

## Energy substrate used by workers of leaf-cutting ants during nest excavation

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**ABSTRACT.** Energy substrate used by workers of leaf-cutting ants during nest excavation. In this study we aimed to ascertain whether leaf-cutting ant workers lose body reserves (fat or sugars) as a function of nest excavation. For each treatment, we isolated 10 workers of *Atta sexdens* into two experimental groups, Control (C- without excavation) and Soil (S- with excavation), which were kept for different time intervals (0, 24, 48 or 72 hours), totaling 700 tested workers. We then determined the concentration of soluble carbohydrates and total lipid content in them. The total carbohydrates were determined colorimetrically, based on the reaction between carbohydrates and sulfuric acid-phenol. For determination of lipids, the insects were immersed in organic solvent until they reached a constant weight. Our results showed that carbohydrates are consumed during nest excavation activities. In the experimental groups S24, S48 and S72, there was an average reduction of 5.82 (20.42%), 14.31 (44.96%) and 13.27 (43.96%)  $\mu\text{.mg}^{-1}$  in soluble sugar when compared with the experimental groups that did not excavate. Furthermore, the lipids were not used during this activity. With respect to dry mass of the workers, their values were C0 = 8%, C24 = 10.4%, C48 = 9.2%, C72 = 10%, S24 = 9.2%, S48 = 8.7% and S72 = 8.5%. Our results show experimentally that the source of energy for nest excavation is carbohydrates, whereas lipids are conserved for other activities.

**KEYWORDS.** Attini; Formicidae; Insecta; lipids; nest.

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In animals, the storage of body reserves results from a positive balance of energy, which is used for daily activities (Willmer *et al.* 1988). In insects, the body reserves are mostly in the body fat (lipid reserves) or in the hemolymph, such as free carbohydrates. The sugars in the hemolymph are mainly in the form of trehalose, a disaccharide composed of two glucose molecules (Thompson 2003).

Social insects transport the food collected in the external environment to the nest, in order to share it with the other nestmates. For this reason, carbohydrate concentrations in the body vary not only with the dietary habits, but also with the social organization of the species. For example, in honeybees, a well-studied organism, the body concentration of carbohydrates depends on the composition of the sugars consumed, the metabolic rate of individuals, and the season (nectar availability of natural origin) (Blatt & Roces 2001). In ants, little is known about the levels of sugars in the hemolymph, as well as its relationship with the energy for behavioral activities.

In *Camponotus rufipes* (Fabricius, 1775), a species that feeds on nectar, the levels of sugars in the hemolymph and the behavioral status of individuals are correlated (Schilman & Roces 2008): immobile ants have higher levels of trehalose and fructose than active ants. This suggests that the concentration of sugars can act as a feedback mechanism, encouraging individuals with different nutritional statuses to forage, and thus promote a rotation in the execution of tasks (Thompson 2003).

In leaf cutting ants, the diet is composed of soluble carbohydrates from hyphae of the fungus garden, cultivated by the colony (Silva *et al.* 2003). Although the fungus garden constitute a high energy food source, some activities performed by ants are probably very energy consuming. Among them stands out the excavation of the nest, initiated by the queen (nest foundation), and later on carried out by the workers. So far, we know more about nest structure than about the effort of digging the nest, and how much energy is expended by those involved in building and maintaining nest structures.

In this study we endeavored to answer the following question: Which storage body reserves are used to fuel digging activity, and to what degree? In order to find out the answer we determined the content of body reserves of workers (total carbohydrates and lipids) before and after nest excavation.

### MATERIAL AND METHODS

**Nest excavation by workers.** Five laboratory colonies of *Atta sexdens* (Linnaeus, 1758) were used as source of workers, with head width varying between 1.2 to 1.6 mm. Workers within this size range are known to be responsible for the excavation of the nest (Camargo *et al.* 2012). The ambient temperature was maintained at approximately  $24 \pm 2^\circ\text{C}$ , with a relative humidity of  $70 \pm 20\%$ . The colonies were fed with *Ligustrum* spp. and *Acalypha* spp. throughout the experiment.

The experimental series, each consisting of 10 workers, are as follows: a) Control 0 (C0): The workers were collected from nests, killed and immediately frozen for determination of total carbohydrates and lipids; b) Control 24 hours (C24): As described above, but workers were collected and maintained isolated for 24 hours in a container; c) Control 48 hours (C48): As described above, but workers maintained isolated for 48 hours; d) Control 72 hours (C72): As described above, but workers maintained isolated for 72 hours; e) Soil 24 hours (S24): The workers were collected from the nests, placed at an environment with soil where they could dig for 24 hours. They were subsequently euthanized and frozen for determination of total carbohydrates and lipids; f) Soil 48 hours (S48): As described above, but workers allowed to dig for 48 hours; g) Soil 72 hours (S72): As described above, but workers allowed to dig for 72 hours. The experimental containers for excavation (10 cm deep by 20 cm in diameter) were filled with soil (Latosol), collected at 60 cm depth (bulk density = 1.6 g/cm<sup>3</sup>; water content: 5.0%) (Stein & Xavier 1984). We used a total of 700 workers for the determination of carbohydrates and lipids. These ants were not fed during experiment.

**Determination of carbohydrates.** The 10 workers from each experimental series were first homogenized in a mortar, and then each sample was suspended, macerated in deionized water and subjected to hot water extraction under reflux for 30 minutes (heat extraction reflux). The extract was filtered and frozen at -20°C until determination. Total carbohydrates were determined colorimetrically by the method of Dubois *et al.* (1956), based on the reaction between carbohydrates and sulfuric acid-phenol. In the presence of carbohydrates, the reaction becomes yellow-orange, being sensitive and stable. For this purpose, aliquots of 400 µL of aqueous extracts received 10 µL phenol 80% and 1000 µL of concentrated sulfuric acid. After cooling to room temperature, the absorbance was determined in Beckman DU640 spectrophotometer at 490 nm, the ideal wavelength for hexoses (Dubois *et al.* 1956). Pure water was used as the reference solution. The values were expressed in µg of total sugars per mg<sup>-1</sup> of worker's wet mass.

**Lipid determination.** We used an adaptation of the extraction method used by Cook *et al.* (2010), in which workers were immersed in organic solvent, until reaching a constant weight. The procedure was as follows: Fresh weight was individually determined; workers were dried for 24 hours at 50°C and their dry weight was determined; lipids were extracted with pentane for 24 hours and then dried and weighed on an analytical balance. All weights were determined close to 4<sup>-10</sup> g. The procedure was repeated for 72 hours of extraction. Values are expressed as a percentage (%) of the dry mass of the workers.

**Statistical Analysis.** The data obtained for carbohydrates were analyzed by ANOVA and subsequent Tukey's test to check for significant differences between the variables measured. The values of percentage of total lipids were submitted to Kruskal-Wallis test. Statistical analyses and graphs were processed by Sigmaplot 11.0.

## RESULTS

The analysis showed a drop in soluble carbohydrates consistent with the excavation activities. In the experimental groups S24, S48 and S72, there was an average reduction of 5.82 (20.42%), 14.31 (44.96%) and 13.27 (43.96%) µg.mg<sup>-1</sup> in soluble sugar when compared with the experimental groups that did not excavate. Statistically, a significant difference was detected by ANOVA ( $F_{6,34} = 6.513$ ,  $p < 0.001$ ), and Tukey's post-test indicated that the experimental groups S48 and S72 differ from the others (Fig. 1 and Table I).

The analysis of total lipids showed a constant percentage of total lipids in all experimental groups (Fig. 2 and Table I). The average values, expressed in relation to the dry mass of the workers, were as follows: C0 = 8%, C24 = 10.8%, C48 = 9.2%, C72 = 10%, S24 = 9.2%, S48 = 8.7% and S72 = 8.5%. The Kruskal-Wallis test did not detect significant differences between the experimental groups ( $H = 3.759$ ,  $df = 6$ ,  $p = 0.709$ ).

## DISCUSSION

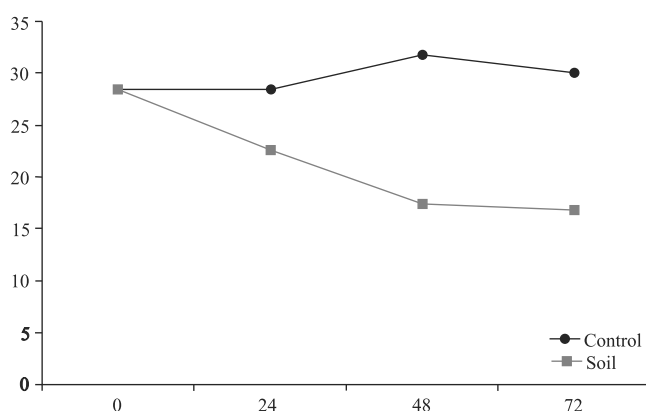
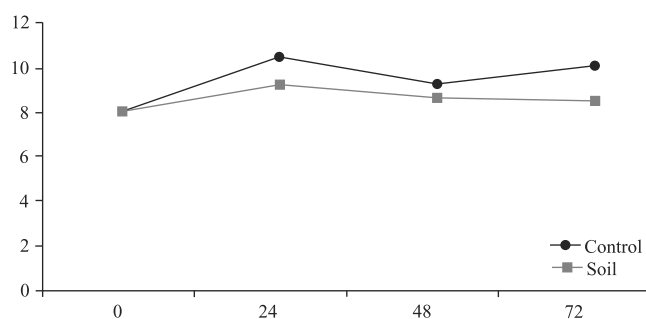
The results showed that workers lose body reserves during nest excavation, and that their soluble carbohydrates are consumed during this activity, whereas their total lipid content remains constant (Figs. 1 and 2). This pattern of use of body reserves had already been described for males of *Atta sexdens* during their nuptial flight (Jutsum & Quinlan 1978). The authors found that 21% of the dry weight of the males corresponds to carbohydrates, and that they are totally consumed after the nuptial flight. Similarly, a study on *Formica lugubris* Zetterstedt, 1838, demonstrated that carbohydrates (stored as glycogen) are the main source of energy for the mating flight (Passera & Keller 1990). However both studies found that the lipids are not used as energy substrates for flight.

The total lipid content of the dry mass of the workers ranged from 8 to 10.5% in the experimental groups, not differing statistically from one another. These percentages were similar to those found for males of *A. sexdens*, about 5.68% (Jutsum & Quinlan 1978) and *Lasius flavus* Fabricius, 7.73% (Peakin 1972). On the other hand, queens of *A. sexdens* have high lipid content in their bodies early in the founding of the colony, with about  $36.50 \pm 2.48\%$  of body fat at the time of the nuptial flight, decreasing to  $9.17 \pm 5.21\%$  after about 3 months (Camargo & Forti 2013), a value similar to that found for workers (Fig. 2).

It is known that the accumulation of body reserves occurs through lipid storage, representing an important role in the evolutionary history of the tribe Attini, developing from a semi-claustral to a claustral founding (Seal 2009). Claustral founding queens do not forage and remain cloistered in their nests, rearing their offspring by metabolizing body reserves (Brown & Bonhoeffer 2003), as in the case of *A. sexdens* (Autuori 1942). Possibly, the active selection pressure for the evolution of such foundations is the high mortality risk during foraging

Table I. Summary statistics for values of concentration of soluble carbohydrates ( $\mu\text{g}\cdot\text{mg}^{-1}$ ) and percentage of total lipids in workers of the leaf-cutting ant *Atta sexdens*.

Experimental groups	Soluble carbohydrates					Total lipids					
	Mean $\pm$ Std Dev	d.f	F	P	Tukey pos test ( $\alpha = 0.05$ )	Median	25%	75%	d.f	H	P
Soil 24 hours	22.6 $\pm$ 3.4	6;34	6.513	<0.001	A	6.8	4.1	12.5	6	3.759	0.709
Soil 48 hours	17.5 $\pm$ 5.8				B	6.6	4.0	12.0			
Soil 72 hours	16.9 $\pm$ 6.1				B	7.4	3.7	11.7			
Control	28.5 $\pm$ 4.2				A	8.2	3.3	12.0			
Control 24 hours	28.5 $\pm$ 4.9				A	8.7	4.0	14.3			
Control 48 hours	31.8 $\pm$ 8.1				A	8.3	4.3	12.8			
Control 72 hours	30.1 $\pm$ 3.1				A	8.1	3.9	13.3			

Fig. 1. Mean concentration of soluble carbohydrates ( $\mu\text{g}\cdot\text{mg}^{-1}$ ) in experimental groups of *Atta sexdens*. Control: 0, 24, 48 and 72 hours; Soil: 0, 24, 48 and 72 hours.Fig. 2. Percentage of total lipids in the dry mass of workers of *Atta sexdens*. Control: 0, 24, 48 and 72 hours; Soil: 0, 24, 48 and 72 hours.

(Hölldobler & Wilson 1990). The most viable adaptation to avoid this would be the ability to store large energy reserves in the body, as shown by Fujihara *et al.* (2012).

Furthermore, Camargo *et al.* (2011) evaluated the excavation effort by the queens of *A. sexdens*, reporting an impact on the queens' survival when they are experimentally subjected to a greater excavation effort. On the other hand, a single worker of *A. sexdens*, in a group of 10 workers isolated for 48 hours, carries approximately 0.56 g of soil in 33 trips (Camargo *et al.* 2012). This corresponds to a large energy cost for the workers, which is reflected in the consumption of their body reserves. This study demonstrates

experimentally that during the activity of digging nests the body uses carbohydrates as an energy resource, keeping constant the percentage of lipids for other activities.

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