

## RESPONSE OF THE GRASS-CUTTING ANT *Atta capiguara* GONÇALVES, 1944 (HYMENOPTERA: FORMICIDAE) TO SUGARS AND ARTIFICIAL SWEETENERS

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**ABSTRACT:** Using of toxic baits made of dehydrated citric pulp to control grass-cutting ants can lead to unsatisfactory results because of the low attractiveness of the substrate to worker ants. This work aimed to identify attractive substances, with potential for incorporation in a matrix of granulated baits for grass-cutting ants, among several kinds of sugars and substances used in artificial sweeteners. Experiments were carried out in mature nests of *Atta capiguara* (Hym.: Formicidae) set in pasture. Studied substances were sucrose, fructose, soluble starch, raffinose, maltose, lactose, sorbose, cellobiose, arabinose, xylose, glucose, galactose, rhamnose, arabinose, melezitose, saccharine and cyclamate (at 5.0% w/v). Later, on maltose, xylose, sucrose, fructose and glucose solutions were included at 5.0%, 7.5%, 10.0% and 20.0% w/v, respectively. Cellulose rectangles were used as vehicle and number of rectangles carried into the colonies was evaluated. Carrying rates were very low with maximum means of 9.6% for lactose and 6.0% for arabinose and cyclamate, at the 5.0% concentration. No differences ( $P > 0.05$ ) were observed relatively to the control (distilled water). No effects were detected for solution, concentration and for the interaction of these factors. Sugars and artificial sweeteners studied were not attractive to *Atta capiguara* workers, turning their inclusion as attractants in toxic ant baits not viable.

Key words: Attini, Myrmicinae, grass-cutting ants, bait

## RESPOSTA DA SAÚVA *Atta capiguara* GONÇALVES, 1944 (HYMENOPTERA: FORMICIDAE) A AÇÚCARES E EDULCORANTES ARTIFICIAIS

**RESUMO:** O uso de iscas tóxicas, formuladas à base de polpa cítrica desidratada, para o controle de formigas cortadeiras de gramíneas pode levar a resultados insatisfatórios devido à baixa atratividade do substrato às operárias. Este trabalho foi realizado com o objetivo de identificar substâncias atrativas e com potencial para incorporação em matrizes de iscas granuladas para formigas cortadeiras de gramíneas, dentre diversos tipos de açúcares e edulcorantes artificiais. Os experimentos foram realizados em ninhos adultos de *Atta capiguara* Gonçalves, em área de pastagem. Inicialmente, as substâncias estudadas foram: sacarose, frutose, amido solúvel, rafinose, maltose, lactose, sorbose, celobiose, arabinose, xilose, glicose, galactose, raminose, arabinose, melizitose, sacarina e ciclamato (5,0% p/v). Posteriormente, estudaram-se soluções de maltose, xilose, sacarose, frutose e glicose a 5,0%, 7,5%, 10,0% e 20,0% p/v. Retângulos de celulose foram usados como veículo. Avaliou-se o número de retângulos carregados para o interior das colônias. De modo geral, as taxas de carregamento foram muito baixas, com médias máximas de 9,6% para lactose e de 6,0% para arabinose e ciclamato, na concentração 5,0%, não diferindo da testemunha (água destilada). No esquema fatorial, não foram verificados efeitos de solução, de concentração e de interação entre esses fatores. Os açúcares e edulcorantes artificiais estudados não são atrativos às operárias de *Atta capiguara*, inviabilizando sua inclusão em iscas tóxicas, com a finalidade de melhorar sua atratividade.

Palavras-chave: Attini, Myrmicinae, formigas cortadeiras de gramíneas, isca

### INTRODUCTION

Ants of the genera *Atta* and *Acromyrmex*, known as leaf-cutting ants, explore a great variety of plant species to grow symbiotic fungi, utilized as food by their

colonies. The species *Atta capiguara* Gonçalves, commonly known as brown leaf-cutting ant, explores monocotyledon plants, basically grasses, and is economically important in sugarcane fields and pastures (Amante, 1967, 1972; Mariconi, 1970; Forti, 1985).

Granulated toxic baits are the main chemical control method utilized against leaf-cutting ants; bait formulation is based on dehydrated citrus pulp and soybean oil. Baits available in the market are attractive for dicotyledon-cutting ants, providing satisfactory control in the field. However, it is common for grass-cutting ant species, especially *A. capiguara*, to reject or carry the bait slowly, as well as to return it over to their loose dirt mounds. This might be related to the low attractiveness of citrus pulp (Robinson et al., 1980; Lapointe et al., 1996; Forti et al., 1998).

Adding chemical substances to the toxic baits can improve their attractiveness, facilitating the encounter of pellets by scout workers (Littleddyke & Cherrett, 1978). Researchers have demonstrated the attractiveness of sugars to leaf-cutting ants, as well as the nutritional importance of such compounds for these insects (Abbott, 1978; Silva et al., 1998 a, b). Robinson et al. (1980) developed alternative, differentiated baits for grass-cutting and dicotyledon-cutting ants of economic importance in Paraguay. Baits for grass-cutting ants were formulated with elephant grass *Pennisetum purpureum*, soybean oil, sugarcane molasses and active ingredient, with satisfactory results in the field. However, the authors did not discuss the effects of molasses in the formulation.

According to Cherrett & Seaforth (1970), certain water-soluble compounds, such as carbohydrates, amino acids, phenols and other glycosides, extracted from grapefruit albedo (*Citrus paradisi*), demonstrated to be attractive to workers of *Atta cephalotes* (L.) and *Acromyrmex octospinosus* (Reich). Mixtures of synthetic sugars were attractive to *A. cephalotes*, but not to *A. octospinosus* (Cherrett & Seaforth, 1970). Preference, in the same ant species, for sucrose and glucose, was observed by Mudd et al. (1978). Nagamoto (1998) verified that the addition of sucrose did not improve the attractiveness of orange juice solutions to *Atta sexdens* L.

Symbiotic fungi degrades cellulose in the leaves and hydrolyses cellobiose, providing ants with assimilable sugars (Bacci Jr. et al., 1995). Silva et al. (1998a) verified that the presence of glucose in artificial diets increased the survivorship rate of *A. sexdens* workers. Xylose, maltose and sucrose can sustain ant metabolism, even being less efficient than glucose (Silva et al., 1998b).

Most sugar-related studies were developed for ants that, preferentially, cut dicotyledons, *A. cephalotes*, *A. sexdens* and *A. octospinosus*, but only scant information is available on grass-cutting ants. Based on the hypothesis that sugars or artificial sweeteners are attractive to leaf-cutting ants, studies were carried out to identify attractants which can be potentially incorporated in bait matrices for grass-cutting ants.

## MATERIAL AND METHODS

Studies were carried out in a pasture area in Botucatu, SP, Brazil, (22°53'09"S and 48°26'42"W; altitude of 786 m). Three experiments were carried out in mature *A. capiguara* nests, selected based on the presence of active trails. Nests were identified by wooden stakes and the area of loose dirt was estimated. Four active foraging holes were marked per nest according to methodology described by Fowler et al. (1993).

The first experiment was performed in September, 1997, involving nests N<sub>c</sub>1, N<sub>c</sub>2, N<sub>c</sub>3, N<sub>c</sub>4 and N<sub>c</sub>6, with loose dirt areas of 128.77; 16.20; 58.76; 389.3; and 78.75 m<sup>2</sup>, respectively. Treatments were: sucrose, fructose, soluble starch, raffinose, maltose, lactose, sorbose, cellobiose, arabinose. Distilled water was used as control. The second experiment, performed in October, 1997, involved nests N<sub>c</sub>1, N<sub>c</sub>2 and N<sub>c</sub>4. Treatments were xylose, glucose, galactose, rhamnose, arabinose, melezitose, saccharine, cyclamate and distilled water as the control. In the third experiment, carried out in May, 1998, sugars studied were maltose, xylose, sucrose, fructose and glucose, at concentrations of 5.0%, 7.5%, 10.0% and 20.0% w/v, with distilled water as control, in nests N<sub>c</sub>1, N<sub>c</sub>2, N<sub>c</sub>4, N<sub>c</sub>6, N<sub>c</sub>7, the latter with a loose dirt area of 58.52 m<sup>2</sup>.

Tests utilized cellulose rectangles (0.3 x 0.6 cm) previously marked with plastic paint, immersed in the different solutions for two minutes, oven-dried at 50° C, and offered to the nests on the same day they were prepared. Treatments were offered according to methodologies described by Forti et al. (1993) for granulated bait attractiveness studies with leaf-cutting ants. Five sequential presentations of lots consisting of 10 cellulose rectangles per treatment were set up, totaling 50 per treatment, in one trail showing worker activity per nest. In the first two trials, treatments were offered on a gridded plate, at the end of the trail, from 16h00 to 19h00. In the third experiment, treatments were offered from 9h00 to 11h30 and from 14h00 to 16h00, alongside the trail, in the vicinity of the foraging hole, with lots arranged in zig-zag. This form of offering was also selected for the third experiment, since during the execution period (May) trails were long, what would imply subjecting treatments to a longer exposure in the field. Before each presentation the position of treatments was randomized, both on the plates and on the trails. Presentations were terminated when all rectangles in the same treatment were carried or, at most, 30 minutes after offering. The remaining rectangles were then counted, to establish the percentage of carried rectangles relative to the total offered per treatment.

Trials were set up in a randomized blocks design, with ten and nine treatments (substances and control) and five and three replicates (nests) for the first and second experiments, respectively. The third experiment was also

organized in a randomized blocks design, with 21 treatments and five replicates (nests). A factorial arrangement was adopted, with factors sugar and concentration at five and four levels, respectively, plus control.

The analyses of variance was performed with the SAEG version 4.0 software package, by the ANOVA procedure. Data were analyzed for normality and homocedasticity, and the transformation  $(x + 0.5)^{1/2}$  was adopted. Comparisons of means were made by Tukey test ( $P \leq 0.05$ ).

## RESULTS AND DISCUSSION

There was effect of treatment in the first experiment (Table 1), sucrose showing the lowest carrying rate. Even though maltose and lactose showed the highest carrying rates, no differences from the control and the other treatments were observed ( $P > 0.05$ ). In the second and third experiments (Table 1), no effects were verified of treatments over carrying rates of substrate. In addition, no effects of concentration and interaction of the two factors were observed in the third experiment, even at the highest concentration (20.0%). Carrying rates were very low in all experiments, and did not indicate attractiveness of the studied substances to *A. capiguara* workers.

Carbohydrates constitute the main energy source for foraging workers, and are probably present in the sap that is ingested during plant cutting and processing and in the symbiotic fungus (Martin et al., 1969; Abbott, 1978; Littleddyke & Cherrett, 1976; Andrade, 1997; Forti et al., 2000). However, grasses have little sap in the leaves, and the source of energy necessary for survival of ants specialized in cutting this group of plants is unknown (Fowler et al., 1991). In addition, grass-cutting ants are particularly distinguished from dicotyledon-cutters, with regard to their cutting and plant substrate processing behavior. Plant fragments are not cut into tiny pieces to the point of forming a mass for later incorporation of the fungus; instead, they are utilized almost without being cut (Fowler et al., 1986).

Studying sucrose, glucose, fructose, xylose and rhamnose, Mudd et al. (1978) observed that *A. cephalotes* and *A. octospinosus* prefer sucrose and glucose. Considering that *A. cephalotes* and *A. octospinosus* preferably cut dicotyledon plants and that *A. capiguara* is a grass-cutting specialist, it can be hypothesized that quantitative differences in carbohydrate composition of these two groups of plants explain the different responses of ant species to sugars. However, even among dicotyledon-cutting ants, responses to sugar-impregnated substrates are variable. Tests with sucrose, fructose and glucose mixtures, conducted by Cherrett & Seaforth (1970), indicated that the substances had attractive effects to *A. cephalotes*, but not to *A. octospinosus*. Barrer & Cherrett (1972) demonstrated that sucrose solution spread over *Ligustrum*

*ovalifolium* leaf disks resulted in significant increases of substrate attractiveness to *A. cephalotes* workers. On the other hand, preference tests of *A. sexdens* to orange juice, revealed that the addition of sucrose did not improve the attractiveness of the solutions relatively to distilled water (Nagamoto, 1998).

A hypothesis could be formulated that the concentrations of studied substances interfered with the results. Cherrett & Seaforth (1970) utilized sucrose and glucose at 2.0% in water and the same solution with the addition of 1.0% fructose, which are concentrations close to the physiological levels found in grapefruit albedo. Barrer & Cherrett (1972) utilized 4.0% sucrose solutions and Mudd et al. (1978) tested 0.5 mol L<sup>-1</sup> sucrose solutions, which correspond to a solution of approximately 17% (w/v). Nagamoto (1998) added 10% sucrose solution in orange juice. When sucrose was studied at 5.0%, 7.5%, 10.0% and

Table 1 - Mean number (No.) and mean carrying rate of cellulose rectangles, impregnated with solutions of sugars (first experiment) and sugars and sweeteners (second experiment), in five and three nests of *Atta capiguara*, respectively, in the field.

Treatment	Carrying	
	No.*	Mean Rate %
Sugars**		
Sucrose	1.0 b	2.0
Fructose	2.2 ab	4.4
Soluble starch	2.4 ab	4.8
Raffinose	1.4 ab	2.8
Maltose	4.2 a	8.4
Lactose	4.8 a	9.6
Sorbose	3.4 ab	6.8
Cellobiose	1.2 ab	2.4
Arabinose	2.2 ab	4.4
Distilled water	1.4 ab	2.8
Sugars and sweeteners***		
Xylose	0.7	1.4
Glucose	1.3	2.6
Galactose	2.0	4.0
Rhamnose	2.0	4.0
Arabinose	3.0	6.0
Melezitose	1.3	2.6
Saccharine	1.3	2.6
Cyclamate	3.0	6.0
Distilled water	1.0	2.0

\*Original data; for statistical analysis they were transformed to  $(x + 0.5)^{1/2}$ .

\*\*CV = 27.22%  $F_{(9,36)} = 3.50$ ,  $p = 3.37 \times 10^{-3}$

\*\*\*CV = 24.93%  $F_{(8,16)} = 1.91$ ,  $p = 1.28 \times 10^{-1}$

Means followed by a common letter do not differ among themselves by Tukey test ( $P \leq 0.05$ )

20.0%, therefore at concentrations both lower and higher than those studied by Mudd et al. (1978) for *A. cephalotes* and *A. octospinosus* (17% w/v), and by Nagamoto (1998) for *A. sexdens* (10% w/v orange juice), no attractive effects were detected for *A. capiguara* workers.

Regarding the form treatments were offered, Cherrett & Seaforth (1970) and Mudd et al. (1978), as well as this work, offered sugar solutions on filter paper or cellulose, while Bass & Cherrett (1972) spread sucrose solution in foliar disks, and Nagamoto (1998) utilized cotton wads imbibed in the solutions. It does not seem likely that the way sugars were offered determined different responses, because sucrose produced positive stimuli in *A. cephalotes* workers either when offered on filter paper (Mudd et al., 1978) or when spread over a plant substrate (Barrer & Cherrett, 1972).

The nutritional importance of mono and disaccharides in ant nutrition, does not necessarily imply that sugars are responsible, alone, for substrates being preferred in tests by grass-cutting ants (Bacci Jr. et al., 1995; Silva et al., 1998 a, b). Little is known about factors which determine the selection of substrates by this group of leaf-cutting ants. Vitória (1996) studied the bromatological composition of several forage grasses and suggested that the nutritional components are not the basis for host specificity in *A. capiguara*. It is more likely that substances studied in this work do not release odors at levels that allow them to be perceived by scout ants. During recruiting, the leaf-cutting ant workers are conditioned to the odor of the substrate that is conducted by the scout ant, utilizing the learned odor as a decision criterion during substrate fragment collection (Roces, 1990, 1994). This hypothesis can also be applied to the sweeteners studied, since, like the sugars, they did not stimulate carrying of substrate by *A. capiguara* workers.

## CONCLUSION

The sugars and artificial sweeteners studied are not attractive to *Atta capiguara* workers, thus making their inclusion in toxic baits for improved attractiveness not viable.

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