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Short Communication

Effect of temperature and substrate moisture on group survival of *Constrictotermes* sp. (Isoptera: Termitidae) under laboratory conditions

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ABSTRACT

Laboratory bioassays should be performed under appropriate conditions that minimize stress and promote greater longevity to tested animals. For social insects, for instance, the stress triggered by removal individuals from nests may result in changes in their survival and behavior. Here we analyzed the effects of variations of different combinations of temperature and substrate moisture (mL of water/g of nest substrate) on group survival of *Constrictotermes* sp. (Termitidae: Nasutitermitinae). In general, lower substrate moisture resulted in faster mortality. On the other hand, groups survival longer at a temperature of 26 °C and under higher substrate moisture (2–3 mL/7 g of nest substrate). The result of this study may contribute to the establishment of bioassay protocols performed with *Constrictotermes* sp. in the laboratory.

Most of social insects harbor their colonies in nests that present controlled microclimate (*e.g.* temperature and humidity), which favors the maintenance of survival and reproduction of individuals. Different from the most terrestrial animals that exhibit adaptations to minimize the body water loss, termites are generally very susceptible to desiccation due the low sclerotization of their cuticle (Noirot and Darlington, 2000). In fact, these insects need environmental conditions with high levels of humidity for their survival and different strategies to circumvent environmental variations can be observed in termites. Termite species that need to leave the nest to search for food resources ("central-place foragers"), for example, build tunnels/galleries to protect themselves or may exhibit foraging habit at the night (Almeida et al., 2016). In spite of such strategies, seasonal variations are known to directly interfere termite forage rates (Iqbal et al., 2015; Moura et al., 2006).

Behavioral and/or ecological studies often require the complete removal of the nest or its parts to obtain individuals to be used in laboratory bioassays. However, the removal of the individuals from their natural environment may already interfere in their behavior and survival (Lenz, 2009). Thus, laboratory bioassays should be based on protocols that allow the maintenance of organisms in conditions as close as possible to their natural environment, so that to minimize stress and keep individuals alive as long as the test requires (Carvalho et al., 2018). In addition, the maintenance of more favorable conditions can also avoid behavioral changes, which could reflect in patterns inconsistent with those observed in natural situations.

Colonies of the genus *Constrictotermes* are common in Brazilian savannah ("*Cerrado*") and semi-arid environments ("*Caatinga*") (Mathews, 1977). The individuals of these species build epigeal and/or arboreal nests and their foraging occurs at night through columns in the open air. Individuals feed predominantly on debris and lichens on the surface of tree barks (Moura et al., 2006; Bourguignon et al., 2011). Colonies of *Constrictotermes* spp. can house a range of termitophiles and obligatory inquiline species (*Inquilinitermes* spp.) (Mathews, 1977; Cunha et al., 2003; Rosa et al., 2018), and therefore were studied in the laboratory in order to verify the mechanisms involved in the cohabitation of their nests (*e.g.* Cristaldo et al., 2016; Cruz et al., 2018).

In the present study, we perform bioassays to analyze the combinations of temperature and substrate moisture most adequate for the survival of individuals of *Constrictotermes* sp. under

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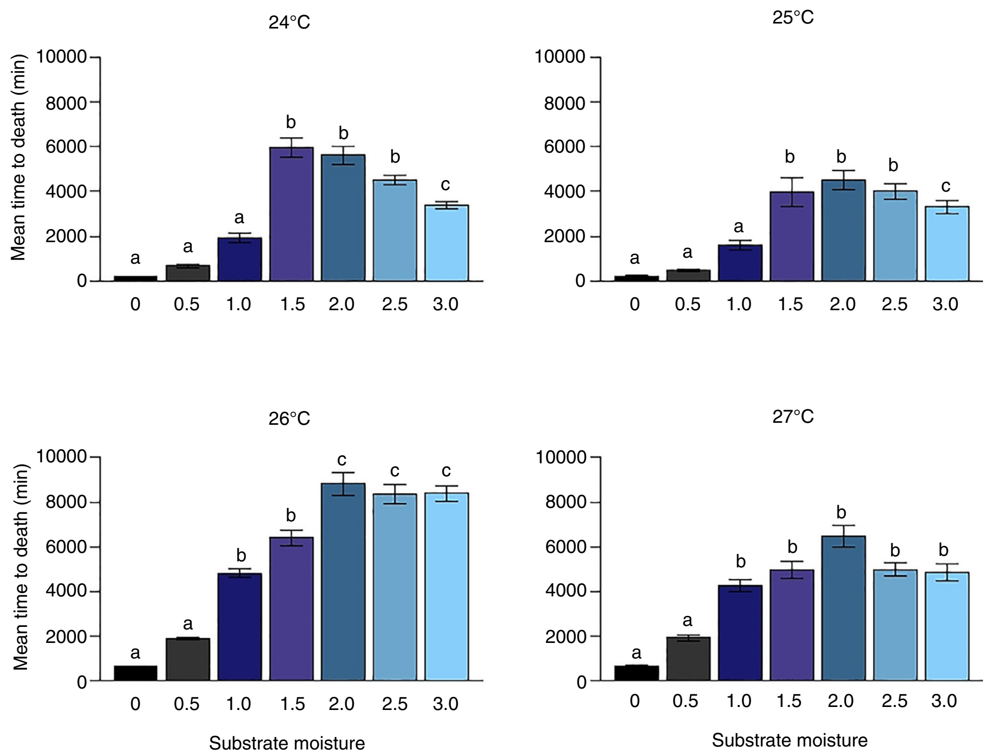

Fig. 1. Survival of *Constrictotermes* sp. in different combinations of temperature and substrate moisture (mL of water/7 g of nest substrate). Different letters in each graphic mean significant difference among treatments of humidity.

laboratory conditions. The study was carried out using six nests that were totally removed from the field, in the municipality of São Cristóvão (11′01°S, 37′12°W), Sergipe, Brazil. The bioassays were conducted in Petri dishes (5.5 × 1.5 cm) covered with 7 g of the substrate from the central part of the nests (*i.e.* dark part with high content of organic matter), that was previously sieved in mesh (6 mm). Twenty individuals (16 workers and 4 soldiers) were placed under the substrate of each Petri dish. During bioassays, no food was offered to the individuals. The treatments consisted of different combinations of temperature (24, 25, 26 and 27 °C) and substrate moisture (0.0, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mL of distilled water/7 g of nest substrate) determined by a complete factorial. The levels of substrate moisture were established with the addition of different volumes of water in a piece of cotton placed in the center of each Petri dish. The temperature was regulated in biochemical oxygen incubator (BOD), kept in the absence of light. For each combination of treatments ($n = 28$) three repetition/nest ($n = 6$) were performed, totaling 504 Petri dishes. The observations consisted in quantifying the number of dead individuals over time. Data were submitted to censored survival analysis under Weibull distribution, in R software (R Development Core Team, 2016). For each treatment, the mean time to death of individuals from each nest was calculated. Then, these data (mean time to death – "*y axis*") were submitted to Deviance Analysis (ANODEV) to determine the effect of combinations of temperature *x* substrate moisture ("*x axis*") on survival of *Constrictotermes* sp.

Our results showed that the mean time to death was significantly affected by temperature and substrate moisture ($F_{9,154} = 26.85$, $p < 0.001$) (Fig. 1). In general, the conditions of lower substrate moisture (0.0–0.5 mL of water/7 g of nest substrate) resulted in faster mortality (885.83 ± 16.77 min). These results corroborate with other studies that showed the importance of maintaining high humidity for termite survival (Gautam and Henderson, 2011; Wiltz, 2012; Zukowski and Su, 2017). Here, we observed that for *Constrictotermes* sp., the temperature of 26 °C combined with higher substrate moisture (2–3 mL of water/7 g of nest substrate) were the conditions that allowed greater survival of the individuals in the laboratory (8525.81 ± 136.84 min [*e.g.* 5.92 ± 0.10 days]). In addition to interfering in the survival of individuals as observed here, other studies have already demonstrated that variations in environmental conditions may also change termite behavior, such as tunneling patterns (Arab and Costa-Leonardo, 2005), feeding (Shelton et al., 2011) and intercolonial aggressiveness (Cornelius and Osbrink, 2009).

In conclusion, here we highlight the importance of maintaining adequate conditions for the survival of *Constrictotermes* sp. under laboratory conditions. The result of this study may contribute to the establishment of laboratory bioassay protocols.

Contributors

PFC and APAA conceived the research. DVF, JSC, JJMS and MLCR collected the colonies in the field and performed the bioassays. APAA performed the statistical analyses. DVF, PFC and APAA wrote the manuscript. All authors approved the final version of this manuscript.

Conflicts of interest

The authors declare no conflicts of interest.

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