MAINTENANCE OF COLONIES OF *CORNITERMES CUMULANS* (BLATTODEA: TERMITIDAE) FOR PREFERENCE TESTING

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ABSTRACT
Termites are social insects, with differentiated castes for specific functions within the colony, and play an important ecological role, contributing to the recycling of nutrients and soil bioturbation. The species Cornitermes cumulans is known for its economic damage to forest plantations, especially eucalyptus seedlings.

Keywords: Termites. Bioturbation.

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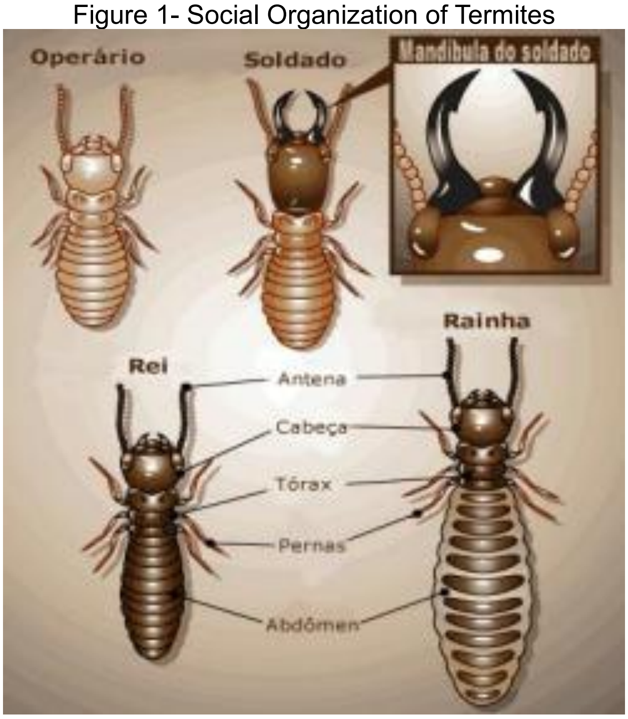
INTRODUCTION

Termites are eusocial insects, they form colonies of individuals with overlapping generations, cooperative care of offspring and division of labor. The social structure of these insects is composed of individuals that develop by paurometabolia, morphologically distinct (polymorphic) and classified into castes with specific functions within the colony (Oliveira et al., 1986).

They were classified as belonging to a separate order (Isoptera), but studies have shown that termites have genetic and molecular similarities to cockroaches, and were included as part of the order Blattodea, with Isoptera being an infraorder or epifamily. Because they are a clearly monophyletic group and the name Isoptera is well established, this terminology is still accepted and can be used to refer to these insects (Constantino, 2020).

In a termite nest three distinct castes are found: 1) Worker caste, the most numerous caste that performs all routine functions such as obtaining food, building and repairing the nest and tunnels, caring for the offspring and providing food to the other castes; 2) Caste of soldiers and are responsible for the defense of the nest and the protection of workers during foraging; and 3) Caste of breeders who are responsible for the generation of new individuals and the multiplication of colonies. According to Costa-Leonardo (2002), among the reproductive individuals is the royal couple, king and queen, who found the nests and remain in them throughout the life of the colony (Figure 1).

Figure 1- Social Organization of Termites



Source: Wilson, T., 2008

Cornitermes cumulans (Kollar, 1932) is a species of termite known as "mound termite" because of the huge termite mounds it builds and its high incidence in pastures, constituting an exception when found in homogeneous plantations with forest species (Perez Filho et al, 2012). In pastures, the term "mound termite" has been associated almost exclusively with this species (Valério et al., 2020).

There are three stages in the development of the termite mound: the initial one, totally underground; the intermediate portion, when a small epigeal portion appears, and the final one, when most of the nest is epigeal. The nest of C. cumulans is fragile and totally underground in the initial stage of growth. Little by little, it emerges at ground level and its surface then becomes resistant and hard (Valério, 2006) (Figure 2).

Figure 2 - Nests of *Cornitermes* sp. in pasture areas of *Brachiaria brizantha*. Parauapebas-PA.

Source: Patrícia Leitão Lima

In areas of natural vegetation, termites play an important ecological role, as they actively participate in the recycling and decomposition of nutrients in the ecosystems where they live. The action of termites can modify the porosity and structure of the soil, improving aeration, which provides greater water infiltration and propagation of plant roots (Costa-Leonardo, 2002).

These activities of digging, building nests, forming tunnels and moving organic matter, called bioturbation, physically alter the soil, which can have important effects on soil structure, nutrient cycling and even ecosystems, so just like ants, beetles and earthworms, termites are called ecosystem engineers.

The basic food of termites is cellulose, but the source of cellulose used varies according to the species (Vasconcellos, 1999). Most species feed on wood in the most varied stages of decomposition, others may feed on humus, lichens, herbivore feces, or fungi grown inside the nests (Medeiros, 2004; Lima and Costa-Leonardo, 2007a; Zorzenon et al., 2011).

However, the feeding habits and damage caused by Cornitermes, in forest nurseries or in transplanted seedlings, are little known (Peres Filho et al., 2012). Termites can feed on wood (living or dead, in different stages of decomposition), grasses, herbaceous plants, leaf litter, fungi, nests built by other species of termites, to even excrement and animal carcasses, as well as lichens, organic material present in the soil, and in some cases, cultivate fungi, thus revealing their ecological importance is as significant as the economic one (Lima & Costa-Leonardo, 2007), becoming pests due to ecological imbalances (Núñez, et al., 2011).

Termites are more destructive in eucalyptus plantations in the seedling phase, as they cut roots and roots, removing the bark from the main roots, causing damage to plants up to one year of age (Junqueira, et al., 2008). In general, the presence of termites of the species C. cumulans on the roots of eucalyptus can cause direct damage, consuming the root system, and, consequently, indirect damage such as the reduction and delay in root growth and in the aerial part of eucalyptus seedlings, where from 60 days after exposure to the attack there is a reduction in variables such as length, root surface and diameter (Leitão-Lima et al, 2013).

USE OF BAITS FOR TERMITE MONITORING

Almeida & Alves (1995) tested different types of cellulosic materials and found that corrugated cardboard was the most attractive to termites of the genera: Cornitermes, Syntermes, Procornitermes, Coptotermes and Nasutitermes. Junqueira (1999) suggests that cardboard baits, monitored in reforestation areas, do not have the same efficiency as in agricultural areas, because in reforestation soils there are roots, bark, wood remains, etc., which are more attractive to termites.

Leitão-Lima et al. (2012) observed that corrugated cardboard was unattractive to the species C. cumulans both in the laboratory and in the greenhouse. Leitão-Lima et al. (2014) in pasture areas tested the standard corrugated cardboard baits of the Termitrap type, where they were cut to the measurements: 50 cm x 30 cm, then rolled in the shape of a "cigar" (Figure 3).

Figure 3 - Corrugated cardboard baits cut to the measurements: 50 cm x 30 cm, then rolled up and in the shape of a "cigar" and secured with masking tape, placed in the field. Parauapebas-PA.

These baits were taken to the field to carry out the monitoring of termites Parauapebas-PA; in three rural properties, where 20 standard cardboard baits were distributed by area, buried vertically in the ground at a depth of approximately 20 cm, distant 4m from each nest (5 nests with 4 baits/nest), in a total of 60 baits, remaining in the field for 30 days in the dry season and 15 days in the rainy season (Figure 4). Termite monitoring consisted of removing the baits from the soil and checking for the presence of C. cumulans, according to the following criterion of scores: (0) absence of termites; (1) 1 to 10 termites; (2) 10 to 100 termites; and (3) presence of more than 100 termites.

Figure 4 - Representation of the placement of corrugated cardboard baits, buried vertically in the ground at a depth of 20 cm.

There was a higher occurrence of termites of the genera Nasutitermes and Heterotermes inside the baits, with a predominance of Heterotermes and absence of species of the genus Cornitermes. The corrugated cardboard bait is efficient for monitoring subterranean termite species such as the genus Heterotermes. However, for mound termite species, corrugated cardboard baits were not efficient.

TERMITE MAINTENANCE IN THE LABORATORY

In the preference tests of Cornitermes cumulans, in the laboratory, using the methodology of Leitão-Lima et al. (2012), for each arena, the following were separated: 300 workers + 50 soldiers + 50 nymphs, to compose each block and its treatments (Figure 5).

Figure 5 - Separation in the laboratory (300 workers, 50 soldiers and 50 nymphs) to perform the foraging tests of *Cornitermes cumulans*.

Source: Patrícia Leitão-Lima

Each arena, consisting of a plastic pot (26 cm in diameter and 12 cm in height), with side holes, for fitting glass tubes (2 cm in diameter and 8 cm in length) containing the tested treatments of eucalyptus root and components for the seedling substrate (Figure 6).

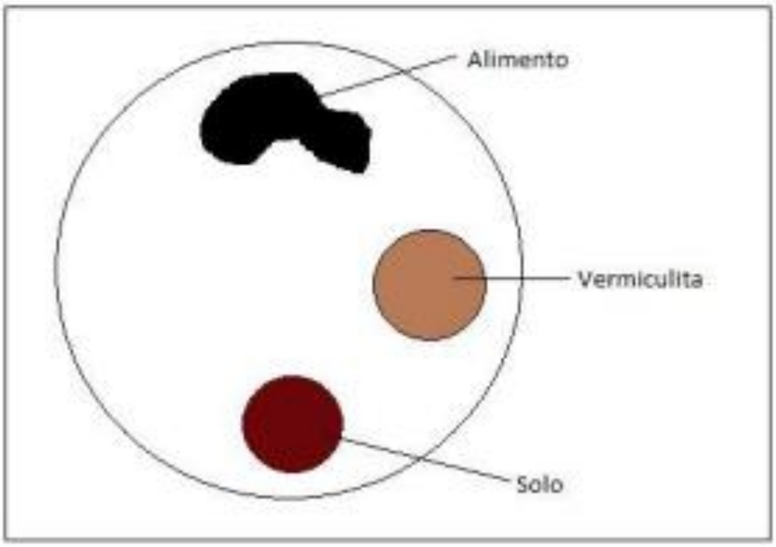
Figure 6 - System called "arena", used for the maintenance of *C. cumulans* in laboratory conditions, where: (a) glass tube containing one of the treatments and (b) nest fragment on a gypsum base and covered by a plastic container, Botucatu-SP, 2003.

Source: Patrícia Leitão-Lima

Leitão-Lima et al. (2012) also evaluated, in the laboratory, the preference of Cornitermes sp to different structures of B. brizantha (leaf, stem and root), "in natura" and dry, from degraded pastures in the Amazon compared to cardboard baits,

Schönhaus (2012) evaluated, in the laboratory, the feeding habits of C. cumulans in relation to different food items, for each experiment five Petri dishes of 9 cm in diameter were used, in which a plastic lid with moistened vermiculite and a small portion of moistened soil were placed. On each plate, 30 workers and 3 soldiers were placed and the food item to be tested, which included four different foods: dry grass leaves, healthy pine sawdust, decomposing pine sawdust, and the food itself that is stored inside the nest (Figure 7).

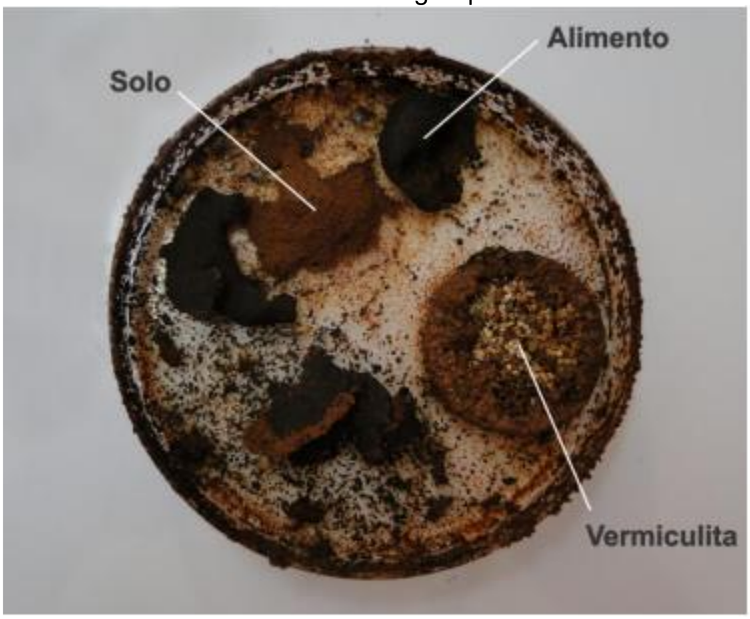
Figure 7 - Scheme representing the arena used for the development of bioassays with different foods.



Source: Schönhaus (2012)

According to Schönhaus (2012), it was not possible to clearly quantify consumption, as the insects showed a behavior of covering food with feces and soil. Often, clumps of soil were observed that surrounded the pieces of food, forming hard spheres of soil and food (Figure 8).

Figure 8 - Maintenance of *Cornitermes cumulans* termite groups in Petri dishes under laboratory conditions.

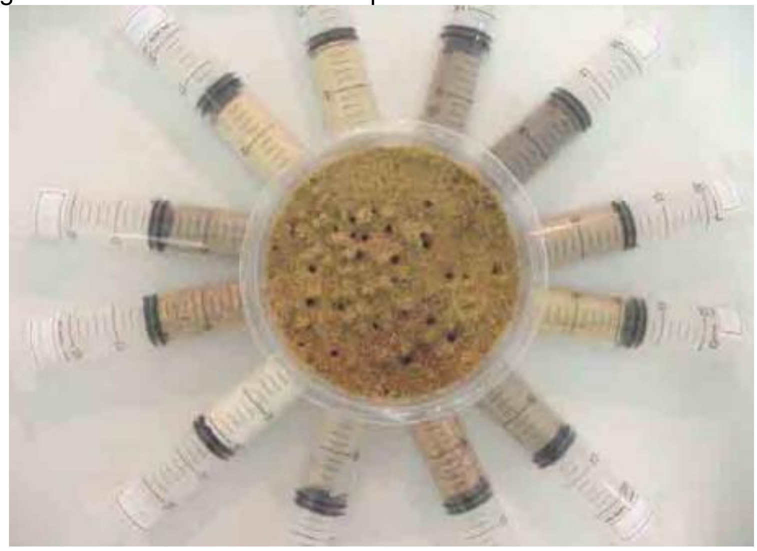


Source: Schönhaus (2012)

For Schönhaus (2012), termites of the species C. cumulans have a very complex eating habit, based mainly on foods of plant origin. Morphological adaptations in the jaws and alimentary canal allow these insects to consume these components. In addition, this species has a feeding preference for decomposing materials, as in the case of aged sawdust, which has shown excellent results for termite survival under laboratory conditions.

Lima (2006), in the selection of attractive cellulosic substrates for the termite Coptotermes gestroi, tested several substrates in arenas composed of a central round plastic container (10cm in diameter and 3.5cm in height - volume equal to 240mL), a "nest" chamber, in which 12 syringes of 20mL each, equidistant from each other - 30º angulation between the syringes, were connected. Each syringe was filled with a different substrate, totaling 12 substrates per repetition (Figure 9)

Figure 9 - Arena used in the multiple-choice tests with 12 substrates.



Source: Lima (2006)

After evaluating the 12 substrates, Lima (2006) performed multiple-choice tests with 4 substrates (ground dry corn stalk, rolled corrugated cardboard, dry cattle manure and chopped cork). Each syringe was filled with a different substrate, totaling 4 substrates per repetition, and the position of each of these substrates was randomly defined by lottery. 10 replications were performed, and in each one 550 forage individuals (500 workers + 50 soldiers) were used (Figure 10).

Corrugated cardboard was the substrate most consumed by Coptotermes gestroi, followed by chopped cork and cattle manure, the least consumed substrate was corn stalk. Corrugated cardboard was the most indicated substrate.

Figure 10 - Arena used in the multiple-choice tests with 4 substrates.

Source: Lima (2006)

MAINTENANCE OF TERMITES IN A GREENHOUSE

Leitão-Lima et al. (2012) conducted preference tests with ten entire colonies of C. cumulans, approximately 20 cm in diameter and 30 cm in height, in a greenhouse. Each colony was kept in 30 L plastic pots, completed with soil, and the set was kept under 50 % shading. In each vessel, a hole was made to fit a transparent plastic hose 2 cm in diameter and 30 cm in length, which connected the colony to the "arena", where there were holes for fitting the glass tubes containing the same treatments evaluated in the laboratory (Figure 11).

Figure 11 - System used for the maintenance of *C. cumulans* in greenhouse conditions, where: a- colony of *C. cumulans*; b- 30 L pot; c- plastic hose and d - "arena", Botucatu-SP, 2003.

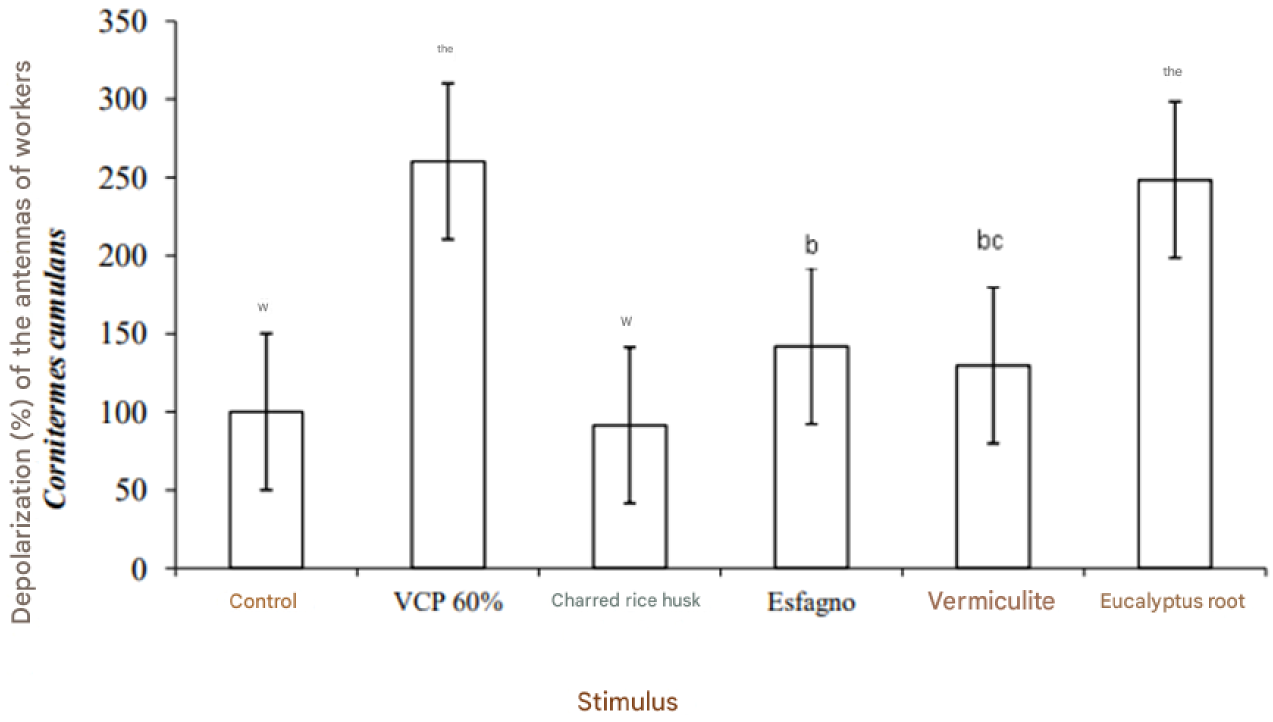
Source: Patrícia Leitão Lima

After the laboratory and greenhouse tests, the electroantenography (EAG) technique was used, which evaluated the sensory response of termite workers, to elucidate the selectivity and sensitivity of the antennal receptors of C. cumulans workers, to the extracts from the materials of the treatments used. It is noteworthy that the materials tested were the same as those used in the experiments carried out in the laboratory and in the greenhouse (Leitão-Lima et al., 2014).

To perform the EAG electroantenography technique according to the methodology described by Batista-Pereira et al. (2004) for the species H. tenuis. When testing the individualized antennae of C. cumulans workers, it was found that they were highly attracted to eucalyptus roots both in the laboratory, with a restricted number of individuals tested, and in entire colonies in the greenhouse condition.

According to the results of the electroantenography (EAG) (Figure 12), the eucalyptus root and the 60% VCP provided the greatest stimuli to the antennae of C. cumulans, due to the greater sensitivity of the antennal receptors to the volatiles present in the root and in the 60% VCP, which corroborates the results of attraction (laboratory and greenhouse) only for the root.

Figure 12 - Electroantnography (EAG) of the antenna of a C. cumulans worker in relation to the control, substrate for eucalyptus forest seedlings (VCP 60%), carbonized rice husks, sphagnum, vermiculite and eucalyptus root. The values are means ± standard errors, where equal letters do not differ from each other by Tukey's test (P< 0.05).



Cornitermes cumulans showed high attraction of eucalyptus roots "in natura", and this attraction can be enhanced by the presence of the substrate for forest seedlings tested. Leitão-Lima et al. (2013) evaluated, in a greenhouse, the damage to Eucalyptus grandis x Eucalyptus urophylla seedlings, using 10 entire colonies, kept in pots, connected to the nests by means of plastic hoses (Figure 13).

Figure 13 - Plastic pot containing an entire colony of *Cornitermes cumulans* connected to four eucalyptus seedlings (PVC pipes), by means of plastic hoses, to determine the damage, with the respective seedlings not exposed to attack (without connections to the colony.

After 24 hours of connection of the pots with the colonies of C. cumulans with the PVC tubes containing the eucalyptus seedlings, it was possible to observe intense activity of the termites between the two structures. Initially, there was the formation of galleries through transparent plastic connection hoses, that is, they were covered with soil, avoiding the direct incidence of light on the termites. Subsequently, before the first evaluations, it was already possible to verify the presence of termites on the soil surface near the eucalyptus seedlings. Also, at the time of removing the seedlings from the pots to separate the roots, the presence of C. cumulans workers in the roots was verified.

The presence of termites in eucalyptus roots interfered in the morphological structure of the root system of the plants where, in general, a reduction in the variables root length, surface and diameter was observed from 60 days after exposure to C. cumulans attack. These changes in the configuration of the root system, due to termite attack, were reflected at 90 days as verified by the decrease in root DM, shoot DM, leaf area and height of eucalyptus seedlings.

FINAL CONSIDERATIONS

The maintenance of termites, in controlled conditions for food preference tests, allows the development of more effective baits for monitoring these insects.

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