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Preliminary studies on the effects of *d*-limonene to workers of the leaf-cutting ant *Atta sexdens rubropilosa* and its implications for control

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Abstract

To better understand the potential of controlling of leaf-cutting ants, a serious pest, with conventional synthetic insecticides as well as botanical pesticides, we propose to evaluate the effect of *d*-limonene on attractiveness and rejection of citric pulp to the leaf-cutting ant *Atta sexdens rubropilosa* Forel. Five treatments were adopted that consisted of fragments of filter paper impregnated with various concentrations of *d*-limonene and with organic extract of citrus pulp. The number of fragments loaded by the ants in the foraging arena and transported to the fungus garden and of fragments rejected in the refuse chamber was counted. It has been shown that *d*-limonene provoked concentration-dependent reduction of attractiveness response to *A. sexdens rubropilosa* workers. No rejection of loaded fragment was observed. We discussed the relationship between substrate selection and its repellent compounds, and also that a repellent substrate component can be overcome or masked by the overall odour of attractive substrates. Also, the repellence of the toxic bait AI (active ingredient) and its implication in control efficiency and the use of repellent AIs in other control methods, emphasizing nebulization, are discussed.

Key words: insecticide, chemical control, repellence, citrus pulp, Attini.

Introduction

Leaf-cutting ants of the genera *Atta* and *Acromyrmex* (Hymenoptera Formicidae Attini) are considered as serious Neotropical pests (Amante, 1967; Mariconi, 1970) due to their ability to consume a greater amount of vegetation than any other local herbivore (Fowler *et al.*, 1986; Hölldobler and Wilson, 1990). They use the cut parts of plants as substrate for the cultivation of their symbiotic fungus as their main food (Weber, 1972; Wilson, 1980).

Due to their economic importance in agriculture, several attempts to develop control methods were undertaken, but as a result, only a few synthetic insecticides have been shown to be highly efficient and practical for use in thermonebulization or in toxic baits (Della Lucia and Vilela, 1993; Forti *et al.*, 1998; Bigi *et al.*, 2004; Camargo *et al.*, 2006). Nowadays, the most used method is toxic bait and, in second place, nebulization (Boaretto and Forti, 1997; Nakano, 1998; Raetano and Wilcken, 1998; Forti *et al.*, 2003). Toxic bait consists of an ant attractant (usually dehydrated citric pulp) impregnated with a delayed-action active ingredient (AI) and vegetable oil (usually soybean) as AI solvent, formulated into pellets (Robinson, 1979; Forti *et al.*, 1998; Nagamoto *et al.*, 2004).

In the face of the growing demand for lower-impact alternatives to the synthetic insecticides for pest control, the use of the botanical pesticides has been growing (Isman, 2006). Some botanical insecticides contain *d*-limonene [(R)-4-isopropenyl-1-methylcyclohexene] as AI. This chemical is a monoterpene that exhibits toxicity against several insects (Coats *et al.*, 1991; Karr and Coats, 1988; Drees, 2002). Usually *d*-limonene is obtained from industrial citric fruit processing because it is the major component of the oil extracted from the peel. Citric oil has been considered efficient in controlling fire ants (Hymenoptera Formicidae Solenopsidini), and thus can be a viable alternative if conventional synthetic insecticides are considered undesirable (Drees, 2002; Vogt *et al.*, 2002).

For leaf-cutting ants, several natural (botanical) pesticides, which usually present both insecticide and fungicide activity, such as leaf extracts of *Ricinus communis* L. (Euphorbiaceae) have been studied for control (Bigi *et al.*, 2004 and references included), but at this moment, none has reached the phase of commercial use. Specifically, *d*-limonene repels the leaf-cutting ants *Atta cephalotes* (L.) and *Acromyrmex octospinosus* Reich (Littledyke and Cherrett, 1978b).

Substrate preference by leaf-cutting ants has been correlated with the nutrition balance for ants and with growth of the fungus (Cherrett, 1968; Rockwood, 1976; Abbott, 1978). According Ridley *et al.* (1996) and North *et al.* (1999), the cultivated fungus can also influence the foraging decision: they observed that citric baits with the fungicide cycloheximide were initially loaded by *Atta sexdens rubropilosa* Forel and incorporated into the fungus culture. Some days later, the baits were rejected by workers (delayed rejection) and the bait foraging ceased. They explained this fact by assuming that the fungus becomes stressed and produces a volatile semiochemical that induces the workers to avoid citric bait. Additionally, in the same species, Verza *et al.* (2007) reported delayed rejection due to the physical resistance of the substrate, and to the absence of nutrients for the fungus growth, on some fragments of polyester film and paper filter mistakenly foraged.

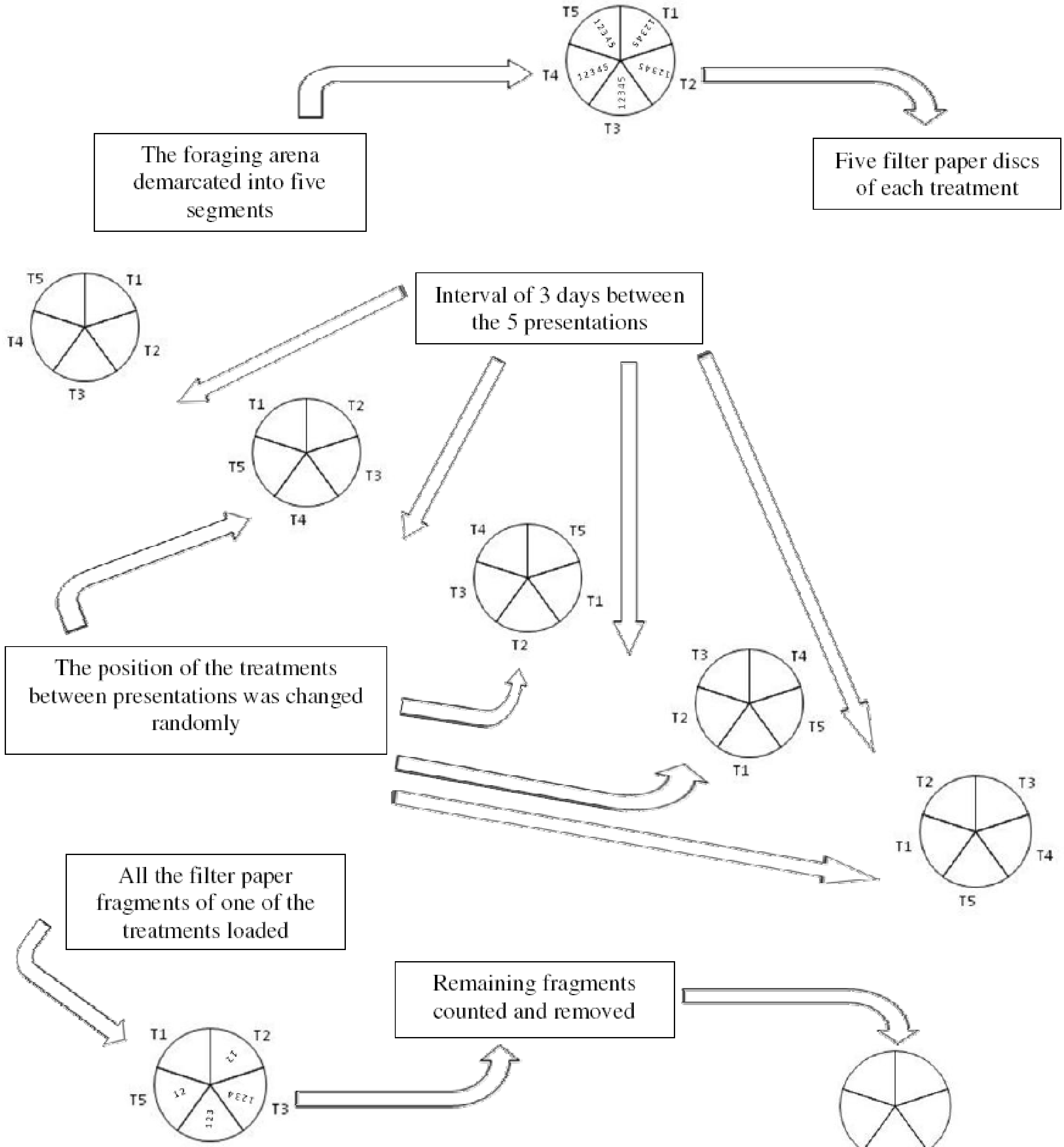

Figure 1. Presentations scheme for each nest.

Recently, it was established that the link between basic knowledge of both biology and toxicology and the control of leaf-cutting ants with baits has been underestimated (Nagamoto *et al.*, 2004; Camargo *et al.*, 2006), and that, in the past, this scenario has slowed the development of new baits (Forti *et al.*, 1998; Nagamoto *et al.*, 2007).

Therefore, a better understanding of the interactions between repellent and attractive compounds of the toxic bait is required. In the present work, we introduce a bioassay to evaluate the effect of *d*-limonene on the attractiveness and rejection of citric pulp extract by workers of *A. sexdens rubropilosa* (model for leaf-cutting ants) and we discuss the possible use of *d*-limonene for controlling leaf-cutting ants by an alternative method, nebulization, instead of toxic bait.

Materials and methods

The present study was carried out in the Social-Pest Insect Laboratory (UNESP/Botucatu). Five colonies of *A. sexdens rubropilosa* were used, which were collected previously in Botucatu (22º53'09"S; 48º26'42"W) and kept in the laboratory (24 ± 2 ºC and RH 60 ± 20%). Each colony was nested in 3 nearly cylindrical pots, linked with pipes (all transparent plastic ones), consisting of a refuse chamber, a fungus chamber and a foraging arena.

From a citrus factory (Sucocitrico Cutrale Ltda., Araraquara, SP, Brazil), technical grade *d*-limonene and citric pulp was obtained. This industrial citrus pulp is the dehydrated residues of the juice extract and peel essential oil (which includes *d*-limonene) from citric fruit. The citric pulp was extracted with the solvent hexane because this extract is highly attractive to leaf-cutting ants (Verza *et al.*, 2006). Using a methodology similar to one employed previously (Verza *et al.*, 2006), the extract was obtained triturating the citric pulp (1 kg) and following chemical extraction: solvent was added (4 l of hexane) and submitted to agitation, decantation, filtering and drying in rotating evaporator (taking one week for this entire process). After the extraction, a solution of 20 ml of this extract was prepared in hexane, at 2% concentration (w/v).

Five filter paper discs (19.6 mm^2 each) of each treatments were impregnated with either citric pulp hexane extract (T1) or citric pulp hexane extract with 100, 300, and 900 ppm (w/w) of *d*-limonene, T2, T3, T4 respectively, or *d*-limonene alone (T5). Each treatment was offered 5 times (presentations) in each nest.

The portion of the foraging arena opposite to the pipe was demarcated into five 36° segments of a semicircle (totaling 180°). After that, for each presentation and all colonies simultaneously, all treatments were supplied. An interval of 3 days between the presentations was adopted and the position of the treatments between presentations was changed randomly. For each colony, when all the filter paper fragments of one of the treatments were loaded (carried away), the remaining fragments were immediately counted and removed (figure 1). The rejection was evaluated in the refuse chamber 24 hours after presentation, for three days.

To determine if there was significant load between treatments, colonies and presentations, the data for number of fragments carried were submitted to three way ANOVA and means were compared by the Student-Newman-Keuls test, at 5% probability (Winer *et al.*, 1991), using the software Sigma Stat 2.03.

Table 1. Comparison of the loaded quantities of filter paper fragments impregnated citrus pulp hexane extract and *d*-limonene, within presentation, in laboratory colonies of *A. sexdens rubropilosa*.

Presentation	Mean + sd *
1	2.56 ± 1.98 ab
2	2.80 ± 1.89 a
3	2.84 ± 1.75 a
4	1.92 ± 1.94 b
5	2.96 ± 1.86 a

* Mean followed by the same letter did not differ significantly from one another (5% probability, Student-Newman-Keuls test).

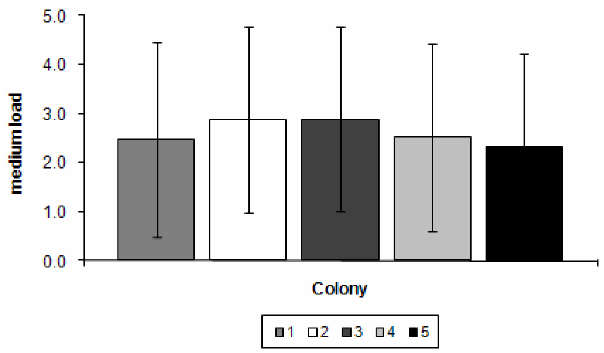

Figure 2. Comparison of the loaded quantities of filter paper fragments impregnated citrus pulp hexane extract and *d*-limonene, within laboratory colonies of *A. sexdens rubropilosa*.

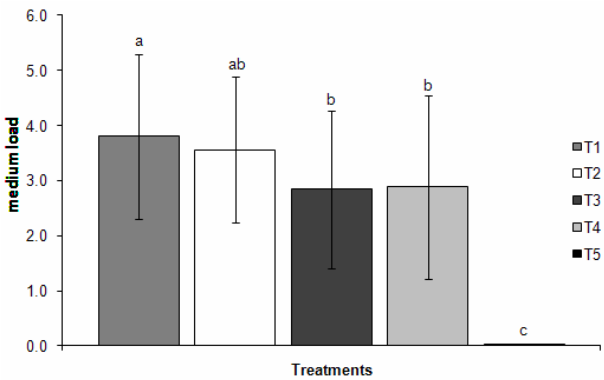

Figure 3. Comparison of the loaded quantities of filter paper fragments (5 fragments by each treatment for each presentation; 5 presentations by colony), impregnated citrus pulp hexane extract and *d*-limonene, within treatments, in 5 laboratory colonies of *A. sexdens rubropilosa*. T1 - citrus pulp; T2 - citrus pulp with 100 ppm (w/w) *d*-limonene; T3 - citrus pulp with 300 ppm (w/w) *d*-limonene; T4 - citrus pulp with 900 ppm (w/w) *d*-limonene; T5 - *d*-limonene. Mean followed by the same letter did not differ significantly from one another (5% probability, Student-Newman-Keuls test).

Results

The number of fragments loaded were not significantly different between colonies ($P = 0.36$; d.f. = 4; $F = 1.109$) (figure 2). No loaded fragment was rejected. The addition of *d*-limonene (figure 3) clearly affected the load of the filter paper fragments ($P < 0.001$; d.f. = 4; $F = 40.294$). Also, it can be stressed that the addition of *d*-limonene to the citrus pulp hexane extract provoked significant reduction of the load (T1 *vs.* T2, T3 and T4).

A significant difference was found between the presentations ($P = 0.025$; d.f. = 4; $F = 3.003$) (table 1), but was not correlated with the elapsed time of this assay (neither reduction nor improvement tendency of foraging was evidenced). The transport duration was very short, with a maximum exposure time of 15 min in each presentation. Possibly this difference was found because the T5 (*d*-limonene only) was extremely repellent, affecting the uniformity of ant disposition in the foraging arena such that the number of presentations possibly was not high enough to overcome totally the effect of T5 on ant disposition.

Discussion and conclusions

Repellent compounds and substrate selection

The substrate repellence and selection is important to the toxic bait method of control because the bait should be highly attractive and infrequently rejected to maximize the time that the workers manipulate it, consequently improving their contamination (Andrade *et al.*, 2002; Nagamoto *et al.*, 2004; Camargo *et al.*, 2006).

Taking into account that citric pulp was one of most attractive substrates to the leaf-cutting ants (Mudd *et al.*, 1978; Mudd and Bateman, 1979; Verza *et al.*, 2006), there was an apparent incongruence in attractiveness of citrus compounds (pulp *vs.* *d*-limonene) - why is the attractiveness so high if one compound is highly repellent?

First, the process of substrate selection for the workers depends on the olfactory stimulation resultant from different chemical substances, repellents and stimulants, present in the substrate (Littledyke and Cherrett, 1978a; 1978b; Mudd *et al.*, 1978). Second, according to Cherrett (1969), the fresh flavedo (peel) of grapefruit was not attractive to *A. octospinosus*, but becomes attractive when it is dried. The same author suggests that the glands of the flavedo can contain repellent volatile compounds which are removed by heating (possibly lowering the concentration of the repellent compounds).

Thus the reduction of attractiveness at low concentrations reported herein was probably an indication that *d*-limonene was repellent to leaf-cutting ants at higher concentrations and also reinforces the argument that the result of different odours in dried citrus pulp could produce a positive effect on attractiveness if concentrations of the repellent compounds were lowered (Cherrett, 1969).

Our finding of no rejection was contrary to previous reports (Ridley *et al.*, 1996; North *et al.*, 1999; Verza *et al.*, 2007), possibly due to absence of adverse effect of *d*-limonene on fungus, so that the fungus did not produce the (previous proposed) semiochemical to inform the ants to stop the foraging (Ridley *et al.*, 1996; North *et al.*, 1999) and that cellulose (filter paper) with citric compounds (*d*-limonene and citrus hexane extract) is suitable for growing fungus, in contrast to inert material (Verza *et al.*, 2007).

Mudd *et al.* (1978) stated that, for leaf-cutting ants in nature, a variety of components present in the plants induces carrying of the substrates by the workers and the absence of repellents or deterrents can be more important than the presence of specific stimulants. But our result suggests that, at least for manmade substrate (bait), this is not completely true because at known concentration (100 ppm w/w) the repellent *d*-limonene did not lower the attractiveness of citrus pulp. This is important because (at least to our knowledge) it is the first empirical demonstration that some repellence of substrate compound, depending on the concentration, can be overcome or masked by the overall odours of the highly attractive substrate. Thus it can be stated that although substrate selection by ant and fungus are general and efficient mechanisms, some gaps in these are natural and perhaps can be explored for development of control methods.

Toxic bait AI repellence and control effectiveness

Applying our general achievement on the role of the repellent compounds in selection for use of toxic baits, it can be indicated that we should choose bait compounds that have no significant repellence, at least for non-insecticide compounds (citric pulp and soybean oil). In the case of AIs, low repellence perhaps can be tolerated.

If the insecticide chlorpyrifos, which presents expressive odour and intermediate to high volatility (Racke, 1993; Tomlin, 2000), is used as AI, the attractiveness and efficiency tend to be low (Cruz *et al.*, 1996; Forti *et al.*, 1998; 2003). However, sulfluramid and fipronil, with much lower volatility (Tomlin, 2000) and probably mild odour (for ants), usually are more frequently carried and highly efficient (Forti *et al.*, 1998; 2003; 2007). Our findings reinforce the general assumption that toxic bait AI should be of little or no repellence to leaf-cutting-ant workers (Forti *et al.*, 2007).

Specifically, the use of *d*-limonene as leaf-cutting-ant toxic bait AI is highly improbable because it provokes reduction of citrus pulp attractiveness at low concentrations although it did not cause any deleterious effect; thus at toxic concentrations, bait with *d*-limonene will not be carried, permitting colony survival. This explanation also reinforces the concept that highly repellent AIs cannot be used in any type of baits for any pest.

Control with repellent AIs

Although repellent, *d*-limonene has been considered efficient for fire ant control. But it should be emphasized that it is in liquid formulation and for superficial mound treatment (Vogt *et al.*, 2002). For deeply underground and huge leaf-cutting-ant nests, with hundreds of fungus chambers at a depth of several meters, drenching with liquid formulation is neither efficient (Boaretto and Forti, 1997) nor viable in field control.

On the other hand, for nebulization methods the use of *d*-limonene will perhaps be possible, because in these methods, the AI is injected into the nest; thus it does not depend on being actively carried by workers like toxic bait and repellence is not important. But as a natural and thermolabile product, *d*-limonene cannot be employed in the most commonly used thermonebulization machines, because this apparatus mixes AI with high temperature gas of diesel oil combustion. Therefore, low-temperature nebulization should be used, such as the so-called “Aero System” machine (FMC Corporation) that uses propane and butane (LPG) as AI carrier (Nakano, 1998; Raetano and Wilcken, 1998).

Given that, in pest control AIs in general, delayed action is relatively rare (Vander Meer *et al.*, 1985; Nagamoto *et al.*, 2007), this same rarity is probably true also for botanical pesticides; thus low-temperature nebulization is probably a much more promising and adequate technology than toxic bait for these natural products.

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References

ABBOTT A. A., 1978.- Nutrient dynamics of ants, pp. 233-244. In: *Production ecology of ants and termites* (BRIAN M. V., Ed.).- Cambridge University Press, Cambridge, UK.

AMANTE E., 1967.- Prejuízos causados pela formiga saúva em plantações de *Eucalyptus* e *Pinus* no Estado de São Paulo.- *Silvicultura*, 6: 355-363.

ANDRADE A. P. P., FORTI L. C., MOREIRA A. A., BOARETTO M. A. C., RAMOS V. M., MATOS C. A. O., 2002.- Behavior of *Atta sexdens rubropilosa* (Hymenoptera: Formicidae) workers during the preparation of the leaf substrate for symbiont fungus culture.- *Sociobiology,* 40 (2): 293-306.

BIGI M. F. M. A., TORKOMIAN V. L., GROOTE S. T., HEBLING M. J. A., BUENO O. C., PAGNOCCA F. C., FERNANDES J. B., VIEIRA P. C., SILVA M. F. G. F., 2004.- Activity of *Ricinus communis* (Euphorbiaceae) and ricinine against the leaf-cutting ant *Atta sexdens rubropilosa* (Hymenoptera: Formicidae) and the symbiotic fungus *Leucoagaricus gongylophorus*.- *Pest Management Science*, 60 (9): 933-938.

BOARETTO M. A. C., FORTI L. C., 1997.- Perspectivas no controle de formigas cortadeiras.- *Série Técnica IPEF*, 11 (30): 31-46.

CAMARGO R. S., FORTI L. C., LOPES J. F. S., NAGAMOTO N. S., 2006.- Studies on leaf-cutting ants, *Acromyrmex* spp. (Formicidae, Attini): behavior, reproduction and control, pp. 161-182. In: *Recent developments in entomology research* (PANDALAI S. G., Ed.).- Research Signpost, Kerala, India.

CHERRETT J. M., 1968.- The foraging behaviour of *Atta cephalotes* (L.) (Hymenoptera, Formicidae). I. Foraging pattern and plant species attacked in tropical rain forest.- *Journal of Animal Ecology*, 37: 387-403.

CHERRETT J. M., 1969.- Baits for the control of leaf-cutting ants I - formulation.- *Tropical Agriculture*, 46 (2): 81-90.

COATS J. R., KARR L. L., DREWES C. D., 1991.- Toxicity and neurotoxic effects of monoterpenoids in insects and earthworms, pp. 305-316. In: *Naturally occurring pest bioregulators* (HEDIN P. A., Ed.).- American Chemical Society, Washington, USA.

CRUZ A. P., ZANNUNCIO J. C., ZANETTI R., GOMES O. S., 1996.- Eficiência de iscas granuladas à base de sulfluramida e de clorpirifós no controle de *Atta sexdens sexdens* (Hymenoptera: Formicidae), no trópico úmido.- *Acta Amazônica*, 26: 145-150.

DELLA LUCIA T. M. C., VILELA E. F., 1993.- Métodos Atuais de Controle e Perspectivas, pp. 163-190. In: *As formigas cortadeiras* (DELLA LUCIA T. M. C., Ed.).- Folha de Viçosa, Viçosa, Brazil.

DREES B. M., 2002.- A new technique for laboratory assessment of red imported fire ant mound drench treatments.- *Southwestern Entomologist*, 27 (2): 177-183.

FORTI L. C., NAGAMOTO N. S., PRETTO D. R., 1998.- Controle de formigas cortadeiras com isca granulada, pp. 113-132. In: *Anais do I simpósio sobre formigas cortadeiras dos países do Mercosul* (BERTI FILHO E., MARICONI F. A. M., FONTES L. R., Eds), FEALQ, Piracicaba, SP, Brazil.

FORTI L. C., NAGAMOTO N. S., RAMOS V. M., ANDRADE A. P. P., SANTOS J. F. L., CAMARGO R. S., MOREIRA A. A., BOARETTO M. A. C., 2003.- Eficiencia de sulfluramida, fipronil y clorpirifos como sebos en el control de *Atta capiguara* Gonçalves (Hymenoptera:Formicidae).- *Pasturas Tropicales*, 25 (3): 28-35.

FORTI L. C., PRETTO D. R., NAGAMOTO N. S., PADOVANI C. R., CAMARGO R. S., ANDRADE A. P. P., 2007.- Dispersal of the delayed action insecticide sulfluramid in colonies of the leaf-cutting ant *Atta sexdens rubropilosa* (Hymenoptera: Formicidae).- *Sociobiology*, 50 (3):1149-1163.

FOWLER H. G., FORTI L. C., PEREIRA DA SILVA V., SAES N. B., 1986.- Economics of grass-cutting ants, pp. 18-35. In: *Fire ants and leaf cutting ants* (LOFGREN C. S., VANDER MEER R. K., Eds).- Westview Press, Boulder, USA.

HÖLLDOBLER B., WILSON E. O., 1990.- *The ants*.- Belknap Press of Harvard University Press, Cambridge, USA.

ISMAN M. B., 2006.- Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world.- *Annual Review of Entomology*, 51: 45-66.

KARR L. L., COATS J. R., 1988.- Insecticidal properties of *d-limonene*.- *Journal of Pesticide Science*, 13: 287-290.

LITTLEDYKE M., CHERRETT J. M., 1978a.- Defence mechanisms in young and old leaves against cutting by the leaf-cutting ants *Atta cephalotes* (L.) and *Acromyrmex octospinosus* (Reich) (Hymenoptera: Formicidae).- *Bulletin of Entomological Research*, 68: 263-271.

LITTLEDYKE M., CHERRETT J. M., 1978b.- Olfactory responses of the leaf-cutting ants *A. cephalotes* (L.) and *Acromyrmex octospinosus* (Reich.) (Hymenoptera: Formicidae) in the laboratory.- *Bulletin of Entomological Research*, 68: 273-282.

MARICONI F. A. M., 1970.- *As saúvas*.- Agronômica Ceres, São Paulo, Brazil.

MUDD A., BATEMAN G. L., 1979.- Rates of growth of the food fungus of the leaf-cutting ant *Atta cephalotes* (L.) (Hymenoptera: Formicidae) on different substrates gathered by the ants.- *Bulletin of Entomological Research*, 69: 141-148.

MUDD A., PEREGRINE D. J., CHERRETT J. M., 1978.- The chemical basis for the use of citrus pulp as a fungus garden substrate by the leaf-cutting ants *Atta cephalotes* (L.) and *Acromyrmex octospinosus* (Reich.) (Hymenoptera: Formicidae).- *Bulletin of Entomological Research*, 68: 673-685.

NAGAMOTO N. S., FORTI L. C., ANDRADE A. P. P., BOARETTO M. A. C., WILCKEN C. F., 2004.- Method for the evaluation of insecticidal activity over time in *Atta sexdens rubropilosa* workers (Hymenoptera: Formicidae).- *Sociobiology*, 44 (2): 413-432.

NAGAMOTO N. S., FORTI L. C., RAETANO C. G., 2007.- Evaluation of the adequacy of diflubenzuron and dechlorane in toxic baits for leaf-cutting ants (Hymenoptera: Formicidae) based on formicidal activity.- *Journal of Pest Science*, 80 (1): 9-13.

NAKANO O., 1998.- A nebulização no controle das saúvas, pp. 105-111. In: *Anais do I simpósio sobre formigas cortadeiras dos países do Mercosul* (BERTI FILHO E., MARICONI F. A. M., FONTES L. R., Eds), FEALQ, Piracicaba, SP, Brazil.

NORTH R. D., JACKSON C. W., HOWSE P. E., 1999.- Communication between the fungus garden and workers of the leaf-cutting ant *Atta sexdens rubropilosa,* regarding choice of substrate for the fungus.- *Physiological Entomology*, 24: 127-133.

RACKE K. D., 1993.- Environmental fate of chlorpyrifos.- *Reviews of Environmental Contamination and Toxicology*, 131: 1-150.

RAETANO C. G., WILCKEN C. F., 1998.- Aerosystem no controle de formigas cortadeiras, pp. 133-139. In: *Anais do I simpósio sobre formigas cortadeiras dos países do Mercosul* (BERTI FILHO E., MARICONI F. A. M., FONTES L. R., Eds), FEALQ, Piracicaba, SP, Brazil.
RIDLEY P., HOWSE P. E., JACKSON C. W., 1996.- Control of the behaviour of leaf-cutting ants by their "symbiotic" fungus.- *Experientia*, 52: 631-635.
ROBINSON S. W., 1979.- Leaf-cutting ant control schemes in Paraguay, 1961-1977. Some failures and some lessons.- *Pans: Pest Articles and News Summaries*, 25: 386-390.
ROCKWOOD L. L., 1976.- Plant selection and foraging patterns in two species of leaf-cutting ants (*Atta*).- *Ecology*, 57: 48-61.
TOMLIN C. D. S., 2000.- *The pesticide manual*, 12th edition.- British Crop Protection Council, Farnham, UK.
VANDER MEER R. K., LOFGREN C. S., WILLIAMS D. F., 1985.- Fluoroaliphatic sulfones: a new class of delayed-action insecticides for control of *Solenopsis invicta* (Hymenoptera: Formicidae).- *Journal of Economic Entomology*, 78: 1190-1197.
VERZA S. S., FORTI L. C., MATOS C. A. O., GARCIA M. G., NAGAMOTO N. S., 2006.- Atractiveness of citrus pulp and orange albedo extracts to *Atta sexdens rubropilosa* (Hymenoptera: Formicidae).- *Sociobiology*, 47 (2): 391-399.
VERZA S. S., FORTI L. C., LOPES J. F. S., CAMARGO R. S., MATOS C. A. O., 2007.- Influence of physical and chemical factors during foraging and culture of the symbiont fungus in *Atta sexdens rubropilosa* (Hymenoptera: Formicidae).- *Insect Science*, 14: 295-300.
VOGT J. T., SHELTON T. G., MERCHANT M. E., RUSSELL S. A., TANLEY M. J., APPEL A. G., 2002.- Efficacy of three citrus oil formulations against *Solenopsis invicta* Buren (Hymenoptera : Formicidae), the red imported fire ant.- *Journal of Agricultural and Urban Entomology*, 19 (3): 159-171.
WEBER N. A., 1972.- *Gardening ants: the attines*.- Memoirs of the American Philosophical Society, Philadelphia, USA.
WILSON E. O., 1980.- Caste and division of labor in leaf-cutter ants (Hymenoptera, Formicidae: *Atta*). I: The overall pattern in *A. sexdens*.- *Behavioral Ecology and Sociobiology*, 7: 143-156.
WINER B. J., BROWN D. R., MICHELS K. M., 1991.- *Statistical principles in experimental design*, 3rd edition.- McGraw-Hill, New York, USA.

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