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RESEARCH ARTICLE - ANTS

Do Atta sexdens rubropilosa workers prepare leaves and bait pellets in similar ways to their symbiotic fungus?

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Abstract

Leaf-cutting ants rely on the obligate symbiosis with a fungus, *Leucoagaricus gongylophorus*, on which they feed. This fungus garden is maintained preferentially with green leaves in combination with other tissues of plant origin. The nutritional composition of this symbiotic relationship is extremely beneficial for the colony, representing the main food source for larvae and an important food component of adult workers. The objective of this study was to observe the behavioral repertoire of *Atta sexdens rubropilosa* during the preparation and incorporation of two different substrates, leaves and bait pellets, as well as the participation of each caste in these processes this process. The first substrate offered to the colonies were *Citrus* sp. leaves cut into disks and the second were pellets of citrus pulp bait formulated without the active ingredient. The observations were made under a stereomicroscope and were started when the first portion of the substrate offered was carried to the fungus garden. The behaviors executed during preparation and incorporation of the leaf disks, and subsequently of the bait pellets, were recorded using three colonies, with six repetitions of 5 and 7 hours per colony, respectively. Eight behavioral acts were recorded for the three castes found, irrespective of the substrate processed by the workers. Licking was the most frequently executed behavioral act, which occurred throughout the observation period, regardless of the substrate offered. Gardeners and generalists were the castes most engaged in the execution of this behavior. Considering the large number of individuals involved in the execution of this task, it can be concluded that this behavior is important for dispersion of the active ingredient and contamination of workers inside the colony.

Introduction

The genus *Atta* is characterized by colonies that contain a large number of workers and shows one of the broadest polymorphism among leaf-cutting ants (Fowler et al., 1986). Among ants of this genus, the lemon ant *Atta sexdens rubropilosa* Forel, 1908, is widely distributed in several Brazilian states (Mariconi, 1970; Della Lucia, 2011) and attacks different crops of agricultural importance, causing significant losses particularly in forest crops (Amante, 1967).

Species of the genus *Atta* forage on a variety of plants which serve as a substrate for culture of the symbiotic fungus. The latter is the primary food source for larvae and is also part of the diet of adults, which is complemented by the ingestion of exudates during the activity of leaf-cutting and manipulation (Littledyke & Cherret, 1976; Hubbell & Wiemer, 1983; Forti & Andrade, 1999; Mueller, 2002; Silva et al., 2003; Schneider, 2003). According to Quinlan and Cherret (1979), the ingestion of exudates accounts for about 95% of the dietary requirements of workers. However, Silva et al. (2003) suggested the fungus to play a much more important role in the worker diet since they observed a decrease in the survival of *A. s. rubropilosa* ants in the absence of the fungus when workers were maintained only on fresh leaves or fungal mycelium. When supplied with pieces of the fungus garden, the ants survived for a longer period of time. The same was

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reported by Benevides (2004) for *Acromyrmex subterraneus* workers. Nevertheless, the fungus is fundamental for feeding of the colony, regardless of whether it is consumed directly or through its exudates (Moreira et al., 2011).

The function of the symbiotic fungus includes the degradation of secondary plant compounds and their transformation into palatable substances. There is evidence that the fungus is able to degrade cellulose (Martin et al., 1969; Bacci et al., 1995; Nagamoto et al., 2011). However, Abril and Bucher (2002) suggested that the fungus *Leucoagaricus gongylophorus* Müller (Singer), whose basic energy reserve is glycogen, which are present in a form that can be readily assimilated by ants (Quinlan & Cherrett, 1979), is unable to degrade cellulose.

The processing and incorporation of plant material are a complex activity and involve a series of different tasks which are performed according to the body size of workers. As a consequence, a high degree of polymorphism may be observed (Wilson, 1980a, 1983). Substrate processing and incorporation can be divided into four different phases. The first consists of the collection of plant material, the second and third of the physical and chemical treatment of the foraged fragments, respectively, and the fourth of incorporation of the substrate itself (Diniz & Bueno, 2009).

After foraging, generalist workers with a head width of 1.3 to 1.6 mm lick and cut the leaves into fragments measuring 1 to 2 mm in diameter. Next, minima workers (ants with a head width of 0.8 to 1.2 mm) chew the leaves along their borders until they are reduced to a moist pulp and incorporate these fragments into the fungus garden (Wilson, 1980a; Fowler, 1983; Andrade et al., 2002; Lopes et al., 2004; Camargo, 2007; Diniz & Bueno, 2009, 2010). Finally, hyphal tufts are inoculated onto the surface of the fragments (Weber, 1956; Lopes et al., 2004).

It is important to note that these behaviors are specialized for culture of the symbiotic fungus and that, somehow, a large number of workers are involved in this process. This high demand may play an important role in the contamination of workers with insecticides, thus demonstrating that plant processing can be a major route of colony contamination. However, the role of oral trophallaxis among adults of leaf-cutting ants in the contamination of workers still remains debatable (Andrade et al., 2002). Therefore, the objective of the present study was to describe and to analyze the behavioral repertoire executed by *A. s. rubropilosa* workers during the preparation and incorporation of substrates into the fungus garden by comparing the processing of leaves and bait pellets

Material and Methods

The colonies used were collected in the municipality of Botucatu, State of São Paulo, Brazil, and kept in the Laboratory of Social Pest Insects (Laboratório de Insetos Sociais-Praga - LISP), School of Agricultural Sciences, UNESP, Botucatu.

For observation, three colonies containing approximately 500 ml of the fungus were transferred to glass boxes (length: 20 cm, width: 10 cm, height: 2.5 cm). The boxes contained a layer of plaster for the maintenance of humidity and at their opposite ends connections to containers destined for foraging and disposal of exhausted waste (Lopes, 2004). The ambient temperature was maintained at approximately 22 ± 2°C and humidity at about 70± 20%.

Prior to the observations, a sample of individuals of variable sizes were removed for the measurement of greatest head width under a stereomicroscope equipped with an ocular micrometer. The sample was classified and the castes were defined as proposed by Wilson (1980a). Since no soldiers were detected, the observations were made based on the behavior of gardening workers (head with of 0.8 to 1.2 mm), generalists (head width of 1.3 to 1.6 mm), and foraging workers (head width of 1.7 to 2.2 mm).

The substrates offered to the colonies consisted of *Citrus* sp. leaves and bait pellets made of citrus pulp and 4% soybean oil and formulated without the active ingredient. The surface of the two substrates was standardized to 63.58 and 62.86 mm², respectively. Standardization of the substrate surface is important for the quantification of behavioral acts, avoiding duplication of the results. The observations were started when the first of the 15 leaf disks or pellets offered was carried to the fungus garden.

The behavioral acts executed during preparation and incorporation of the leaf disks were recorded using three colonies with six repetitions of 5 hours each, for a total of 90 hours of observation. In view of the physical nature of the bait pellets, six repetitions of 7 hours each were performed per colony to record the behavioral acts executed during bait preparation and incorporation, for a total of 105 hours of observation. The ants were observed *ad libitum*, i.e., there was no time interval between recordings.

The main behaviors executed by *A. s. rubropilosa* workers during preparation and incorporation of the different substrates into the fungus garden were recorded and the percentage of individuals performing the different behavioral acts was calculated according to size class of the workers. The results were compared by analysis of residuals standardized in contingency tables (Z) (Pereira, 2010). The Wilcoxon-Mann-Whitney test was used to compare the behaviors between bait versus leaves (p=0.05).

Results

Processing and incorporation of leaf disks

At the time when the leaf disks were carried by foraging workers into the fungal chamber, other workers of different size classes touched the leaf surface and borders with their antennae and mouthparts. These ants then started to lick the leaves continuously, while other workers transported and held the disks on the surface of the fungus culture. The disks were reduced to increasingly smaller fragments. These fragments, in turn, had their borders pleated and were then chewed by the workers, which occasionally deposited fecal fluid on the fragments to induce their softening and moistening. After this sequence of behaviors, the substrate was finally incorporated into the outer region of the fungal sponge and inoculated with a small quantity of fungus.

The most commonly observed behavior was licking the surface and borders of the leaf disk, which accounted for 38.88% of all behavioral acts executed (Fig 1). Basically, all castes contributed to the execution of this activity, although at different proportions (Table 1). Foraging workers performed 13.33% of this activity ($Z_{LD}1=5.311994$;$Z_{LD}2= -0.14412$; $Z_{LD}3 = 7.43419$), generalists 25.05% ($Z_{LD}1=28.65059$;$Z_{LD}2=19.27417$; $Z_{LD}3 =21.34883$), and gardeners 61.62% ($Z_{LD}1=78.58434$;$Z_{LD}2=84.38611$; $Z_{LD}3 =83.21173$).

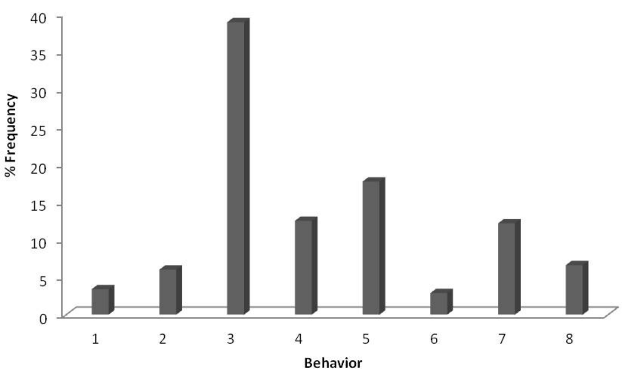

Fig 1. Percentage of each behavioral act performed by *Atta sexdens rubropilosa* workers during preparation of the leaf disk substrate and its incorporation into the fungus garden. 1: Transporting the disk to the surface of the fungus garden; 2: holding the disk on the surface of the fungus culture; 3: licking the surface and borders of the leaf disk; 4: cutting the leaf disk into smaller fragments; 5: pleating the borders; 6: deposition of fecal fluid; 7: incorporation into the fungus garden; 8: deposition of hyphae on the incorporated fragments.

The second most observed behavior was pleating the borders of the leaf disk, which accounted for 17.7% of the acts executed by workers (Fig. 1 and Table 1), with the observation of excess of significant events in the groups of gardeners and generalists. Gardening workers were effectively responsible for 54.94% of this activity ($Z_{LD}1= 22.68025$;$Z_{LD}2= 25.2817$;$Z_{LD}3= 25.56538$), followed by generalists responsible for 28.57% ($Z_{LD}1=0.185945$;$Z_{LD}2=5.377955$;$Z_{LD}3=5.506622$) and foragers responsible for 14.49% ($Z_{LD}1= -9.282169$; $Z_{LD}2= -4.69528$; $Z_{LD}3 = - 5.93832$).

The behaviors of cutting the disks into smaller fragments and incorporating them into the fungus garden corresponded to 12.47% and 12.14% of the acts executed, respectively, and were performed mainly by gardening workers (Table 1). Holding the disks and depositing hyphae on the incorporated fragments corresponded to 6.58% and 5.98% of all behavioral acts executed by workers, respectively. The first activity was performed equally by generalists and foragers (33.98%) and the second by gardening workers (91.58%).

The second least frequent behavior was transporting the disk to the surface of the fungus garden, accounting for 3.39% of all acts observed. However, the percentage of this behavior compared to the previously cited acts was low, corresponding to only 2.87% of all tasks.

Processing and incorporation of bait pellets

The pellets were processed in a similar manner as the *Citrus* sp. disks, with each size category contributing to at different proportions to the execution of these acts. However, the time spent on the complete incorporation of the bait into the fungus garden was longer than the time spent by workers to perform the whole process using leaves as substrate.

Once the bait pellets had been offered, the workers were immediately attracted and started touching the pellets with their antennae and palps. The pellets were then carried into the fungal chamber where workers started to lick them continuously for hydration. While some workers licked the pellets, others carried and held them on the surface of the fungus. Once the pellets were well hydrated, they were fractionated into very small portions and incorporated into the fungus garden.

As observed in the previous experiment, the most common behavior was licking the surface of the bait pellet, accounting for 38.68% of all behavioral acts executed (Fig. 2). Gardening workers were the main size class executing this behavior, which were responsible for 51.73% of this activity ($Z_B1= 97.2501$; $Z_B2= 72.1057$; $Z_B3= 51.4991$), followed by generalists performing 24.54% of this activity ($Z_B1=36.6324$; $Z_B2= 14.7516$; $Z_B3=22.4027$) and foraging workers performing 23.73% ($Z_B1=23.8329$; $Z_B2= 18.6163$; $Z_B3= 4.95512$) (Table 2).

Observed as the second most frequent behavior, pressing the pellet fragment corresponded to 14.37% of all acts executed by workers. Gardening workers were

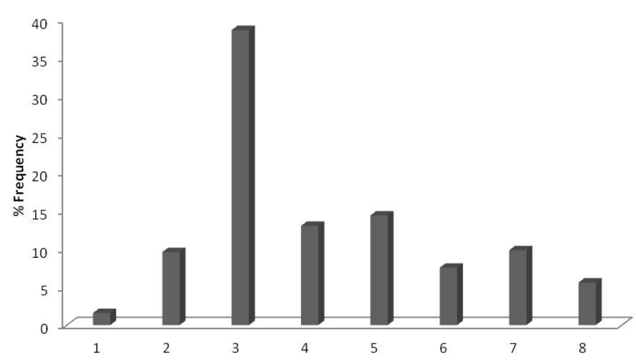

Fig 2. Percentage of each behavioral act performed by *Atta sexdens rubropilosa* during preparation of the bait substrate and its incorporation into the fungus garden. 1: Transporting the pellet to the surface of the fungus garden; 2: holding the pellet; 3: licking the surface; 4: pellet fragmentation; 5: pressing the pellet fragment; 6: deposition of fecal fluid; 7: incorporation into the fungus garden; 8: deposition of hyphae on the incorporated fragments.

Table 1.Standardized residuals in contingency table analysis of the behaviors executed by *Atta sexdens rubropilosa* during the preparation and incorporation of leaf disksas substrate.

Behavior	Head width (mm)	Observed frequency	% Behavior	Leaf disk substrate (Z_{LD})		
				$Z_{LD}1$	$Z_{LD}2$	$Z_{LD}3$
Transporting the disk to the surface of the fungus garden	0.8 - 1.2	43	6.17	*-16.94109*	*-15.8001*	*-16.4797*
	1.3 - 1.6	289	41.46	*-12.77994*	*-10.096*	*-11.4801*
	1.7 - 2.2	365	52.37	*-8.679104*	*-10.5815*	*-10.5163*
Total		697	100.00			
Holding the disk	0.8 - 1.2	465	32.05	*-5.121023*	*-10.3994*	*-8.2273*
	1.3 - 1.6	493	33.98	*-8.618798*	*-7.30461*	*-6.11903*
	1.7 - 2.2	274	33.98	*-11.21198*	*-14.5258*	*-9.55251*
Total		1,232	100.00			
Licking the surface and borders	0.8 - 1.2	4,933	61.62	**78.58434**	**84.38611**	**83.21173**
	1.3 - 1.6	2,005	25.05	**28.65059**	**19.27417**	**21.34883**
	1.7 - 2.2	1,067	13.33	**5.311994**	*-0.14412*	**7.43419**
Total		8,005	100.00			
Cutting leaf pieces into smaller fragments	0.8 - 1.2	980	38.16	**4.648623**	0.766112	1.952667
	1.3 - 1.6	903	35.16	**4.829542**	*-0.44753*	*-1.66152*
	1.7 - 2.2	685	26.67	*-6.447765*	*-1.53981*	*-2.44460*
Total		2,568	100.00			
Pleating the borders	0.8 - 1.2	2,075	56.94	**22.68025**	**25.2817**	**25.56538**
	1.3 - 1.6	1,041	28.57	0.185945	**5.377955**	**5.506622**
	1.7 - 2.2	528	14.49	*-9.282169*	*-4.69528*	*-5.93832*
Total		3,644	100.00			
Deposition of fecal fluid	0.8 - 1.2	512	86.78	*-3.975201*	*-8.39689*	*-8.52849*
	1.3 - 1.6	64	10.85	*-15.91588*	*-15.8001*	*-16.2388*
	1.7 - 2.2	14	2.37	*-16.94109*	*-16.8924*	*-17.1423*
Total		590	100.00			
Incorporation into the fungus garden	0.8 - 1.2	1,926	77.07	**24.36883**	**20.30576**	**19.84291**
	1.3 - 1.6	469	18.77	*-7.412669*	*-7.48666*	*-8.58872*
	1.7 - 2.2	104	4.16	*-14.89067*	*-14.5865*	*-16.0581*
Total		2,499	100.00			
Depositionof hyphae on the incorporated fragments	0.8 - 1.2	1,240	91.58	**1.693606**	**14.29823**	**7.133007**
	1.3 - 1.6	93	6.87	*-15.91588*	*-14.101*	*-16.1785*
	1.7 - 2.2	21	1.55	*-16.82048*	*-16.8924*	*-16.8411*
Total		1,354	100.00			
Overall total	0.8 - 1.2	12,174	59.13			
	1.3 - 1.6	5,357	26.02			
	1.7 - 2.2	3,058	14.85			
Total		20,589	100.00			

Z: Residuals standardized in contingency tables.

(*) Z >1.96 excess of significant events (alpha = 0.05); Z < -1.96 scarcity of significant events (alpha = 0.05).

Table 2.Standardized residuals in contingency table analysis of the behaviors executed by *Atta sexdens rubropilosa* during the preparation and incorporation of bait pellets as substrate.

Behavior	Head width (mm)	Observed frequency	% Behavior	Bait substrate (Z_B)		
				Z_B1	Z_B2	Z_B3
Transporting the pellet to the surface of the fungus garden	0.8 - 1.2	4	1.07	*-18.721*	*-18.518*	*-20.155*
	1.3 - 1.6	69	18.4	*-18.001*	*-16.614*	*-19.223*
	1.7 - 2.2	302	80.53	*-13.347*	*-12.973*	*-14.874*
Total		375	100			
Holding the pellet	0.8 - 1.2	986	40.44	**8.09667**	*-1.3232*	*-10.266*
	1.3 - 1.6	723	29.66	**2.88821**	*-12.077*	*-9.0754*
	1.7 - 2.2	729	29.9	*-2.5419*	*-12.301*	*-3.4839*
Total		2,438	100			
Licking the surface	0.8 - 1.2	5,099	51.73	**97.2501**	**72.1057**	**51.4991**
	1.3 - 1.6	2,419	24.54	**36.6324**	**14.7516**	**22.4027**
	1.7 - 2.2	2,339	23.73	**23.8329**	**18.6163**	**4.95512**
Total		9,857	100			
Pellet fragmentation	0.8 - 1.2	1,504	45.37	**8.2629**	**11.559**	**4.48916**
	1.3 - 1.6	873	26.34	*-5.257*	*-3.6756*	*-1.4647*
	1.7 - 2.2	938	28.3	*-10.909*	*-1.2672*	**4.95512**
Total		3,315	100.01			
Pressing the pellet fragment	0.8 - 1.2	2,087	56.98	**8.53995**	**19.9605**	**26.648**
	1.3 - 1.6	959	26.18	*-7.0855*	*-3.3956*	**4.43739**
	1.7 - 2.2	617	16.84	*-13.845*	*-7.7084*	*-2.9662*
Total		3,663	100			
Deposition of fecal fluid	0.8 - 1.2	1,596	83.3	**9.4265**	**14.6956**	**5.26576**
	1.3 - 1.6	179	9.34	*-15.674*	*-14.934*	*-17.256*
	1.7 - 2.2	141	7.36	*-17.281*	*-16.278*	*-16.479*
Total		1,916	100			
Incorporation into the fungus garden	0.8 - 1.2	1,871	74.87	**4.99376**	**15.5917**	**22.8168**
	1.3 - 1.6	423	16.93	-13.5129	-11.181	-10.1109
	1.7 - 2.2	205	8.2	-17.5024	-12.5812	-16.3754
Total		2,499	100			
Deposition of hyphae on the incorporated fragments	0.8 - 1.2	1,372	96.62	*-8.6369*	**13.7434**	**11.4268**
	1.3 - 1.6	41	2.89	*-18.777*	*-17.678*	*-18.964*
	1.7 - 2.2	7	0.49	*-18.832*	*-18.518*	*-19.896*
Total		1,420	100			
Overall total	0.8 - 1.2	14,519	56.98			
	1.3 - 1.6	5,686	22.31			
	1.7 - 2.2	5,278	20.71			
Total		25,483	100.00			

Z: Residuals standardized in contingency tables.

(*) Z > 1.96 excess of significant events (alpha = 0.05); Z < -1.96 scarcity of significant events (alpha = 0.05).

effectively responsible for 56.98% of this activity (Z_B1= 8.53995; Z_B2= 19.9605; Z_B3= 26.648), generalists for26.18% (Z_B1= -7.0855; Z_B2= -3.3956; Z_B3= 4.43739), and foraging workers for 16.84% (Z_B1= -13.845; Z_B2= -7.7084; Z_B3= -2.9662).

The behaviors of fragmenting the pellet, incorporating the pellet into the fungus garden and holding the pellets were recorded at the following frequencies, respectively: 13.01%, 9.81% and 9.57% (Fig. 2).The significant participation of gardening workers in the activities of pellet fragmentation, responsible for 45.37% of this activity (Z_B1 = 8.2629; Z_B2 = 11.559; Z_B3 = 4.48916) and pellet incorporation into the fungus garden, responsible for 74.87% (Z_B1 = 4.99376; Z_B2 = 15.5917; Z_B3 = 22.8168), should be highlighted. Holding the pellets on the fungus garden was a behavior shared by the different size classes, with gardening workers performing 40.44% of this activity (Z_B1= 8.09667; Z_B2= -1.3232; Z_B3= -10.266), generalists 29.66% (Z_B1= 2.88821; Z_B2= -12.077; Z_B3= -3.4839),and foraging workers29.9% (Z_B1= -2.5419; Z_B2= -12.301; Z_B3= -3.4839).

Deposition of fecal fluid and deposition of hyphae on the incorporated fragments accounted for 7.52% and 5.57% of all behavioral acts recorded. Gardening workers were the main size class executing these two acts, being responsible for 83.3% (Z_B1=9.4265; Z_B2= 14.6956; Z_B3= 26.648)and 96.62% (Z_B1= -8.6369; Z_B2= 13.7434; Z_B3= 11.4268) of these activities, respectively.

The least common behavioral act was transporting the pellet to the surface of the fungus garden, which accounted for only 1.57% of all acts observed. Foraging workers were the main size class responsible for the execution of this activity (80.53%) (Z_B1= -13.347; Z_B2= -12.973; Z_B3= -14.874).

Comparison of behaviors between bait versus leaves

In general, the workers performed more behavioral acts during processing of the bait pellets compared to leaf processing (Fig. 3). The frequency of the behavior of leaf disk/pellet transportation was higher for leaves (39.22±7.38) compared to bait (20.78±5.50), with the difference being statistically significant (U=9.00; p-value (bilateral)< 0.0001).

On the other hand, the behavior of holding the leaf disk/pellet was significantly more frequent for bait (133.50±69.32) than leaves (67.94±22.83) (U =59.50; p-value (bilateral) =0.0012). The frequency of the licking behavior was higher for bait (546.22±87.76) than leaves (444.72±145.76), with the difference being significant (U =91.50; p-value (bilateral) =0.0257). The same was observed for cutting the leaf pieces into smaller fragments/ pellet fragmentation, with a significantly higher frequency for bait (184.22±60.58) compared to leaves (142.67±36.77) (U =94.00; p-value (bilateral) =0.0314). In contrast, the frequency of pleating the leaf borders/pressing the pellet fragments was similar for leaves (202.44±66.00) and bait (194.61±102.01) (U =157.50; p-value (bilateral)=0.8868).

The behavior of fecal fluid deposition was more frequent for bait (106.44±30.53) than leaves (32.78±12.53), with the difference being significant (U =0.00; p-value (bilateral)< 0.0001). In contrast, the frequency of the behavior of incorporation into the fungus garden did not differ significantly between bait (138.83±57.85)and leaves (138.83±54.79) (U =160.00; p-value (bilateral)=0.9495). There was also no significant difference in the frequency of hyphal deposition (bait: 78.67±41.39 and leaves: 75.22±29.27) (U =148.50; p-value (bilateral)=0.9495).

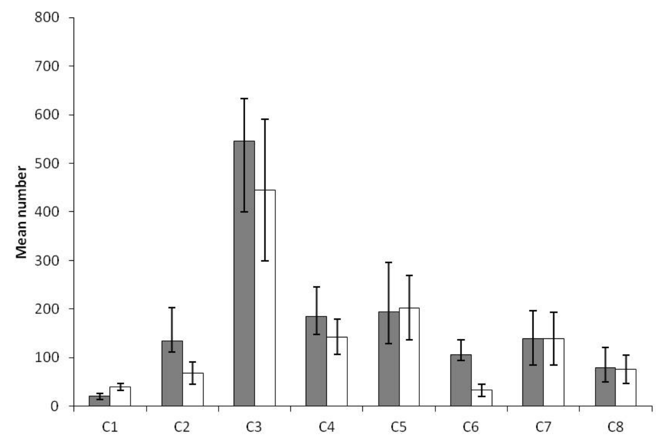

Fig 3. Mean number and standard deviation of the frequency of behaviors executed by *Atta sexdens rubropilosa* workers during culture of the symbiotic fungus on bait pellets (gray bars) and leaf disks (white bars). C1: Transporting the disk/pellet to the surface of the fungus garden; C2: holding the disk/pellet; C3: licking the surface and borders of the disk/pellet; C4: cutting the leaf disk into smaller fragments/pellet fragmentation; C5: pleating the borders/ pressing the pellet fragment; C6: deposition of fecal fluid; C7: incorporation into the fungus garden; C8: deposition of hyphae on the incorporated fragments.

Discussion

Processing and incorporation of leaf disks

The present results show that the act of substrate licking wasin general the most frequently executed behavior, regardless of size class. By licking the surface of the substrate, workers remove deleterious impurities and thus guarantee the integrity of the fungus garden (Stahel, 1943; Andrade et al., 2002; Andrade, 2002; Mueller, 2002; Diniz & Bueno, 2009, 2010). Furthermore, licking the leaves permits removal of the existing layer of wax, thus facilitating the growth of hyphae of the symbiotic fungus and increasing their inoculation potential. Similar observations have been made for *Acromyrmex octospinosus* (Quinlan & Cherret, 1977).

The activity of gardening workers and generalists is unquestionably essential during execution of this behaviorand the demand of individuals of these size classes for the execution of this behavior is high. Studying the behaviors of *A. s. rubropilosa* during substrate preparation, Andrade et al. (2002) reported that gardening workers were the main caste involved in the behavior of leaf licking, which were responsible for 52% of this task, followed by generalists (38%). In a similar study, Diniz and Bueno (2009) observed that in the grass-cutting ant species *Atta bisphaerica*, gardening workers and generalists were responsible for 49% and 48% of this task, respectively. In the same study, but using *Atta laevigata*, the same authors offered leaves of monocot and dicot plants and observed that gardening workers were the main caste involved in this task, irrespective of the substrate offered.

In addition to being the most frequent, the act of licking also required most of the time of the workers involved, as demonstrated by the fact that they started to execute the task as soon as the disks were transported to the fungus chamber. Similar results have been reported by Andrade et al. (2002). The smaller size classes were found to execute this behavior throughout the observation period. The smaller castes, gardening workers and generalists, are the main castes responsible for preparation of the substrate and represent the majority of ants in a colony (Weber, 1972). According to this author, gardening workers correspond to 60-67% of ants present in the fungus gardens, generalists to 30-33%, foraging workers to 1-2%, and soldiers (limited to the genus *Atta*) to less than 1% of a colony. Hence, the symbiotic balance is disturbed when workers directly involved in substrate processing are affected to an extent that compromises the care and maintenance of the main energy source of the colony.

It is known that workers ingest exudates during substrate processing (Peregrine & Mudd, 1975; Littledike & Cherret, 1976; Forti & Andrade, 1999). Workers are therefore able to simultaneously feed and eliminate microorganisms that are harmful to them and to the symbiotic fungus. The mechanism of substance exchange in leaf-cutting ants is widely debated. In addition to permitting food exchange, trophallaxis is an important and complex mechanism of communication among nest mates (Hölldobler & Wilson, 1990). Abdominal or proctodeal trophallaxis is the consumption of anal excretion by the larvae (Jaffé, 1993; Schneider, 2003; Lopes et al., 2005) and oral trophallaxis, which occurs among adult workers, consists of the transfer of liquid food stored in the crop to nestmates. However, some authors argue that oral trophallaxis is rare or even absent in this group (Andrade et al., 2002; Paul & Roces, 2003), although subsequent studies have shown oral trophallaxis in leaf-cutting ants (Moreira et al., 2006, 2010; Da Silva et al., 2009; Richard & Errard, 2009; Moreira et al., 2014). One may therefore speculate that the transfer of substances between castes in the colony occurs during mutual cleaning and by oral trophallaxis.

Processing and incorporation of bait pellets

During processing of the pellets, as observed in the previous experiment, licking the substrate was the most frequently executed task throughout the observation period. As observed for the leaf substrate, gardening workers and generalists more effectively participated in this behavior, although a larger number of ants exerted this function during pellet processing (Table 2). In agreement with the present study, Andrade (2002) also found that the size classes of *Acromyrmex* workers involved in the process were the smallest of the colony.

The larger number of workers performing this task may be attributed to the fact that ants are most attracted to the citrus pulp present in the pellet formulation (Carlos et al., 2009). As a consequence, a large number of workers are attracted and come in direct contact with the active ingredient. These ants then become contaminated and die and no longer exert the functions attributed to them within the colony, causing an imbalance in the symbiotic relationship.

Baits are one of the most important control methods of leaf-cutting ants because of their efficiency. The bait formulated as pellets consists of a mixture of dehydrated citrus pulp meal (vehicle) and active ingredient dissolved in soybean oil (Robinson, 1979; Forti et al., 1998). The citrus pulp used as vehicle and attractant in toxic baits is appropriate for growth of the fungus cultured by leaf-cutting ants since it is slightly acid, has a high carbohydrate content and contains nitrogen, vitamins and microelements that provide good conditions for development of the symbiotic fungus (Boaretto & Forti, 1997). Other factors such as contamination and humidity also play an important role in this control method, but attractiveness is the key factor for the efficiency of bait pellets, guaranteeing that the baits are carried by workers to the nest (Etheridge & Phillips, 1976, Forti et al., 1998, Nagamoto et al., 2004; Verza et al., 2006).

Comparison of behaviors between bait versus leaves

Comparison of the behavioral acts executed during preparation of the two different substrates offered showed that physical factors influenced both the time spent on this process and the number of workers involved. Physical factors of the substrate are known to influence the selection of plants and post-selection for incorporation of forage material into the fungus garden by leaf-cutting ants (Camargo et al., 2004). For example, when the acceptance of new and old leaves by leaf-cutting ants are compared, a higher frequency of workers showing a preference for young leaves is noted, probably as a result of physical and chemical factors (Cherrett & Seaforth, 1970; Barrer & Cherrett, 1972; Cherrett, 1972; Rockwood, 1976; Littledyke & Cherrett, 1978; Waller, 1982).

The procedures involved in the preparation of the substrate for culture of the symbiotic fungus imply the manipulation of this substrate by workers (licking, cutting, ingesting exudates) and ants are able to perceive the composition of the materialduring the execution of these behavioral tasks (Camargo et al., 2004). Bait pellets are obviously more resistant than leaf disks and their processing by workers is therefore more time consuming. This is likely due to the fact that the bait needs to be hydrated for incorporation into the fungus garden considering the absorption of water from the medium (fungal chamber). In the case of pellets, in addition to asepsis, hydration can be performed during licking and can be complemented subsequently by the deposition of fecal fluid.

Even considering the attempt to standardize the area of the substrates offered for foraging (see Material and methods), an increase in the size of the pellets was visually observed after hydration, a fact that may explain the large number of ants involved in their processing. However, further investigation is necessary to test this hypothesis. On the basis of the behavioral repertoire obtained, it can be concluded that the behavioral acts executed during processing of the plant material and bait pellets were similar (e.g., licking and reducing the substrates tested to minute fractions) despite differences in worker demand and frequency.

The present study described and analyzed the behavioral repertoire executed by *A. s. rubropilosa* workers during culture of the symbiotic fungus with leaves and bait pellets. The results showed that baits are more laboriously processed than leaves and can therefore act as a contaminating agent of workers in the colony during processing, with the consequent contamination of a large number of gardening workers and generalists by the active ingredient.

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