ECOLOGY, BEHAVIOR AND BIONOMICS

Seasonal Variations of Metapleural Secretion in the Leaf-Cutting ant *Atta sexdens piriventris* Santschi (Myrmicinae: Attini), and Lack of Fungicide Effect on *Beauveria bassiana* (Bals.) Vuillemin

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Variações Sazonais da Secreção Metapleural da Formiga Cortadeira *Atta sexdens piriventris* Santschi (Myrmicinae: Attini), e Ausência de Efeito Fungicida Sobre o Entomopatógeno *Beauveria bassiana* (Bals.) Vuillemin

RESUMO – Os mecanismos envolvidos na manutenção das colônias de insetos sociais livres de patógenos são aspectos importantes relacionados à biologia evolutiva do grupo. No caso das formigas, tem sido sugerido que a secreção das glândulas metapleurais teria importante papel na assepsia da colônia. Neste trabalho, as diversas subcastas de operárias da formiga cortadeira *Atta sexdens piriventris* Santschi foram comparadas quanto à presença de secreção metapleural ao longo de um ano. Simultaneamente, a atividade tópica da secreção foi testada em operárias tratadas com o fungo entomopatogênico *Beauveria bassiana* (Bals.) Vuillemin. De acordo com a largura média da cápsula cefálica, foram definidas seis subcastas de operárias: jardineira, mínima, média, grande, máxima e soldado. Foi encontrada uma correlação positiva entre subcasta e comprimento do reservatório metapleural. As subcastas apresentaram diferenças significativas na presença de secreção metapleural ao longo do ano exceto nos meses de abril, maio, julho e novembro. Foi observada uma correlação positiva entre as temperaturas médias sazonais e presença de secreção metapleural. Foram registradas altas freqüências de operárias de todas subcastas infectadas por *B. bassiana* durante os bioensaios, indicando que a secreção metapleural não tem atividade fungicida contra esse patógeno. Os resultados sugerem que a secreção metapleural não está entre os principais mecanismos de assepsia das colônias de *A. sexdens piriventris*.

PALAVRAS-CHAVE: Insecta, formigas cultivadoras de fungo, glândula metapleural, fungo entomopatogênico, controle biológico.

ABSTRACT – Mechanisms involved in maintaining colonies of social insects free of pathogens are among the main aspects of interest in the evolutionary biology of this group. For ants, it has been suggested that secretion from the metapleural glands play an important role in colony asepsis. In this study, different worker subcastes of the fungus-growing ant *Atta sexdens piriventris* Santschi were compared in relation to presence of metapleural secretion during a year. At the same time, the topical activity of the secretion was tested on workers treated with the entomopathogenic fungus *Beauveria bassiana* (Bals.) Vuillemin. Based on the mean width of the cephalic capsule, six worker subcastes were defined: gardener, minor, media, large, major and soldier. A positive correlation between worker subcaste and metapleural reservoir length was observed. Significant differences for presence of metapleural secretion were found throughout the year among subcastes, except in April, May, July and November. A positive correlation between seasonal mean temperature and presence of the metapleural secretion was observed. High frequencies of infected workers of all subcastes were observed during bioassays with inoculation of *B. bassiana*, indicating that metapleural secretion has no fungicidal activity against this pathogen. The results suggest that metapleural secretion of workers is not the main mechanism of *A. sexdens piriventris* colony asepsis.

KEY WORDS: Insecta, fungus-growing ants, metapleural gland, entomopathogenic fungus, biological control.

In most terrestrial habitats, social insects have higher biomass and energy consumption than vertebrates. Among the eusocial insects, ants are dominant, widely distributed throughout the world, excepting the polar regions. According to Hölldobler & Wilson (1990), the eusocial behavior is an ecological strategy of high efficiency. The ants' evolutionary success may be attributed to the appearance of small and wingless workers, capable of exploiting hardly accessible sites, gathering a variety of energetic resources and nesting in the soil. The occurrence of metapleural glands whose acid secretion inhibits microrganism growing (Maschwitz *et al*. 1970) was an adaptation which allowed the ants to explore other habitats (Hölldobler & Wilson 1990).

In some ant species, the metapleural secretion is active against bacteria and fungi (Maschwitz 1974, Beattie *et al*. 1986, Veal *et al*. 1992, Mackintosh *et al*. 1995, Nascimento *et al*. 1996) besides having insecticide properties (Attygalle *et al*. 1989). For some authors, it may reduce spore germination and colony growth of some soil fungi, and the pollen quality of certain plants is also reduced (Beattie *et al*. 1985). However, Ramsey (1995) found a species of *Iridomyrmex* pollinating *Blandfordia grandiflora* (Liliaceae), suggesting that metapleural secretion has no such great effects on pollen activity. Other roles of the metapleural gland have been reported, such as intraspecific recognition (Brown 1968) and territorial marker, increasing workers aggressiveness in the areas where present (Jaffé & Puche 1984, Cammaerts & Cammaerts 1998).

Atta sexdens piriventris Santschi is a fungus-growing ant widely distributed in Rio Grande do Sul state, Brazil, and responsible for serious damage to crops. Microbial control has been suggested as an alternative control method for this pest (Diehl-Fleig *et al*. 1993, Specht *et al*. 1994). However, the metapleural secretion and its possible antifungal effect should be an obstacle to the use of entomopathogenic fungi as biological control agents (Kermarrec *et al*. 1986). Wilson (1980) has suggested that the development of glands should be higher on ant castes where they would be more needed. Then, if the metapleural glands are responsible by colony asepsis one should expect to find larger and more active glands in minor workers and gardener ants, which take care and protected the symbiontic fungus, the immatures and the queen. Thus this study was carried out aiming: a) to relate the size of metapleural reservoir according to the subcastes (defined from the cephalic capsule mean width) of *A. sexdens piriventris* workers; b) to evaluate the frequencies of workers by subcaste with metapleural secretion in the gland reservoirs throughout a year; and c) to test the topical activity of metapleural secretion of workers against the entomopathogenic fungus *Beauveria bassiana* (Bals.) Vuillemin.

Material and Methods

The ant workers were collected from an *Atta sexdens piriventris* colony located situada in the municipality of São Leopoldo (29º45'S; 51º08'W), Rio Grande do Sul state, Brazil. The climate is subtropical with a short dry season in the summer and an annual rainfall of 1347.4 mm. The annual mean temperature is 19.5ºC, with the mean maximum of 30.2ºC in January and the mean minimum of 10.7ºC in June and July (MARA/SNI/DNM 1992, Pereira *et al.* 2001).

Morphometric Measurements of Worker Subcastes. The width of the cephalic capsule was determined as the distance between eyes, and the length of metapleural gland reservoir were measured in *A. sexdens piriventris* workers (N=1000) collected, separated and arbitrarily grouped into subcastes according to their size (see Wilson, 1980). To set the subcastas, the workers were arbitrarily distributed in six classes of cephalic capsule width: gardener (0.85 mm - 1.15 mm), minor (1.16 mm - 1.45 mm), media (1.46 mm - 1.75 mm), major (2.36 mm - 2.65 mm), large (2.96 mm - 3.25 mm) and soldier (3.26 mm - 3.65 mm). Thirty individuals from each subcaste, randomly chosen, were measured using reticulated micrometer attached to an optical microscope.

Mean size of the cephalic capsule and mean length of the metapleural gland reservoir and their standard deviations were calculated for each subcaste. Pearson correlation coefficient (r) was used when analyzing the relation between these two measures.

Evaluation of Metapleural Reservoir. From January to December 1994, 50 workers/subcaste in the same colony, were collected monthly and had their reservoir perforated in the median-superior portion with micro-needle under stereomicroscope (40X). Presence or absence of secretion in the reservoirs was individually registered for both left and right glands. Considering that production of metapleural secretion, such as occurs in others glands of several organisms may be related to climatic factors, possible relations between frequencies of workers with secretion in the metapleural reservoirs, seasonal mean temperature and relative humidity were analyzed through Pearson correlation coefficient (r).

Bioassays with Workers. To determine whether the metapleural secretion is active against the entomopathogenic fungus *Beauveria bassiana*, and to evaluate this action throughout a year, bioassays were conducted during middle months (January, April, July and October) of each season with all six subcastes of fresh collected *A. sexdens piriventris* workers.

For the bioassays it was used the B_{SA} strain of *B. bassiana* that is a microbial agent for leaf-cutting ants control in some experimental areas of Rio Grande do Sul. A suspension of 1.6×10^6 conidia/ml of this strain was applied, using a microcapillary, either to the metapleural gland atrium or to the antero-superior portion of the metathorax (sites where the ant is unable to spread the metapleural secretion). Fifty ants/subcaste were used for each application. As a control, sterile saline solution was applied in equal number of individuals of each subcaste.

Ants were individually distributed in sterile vials containing filter paper with 0.05 ml of sugar solution and kept in humid chamber at 27±1ºC, 80% RH and 12h photoperiod. The frequencies of *B. bassiana* infected ants by treatment (site of pathogen application), the season on the year and subcaste were compared by the chi-square test.

Results

Morphometric Measurements of Worker Subcastes. Based on cephalic capsule mean width (Table 1), six subcastes of *A. sexdens piriventris* workers (gardener, minor, media, large, major and soldier) were defined. Significant positive correlation between cephalic capsule size and metapleural reservoir length was found for gardener, large and soldier subcastes (Table1).

Evaluation of Workers Metapleural Reservoirs. Monthly comparisons among subcastes showed that they significantly differ for metapleural secretion production along the year, except for April, May, July and November (Table 2). In general, a larger number of soldiers, compared to other subcastes, had secretion in their metapleural reservoirs.

Table 1. Mean values (± standard deviation) for cephalic capsule width and metapleural reservoir length of the worker subcastes of *A. sexdens piriventris*.

Subcaste	Cephalic capsule (mm)	Metapleural reservoir (mm)	r	P
Gardener	0.97 ± 0.10	0.38 ± 0.02	0.4764	<0.01
Minor	1.35 ± 0.07	0.40 ± 0.05	0.3322	ns
Media	1.62 ± 0.13	0.56 ± 0.03	0.1253	ns
Large	2.45 ± 0.16	0.69 ± 0.06	0.6051	<0.001
Major	3.04 ± 0.11	0.89 ± 0.04	0.2424	ns
Soldier	3.46 ± 0.16	0.97 ± 0.07	0.8247	<0.001

(r) Pearson Correlation.

Throughout the year the frequencies of workers with secretion in the metapleural reservoir varied from 13.0% in July to 61.7% in November (Fig. 1).

Considering the number of workers of all subcastes, for each month, there was a significant positive correlation between the presence of secretion in the metapleural reservoir and the seasonal mean temperature (r=0.6967; P<0.01), However, there was no correlation between presence of secretion and seasonal mean relative humidity during the sampled period (r=-0.1559, P>0.05).

Bioassays with Workers. During winter, spring and summer, independent of the inoculation site (metapleural gland or metathorax antero-superior portion), there were no significant differences for infection frequencies among subcastes. Only during autumn, a lower frequency of infected gardeners was found after inoculation in the metathorax antero-superior portion (Table 3). However, when infection frequencies of each inoculated subcaste were compared to their respective controls (inoculated by sterile saline solution), differences were significant except for gardeners, minors and media ants that had no difference during winter (Table 3). Despite carefull handing during manipulations of all four seasons, pathogen contamination occurred in some control groups.

In relation to seasonal data, during spring and summer, we found the highest frequencies of workers infected with *B. bassiana*, all subcastes together (Table 3). A lower occurrence of saprophytes was observed in the bioassays during these seasons if compared with autumn and winter.

Discussion

In the study of social insects, descriptive and numeric data are necessary for recognizing species-specific characteristics (Tschinkel 1991). Thus, for this study, tests were designed considering the mean size of *A. sexdens*

Table 2. Comparison of the percentage of workers subcastes of *A. sexdens piriventris*, presenting secretion in their metapleural reservoirs during evaluation period.

	Subcastes						χ^2	d.f.	P
	GAR	MIN	MED	LAR	MAJ	SOL			
January	26.0	40.0	28.0	30.0	58.0	76.0	40.922	5	<0.001
February	32.0	32.0	64.0	60.0	48.0	58.0	20.128	5	<0.01
March	22.0	54.0	50.0	44.0	46.0	58.0	16.188	5	<0.01
April	34.0	46.0	46.0	32.0	40.0	56.0	8.097	5	ns
May	40.0	64.0	52.0	56.0	62.0	62.0	8.279	5	ns
June	54.0	62.0	42.0	34.0	34.0	34.0	14.769	5	<0.05
July	8.0	12.0	14.0	8.0	12.0	24.0	7.692	5	ns
August	10.0	14.0	14.0	6.0	26.0	44.0	31.644	5	<0.001
September	48.0	38.0	32.0	30.0	18.0	38.0	11.230	5	<0.05
October	30.0	32.0	20.0	18.0	38.0	58.0	23.944	5	<0.001
November	62.0	56.0	64.0	58.0	62.0	68.0	1.932	5	ns
December	56.0	44.0	44.0	54.0	64.0	76.0	15.353	5	<0.01

* N = 50/subcaste/month; GAR = gardener; MIN = minima; MED = media; LAR = large; MAJ = major; SOL = soldier.

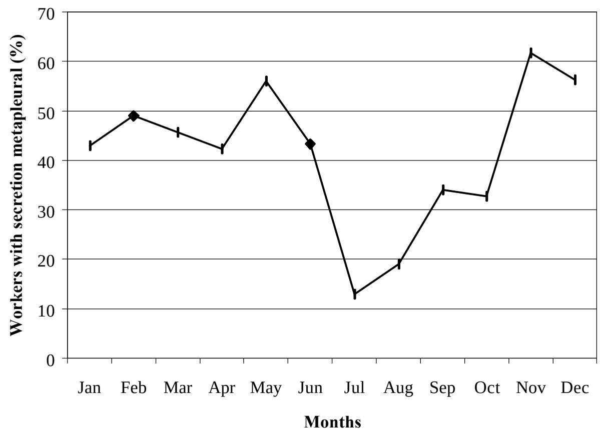

Figure 1. Comparison of the percentage of workers of *A. sexdens piriventris*, presenting metapleural secretion in their reservoir throughout the year.

piriventris subcastes of workers. Differential production of metapleural secretion was observed among the six worker subcastes, and often, the highest frequencies of reservoirs with secretion were found in the larger subcastes. In relation to the season of the year, there was a positive correlation between secretion presence and seasonal mean temperature.

Metapleural secretion has been studied in several ant species, with demonstration of its activity against bacteria and fungi (Maschwitz *et al*. 1970, Maschwitz 1974, Beattie *et al*. 1986, Veal *et al*. 1992, Nascimento *et al*. 1996), insects (Attygalle *et al*. 1989) and pollen (Beattie *et al*. 1985). However, none of this authors reported seasonal differences or differences among production from subcastes, possibly because their studies were conducted with ant colonies kept under laboratory conditions, tests performed during only one period of the year, with a small ant number, and perhaps, with the whole ants belonging to the same subcaste. Recently, Bot & Boomsma (1996) evaluating workers from colonies of two *Acromyrmex* species of Panama, found that the metapleural gland relative size gradually decreases as body size increases. According to them, their data follow what has been proposed by Wilson (1980) that organs achieve maximum development in those ant castes where they would be more important. In other words, if metapleural gland is associated with colony asepsis, it would be expected to be larger and to produce more secretion in the gardeners and minor workers, based on their colony role. However, for *A. sexdens piriventris*, a correlation between cephalic capsule size and metapleural reservoir length was found. A higher frequency of secretion in the reservoirs was found in the soldiers which, according to Wilson (1980), are more involved in colony defense than in hygiene.

Maschwitz *et al*. (1970) found that workers of *A. sexdens* poorly fed have an air bubble in their metapleural reservoirs. Variation in metapleural secretion presence in *A. sexdens piriventris* should be related to environmental factors, such as temperature, not excluding genetic factors as proposed by Bot & Boomsma (1996).

High frequencies of *A. sexdens* workers infected during the bioassays were observed, indicating that metapleural secretion has no fungicidal and/or fungistatic activity against the strain of *B. bassiana*. All subcastes were equally sensible to the pathogen, thus, it seems to have no relationship between the role of each subcaste (which could be more or less exposed to entomopathogens) and the antimicrobial effect of metapleural secretion, as suggested by Wilson (1980). However Alves & Sosa-Gómes (1983) observed greater resistance of *A. sexdens rubropilosa* (Forel) soldiers to the fungi *B. bassiana* and *Metarhizium anisopliae* in relation to the other workers.

During bioassays with *A. sexdens piriventris*, high incidence of saprophytic fungi occurred through fall and winter in dead workers, which are commonly present during wet periods specially in soil dwelling insects. Considering that the metapleural gland, which is found exclusively in ants, has been suggested as responsible for colony asepsis, growth of saprophytes suggests that *A. sexdens piriventris* secretion is not an efficient fungicide. The hypothesis of metapleural secretion not presenting fungicidal activity is also supported by the occurrence of high frequencies of infected workers during the periods with the greatest number of workers presenting secretion in their reservoirs.

Beattie *et al*. (1985) found that the secretion of a species

Table 3. Percentage of *A. sexdens piriventris* workers infected by *B. bassiana* in the metapleural glands (MPG) and in the metathorax antero-superior portion (MAS) and respective controls (CON).

Subcastes	Winter[a]				Spring[a]				Summer[b]				Autumn[b]			
	MPG	MAS	CON	P	MPG	MAS	CON	P	MPG	MAS	CON	P	MPG	MAS	CON	P
Gardener	20.0	30.0	0	ns	90.0	90.0	0	<0.05	80.0	86.0	0	<0.001	52.0	30.0	0	<0.001
Minor	40.0	40.0	0	ns	90.0	80.0	0	<0.05	92.0	90.0	0	<0.001	58.0	62.0	2.0	<0.001
Media	60.0	50.0	0	ns	100.0	100.0	0	<0.001	86.0	92.0	2.0	<0.001	54.0	46.0	0	<0.001
Large	50.0	70.0	0	<0.05	90.0	100.0	0	<0.001	90.0	96.0	12.0	<0.001	54.0	66.0	0	<0.001
Major	60.0	60.0	0	<0.05	100.0	80.0	0	<0.001	90.0	88.0	8.0	<0.001	78.0	78.0	6.0	<0.001
Soldier	80.0	90.0	10.0	<0.05	70.0	90.0	20.0	ns	92.0	94.0	4.0	<0.001	70.0	74.0	0	<0.001
P	ns	ns			ns	ns			ns	ns			ns	<0.05		

P for χ^2 test; a - N = 10/subcaste; b - N = 50/subcaste.

of *Myrmecia* has fungicidal activity against *B. bassiana* and other entomopathogens. More recently, Nascimento *et al*. (1996) confirmed the presence of substances with bactericidal and fungicidal activities in the metapleural secretion from *A. sexdens rubropilosa*, *Atta cephalotes* (Linn.) and *Acromyrmex octospinosus* (Reich). However, our results with *A.sexdens piriventris* workers does not show any fungicidal activity, corroborated by Billen (1990) citing Vander Meer (unpubl. data) who found that *Solenopsis invicta* Buren metapleural secretion has no antibiotic activity.

It seems that the metapleural secretion of *A. sexdens piriventris* workers, by itself, cannot affect biological control programs using *B. bassiana* as agent. Other chemical mechanisms should also be considered, such as, for example, mandibular glands secretion (Brough 1983) or pathogen recognition and/ or hygiene behavior (Kermarrec *et al*. 1986, Machado *et al*. 1988, Diehl-Fleig & Lucchese 1991) as well as the occurrence of a bacterium which produces antibiotic substances on the ant body (Currie *et al.* 1999, Schultz 1999). These mechanisms could be more effective than metapleural secretion in protecting the ant against pathogens.

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Literature Cited

Alves, S.B. & D.R. Sosa-Gómes. 1983. Virulência de *Metarhizium anisopliae* (Metsch) Sorok. e *Beauveria bassiana* (Bals.) Vuill. para duas castas de operárias de *Atta sexdens rubropilosa* (Forel, 1908). Poliagro 5: 1-9.

Attygalle, A.B., B. Siegel, O. Vostrowsky, H.J. Bestmann & V. Maschwitz. 1989. Chemical composition and function of metapleural gland secretion of the ant *Crematogaster deformis* Smith (Hymenoptera: Myrmicinae). J. Chem. Ecol. 15: 317-329.

Beattie, A.J., C.L. Turnbull, T. Hough & R.B. Knox. 1986. Antibiotic production: A possible function for the metapleural glands of ants (Hymenoptera: Formicidae). Ann. Entomol. Soc. Am. 79: 448-450.

Beattie, A.J., C.L. Turnbull, T. Hough, S. Jobson & R.B. Knox. 1985. The vulnerability of pollen and fungal spores to ant secretions: Evidence and some evolutionary implications. Amer. J. Bot. 72: 606-614.

Billen, J. 1990. A survey of the glandular system of fire ants, pp. 85-94. In Vander Meer, R.K., K. Jaffe & A. Cedeno (eds.), Applied Myrmecology**:** A world perspective. Westview Press. Boulder, San Francisco. 741p.

Bot, A.N.M. & J.J. Boomsma. 1996. Variable metapleural gland size-allometries in *Acromyrmex* leafcutter ants (Hymenoptera: Formicidae). J. Kansas Entomol. Soc. 69: 375-383.

Brough, E.J. 1983. The antimicrobial activity of the mandibular gland secretion of a Formicidae ant, *Calomyrmex* sp. (Hymenoptera: Formicidae). J. Inv. Pathol. 42: 306-311.

Brown, W.L. 1968. An hypothesis concerning the function of the metapleural glands in ants. Amer. Natur. 102: 188-191.

Cammaerts, M.C. & R. Cammaerts. 1998. Marking of nest entrance vicinity in the ant *Pheidole pallidula* (Formicidae, Myrmicinae). Behav. Proc. 42: 19-31.

Currie, C.R.; J.A. Scott; R.C. Summerbell & D. Malloch. 1999. Fungus-growing ants use antibiotic-producing bacteria to control garden parasites. Nature 398: 701-704.

Diehl-Fleig, E., M.E. da Silva, A. Specht & M. Valim-Labres. 1993. Efficiency of *Beauveria bassiana* for *Acromyrmex* spp. control (Hymenoptera: Formicidae). An. Soc. Entomol. Brasil 22: 281 - 285.

Diehl-Fleig, E. & M.E. de P. Lucchese. 1991. Reações comportamentais de operárias de *Acromyrmex striatus* (Hymenoptera, Formicidae) na presença de fungos entomopatogênicos. Rev. Bras. Entomol. 35: 101-107.

Hölldobler, B. & E.O. Wilson. 1990. The Ants. The Belknap Press of Harvard University Press. Cambridge, 732 p.

Jaffé, K. & H. Puche. 1984. Colony-specific territorial marking with the metapleural gland secretion in the ant *Solenopsis geminata* (Fabr.). J. Insect Physiol. 30: 265-270.

Kermarrec, A., G. Febvay & M. Decharme. 1986. Protection of leaf cutting ants from biohazards: Is there a future for microbiological control? p. 339-355. In Lofgren, S. & R.K. Vander Meer (eds.), Fire ants and leaf-cutting ants: biology and management, Westview Press, Boulder and London, 433p.

Machado, V., E. Diehl-Fleig, M.E. da Silva & M.E. de P. Lucchese. 1988. Reações observadas em colônias de algumas espécies de *Acromyrmex* (Hymenoptera; Formicidae) quando inoculadas com fungos entomopatogênicos. Ci. Cult. 40: 1106-1108.

Mackintosh, J.A., J.E. Trimble, M.K. Jones, P.H. Karuso, A.J. Beattie & D.A. Veal. 1995. Antimicrobial mode of action of secretions from the metapleural gland of *Myrmecia gulosa* (Australian bull ant). Can. J. Microbiol. 41: 136-144.

MARA/SNI/DNM. 1992. Normais climatológicas (1961-1990). 84p.

Maschwitz, V. 1974. Vergleichende untersuchungen zur funktion der ameisenmetathorakaldrüse. Oecologia 16: 303-310.

Maschwitz, V., K. Koob & H. Schildnecht. 1970. Ein beitrag zur funktion der metathoracaldrüse der ameisen. J. Insect Physiol. 16: 387-404.

Nascimento, R.R. do, E. Schoeters, E.D. Morgan, J. Billen & D.J. Stradling. 1996. Chemistry of metapleural gland secretions of three attine ants, *Atta sexdens rubropilosa*, *Atta cephalotes*, and *Acromyrmex octospinosus* (Hymenoptera: Formicidae). J. Chem. Ecol. 22: 987-1000.

Pereira, A.R., L.R. Angelocci & P.C. Sentelhas. 2001. Agrometeorologia: fundamentos e aplicações práticas. Ed. Agropecuaria, Guaíba. 480p.

Ramsey, M. 1995. Ant pollination of the perennial herb *Blandfordia grandiflora* (Liliaceae). Oikos 74: 265-272.

Schultz, T.R. 1999. Ants, plants and antibiotics. Nature 398: 747-748.

Specht, A., E. Diehl-Fleig & M.E. da Silva. 1994. Atratividade de iscas de *Beauveria bassiana* (Bals.) Vuill. a formigas do gênero *Acromyrmex* (Hymenoptera: Formicidae). An. Soc. Entomol. Brasil 23: 99-104.

Tschinkel, W.R. 1991. Insect sociometry, a field in search of data. Insectes Sociaux 38: 77-82.

Veal, D.A., J.E. Trimble & A.J. Beattie. 1992. Antimicrobial properties of secretions from the metapleural glands of *Myrmecia gulosa* (the Australian bull ant). J. Appl. Bacteriol. 72: 188-194.

Wilson, E.O. 1980. Caste and division of labor in leaf-cutter ants (Hymenoptera: Formicidae: *Atta*) 1. The overall pattern in *A. sexdens*. Behav., Ecol. Sociobiol. 7: 143-156.

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