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Influence of Food Resource Size on the Foraging Behavior of *Nasutitermes corniger* (Motschulsky)

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Abstract

In general, termite foraging can be affected by physical and chemical factors linked to food. This study investigated if the wood length of *Eucalyptus grandis* W. Hill ex Maiden, as a food resource, influences the behavior of foraging events of *Nasutitermes corniger* (Motschulsky). Nests with mature and active colonies were collected in the field and transferred to glass cubes connected to a test arena under laboratory conditions. Wooden blocks of *E. grandis*, with a 2.5 x 2.0 cm rectangular cross section, were offered to termites in three different lengths: 5, 10 and 15 cm. Each test was repeated with 20 nests and lasted 60 minutes, when the following behavioral events and their duration were observed: initial exploration, initial recruitment and mass recruitment. At the end of each test, the quantities of termites (total, workers and soldiers) and gnawing workers were determined. The results show that longer blocks favored a higher occurrence of exploration and initial recruitment. However, the highest mass recruitment occurred with the 10 cm blocks. The length of the wood influenced the total number of termites recruited and gnawing workers; both were highest for the 10 cm blocks. There was no significant difference in relation to exploration time of the blocks and number of workers and soldiers recruited. Therefore, we conclude that wood length is a factor that can affect *N. corniger* foraging.

Introduction

Termite foraging is a group activity composed of individual actions integrated with patterns organized towards a new food source, which involves communicating the location of the source to other members of the colony using chemical and tactile signals that stimulate the foragers to leave the nest and guide them to the discovered food (Traniello & Busher, 1985; Traniello & Leuthold, 2000; Costa-Leonardo, 2002; Andara et al., 2004). This activity comprises recruitment and aggregation events of individuals that can change over time and, therefore, is a dynamic process governed by the nutritional requirements of the colony and quantity and/or quality of available food resources,

as observed in some species of the genus *Nasutitermes* Dudley (Traniello & Busher, 1985; Traniello & Leuthold, 2000; Andara et al., 2004; Gazal et al., 2014a, b).

The arboreal termite *Nasutitermes corniger* (Motschulsky) is widely distributed in Meso-America, from southern Mexico to Panama, and South America (Atkinson & Adams, 1997; Torales, 2002; Constantino, 2002; Scheffrahn et al., 2005). In the last decades, *N. corniger* has become of great economic importance due to increasing reports of damage to residences in various Brazilian and Argentinian cities, which have confirmed its status as a pest (Mill, 1991; Menezes et al., 2000; Constantino, 2002; Costa-Leonardo, 2002; Fontes & Milano, 2002; Torales, 2002; Albuquerque et al., 2012).

As urban pests, *Nasutitermes* termites have been controlled using chemical insecticides on the nests or by removing the nests (United Nations Environment Programme [UNEP], 2000; Gerozisis et al., 2008), which are often high up in trees and difficult to access or sometimes impossible to locate and only the tunnels on walls of infested buildings are visible (Menezes et al., 2000; Fontes & Millano, 2002).

New technologies for termite control, which are more environmentally friendly, include a toxic bait system that is efficient for subterranean termites, especially against *Coptotermes formosanus* Shiraki and *Reticulitermes flavipes* (Kollar) (Rhinotermitidae); however, the effectiveness of this system is still unknown for arboreal termites (Su et al., 1995; United Nations Environment Programme [UNEP], 2000; Lee, 2002; Su, 2002; Potter, 2004; Dow AgroSciences, 2013). In this system, particularly in the Sentricon™ Colony Elimination System, two pieces of untreated wood of equal size (approximately 2 cm x 20 cm) are placed in a tube-shaped artefact in the soil to monitor the insects. When the insects are detected on the wood, the pieces are substituted with bait impregnated with insecticide that inhibits insect growth, such as hexaflumuron (Potter, 2004; Ogg et al., 2006). Su et al. (1995) described a prototype of a monitoring station that uses wooden pieces of *Picea* sp. (Pinaceae) that are approximately 2.5 x 4.0 x 28 cm and baited with hexaflumuron, which was found to be efficient at controlling subterranean termite colonies near buildings in Florida. However, the lack of information about the mechanisms of food finding by termites, particularly for recruitment and orientation behavior, limits the use of a toxic bait system for *Nasutitermes* (Waller & La Fage, 1987).

Despite the increasing importance of *N. corniger* as a pest, little is known about its foraging behavior in relation to exploration of food resources; although, there is a record that *N. corniger* selects wood based on the species and that it prefers the wood of *Eucalyptus grandis* W. Hill ex Maiden (Myrtaceae) more than *Pinus elliottii* Engelm (Pinaceae) (Gazal et al., 2010). In addition, the degree of decomposition (Gazal et al., 2012) and chemical stimuli of wood (Gazal et al., 2014a) also influence attraction, as well as substances of the salivary gland of *N. corniger* workers, which can have an aggregation or arrestant effect, and possibly pheromone substances in the feces that aid this species in the exploratory orientation of cellulosic substrates (Gazal et al., 2014b).

Other factors can also affect the selection of a food resource by xylophagous termites, such as size, shape, volume and position of the food, surface area of the wood, presence of chemical substances in the food that can act as nutrients or allelochemicals, and density, hardness and humidity of the wood (Usher & Ocloo, 1974; Howick, 1975; Waller & La Fage, 1987; Gerozisis et al., 2008). In relation to size of the food resource, certain xylophagous termites prefer large logs or trees and others like small branches (Wood, 1978). Field tests conducted in Ghana demonstrated that *C. formosanus* prefers to attack pieces of wood that have smaller volumes

and larger surface areas (Usher & Ocloo, 1974; Waller & La Fage, 1987).

In the laboratory, Howick (1975) evaluated the preference of three termite species for different lengths of *Eucalyptus regnans* F. Muell. wood (with the same shape), which varied from 20 to 100 mm, and observed that *Coptotermes acinacicormis* (Froggatt) (Rhinotermitidae) and *Nasutitermes exitiosus* (Hill) (Termitidae) prefer longer pieces of eucalyptus, unlike *Mastotermes darwiniensis* (Froggatt) (Mastotermitidae). Cornelius and Osbrink (2001) observed the effect of wooden pieces of *Picea* sp., which had the same dimensions in cross section (3.5 x 1 cm) but varied in length (4 and 11 cm), on two species of subterranean termite and concluded that *R. flavipes* consumption was much higher for the longer pieces and that *C. formosanus* consumption was not affected by this factor.

With the goal of selecting *E. grandis* wood of adequate size to use as monitoring bait for *N. corniger*, we verified if this physical factor influences the foraging behavior of this termite.

Material and Methods

Collection of termite nests

Nests of *N. corniger* that had active and mature colonies (with the presence of winged individuals) and were 40 cm wide and 60 cm tall were removed from trees in the park Parque Frei Leão Vellozzo between December 2016 and May 2017. The park is an Atlantic Forest reserve maintained by the Universidade Federal do Rio de Janeiro (UFRJ) that is located on the Catalão Peninsula in the municipality of Rio de Janeiro, RJ, Brazil (22°50'44"S, 43°13'19"W). Taxonomic identification was made using the nest architecture described by Thorne (1981) and collected soldiers (Scheffrahn et al., 2005). The nests were collected in black, 100-liter plastic bags and placed in cardboard boxes to avoid them from being damaged during transport to the laboratory at the Centro Integrado de Manejo de Pragas (CIMP) of the Departamento de Entomologia e Fitopatologia at Universidade Federal Rural do Rio de Janeiro (UFRRJ), in the municipality of Seropédica, RJ, Brazil.

Termite maintenance in the laboratory

In the laboratory each collected nest was placed in a transparent glass cube (50.0 cm x 50.0 cm x 60.0 cm tall), supported on a Styrofoam plate, containing a 5.0 cm layer of sterilized sand (Fig 1). The termites had free access to the foraging arena through a silicone hose (Ø = 8.0 mm; length = 10 cm) connected to a PVC pipe (black) inserted in the exit hole of the cube. Each arena consisted of a glass plate bottom (50.0 x 40.0 cm) and perimeter wall (5.0 cm tall). The arena was placed on an acrylic tube (Ø = 10.0 cm and length = 20.0 cm) so that the exit of the cube was at the same height as the top of the arena wall (Gazal et al., 2010). To allow the termites to access the foraging arena, a glass ramp was connected from the end of the silicone hose to the arena. The ramp consisted

of two, transparent, glass plates (4.0 cm x 4.0 cm and 6.0 cm x 4.0 cm) fixed together with adhesive epoxy (Durepoxi®); the longer plate was placed parallel to the termite exit.

Termites were prevented from escaping by placing transparent adhesive tape (5 cm wide) on the upper edges of the walls of the cube; the adhesive part was facing inward. Pieces of moistened *Pinus* sp. wood were placed in the arena as food. Nearby, a PET bottle cap was placed that contained water to maintain the humidity of the foraging arena. The nests were maintained in a room, at a temperature of 25 ± 5ºC, relative humidity of 80 ± 5% and photoperiod of 10:14 hours (light:dark), at CIMP/UFRRJ. The pieces of wood and the sand in each box were moistened with distilled water each day. The experiments were conducted a week after the nests were brought to the laboratory.

Termite foraging arena setup

The tests were conducted in the foraging arenas based on the methodology described by Gazal et al. (2010). Thirty minutes before the start of each test, the connection between the nest and arena was blocked with hydrophilic cotton to impede the termites from accessing the arena. Then, the food was removed from the arena and replaced with the treatments (eucalyptus blocks that were 5, 10 and 15 cm long) on glass plates (5.0 x 4.0 cm, 10.0 x 4.0 cm and 15.0 x 4.0 cm, respectively). The treatments were placed in a situation of choice and equidistant from the termite access point to the arena; that is, the blocks were inside the arena, 19 cm from the base of the access ramp. For each test, the position of the treatments in the arena was randomized.

Fig 1. Cube, for the colonies of *Nasutitermes corniger*, connected to the foraging arena.

Bioassay

Three lengths of blocks of *E. grandis* wood (heartwood with 6 year old and 14% of humidity), the same size in cross section (2.5 cm x 2.0 cm), were tested: 5 cm, 10 cm and 15 cm. Each test lasted 60 minutes and started when the access to the arena was reopened. The tests were conducted with 20 termite nests. During the test, the occurrence time of the following behavioral events was recorded with the ID, of a chronometer (Traniello, 1981; Gazal II, 2010): initial exploration (random arrival of the first soldier to the treatments); initial recruitment (arrival of the first worker); mass recruitment, which was when there was a continuous flow of workers to the substrate, demarcated by a trail on the glass plate of feces (mass arrival of workers to the treatments). The time to each behavioral event was measured, with the ID, of a chronometer, from the beginning of the test to the corresponding event. The occurrence percentages of these events, for each treatment, were calculated by the total number of blocks with the occurrence event divided by the total number of blocks available in each treatment (n = 20), multiplied by 100.

After 60 minutes of observation, the glass plates with the wood and respective recruited termites were removed. The number of termites present on each treatment, the number of termites (soldiers and workers) recruited to each plate of glass+treatment, and the number of gnawing workers (chewing on the wood) present on the treatments were recorded.

Statistical analysis

The experimental design was of randomized blocks, where each nest represents a block, using 20 nests (20 blocks). The following dependent variables used to evaluate each treatment were: percentage of occurrence of each behavioral event (initial exploration, initial recruitment and mass recruitment) along the 20 nests tested, total number of termites recruited, time (in minutes) until the occurrence of each behavioral event, number of soldiers recruited, number of workers recruited, and number of workers gnawing wood. Percentage data were obtained by summing the occurrence of the behavioral event in each repetition divided by the total number of repetitions (n = 20). The occurrence percentages of each behavioral event, for each treatment, were not transformed and compared using a chi-squared test. Due to the absence of a normal distribution, the time data for each behavioral event and number of recruited workers were compared using the Kruskal-Wallis test (p < 0.05). The total number of recruited termites, the number of recruited soldiers and the number of gnawing workers met the assumptions of the analysis of variance (Kolmogorov-Smirnov and Lilliefors at 5%) and was analyzed using ANOVA and the averages were compared using Tukey's HSD test (p < 0.05). The data were analyzed by STATISTICA® 10.0 and BioStat® 5.3 programs.

Results

Nasutitermes corniger exhibited the behavior of initial exploration, initial recruitment and mass recruitment for all sizes of the *E. grandis* wood. However, the occurrence of initial exploration of the 10 and 15 cm blocks of wood was higher than the 5 cm blocks (test $\chi^2 = 13.13$; df = 2; p < 0.01). For the initial recruitment of the *N. corniger* workers, a higher occurrence for the 10 and 15 cm blocks was also observed when compared to the 5 cm blocks (test $\chi^2 = 4.18$; df = 2; p < 0.05). On the other hand, for mass recruitment of *N. corniger* workers the occurrence was higher for the 10 cm blocks (13/20) than the 5 cm (7/20) and 15 cm (6/20) blocks. For the 5 and 15 cm blocks, mass recruitment was similar (test $\chi^2 = 16.82$; df = 2; p < 0.01).

The times for initial exploration, initial recruitment and mass recruitment of workers were not significantly different among the treatments (Table 1). On average, initial exploration lasted 8.8 ± 0.5 min after the beginning of the test, initial recruitment lasted 17.7 ± 0.6 min, and mass recruitment lasted 34.9 ± 1.1 min.

Table 1. Elapsed time (average ± SD) from the beginning of the test to the occurrence of initial exploration, initial recruitment and mass recruitment exhibited by the workers of *Nasutitermes corniger* (n = 20) on the wood of *Eucalyptus grandis* offered in three lengths in a situation of choice during 60 minutes of observation under laboratory conditions.

Length of wood (cm)	Behavioral event[1]		
	Initial exploration (min)	Initial recruitment (min)	Mass recruitment (min)
5.0 x 2.5 x 2.0	5.0 ± 0.4 a	10.5 ± 0.4 a	25.0 ± 1.1 a
10.0 x 2.5 x 2,0	11.0 ± 0.6 a	19.5 ± 0.5 a	42.4 ± 1.0 a
15.0 x 2.5 x 2.0	10.4 ± 0.6 a	23.1 ± 0.8 a	37.3 ± 1.2 a

[1]Averages followed by the same letter in the column did not differ from each other by the Kruskal-Wallis test, p <0.05.

The total number of termites recruited to the 10 cm blocks (183.4 ± 6.2) was higher than the number for the 5 cm blocks (73.0 ± 8.6). However, the 15 cm blocks (111.2 ± 5.3) exhibited a recruitment of termites that was similar to the 5 cm blocks ($F_{2.48} = 3.3$; p < 0.05; Fig 2). The total number of recruited soldiers to the *E. grandis* wood did not differ between the 10 cm (35.1 ± 1.6), 15 cm (30.2 ± 1.5) and 5 cm (16.8 ± 1.2) ($F_{2.48} = 2.2$; n.s.) blocks. The total number of recruited workers to the *E. grandis* wood for the 10 cm (148.4 ± 5.1), 15 cm (81.0 ± 4.2) and 5 cm (55.7 ± 7.3) blocks was similar (Kruskal-Wallis $H_{2.48} = 4.7$, n.s.).

The total number of workers gnawing on the 10 cm (144.7 ± 4.9) *E. grandis* blocks was higher than the 5 cm (54.7 ± 7.0) blocks. However, for the wood that was 15 cm long the *N. corniger* workers exhibited a gnawing behavior that was similar to the 5 cm and 10 cm blocks (77.6 ± 4.1) ($F_{2.48} = 3.6$; p < 0.05; Fig 3).
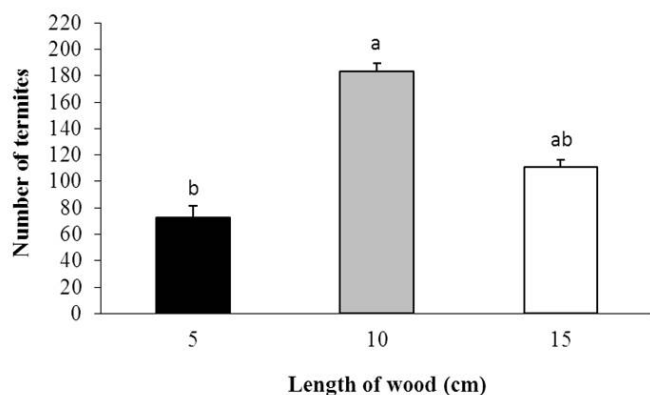
Fig 2. Number of termites of *Nasutitermes corniger* recruited (average ± SD) after 60 minutes of the test (n = 20) of three lengths of *Eucalyptus grandis* wood: 1) 5.0 x 2.5 x 2.0 cm (Size 1); 2) 10.0 x 2.5 x 2.0 cm (Size 2); and 3) 15.0 x 2.5 x 2.0 cm (Size 3). Different letters indicate a significant difference between the treatments based on Tukey's HSD test, p < 0.05.
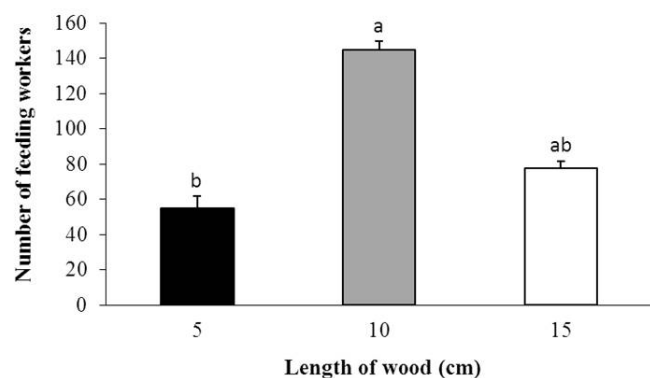

Fig 3. Number of feeding *Nasutitermes corniger* workers (average ± SD) after 60 min. of the test (n=20) for the wooden blocks *Eucalyptus grandis*: 1) 5.0 x 2.5 x 2.0 cm (Size 1); 2) 10.0 x 2.5 x 2.0 cm (Size 2); and 3) 15.0 x 2.5 x 2.0 cm (Size 3). Different letters indicate a significant difference between the treatments based on Tukey's HSD test, p < 0.05.

Discussion

Independent of the size of the *E. grandis* wood, the three behavioral events (initial exploration, initial recruitment and mass recruitment of workers) were conducted by *N. corniger* while foraging for this food resource under laboratory conditions, which corroborates observations made by other authors for this species and other species of the genus (Traniello, 1981; Traniello & Busher, 1985; Arab & Issa, 2000; Gazal et al., 2010).

In the study, mass recruitment of workers and some soldiers to the eucalyptus blocks occurred, which indicates the blocks were recognized as food, seeing that, according to Traniello (1981), this behavioral event is only triggered when the food source is appropriate for consumption.

In relation to the length of the blocks of *E. grandis*, initial exploration and initial recruitment of workers were more intense for the 10 cm (medium length) and 15 cm (longest) pieces, which suggests that these sizes of wood

represent, at the same distance, more attractive food resources for *N. corniger*. It is possible that the individuals were directed by a higher concentration of volatiles released by the wood, but this chemical stimulus needs to be further investigated. Mass recruitment of workers was greater for the medium length wood (10 cm), which allows us to infer that, after the termites begin to explore this food resource, discrimination occurs during the third stage of foraging.

Termites can measure the volume of a food source in a number of ways. For smaller food sources on the ground, some individuals need to walk or establish trails throughout the substrate so they can perceive the volume, since there is a difference in the threshold of perception for these insects in relation to variation in food size (Lenz, 1994). Howick (1975) studied how *N. exitiosus* consumed wooden blocks of *E. regnans*, which differed in length (20 to 100 x 1.5 x 2.5 mm), and found that consumption was greater as the length of the blocks increased. Esenther (1970) and French et al. (1986) observed that *R. flavipes* modified its rate of consumption according to the size of the available food. Studies of subterranean termites conducted by Waller (1988; 1991) and Lenz (1994) showed that the larger the food source, the greater the consumption, independent of the number of termites present. However, in the present work, we found that *N. corniger* clearly discriminated between the distinct sizes of wood, which was based on differences in total recruitment of termites and the number of gnawing workers.

Termite foraging is a group activity and composed of unified individual actions mediated by chemical and tactile stimuli (Traniello & Leuthold, 2000; Costa-Leonardo, 2002). The tactile stimuli come from the edges of solid objects present along the trail and of the food, and help the termites establish the trail of the colony to the food source (Swoboda & Miller, 2004). In the case of drywood termites, some species produce acoustic stimuli by drumming their head against the substrate or shaking their body as a mechanism to attract foragers and to assess the size of the food source (Evans et al., 2005; 2007).

Hedlund and Henderson (1999) verified that for *C. formosanus* the larger the size of the food source, the greater the consumption. In the present work, the shortest block (5 cm) had the lowest number of gnawing workers when compared to the medium length (10 cm) block; although, for the longest block (15 cm), the number of feeding workers was equivalent to the 5-cm block. This suggests that, for *N. corniger*, size is a factor that influences the exploration of a food source. However, apparently, starting at a certain size of the food source, the attributes responsible for recruitment would be reduced.

On the other hand, the recruitment of *Cryptotermes domesticus* (Haviland) (Kalotermitidae) workers is greater for smaller pieces of wood, a preference that could be related to food competition (Evans et al., 2005). Experiments conducted with the arboreal termites *Microcerotermes turneri* (Froggatt) (Termitidae), *Nasutitermes graveoleus* (Hill) (Termitidae) and

Nasutitermes walkeri (Hill) (Termitidae), and two lengths of wood (2.5 cm and 10 cm), found that the three species preferred the longer pieces (Gerozisis et al., 2008).

In general, the present study found that wood of distinct sizes, which are potential food sources, present differences in attractiveness and can be discriminated by *N. corniger*. This discrimination occurs during the process of exploring the resource through a preference mechanism that results in differences in the total number of termites recruited and the number of workers feeding on the wood. Wood size could play an important role in determining the food preference of *N. corniger.* Future research should focus on quantifying this possible importance, as well as the role of the number (quantity) of wooden pieces in relation to *N. corniger* foraging.

However, additional observations made while collecting the nests found that *N. corniger* forages in tunnels built on and a few centimeters below the soil surface, which allows us to infer that buried pieces of eucalyptus wood could be used as attractive bait. In addition, Fontes and Millano (2002) cite that, similar to subterranean termites, *N. corniger* can infest buildings through the soil. Thus, this bait could aid in surveys of this species under field conditions, as occurs for some subterranean species, such as *Coptotermes gestroi* (Wasmann) (Santos et al., 2010), and could also be used in monitoring stations in a toxic bait system to detect this termite in urban centers. Based on the results of the laboratory tests, 10 cm pieces would be the most adequate size for this because they attracted the greatest mass recruitment. However, more studies are needed to support these hypotheses, since in addition to foraging behavior inherent to the species, other factors might interfere with termite foraging, such as edaphoclimatic conditions, presence of other food sources and predators, soil structure and humidity, and level of soil disturbance (Hu & Appel, 2004; Souza et al., 2009; Santos et al., 2010; Dow AgroSciences, 2013).

Acknowledgments

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