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RESEARCH ARTICLE - TERMITES

Survival of *Coptotermes testaceus* (Isoptera: Rhinotermitidae) to Environmental Conditions (Relative Humidity and Temperature) and Preference to Different Substrates

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Abstract

Coptotermes testaceus (L.) (Rhinotermitidae) is a subterranean termite species that causes damage in urban and agricultural areas in the neotropics. Despite its economic importance, there are no studies on its basic biological aspects for laboratory management and the development of strategies for its control. The objective of the present study was to evaluate the relative humidity, temperature, substrate moisture and preference to different wood substrates for the best *C. testaceus* survival under laboratory conditions. For this, a range of eight relative humidity (from 9 to 100%), three temperatures (20, 25 and 30 °C), six substrates (*Pinus* sp, *Cedrela odorata* (L.), *Cocos nucifera* (L.), *Eucalyptus urophylla* (S. T. Blake), *Haematoxylum campechianum* (L.) and *Tabebuia rosea* [Bertol.] DC) and four substrate moistures, (0 to 60%) were tested. The results of this study indicated a significant effect of all factors on termite survival or termite preference. When tested independently, the highest survival percentage of *C. testaceus* was obtained with humidity of 100%, temperature of 20 °C, substrate moisture of 60% and the *Eucalyptus urophylla* substrate, reaching 83.33% survival at 21 days of observation. With these preliminary assays on small termite groups, it is concluded that with the appropriate percentages of humidity, temperature and substrate and the interaction of these three factors, further research can be conducted using larger termite groups in biologically relevant conditions, in order to study various aspects of *C. testaceus* biology.

Introduction

Coptotermes Wasmann (Isoptera: Rhinotermitidae), is a genus of subterranean termites that currently has 21 validated species (Chouvenc et al., 2016), of which 16 are classified as pests of economic importance in various parts of the world (Krishna et al., 2013). While only two of these species, *C. formosanus* Shiraki, and *C. gestroi* (Wasmann) have demonstrated remarkable invasive abilities, most *Coptotermes* species still have an economic impact in their native range. *Coptotermes* have the ability to infest live trees, create large, populous colonies, with extensive foraging abilities, which can reach more than 100 m of underground galleries (Greaves, 1962; King & Spink, 1969; Su & Scheffrahn, 1988). *Coptotermes testaceus* (L.) is the only endemic *Coptotermes* species in the new world and is established throughout most of the neotropics (Scheffrahn et al., 2015; Chouvenc et al., 2016). This species has been found affecting plantations of forest importance such as rubber (*Hevea brasiliensis* [Willd. ex A.Juss.] Müll.Arg.) (Apolinário & Martius, 2004; Krishna et al., 2013) and eucalyptus (*Eucalyptus urophylla* S. T. Blake) (Santos et al., 1990; Amaral-Castro, 2004); agricultural crops such as cassava (*Manihot esculenta* C.), cocoa (*Theobroma cacao* L.) and sugar cane (*Saccharum officinarum* L.) (Krishna et al., 2013); and wooden constructions in urban areas (Bandeira et al., 1989). In Mexico, the *C. testaceus* population represents the northern most distribution of the species (Light, 1933; Sheffrahn et al., 2015) and was reported to have an agricultural impact, damaging roots and stems of crops of economic, social and cultural importance (López-Vera et al., 2018; Capetillo-Concepción et al., 2019).

Due to the economic importance of *C. testaceus* as a pest, it is necessary to find strategies for its control. However, to date, the environmental requirements to keep populations alive for prolonged periods of *C. testaceus* in the laboratory that allow the establishment of bioassays to evaluate their control, have not been reported. On the other hand, there is a continuous search for friendly alternatives to the environment and human health, for termite control. These studies generally start with laboratory bioassays, for example, the use of target-specific chemicals (baits and termiticides). While a lot of research efforts have been focusing on botanicals (essential oil, seed, bark, leaf, fruit, root, wood, resin) and entomopathogens (fungi, bacteria and nematodes), none has resulted in practical or commercial application (Verma et al., 2009; Chouvenc et al., 2011).

However, achieving the establishment of live subterranean termite colonies in the laboratory is logistically complicated, time consuming and challenging (Chouvenc, 2018), due to factors such as temperature (Fei & Henderson, 2002; Nakayama et al., 2004; Gautam & Henderson, 2011; Wiltz, 2012), humidity (Nakayama et al., 2004; Wong & Lee, 2010; Gautam & Henderson, 2011; Wiltz, 2012) and the food source (Smythe & Carter, 1969; Su & Tamashiro, 1986). Particularly, desiccation is determining factor for termite survival, so it is important that in laboratory survival studies, not only relative humidity is considered, but also the substrate moisture and food (Hu et al., 2012; Zukowski & Su, 2017).

In order to provide preliminary information of the basic biological requirements for the survival and maintenance of *C. testaceus* in laboratory conditions, the aim of this study was to evaluate the survival of *C. testaceus* at various levels of relative humidity, temperature, substrate moisture, and preference for different substrates, to obtain the appropriate conditions that allow establishing live termite colonies in the laboratory, to ultimately carry out bioassays to study the control of *C. testaceus*.

Materials and methods

Obtaining C. testaceus

The specimens were collected in the botanical garden of the Academic Division of Biological Sciences of the Universidad Juárez Autónoma de Tabasco. Traps (modified from Tamashiro et al., 1973) built with a metal cylinder (18.5 cm high x 15.5 cm in diameter) with exposed ends and internal walls lined with wood were used, a roll of corrugated paper 10 cm in diameter was placed in the center. The trap was placed in the basal part of trees that had damage caused by *C. testaceus*, buried in the ground 20 cm deep with the top of the trap covered. The corrugated paper cylinder with termites was removed after five days and transferred to the laboratory. In this study only workers were used of a single colony (a total of 1740).

C. testaceus survival at different percentages of relative humidity (RH) and temperatures

The methodology for this section was proposed by Zukowski and Su (2017), with modifications. Environmental chambers (EC) were conditioned using plastic containers with lid (24.7 × 17 × 6.4 cm), with a 2.7 cm diameter hole in the central part of the lid to introduce a digital hydrometer. The RH inside the EC was stabilized using various stabilizing materials (RHSM) in different amounts (Table 1). To achieve a high RH, the water was placed in plastic containers (6 cm diameter x 3.6 cm height) and cotton, covering the bottom of the EC; the salts and the silica gel were placed in the plastic containers described above and for the lowest RH, the $CaCl_2$ was spread at the bottom of the EC. The RH in the EC was assessed for 15 days with a digital hygrometer (VWR; TRACEABLE ™). The temperature was stabilized at 20, 25 and 30 °C in an incubator (NOVATECH, MOD. DBO-200).

The bioassay was carried out using five Petri dishes in each stabilized EC, the Petri dishes (60 mm x 15 mm) contained a filter paper disk (60 mm) to facilitate locomotion of the termites and as a source of food was pine sawdust (200 mg), 10 termite workers were placed in each box. The daily record of live termites was monitored, reporting the survival percentage. Dead termites were removed daily. The bioassay required five repetitions with ten workers in each repetition for the eight RH at the three temperatures tested (a total of 1,200 workers).

Table 1. RHSM and amounts used to obtain different RH.

RHSM	Formule	Amount	RH obtained (%)[a]
Water, in cotton	H_2O	200 ml	100 ± 0.25
Water	H_2O	100 ml	83.25 ± 0.55
Undiluted salt	$Mg(NO_3)_2$	100 g	75.28 ± 0.77
Saturated saline solution	NaCl	100 ml	64.88 ± 0.59
Silica gel	Silica gel	100 g	61.12 ± 0.83
Saturated saline solution	$MgCl_2$	50 ml	42.98 ± 2.43
Undiluted salt	$CaCl_2$	100 g	23.88 ± 1.55
Undiluted salt	$CaCl_2$	300 g	9.89 ± 0.11

[a]Mean (M) ± Standard Error (SE). n = 18.

C. testaceus preference towards different substrates (woods)

Sawdust from pine (*Pinus* sp.), cedar (*Cedrela odorata* L.), coconut fiber (*Cocos nucifera* L.), eucalyptus (*Eucalyptus urophylla* S. T. Blake), blackwood (*Haematoxylum campechianum* L.) and pink poui (*Tabebuia rosea* [Bertol.] DC) were used with a homogeneous particle size (sieve No. 16, 1.13 mm spacing). They were dehydrated in a drying oven (Felisa® Brand) at 50 °C for 24 h and stored in a desiccator with dehydrator until use for bioassays.

A device was designed to allow a high RH and for termites to choose between different substrates (Fig 1). Small environmental chambers (sEC) were used, which consisted of 8 x 5.5 cm (diameter x height) circular plastic containers, which inside contained plastic vials of 4 x 2 cm, suspended inside each sEC. Cotton was placed at the bottom of each radial sEC with water to obtain the RH of 100% and the substrates were placed in the vials (Fig 1a). Using 6 x 1 cm plastic tubes, six radial sEC were connected to a central chamber. The device was placed in the incubator at 20 ºC and the RH and temperature were stabilized for 4 h. Once the RH and temperature had stabilized, 30 termites were deposited in the central chamber and at 24 h the number of termites within each sEC with substrate was recorded, the termites found in the connection tubes were also included in the counts. The aggregation of termites to substrates was considered as preference. The RH and temperature were monitored during the bioassay and the percentage of preference was reported. The bioassay required ten repetitions with 30 workers in each repetition (a total of 300 workers).

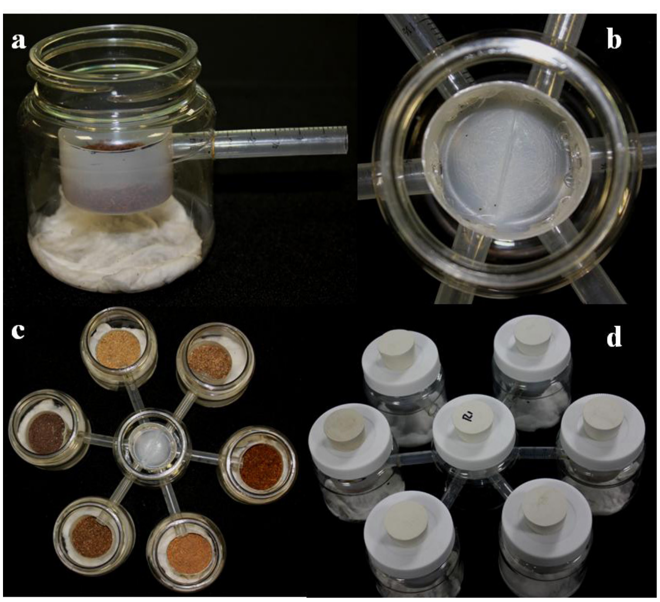

Fig 1. Device for high RH and preference of termites towards different substrates. a) Radial small environmental chamber, with suspended vial; b) Central chamber for release of termites with vial connected to six radial small environmental chambers; c) device with the six substrates for multiple choice; d) device with modified caps for HR monitoring.

Survival of C. testaceus at different substrate moisture

The most preferred substrate previously conditioned was used. In Petri dishes (60 mm x 15 mm) 3 g of the substrate were placed and sterile distilled water was used to obtain substrate moistures (SH) of 0, 20, 40 and 60%.The formula used to obtain the desired SH (NMX-AA-16-1984) was as follows: $H = \frac{G - G1}{G} \times 100$

Where: H = % of humidity

G = Wet sample weight in g

G1 = Dry sample weight in g

Six Petri dishes were placed in environmental chambers (used in the survival test at different RH and temperature) with the stabilized RH of 100 ± 0.25% (Fig 2a). Groups of ten termites were placed in each Petri dish with the substrate at the required humidity (Fig 2b). This bioassay was incubated at 20 °C in total darkness. The daily count of living termites was recorded, and the survival (percentage of live termites) was reported. The bioassay required six repetitions with ten workers in each repetition for the four SH evaluated (in total 240 workers).

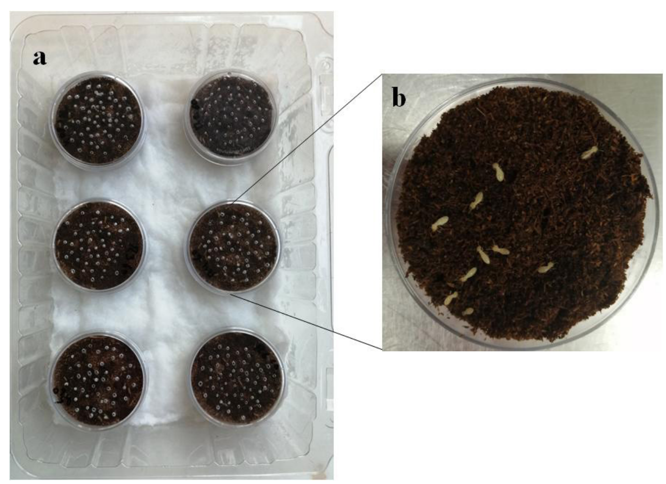

Fig 2. a) Petri dishes in environmental chambers with the stabilized RH of 100 ± 0.25%; b) Group of ten termites placed in the Petri dish with the substrate at the required humidity.

Experimental design and statistical analysis

For all bioassays, a completely randomized simple design was used. The statistical analysis used for the termite survival test at different RHs and temperatures was an analysis of variance (ANOVA) for an 8 by 3 factorial experimental design (8 RHs and 3 temperatures), the response variable was the percentage of survival. Statistical analysis for preference towards different substrates was performed with a simple analysis of variance (ANOVA) and the percentage of preference as the response variable. A comparison of means with Fisher's LSD with an α = 0.05 was performed for these two trials. Finally, for the survival of termites towards different SH, a Kaplan-Meier LogRank analysis and the comparison of Holm Sidak means with an α = 0.05 were used, where the highest median lethal time (LT_{50}) was considered to select the best treatment. Because the results of the first two trials were expressed as a percentage, it was necessary to transform the data to the square root of the arcsine prior to ANOVA. The statistical package used for all analyzes was SigmaPlot 12.0.

Results

C. testaceus survival at various RH levels and temperatures

Table 2 shows the survival rate of *C. testaceus* 24 hours after being exposed to eight RH and three temperatures. The RH of 100% presents the highest percentage of survival at 24 hours of observation for the three temperatures evaluated (F = 109,313; df = 7; P <0.001). There was a greater survival of termites at a temperature of 20 °C (F = 192,213; df = 2; P < 0.001). On the other hand, the interaction between the two studied factors, in the same way, generated a significant effect (F = 7,885; df = 14; p < 0.001), being the combination of 20 °C and 100% RH the treatment that provided the highest termite survival, with 88.4 ± 0.07%. Likewise, it was observed that as the RH decreases, the termite survival also does it gradually, while at higher temperatures, it's a lower survival.

C. testaceus preference for various substrates (woods)

Termites showed a statistically different response in their aggregation toward a substrate after 24 hours of observation (F = 4,630; df = 5; p < 0.001, Fisher's LSD α = 0.05). The most preferentially aggregated on eucalyptus, with 68.75%, followed by blackwood with 12.5% and pine with 10%. The substrates that obtained the lowest preferences were coconut fiber, pink poui and cedar, with 4.38, 4.37 and 0.0% respectively (Fig 3).

Survival of C. testaceus at different substrate moisture (SH)

The four SHs showed a significant difference (Statistic = 385,312; df = 3; P < 0.001) with respect to *C. testaceus* survival during the bioassay. The SH of 60% obtained the highest survival, a LT_{50} value was not recorded, because after 21 days of the bioassay the percentage of live termites was 83% (LT_{50} > 21 days). The SH of 40%, 20% and 0% obtained a LT_{50} of 21, 13 and 1 days, respectively (Fig 4).

Table 2. Percentage of survival (M ± SE) of *Coptotermes testaceus* 24 hours after being exposed to eight RHs and three temperatures. n = 5.

	RH[a]							
	H_2O	H_2O	$Mg(NO_3)_2$	NaCl	SÍL GEL	$MgCl_2$	$CaCl_2$	$CaCl_2$
T°C	(100 ± 0.3%)	(83.25 ± 0.5%)	(75.28 ± 0.8%)	(64.88 ± 2.5%)	(61.12 ± 3.5%)	(42.98 ± 2.4%)	(23.88 ± 0.4%)	(9.89 ± 0.1%)
20	88.4 ± 0.07Aa	71.8 ± 0.07Ab	70.5 ± 0.07Ab	69.8 ± 0.07Ab	39.6 ± 0.07Ac	31.5 ± 0.07Acd	31.5 ± 0.07Acd	20.7 ± 0.07Ad
25	74.7 ± 0.06Ba	59.5 ± 0.06Ab	9.3 ± 0.06Bc	9.3 ± 0.06Bc	6.7 ± 0.06Bc	0.0 ± 0.0Bc	0.0 ± 0.0Bc	0.0 ± 0.0Bc
30	74.7 ± 0.05Ba	23.2 ± 0.05Bb	13.9 ± 0.06Bbc	9.26 ± 0.05Bcd	4.63 ± 0.05Bcd	0.0 ± 0.0Bd	0.0 ± 0.0Bd	0.0 ± 0.0Bd

[a] Different lowercase letters within a row or different uppercase letters within a column indicate significant differences between Means.

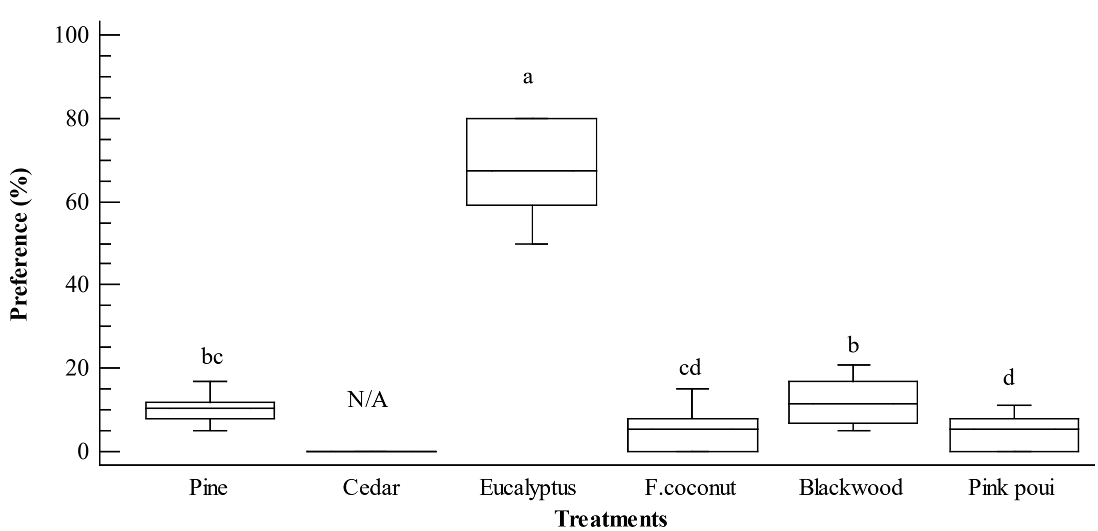

Fig 3. Percentage of preference of *Coptotermes testaceus* workers 24 hours after being exposed to six substrates (woods), at an RH of 100% and a temperature of 20 °C, n = 10. Different letters indicate significant differences between treatments.

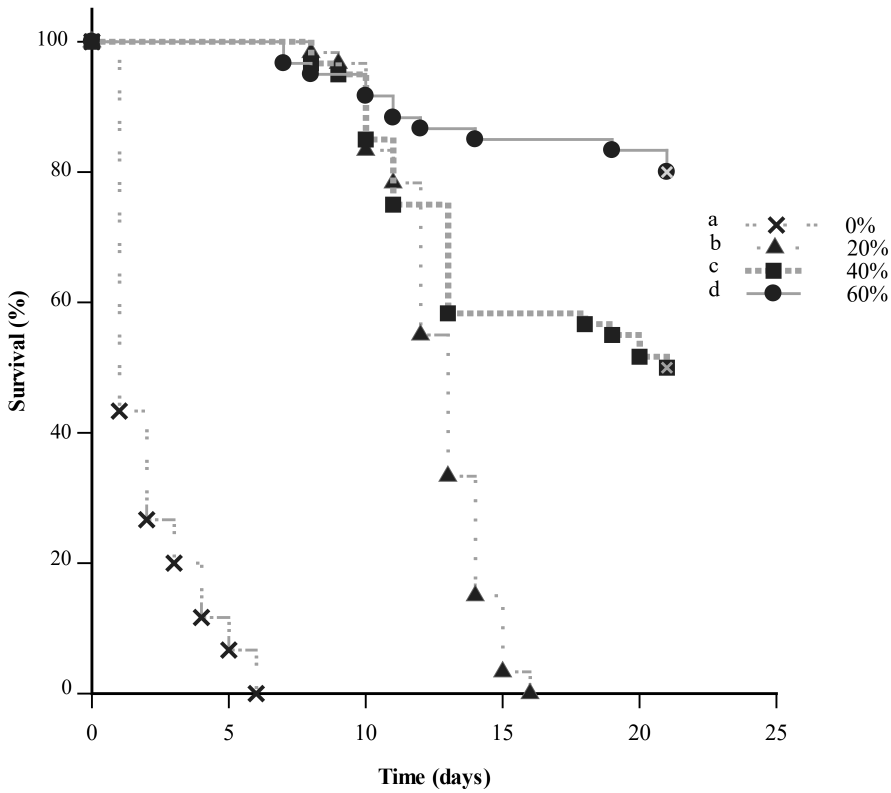

Fig 4. Percentage of survival of *Coptotermes testaceus* workers after being exposed to four substrate moistures (0%, 20%, 40% and 60%) at 20 °C and 100% RH, n = 6. Different letters on substrate moisture indicate significant differences (Holm Sidak, α = 0.05).

Discussion

The results of this study showed that the highest survival (88.4%) of *C. testaceus* was at 100% RH and temperature of 20 °C. This shows that both RH and temperature are important for the survival of *C. testaceus*, but they are even more so when the interaction between these factors occurs. This agrees with that reported by Gautam and Henderson (2011) as well as Wiltz (2012), who found the highest survival percentages for *C. formosanus* at low temperatures (20 and 10 °C) and high RH (98 and 99%) respectively. The results of this study show an effect of RH and temperature, however, these two factors alone cannot maintain the *in vitro* survival for long periods of time (24 h for this study), which is necessary for studies focusing on control aspects of this pest.

Lenz (2005) points to substrate as another factor that must be considered to obtain successful results in bioassays. The substrate can be used for the construction of their galleries, food or as indicated by Hu et al. (2012) and Zukowski and Su (2017), a resource for obtaining moisture. That is why, in this study, the preference that *C. testaceus* had towards six different woods that could function as a substrate was also evaluated. The results obtained in this work indicated that eucalyptus was the substrate with the greatest choice. This coincides with studies carried out in agricultural systems where it was reported that *C. testaceus* was the main responsible for infestations in eucalyptus plantations (Amaral et al., 2004). This could be due to the fact that, as Zabel and Morrel (1992) points out, the different kinds of wood have the presence of extractable (chemicals). The part of the wood in which the extractables are produced is what determines if they play a role as attractants or repellents, i.e. in the sapwood, there is a higher concentration of starch and carbohydrates which makes this part of the wood more palatable to the biological agents that attack it. On the other hand, the heartwood has a series of components that make it less preferred to these agents (Kollmann, 1959). This leads us to think that eucalyptus was the most attractive substrate due to its chemical composition, in addition to cellulose, which is the main food source of xylophagous insects, such as termites (Bignell & Eggleton, 2000; Ramírez & Lanfranco, 2001; Shimada & Maekawa, 2010). This coincides with that reported by Scheffrahn (1991), who likewise attributes the preference of termites to this type of (extractable) substances.

Finally, and once the most preferred substrate was identified, different humidity levels were evaluated in this substrate. McManamy et al. (2008) pointed out that the humidity of the substrate is an important factor for the prolonged survival of species of subterranean termites, this due to the fact that after subjecting *Reticulitermes flavipes* (Kollar) (Isoptera: Rhinotermitidae) to ideal conditions of temperature and RH, but exposed to low moisture wood, the termites did not survive. Our results indicated that the humidity of the substrate of 60% (the highest tested), was the one with which the longest survival time of the termite (21 days) was reached, reaching 83.33% of live insects at the end of this time. This coincides with that reported by Zukowski and Su (2017) for the termite species *C. formosanus*, which, after three weeks of observation (21 days), reached 90% survival with wet food, but when the food source provided was dry, survival was 0%. These results lead us to agree with what was pointed out by Gautam and Henderson (2014), who pointed out that subterranean termite species are extremely susceptible to desiccation, therefore, they require not only high RH, but also other sources of humidity for its greatest survival.

At a global level, although studies of this type were conducted on pest species of subterranean termites (Rhinotermitidae), the current study provides preliminary information on optimal experimental and rearing conditions in the laboratory for *C. testaceus*. As previously shown, the importance of the biological relevancy of a bioassay when testing control method against subterranean termites in laboratory conditions is critical (Su, 2005; Chouvenc, 2018), to study the applicability of such approach in a field situation. The current study therefore provides initial guidelines for the manipulation of *C. testaceus* in the laboratory, thus being able to carry out future studies aimed at testing control approach for this species.

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Contribution of the authors

CO Pozo-Santiago, conception, design, data collection, analysis, interpretation of results and document writing; M Pérez-De la Cruz, conception, design, critical review for important intellectual content, interpretation of results and final approval of the version to be published; JR Velázquez-Martínez, conception, design, critical review for important intellectual content, interpretation of results and final approval of the version to be published; M Torres-De la Cruz, design, critical review for important intellectual content, interpretation of results and final approval of the version to be published; A De la Cruz-Pérez, design, critical review for important intellectual content, and final approval of the version to be published; S Capello-García, design, critical review for important intellectual content, and final approval of the version to be published; MA Hernández-Gallegos, critical review for important intellectual content, and final approval of the version to be published.

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