methods, (e) mechanicals and physical methods, and (f) changing the habitat should be available in the market and be operationally efficient in order for us to have a feasible Integrated Management of leaf-cutting ants. Obviously we recommend the use of innovative techniques of control of leaf-cutting ants, however, the cost of implementation of new techniques is very high, such as with synthetic pheromones (Vasileiadis et al., 2011).

PROCEDURES RECOMMENDED FOR PRODUCT DEVELOPMENT FOR CONTROL OF LEAF-CUTTING ANTS

Based on research conducted on control of leaf-cutting ants, the Brazilian government represented by the SDA/MAPA (Secretary of Agribusiness Defense/Ministry of Agriculture, Livestock and Food Supply), established in Brazil through Annex VI of the Normative Instruction SDA no. 42, from December 5th, 2011, the “additional requirements and content for conducting tests aimed at the control of leaf-cutting ants” (Brasil, 2011).

Despite being a country specific norm and focused in the toxic bait formulation, considering the serious inconsistencies in the complementary or supposed alternative control proposals, which were criticized in the publications from Forti et al. (1998) and Schoereder et al. (2012), it is recommended that technical requirements should be widely taken into account, regardless of the formula or application technique, thus promoting a higher reliability of work conducted based on proposals of new products that can be effectively used as alternatives. Therefore, if necessary, due to environmental or public health issues, to discontinue the use of standard products, those new products will have a better potential for replacement. Considering the above, one recommends that these procedures are followed in the attempts to develop alternatives to the control of leaf-cutting ants:

1) Laboratory tests, when technically relevant, are very welcome, and might be used as preliminary or complementary studies. In the laboratory, some parameters may be better evaluated than in the field, e.g. whether the insecticide acts fast or in a delayed manner.
2) Field tests are essential and must be laid out in realistic and scientifically valid ant fighting conditions. Those field experiments should follow, if not only, at least necessarily, these parameters and procedures:
   - Choice of ant nest: for each treatment use at least ten nest in a minimum 10 square meters area of loose soil for the genus *Atta*, and for the *Acromyrmex* the nest should be adults. These nest should be isolated from each other and should not have previously received any insecticides.
   - Treatments: control (without active ingredient), standard (registered market product, proven effective), experimental product to be tested in at least three doses or concentrations. The experimental layout must be be the completely randomized.
   - Formulation: ensure the complete decontamination of the equipment and materials before each formulation.
   - Application: products will be mandatorily evaluated in experimental situation of single application.
   - Data evaluations and analyses: (i) periodically evaluate nests up to at least 150 days for baits in the *Atta* and 90 days for other formulations or for the *Acromyrmex* (or less, provided it is technically well justified), considering that in the final evaluation the fungus chambers inspection must be included, as well as the ants in the interior of the nests, preferably through manual digging. The chambers must be successively verified until it remains no doubts whether the colony is alive or dead, considering that the death confirmation should come with a verification of degraded fungal culture chambers, and no live ants whatsoever, taking into account the architecture of the colonies: (i) for the *Atta*, one must verify several chambers at least down to 2 m deep, considering that in the event any doubts remaining, one must verify at least down to 3 m, (ii) for the *Acromyrmex*, on each colony, a substantial proportion of the fungus chambers must be evaluated.
3) As a component for proposing an alternative product,

the main results must be published in: (i) scientific journals, and/or (ii) summaries or annals of a scientific event (congress, symposium, etc.), which must have prestige and tradition, of national or international nature, and where the work has been effectively presented.

4) Consider that products that are able to meet only small niches, such as, for example, organic agriculture, although being welcome, many times they cannot be considered as replacement alternatives for wide standard use products. In order to be considered as alternatives, products must meet the following parameters (UNEP/POPS/POPRC.5/10/Add.1): technical feasibility, cost-effectiveness, effectiveness, availability, accessibility.

FUTURE OF THE CONTROL OF LEAF-CUTTING ANTS WITHIN THE INTEGRATED PEST MANAGEMENT APPROACH

It is believed that the future of the control of leaf-cutting ants will continue to be the chemical method, taking into account that its still the only one with proven efficiency, available in the market at low cost and accessible to a wide range of consumers.

Candidate alternative methods to chemical control, such as biological control using predators, parasitoids, microorganisms or way to increase the action of the natural enemies, use of extracts of plants or insecticides/ fungicides of botanical origin, or non-preferred species cultivars, use of crop control (diversification of crop systems, trap crops, crop rotation, soil plowing and harrowing, use of fertilizers, etc.) are still being researched, and none of them are currently available in the market, and they do not have its efficiency proven in pilot tests and in operational conditions.

Methods that do not use synthetic chemicals, as biological applied or conservative, non-preferred ("resistant") plants, extracts of toxic plants or active ingredients of botanical origin, crop control, have produced poor, inconsistent results, without the indication of technical, economical and operational feasibility, although many projects have been developed in research institutions and universities. It seems there will be no recommendations and products available on a short term. For example, a project developed in Brazil for studies on natural products of botanical origin with insecticidal/ fungicidal potential, financed by an institution that incentives research in partnership with universities, after 25 years and despite many efforts spent on research, they found substances with low success expectations, considering that 98% of the experiments were conducted in laboratory. Studies with biological control are more focused in the entomopathogenic fungi, and the results obtained, however, having been showing inconsistent, also demonstrating their technical, economical and operational infeasibility.

The remaining chemical control formulations, such as dry powder and thermal fogging, may not be considered as substitutes to toxic baits, but as their supplement in very specific situations. The toxic bait, like the sulfluramid is the most used formulation and shows full efficiency in the control of leaf-cutting ants and grass-cutting ants.

An active ingredient candidate to replace the sulfluramid in the manufacturing of toxic baits should show particular features in its control efficiency: must act on ingestion, be odorless and non repellent, show delayed toxic action, be lethal in low concentrations and paralyze the cutting activity (injuries or damage caused by the ants), right after the first days of its application. Active ingredients, such as the fipronil and other phenylpyrazoles used in the toxic bait formulation, do not show chances of replacing the sulfluramid because they add limitations. Therefore, the need for maintenance in the use of sulfluramid until another active ingredient is found with the desirable features, like other products registered and marketed for that control, are not efficient options.

Probably, monitoring (survey of the number of nests/ha) will continue to be used exclusively in planted forests with species of *Eucalyptus* and *Pinus* with the purpose of reducing the need for use of hand labor and insecticide in the toxic bait formulation.

Decision making in the control of the cultivation of species of *Eucalyptus* has the purpose of determining the moment to perform chemical control, therefore it is just the rational use of chemical control, and is not considered within the ideal approach of IPM. Thus, that "supervised control" is not necessarily bad, taking into account that the careful use of the insecticides should be encouraged. The biggest issue in the future with that vision is that it perpetuates the idea of the "quick-fix mentality" and so we are not able to implement the true "Integrated Pest Management". Thus, in the future, one must invest in research so that other technologies such as: biological control, insecticides of botanical origin, resistant plants, crop methods and others, may become efficient, market competitive and be available to consumers. Thus, only in the future, when other control methods are available in the market with proven efficiency, will we be able to use the integration of various methods as advocated by the principles of IPM.

Taking into account that chemical control is the only efficient control method available, so it is not possible to use the principles of IPM in order to promote the control of leaf-cutting ants within that approach in light of the current knowledge.

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