

Influence of Different Substrate Moistures on Wood Consumption and Movement Patterns of *Microcerotermes crassus* and *Coptotermes gestroi* (Blattodea: Termitidae, Rhinotermitidae)

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ABSTRACT Moisture is an important physical factor for the survival of termites. The effects of different moisture levels (0, 5, 10, 15, 20, and 25%) of a sand substrate on the behavior of laboratory groups of *Microcerotermes crassus* Snyder and *Coptotermes gestroi* (Wasmann) (Blattodea: Termitidae: Rhinotermitidae) were evaluated. Moisture content of sand affected wood consumption and influenced termite distribution across a moisture gradient for *M. crassus*. Changing the moisture parameters affected the location preference of *C. gestroi*, but the effect on wood consumption was not significant. Nonetheless, *M. crassus* and *C. gestroi* showed a similar distribution pattern of association with particular moisture levels.

KEY WORDS *Microcerotermes crassus*, *Coptotermes gestroi*, moisture, tunneling activity, wood consumption

Moisture can affect foraging behavior of subterranean termites in soil. Termite foraging is expected to vary depending on the moisture preference in a given species (Green et al. 2005). Studies on *Coptotermes* spp. [*Coptotermes frenchi* Hill, Evans 2003; *Coptotermes formosanus* Shiraki, Su and Puche 2003; *Coptotermes gestroi* (Wasmann), Kulis et al. 2008] showed that the termites were active and tunneled the most when offered more humid conditions. Termite feeding behavior also can be influenced by moisture levels. Kulis et al. (2008) indicated that *C. gestroi* gathered and fed more in wetter substrates. Moist wood was more preferred by groups of *C. formosanus* with the highest initial moisture content ($\approx 96\%$) compared with dry wood (Delaplane and LaFage 1989).

Microcerotermes crassus Snyder is widely distributed in the lowlands of Malaysia, especially along coastal areas and around rural dwellings (Tho 1992). In northern peninsular Malaysia, *M. crassus* attacks premises and living trees resulting in economic losses (Lee et al. 2007). It is considered a secondary pest of structures. However, *C. gestroi* and other species of *Coptotermes* are the dominant pest species in urban areas. In peninsular Malaysia, >80% of buildings infested are caused by *C. gestroi* (Kirton and Azmi 2005).

Recently, termite species from other genera usually considered as secondary pest species or nonpest species, have been found to infest buildings or structures after the elimination or reduction of rhinotermitid populations (e.g., *Coptotermes* spp.) with baits (Lee

2002a,b; Kirton and Azmi 2005; Lee et al. 2007). The residual tunnels constructed by *C. gestroi* before elimination create easy pathways for other termite genera to enter the infested structures. Therefore, more opportunities for entering buildings are available. However, secondary pest species can also invade buildings via their self-constructed tunnels.

In this study, we examine the effects of different sand moisture levels on the wood consumption, tunneling activity, and distribution patterns in *M. crassus* groups and compare it with those of *C. gestroi* groups.

Materials and Methods

Insects. *M. crassus* were collected from the field on the Universiti Sains Malaysia, Minden campus, Penang Island, Malaysia, by partially excavating a nest. Three different *M. crassus* colonies were selected for this experiment. Nest debris was brought back to the laboratory, and termites were separated by lightly tapping the nest carton. Termites were carefully isolated from the nest and soil debris by using a piece of moistened and crumpled filter paper.

Two colonies of *C. gestroi* were collected from in-ground aggregation devices that were established earlier at Minden campus. A plastic container measuring 30 by 24 by 10.5 cm served as an aggregation device. Nine rubber (*Hevea brasiliensis* Müll.Arg.) wood stakes (2.2 by 2.2 by 17 cm) were placed in each station to serve as the food source. Termites were brought to the laboratory and separated from the soil debris with the method described by Tamashiro et al. (1973).

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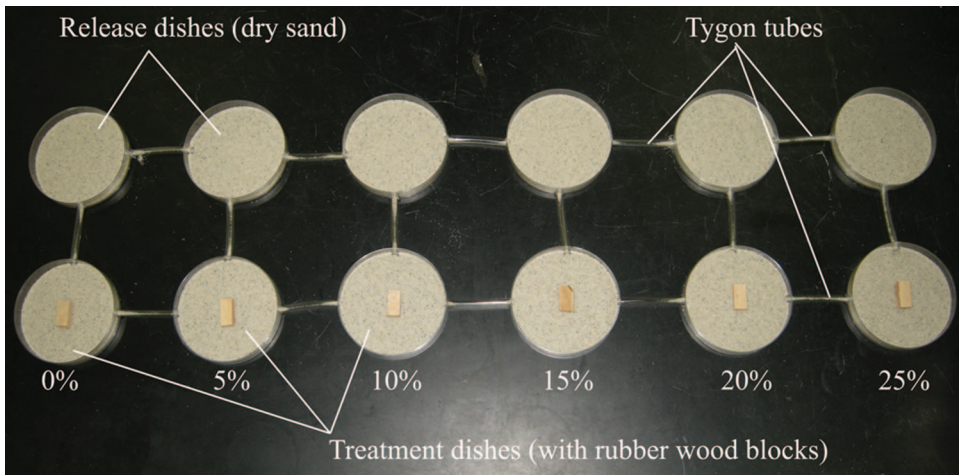


Fig. 1. Experimental setup for effects of various sand moisture levels against *M. crassus* and *C. gestroi*. (Online figure in color.)

Assay. Twelve petri dishes (90 mm in diameter) were connected to one another with Tygon tubes measuring 5 cm in length and 0.4 cm in diameter (Fig. 1). Release dishes contained 40 g of dry sand only, whereas treatment dishes contained sand at different moisture levels. Distilled water of different amounts, was evenly stirred into the sand to give moisture levels of 5, 10, 15, 20, and 25% (wt:wt). No water was added to achieve moisture level 0%. At a moisture level of 25%, the substrate was saturated. To reduce water evaporation, lids and tubes were checked to ensure a tight fit. A piece of preweighed rubber wood (2 by 1 by 1 cm) was placed in each treatment dish to serve as food source. No wood blocks were placed in the release dishes. In each replicate, 270 workers and six soldiers were released; forty-five workers and one soldier in each of the six release dishes. The experiment was replicated five times with termites from each colony. The experimental set-up was kept in the dark at $25.2 \pm 0.2^\circ\text{C}$ and $56.3 \pm 0.7\%$ RH.

On the seventh day, the bases of all dishes were scanned using a flat-bed scanner in grayscale mode (Canon CanoScan LiDE20, Canon Inc., Beijing, China) before the experiment was dismantled. The numbers of termites located in each of the interconnected dishes were counted and recorded to determine termite distribution. The rubber wood blocks were cleaned and oven-dried at 50°C for 2 d before being weighed to obtain the amount of wood consumed. Controls without termites were set up and wood was weighed before and after 7 d.

Data Analysis. Tunneling distances and wood consumption of each colony of *M. crassus* and for the one colony of *C. gestroi* were analyzed using a Kruskal-Wallis multiple range test at $\alpha = 0.05$. The termite counts from each treatment dish were computed to determine the location preference and also subjected to a Kruskal-Wallis multiple range test at $\alpha = 0.05$. The survival rate (percentage) was transformed into arc-sine values and analyzed using a Kruskal-Wallis mul-

tiply range test at $\alpha = 0.05$. All analyses were performed using Statistix version 7.0 (Analytical Software, Tallahassee, FL).

Results and Discussion

General Observations. The workers and soldiers of *M. crassus* did not immediately leave the release areas. Both castes moved toward the treatment dishes in a consistent manner. A small amount of moist sand was found around the Tygon tube openings at the release dishes. *M. crassus* built fewer shelter tubes to the food source on the surface of the treatment dishes than *C. gestroi* did. Instead, *M. crassus* tunneled to the base of the treatment dishes and made their way to the rubber wood blocks from below.

After release, both the soldiers and workers of *C. gestroi* immediately explored the release dishes and moved through the Tygon tubes to the treatment dishes. It is normal for termites to abandon the release dishes but the behavior of sealing the entrance at the release dishes can differ according to species (Green et al. 2005). In our study, *C. gestroi* did not seal the entrances of the Tygon tubes. The termites tunneled to the base of the treatment dishes and moist sand was transported to the surface.

Termites have the ability to transport water to a substrate, such as soil or wood, with lower moisture content (Delaplane and LaFage 1989, Su and Puche 2003) by using their salivary reservoirs as "water sacs" (Grube and Rudolph 1999b). *Reticulitermes santonen-sis* Feytaud uses its salivary reservoirs to store and transport imbibed water (Grube and Rudolph 1999b). The pseudergates of *R. santonen-sis* would moisten the building material they were using with the contents of their water sac and also apply the fluid to maintain a favorable microclimate (Grube and Rudolph 1999a). The alates of *Hodotermes mossambicus* (Hagen) store free water in their two large water sacs (Hewitt et al. 1971, Watson et al. 1978) and workers of *Macrotermes*

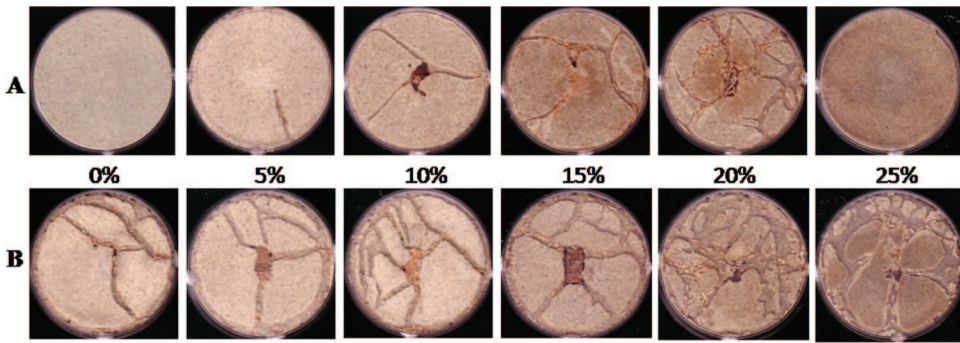


Fig. 2. Tunneling activities of *M. crassus* (A) and *C. gestroi* (B) in sand at 0, 5, 10, 15, 20, and 25% moisture. (Online figure in color.)

michaelseni (Sjöstedt) imbibe free water and release it onto the fungus comb (Sieber and Leuthold 1981). It can be assumed that the use of the reservoirs as water sacs represents an important morphological and physiological adaptation to dry and warm habitats (Grube and Rudolph 1999b). In our study, *C. gestroi* added water to the dishes containing lower moisture levels. This effort would help create a larger area (number of dishes) with the preferred moisture conditions by moving water from dishes with wetter sand into dishes with drier sand. Shelter tubes made of moist sand were found on the surface of each treatment dish connecting the openings of the Tygon tubes toward the wood block.

Tunneling Activities. Different termite species have been found to exhibit different tunneling geometries or patterns (Campora and Grace 2001, Cornelius and Osbrink 2001, Su 2001).

M. crassus formed narrower tunnels with fewer branches (Fig. 2), whereas *C. gestroi* constructed wider and highly branched tunnels (Fig. 2). The results show that *C. gestroi* is more aggressive and tunnels more extensively compared with *M. crassus*. After the seventh day, *M. crassus* had tunneled on average a total distance of 119.6, 64.5, and 118.2 cm for colonies A, B, and C, respectively, whereas colony A and colony B of *C. gestroi* had built tunnels with an average length of 315.4 and 295.4 cm, respectively.

Higher tunneling activities were found in soils with higher moisture content. Haagsma and Rust (1995) and Khan (1980) stated that such abiotic factors are important determinants in the survival of termites.

Some species can still survive under unfavorable conditions as they have the capability to change their ecological niches to survive (Su and Puche 2003, Kulis et al. 2008).

M. crassus from colony A tunneled significantly further in sand with 20% moisture than compared with sand with 0, 5, and 25% moisture levels ($P < 0.05$), whereas colonies B and C tunneled significantly further in sand with 20% moisture compared with sand with 0% moisture. Tunnel formation was not significantly different between dishes containing sand moistened to 10 or 15% for colony A, whereas colonies B and C did not show any difference in tunneling formation in dishes containing 5, 10, 15 or 25% moisture ($P > 0.05$). In general, most tunnels were built in dishes with a 20% moisture content, whereas in dishes with 25% water content termites were only concentrated around the wood block and hardly tunneled in the wet sand at all (Table 1; Fig. 2).

Both colonies of *C. gestroi* also tunneled significantly more in the 20% moisture level dishes compared with dishes with 0% moisture levels ($P < 0.05$). In contrast to *M. crassus*, *C. gestroi* also tunneled extensively in the wet sand with a water content of 25% (Table 1; Fig. 2). Arab and Costa-Leonardo (2005) reported that a rise in soil moisture increased the exploration of *C. gestroi* workers, which resulted in construction of more tunnels.

Wood Consumption. Termites fed on wood located in all treatment dishes although at different proportions depending on the moisture level of the sand. Wood consumption of *M. crassus* was greatest in the

Table 1. Tunneling distance (mean \pm SE) of *M. crassus* and *C. gestroi* in sand at different moisture levels at day 7

Moisture level (%)	Mean tunnelled distance (cm)						
	<i>M. crassus</i>				<i>C. gestroi</i>		
	Colony A	Colony B	Colony C	Combined	Colony A	Colony B	Combined
0	0.00 \pm 0.00a	0.00 \pm 0.00a	0.00 \pm 0.00a	0.00 \pm 0.00a	40.74 \pm 3.12a	33.58 \pm 4.37a	37.16 \pm 8.01c
5	5.70 \pm 5.38a	3.12 \pm 1.94ab	5.02 \pm 4.07ab	4.61 \pm 1.73ab	40.78 \pm 2.72ab	39.52 \pm 2.35a	40.15 \pm 1.41bc
10	23.86 \pm 6.14ab	12.58 \pm 5.78ab	29.46 \pm 8.19ab	21.73 \pm 10.7ab	42.50 \pm 7.42ab	43.48 \pm 6.68ab	42.99 \pm 1.10bc
15	35.96 \pm 2.45ab	16.82 \pm 8.82ab	28.74 \pm 7.38ab	27.41 \pm 12.57ab	49.64 \pm 1.92ab	45.58 \pm 3.50ab	47.61 \pm 4.54bc
20	50.52 \pm 6.50b	30.36 \pm 6.25b	52.86 \pm 7.03b	44.58 \pm 15.97b	87.12 \pm 4.09b	79.70 \pm 5.13b	83.41 \pm 8.30a
25	3.60 \pm 3.60a	1.60 \pm 1.13ab	2.14 \pm 2.14ab	2.44 \pm 2.29ab	54.60 \pm 3.61ab	53.58 \pm 3.00ab	54.09 \pm 1.14ab

Means followed by same letter within the same column are not significantly different at $\alpha = 0.05$ (Kruskal-Wallis multiple range test).

Table 2. Mean wood consumption of *M. crassus* and *C. gestroi* in sand at different moisture levels at day 7

Moisture level (%)	Mean wood consumed (g)						
	<i>M. crassus</i>				<i>C. gestroi</i>		
	Colony A	Colony B	Colony C	Combined	Colony A	Colony B	Combined
0	0.0046 ± 0.0004c	0.0059 ± 0.0010a	0.0039 ± 0.0010a	0.0039 ± 0.0015a	0.0466 ± 0.0053a	0.0928 ± 0.0027a	0.0697 ± 0.0040a
5	0.0229 ± 0.0012abc	0.0265 ± 0.0102ab	0.0240 ± 0.0072ab	0.0170 ± 0.0066ab	0.0622 ± 0.0079a	0.1042 ± 0.0113a	0.0832 ± 0.0096a
10	0.0348 ± 0.0059ab	0.0217 ± 0.0077ab	0.0308 ± 0.0095ab	0.0275 ± 0.0105ab	0.0812 ± 0.0061a	0.1037 ± 0.0131a	0.0925 ± 0.0096a
15	0.0256 ± 0.0030ab	0.0348 ± 0.0161ab	0.0375 ± 0.0126ab	0.0310 ± 0.0067ab	0.0850 ± 0.0132a	0.1030 ± 0.0063a	0.0940 ± 0.0097a
20	0.0494 ± 0.0084a	0.0434 ± 0.0103b	0.0467 ± 0.0068b	0.0384 ± 0.0131c	0.0665 ± 0.0051a	0.1057 ± 0.0096a	0.0861 ± 0.0073a
25	0.0070 ± 0.0018bc	0.0150 ± 0.0038ab	0.0162 ± 0.0042ab	0.0098 ± 0.0031bc	0.0526 ± 0.0094a	0.0948 ± 0.0056a	0.0727 ± 0.0075a

Means followed by same letter within the same column are not significantly different at $\alpha = 0.05$ (Kruskal-Wallis multiple range test).

20% moisture level dishes for all three colonies. Overall, consumption for each *M. crassus* colony was significantly different in the 20% moisture dishes (Table 2) compared with the 0% moisture dishes ($P < 0.05$). *M. crassus* from colony A also consumed significantly more wood from the 20% moisture level dishes compared with that consumed in the 25% moisture level dishes. In addition, wood consumption was also significantly different between the 0% moisture level dishes with 10 and 15% moisture level dishes.

Remarkably, there were no significant differences in wood consumption for both colonies of *C. gestroi* tested ($P > 0.05$) in spite of the different sand moisture levels (Table 2). This illustrates the ability of *C. gestroi* to render even a dry food source/environment more suitable for feeding by transporting water from elsewhere, thus optimizing food exploitation under all offered conditions.

Termite Distribution. Moisture significantly affected termite location as shown by the counts ($P <$

0.05). By day 7, the majority (42.5%) of *M. crassus* had aggregated in the 20% moisture level dishes (Fig. 3). *M. crassus* from colony A were found significantly less often in the 25% moisture level dishes. Termites from *M. crassus* colonies B and C concentrated in the 20% moisture level dishes significantly more than in the 0% moisture level dishes ($P < 0.05$). Generally, *M. crassus* preferred not to reside in the 0% level moisture dishes; these recorded the least number of termites among the moisture levels tested. The soldier caste of *M. crassus* were distributed in the treatment dishes except in the 0% moisture level dishes and release dishes that contained dry sand only.

The majority of *C. gestroi* from colonies A and B were found in the treatment dishes. Occasionally (in six of 30 possible dishes of colony A, and in nine of 30 possible dishes of colony B), a small number of individuals (0.34–2.04% of the total number of colony A, and 0.37–2.96% of the total number of colony B) also were found in the release dishes, which contained dry

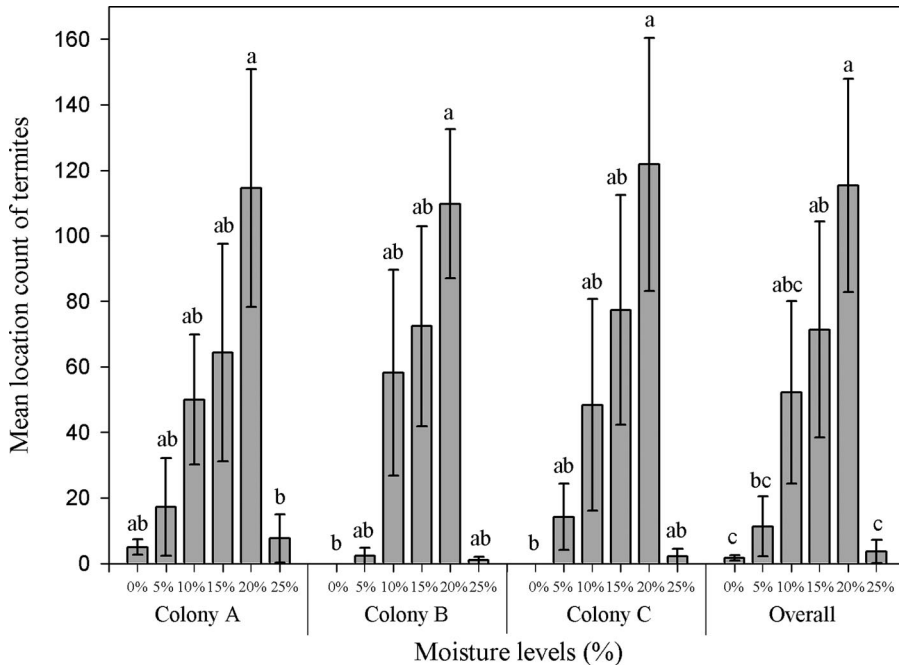


Fig. 3. Distribution of *M. crassus* from colonies A, B, and C (mean ± SEM) and overall means across dishes with different sand moisture levels on the seventh day at $\alpha = 0.05$ (Kruskal-Wallis multiple range test).

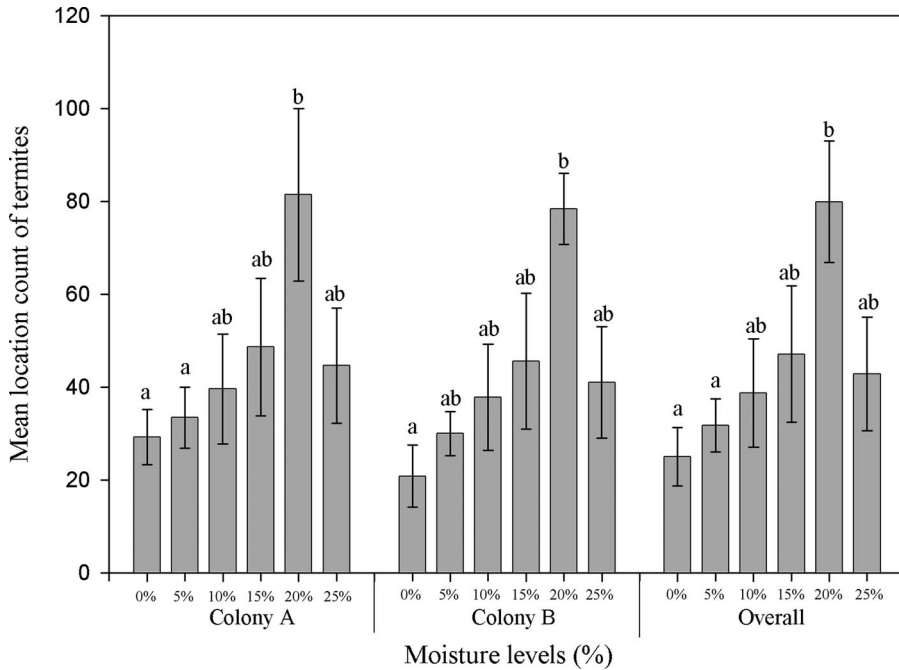


Fig. 4. Distribution of *C. gestroi* from colonies A and B (mean ± SEM) and the overall means across dishes with different sand moisture levels on the seventh day at $\alpha = 0.05$ (Kruskal-Wallis multiple range test).

sand only. In general, both colonies of *C. gestroi* preferred 20% moisture levels compared with the 0 and 5% moisture levels (Fig. 4). Soldiers of *C. gestroi* were found in both the treatment and release dishes at the end of the experiment.

Both *M. crassus* and *C. gestroi* showed an overall similar distribution pattern across the different moisture levels. Both species were located least in the 0% moisture level dishes. As moisture levels increased, the number of termites in the particular moisture level dish also increased. However, in the 25% moisture level dishes, neither species tunneled as much as they did in the 20% moisture level dishes. This could be due to the saturation of substrate (sand) with water, making the conditions unfavorable to the termites. As reported by Su and Puche (2003), given that all other factors are the same, subterranean termites prefer to tunnel in soil with a higher moisture level unless the soil is oversaturated with water.

Survival. At the end of the experimental period, groups from colonies A, B, and C of *M. crassus* recorded a survival rate of 88.4, 86.7, and 89.8%, respectively. Groups of *C. gestroi* from colonies A and B had a survival rate of 95 and 93.9%, respectively. There were no significant differences for the survival rate between the two termite species ($P > 0.05$).

C. gestroi can survive well under adverse conditions, such as dry conditions by transporting water into their nests (Arab and Costa-Leonardo 2005) and even to the food source. This helps maintain a highly humid environment. Both *C. gestroi* and *M. crassus* tend to accumulate and tunnel more in dishes with higher moisture unless the substrate is oversaturated with

water. The feeding rate for *M. crassus* was significantly influenced by moisture levels, whereas *C. gestroi* showed no preference. Water that was brought to the wood blocks by *C. gestroi* created favorable conditions for their feeding irrespective of sand moisture content.

The ability of *C. gestroi* to adapt to drier conditions and their aggressive tunneling explain their success over other termite species and certainly over *M. crassus*. The aggressive food searching pattern by *C. gestroi* results in extensive branching of tunnels. If elimination of *Coptotermes* using bait is successful, the tunnels previously constructed would provide other termite species with the opportunity to take them over for their own use and start an infestation of their own.

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