Colony Size Affects the Efficacy of Bait Containing Chlorfluazuron Against the Fungus-Growing Termite *Macrotermes gilvus* (Blattodea: Termitidae)

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ABSTRACT The efficacy of chitin synthesis inhibitors (CSIs) against fungus-growing termites is known to vary. In this study, 0.1% chlorfluazuron (CFZ) cellulose bait was tested against medium and large field colonies of *Macrotermes gilvus* (Hagen). The termite mounds were dissected to determine the health of the colony. Individual termites (i.e., workers and larvae) and fungus combs were subjected to gas chromatography–mass spectrometry (GC–MS) analysis to detect the presence of CFZ. In this study, 540.0 ± 25.8 g (or equivalent to 540.0 ± 25.8 mg active ingredient) and 680.0 ± 49.0 g (680.0 ± 49.0 mg active ingredient) of bait matrix were removed by the medium- and large-sized colonies, respectively, after baiting. All treated medium-sized colonies were moribund. The dead termites were scattered in the mound, larvae were absent, population size had decreased by 90%, and the queens appeared unhealthy. In contrast, no or limited effects were found in large-sized colonies. Only trace amounts of CFZ were detected in workers, larvae, and fungus combs, and the population of large-sized colonies had declined by only up to 40%. This might be owing to the presence of large amount of basidiomycete fungus and a drastic decrease of CFZ content per unit fungus comb (a main food source of larvae) in the large-sized colonies, and hence reduced the toxic effect and longer time is required to accumulate the lethal dose in larvae. Nevertheless, we do not deny the possibility of CSI bait eliminating or suppressing the higher termite if the test colonies could pick up adequate lethal dose by installing more bait stations and prolonging the baiting period.

KEY WORDS fungus-growing termite, *Macrotermes gilvus*, termite baiting, chlorfluazuron, colony size

Subterranean termites are important structural pests that cause severe damage to structures in the tropics, subtropics, and temperate regions. An estimated US$40 billion is spent annually to control termites and repair losses caused by pest termites around the world (Rust and Su 2012). Baiting systems have gained significant popularity and have been widely used to manage subterranean termites in recent years. Baiting uses a relatively small amount of insecticide compared with conventional chemical soil treatment, and the active ingredients are of low mammalian toxicity and are target specific (Broadbent 2011). In the mid-90s, benzoylphenylurea (BPU) compounds such as hexaflumuron were proven to be effective as an active ingredient in bait, as they were able to suppress and eliminate termite colonies (Su 1994). BPUs are chitin synthesis inhibitors (CSIs), which mean that they act by disrupting the synthesis of chitin in the insect cuticle during molting (Yu 2008). To date, various studies have documented the successful elimination or suppression of lower termite species (rhinotermitids) using CSIs (Su et al. 1997, Tsunoda et al. 1998, Peters and Fitzgerald 2003, Cabrera and Thoms 2006, Evans 2010, Haverty et al. 2010). In contrast, relatively few studies have evaluated the effectiveness of CSI-based baits against higher termites (termitids) (Peppuy et al. 1998, Neoh et al. 2011).

The fungus-growing termites of the subfamily Macrotermitinae originated in the African rain forest and later dispersed into South and Southeastern Asia region (Aanen and Eggleton 2005). *Macrotermes gilvus* (Hagen) (Isoptera: Termitidae: Macrotermitinae) is a common species of mound-building termite that is usually found along the perimeters of structures and buildings. Lee et al. (2007) found that once the predominant species, *Coptotermes* spp. (Rhinotermitidae), were eliminated with termite baits, the incidence of *M. gilvus* infestations became increasingly common. Unlike the rhinotermitids, *M. gilvus* generally responds poorly to termite baits (Lee 2002b, Lee et al. 2007, Neoh et al. 2011). Thus, pest control op-

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erators currently use spraying of chemicals and physical removal of termite mounds to manage the higher termites.

The lower and higher termites vary biologically. First, the lower termites depend on their symbiotic intestinal protozoa for cellulose digestion. In contrast, macrotermitines lack symbiotic protozoa and instead process food via the combined action of endogenous enzymes from bacteria and a symbiotic fungus (Wood and Thomas 1989). In *Macrotermes*, plant materials ingested by young major workers are deposited as fecal pellets on the fungus comb (Badertscher et al. 1983). Young workers consume both the fungus nodules and the aged fungus comb. Subsequently, fungus nodules and salivary secretions rich in nutrients are delivered to the dependent castes (i.e., larvae, nymphs, and primary reproductives; Hinze et al. 2002). Fungus nodules, which are enriched with nitrogen, are an important nutrient source for macrotermitine species (Rohrmann and Rossman 1980).

Second, rhinotermitid workers undergo a series of molting processes, and several worker instars exist inside the colony (Roisin 2000). In macrotermitines, worker caste is in the terminal form and unable to undergo further molting, and only a single stage of worker caste exists inside the colony (Neoh and Lee 2009). This worker caste appears to be invulnerable to CSI-based baits, which impacts the effectiveness of baits. Termite baits may only show deleterious effects on macrotermitine colonies if toxicants are successfully delivered to significant proportions of larvae (larvae constituted 42.60% of the total population in *M. gilvus;* Lee et al. 2012).

Several hypotheses have been proposed to explain the ineffectiveness of CSI-based baits against *Macrotermes*, but the exact cause has not yet been determined. One possibility is that the termites do not feed on the baits because they find the bait matrix to be unpalatable (Lee 2002b, Lee et al. 2007). A second possibility has to do with their symbiotic relationship with basidiomycete fungi of the genus *Termitomyces*, which belongs to the white rot fungus group (Darlington 1994). White rot fungi are known for being able to degrade a wide variety of organic pollutants such as polyaromatic hydrocarbons, several pesticides, chlorinated hydrocarbons, and other toxic organic compounds (Mougin et al. 1996, Bending et al. 2002, Gao et al. 2010). In fungus-growing termites, ingested food passes rapidly through the gut without much digestion and subsequently is deposited on the fungus comb. It is possible that *Termitomyces*, like other white rot fungi, may be able to degrade the ingested bait toxicant so that it does not reach the termites in the colony (Neoh et al. 2011). If it is true, the efficacy of baits against macrotermitines is thought to depend on the colony size because the number of fungus comb increase parallels the colony size, and the phenomena may decrease the content of chlorfluazuron (CFZ) per unit fungus comb. Consequently, larger colonies may require higher uptake of the active ingredient to achieve colony elimination.

In this study, CFZ bait was tested for its efficacy in eliminating medium- and large-sized *M. gilvus* colonies. CFZ is a BPU insecticide that has been shown to be effective against termites both in the laboratory and in the field (Rojas and Morales-Ramos 2001, Peters and Fitzgerald 2003). Treated mounds were destructively sampled at the end of the experiment to evaluate colony status. Termite numbers, appearance of workers and queens, presence or absence of immature castes, and structure of fungus combs were evaluated to assess the effects of the bait on the colonies. We also investigated whether the bait toxicant was successfully transferred or delivered among colony members. We used gas chromatography–mass spectrometry (GC-MS) to measure the amount of CFZ present in different termite castes and fungus combs.

Materials and Methods

Study Site. Mounds of *M. gilvus* were sampled in Bayan Lepas, Penang in northern Peninsular Malaysia (5° 32′ N, 100° 29′ E). Four medium (mound A–D; height, 21–40 cm; diameter, 51–80 cm) and four large (mound E–H; height, 41–80 cm; diameter, 81–130 cm) mounds were selected for treatment in this study. In addition, two mounds from each size group (medium, mound I and J; large, mound K and L) were used as controls.

Bait Stations. A termite trap was installed adjacent to each mound before testing. Each termite trap consisted of a bottomless polyethylene container (26 by 19.5 by 11 cm^3) that was placed over a wooden block consisting of Jelutong (*Dyera costulata* (Miq.) Hook.f.) wooden stakes (15 by 4 by 2.2 cm^3). Once the termites attacked the wooden block, it was replaced with the bait station. Each bait station consisted of a plastic container (16 by 10.5 by 7 cm^3) with holes (0.8 cm i.d.) drilled in each side to allow termites access to the bait matrix.

Application of Bait Matrix. Cellulose powder (Ensystex Sdn. Bhd., Kuala Lumpur, Malaysia) containing 0.1% wt:wt CFZ was used as the bait matrix for treated colonies, whereas blank cellulose powder was used for the control colonies. Approximately 80 g of bait matrix mixed with 400 ml of water (1:5 wt:wt ratio) was introduced into the bait stations. The experiment started on 30 March 2012. The bait stations were inspected weekly, and the percentage of the bait matrix removed was estimated visually. The baits were replenished at each inspection until termite feeding activity ceased or no termite activity was found in the bait stations.

Assessment of Colony Status. Colony status was evaluated at month 4 (17 July 2012). Treated and control mounds were destructively excavated to obtain reproductive individuals whenever possible, and the royal chambers were opened to obtain the primary reproductives. The fresh weights of queens and kings were recorded using an analytical balance (Sartorius AG, Göttingen, Germany). The appearance of termites and condition of fungus combs were examined. Surviving termites were collected and counted. The

number of termites after baiting was compared with the estimated number of workers before baiting using the following regression equation derived by Lee et al. (2012): $y = 586x - 4608$, where y = population size and x = mound diameter (20 cm $<$ x $<$120 cm). Colony status was assessed as—1) healthy, a normal and active colony; 2) weakening, a colony with reduced termite numbers; 3) moribund, a colony with impaired reproductive capacity and absence of immature castes; or 4) eliminated, a colony in which all termite individuals were dead.

Chemical Analysis. Larvae and worker termite samples from different parts of each of the mounds (i.e., peripheral zone, nursery zone, and royal chamber) were collected and subjected to GC-MS analysis. Three replicates were analyzed for each caste from each zone of the mound. In addition, six fungus nodules and six fungus comb samples from each mound were analyzed.

The termites, fungus combs, and fungus nodules were analyzed by GC-MS using methods specifically developed by Lee et al. (2013) for better detection of CFZ in the samples. Termite samples were rinsed with acetonitrile to remove external CFZ contamination. Termites, fungus nodules, and fungus comb samples were hand homogenized and crushed using a mortar and pestle. Homogenized samples (0.2 g) were transferred into a test tube, and 50 μl of the internal standard (hexaflumuron, 0.5 mg/ml) and 50 μl of 0.1 M sulfuric acid were then pipetted into the tube. Sample extraction was performed by adding 200 μl of isopropanol to termite samples and 500 μl of isopropanol to fungus nodule and fungus comb samples. The mixture was vortexed for 1 min, followed by centrifugation at 2,500 rpm for 5 min. The recovered supernatant was dried in anhydrous sodium sulfate and then placed in an autosampler vial for GC-MS analysis. GC-MS analysis was performed using an Agilent 7,890A gas chromatograph equipped with an Agilent 7,693 autosampler interfaced with an Agilent 5975C mass spectrometer detector (Agilent Technologies, Bayan Lepas, Penang, Malaysia). One microliter of sample was injected into a fused-silica capillary column (30 m $\times$ 0.25 mm i.d., 0.25 μm film thickness) using the splitless mode. The carrier gas was helium. The injector and interface temperatures were set at 250°C and 280°C, respectively. Oven temperature was programmed at 90°C initially, then ramped at 38°C/min to 280°C and held for 5 min. BPUs are thermally liable compounds. Screening of CFZ and the internal standard (IS) was based on the detection of specific aniline derivatives or major degradation products. The quantifying ions used in the selective ion monitoring mode were m/z 321 (CFZ) and m/z 176 (IS). Under these conditions, the relative retention time of CFZ was 1.40. The limit of detection was 0.02 nanogram per termite, 0.3 ng/g of fungus comb, and 0.3 ng/g of fungus nodule.

Statistical Analysis. CFZ concentrations of each termite caste and nest area were subjected to logarithmic transformation to normalize the data. The transformed values were then analyzed using one-way analysis of variance, and the means were separated using Tukey's honestly significant difference (HSD) test. The concentrations of CFZ detected in termite workers and fungus combs from medium mounds (excluded mound B) were compared with those detected in large mounds using Student's t-test. Differences in fresh weights of reproductives between treated and control mounds were also compared using Student's t-test. All analyses were performed using SPSS, v.11.0 (SPSS Inc., Chicago, IL) at $\alpha = 0.05$.

Results

Changes in Colonies After Bait Application. Over the course of the experiment, all bait stations in both the treated and control colonies were infested by termites. In general, termites covered the inner top surface of the bait station and termite trap with mud, and the bait matrix inside was either fully removed or displaced with mud. During weekly inspections, termite workers were absent in some of the bait stations when the bait matrix inside was fully removed. However, termite workers reappeared in the bait stations once the bait matrix was replenished. Approximately 540.0 ± 25.8 g (equivalent to 540.0 ± 25.8 mg active ingredient) and 680.0 ± 49.0 g (equivalent to 680.0 ± 49.0 mg active ingredient) of the CFZ-based bait matrix were removed by medium-sized and large-sized colonies, respectively. Trace amounts of bait were found in a number of spots inside the mounds, including the food stores (Fig. 1d). A total of 755.0 ± 5.0 g of blank bait was removed by control colonies.

Medium-Sized Mounds. In treated colonies, some termites found inside the bait stations were marbled white in color (Fig. 1a). A strong malodor was emitted from the inner nest due to the presence of dead and decaying termite cadavers in a number of spots inside the mounds, especially in the carton structure near the nursery zone. Small numbers of workers and soldiers were observed, whereas none of the immature castes (e.g., larvae and nymphs) were found inside the nest. Overall, medium-sized treated colonies experienced a 90–96% decrease in population size after 4 mo of baiting (Table 1). Fungal combs were heavily consumed (Fig. 1c). Fast-growing fungus was found growing on the carton material in the nursery zone or growing on termite cadavers.

In all treated medium-sized mounds (except mound B), the queens were dark yellow in color, flaccid, and less physogastric (Fig. 1b) compared with those in the untreated control colonies, which were turgid and creamy white in color. The body weight of queens from the treated medium colonies (0.7377 ± 0.0048 g) was significantly lower than that of queens from the control colonies (2.5287 ± 1.1805 g; $t = 3.034$; df = 6; $P < 0.05$). The weights of kings from both medium- and large-sized treated colonies (0.0722 ± 0.0033 g) did not differ significantly from that of the control colonies (0.0736 ± 0.0058 g; $t = 0.260$; df = 10; $P = 0.80$).

Large-Sized Mounds. On average, the population size decreased by 15–40% in large-sized treated col-
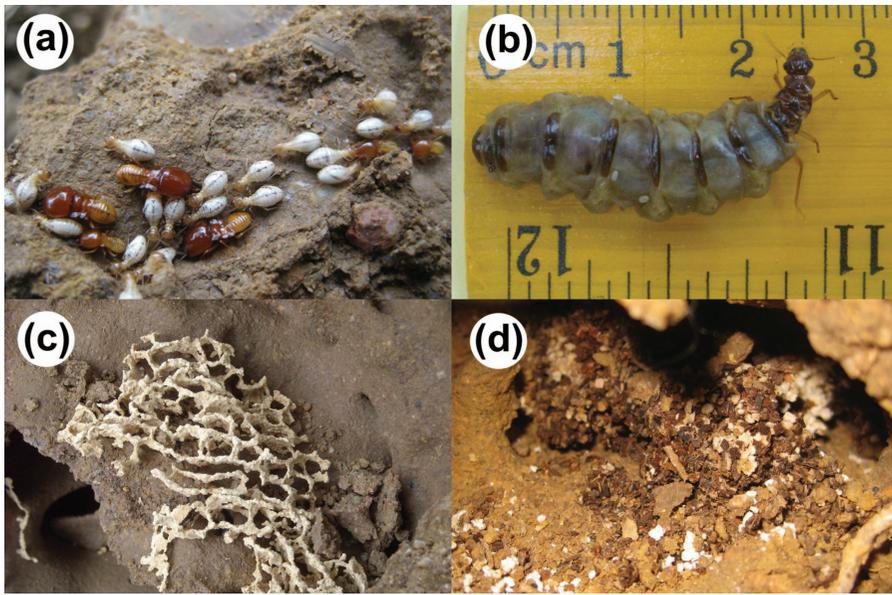
Fig. 1. Conditions in a treated medium-sized colony of *M. gilvus* (colony A) after the 4-mo baiting period. (a) Worker termites had a marbled white body. (b) The unhealthy queen was dark yellow in color, flaccid, and less physogastric. (c) Much of the fungus comb was consumed following the drastic reduction in number of worker termites. (d) The bait matrix was incorporated into the food store. (Online figure in color.)

onies after 4 mo of baiting (Table 1). However, dead workers, soldiers, and larvae were rarely encountered in the test colonies compared with those in medium-sized colonies. In some instances, the fungus combs inside the nests were moderately consumed (especially in the mound E, in which the colonies experienced a 40% population decrease). The queens appeared healthy and were creamy white in color with the presence of eggs. There were no significant differences in body weights between queens from the treated large colonies (2.5258 ± 0.7374 g) and queens from control colonies ($t = 0.003$; df = 6; $P = 0.998$).

In some of the bait stations, ant invasions precluded foraging termites from continuing to feed inside the

station, and the baiting process stopped early. For example, the presence of *Anoplolepis gracilipes* (Fr. Smith) at the bait station of mound G prevented termites from entering the station, and the baiting process was stopped at week 10.

CFZ in Termite Colonies. In general, a significantly higher concentration of CFZ was detected in workers from the royal chamber compared with workers from the peripheral zone and the nursery zone ($F = 572.47$; df = 3, 8; $P < 0.05$; Table 2). In treated large-sized colonies, 1.02 ± 0.14–2.63 ± 0.31 ng CFZ per termite was detected in termite larvae. CFZ was also detected in fungus combs, where the CFZ levels ranged from 2.29 ± 0.25–204.83 ± 12.45 mg/kg of fungus comb. However, CFZ was not detected in the fungus nodules. This indicated that the phenomenon of larvae feeding on fungus nodules did not deliver the toxicant to the larvae.

Overall, the mean concentration of CFZ detected in termite workers in medium-sized colonies ($9,385 \pm 1,235$ ng/g) was significantly higher than that of termite workers in the large-sized colonies ($2,529 \pm 310$ ng/g; $t = 7.003$; df = 5; $P < 0.05$). Similarly, the mean CFZ concentration detected in fungus combs from medium-sized colonies (117.47 ± 45.36 mg/kg) was significantly higher than that of the large-sized colonies (12.03 ± 4.15 mg/kg; $t = 2.756$; df = 5; $P < 0.05$).

Table 1.   Prebaiting and postbaiting population size estimates of *M. gilvus* colonies

Mound size	Colony	Number of termite individuals		Changes (%) in pop size
		Prebaiting[a]	Postbaiting[a]	
Treated medium	A	37,600	1,550	−96
	B	33,500	1,780	−95
	C	41,100	3,930	−90
	D	35,800	2,840	−92
Large	E	43,400	26,170	−40
	F	48,100	28,500	−41
	G	71,000	49,000	−31
	H	71,600	61,200	−15
Control medium	I	31,100	34,200	+10
	J	39,300	43,110	+10
Large	K	45,200	49,050	+9
	L	62,200	65,070	+5

[a] Population numbers before baiting period were estimated based on the regression equation for mound diameter versus population size derived by Lee et al. (2012); population numbers after baiting period were determined using the direct counting method.

Discussion

Table 3 summarizes the published data on efficacy of termite baits against rhinotermitids and termitids. Although Peters et al. (2008) and Dhang (2011) reported successful colony suppression and elimination

Table 2. CFZ concentrations in medium and large termite colonies and colony status after baiting

Mound size	Colony	Mound ht and diam (cm)	CFZ concn (ng/termite) in different termite castes from different nest areas				CFZ concn (mg/kg) in fungus comb	Colony status
			P.Z. wk[a]	N.Z. wk[a]	R.C. wk[a]	N.Z. lv[a]		
Medium	A	39 & 72	ND	74.15 ± 2.15a	98.3 ± 15.61b	ND[b]	204.83 ± 12.45	Moribund
	B[c]	40 & 65	3.26 ± 0.22a	3.61 ± 0.22a	5.53 ± 0.31b	ND	2.68 ± 0.18	Moribund
	C	31 & 78	42.57 ± 2.35a	45.33 ± 1.74a	76.2 ± 5.5b	ND	52.61 ± 1.08	Moribund
	D	34 & 69	53.33 ± 2.67a	58.89 ± 0.59a	90.67 ± 1.43b	ND	94.97 ± 1.56	Moribund
Large	E	44 & 82	ND	18.71 ± 1.71b	25.98 ± 0.37c	1.41 ± 0.03a	21.12 ± 1.07	Weakening
	F	56 & 90	16.72 ± 0.50b	15.90 ± 3.38b	35.63 ± 0.86c	1.02 ± 0.14a	16.13 ± 1.22	Weakening
	G[c]	75 & 129	10.64 ± 0.97b	10.19 ± 0.89b	16.50 ± 1.24c	2.63 ± 0.31a	2.29 ± 0.25	Weakening
	H	51 & 130	18.79 ± 1.14b	16.78 ± 1.03b	25.05 ± 3.39c	1.25 ± 0.14a	8.57 ± 0.70	Healthy

Values represent mean ± SD, and mean followed by different letters in the same row are significantly different as determined by Tukey's HSD test ($P < 0.05$).

[a] P.Z. wk, workers from peripheral zone; N.Z. wk, workers from nursery zone; R.C. wk, workers from royal chamber; N.Z. lv, larvae from nursery zone.

[b] ND, not determined due to absence of the caste.

[c] Termites abandoned the bait station on week 10 due to ant invasion.

of termitids (including *M. gilvus*) with CFZ baits, the sizes of the *M. gilvus* colonies that were baited in their studies were not recorded. It is possible that small- to medium-sized colonies were baited in those studies. In the current study, on average, ≈615 mg of CFZ was ingested by treated colonies during the 4-mo baiting period, but none of the colonies was completely eliminated. This finding demonstrates the complexity and challenges faced when trying to manage fungus-growing termites using baiting systems. Although a considerable amount of bait was removed by worker termites in our study (medium-sized colony: 540.0 ± 25.8 g; large-sized colony: 680.0 ± 49.0 g), the bait was not entirely digested and shared among nestmates (Duncan 1997). As a rule, the bait can be delivered to larvae via at least three routes, i.e., from salivary secretion of workers who feed on the CSI-contained fungus combs and foraging workers who feed plant materials and bait, and from fungus nodules fed by workers (see below for discussion). However, the former route should be seen as major route, as it is associated with the food habits of macrotermitines (Hinze et al. 2002). The bait matrix was detected in food stores and the fungus comb, indicating that the baits were integrated into the food processing pathway of *M. gilvus.* Food stores, which consist of comminuted food, were ingested by worker termites after approximately a week and subsequently excreted as fecal pellets on the fungus comb (Wood and Thomas 1989). This time lag of bait distribution to the targeted caste (larvae) explains why CSIs take longer to affect macrotermitines than wood-feeding rhinotermitids. Furthermore, some of the bait in our study was used to construct the mound structure, as was also reported by Peters and Broadbent (2005). This further decreased the amount of active ingredient available for distribution to the targeted termites.

Although none of the treated colonies was completely eliminated during the 4-mo baiting period, the medium-sized colonies were moribund. These colonies lost their reproductive capacity, as evidenced by the unhealthy queen and the absence of newly produced offspring. This could have happened as early as a month after the baiting began, as a large proportion of aged marbled white worker termites were observed in the test colonies at this time point. Two possible explanations may account for the low worker output in the medium-sized treated colonies. First, CSIs are known to function as a larvicide, which causes abortive molting in larvae, and this could have indirectly caused the worker population in treated colonies to decline over time. This premise is supported by the observation that much of the fungus comb in the treated medium-sized colonies was consumed and no new fungus comb was constructed, suggesting that foraging activity by workers declined because the number of workers present was insufficient to initiate foraging activity (Darlington 1991). Thus, the fungus comb became the main food source. In this scenario, the uptake of CFZ by larvae could have

Table 3. Summary of previous field evaluations of effects of termite baits against rhinotermitids and termitids

Termite species	Bait toxicant	Min. duration required to eliminate a test colony (wk)	Min. amt of toxicant required for colony elimination (mg)	Reference
Rhinotermitidae				
Coptotermes formosanus	Hexaflumuron (0.5%)	16	233	Su (1994)
	Hexaflumuron (0.1%)	12	699	Su et al. (1995)
	Hexaflumuron (0.1%)	6	114	Grace et al. (1996)
	Hexaflumuron (0.5%)	8	30	Su et al. (1997)
	Hexaflumuron (0.5%)	12	390	Su et al. (2000b)
	Hexaflumuron (0.5%)	12	550	Messenger et al. (2005)
	Hexaflumuron (0.1%)	63	419	Tsunoda et al. (2005)
Coptotermes acinaciformis	Hexaflumuron (0.5%)	23	670	Peters and Fitzgerald (1999)
	Chlorfluazuron (0.05% & 0.25%)	12	200	Peters and Fitzgerald (2003)
	Noviflumuron (0.5%)	10	13	Cabrera and Thoms (2006)
	Chlorfluazuron (0.1%)	15	400	Peters et al. (2008)
	Bistrifluron (0.5% & 1.0%)	8	180	Evans (2010)
Coptotermes curvignathus	Hexaflumuron (0.5%)	2	138	Sajap et al. (2000)
	Chlorfluazuron (0.1%)	6	420	Sukartana et al. (2009)
Coptotermes gestroi	Hexaflumuron (0.5%)	12	200	Su et al. (2000a)
	Hexaflumuron (0.5%)	7	924	Lee (2002a)
Reticulitermes flavipes	Hexaflumuron (0.5%)	8	4	Su (1994)
	Hexaflumuron (0.1% & 0.2%)	12	827	Su et al. (1995)
	Hexaflumuron (0.1% & 0.5%)	8	85	Stansly et al. (2001)
Reticulitermes hesperus	Lufenuron (0.15%)	6	3	Haverty et al. (2010)
Reticulitermes speratus	Hexaflumuron (0.5%)	70	33	Tsunoda et al. (1998)
Reticulitermes virginicus	Hexaflumuron (0.1% & 0.5%)	8	120	Stansly et al. (2001)
Termitidae				
Macrotermes gilvus	Chlorfluazuron (0.1%)	6	900	Peters et al. (2008)
	Chlorfluazuron (0.1%)	16	2,600	Dhang (2011)
Odontotermes formosanus	Fipronil (0.004%)	16	3	Huang et al. (2006)
	Chlorfluazuron (0.1%)	31	4,800	Peters et al. (2008)
Globitermes sulphureus	Chlorfluazuron (0.1%)	11	625	Peters et al. (2008)
	Bistrifluron (1.0%)	16	143	Neoh et al. (2011)

been facilitated, thereby multiplying its impact on the medium-sized colonies.

Second, in all of the treated medium-sized colonies (except mound B), the queens appeared unhealthy and weighed much less than queens in control colonies. This likely reflects the fact that these queens received less food, nursing, and grooming by workers following the drastic decrease in the worker population, which in turned reduced their reproductive capacity. In addition, although the level of CFZ in the reproductive castes was not quantified in the current study, the high level of the compound detected in workers in the royal cell suggested that CFZ may have been acquired by the reproductive caste. CSIs can be transovarially diffused to eggs, which subsequently inhibits egg development (Peppuy et al. 1998, Rojas and Morales-Ramos 2004, Haagsma and Rust 2005). Eggs produced by reproductives that contain a lethal amount of CFZ may fail to hatch into larvae.

The present results agree with the hypothesis proposed by Neoh et al. (2011) that the basidiomycete fungus in macrotermitines potentially filters the CSIs in the same way that other white rot fungi degrade environmental pollutants as evidenced by the absence of CFZ in fungus nodules. In all likelihood, larvae did not pick up the CFZ via feeding on the fungus nodules. However, the current result indicated that the number of basidiomycete fungus in the medium-sized colonies might not be sufficient or too slow to filter all the CFZ in the fungus combs before the combs were reconsumed and distributed to larvae by workers. Further

studies are needed to determine the degree of degradation of CFZ by the white rot fungi.

Nevertheless, CSI-based baits had few adverse effects on large-sized colonies compared with medium-sized colonies, as the large colonies experienced a smaller decrease in population size (15–41%), at least with the total bait consumed (680.0 ± 49.0 g) in the current study. It is worth noting that the amount of fungus comb is positively correlated with population parameters and mound size (Darlington 1986, Darlington and Dransfield 1987, Lee et al. 2012). With the amount of CFZ incorporated in the large amount of fungus comb, this might decrease the content of CSI per unit fungus comb. It was supported by the finding that fungus combs from medium-sized colonies (>53 mg/kg of fungus comb) contained much more CFZ per unit fungus comb than did combs from large-sized mounds (<22 mg/kg of fungus comb).

In the only comparable result in the literature, Peppuy et al. (1998) reported that 59 mg of hexaflumuron per kilogram of fungus combs accumulated in a *Pseudocanthotermes spiniger* (Sjöstedt) colony that was exposed to treated food for 15 d. Only 4–21% of the CFZ acquired by the worker termites in our study was successfully transferred to the larval termites. In conjunction with the ability of basidiomycete fungus filtering the CFZ, these have important implications for large colonies of *M. gilvus* on baiting. Kubota et al. (2009) reported that the amount of bistrifluron that was recovered from foraging *Coptotermes formosanus* (Shiraki) workers during the few days before colony elimination was in the range of 483–1,380 nanogram

per termite, and ≈400 ng bistrifluron per termite was required to achieve the lethal effect (Kubota et al. 2008). Karr et al. (2004) found that ≈300 ng noviflumuron per termite was detected in dead *Reticulitermes flavipes* (Kollar) workers when they were continuously exposed to noviflumuron-treated food at 625 ppm (wt:wt) for 30 d. In the current study, the minimum threshold level of CFZ needed to cause mortality in larvae was not determined, but we postulate that the CFZ concentration accumulated in larvae (1.58 ± 0.36 nanogram per termite) in the treated large-sized colonies was too low to cause the widespread mortality required to have a colony-level impact. Nevertheless, we do not deny the possibility of CSI bait eliminating or suppressing the higher termite if the test colonies are given more time to accumulate the toxicant up to lethal level.

To the best of our knowledge, this is the first study to have successfully quantified the amount of bait toxicant transferred among termite individuals and fungus combs of *Macrotermes* in the field. We have also shown that basidiomycete fungus potentially degrade the CFZ. The results help explain the mixed performance of baits against macrotermitids. CFZ significantly suppressed medium-sized colonies of *M. gilvus* within 4 mo after bait introduction. In contrast, CFZ had limited detrimental effects on large-sized colonies, as indicated by the comparatively lower levels of CFZ in targeted termites (larvae) and fungus combs.

One of the factors that limit the widespread impact of CSIs in macrotermitids is the unique caste developmental pattern (i.e., nonmolting workers); because of this, CSIs can only target the larval caste. The interplay of the caste developmental pathway and the incorporation of CSIs into fungus combs pose a great challenge to higher-termite management programs. Our results suggested that large-sized colonies require intensive baiting effort to allow sufficient active ingredient diffuse into the colony, either by installing more bait stations or extending the baiting period. In addition, we strongly believe that more studies are required to focus on various types of bait toxicant (e.g., stomach poisons) as well as soil treatment to find ways to target both the immature stages and the worker population. Such treatments are needed to increase the chances of successful termite colony elimination in fungus-growing termites.

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