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Effect of food resource and carton nest material on laboratory group survival of *Nasutitermes corniger* (Motschulsky, 1855) (Termitidae: Nasutitermitinae)

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Abstract. Ecological, behavioral, and toxicological studies with termites are frequently difficult to conduct under field conditions because their cryptic lifestyle. It is important to know the factors that can limit survival to ensure that laboratory studies will reflect the natural survival of these insects. Here, we tested the effect of a food resource and carton nest material on group survival of *Nasutitermes corniger* (Motschulsky, 1855) (Termitidae: Nasutitermitinae) in the laboratory. In general, termites survived longer in treatments with a food resource (sugarcane baits) compared to groups with carton nest material and control. However, groups with food resources exhibited high mortality due to fungal infection. The result of this study may contribute to the establishment of bioassay protocols performed with *N. corniger* in the laboratory.

Keywords: manipulative bioassays, protocols, termite, survivorship.

Termites (Blattodea: Isoptera) are considered one of the most important macrodetritivores of tropical forests due to their active participation in the decomposition and cycling of nutrients (Jouquet et al. 2011). These insects are also of great economic importance, due to damage caused by some species in agricultural and urban environments (Constantino 2002). Much of the ecological and economic importance of this group is due to their feeding habits. Termite species feed on cellulose in different stages of humification (from dry wood to soil) (Donovan et al. 2001).

Despite the high ecological and economic importance of termites, ecological, behavioral and toxicological studies are frequently difficult to conduct in the field because their cryptic lifestyle. It is important to know factors that can limit survival to ensure that laboratory tests will reflect the natural survival of these insects. While previous studies have been extensively tested the effect of markers (*e.g.*, Loreto et al. 2009; Marins et al. 2023) and abiotic factors (*e.g.*, Ferreira et al. 2019; Zukowski & Su 2017) on the survival of termites to develop bioassays protocols; the effects of food resources and nest material on group survival of Nasutitermitinae are still poorly studied. Only one study has been conducted to investigate the better substrate to survival of *Nasutitermes coxipoensis* (Holmgren, 1910) (Termitidae: Nasutitermitinae) (Albuquerque et al. 2008). It is important to highlight that, different from other termite groups, species of Nasutitermitinae subfamily are not easy to maintain in laboratory conditions. Such difficult in the maintenance of Nasutitermitinae species in laboratory impair the conduction of studies.

Colonies of the subfamily Nasutitermitinae, notably genus *Nasutitermes,* has the highest incidence of arboreal nesting. *Nasutitermes corniger* (Motschulsky, 1855) (Termitidae, Nasutitermitinae) is an urban and agricultural pest, which is widely distributed in the Neotropical region (Boulogne et al. 2017; Scheffrahn et al. 2005). The nests of this species are constructed from carton, masticated wood or feces, and other local materials cemented with salivary secretions (Noirot 1970; Thorne & Haverty 2000). The individuals of *N. corniger* feed on a wide variety of wood, and also with a capability to adapt to a variety of habitats and resources sources (Boulogne et al. 2017). According to Boulogne et al. (2017), *N. corniger* is the most important pest species of this genus in the New World.

In the present study, we evaluated the effect of a food resource and carton nest material on the group survival of *N. corniger* under laboratory conditions. We test the hypothesis that the combination of a food resource and carton nest material in the experimental arenas increase the survival of termite groups.

The study was carried out using five colonies collected at the Universidade Federal Rural de Pernambuco (UFRPE) - *campus* SEDE (8º04'03'' S, 34º55'00'' W), in Recife, Pernambuco, Brazil. The bioassays were conducted in Petri dishes (80 × 15 mm) with a filter paper. The following conditions were established: *(1)* termite groups (seven workers and three soldiers) alone (control), *(2)* termite groups with 3 g of carton nest material (NW), *(3)* termite groups with 5 g of freshly cut sugarcane baits (a surrogate of food resource) (SC); and *(4)* termite groups with 3 g of carton nest material and 5 g of freshly cut sugarcane baits (NW+SC). Nest material used in the bioassays was collected from the central part of the nest and it was kept whole without add water. All pieces of nests used were recently sampled. The choice of sugarcane as food resource was based considering previous studies with *N. corniger* that used sugarcane as bait in laboratory experiments (Sacramento et al. 2020; Silva et al. 2021). The caste proportion in the group was chosen to account for the natural proportion of soldiers in *N. corniger* colonies (*i.e.,* 30%) (Haverty 1977). Bioassays were conducted in laboratory under controlled conditions (25 ºC, 70% r.h.), kept in the absence of light. The number of dead individuals was determined at one-hour intervals for the first eight hours, and then daily until the death of all individuals. Three repetitions per treatment were performed for each of the five colonies tested, totaling 60 repetitions/bioassay.

Data were subjected to censored survival analysis under Weibull distribution, using R software (R Development Core Team 2019). Statistical analysis was used to check whether treatments (control, NW, SC and NW+SC) would affect the group survival of *N. corniger*. Contrast analysis was conducted to check differences among treatments.

To confirm whether the death of termites occurs by fungal pathogen, dead termites were placed in a humid chamber in a biochemical oxygen demand (B.O.D.) incubator (25 ºC, 12h photoperiod). The fungus sporulation was assessed after five days to allow sufficient time for

termite corpses to sporulate. The proportion of termites killed by fungi was assessed using Generalized Linear Model (GLM) with Binomial error distribution. Models were validated by inspecting residuals and testing for overdispersion. Differences among treatments were tested by Analysis of Deviance (ANODEV; a maximum-likelihood equivalent of ANOVA) followed by Contrast Analysis.

In general, the mean time to death of termites was 121.30 hours. The survival of termite groups was significantly affected by treatments (*Likelihood*= 5,983.9, *d.f.*= 600, $P < 0.001$). The survivorship of termite was lower in the treatments without sugarcane baits (control and NW) compared to treatments with sugarcane baits (SC and NW+SC) (Tab. 1; Fig. 1). A higher survival of groups kept with sugarcane compared to others food resources (decomposed wood, dry wood, and wood paper) was already observed for *N. coxipoensis* (Albuquerque et al. 2008). After a few days from the beginning of our bioassays, however, sugarcane baits showed a fungal appearance. Our study showed that proportion of termites killed by fungi was significantly affected by treatments (Deviance= 416.9, *d.f.*= 20, $P < 0.001$). The proportion of termites dead by fungi in the control and NW significantly differ from those in the SC and NW+SC groups. Termite mortality reached 68% and 72% due to the fungal pathogen in SC and NW+SC, respectively (Tab. 2). Such result can indicate that the presence of sugarcane baits as food resource in laboratory bioassays impaired the experiment because death occurs due to the presence of the fungus in the sugarcane and not because natural factors. Thus, to conduct experiments with sugarcane baits we suggest that sugarcane must be replace every two days to avoid proliferation of fungi or even used some antifungal.

Table 1. Mean time to death of termite groups among treatments (control, carton nest material [NW], sugarcane baits [SC] and carton nest material + sugarcane baits [NW + SC]).

Treatments	Mean time to death (hours)
Control	63.36
NW	72.89
SC	189.92
NW + SC	213.16

Table 2. Proportion of termites dead by fungi among treatments (control, carton nest material [NW], sugarcane baits [SC] and carton nest material + sugarcane baits [NW + SC]).

Treatments	% of termites dead by fungi
Control	0.00±0.00 a
NW	0.00±0.00 a
SC	0.72±0.04 b
NW + SC	0.68±0.02 b

Mean followed by the same letter did not differ significantly by ANODEV followed by Contrast Analysis.

Even though the nests of *N. corniger* are constructed from stercoral carton, and other local materials cemented with saliva, thus, incorporating antimicrobial substances, and provide a significant benefit to termites (Noirot 1970; Thorne & Haverty 2000; Bulmer et al. 2009). Our results indicate that the presence of carton nest material did not increase the termite survival compared to the control. This absence of significant variation can be explained with two factors: *i)* the size of carton nest material used in the present study did not provide enough chemical signal to increase group cohesion, and, therefore, increase survival; or *ii)* for *N. corniger* carton nest material did not enhance survival.

In conclusion, the results of this study may contribute to the establishment of bioassay protocols, maintaining adequate conditions for the survival of *N. corniger* under laboratory condition. Future studies must test the alternative food sources which can kept termite alive without proliferation of fungi. Another point that can be improved is use nest material in different conditions and the combination of temperature and substrate moisture (mL of water/g of nest material).

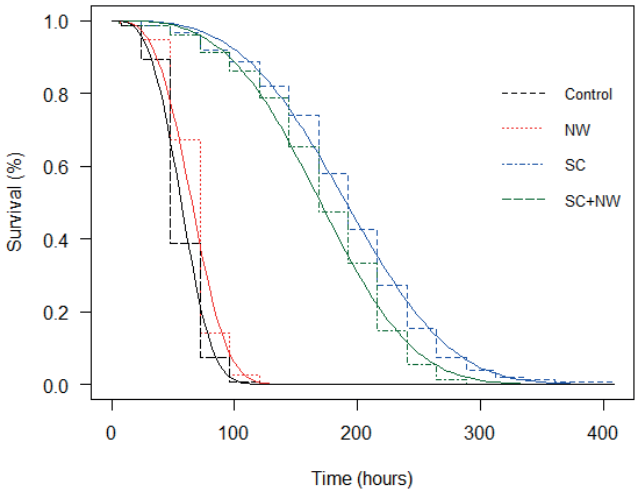

Figure 1. Survival curves for *Nasutitermes corniger* (Termitidae, Nasutitermitinae) groups under different treatments (control, carton nest material [NW], sugarcane baits [SC] and carton nest material + sugarcane baits [NW + SC]). All treatments significantly differ from each other by Contrast Analysis.

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Authors' Contributions

PFC conceived the experiment. LFF, CRS and RECS collected the colonies in the field and performed the bioassays. LFF and PFC performed the statistical analyses. All authors wrote the manuscript.

Conflict of Interest Statement

The authors declare no conflicts of interest.

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