

# DOES THE DISTANCE BETWEEN THE NEST AND THE FOOD SOURCE AFFECT THE FORAGING BEHAVIOR OF *Nasutitermes corniger* (TERMITIDAE)?

Thiago Sampaio DE SOUZA<sup>1</sup> , Vinícius Siqueira GAZAL<sup>2</sup> ,  
Elen de Lima AGUIAR-MENEZES<sup>2</sup> , Vinicius José FERNANDES<sup>1</sup> 

<sup>1</sup> Postgraduate Program in Phytosanitary and Applied Biotechnology, Federal Rural University of Rio de Janeiro, Seropédica, Rio de Janeiro, Brazil.

<sup>2</sup> Department of Entomology and Phytopathology, Federal Rural University of Rio de Janeiro, Seropédica, Rio de Janeiro, Brazil.

## Corresponding author:

Thiago Sampaio de Souza  
thiagosampaio.agro@gmail.com

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## Abstract

Research on food finding by pest termites can be used to inform the development of techniques to control their population; however, there is a paucity of information available on the foraging behavior of *Nasutitermes corniger*, an urban pest in South America. In the present study, we analyzed the effect of the distance between the nest and food on the exploration and recruitment of *N. corniger* during foraging behavior under laboratory conditions. Nests containing mature colonies were collected in the field and placed in a glass cube connected to a test arena (50.0 × 40.0 cm) in which *Eucalyptus grandis* blocks were supplied at three different distances: 10, 20 and 30 cm. In each test, the occurrence of the following events were recorded: initial exploitation, initial recruitment, and mass worker recruitment. Individuals in the blocks were counted at the end of each test and divided into the total number of recruited termites, recruited workers, consuming workers and recruited soldiers. Each test lasted 60 minutes and was repeated with 20 colonies. *Nasutitermes corniger* foragers showed the three behavioral events of interest at all three distances. The occurrences of initial exploitation and initial recruitment, the latency of the three events and the number of foragers were not affected by the distance between the nest and food. The occurrence of mass worker recruitment was the only event affected by this distance, with higher recruitment at shorter distances.

**Keywords:** Arboreal termites. *Eucalyptus grandis*. Food Location. Nasutitermitinae.

## 1. Introduction

*Nasutitermes corniger* (Motschulsky) is widely distributed in Mesoamerica, from southern Mexico to Panama, and in South America (Atkinson and Adams 1997; Torales 2002; Constantino 2002; Scheffrahn et al. 2005). In recent decades, the species has been of high economic importance due to increasing reports of damage to residences and historic buildings in several cities in Brazil and Argentina, which confirmed its pest status (Menezes et al. 2000; Constantino 2002; Costa-Leonardo 2002; Fontes and Milano 2002; Torales 2002; Albuquerque et al. 2012; Mello et al. 2014; Gazal et al. 2019).

Controlling *N. corniger* is a difficult task due to the high incidence of reinfestations resulting from polycalic nests and distant nest locations, which are often far from the attacked area (Costa-Leonardo 2002). However, in urban areas, this pest usually causes problems in buildings near to the trees in which its

nests are built. Nests may even be constructed inside the building, under the roof or inside the walls (Menezes et al. 2000; United Nations Environment Programme [UNEP] 2000; Mello et al. 2014; Gazal et al. 2019).

Toxic bait systems are new, environmentally friendly technologies for controlling subterranean termites, especially *Coptotermes formosanus* Shiraki and *Reticulitermes flavipes* (Kollar) (Rhinotermitidae) (Su et al. 1995; UNEP 2000; Lee 2002; Su 2002; Potter 2004; Dow AgroSciences 2013). This method involves the establishment of a monitoring station near buildings to intercept termites for use as a control. The mass recruitment of foragers to toxic bait (food) is important for the efficacy of this control technology (Potter 2004; Ogg et al. 2006). However, there is a paucity of information available on the mechanisms by which *Nasutitermes* find food sources (including the recognition, acceptance and assessment of adequacy of available food sources), which is needed to help develop new arboreal termite control techniques, including baiting systems (Jurd and Manners 1980; Traniello 1981; Evans and Iqbal 2014).

The arboreal termite *N. corniger* is increasingly recognized as an important pest species, although little is known about its foraging behavior in the exploitation of food resources. However, records on the food selection process of this termite show that it is based on the wood species – these termites prefer *Eucalyptus grandis* W. Hill ex Maiden (Myrtaceae) to *Pinus elliottii* Engelm (Pinaceae) (Gazal et al. 2010). In addition, wood decomposition levels (Gazal et al. 2012), chemical stimuli (Gazal et al. 2014a) and size (Souza et al. 2018), as well as fecal pheromonal substances that orient individuals toward cellulosic substrates (Gazal et al. 2014b), influence its attraction process. Thus, it is essential to understand the foraging dynamics of *N. corniger* to develop new control techniques for this termite species.

The foraging behavior of *N. corniger* starts with small groups of soldiers (2 to 5 individuals) who leave the nest in different directions. When a given soldier finds a food source, it returns to the nest while intermittently pressing its abdomen onto the substrate to leave a chemical trail (trail pheromone). In the second phase, new soldiers and workers are recruited and head toward the food source by following the chemical trail. In the third phase, mass worker recruitment occurs if the food source is appropriate, and then workers begin to consume this food (Traniello 1981; Arab and Issa 2000; Andara et al. 2004). Different wood species can represent equally attractive food sources for *N. corniger* throughout the food exploitation and recognition phases; however, food source discrimination occurs during mass worker recruitment, in which preference for one of the available sources is established gradually (Gazal et al. 2010).

The distance between the nest and food source may increase the cost of the food exploitation and recognition phases due to increased energy expenditure and possible predation by natural enemies (Lima and Dill 1990). However, the arboreal nests of *N. corniger* termites contain a large population, harboring over 1 million individuals; these individuals may build several tunnels between their nest and the food source, and thus can forage for food at large distances from the nests (Andara et al. 2004; Scheffrahn et al. 2014), although it remains unknown whether foragers explore nearby sources. Thus, we tested the hypothesis that the foraging behavior events of *N. corniger*, the number of foragers (workers and soldiers) leaving the nest and the number of workers consuming the food source are increased when the food source is closer to its nest, while the latencies of each behavioral event are decreased.

The aim of the current study was to evaluate the effects of distances between eucalyptus wood (a suitable food source) and the nests of *N. corniger* on its foraging behavior under laboratory conditions.

## 2. Material and Methods

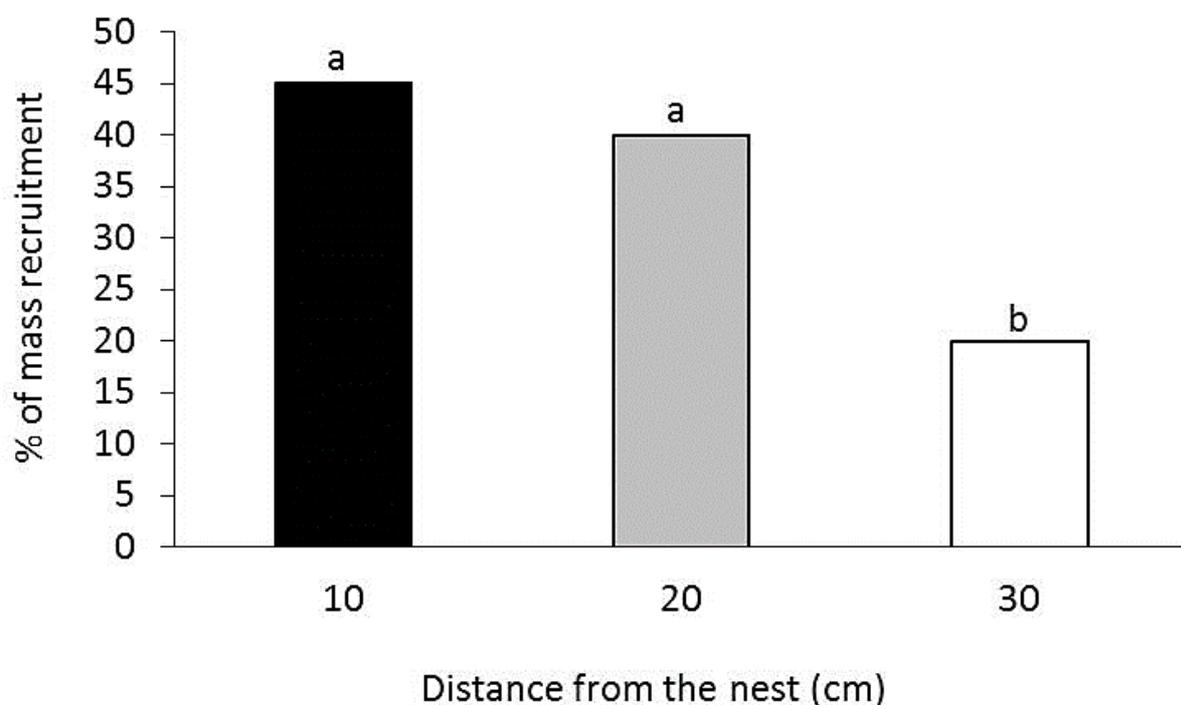
### Termite nest collection

Arboreal nests (approximately 40 cm wide and 60 cm tall) containing active and mature (i.e., winged termite) colonies of *N. corniger* were collected from trees grown at Frei Leão Vellozzo Park, which is maintained by the Federal University of Rio de Janeiro (UFRJ), from February to May 2017. The park is located in Catalão Peninsula, Rio de Janeiro City, Rio de Janeiro State, Brazil (22° 50' 44" S; 43° 13' 19" W). Taxonomic identification was based on the nest architecture described by Thorne (1981) and on the morphology of collected soldiers described by Scheffrahn et al. (2005). Collected nests were placed in 100-

L black plastic bags and stored in cardboard boxes to prevent damage during transportation to the laboratory in the Integrated Center for Pest Management (CIMP - Centro Integrado de Manejo de Pragas) of the Entomology and Phytopathology Department of Federal Rural University of Rio de Janeiro (UFRRJ), Seropédica County, Rio de Janeiro State, Brazil.

### Termite maintenance in the laboratory

The collected nests were individually placed in transparent glass cubes (50.0 cm × 50.0 cm × 60.0 cm tall) filled with a layer (5.0 cm) of sterilized sand and supported by Styrofoam plates (Figure 1). Termites had free access to the foraging arena through a silicone hose ( $\varnothing = 8.0$  mm, length = 10 cm), which was connected to a black PVC tube inserted in the exit hole of the cube. Every arena comprised a glass plate base (50.0 × 40.0 cm) and a perimeter wall (5.0 cm tall). Sand was placed on an acrylic tube ( $\varnothing = 10.0$  cm and length = 20.0 cm) such that the outlet of the vat was at the same height as the top of the arena wall (Gazal et al. 2010). A glass ramp connected the end of the silicone hose to the arena to allow the termites to access the foraging arena. The ramp comprised two transparent glass plates (4.0 cm × 4.0 cm and 6.0 cm × 4.0 cm) fixed to one another with epoxy adhesive (Durepoxi®).



**Figure 1.** The mass recruitment rate of *Nasutitermes corniger* to *Eucalyptus grandis* wood placed at the following distances from the nest in the test arena under laboratory conditions: 1) 10 cm, 2) 20 cm, and 3) 30 cm (n = 20 colonies at each distance). Different letters indicate significant differences in mass recruitment rates, according to the  $\chi^2$  test;  $p < 0.01$ .

Termites were prevented from escaping due to the placement of transparent adhesive tape (5 cm wide) at the upper edges of the vat walls; the adhesive side of the tape faced the inner side of the vat. Pieces of *Pinus* sp. wood were moistened and provided as food in the arena; a PET bottle cap filled with water was placed next to the wood pieces to humidify the foraging area. The nests were kept in a room at CIMP/UFRRJ under the following conditions: mean temperature of  $25 \pm 5$  °C, relative humidity of  $80 \pm 5\%$  and a light:dark period of 10:14 hours. The wood pieces and the sand of each vat holding a nest were moistened with distilled water daily.

### Bioassay

The experiments were conducted a week after the nests were taken to the laboratory and were

performed in the foraging arenas, based on the methodology described by Gazal et al. (2010). The connection between the nest and foraging arena was blocked with hydrophilic cotton 30 minutes before the beginning of each test to prevent termites from gaining access to the arena. Next, the food (*Pinus* wood) was removed from the arena and replaced with the test food sources, which comprised blocks (5.0 cm × 2.5 cm × 2.0 cm) of *Eucalyptus grandis* wood placed at 3 different distances in the arena. These test food sources were placed on glass plates (7.0 cm × 4.0 cm) to allow their selection by termites and connected to glass plates with the following dimensions: 1) 10.0 cm × 4.0 cm; 2) 20.0 cm × 4.0 cm; and 3) 30.0 cm × 4.0 cm. These plates were in contact with a 35.0 cm × 4.0 cm glass plate, which was placed perpendicular to the termites' access to the arena; thus, the blocks were supplied to termites at 10, 20 and 30 cm away from the wall of the arena (i.e., 35, 45 and 55 cm from the nest in a straight line, respectively). The glass plates were laid on plastic caps (2.8 cm × 2.8 cm × 2.0 cm) to prevent termites found at the base of the foraging arena from accessing the experimental plates. The position of the test food sources in the arena was randomly chosen at all tests, which comprised 20 termite nests and started when the termites were allowed to access the test arena.

All tests lasted 60 minutes and started when termites were allowed to access the arena. A timer was used to record the latency to the following behavioral events (Traniello 1981; Gazal et al. 2010): initial exploitation (random arrival of the first soldier to the test food source), initial recruitment (arrival of the first worker), and mass recruitment (mass arrival of workers to the test food source). Mass recruitment was characterized by continuous flow of workers on the substrate, which resulted in a trail of feces on the glass plate. The time elapsed until the observation of behavioral events was cumulatively recorded as these events took place. The incidence rates of behavioral events in each treatment were calculated by dividing the number of behavioral events by the total number of termite nests at each distance (n = 20) multiplied by 100.

The glass plates holding the wood pieces and their recruited termites were removed after 60 minutes of observation. The number of termites found in each test food source, the number of termites (soldiers and workers) recruited to each glass plate + food source and the number of gnawing workers (chewing the wood) in each food source were recorded.

### Statistical analysis

Initial exploitation, initial recruitment, and mass worker recruitment rates in each treatment were compared with the chi-square test. The latency to each behavioral event and the number of recruited workers were compared through the Kruskal–Wallis test ( $p < 0.05$ ) due to the nonnormal distribution of these variables. However, the total number of recruited termites, the number of recruited soldiers and the number of workers exhibiting gnawing behavior met the assumptions for analysis of variance (Kolmogorov–Smirnov and Lilliefors tests at 5%) and were analyzed through ANOVA; means were compared through Tukey's HSD test ( $p < 0.05$ ). STATISTICA® 10.0 and BioStat® 5.3 were used to perform the data analysis.

### 3. Results

*Nasutitermes corniger* foragers exhibited initial exploitation, initial recruitment and mass recruitment behaviors to wood pieces placed at three distances from their nest. However, the rate of initial exploitation by *N. corniger* foragers was higher at wood pieces located 10 cm (14/20) and 20 cm (14/20) from the nest than at those 30 cm (11/20) from the nest ( $\chi^2 = 4.2$ ;  $df = 2$ ;  $p < 0.05$ ).

Similarly, the rate of initial recruitment by *N. corniger* was higher for wood pieces 10 cm (13/20) and 20 cm (13/20) from the nest than for those 30 cm (8/20) from the nest ( $\chi^2 = 11.6$ ;  $df = 2$ ;  $p < 0.01$ ). The rate of mass worker recruitment was also higher for wood pieces 10 cm (9/20) and 20 cm (8/20) from the nest than those placed 30 cm (4/20) from the nest ( $\chi^2 = 8.6$ ;  $df = 2$ ;  $p < 0.01$ ; Figure 1).

The latencies to initial exploitation, initial recruitment and mass worker recruitment did not significantly differ among the distances (Table 1). Initial exploitation, initial recruitment, and mass worker

recruitment occurred an average of  $13.8 \pm 0.8$  min,  $25.7 \pm 0.8$  min and  $40.0 \pm 1.0$  min after the beginning of the test, respectively.

The total number of recruited termites was similar ( $H_{2,57} = 3.7$ , n.s.) in wood pieces located 10 cm ( $92.0 \pm 4.5$ ), 20 cm ( $106.3 \pm 7.3$ ) and 30 cm ( $41.2 \pm 3.2$ ) from the nest. Similarly, the total number of recruited soldiers in wood pieces located 10 cm ( $27.6 \pm 1.3$ ), 20 cm ( $33.0 \pm 1.6$ ) and 30 cm ( $15.6 \pm 1.1$ ) from the nest did not differ ( $H_{2,57} = 3.2$ , n.s.). The total number of recruited workers was similar ( $H_{2,57} = 4.3$ , n.s.) in wood pieces located 10 cm ( $64.7 \pm 3.4$ ), 20 cm ( $73.1 \pm 6.0$ ) and 30 cm ( $25.6 \pm 2.3$ ) from the nest.

The number of workers exploiting (gnawing) wood pieces located 10 cm ( $61.2 \pm 3.3$ ), 20 cm ( $69.7 \pm 5.7$ ) and 30 cm ( $24.8 \pm 2.2$ ) from the nest was also similar ( $H_{2,57} = 4.3$ , n.s.).

**Table 1.** Latencies (mean  $\pm$  SE, in minutes) from the beginning of the test to the initial exploitation, initial recruitment and mass recruitment of *Nasutitermes corniger* workers in *Eucalyptus grandis* wood located at three distances from the nest inside the test arena during a 60-minute observation period in laboratory conditions (n = 20 colonies at each distance).

Distance to the available wood	Behavioral event <sup>1</sup>		
	Initial exploitation	Initial recruitment	Mass recruitment
10 cm	13.0 $\pm$ 0.8a	25.9 $\pm$ 0.8a	44.9 $\pm$ 1.1a
20 cm	9.5 $\pm$ 0.5a	19.6 $\pm$ 0.6a	34.2 $\pm$ 1.0a
30 cm	18.8 $\pm$ 1.2a	31.7 $\pm$ 1.0a	40.8 $\pm$ 1.0a

<sup>1</sup>(Different letters indicate significant differences in behavioral events, according to the Kruskal–Wallis test; p < 0.05).

#### 4. Discussion

As expected, *N. corniger* foraged for *E. grandis* wood. Some studies have shown that wood of other *Eucalyptus* species is also sought and consumed by *Nasutitermes* termites under laboratory conditions. In a natural resistance test of four wood species in the laboratory, wood of *Eucalyptus camaldulensis* Dehnh. exhibited the greatest mass loss (100.0% of the sapwood and 96.25% of the heartwood) due to *N. corniger* (Paes et al. 2013). *Nasutitermes corniger* preferentially consumed sapwood from *Eucalyptus cloeziana* F. Muell. compared to that of *Acacia mangium* Willd., *Casuarina equisetifolia* L., *Corymbia torelliana* F. Muell, *Tectona grandis* L. f. and *Caesalpinia echinata* Lam. under laboratory conditions (Paes et al. 2015). Gallio et al. (2018) observed that *Nasutitermes* termites preferred wood of *Eucalyptus dunnii* Maiden over that of *Eucalyptus saligna* Sm.

Under laboratory conditions, *N. corniger* foragers exhibited three typical foraging behavior events (initial exploitation, initial recruitment and mass worker recruitment) for *E. grandis* wood, regardless of the distance of this food source from the termite nest. Based on this outcome, the distance from the food source did not restrict *N. corniger* foragers from finding, recognizing and initially accepting equivalent food sources.

On the other hand, the three behavioral events of *N. corniger* foragers occurred at lower rates in the furthest wood pieces, which suggests that this termite prefers food sources closer to the nest. Andara et al. (2004) found similar results for *N. corniger* and *Nasutitermes ephratae* (Holmgren, 1910) (Termitidae), with lowest worker recruitment at the farthest food source (*Erythrina* wood) from the colony in laboratory experiments. These results may be linked to termites' heightened perception of allelochemicals in the wood with proximity to these food sources. Termites are attracted to areas with chemical exudates released from food sources; their perception of these chemical compounds can be affected by the distance between the food source and their nest (Grace and Campora 2005). In addition, Robson et al. (1995) and Campora and Grace (2001) conducted experiments with subterranean termites and observed that during foraging, termites did not exhibit directional orientation unless in close proximity to the food source, due to allelochemicals released from the wood.

Delaplane and La Fage (1987) examined the feeding behavior of *C. formosanus* in laboratory conditions and found that foraging individuals that discover multiple equivalent food sources concentrate their efforts on the first source located, i.e., chronological priority. However, this chronological priority was not observed in the current study, since a similar number of workers exploited the wood pieces (exhibited gnawing behavior) regardless of distance from the nest. This outcome indicates that *N. corniger* did not

select the available food sources based on distance from the nest. Therefore, the distance between equivalent wood pieces and the nest did not influence the food exploitation behavior of this termite. According to experiments by Campora and Grace (2001) and Puche and Su (2001) on *C. formosanus*, proximity to the wood did not influence the size of the tunnel or the number of foraging individuals. Nobre et al. (2007) reported similar findings for *Reticulitermes grassei* Clément, 1977 (Rhinotermitidae).

## 5. Conclusions

Under laboratory conditions, all foraging behavior events (i.e., initial exploitation, initial recruitment, and mass worker recruitment) of *N. corniger* were affected by the distance between a suitable food source (e.g., *Eucalyptus grandis* wood) and the nest; however, these events occurred at higher rates when the food source was located closer to the nest.

The latencies to these events and total numbers of recruited termites, recruited soldiers and workers, and workers exploiting (gnawing) the wood were not affected by the distance from a suitable food source to the nest of *N. corniger*.

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**Ethics Approval:** Not applicable.

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