Toxicity of *Cedrela fissilis* to *Atta sexdens rubropilosa* (Hymenoptera: Formicidae) and its Symbiotic Fungus

by

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ABSTRACT

Crude extracts from roots, stems, branches, fruits and leaves of *Cedrela fissilis* were tested to verify their toxicity to *Atta sexdens rubropilosa* workers and to their symbiotic fungus *Leucoagaricus gongylophorus*. The workers that were fed daily on an artificial diet to which crude extracts from this plant were added had a higher mortality rate than the controls, especially for the hexane, dichloromethane and methanol crude extracts from roots (RH, RD and RM) and from leaves (LH, LD and LM). Fungal growth was inhibited by the hexane (RH) and dichloromethane crude extract from roots (RD). The RH, RD and FD crude extracts were fractioned and their fractions were tested. All the fractions tested presented toxicity to the ants and some fractions (RH-H, RH-D, RD-4 and RD-5) completely inhibited fungus development. The possibility of controlling these insects in the future using *Cedrela fissilis* compounds that can simultaneously target both organisms is discussed.

Keywords: insecticidal activity, antifungal activity, leaf-cutting ant, *Leucoagaricus gongylophorus*.

INTRODUCTION

More than 11 thousand species of ants are present in nearly all habitats on Earth, acting on the environment's stability, soil conditions, pollination of plants and seed dispersion (Hölldobler & Wilson 1990; Brown Jr. 2000). However, when the environment is altered, mainly by human activity, these insects can become difficult pests to control. Especially difficult to control are leaf-cutting ants of the genera *Atta* and *Acromyrmex* which are distributed from Argentina to the southern United States, cause serious damage to a wide variety of

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plants and are a serious crop pest (Weber 1972; Della Lucia & Fowler 1993).

In natural ecosystems, these ants show preference for some plant species which are constantly defoliated, whereas others are not attacked even though they are abundant and located close the nests (Cherrett 1968; Hubbell & Wiemer 1983; Littledyke & Cherrett 1975; Rockwood 1975, 1976; Garcia *et al.* 2003). Attraction or repellency may be influenced by different properties of plants, such as density, hardness, moisture level, nutritional quality, and secondary metabolites (Hubbell & Wiemer 1983; Hubbell *et al.* 1984).

The complex interrelationship between phytophagous insects and plants are the result of a long and continuous evolutionary process which involves the development of defense mechanisms by the plants and adaptation of leaf-cutting ants to such mechanisms (Febvay & Kermarrec 1986). These interactions involve numerous secondary plant metabolites that may act on insect growth and development (Whittaker & Feeny 1971; Beck & Reese 1976) and are more complex for Attine ants because the plants are used by them to grow their symbiotic fungus (Febvay *et al.* 1985).

These secondary compounds may serve as powerful toxicants to deter insects and others herbivores from feeding and in some cases they serve as a starting point for developing novel insecticides, as in the case of the synthetic pyrethroids (Elliott & Potter 1978). Finally, there is a great interest in the study of species of the Meliaceae family due to the presence of some limonoids, belonging to the terpenoids class, that have displayed biological activity on a variety of insects acting as feeding deterents, ovipositional repellents and growth inhibitors (Champagne *et al.* 1989).

The main objective of this study was to determine the toxicity of *Cedrela fissilis* to leaf-cutting workers of the species *Atta sexdens rubropilosa* and to their symbiotic fungus *Leucoagaricus gongylophorus*.

MATERIALS AND METHODS

Preparation of *Cedrela fissilis* crude extracts

Roots, stems, branches, fruits and leaves of *Cedrela fissilis* Vell. (Rutales: Meliaceae) were collected at the Campus of Universidade Federal de São Carlos, São Carlos, SP, Brazil in June 2001, dried in an air-circulation sterilizer at 40°C, for approximately 24 hours and pulverized in an electric grinder. A known mass of dried and ground material was macerated for three days, three times, at room temperature and resting with solvents of increasing polarity (hexane, dichloromethane and methanol). At the end of this period, the extracts

were filtered in paper, resulting in:

Root - hexane extract (RH), dichloromethane extract (RD) and methanol extract (RM)

Stem - hexane extract (SH), dichloromethane extract (SD) and methanol extract (SM)

Branch - hexane extract (BH), dichloromethane extract (BD) and methanol extract (BM)

Fruit - hexane extract (FH), dichloromethane extract (FD) and methanol extract (FM)

Leaf - hexane extract (LH), dichloromethane extract (LD) and methanol extract (LM)

Chemical fractioning of crude extracts

RH extract was submitted to liquid chromatography in a vacuum syntherized plate funnel, using silica gel as the stationary phase and eluents with increasing polarity (hexane, dichloromethane, ethyl acetate and methanol), obtaining 4 fractions: RH-H, RH-D, RH-E and RH-M.

RD extract was done with successive chromatographic columns, having silica as the stationary phase. Initially a column was made using solvents with increasing polarity (hexane, dichloromethane, ethyl acetate and methanol). The other columns were made isocratically: hexane/dichloromethane/acetone (6:3:1). The fractions obtained were RD-3, RD-4, RD-5, RD-6, RD-8 and RD-9.

For the LD extract, a similar procedure to the fractioning of the RH extract was done, obtaining the following fractions: LD-H, LD-D, LD-E and LD-M.

Ants' bioassays

The *Atta sexdens rubropilosa* workers used in the assays were randomly picked up from laboratory nests. They had a body mass of 20-25 mg. Before the assays the nests were supplied daily with leaves of *Eucalyptus* sp., oat seeds and occasionally with leaves of other plants such as *Hibiscus* sp., *Ligustrum* sp. or rose petals. Fifty ants were put into five Petri dishes (ten ants each) for each treatment. During the assays the ants were maintained on an artificial diet prepared with glucose (50 g liter^{-1}), bacto-peptone (10 g liter^{-1}), yeast extract (1.0 g liter^{-1}) and agar (15 g liter^{-1}) in distilled water (100 ml) (Bueno *et al.* 1997). The diet (0.4-0.5 g per dish) with the addition of *Cedrela fissilis* extracts (experimental) or without (control) were offered daily in a small plastic cap. The control was prepared with the diet and the solvent. To ensure that undetectable remaining amounts of the solvent did not affect the ants, a comparison was made with another set of dishes in which water

was used instead of solvent. As expected, the same survival rates were obtained with both systems (data not shown). The compounds were poured into the hot diet immediately after sterilization. The final concentrations of material added to the diet were 2000 mg ml^{-1} for the crude extracts and between 700 – 2000 mg ml^{-1} for the fractions, according to the amount of material available (Table 2). During the assays the ants were maintained in an incubator at temperature of 25 (±1) °C and relative humidity ranging between 70-80% for maximum length of 25 days and the number of dead was registered daily. The survival average 50% (S_{50}) was calculated and survival curves were compared by the computer-assisted software Graph-Pad™ using the log-rank test.

Fungus' bioassays

The fungus *Leucoagaricus gongylophorus* (Singer) Möller (syn *Rozites gongylophorus*) was isolated from a nest of *Atta sexdens rubropilosa*. As previously described by Pagnocca *et al.* (1996) the culture medium used for both culture maintenance and experimental assays had the follow-

Table 1. Mortality (%) of *Atta sexdens rubropilosa* workers fed on *Cedrela fissilis* crude extracts*

	Day										
Extracts	1	2	3	6	8	10	14	17	21	25	S_{50}**
Control H	0	0	0	4	6	14	38	56	74	86	17*a*
RH	0	0	10	26	56	86	100	-	-	-	8*b*
SH	0	4	4	4	10	20	32	44	76	96	18*a*
BH	0	0	2	8	20	26	42	62	72	90	15*a*
FH	0	4	10	16	22	26	36	56	86	96	17*a*
LH	0	0	2	30	46	60	90	100	-	-	9*b*
Control D	0	0	0	4	6	14	38	56	74	86	17*a*
RD	0	4	8	34	48	56	74	90	92	92	9*b*
SD	0	2	2	6	18	26	44	62	88	92	16*a*
BD	0	2	2	10	12	24	42	74	84	88	15*a*
FD	0	2	2	8	16	26	46	58	76	94	15*a*
LD	0	0	2	36	50	76	96	98	100	-	9*b*
Control M	0	0	4	14	20	26	48	60	84	94	15*a*
RM	0	2	6	12	20	34	66	86	92	100	13*b*
SM	0	4	10	28	30	40	56	76	90	100	12*a*
BM	0	2	4	10	22	28	48	72	94	100	15*a*
FM	2	4	4	8	12	22	58	76	82	92	13*a*
LM	0	0	4	32	58	76	98	100	-	-	8*b*

*Ants were fed on a solid diet without addition (control) or with addition of *Cedrela fissilis* compounds (experimental) at concentration of 2000 mg ml^{-1}.

**S_{50} = survival median 50%. Different letters after the S_{50} values show a significant difference according to the log-rank test ($p < 0.05$).

R = roots; S = stems; B = branches; F = fruits; L = leaves; H = hexane; D = dichloromethane; M = methanol.

Table 2. Mortality (%) of *Atta sexdens rubropilosa* workers fed on *Cedrela fissilis* extracts partially purified *

Fraction	Concentration (mg ml^{-1})	Day 1	2	3	6	8	10	14	17	21	25	S_{50}**
Control H	-	0	0	2	14	26	38	62	84	90	96	13*a*
RH-H	1000	0	4	4	36	56	80	96	98	100	-	8*b*
RH-D	2000	0	6	14	34	60	70	98	100	-	-	7*b*
RH-E	2000	0	4	10	48	62	78	96	100	-	-	7*b*
RH-M	2000	0	4	12	70	78	98	100	-	-	-	5*b*
Control D	-	0	0	4	14	28	42	78	86	98	100	11*a*
RD-4	700	0	4	10	48	62	70	96	96	100	-	7*b*
RD-5	700	0	2	6	42	64	86	100	-	-	-	7*b*
RD-6	1200	0	6	18	52	66	88	100	-	-	-	6*b*
RD-8	500	2	2	12	42	58	74	96	98	100	-	8*b*
RD-9	900	0	6	8	38	48	68	94	98	100	-	9*b*
LD-H	2000	0	2	2	40	52	68	92	96	100	-	8*b*
LD-D	2000	0	0	2	42	64	82	100	-	-	-	7*b*
LD-E	2000	0	0	4	34	60	80	98	100	-	-	8*b*
LD-M	2000	0	12	16	46	68	76	90	96	100	-	8*b*

* Ants were fed on a solid diet without addition (control) or with addition of *Cedrela fissilis* compounds (experimental) in different concentrations.
** S_{50} = survival median 50%. Different letters after the S_{50} values show a significant difference according to the log-rank test ($p < 0.05$).
R = roots; L = leaves; H = hexane; D = dichloromethane; E = ethyl acetate; M = methanol.

ing composition: peptone (5.0 g liter^{-1}); sodium chloride (5.0 g liter^{-1}); malt extract (10.0 g liter^{-1}); glucose (10.0 g liter^{-1}) and agar-agar (15.0 g liter^{-1}). The fungal growth of one-month-old slant culture (five or six tubes) was aseptically transferred to an all-glass tissue grinder containing sterile peptone (1 g liter^{-1}; 30 ml) and weakly fragmented. After that it was diluted to about 120 ml with a sterile saline solution in order to obtain a mycelial suspension for inoculation. For the assays, a methylene chloride suspension of the test compound (1.0 ml) was added to the culture medium (9.0 ml) in screw-cap test tubes (20 mm x 150 mm). The final concentrations of crude extracts obtained from *Cedrela fissilis* in the test tubes were 1000 mg ml^{-1}, whereas the final concentrations of the fractions were variable between 138 – 579 mg ml^{-1}, according to the amount of material available (Table 4). The material was sterilized at 121 °C for 10 min and slanted. The mycelial suspension was gently spread onto the surface of the agar slant, and incubated at 25 (±1) °C for 30 days. The assays were run twice using five tubes of each concentration every time. A set of ten tubes containing 1.0 ml of methylene chloride and 9.0 ml of the culture medium was used as control during the assays. To certify that undetectable remaining

Table 3. Inhibitory effect of crude extracts obtained from *Cedrela fissilis* on symbiotic fungus of leaf-cutting ants

Crude Extract *	Tissue	Inhibition of fungal growth (%) **
Hexane	Roots	100
	Stems	20
	Branches	40
	Fruits	0
	Leaves	40
Dichloromethane	Roots	100
	Stems	0
	Branches	20
	Fruits	0
	Leaves	0
Methanol	Roots	40
	Stems	0
	Branches	20
	Fruits	0
	Leaves	20

* Concentration = 1000 mg ml^{-1}
** Control with and without solvent = 0% inhibition of fungal growth
** Dry-weight of inoculum = 7.0 $\pm$ 0.2 mg ml^{-1}

amounts of the solvent did not affect fungal growth, a comparison was made with another set of tubes in which water was used instead of solvent. Both systems produced the same results (100% fungus growth; see footnote, Tables 3 and 4). Fungal growth was estimated macroscopically on the basis of the mycelial surface and density after 30 days of incubation using the same pattern previously described by Pagnocca *et al.* (1996). The modal values were registered.

RESULTS

Bioassays with crude extracts

The survival of the workers was significantly reduced ($p < 0.05$) in the diets containing the crude extracts RH, LH, RD, LD and LM (Table 1). Furthermore, the RH and RD extracts completely inhibited the development of the symbiotic fungus while the other extracts were less powerful or showed no activity (Table 3).

Bioassays with fractions of roots and leaves

The fractions obtained from RH (RH-H; RH-D; RH-E; RH-M), from RD (RD-4; RD-5; RD-6; RD-8; RD-9) and from LD (LD-H; LD-D; LD-E; LD-M) were tested in bioassays with ants and all of them were toxic (Table 2).

The same fractions derived from RH and RD were used in tests with the symbiotic fungus and some (RH-H; RH-D; RD-4; RD-5) completely inhibited its development (Table 4).

DISCUSSION

Traditional control of ants with insecticides, in spite of its efficiency, is still a problem because of their nonselective toxicity (Loeck & Nakano 1982; Vilela & Howse 1988). There is a growing necessity for ant control without ecological injury, one that does not lead to selection of resistant

Table 4. Inhibitory effect of fractions of hexane and dichloromethane crude extracts (root) of *Cedrela fissilis* on symbiotic fungus of leaf-cutting ants

Fraction	Concentration (mg ml^{-1})	Inhibition of fungal growth (%) *
RH-H	300	100
RH-D	270	100
RH-E	138	60
RH-M	579	0
RD-3	430	80
RD-4	570	100
RD-5	424	100
RD-6	412	80
RD-7	160	20
RD-8	214	0
RD-9	336	40

* Control with and without solvent = 0% inhibition of fungal growth
** Dry-weight of inoculum = 7.4 ± 0.4 mg.ml^{-1}
R = roots; H = hexane; D = dichloromethane; E = ethyl acetate; M = methanol.

populations, and that can be effective, specific and enduring (Diehl-Fleig *et al.* 1988; Silva & Diehl-Fleig 1988). The natural products of higher plants may represent new alternative methods for the control of these economically important insects (Balandrin *et al.* 1985; Bueno *et al.* 1990) and the tropical plants have proved to be a rich source of biologically active substances (Isman 1989).

The present results show that there was a strong mortality of *Atta sexdens rubropilosa* workers which were fed daily with an artificial diet containing crude extracts and all fractions from roots and leaves of *Cedrela fissilis*. In addition, an inhibitory effect on fungal development was observed to crude extracts and some fractions of roots from this plant. Similar toxic effects to leaf-cutting ants and their symbiotic fungus were observed in studies with *Vismea baceifera* (Howard 1988), *Sesamum indicum* (Pagnocca *et al.* 1990; Bueno *et al.* 1995; Ribeiro *et al.* 1998; Bueno *et al.* 2004 a, b; Morini *et al.* 2005), *Virola sebifera* (Pagnocca *et al.* 1996), *Ricinus communis* (Hebling *et al.* 1996; Bigi *et al.* 1998, 2004), *Canavalia ensiformis* (Monteiro *et al.* 1998; Hebling *et al.* 2000a), *Ipomea batatas* (Hebling *et al.* 2000b) and *Picramia teapensis* (Rodrígues-Gamboa *et al.* 2000).

Probably the effects observed are due the action of some secondary metabolites present in the tissue of this plant. Plant compounds toxic for insects have long been known and used as insecticides, such as pyrethrin, nicotine and rotenone, which were obtained, respectively, from *Chrysanthemum*, *Nicotina* and several species of Leguminosae family (Godfrey 1994; Vieira & Fernandes 1999). Recently, Bigi *et al.* (2004) studied the effects of ricinine isolated from *Ricinus communis* leaves to *Atta sexdens rubropilosa* workers and their symbiotic fungus and related toxic effects for the former but not for the fungus.

Further research is needed to identify the chemical compounds from *Cedrela fissilis* roots and leaves responsible for the toxic effects and to evaluate their potential as insecticides and/or fungicides for the control

of leaf-cutting ants. However, this process of isolation and structural determination devoted many years and, sometimes, the separation of these compounds during the different steps of purification can result in no or lower inhibitory effect for most of the fractions, indicating that the inhibitory activity could be due to the joint action of these compounds rather than to the action of a single substance (Ribeiro *et al.* 1998; Morini *et al.* 2005).

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REFERENCES

Balandrin, M.F., J.A. Klocke, E.S. Wurtele & W.N.H. Bollinger 1985. Natural plant chemicals: sources of industrial and medicinal materials. Science 228: 1154-1160.

Beck, S.D. & T.C. Reese 1976. Insect-plant interactions, nutrition and metabolism. *In*: Biochemical interaction between plants and insects. Recent advances in phytochemistry. Wallace, J.W. & R.L. Mansell, eds. Plenum Press, New York, USA. Pp. 41-92.

Bigi M.F.M.A., M.J.A. Hebling, O.C. Bueno, F.C. Pagnocca, O.A. Silva, J.B. Fernandes & P.C. Vieira 1998. Toxicidade de extratos foliares de *Ricinus communis* L. para operárias de *Atta sexdens rubropilosa* Forel, 1908 (Hymenoptera, Formicidae). Revista Brasileira de Entomologia 41(2-4): 239-243.

Bigi M.F.M.A., V.L.V. Torkomian, S.T.C.S. Groote, M.J.A. Hebling, O.C. Bueno, F.C. Pagnocca, J.B. Fernandes, P.C. Vieira & M.F.G.F. Silva 2004. Activity of the *Ricinus communis* (Euphorbiaceae) and ricinine to the leaf-cutting ants *Atta sexdens rubropilosa* Forel, 1908 (Hymenoptera, Formicidae) and the symbiotic fungus *Leucoagaricus gongylophorus* (Singer) Möller. Pest Management Science 60 (9): 933-938.

Brown Jr, W.L. 2000. Diversity of Ants. *In:* Ants: standard methods for measuring and monitoring biodiversity. Agosti, D., J.D. Majer, L.E. Alonso & T.R. Schultz, eds. Smithsonian Institution Press, Washington and London. Pp. 45-79.

Bueno, O.C., M.J.A. Hebling-Beraldo, O.A. Silva, F.C. Pagnocca & J.B. Fernandes 1990. Toxic effect of plants on leaf-cutting ants and their symbiotic fungus. *In*: Applied myrmecology: a world perspective. R.K. Vander Meer, K. Jaffe & A. Cedeno, eds. Westview Press, Boulder, USA. Pp.420-426.

Bueno, O.C., M.J.A. Hebling, O.A. Silva & A.M.C. Matenhauer 1995. Effect of sesame (*Sesamum indicum* L.) on nest development of *Atta sexdens rubropilosa* Forel (Hym., Formicidae). Journal Applied Entomology 119: 341-343.

Bueno, O.C., M.S.C. Morini, F.C. Pagnocca, M.J.A. Hebling & O.A. Silva 1997. Sobrevivência de operárias de *Atta sexdens rubropilosa* Forel (Hymenoptera, Formicidae) isoladas do formigueiro e alimentadas com dieta artificial. Anais da Sociedade Entomológica do Brasil 26: 107-113.

Bueno, O.C., F.C. Bueno, J. Brochini, K. Sinhori, M.S.C. Morini, M.J.A. Hebling, F.C. Pagnocca, A.C. Leite, P.C. Vieira & J.B. Fernandes 2004a. Activity of sesame leaf extracts to the leaf-cutting ant *Atta sexdens rubropilosa* (Hymenoptera: Formicidae). Sociobiology 44 (3): 511-518.

Bueno, O.C., F.C. Bueno, G. Betella, M.S.C. Morini, M.J.A. Hebling, F.C. Pagnocca, A.C. Leite, P.C. Vieira & J.B. Fernandes 2004b. Toxicity of sesame extracts to leaf-cutting ant *Atta sexdens rubropilosa* (Hymenoptera: Formicidae). Sociobiology 44 (3): 599-606.

Champagne D.E., M. Isman & G.H.N. Towers 1989. Insecticidal activity of phytochemical and extracts of the Meliaceae. *In:* Insecticides of plant origin. Arnason, J.T., B.J.R. Philogéne & P. Morand, eds. The American Chemical Society, Washington. Pp. 95-109.

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

Della Lucia, T.M.C. & H.G. Fowler 1993. As formigas cortadeiras. *In:* As Formigas Cortadeiras, Della Lucia, T.M.C. ed. Viçosa, Minas Gerais, Folha de Viçosa. Pp. 1-3.

Diehl-Fleig, E., M.E. da Silva & M.R.M. Pacheco 1988. Testes de patogenicidade dos fungos entomopatogênicos *Beauveria bassiana* e *Metarhizium anisopliae* em *Atta sexdens piriventris* (Santschi, 1919) em diferentes temperaturas. Ciência e cultura 40: 1103-1105 (1988).

Elliott, M., N.F. Janes & C. Potter 1978. The future of pyrethroids in insect control. Annual Review Entomology 23: 443-469.

Febvay, G., P. Bourgeois & A. Kermarrec 1985. Antiappétants pour la fourmi attine, *Acromyrmex octospinosus* (Reich) (Hymenoptera, Formicidae), chez certaines espèces d'igname (Dioscoreaceae) cultivées aux Antilles. Agronomie 5: 439-444.

Febvay, G. & A. Kermarrec 1986. Prevention of feeding by *Acromyrmex octospinosus* with antifeedants from yams. *In*: Fire ants and leaf-cutting ants. Lofgren, C.S. & R.K. Vander Meer, eds. Westview Press, Boulder, USA. Pp.247-259.

Garcia, I.P., L.C. Forti, V.L. Engel, A.P.P. Andrade & C.F. Wilcken 2003. Ecologycal interations between *Atta sexdens* and the vegetation of a mesophyll semideciduous forest fragment in Botucatu, SP. Sociobiology 42: 265-283.

Godfrey C.R.A. 1994. Agrochemicals from natural products, Marcle Dekker, New York.

Hebling, M.J.A., P.S. Maroti, O.C. Bueno, O.A. Silva, & F.C. Pagnocca 1996. Toxic effects of leaves of *Ricinus communis* (Euphorbiaceae) to laboratory nests of *Atta sexdens rubropilosa* (Hymenoptera: Formicidae). Bulletin Entomological Research 86: 253-256.

Hebling, M.J.A., O.C. Bueno, F.C. Pagnocca, O.A. Silva & P.S. Maroti 2000a. Toxic effects of *Canavalia ensiformis* L. (Leguminosae) on laboratory colonies of *Atta sexdens* L. (Hym., Formicidae). Journal Applied Entomology 124: 33-35.

Hebling, M.J.A., O.C. Bueno, P.S. Maroti, F.C. Pagnocca & O.A. Silva 2000b. Effects of leaves of *Ipomoea batatas* (Convolvulaceae) on nest development and on respiratory metabolism of leaf-cutting ants *Atta sexdens* L. (Hym., Formicidae). Journal Applied Entomology 124: 249-252.

Hölldobler, B. & E.O. Wilson 1990. The ants. Havard University Press, Massachusetts, 732p.

Howard, J.J. 1988. Leaf cutting ant diet selection: relative influence of leaf chemistry and physical features. Ecology 69:250-260.

Hubbell, S.P. & D.F. Wiemer 1983. Host plant selection by an Attine ant. In: Social Insects in the Tropics. Jaisson, P., ed., vol.2. University of Paris Press, Paris, France. Pp. 133-154.

Hubbell, S.P., J.J. Howard. & D.F. Wiemer 1984. Chemical leaf repellency to an Attine ant: Seasonal distribution among potential host plant species. Ecology 65: 1067-1076.

Isman, M.B. 1989. Toxicity and fate of acetylchromenes in pest insects. *In*: Insecticides of plant origin. Arnason, J.T., J.R. Philogene & P. Morand, eds. Washington DC, American Chemical Society. Pp. 44-58.

Littledyke, M. & J.M. Cherrett 1975. Variability in the selection of substrate by the leaf-cutting ants, *Atta sexdens rubropilosa* (L.) and *Acromyrmex octospinosus* (Reich) (Hymenoptera: Formicidae). Bulletin Entomological Research 65: 33-47.

Loeck, A.E. & O. Nakano 1982. Distribuição de substratos no interior de um sauveiro de *Atta sexdens rubropilosa* Forel, 1908 (Hymenoptera - Formicidae). O solo 74: 36-42.

Monteiro M.R., V.L.V. Torkomian, F.C. Pagnocca, P.C. Vieira, J.B. Fernandes, F.G.F. Silva, O.C. Bueno & M.J.A. Hebling 1998. Activity of extracts and fatty acids of *Canavalia ensiformis* (Leguminosae) against the symbiotic fungus of the leaf-cutting ants *Atta sexdens*. Anais da Acadademia Brasileira de Ciências 70 (4): 733-736.

Morini, M.S.C., O.C. Bueno, F.C. Bueno, A.C. Leite, M.J.A. Hebling, F.C. Pagnocca, J.B. Fernandes, P.C. Vieira & M.F.G.F. Silva 2005. Toxicity of sesame seed to leaf-cutting ant *Atta sexdens rubropilosa* (Hymenoptera: Formicidae). Sociobiology 45(1): in press.

Pagnocca, F.C., O.A. Silva, M.J.A.H Beraldo, O.C. Bueno, J.B. Fernandes & P.C. Vieira 1990. Toxicity of sesame extracts of the symbiotic fungus of leaf-cutting ants. Bulletin of Entomological Research 80: 349-352.

Pagnocca F.C., V.L.V. Torkomian, M.J.A. Hebling, O.C. Bueno, O.A. Silva, J.B. Fernandes, P.C. Vieira, M.F.G.F. Silva & A.G. Ferreira 1996. Toxicity of lignans to symbiotic fungus of leaf-cutting ants. Journal of Chemical Ecology 22: 1325-1330.

Hebling, M.J.A., O.C. Bueno, F.C. Pagnocca, O.A. Silva & P.S. Maroti 2000a. Toxic effects of *Canavalia ensiformis* L. (Leguminosae) on laboratory colonies of *Atta sexdens* L. (Hym., Formicidae). Journal Applied Entomology 124: 33-35.

Hebling, M.J.A., O.C. Bueno, P.S. Maroti, F.C. Pagnocca & O.A. Silva 2000b. Effects of leaves of *Ipomoea batatas* (Convolvulaceae) on nest development and on respiratory metabolism of leaf-cutting ants *Atta sexdens* L. (Hym., Formicidae). Journal Applied Entomology 124: 249-252.

Hölldobler, B. & E.O. Wilson 1990. The ants. Havard University Press, Massachusetts, 732p.

Howard, J.J. 1988. Leaf cutting ant diet selection: relative influence of leaf chemistry and physical features. Ecology 69:250-260.

Hubbell, S.P. & D.F. Wiemer 1983. Host plant selection by an Attine ant. In: Social Insects in the Tropics. Jaisson, P., ed., vol.2. University of Paris Press, Paris, France. Pp. 133-154.

Hubbell, S.P., J.J. Howard. & D.F. Wiemer 1984. Chemical leaf repellency to an Attine ant: Seasonal distribution among potential host plant species. Ecology 65: 1067-1076.

Isman, M.B. 1989. Toxicity and fate of acetylchromenes in pest insects. *In*: Insecticides of plant origin. Arnason, J.T., J.R. Philogene & P. Morand, eds. Washington DC, American Chemical Society. Pp. 44-58.

Littledyke, M. & J.M. Cherrett 1975. Variability in the selection of substrate by the leaf-cutting ants, *Atta sexdens rubropilosa* (L.) and *Acromyrmex octospinosus* (Reich) (Hymenoptera: Formicidae). Bulletin Entomological Research 65: 33-47.

Loeck, A.E. & O. Nakano 1982. Distribuição de substratos no interior de um sauveiro de *Atta sexdens rubropilosa* Forel, 1908 (Hymenoptera - Formicidae). O solo 74: 36-42.

Monteiro M.R., V.L.V. Torkomian, F.C. Pagnocca, P.C. Vieira, J.B. Fernandes, F.G.F. Silva, O.C. Bueno & M.J.A. Hebling 1998. Activity of extracts and fatty acids of *Canavalia ensiformis* (Leguminosae) against the symbiotic fungus of the leaf-cutting ants *Atta sexdens*. Anais da Acadademia Brasileira de Ciências 70 (4): 733-736.

Morini, M.S.C., O.C. Bueno, F.C. Bueno, A.C. Leite, M.J.A. Hebling, F.C. Pagnocca, J.B. Fernandes, P.C. Vieira & M.F.G.F. Silva 2005. Toxicity of sesame seed to leaf-cutting ant *Atta sexdens rubropilosa* (Hymenoptera: Formicidae). Sociobiology 45(1): in press.

Pagnocca, F.C., O.A. Silva, M.J.A.H Beraldo, O.C. Bueno, J.B. Fernandes & P.C. Vieira 1990. Toxicity of sesame extracts of the symbiotic fungus of leaf-cutting ants. Bulletin of Entomological Research 80: 349-352.

Pagnocca F.C., V.L.V. Torkomian, M.J.A. Hebling, O.C. Bueno, O.A. Silva, J.B. Fernandes, P.C. Vieira, M.F.G.F. Silva & A.G. Ferreira 1996. Toxicity of lignans to symbiotic fungus of leaf-cutting ants. Journal of Chemical Ecology 22: 1325-1330.

Ribeiro S.B., F.C. Pagnocca, S.R. Victor, O.C. Bueno, M.J.A. Hebling, M. Bacci Júnior, O.A. Silva, J.B. Fernandes & P.C. Vieira 1998. Activity of sesame leaf extracts against the symbiotic fungus of *Atta sexdens* L. Anais da Sociedade Entomológica do Brasil 27(3): 421-426.

Rockwood, L.L. 1976. Plant selection and foraging patterns in two species of leaf-cutting ants (*Atta*). Ecology 57: 48-61.

Rockwood, L.L. 1977. Foraging patterns and plant selection in Costa Rica leaf cutting ants. Journal of the New York Entomological Society 85: 22-233.

Rodrígues-Gamboa T., S.R. Victor, J.B. Fernandes, M.F.G.F. Silva, P.C. Vieira, F.C. Pagnocca, O.C. Bueno, M.J.A. Hebling & O.C. Castro 2000. Anthrone and oxanthrone C, O – diglycosides from *Picramnia teapensis*. Phytochemistry 55: 837-841.

Silva, M.E. & E. Diehl-Fleig 1988. Avaliação de diferentes linhagens de fungos entomopatogênicos para controle da formiga *Atta sexdens piriventris* (Santschi, 1919) (Hymenoptera: Formicidae). Anais da Sociedade Entomológica do Brasil 17: 263-269.

Vieira, P.C. & J.B. Fernandes 1999. Plantas inseticidas. In: Farmacognosia, da planta ao medicamento. Simões, C.M.O. *et al.*, eds. Editora da Universidade/ UFRS, Rio Grande do Sul, Brasil. Pp. 739-754.

Vilela, E.F. & P.E. Howse 1988. Pheromone performance as an attractive component in baits for the control of the leaf cutting ant *Atta sexdens rubropilosa* Forel, 1908 (Hymenoptera: Formicidae). Anais da Sociedade Entomológica do Brasil 17: 107-124.

Weber, N.A. 1972. Gardening ants, the attines, The American Philosophical Society, Philadelphia, USA, 146p.

Whittaker, R. & P. Feeny 1971. Allelochemis: chemical interaction between species. Science 171: 757-770.