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APPROACH ON THE ANT`S BIOLOGY, SCREENING AND DESIRABLE FEATURES OF ACTIVE INGREDIENTS AND INSECT GROWTH REGULATORS FOR CONTROL OF LEAF-CUTTING ANTS

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APPROACH ON THE ANT'S BIOLOGY, SCREENING AND DESIRABLE FEATURES OF ACTIVE INGREDIENTS AND INSECT GROWTH REGULATORS FOR CONTROL OF LEAF-CUTTING ANTS.

1) Differences among leaf - cutting ants and tramp ants

The ants from the Attini tribe (Myrmicinae) are exclusively American and all species are in symbiotic association with fungus they grow to feed grown ants and larvae. Such symbiotic association allows the less derived (primitive) Attini to use substrates such as insect excrements and even substrates already used by other cutting ants to grow fungus but, to our knowledge, such substrates are vegetal tissues and rarely from animal origin (WEBER, 1972; FOWLER *et al.*, 1991). The ants from this tribe, of the *Atta* and *Acromyrmex* genera, known as leaf-cutting ants, use live tissue as substrate to cultivate the fungus, which they eat, as mentioned before, often cutting different kinds of vegetal species (BELT, 1874; SOUZA, 1965; CRERRETT, 1968 and MARICONI, 1970). Occasionally Attini species of the *Sericomyrmex* and *Trachyrmymex* genera may also use live tissues from leaves. (WEBER, 1972).

The leaf-cutting ants of the *Atta* genus, popularly known as "saúva" and the *Acromyrmex*, called "quenquéns" in Brazil, are recognized as agricultural pests of planted forests and cattle (Figure 1). The success of this group of insects is related both their social organization and to the ant-plants-fungus interaction. They cut about 29 to 77% of the leaves in the natural habitats (CHERRETT, 1968; FORTI, 1985, DEJEAN et al., 1992 and GARCIA et al., 2003). Their foraging behavior, in the ecological sense, can be classified as polyphagous, due their exploitation of a large numbers of plant species used for fungus cultivation. Because of their exploitation of a large number of plant species, leaf-cutting ants may be considered the greatest herbivores in the Neotropical region (FOWLER et al., 1991).

The leaf-cutting ants' species, their distribution and their relative pest status, are listed in table 1 according (FOWLER *et al.* 1990).

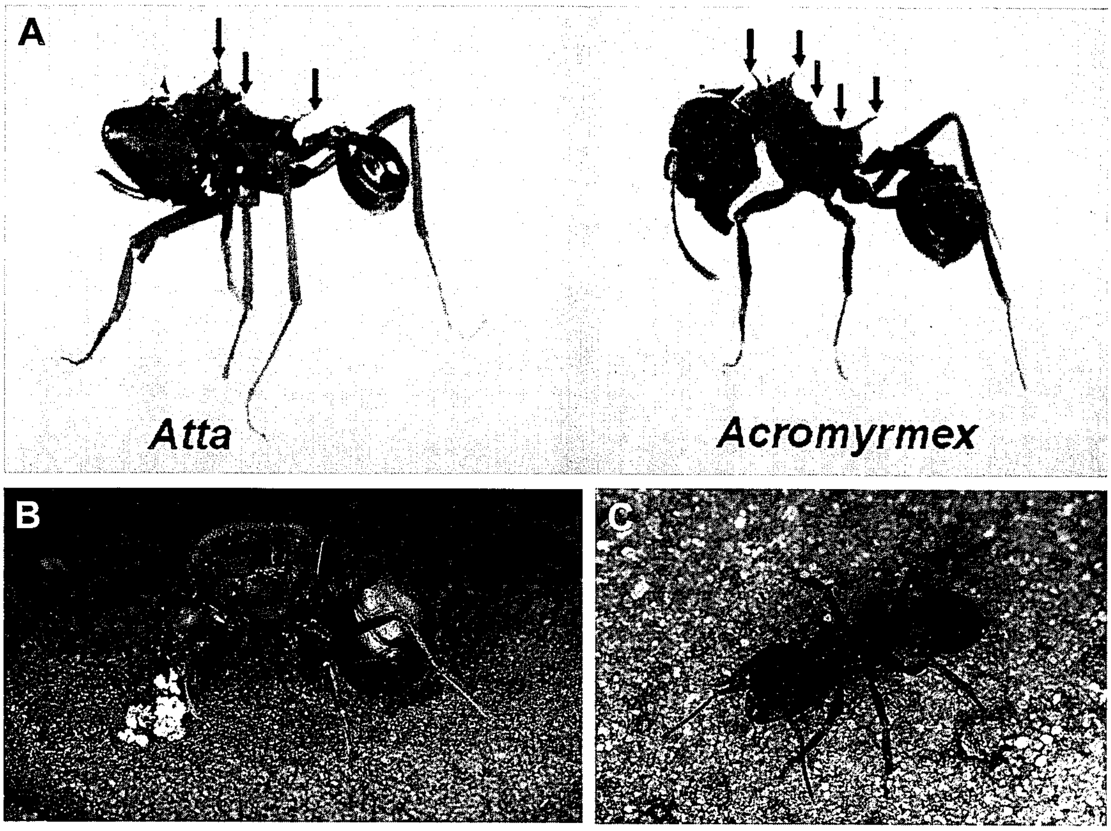

Figure 1. Differences between *Atta* ant *Acromyrmex.* A) The bigger workers of *Atta* have 3 pairs of spines in the thorax while the *Acromyrmex* may have 4 or 5 pairs. B) *Atta* queen with initial culture of fungus and eggs. C) *Acromyrmex* queen is significantly smaller than the *Atta* queen (FORTI & BOARETTO, 1997). Original pictures of the author.

In Brazil, we find 9 species of *Atta* (Figure 2) and 21 of *Acromyrmex* (FORTI *et al.*, 2006). As can be observed in table 1, the ants from both genera can explore and cut both dicotyledoneous and monocotyledons. Therefore there are species that cut preferably dicotyledoneous with big pest potential (pest status), such as *Atta sexdens, Atta cephalotes* while others, such as *Atta capiguara* and *A. bisphaerica,* which use grasses (MARICONI, 1970; FORTI, 1985; FOWLER *et al.*, 1986 a), cause significant losses in pastures (AMANTE, 1967 a, b, c.) and in cereal and sugar cane crops (AMANTE, 1972; PRECETTI *et al.*, 1988). The *Atta laevigata*, which may cut both dicotyledoneous and grasses is a very interesting species due to this biological aspect and is a very important species economically due to this biological plasticity.

Table1. The 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)	Vegetation 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
lundi	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

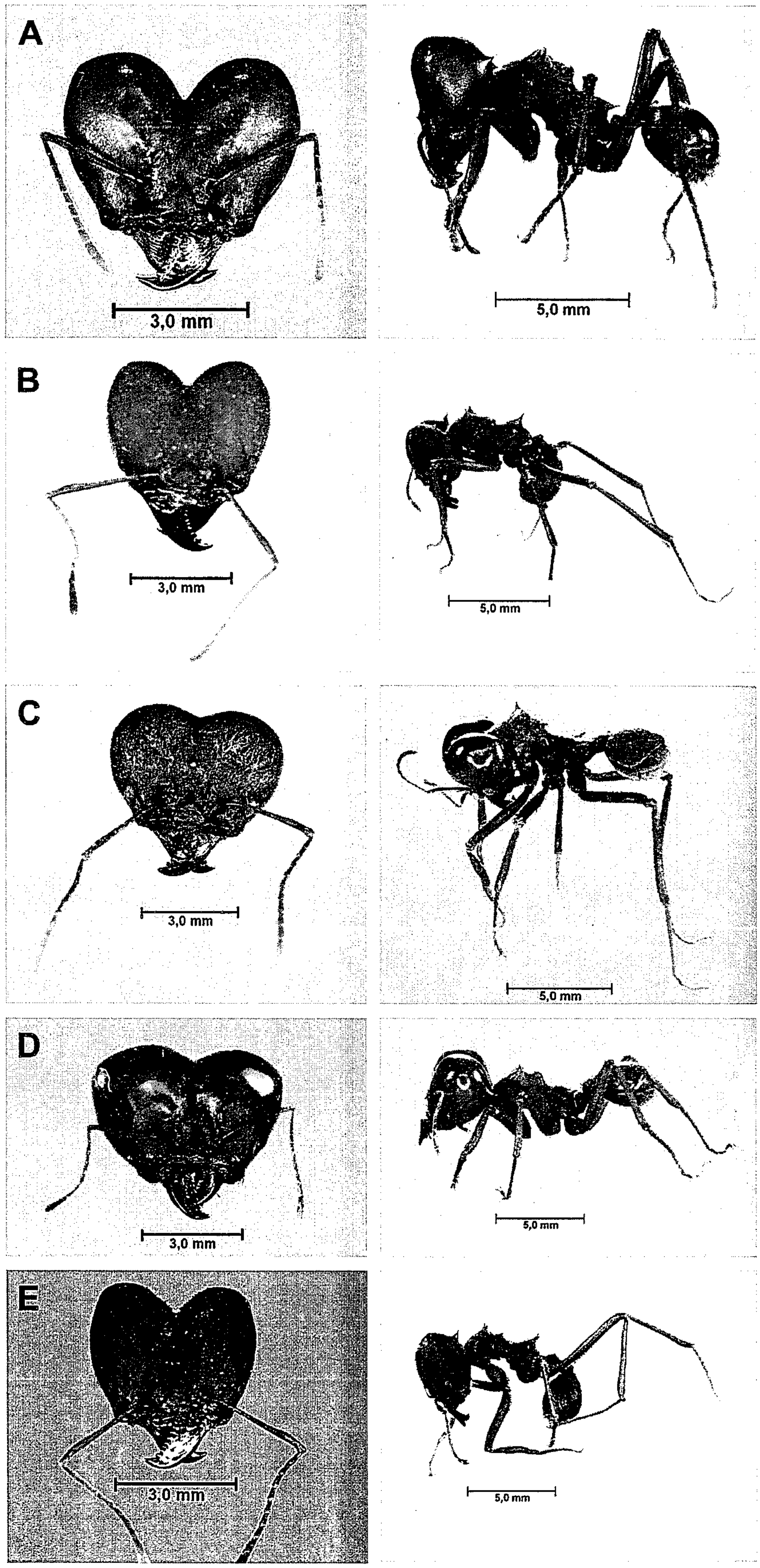

Figure 2. The five most important species of *Atta* (saúva ants) in Brazil. A) *Atta bisphaerica*; B) *Atta sexdens*. C) *Atta cephalotes*; D) *Atta laevigata* and E). *Atta capiguara*. Original pictures of the author.

Among the *Acromyrmex* there are also species that cut preferably grasses and others that cut dicotyledoneous, as well as species that cut both groups of plants (Table1).

Depending on the part of the country and the plant cultivated, some *Acromyrmex* may cause severe damages to the cultivated leaves and even limit their planting. For example, *Acromyrmex crassispinus, Ac. subterraneus, Ac. octospinosus, Ac. rugosus* and *Ac. lobicornis* may be found in vast areas planted with *Eucalyptus* and are severe pests that limit production. Among the grass-cutting ants, *Acromyrmex landolti, Ac. striatus, Ac. labicornis* and *Ac. heyery* are the most important pests. As mentioned before, the *Acromyrmex* may cause economic damages as severe as the ones caused by the *Atta,* but there are few studies on the *Acromyrmex,* and that is the reason they are not mentioned so often in the literature, being this knowledge restricted to the people and companies in agricultural and forest sectors and therefore such data is recorded in literature.

Those species which harvest grasses for use as fungal substrate differ in behavior and morphology from those that use dicots as a fungal substrate. Species which live in forests cut dicots almost exclusively, while species which live in open habitats cut either dicots or grasses, or both. For the species which cut dicots, the harvested vegetation is cut into smaller and smaller pieces until it is the form of pulp which is then used as the substrate of the fungal garden (Figures 6 and 7) (FOWLER *et al.*, 1986b; ANDRADE *et al.*, 2002) consequently, the conversion of fungal into ant biomass, starting from the plant base, is probably much higher in dicot-cutting than grass-cutting ants, as the latter do not heavily process the grass blades which they bring into the nest (FOWLER & ROBINSON, 1979; FOWLER *et al.*, 1986b) (Figure 3).

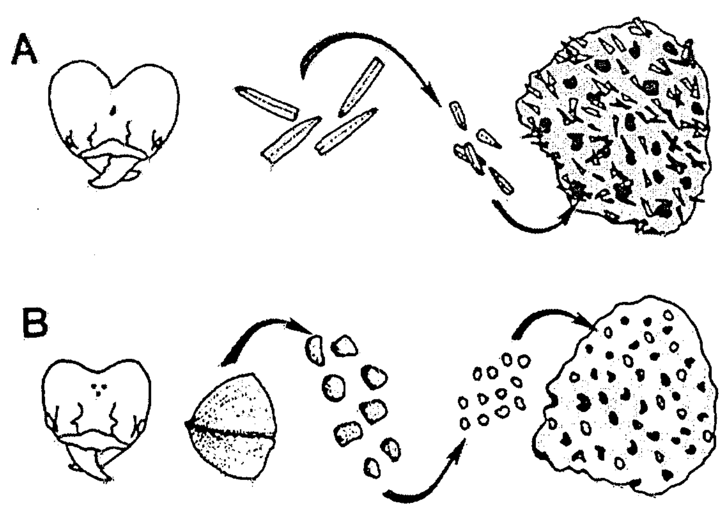

Figure 3. Differences in fungal substrate preparation between grass-cutting (A) and leaf-cutting ants (B). Note how vegetation is triturated for fungal substrate in leaf-cutters, while only smaller pieces are planted into the fungal garden of grass-cutters (FOWLER *et al*, 1986a).

Correspondingly, morphological differences are evident in the two ant types. Species wich harvest primarilly grasses tend to have massive, short mandibles, while those species which harvest dicots tend to have longer, but less massive mandibles. Grass – cutters also tendo to harvest vegetation differently (FOWLER *et al.*, 1991; GARCIA, 2003). In particular, they tend to have shorter metathoracic legs than leaf – cutters use these legs as pivots while cutting, the grass cutters do not (FOWLER *et al.*, 1986 b) (Figure 4).

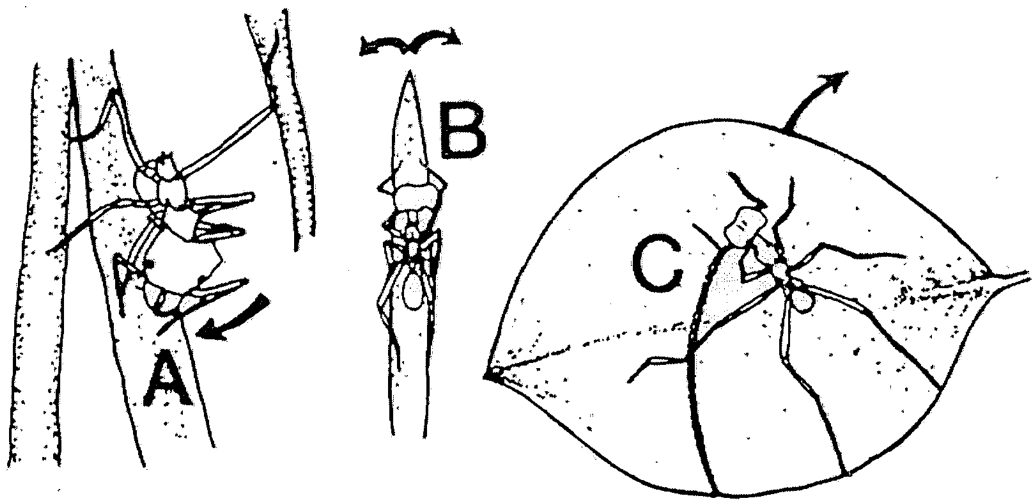

Figure 4. Cutting behaviors of leaf-cutting and grass-cutting ant compared. A. *Atta capiguara* orients downward, cutting in one direction. When the grass blade is finally severed, it is transferred to the mandibles and the ant carries it to the nest. B. Cutting behavior of *Acromyrmex fracticornis.* The worker orients upwards and cuts the grass blade by alternating the force of its mandibles on lither side of the blade. C. Cutting behavior of *Atta sexdens.* Note mandibles are used like scissors with the metathoracic legs serving as a pivotal point while cutting. (FOWLER *et al.*, 1986a).

All Attini grow symbiont fungus to serve as food to the larvae and working ants. The leaf-cutting ants of the genera *Atta* and *Acromyrmex* evolved the ability to cultivate *Leucoagaricus gongyllophorus* fungus as major source of food for the larvae, while the workers can obtain part of their diet from the fluids extravasated by the leaves during the cutting activity (PEREGRINE & MUDD, 1975; LITTLEDYKE & CHERRETT, 1976; FORTI & ANDRADE, 1999). Consequently, leaf-cutting ants starts to ingest substance at the time when they cut the plant, (ANDRADE *et al.*, 2002). The fungus cultured by the ants degrade cellulose (BACCI *et al.*, 1995)

Table 2. Ant pest of South America, given in alphabetical order, with their origin and principle activity of economic importance. (Modified from FOWLER *et al.*, 1990).

Ant Taxa	**Origin**	**Principe activity of the pest status**						
	Native (N) of Introduced (1)	**Cut leaves or flowers**	**Other plant damage**	**Tending of Homoptera**	**Aggressive?**	**Public Health importance**	**Domestic pest**	**Material damage**
Acropyga spp.	N			X				
Acromyrmex spp.	N	X	X					
Atta spp.	N	X	X					X
Azteca spp.	N		X	X	X			
Camponotus spp.	N		X	X	X		X	X
Crematogaster spp.	N		X	X		?	X	
Linepithema humile	N/I			X		X	X	
Monomorium pharaonis	I					X	X	
Monomorium destructor	I					X	X	
Monomorium floricola	I					?	X	
Paratrechina fulva	IN		X	?			X	
Pheidole spp.	N					?	X	
Pheidole megacephala	I			X			X	?
Solenopsis spp.	N/I		X	X	X	?	X	X
Tapinoma melanocephalum	I			X		?	X	
Wasmania auropunctata	N/I			X	X	?	X	?

From practical viewpoint, the preparation and incorporation of the leaf substrate for fungus culture are intimately linked to workers contamination with insecticides. Thus, PEREGRINE & CHERRETT (1974) and LITTLEDYKE & CHERRETT (1976) observed that medium-sized workers of *Atta cephalotes* and *Acromyrmex octospinosus* were those most intensely labeled with ^{32}P, and conduded that workers of this caste were those most responsible for the preparation of the leaf substrate. Studies with *Atta sexdens rubropilosa*, (ANDRADE *et al.*, 2002) conclude that a caste preponderantly engaged in the activity of liking the substrate was the minima workers, which were responsible for 52% of this task. The smaller castes, minima and generalists, are those most responsible for the preparation of the leaf substrate and predominate within a colony (ANDRADE *et al.*, 2002).

On the other hand, other ants that do not belong to the *Attini* tribe do not grow fungus (Figure 5) and show some behavioral patterns from the feeding point of view that can be catalogued. Among the Myrmicinae, the ants from the Dacetini and Basicerotini tribes inhabit the interstitial humus, where they eat mainly insects of the Collembola Order.

The Solenopsidini and Crematogastrini tribes may present association with plants and homopterous. In the Solenopsidini tribe one may find some species of economic importance in South America, such as the *Solenopsis* spp., which is very important economically in North America, and in Brazil its importance is associated to the time of the harvest of certain cultivated leaves or when they occur in gardens (FOWLER *et al.*, 1990), biting people or even injuring plants. The *Wasmannia auropunctata* species, due to their ability to colonize quickly, fast colony growth with multiple queens, expand quickly. This species prefers disturbed regions. In the cocoa-growing region of Bahia, Brazil, *W. auropunctata* lives in the cocoa canopy, and has apparently expanded following massive control programs against *Azteca* spp. In the 1950s and 1960s (DELABIE, 1988). This ant is active predator of herbivorous insects and other ants and also depends upon honeydew of scales. Because of their agressivity and strong stings, which can result in allergic reactions, fruit-pickers are reluctant to work in areas in which these ants occur (FOWLER *et al.*, 1991).

Subfamily Formicinae has some peculiarities. A good portion of their kinds are widely spread around the world. Some kinds gather hundreds of species, which are mostly described improperly. Generally speaking one may say that the Formicinae have a liquid diet, associate with homopterous and do not have a functional sting. Their poison glands are altered to produce important substances in the chemical communication (pheromones): path marking, colony recognition, recruiting among working ants and alarm (FOWLER *et al.*, 1991). The *Camponotus* is one the largest and most heterogeneous in the Neotropics (KEMPF, 1972) and, consequently it is difficult to determine which species may be of economic importance, especially since identification of species is difficult. Of the larger species, such as *Componotus rufipes* and *C. brasiliensis* ants protecting associated Homoptera may create problems in fruit producting areas due to their aggressiveness (SCAR PELLINI *et al.*, 1986).

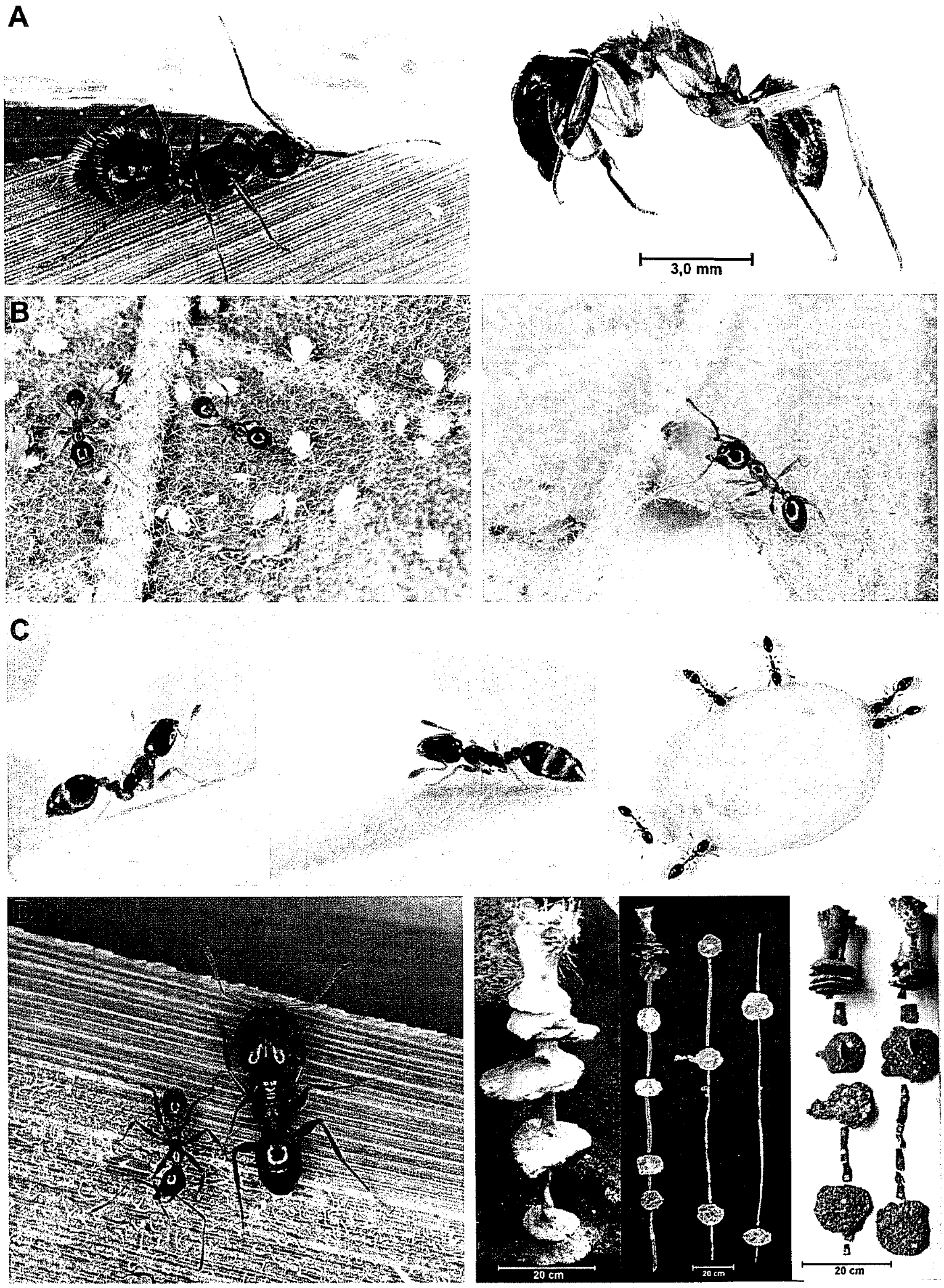

Figure 5. Some ants genera that do not cultivate fungus. A) *Camponotus* spp.; B) *Pheidole* spp. associated to Homoptera; C) *Monomorium* spp. feeding with honey and D) *Pheidole* spp. with small worker and soldier that can build nests with depth of up to 5 m. The nests are of Brazilian *Pheidole oxyopes* (FORTI *et al.*, 2007). Original pictures of the author.

Dolichoderinae are also frequently associated to Homopterous. For exemple, the Argentine ant *Linepithema humile*, has spread from South America throughout the world. Even though species are generally more problematic than native species, *L. humile* can be pestiferous in South America. (FOWLER *et al.*, 1990). This polygynous Dolichoderinae is a recorded tender of Homoptera (GALLO *et al*, 1978). The Dolichoderinae *Tapinoma melanocephalum* (ghost ant) was introduced in Brazil. These species is a domestic pest, and may transmit hospital infections, like *Linepithema humile*. Boths species of the small size and has capacity of colonies to divide easily.

The Dolichoderinae *Azteca paraensis* and *A. chartifex* depend heavily upon Homoptera honeydew, especially the dew produced by fruit-attacking Membracids. In cocoa plantations, the heavy nest of *A. chartifex* break branches. By building carton galleries on branches, the ants interfere with flowering and reduce potencial fruit yield. Both species are aggressive, and rural workers are wary about working on plantations where these ants are present, thus making fruit harvest and plantation maintenance more costly and irregular (FOWLER *et al.*, 1990). However this ant dominates the canopy, increased fruit production may be due to its role a predator of phytophagous insects. More evidence is needed to determine if *Azteca* species are pestiferous or beneficial. (FOWLER *et al.*, 1990).

The *Monomorium* exotic species of the myrmicinae are generally associated only with structures (FOWLER *et al.*, 1990). Like *Wasmannia auropunctata* and *Linepithena humile,* these ants form polygynous colonies and fragment easily. Studies reveal that *M. pharaonis* is common in hospitals and health care centers, and is associated with hospital infections. *Monomorium* species are notorious household pests and colonies are often transported from are local to another in boxes for the supermarket. (FOWLER *et al.*, 1990).

Some of the smaller *Pheidole* species can be of occasional importance in household as domestics pests, but the introduced *Pheidole megacephala* is undoubtedly the genus' most important representative pest. This Myrmicinae ant, often damaging structures such as electrical cables (FOWLER, 1988) and becomes a domestic and household pest.

2) Screening of active ingredient (a.i.) for leaf-cutting ants

The main method for the control of leaf-cutting ants (*Atta* and *Acromyrmex* genera) is the use of granulated toxic baits. For a bait to be affective, it requires on active ingredient (a.i.) which: a) does not provoke significant initial rejection (carrying) or delayed rejection (after carrying) of the baits (AMANTE, 1968; PEREGRINE & CHERRETT, 1974; FORTI *et al.*, 1998, 2003; CAMARGO *et al.*, 2003; LOPES *et al.*, 2003; NAGAMOTO *et al.*, 2004; 2007). b) presents a delayed action sufficient to permit good dispersal of the insecticide inside the colony (FORTI *et al.*, 1998).

Now a is are generally selected by applying insecticides already formulated in granulated baits directly to field of laboratory colonies (CHERRETT 1986; FORTI *et al.*, 1993a), situations in which it is difficult to evaluate insecticide activity in detail. In the laboratory, the quantification of worker mortality is intrinsically difficult since the colonies generally posses thousands of workers, with the presence of a fungus garden (MARICONI, 1970; WEBER, 1972) representing on additional difficulty compared to other ant pest species because part of the workers remain inside the fungus garden. For field colonies, evolution of the mortality of colonies is also difficult because even though the colonies appear to be dead, when the nests are excavated the colonies may actually be found to be alive (FORTI *et al.*, 1993a).

Another possible problem impairing the assessment of new a.i.s is the accidental contamination of experimental baits with another insecticides residues, like dechlorane (FORTI *et al.*, 1993b) a fact that contributed to the equivocal selection of copper oxychloride and diflubenzuron, which were considered to be excellent a.i.s (LOECK & NAKANO, 1983; 1984; BUSOLI *et al.*, 1992)., leading to the commercialization of the Formilin® bait containing 40 ppm diflubenzuron (LOECK *et al.*, 1993). However, these a.i.s are not actually toxic to workers (FORTI *et al.* 1993b) and do not Kill colonies (without contamination).

One of the main characteristics to be taker into account when selecting a.i.s for the use in toxic baits is their delayed action on adult workers (STRINGER *et al.*, 1964; WILLIANS *et al*; 2001; RUST *et al.*, 2003). The term delayed action was first used for the fire ants *Solenopsis richteri* and *S. invicta* (Hym, Formicidae), and was defined as an insecticidal activity whereby mortality is <15% during the first 24 hours and >89 or 90% at the end of the assay (24 or 21 days) (STRINGER *et al.*, 1964; WILLIANS, 1983; VANDER MEER *et al.*, 1985.NAGAMOTO *et al.*, 2004) developed an insecticides classification method by evaluating formicidal activity over time in groups of workers of *Atta sexdens rubropilosa* isolated from colonies; used a model for leaf-cutting ants. An attractive formulation in terms of ingestion prepared on the basis of citrus pulp was used as vehicle for the a.i.s.

2.1) Insecticide formulation

The insecticides were formulated as palatable pastes based on citrus pulp (industrial residue of juice extraction and essential oil of citric fruits, mainly orange, dehydrated), because this is also the basics of commercial toxic baits. This component was obtained in the form of pellets, triturated in a blender and sieved various times through a fine mesh, separating the powder. In addition, we used sucrose (p.a.) witch, like citrus pulp, is considered to be very attractive to leaf-cutting ants (CHERRETT & MERRETT, 1969; MUDD *et al.*, 1978; JUTSUM & CHERRETT, 1981), and distilled water to permit spontaneous ingestion, since workers feed on fluids.

The a.i.s used were purchased as technical grade preparations. In the presents study, all formulations were prepared based on the weight/weight (w/w) ratio and the grade of purity of each technical grade product was taken into account.

Previous formulations were initially prepared with a concentration of the a.i. 10 times higher than that determined for the final formulation. For formulations with higher a.i concentrations (10 and 1%), the technical grade products were dissolved in acetone p.a. in a 50-ml beakers and the citrus pulp powder was added, followed by homogenization, with frequent mixing as evaporation of the solvent occurred until loss of the moist aspect. For evaporation of the residual solvent, these formulations were maintained under laboratory conditions for at least and additional 24 hours before preparation of the final formulation or of less concentrated formulations.

For formulations with a.i. concentrations equal to or lower than 0.1%, an aliquot of the previous formulations with the immediately higher concentrations was weighed instead of the technical grade product, thus diluting the concentration 10 times, the aim of this difference in preparation was to prevent a weight measurement with less than 3 significant figures on the scale used (of 4 figures).

Immediately before application, the previous formulations were finalized by the parsimonious alternate addition of various aliquots of aqueous sucrose solution

(10%) and citrus pulp powder, in such a way as to simultaneously: a) form a homogenous paste as moist as possible but without separation of the liquid phase when manipulated, and b) to obtain a final weight exactly 10 times higher than in the previous formulation. The amount of sucrose solution added was about 77.5% of the final formulation. Thus, pasty formulations were obtained, in which the insecticide concentrations decreased at 10-fold rates from 1%.

2.2) Assays

Concentrations of 1, 0.1 and 0.01% of the a.i. were initially evaluated. If a fast action was observed at all of these concentrations, a new experiment was performed to determine the possible existence of a delayed action at lower concentrations, i.e. 0,001, 0.0001 and 0.00001% the experimental design used was that of random blocks (a total of 10 blocks), with each block consisting of workers isolated from a distinct colony.

Application of the insecticide was done by adding 1.5 to 2 g of the formulation per pot, separated from the plaster by a 2x2 cm polyethylene film. These formulations were removed after 24 hours, approximately20 garden workers (2 mm body length) per group. Garden workers were included because medium-size workers have difficulties in maintaining the fungus garden (BASS & CHERRET, 1995)..

If necessary more fungus garden was added during the experimental period and the waste produced was removed periodically. Mortality (cumulative) and intoxication symptoms (now-cumulative) were evaluated in medium-size workers at 1, 2, 3, 5, 7, 9, 11, 14, 17 and 21 day after application. All experiments were carried out under laboratory conditions at a temperature ranging from 23 to 25°C. Intoxication symptoms were analyzed by assigning one of the following scores: O for each group of live ants without symptoms, 0.5 for groups in which part of the individuals showed symptoms, and 1.0 when all individuals presented symptoms.

2.3) Data analysis

On the basics of the classification used for *Solenopsis* spp. (WOJCIK *et al.*, 1976; WILLIANS, 1983; VANDER MEER *et al.*, 1985), the following classes of insecticidal activity over time were proposed for *A. sexdens rubropilosa*: a) class I –

compound that causes > 90% mortality at 21 days even at a higher concentration (1%); b) class II – compound with a fast action (mortality > 15% at 24 hours and > 20% at 21 days) at least one concentration; c) class III – compound with a delayed action (mortality≤15% at 24 hours and ≥90% at 21 days) at a concentration range of ≥ 1 time to < 10 times; d) class IV – compound with a delayed action at a concentration range of ≥ 10 to < 100 times, and e) class V – compound with delayed action a concentration range of 100 times. The classes with a delayed action do not depend on the occurrence of an action defined as fast at higher concentrations because what is important is presence of a delayed action and its respective range of concentrations.

Median mortality rates corrected by the Abbott formula (ABBOTT, 1925) were used to fit the result obtained with this classification. Statistical analysis was performed using the SigmaStat 2.03 software. Since the data showed no normal distribution, parametric statistics could not to be used. Therefore, to determine the significance of differences in mortality between treatments, the corrected data obtained for each evaluation were submitted to nonparametric analysis of variance (Friedman test), and medians were compared by the Student-Newman-Keuls test, at a probability of 5% (WINER *et al.*, 1991).

3) Desirable features in active ingredients to control cutting ants

The active ingredients (a.i.s) used to control cutting ants are incorporated in toxic baits. The commercial toxic baits are composed of an attractive substrate (vehicle) mixed with the active ingredient and formulated in pellets. The active ingredient is dissolved in vegetal oil (4 to 6%) and incorporated into the substrate. The material widely used as vehicle is the dehydrated citric pulp form the orange juice industry. This citric pulp is composed mainly of peel, seed, albedo and bagasse. It should be pointed out that the industry extracts several essential oils from the peel, pectin and other substances (CHERRETT, 1972; ROBINSON, 1979; LOECK & NAKANO, 1984; FORTI *et al.*, 1998 e FORTI *et al.*, 2007.)

The toxic bait for cutting ants, according to ETHERIDGE & PHILLIPS (1976) must be attractive to the ants, if possible at a certain distance from the nest, be carried by the working ants whenever found, to have a sufficiently retarded action to be conducted for long distances by the ant, have active ingredient widely distributed within the nest, present specificity to the target species and have low toxicity to mammals and other animals.

The continuous use of toxic baits in Brazil for over 50 years shows that the formulation with citric pulp is indisputably efficient in terms of attractiveness to the *Atta* and *Acromyrmex* target species. (FORTI *et al.*, 1998). Toxic baits containing 0.3% sulfluramid are uniformly distributed within the nest, according to studies developed by PRETTO & FORTI, 2000 and MOREIRA *et al.*, 2003 for different *Atta* species. This aspect is very important to the good efficacy of the toxic bait.

The ideal insecticide for the toxic bait should present the following features according to FORTI *et al.* (1998); NAGAMOTO *et al.* (2004):

1. It must be lethal in low concentration and not kill ants in high concentrations quickly;
2. It must be an ingestion insecticide with retarded action and little or no contact action;
3. It must be inodorous and non-repellant, low steam pressure, i.e, sulfluramid present.
4. It must be easily dispersed by the cleaning process (grooming and self-grooming) and by trophallaxys during the whole substrate preparation process.
5. It must have a relatively quick degradation, low toxicity to animals and be environmentally accepted.

The insecticides that operate by ingestion and present retarded action, such as the sulfluramid, disperse themselves to all the colony ants by grooming, self-grooming and trophallaxys. Retarded action insecticides do not interrupt the grooming, self-grooming and trophallaxys processes and kill even in low concentrations (Figure 9). The bait pellets are carried by larger workers to the fungus chamber, where smaller ants work with the pellets (ANDRADE *et al.*, 2002) the fact that smaller workers are less contaminated initially can be explained by the fact that larger ants are involved in primary activities with substrates, like foraging, and that smaller workers are those more involved in implanting the fungus hyphae on substrates (ANDRADE *et al.*, 2002).

Because the smaller workers are involved in microfungi control, their contamination can be very important in loss of microfungi (fungi do not cultivated for feed) control, commonly reported in baited colonies (FORTI *et al.*, 2007; NAGAMOTO *et al.*, 2003; RODRIGUES *et al.*, 2005b).

To attempt to control detrimental microorganism, the workers spend a large amount a time licking: a) the symbiotic fungus hyphae, b) themselves and each other (grooming and self-grooming), and c) the substrate (STAHEL & GEIJSKES, 1939; CURRIE & STUART, 2001; CURRIE 2001; ANDRADE *et al.*, 2002). For the substrate, licking activity also important to remove wakes waxes which can impair the fungal cultivation (GARCIA *et al.*, 2005). This pattern of intensive substrate licking and treatment applies to all attractive substrates, including toxic baits, so it can be states these activities are crucial for the toxic baits' a.i to contaminated the workers (ANDRADE *et al.*, 2002; NAGAMOTO *et al.*, 2004). These intensive activities also to explain the high percentage of the great level of contamination, about 50% to 70% at 48 hours, in all sizes of workers demonstrates that workers contact with toxic bait is intense within this period (FORTI *et al.*, 2007) trophallaxis could be an alternative explanation for dispense in other ants; however, most authors states the trophallaxis is less prevalent in leaf-cutting ant when compared with ant genera that typically forage on liquids (ANDRADE *et al.*, 2002; PAUL & ROCES, 2003; NAGAMOTO *et al.*, 2004).

Although it is know that workers distribute bait pellets uniformly trough almost all fungus chambers (PRETTO & FORTI, 2000; MOREIRA *et al.*, 2003), colony size under field conditions is a trait that must not be overlooked, as some of the colonies may contain thousands of fungus chambers (AUTUORI, 1942; MOREIRA *et al.*, 2004) (Figure 8). The *Acromyrmex* nests, are smaller than the *Atta* nests. However, in some species, such as *A. rugosus,* may build much deeper and more complex nests (VERZA *et al.,* 2007) (Figure 9). Faster acting a.i.s my decrease the bait distribution and also improve its rejection (CAMARGO *et al.*, 2003; FORTI *et al.*, 2003; LOPES *et al.*, 2003), lowering the efficiency to these bait in larger field colonies, i.e. with clorpiriphos, propoxur, endosulfan and others tested in laboratory and field (FORTI *et al.* 1998).

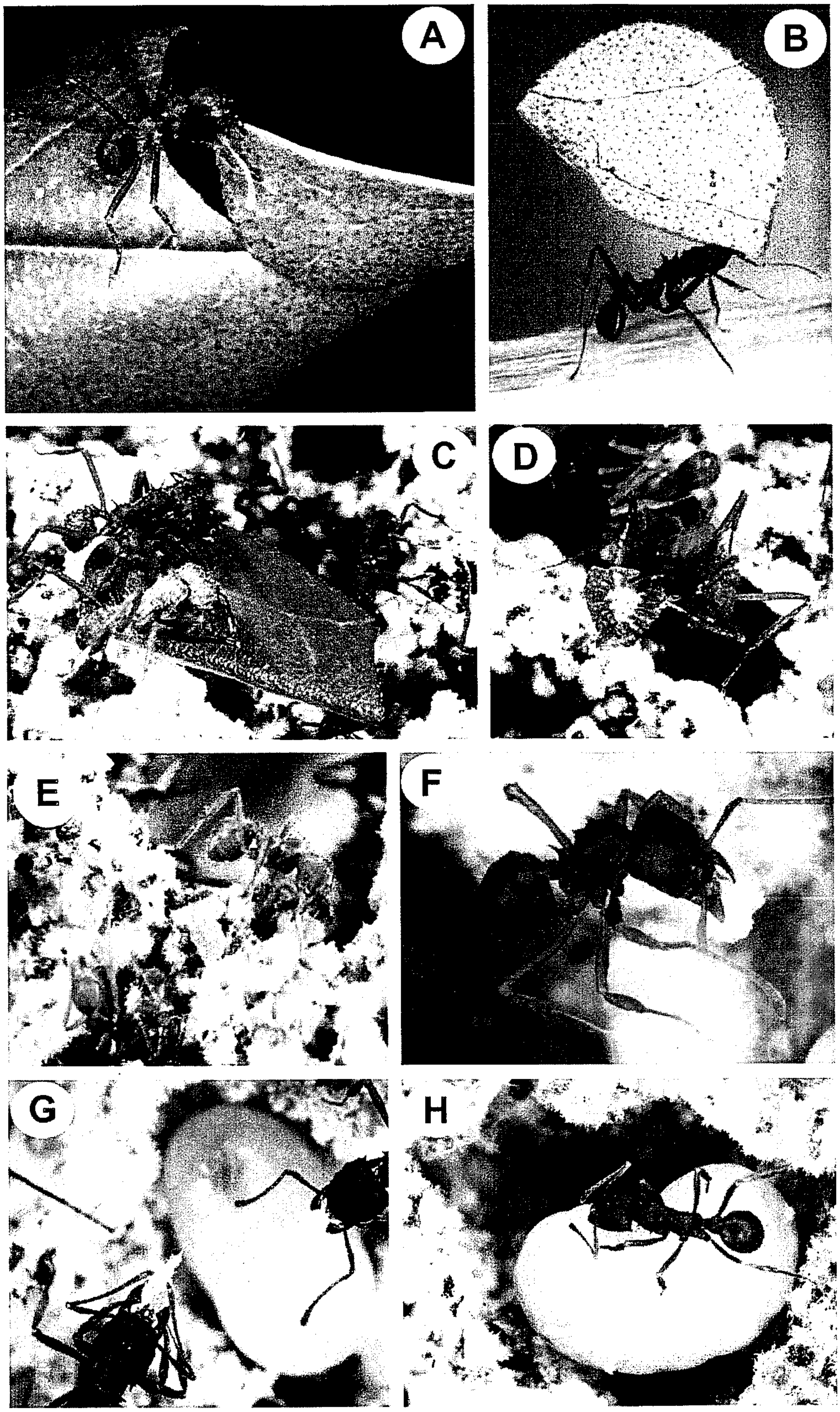

Figure 6. Behavioral sequence since the cut of leaves, preparation for inoculation of fungus cultivated until the larvae feed in *Acromyrmex* spp. A) Leaves cutting (substrate); B) Transport of leaves by worker; C) Licking substrate; D) Chewing and crimping edges of fragments of leaves E) Incorporating and licking(licking and touching) the fungus culture; F) Bunch of hyphae; G and H). Feeding larvae with fungal hyphae previously prepared. (CAMARGO, 2007).

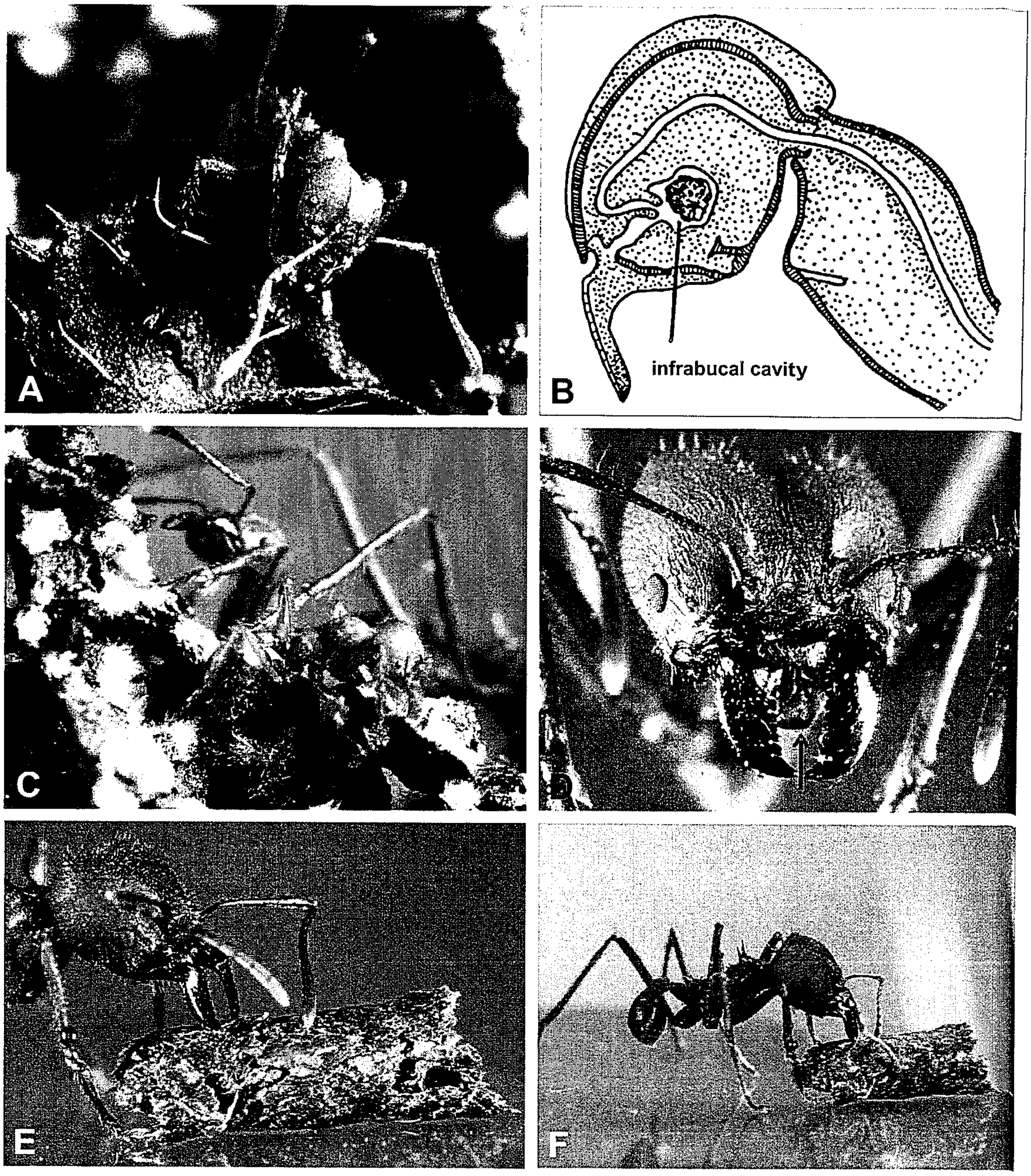

Figure 7. Preparation of substrate (leaves and toxic baits) and care with fungus garden in *Atta sexdens*. A) Behavioral act of licking substrate (leaf); B) solid particle in the infrabucal cavity; C) Touching and licking fungus culture; D) Glossae (arrow) and E) Preparation of toxic bait pellet to be incorporated to the fungus garden.

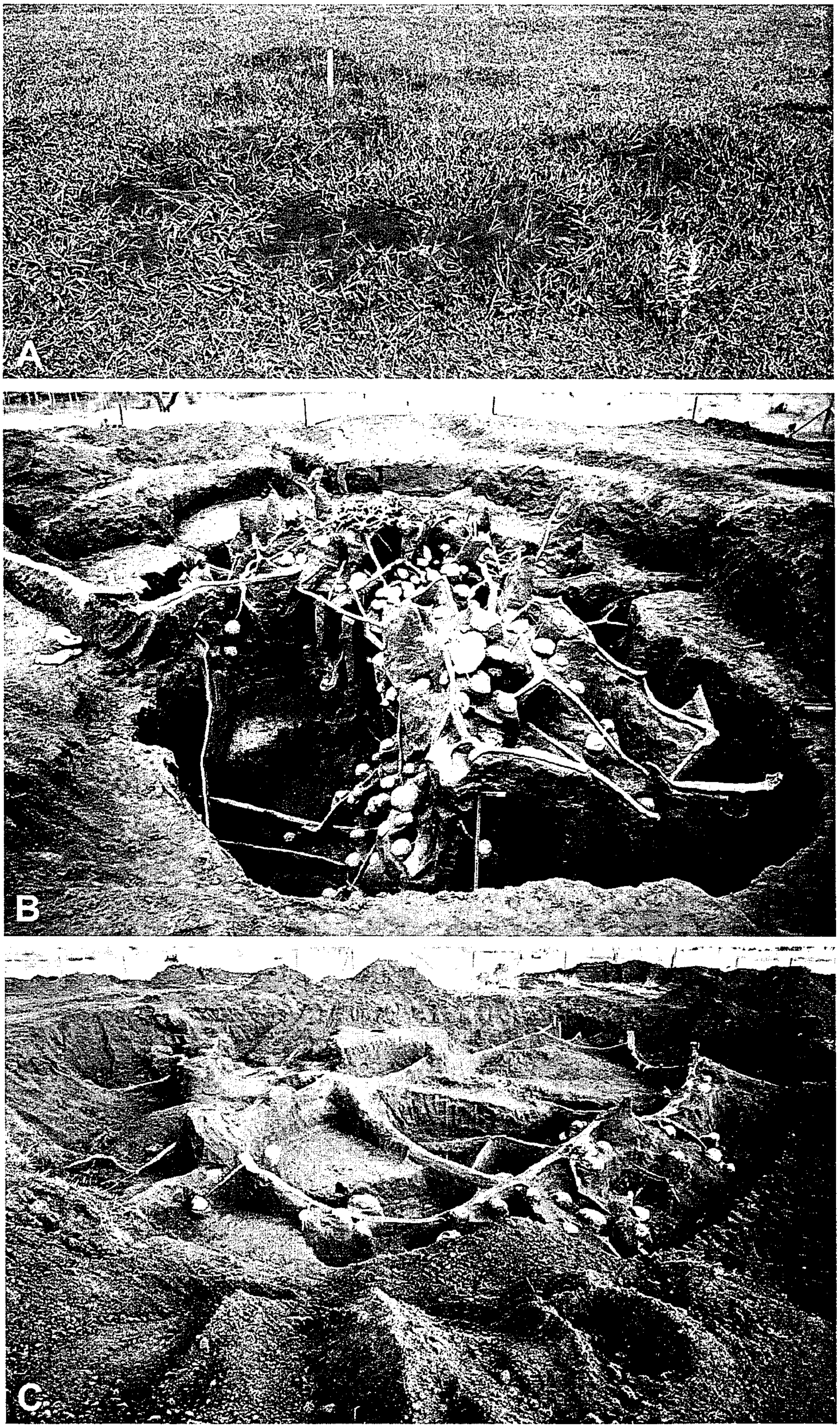

Figure 8. *Atta* spp. colonies molded with cement. A) View of refuse soil B) Nest of *Atta laevigata* molded with cement. Chamber of fungus culture, spherical structures interconnected by channels are observed. C) Nest of *Atta capiguara* molded with cement. Note the *Atta laevigata* nest contains a greater number of chambers of fungus culture and are more concentrated than in the *A. capiguara* nests. Original pictures of the author.

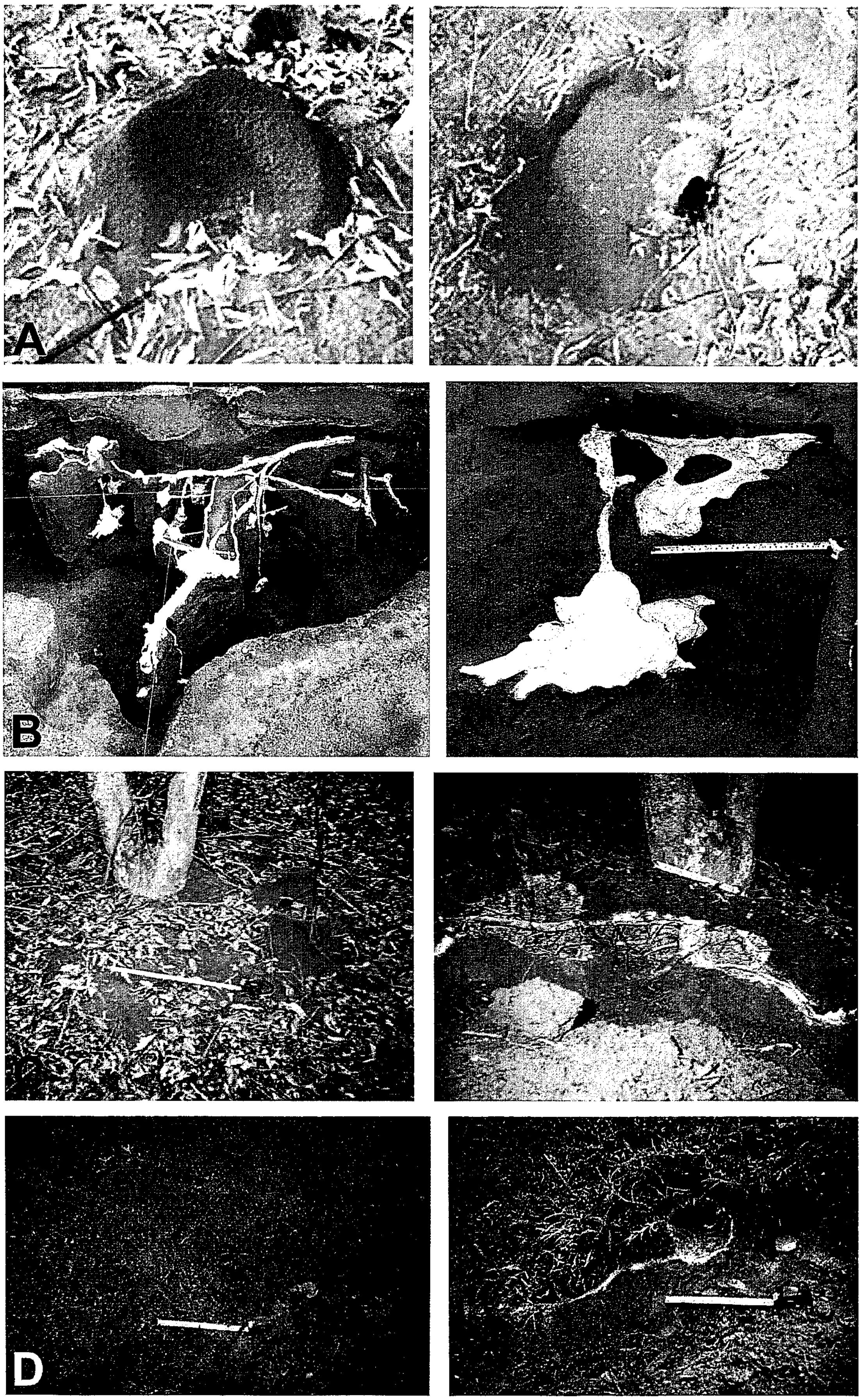

Figure 9. *Acromyrmex* nest A and B). *Acromyrmex rugosus*, and C and D). *Acromyrmex subterraneus*. (VERSA, *et al*. 2007 and ANDRADE, 2002).

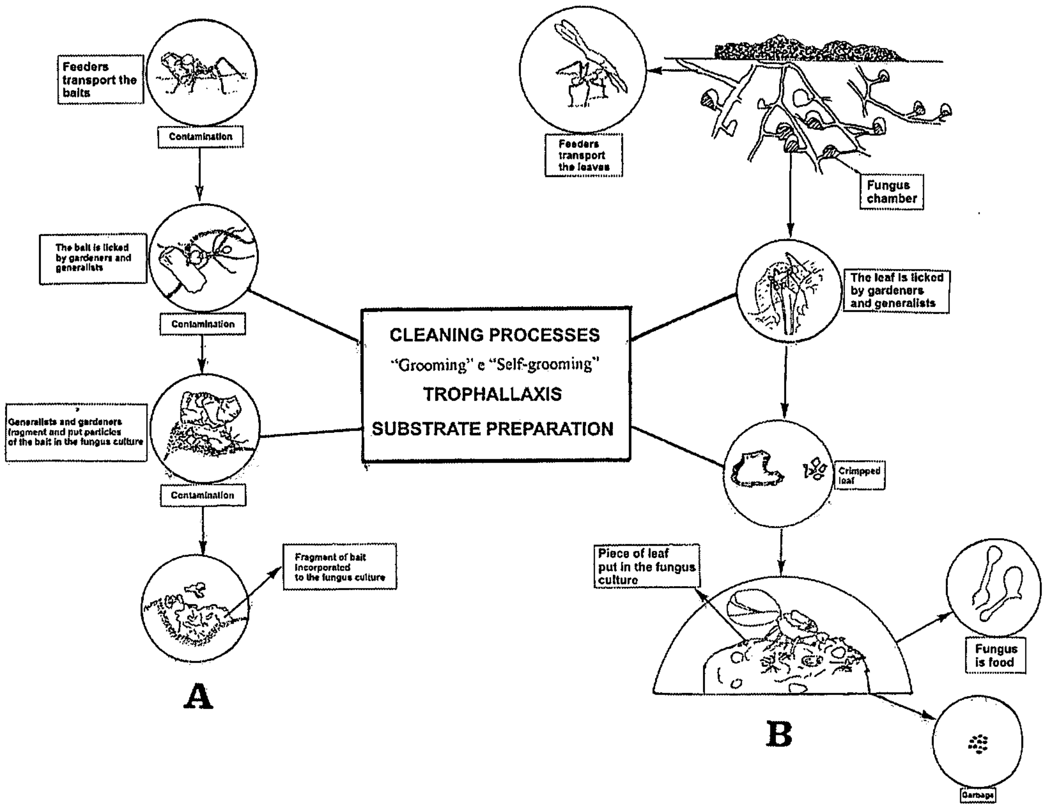

Figure 10. Similarities between the preparation of toxic baits (A) and leaves (B) by *Atta* spp. (leaf-cutting ant). The workers prepare the baits pellets (a) and are contaminated with sulfluramid by cleaning processes and trophallaxis. (FORTI *et al.*,1999).

4) The feeding of other ant species

This knowledge is very important for us to understand why the non leaf-cutting or fungus growing ants may ingest active ingredients from toxic baits, unlike the leaf-cutting (*Atta* and *Acromyrmex*) ones. The *Formicinae,* which comprise several different kinds, being the *Componotus* very common as urban pest and *Dolichoderinae* such as *Azteca* spp. *Tapinoma melanocephalum* (ghost ant) and *Linespithema humile* (Argentine ant) have perfect adaptation to liquid food. Both subfamilies have developed, during their evolution, processes to retain fluids in their crop (EISNER, 1957). The crop' content may be emptied through the mouth by regurgitation, feeding other colony members (FOWLER *et al.*, 1991). The anatomic structures of these ants allow them to carry big quantities of fluid in the crop. The extreme of this development is in the "full working ants" in Dolichoderinae and Formicinae. Leaf-cutting ants do not have the mechanism to reserve fluid in the crop to regurgitate it afterwards to the other working ants and larvae.

One of the most common foods among non-cutting ants is the "honeydew", which in some cases is the main diet item. Honeydew is a fluid excretion expelled by the aphids, coccids and other homopterous' anus. This excretion contains, besides sugars such as fructose, glucose and sacharose, a complex mixture of nutrients, including lipids, free amino acids and starches (MALTAIS & AUCLAIR, 1952).

The Myrmicinae most known pests are of the *Solenopsis spp* (fire ants) , *Wasmania auropunctata* (little fire ant) and some species of the *Pheidole* genera. Many Myrmicinae feed their larvae with pieces of food that the working ants bring to the nest. Ants such as the fire-ants (*Solenopsis*) and little fire ants (*Wasmania auropunctata*) give their larvae pieces of insects and regurgitated food as well. Therefore we can say that these ants are little insects' predators and also associate to the Homoptera combining ways of feeding. Among the *Pheidole*, the most important species is undoubtedly the *Pheidole megacephala* (big head ant). *Pheidole* normally predate insects, collect seeds and use insects' corpses. Some of the smaller *Pheidole* species can be of occasional importance in households as domestic pest. (FOWLER *et al.*, 1991). Some beneficial species such as the *Pheidole oxyops* may build nests that are up to 5m deep, but with a few flattened chambers (similar to dishes) connected by thin channels perpendicular to the ground level. (FORTI *et al.*, 2007).

5) Why don't insect growth regulators control leaf-cutting ants?

The efficiency of some insect growth regulators (IGR) and chitin synthesis inhibitory substances is quite contradictory in literature both as for leaf-cutting ants (FORTI *et al.*, 1998) and *Solenopsis* spp. (BANKS, 1986). In ants other than leaf-cutting, those substances may cause morphological changes in male and female subjects, but do not sterilize the queen (GLANCEY*et al.*, 1990).

Fenoxycarb is a carbamate considered an insect growth regulator, with properties that differ from other carbamates. In the United States, it was registered for the red imported fire ants (GLANCEY & BANKS, 1988). When tested in Brazil, there was no success for controlling leaf-cutting ants. (FORTI *et al.*, 1998). Studies accomplished in the USA with fenoxycarb by GLANCEY *et al.* (1990), to Solenopsis spp, shown that such substance just changes the tissue of the queen ants' ovary. Even though, among a number of growth regulators tested for *Solenopsis* spp. in the USA, a few have been relatively promissory and effective. In recent years, just fenoxycarb and pyriproxiphen have been commercialized in the USA.

TROISI & RIDDIFORT (1974) and studies performed at Texas Agricultural Experiment Station (BANKS, 1986) tested methoprene and hydropene to *Solenopsis invicta* and they reached very erratic results against field populations, despite the intervention of such substances in the maturation and development of larvae, regular metamorphosis, and caused worker mortality. The inconsistent results have been attributed to the short experience of the a.i.s in the colony, and the fact as those ants quickly metabolize and excrete those IGRs, facts verified by (WENDEL & VINSON, 1978; BIGLEY & VINSON, 1979). Studies performed by BANKS (1986) suggest that some IGRs may quickly be metabolized by *Solenopsis* spp; in the first week, after the application, researchers detected some methoprene and hydroprene metabolites in experiments with 14c (BANKS, 1990).

The IGRs tested for leaf-cutting ants such as fenoxycarb, pyriproxyfen, diflubenzuron, teflubenzuron, silafluofen, tidiazuron, tefluron, prodrone, methoprene has not cause mortality in colonies of leaf-cutting ants and results has not differed from the witnesses (FORTI *et al.*, 1998; NAGAMOTO *et al.*, 2004; NAGAMOTO *et al.*, 2007). Recent studies using piryproxyfen 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) (Figure 11).

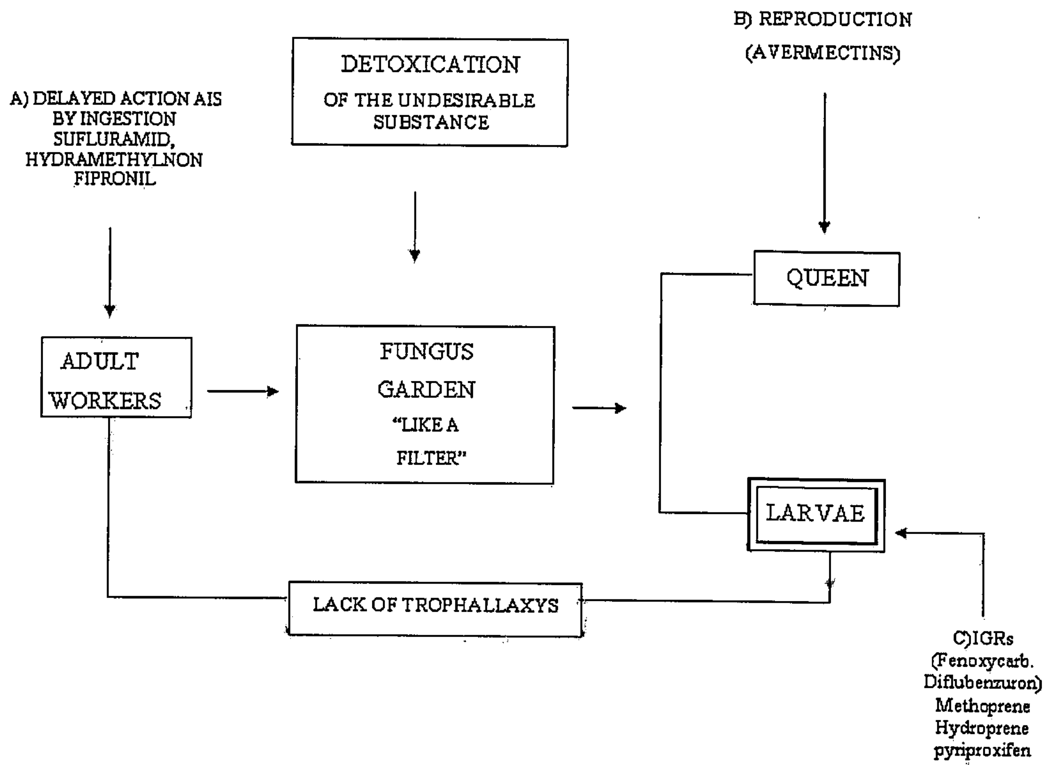

Figure 11. Target of action of different substances in colonies of *Atta* and *Acromyrmex.* A) Active ingredients of ingestion kill just adult ants and the highest mortality of workers occurs in the first week. (FORTI *et al.*, 2007, NAGAMOTO *et al.*, 2007). B) reproduction inhibitors have to keep their characteristics even after being incorporated at the fungus garden for a long period of time, from weeks to months. (DREES & VINSON, 1991). C) Growth regulators should also count on similar features as those of the reproduction inhibitors. (FORTI *et al.*, 1998; BARR *et al.*, 2005).

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 cutting-leaf ants, because they suggest 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 *Atta 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 by intoxication, once it is worthy to remind that both larvae and adults ingest hyphae of the fungus cultivated *Leucoagaricus gongylophorus.*

The most efficient insecticides against cutting-leaf ants are those that slay adult workers (Figure 11) and have delayed action as characteristic, have low steam pressure; therefore, are not detected at toxic baits by the ants, slay in small concentrations, are not quickly detoxified, very high DL_{50}, besides other characteristics already discussed in item 11, when the characteristics of the ideal bait were treated. The insecticide that contemplates all these characteristics is sulfluramid (FORTI *et al.*, 1998; FORTI *et al.*, 2007). As described before, the contamination of small, medium and big workers by sulfluramid occurs during the licking and rubbing the pellets of toxic baits processes, during behavioral acts of incorporation of small fragments of toxic baits in the fungus garden (ANDRADE *et al.*, 2002; FORTI *et al.*, 2007).

The ants that are not leaf cutters, and then do not cultivate fungus as food, ingest liquids, I), i.e., *Linepithema humile* consuming approximately 99% of the food forages brought to the nest is honeydew and nectar (MARKIN, 1970) retaining them in the maw or taking small parts of solid particles (toxic baits) and directly providing it to the larvae. Therefore, the larvae receive liquids or solids containing active ingredients. II) BAKER *et al.* (1985) found that honey or sucrose water was preferred over solid foods and the workers were billed when fed sucrose water containing 10 or 100 ppm of avermectin to *Linepithema humile*. With this distinguished feeding habit of the leaf-cutting ants, they may directly contaminate their larvae or other workers with active ingredients, such as growth regulators (piryproxyfen, methoprene), toxics of delayed action (sulfluramid, hydramethylnon) and those that may cause reproduction inhibition as well (avermectins).

Ingestion insecticides with delayed action, such as sulfluramid, slay a great quantity of *Atta* workers in a week, considering that *Atta* colonies may have from 3.5 to 7 million subjects in the nests and the nests having reached a depth of up to 8 m with up to 8 thousand chambers. (FORTI *et al.*, 1998). An even major factor is that in colonies that receive sulfluramid, the workers cease cutting leaves or damaging plants after the 4th day of application. As for growth regulators when administered to non-leaf-cutter ants, where they have relative efficiency, take months to slay a colony, once they act over the younger forms, that is, preventing the reposition of workers. As for the *Solenopsis* spp., IGRs recently tested in field conditions, such as fenoxycarb, methoprene and pyriproxyfen have taken up to 7 months to start reducing the number of colonies and do not kill workers or queens as well.

The IGRS methoprene 0.4% at toxic baits, applied to control *Wasmania auropunctata* in Galapagos Islands, after six weeks, did not reduce the number of nests, queens and workers, but just the number of eggs laid by the queens, and the reduction occurred only after 3 to 4 applications (ULLOA – CHACÓN & CHERIX, 1994). At these same islands, hydramethylnon was also used to *W.auropunctata,* and the results were not satisfactory either (ROQUE-ALBEDO *et al.*, 2000; WETTERER & PORTER, 2003) event though this is a delayed action insecticide and ingestion is not enough in many situations, which is one of the reasons attributed to the rapid UV breakdown of hydramethylnon, and quick foragers mortality (KRUSHELNYCKY & REIMER, 1998 and Harris, 2002). Methoprene and hydramethylnon baits were not successful and boric acid based baits had a mixed result to *Tapinoma megalocephalum* (ghost ant) (SHAH & PINNIGGER, 1996; ESPADALER & ESPEJO, 2002). On the other hand promising result have been achievel by using sulfluramid baits in Berlin (SCHEURER *et al.*, 1999; ESPADALER & ESPEJO, 2002). We should register that boric acid was tested for leaf-cutting ants to the concentration of 5% (FORTI *et al.*, 1998) and there was no mortality of colonies, even under laboratory conditions, where such colonies are under stressing conditions and then sensitized. It is advisable to remember that some a.i.s that kill colonies in laboratory, when tested in field may cause no mortality or very low mortality. Such fact is quite understandable once the *Atta* colonies in field are extremely populous relying in a complex organization system (Figure 8) (FORTI *et al.*, 1998). Even if the IGRs were effective over leaf-cutting ants, this period of time is very long to slay a colony and no rural producer or production facilities of wood destined to celluloses, or any other purpose in which controlling leaf-cutting ants is required, would use such bait containing those growth regulators, due to the fact that the ants would persist for a long time, possibly months, cutting leaves and causing irreversible damages such as death (FORTI & BOARETTO, 1997).

All researches taken together with those reported active ingredients in a previous study by our group (NAGAMOTO *et al.*, 2004), support the proposal that a.i.s in toxic baits to be efficient in the control of leaf-cutting ants, as shower by the high control efficiency of dechlorane (commercial use has prohibited) and sulfluramid (CHERRETT, 1986; FORTI *et al.*, 1998; GROSSMAN *et al.*, 2002; FORTI *et al.*, 2003; NAGAMOTO *et al.*, 2004; NAGAMOTO *et al.*, 2007).

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