

INFLUENCE OF THE AMOUNT OF FOOD RESOURCES IN THE FORAGING BEHAVIOR OF *Nasutitermes corniger* (MOTSCHULSKY)

INFLUÊNCIA DA QUANTIDADE DE MADEIRA NO COMPORTAMENTO DE FORRAGEAMENTO DE *Nasutitermes corniger* (MOTSCHULSKY)

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ABSTRACT: Food scarcity or abundance are factors regulating termites' foraging behavior in general. The aim of the present study is to evaluate the influence of four amounts of *Eucalyptus grandis* on foraging behavior events shown by worker and soldier of *Nasutitermes corniger* during laboratory tests. The tests were carried out with adult and active *N. corniger* colonies found in nests collected in the field, which were stored in glass cubes connected to the test arenas. Four different amounts of wood blocks were used in the tests and each amount concerned a treatment and defined a different experimental group: 1, 2, 3 and 4 blocks/arenas, with 5 repetitions. Each test lasted 60 minutes and consisted in observing, or not, the occurrence of behavioral events shown by foragers when they had contact with the treatment. The duration of each event was recorded, whenever it was observed. The number of recruited foragers and the number of workers consuming the blocks were recorded at the end of each test applied to each treatment. *Nasutitermes corniger* presented the three behavioral events in all treatments; however, there was not significant difference between treatments in the occurrence of the two first events, in the time taken from test start to the occurrence of a new event, in the number of recruited termites and in the number of gnawing workers. Only workers' mass recruiting was influenced by the amount of wood available. The occurrence of this event was significantly higher in treatments with greater amounts of wood. Thus, *N. corniger* adjusts its mass recruitment behavior in response to available food amount, which should be considered when developing baiting system for its control.

KEYWORDS: Arboreal-nesting termite. Food availability. Foraging decisions. Xylophagous termite.

INTRODUCTION

The arboreal termite species *Nasutitermes corniger* (Motschulsky) (Termitidae: Nasutitermitinae) is the most widely distributed one, and it is found in the Meso-American region, from Southern Mexico to Panama, and also in South America (ATKINSON; ADAMS, 1997; CONSTANTINO, 2002; TORALES, 2002; SCHEFFRAHN et al., 2005; SANTOS et al., 2017; CONSTANTINO, 2019). Moreover, *N. corniger* causes great damage in urban zones in the entire Neotropical region; and it is seen as a severe pest in some South American countries, such as Argentina, and in many Brazilian states, where it causes damage to timber used in construction sites and for furniture manufacture (BANDEIRA et al., 1989; BANDEIRA; MIRANDA; VASCONCELLOS, 1998; MILL, 1991; MENEZES; AGUIAR-MENEZES; BICALHO, 2000; COSTA-LEONARDO, 2002; TORALES, 2002; ALBUQUERQUE et al., 2012). Originally, this

termite was considered non-discriminatory among wood of different plant species (BUSTAMANTE, 1993); however, Gazal, Bailez and Viana-Bailez (2010) have shown that wood of *Eucalyptus grandis* W. Hill ex Maiden (Myrtaceae) was more severely attacked than woods of *Pinus elliottii* Engel. (Pinaceae) and *Manilkara huberi* (Ducke) A. Cheval (Sapotaceae), when they were simultaneously offered.

It is hard controlling *N. corniger* because of the high rates of infestation relapses, which are mainly caused by the construction of polycalic nests, which are often far from where they attack and damage the cellulosic materials (COSTA-LEONARDO, 2002). Thus, urban infestations with *N. corniger* have been controlled through the adoption of methods that compromise environmental sustainability, since they are based on preparing chemical barriers in the soil through the application of high-residual effect insecticides and on chemically treating the timber (United Nations Environment Programme [UNEP], 2000;

FONTES; MILANO, 2002; GEROSIS; HADLINGTON; STAUNTON, 2008).

Understanding termites' process to select food sources demands knowing the mechanisms responsible for the repellency or attraction of a food stimulus (SUOJA et al., 1999). Attraction is defined as the action that triggers movements driven towards the stimulus and repellency is the very opposite of it (KENNEDY, 1978; MATTHEWS; MATTHEWS, 1978). Often times, these two actions take part in the process to recruit termites to the food source (SUOJA et al., 1999); besides, they depend on the chemical and physical properties of the wood (PERALTA et al., 2003; SOUZA et al., 2009; PAES et al., 2015).

Laboratory studies revealed that the foraging process carried out by *N. corniger* is selective when it comes to wood species, this termite rather attacks intermediate-density wood, as well as wood presenting high amounts of secondary metabolites, such as *E. grandis* (GAZAL; BAILEZ; VIANA-BAILEZ, 2010; GAZAL et al., 2012). This termite also attacks both dry and moistened wood, either manufactured or not (BANDEIRA; MIRANDA; VASCONCELLOS, 1998). Nevertheless, this termite species prefers wood that has undergone some sort of deterioration, and that is why it is considered a selective species when it comes to the degree of wood deterioration (BUSTAMANTE, 1993; GAZAL et al., 2012). However, any information was not found about whether the foraging behavior of *N. corniger* can be regulated by volume or amount of the wood.

However, the amount of food available also seems to be one of the main criteria for food resource selection and finding by some termite species (SUOJA et al., 1999, EVANS et al., 2005, INTA et al., 2007, OBERST; LAI; EVANS, 2018). For example, the amount of wood available regulates how severely *Coptotermes formosanus* Shiraki (Rhinotermitidae) build foraging tunnels (HEDLUND; HENDERSON, 1999). Similarly, Cristaldo et al. (2018) have observed that the number of worker of *Nasutitermes* aff. *coxipoensis* (Holmgren) (Termitidae) has linearly increased due to increased food-source density (0.32, 0.64 and 1.92 baits/m²). The information about the mechanisms of food exploration by termites can help develop more environmentally friendly control methods, such as toxic bait system, whose efficiency depending on information about their recruitment and orientation behavior.

In this context, the aim of the present study was to evaluate the effects of the amount of wood available on the foraging behavior of *N. corniger*

under controlled environmental conditions in laboratory.

MATERIAL AND METHODS

Collection of termite colonies

Nasutitermes corniger nests (40 cm wide and approximately 60 cm tall) with adult and active colonies (with winged termites) were removed from trees at Frei Leão Vellozzo Park, between December 2016 and May 2017. The park is managed by UFRJ and is located in Península do Catalão, Rio de Janeiro County, Rio de Janeiro State, Brazil (22°50'44" S, 43°13'19" W). Taxonomic identification was carried out based on the nest architecture described by Thorne (1981) and on examining the collected soldiers, as well as on the description by Scheffrahn et al. (2005). The collected nests were placed in 100-l black plastic bags and stored in cardboard boxes, in order to avoid damages during transportation to the laboratory of *Centro Integrado de Manejo de Pragas (CIMP) do Departamento de Entomologia e Fitopatologia da Universidade Federal Rural do Rio de Janeiro (UFRRJ)* – Integrated Pest Management Center of Entomology and Phytopathology Department of Federal Rural Rio de Janeiro University (UFRRJ), in Seropédica County, Rio de Janeiro State, Brazil.

Termite colony maintenance in laboratory

Each of the collected nests was placed in transparent glass cube (50.0 cm x 50.0 cm x 60.0 cm height) connected to foraging arenas after they were taken to the laboratory. The cube was held by a styrofoam plate covered with a layer (5.0 cm) of sterilized sand. The termites had free access to the foraging arena through a silicone hose ($\varnothing = 8.0$ mm; length = 10 cm) connected to a black PVC pipe inserted in the exit of the glass cube. Each foraging arena consisted of a basis made out of a glass plaque (50.0 x 40.0 cm) and a perimeter wall (5.0 cm tall). The arena was placed over an acrylic tube ($\varnothing = 10.0$ cm and length = 20.0 cm) so that the cube exit was at the same height of the top of the arena's wall (GAZAL; BAILEZ; VIANA-BAILEZ, 2010). A glass ramp was connected from the tip of the silicon hose to the arena in order to allow termites to have access to foraging arena. The ramp consisted of two transparent glass plaques (4.0 cm x 4.0 cm and 6.0 cm x 4.0 cm) fixed with epoxy adhesive (Durepoxi®).

Termites were stopped from escaping by a transparent adhesive tape (5 cm wide) glued on the upper edge of the glass cube wall – the sticky side

of the tape was turned to the inside of the cube. Pieces of *Pinus* sp. wood were moistened and made available as food in the arenas. A PET bottle cap, filled with water, was placed by the wood blocks in order to provide humidity to the foraging arena. The nests were stored in a room under controlled temperature ($25 \pm 5^\circ\text{C}$), relative humidity ($80 \pm 5\%$) and 10:14-hour (light/dark) photoperiod at CIMP/UFRRJ. The wood blocks and the sand in each glass cube (holding a nest) were moistened with distilled water on a daily basis. The experiments were carried out one week after the nests were taken to the laboratory.

Bioassay

Tests were carried out in the foraging arenas of *N. corniger*, based on the methodology described by Souza et al. (2018). The connection between nest and foraging arena was blocked with hydrophilic cotton, 30 minutes before the test had started, in order to block termites' access to the arena. Next, the food was removed from the arena and replaced by blocks of *E. grandis* wood (14% humidity) (5.0 cm x 2.5 cm x 2.0 cm) at four different amounts: 1) One wood block, 2) Two wood blocks, 3) Three wood blocks and 4) Four wood blocks. Treatments were based on situation of choice over glass plaques (7.0 cm x 4.0 cm); the plaques were connected to other glass plaques (10.0 cm x 4.0 cm). These plaques were in contact to another glass plaque (35.0 cm x 4.0 cm), which was placed perpendicular to termites' access to the arena. The glass plaques were arranged over plastic caps (2.8 cm x 2.8 cm x 2.0 cm) in order to avoid the access of termites on the basis of the foraging arena to the experimental plaques. Treatments' position in the arena was randomly changed between tests. Tests started when termites' access to the arena was free, they were carried out with 20 *N. corniger* nests.

The time needed to observe the following behavioral events was recorded throughout each test with a chronometer (TRANIELLO, 1981; GAZAL; BAILEZ; VIANA-BAILEZ, 2010): initial exploration (random arrival of the first soldier to the treatment), initial recruiting (arrival of the first worker to the treatment) and mass recruiting, which was observed when there was a continuous flow of workers to the treatment and a delimited track over the glass plaque due to feces deposition (arrival of a

massive number of workers at the treatment). Occurrence rates recorded for these events in each treatment were calculated through the total number of blocks and the occurrence of behavioral events divided by the total number of blocks available in each treatment ($n = 20$), multiplied by 100. The time taken to observe the behavioral events was counted in a cumulative way, based on the occurrence of events.

The recruited termites found over the glass plaques and the wood blocks were removed after 60 minutes of observation; the number of termites on each treatment was determined, as well as the number of termites (soldiers and workers) recruited to the glass plaque+treatment and the number of gnawing workers (when they were chewing on the wood) in the treatments.

Statistical analysis

The rate of initial exploration occurrences, of initial recruiting and of workers' mass recruiting was compared through Chi-squared test. Due to lack of normal distribution (Kolmogorov-Smirnov/Lilliefors at 5%), data about time, number of gnawing workers, number of workers, number of soldiers and the total number of recruited termites (glass plaques+treatment) were compared through Kruskal-Wallis test ($p < 0.05$). The analyses were carried out in the STATISTICA 10.0 and BioStat[®] 5.3 software.

RESULTS

The three behavioral events (initial exploration, initial recruiting and mass recruiting) were shown by *N. corniger* foragers regardless of the amount of *E. grandis* wood. However, the occurrence recorded for initial exploration and initial recruiting was similar between different wood amounts ($\chi^2 = 2.2$; g.l.= 3; n.s. and $\chi^2 = 0.4$; g.l.= 3; n.s., respectively). On the other hand, the mass recruiting of *N. corniger* workers was greater in the treatment with four wood blocks (13/20) than in treatments with two blocks (9/20) and 1 block (7/20) ($\chi^2 = 7.3$; g.l.= 3. $p < 0.01$, Figure 1). Nevertheless, the mass recruitment of *N. corniger* was similar between the treatment with two wood blocks (9/20) and the one with only one block of it (7/20).

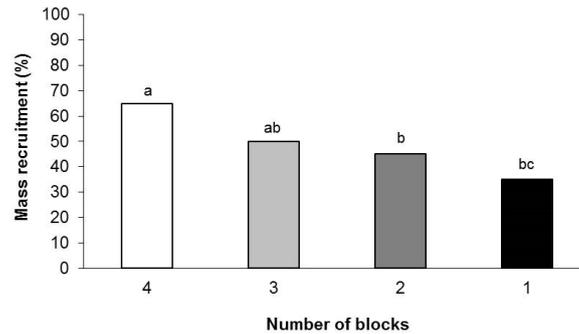


Figure 1. The rate of mass recruitment of *Nasutitermes corniger* (n = 20) on *Eucalyptus grandis* wood blocks (5.0 cm x 2.5 cm x 2.0 cm) provided at four different amounts.

Different letters indicate significant difference between the treatments based on the χ^2 Test; $p < 0.01$.

The amount of wood did not influence time latency for the performance of the three behavioral events. The initial exploration happened 15.7 ± 0.8 min after the test had started, on average; initial

recruiting started after 28.1 ± 0.8 min, and workers' mass recruiting started 46.3 ± 1.0 min after the test had begun (Table 1).

Table 1. Elapsed time (mean \pm SE, in minutes) from the beginning of the test until the occurrence of foraging behavior events by *Nasutitermes corniger* (n = 20) on wood blocks of *Eucalyptus grandis* provided at four different amounts under situation of choice throughout 60 minutes of observation.

Amount of wood blocks (5.0 x 2.5 x 2.0 cm)	Behavioral event ¹		
	Initial exploration	Initial recruitment	Mass recruitment
1 block	16.5 ± 1.0 a	28.4 ± 0.8 a	39.3 ± 0.9 a
2 blocks	18.0 ± 0.8 a	31.7 ± 0.8 a	50.5 ± 1.1 a
3 blocks	13.2 ± 0.7 a	25.9 ± 0.7 a	44.0 ± 1.0 a
4 blocks	14.9 ± 0.8 a	26.5 ± 0.7 a	51.3 ± 1.1 a

¹Means followed by the same letter in the column do not differ from each other in the Kruskal-Wallis test ($p < 0.05$).

The total number of recruited termites after 60 minutes for the treatments with one (73.6 ± 5.7), two (113.9 ± 6.3), three (219.7 ± 15.1) and four (243.8 ± 12.9) wood blocks was similar ($H_{3,48} = 4.6$, n.s.). The total number of recruited soldiers and workers after 60 minutes was also similar ($H_{3,48} = 5.0$, n.s. and $H_{3,48} = 4.1$, n.s., respectively) (Table 2).

Out of the total number of workers, the number of gnawing workers exploring the treatment

was 56.8 ± 4.8 , 91.3 ± 5.5 , 187.6 ± 12.9 and 208.8 ± 11.5 for treatments with 1, 2, 3 and 4 wood blocks, which corresponds to 92.7%, 95.6%, 99.3% and 98.7% of the total number of recruited workers, respectively. However, the number of gnawing workers on the treatments with 1, 2 3 and 4 wood blocks was similar ($H_{3,48} = 4.5$, n.s.) (Table 2).

Table 2. Total number of termites, number of soldiers, number of workers and number of exploring workers from the beginning of the test until the occurrence of foraging behavior events shown by *Nasutitermes corniger* (n = 20) on the wood species *Eucalyptus grandis* provided at four different amounts under situation of choice throughout 60 minutes of observation.

Amount of wood blocks (5.0 x 2.5 x 2.0 cm)	Number of termites (mean \pm SE)	Number of soldiers (mean \pm SE)	Number of workers (mean \pm SE)	Number of exploring workers (mean \pm SE)
1 block	73.6 ± 5.7 a	12.3 ± 0.7 a	61.3 ± 5.1 a	56.8 ± 4.8 a
2 blocks	113.9 ± 6.3 a	18.5 ± 0.9 a	95.5 ± 5.6 a	91.3 ± 5.5 a
3 blocks	219.7 ± 15.1 a	30.8 ± 2.1 a	188.9 ± 13.0 a	187.6 ± 12.9 a
4 blocks	243.8 ± 12.9 a	32.3 ± 1.5 a	211.5 ± 11.7 a	208.8 ± 11.5 a

Means followed by the same letter in the column did not differ from each other in the Kruskal-Wallis test ($p < 0.05$).

DISCUSSION

The similarity between the initial exploration and initial recruiting shown by *N. corniger* suggests that the amount of wood available does not influence the location and recognition of food sources. However, the greatest occurrence of mass recruitment of workers to the largest quantity of wood indicates that after the recognition of the wood, discrimination occurs in the acceptance of this food source. The amount of food is considered as one of the essential criteria used by underground and dry-wood termites at the time to select a food source (SUOJA et al., 1999; GRACE; CAMPORA, 2005; EVANS et al., 2005; INTA et al., 2007; OBERST; LAI; EVANS, 2018). According to Grace and Campora (2005), after the underground termite species *C. formosanus* sets many eating locations, workers' efforts are triggered, since not all food sources can be equally accepted by representatives of this species. Thus, *C. formosanus* changes its recruiting behavior, depending on the quality and amount of food (WALLER; LA FAGE, 1987). Almeida et al. (2018) showed that the foraging behavior of *Nasutitermes* aff. *coxipoensis* is regulated by food resource availability in experiments carried out in the Brazilian Northeastern region. It happened due to the increased food density, which reduced the foraging area available to these termites. The same behavior was observed in *Cornitermes cumulans* (Kollar) representatives, when the amount of food resources increased (ARAÚJO; ARAÚJO; DE SOUZA, 2011).

Soldiers' recruitment in treatments with different amounts of wood was also equivalent, and this outcome indicates that this caste has actively participated in the foraging process at all amounts of wood available, due to the escort by the recruited workers and the gnawing workers who explored the wood blocks. This soldier behavior was also observed by Lima and Costa-Leonardo (2014) in underground termite species and by Souza et al. (2018) in *N. corniger* colonies.

According to Delaplane and La Fage (1987) and Jones, Trosset and Nutting (1987), when termites cover a location where there is a favorable food source, they become quite loyal to this source; however, the similar number of recruited *N. corniger* foragers in treatments with different amounts of wood blocks suggests that this species does not show recruiting loyalty to certain amount of wood. On the other hand, the underground termite species *Coptotermes gestroi* (Wasmann) (Rhinotermitidae) and *Heterotermes tenuis* (Hagen)

(Rhinotermitidae) concentrated their efforts on the first food found when they did not have access to other food sources (LIMA; COSTA-LEONARDO, 2014).

The similar number of gnawing workers exploring the wood blocks is not associated with the amount of wood available. This outcome indicates that *N. corniger* does not set the adequacy of food sources available, based on the amount of such sources. Thus, the amount of wood does not regulate wood exploration by *N. corniger*, likely because workers lay similar saliva concentrations on the wood, regardless of the amount of wood available. *N. corniger* workers have substances in their saliva that have phage- or arresting-stimulating action in the location where it is laid on, and these substances can be the trigger to increase the number of gnawing workers exploring the wood blocks (SILVA, 2008). Some termites species, such as those living in moistened wood like *Schedorhinotermes lamanianus* (Sjöstedt) (Rhinotermitidae) and underground species such as *Reticulitermes flavipes* (Kollar) (Rhinotermitidae) and *C. gestroi*, also have non-volatile substances in their saliva, which are phage-stimulants that increase workers gathering on the food source (KAIB; ZIESMANN, 1992; REINHARD; HERTEL; KAIB, 1997; REINHARD; KAIB, 2001; CASARIN; ARAB; COSTA-LEONARDO, 2003). These chemical signs facilitate the location, recruitment and exploration of food sources (LIMA; COSTA LEONARDO, 2012).

In this work it was verified that when timber in different quantities are offered to the termites simultaneously, these initially represent for *N. corniger* sources of food of equivalent interest. However, in the process of accepting these sources, there is competition that causes differences in the proportion of mass recruitment. In the last phase of foraging, termites again lack preference in the exploitation of these woods.

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RESUMO: A escassez ou abundância de alimento são fatores que regulam o forrageamento do térmitas em geral. No presente estudo, avaliou-se a influência de quatro quantidades de madeira de *Eucalyptus grandis* em eventos comportamentais do forrageamento exibidos por operárias e soldados de *Nasutitermes corniger* em testes de laboratório. Os testes foram realizados com colônias maduras e ativas de *N. corniger* presentes em ninhos coletados a campo, os quais foram acondicionados em cubas de vidro conectadas a arenas testes. Blocos de madeira de mesma dimensão foram ofertados aos térmitas em quatro quantidades: 1, 2, 3 e 4 blocos/arena, com 5 repetições. Cada teste durou 60 minutos e consistiu na observação ou não da ocorrência de eventos comportamentais exibidos pelos forrageadores quando foi dado acesso a cada tratamento. Quando cada evento foi observado, o tempo de sua duração foi registrado. Ao final de cada teste registrou-se, em cada tratamento, o número de térmitas forrageadores recrutados e o número de operários consumindo os blocos. Observou-se que *N. corniger* exibiu os três eventos comportamentais em todos os tratamentos. Todavia, não houve diferença significativa entre os tratamentos com relação à ocorrência dos dois primeiros eventos, aos tempos transcorridos desde o início do teste até a ocorrência de cada evento, aos números de térmitas recrutados e ao número de operários em roedura. Apenas a ocorrência de recrutamento em massa dos operários foi influenciada pela quantidade de madeira ofertada, sendo que foi significativamente superior quando se ofertou os blocos de madeira na maior quantidade. Dessa forma, *N. corniger* ajusta seu comportamento de recrutamento em massa em resposta a quantidade de alimento disponível, o que deve ser considerado ao se desenvolver um sistema de isca para seu controle.

PALAVRAS-CHAVE: Cupim de nidificação arbórea. Cupim xilófago. Decisões de forrageamento. Disponibilidade de alimento.

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