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Elimination of Field Colonies of a Mound-Building Termite *Globitermes sulphureus* (Isoptera: Termitidae) by Bistrifluron Bait

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ABSTRACT The efficacy of Xterm, which contains 1% bistrifluron, in the form of cellulose bait pellets was evaluated for its efficacy in eradicating field colonies of the mound-building termite *Globitermes sulphureus* (Haviland) (Isoptera: Termitidae). The termite mounds were dissected at the end of the experiment to determine whether the colonies were eliminated. By ≈2 mo postbaiting, the body of termite workers appeared marble white, and mites were present on the body. The soldier–worker ratio increased drastically in the colonies, and the wall surface of the mounds started to erode. Colony elimination required at least a 4-mo baiting period. Mound dissection revealed wet carton materials (food store) that were greatly consumed and overgrown by fast-growing fungi. Decaying cadavers were scattered all over the nests. On average, 84.1 ± 16.4 g of bait matrix (68.9 ± 13.4%, an equivalent of 841 ± 164 mg of bistrifluron) was consumed in each colony. Moreover, we found that a mere 143 mg of bistrifluron was sufficient to eliminate a colony of *G. sulphureus*.

KEY WORDS bistrifluron, baiting, *Globitermes sulphureus*, colony elimination, secondary pest

Bait is commonly used for termite management. Chitin synthesis inhibitors (CSIs) generally are used as the toxicant in the bait as they interfere with the formation of chitin during the molting process, thereby particularly targeting the immature stages (i.e., larvae, nymphs, and workers). The effects of CSIs on lower termite species (rhinotermitids), e.g., *Coptotermes gestroi* (Wasmann), Su et al. (2000a); *Coptotermes curvinagthus* Holmgren (Sajap et al. 2000; *Coptotermes travians* (Haviland), Lee 2002a; *Coptotermes formosanus* Shiraki (Su et al. 1997, 2000c; Rojas and Morales-Ramos 2001; Kubota et al. 2006); *Reticulitermes hesperus* Banks (Haagsma and Rust 2005, Haverty et al. 2010), and *Reticulitermes flavipes* (Kollar) (Su et al. 2000b, Stansly et al. 2001, Su 2007) have been well documented. Despite the promising performance of CSIs in managing rhinotermitids, documentation of successful elimination or suppression of higher termites (termitids) by using CSI-containing baits is limited.

Studies using paper-based hexaflumuron baits revealed that several pest termitids (e.g., *Macrotermes, Microcerotermes, Microtermes, Nasutitermes,* and *Globitermes*) did not respond well to the baits (Lee 2002b, Ngee et al. 2004, Lee et al. 2007). Furthermore, the treated colonies showed no visible detrimental effects (Ngee et al. 2004). The lack of response of termitids to termite baits has caused numerous problems in termite management among the pest management professionals in Southeast Asia. To date, they resort to insecticide spraying to repel termitids when they are found inside buildings and other structures.

Bistrifluron is a benzoylphenylurea CSI compound with the following International Union of Pure and Applied Chemistry name: 1-[(2-chloro-3,5-bis(trifluoromethyl)phenyl)-3-(2,6-difluorobenzoyl)] urea. It was developed by Dongbu Hannong Chemical Co., Ltd., Seoul, Korea (Kim et al. 2000) and was later incorporated into cellulose bait pellets under the trade name Xterm by Sumitomo Chemical for use in termite management. Because of the greater contact surface of the bait matrix, greater toxicity of bistrifluron compared with other toxicants, or both, the product can eliminate rhinotermitid colonies within a relatively shorter time (≈3–8 wk) with less bait toxicant (Evans 2010) compared with other bait products that have been tested previously (Peters and Fitzgerald 1999, Sajap et al. 2000, Su et al. 2000a, Peters and Fitzgerald 2003).

Globitermes sulphureus (Haviland) of the subfamily Termitinae occurs at high densities in the Indo-Malayan region, from Myanmar to peninsular Malaysia (Roonwal 1970, Tho 1992). It is a common mound-building termite that forms a dome-shape earthen mound that can be up to 80 cm in height and 60 cm in diameter. One main characteristic of this species is the bright yellow-colored body of the soldier termite in which the salivary gland extends to the end of the abdomen (Noirot 1969, Ngee and Lee 2002). It is highly destructive to agricultural crops and sometimes

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attacks building structures, particularly in rural and suburban areas (Lee 2002b, Kirton and Azmi 2005). *G. sulphureus* recently has been receiving closer attention from general public because of its secondary pest status. It has been found in premises that previously were treated with termite bait (i.e., *G. sulphureus* can appear after the predominant species *Coptotermes* spp. has been suppressed or eliminated by bait) (Lee et al. 2007).

In this study, we evaluated the effectiveness of bistrifluron bait in eliminating field colonies of *G. sulphureus.* The treated nests were dissected to obtain direct evidence that a given colony was eliminated. In light of our results, we also discuss several factors that may contribute to the ineffectiveness of CSI-based baits against the fungus-growing termite *Macrotermes* (subfamily Macrotermitinae), as noted previously (Lee 2002b, Ngee et al. 2004, Lee et al. 2007).

Materials and Methods

Study Sites. The study was conducted at the Minden Campus of Universiti Sains Malaysia and at Bayan Lepas, Penang in northern Peninsular Malaysia (5° 21′ N, 100° 18′ E). Penang experiences an equatorial climate that is uniformly warm and humid. In general, the study area has a constant temperature (29–35°C during the day and 26–29°C at night) throughout the year with pronounced rainy season from September to November and a dry season from January to February.

G. sulphureus colonies with mound size ranging from 30 to 50 cm in height and 35–55 cm in diameter were chosen for this study. Two colonies (designated as colonies 1 and 2) and five colonies (colonies A–D and untreated control colony) were baited on the 4 February 2008 and 10 August 2009, respectively. The colony 1 and colony 2 were evaluated during the preliminary study.

Monitoring Stations. Independent underground monitoring stations were set up for colonies A–D. A polyethylene container measuring 370 by 300 by 150 cm (with its base removed) was installed adjacent to the termite mound (≈5 cm). It contained pieces of oven-dried (60°C for 24 h) rubber wood (*Hevea brasiliensis* Mueller) that were bundled together (15 by 21 by 8.5 cm). We used these blocks to observe the colony activity by counting termites, because termites from the adjacent colony would enter the blocks and consume the wood. The wooden blocks within the monitoring stations were replaced at each monthly inspection. The infested wood blocks were brought back to the laboratory for counting of termites. The counted termites were released back to the station from which they were collected. Colonies 1, 2, and untreated control colony were evaluated without the use of independent monitoring stations.

Bait Installation (mo 0). The bait used in this study was 1.0% (wt:wt) bistrifluron-based cellulose solid pellets (Sumitomo Chemical Enviro-Agro Asia Pacific Sdn. Bhd., Senawang, Malaysia). A hole (11 cm in diameter and 20 cm in depth) was drilled into each mound, and a bait station (9 cm in diameter and 22 cm in height) was installed directly into each nest. The bait cartridge (7.5 cm in diameter and 6 cm in height) containing ≈122.0 ± 0.3 g (dry weight; $n = 5$) of 1% bistrifluron cellulose bait pellets was introduced into the bait station. For the untreated control mound, only blank bait (without toxicant) was introduced. Each bait cartridge was weighed before installation and at the end of experiment to determine the bait consumption.

Colony Health Evaluation (mo 1–4). Each of the seven colonies was monitored monthly. At each monthly inspection, colonies were assessed based on the presence of termites in the bait station (by opening the cover of bait station), in the monitoring station (colonies A–D) or the presence of termite activity when three small holes (1 cm in diameter) were drilled into the nest (colonies 1 and 2 and untreated control colony). Once termite activity was no longer visible, the mounds were dissected and examined.

At the end of experiment, the degree of mound erosion was ranked using the following scoring system: 1, no visible mound surface erosion (intact); 2, ≤25% of the wall surface was eroded; 3, between 25 and ≤50% of the mound surface was eroded; 4, between 50 and ≤75% of the mound surface was eroded; and 5 ≥75% of the mound surface was eroded.

Results

Number of Termites in Monitoring Stations. The number of termites present in the independent monitoring stations declined after 1 mo of baiting in treated colonies B, C, and D (Fig. 1). In colony A, *Coptotermes* sp. invaded the independent station after 2 mo baiting. No *G. sulphureus* was observed the station in the subsequent months, but the termites still remained in the nest. In all instances, the body of workers was marble white and showed decreased movement ≈2 mo after baiting. Complete reduction of workers in monitoring stations was achieved after 4 mo of baiting.

At the final inspection (mound dissection after 4 mo of baiting), all treated colonies were either moribund or dead. We observed a small number of larvae, marble white-bodied workers, and decaying cadavers. Occasionally, mites were observed on the bodies of the workers. Colony C may have been completely eliminated, because we did not find any termites inside the mound and the mound was invaded by ants. *G. sulphureus* stores its clump-shaped food (carton materials) in the central cavity of the nests (Noirot 1959). In all cases, the carton materials inside the nests were moist and overgrown with fast-growing fungus. Most of these carton materials had been consumed. No identifiable royal cells and nursery zones were found.

In colony A and colony 2, other termite species [i.e., *Coptotermes* sp., *Amitermes* sp., and *Macrotermes gilvus* (Hagen)] were found in the nest. We also found a female reproductive in colony A. This reproductive was weak, dark yellow, wrinkled, flaccid, and had a reduced body compared with that in the untreated control colony, which was creamy in color, turgid, and glossy.

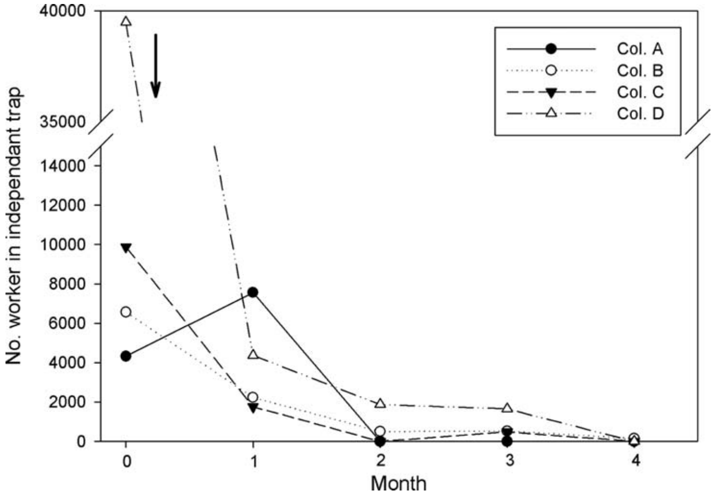

Fig. 1. Number of *G. sulphureus* workers trapped in the monitoring station from the prebaiting to the end of the baiting period. The arrow indicates when baiting began.

Mound Erosion. The erosion of treated mounds was observed at the 2-mo inspection (Table 1). At the 4-mo inspection, the wall surface of all treated mounds was eroded. The erosions ranked from category 3 and 4. The workers were unable to repair the damage, thus the inner sections of the mounds were exposed (Fig. 2). In some instances, the nests were overgrown with vegetation. In contrast, the mound of the untreated control colony remained intact (Fig. 2) throughout the experimental period.

Soldier–Worker Ratio. For colonies C and D, the proportion of soldiers found inside the independent monitoring station increased from 6 and 4% during the prebaiting period to 64 and 81%, respectively, after 4 mo of baiting (Table 1). This did not occur in colony B, where the proportion of soldiers equalized around 6% in the monitoring station. However, the number of live soldiers found during mound dissection for colony B was exceptionally large (Fig. 3). No data obtained from colony A because no termites were present because of the monitoring station was invaded by other termite species.

Table 1. Effects of bistrifluron bait against *G. sulphureus* and total bait consumption

Colony	Degree of nest erosion	Soldier–worker ratio	Bait matrix consumption (g)	Bistrifluron bait taken (mg)
1	3	N.A.	71.2	712
2	4	N.A.	93.2	932
A	4	N.A.	122.3	1,223
B	4	1:16	14.3	143
C	3	1:1.6	81.1	811
D	4	1:1.2	122.6	1,226
Untreated control	1	1:10[a]	118.9[b]	

N.A., not applicable.

[a] Soldier–worker ratio in a healthy colony (after Bordereau et al. 1997).

[b] Blank bait.

Bait Consumption. Approximately 68.9 ± 13.4% of the total bait matrix (equivalent to 841 ± 164 mg of bistrifluron) were removed by each colony (Table 1). Among the treated colonies, the highest consumption rate was recorded in colonies A and D (100% of the total amount of bait offered). In contrast, colony B had the lowest consumption rate (14.3 g [11.7%]).

Discussion

The bistrifluron bait eliminated all six treated *G. sulphureus* colonies by ≈4 mo after the bait was introduced. This result agrees well with that of a previous field evaluation of the effectiveness of chlorfluazuron against *G. sulphureus* in Thailand (Peters and Broadbent 2005). Huang et al. (2006) reported that a similar amount of time was required to suppress a colony of another macrotermitine, *Odontotermes formosanus* Shiraki, when using fipronil bait. The time required to eliminate a colony of higher termites generally is longer than that required for rhinotermitids [e.g., *Coptotermes acinaciformis* (Froggatt), Evans (2010) and *C. formosanus* and *Reticulitermes speratus* (Kolbe), Kubota et al. (2007)]. One possible explanation for this difference is that rhinotermitids are wood-feeding termites that readily consume food and share it among colony members. Buczkowski et al. (2007) revealed that food is delivered through trophallaxis to ≈50% of various castes of *R. flavipes* within 3 d.

Food processing in termitids is more complicated and rigorous compared with that in rhinotermitids. For example, in the case of *Globitermes*, workers often store balls of vegetation within the nest without undergoing digestion process (Noirot 1959), and the food is fed upon a certain period of time. Similarly, the macrotermitines deposit food as pellets in a comb-like structure in the nest (Pearce 1997). Boutton et al. (1983) suggested that the food movement path of

Fig. 2. Nest of *G. sulphureus* (colony 2). (A) Healthy nest before treatment. (B) Appearance of nest after the 4-mo baiting period. The arrow indicates the bait station. (Online figure in color.)

Macrotermes is as follows: plant material → major workers → fungus comb → nonreproductive castes → reproductive caste. In *Macrotermes bellicosus* (Smeathman), the foods that were taken by major workers were deposited and remained as fungus combs for >2 wk (Collins 1981). Subsequently, they were delivered to the nonreproductive and reproductive castes. Thus, we hypothesize that the low turnover rate of the food store (e.g., balls of vegetation and fungus combs) may delay the delivery of toxicant among colony members. In addition, Duncan (1997) reported that the bait that were removed may not be consumed. In many cases, the baits also may be used for mound construction rather than being consumed (Peters and Broadbent 2005). These factors may explain why it took longer for the bait to eliminate *G. sulphureus* colonies compared with the rhinotermitid colonies, i.e., *C. acinaciformis* (Evans 2010).

In our study, after weeks of bait treatment the worker termites became marble white in color due to uric acid accumulation in their bodies (Peters and Broadbent 2005). An increase in the soldier–worker ratio also occurred in colonies C and D. A *G. sulphureus* colony consists of tens of thousands of individuals (Ngee and Lee 2002), and usually the soldiers only account for ≈5–10% of the population (Bordereau et al. 1997). Thus, the soldier–worker ratio increased by six-fold within 4 mo of baiting in colonies C and D compared with a normal colony; this disrupted the caste balance and eventually led to the collapse of the colony.

Kubota et al. (2006) demonstrated that *C. formosanus* movements were affected by exposure to 5,000 ppm bistrifluron. They became weak and were unable to carry out routine colony maintenance and allogrooming. If this was true in our experiment, it could explain the presence of mites on the termite body and why fast-growing fungi were observed inside the nests. It also could explain the inability of termites to repair the eroded mounds. When working with *Macrotermes*, Darlington (1991) observed highly degenerated food stores (fungus comb) in the collapsed

Fig. 3. Soldiers (yellow) outnumber worker termites (white) (colony B). (Online figure in color.)

mound, and we found the same in this study. Darlington (1991) suggested that this may have resulted from the lack of workers maintaining the food stores in the mound.

In the current study, *G. sulphureus* responded well to the bistrifluron cellulose bait pellets, as shown by the high bait intake. This may be due to the large bait surface area, which increases bait consumption (Evans and Gleeson 2006). Depending on the size of the colony, a mere 143 mg of bistrifluron was sufficient to eliminate the *G. sulphureus* colony. This result may indicate that bistrifluron has a greater termiticidal activity compared with hexaflumuron, chlorfluazuron (*C. acinaciformis*, Evans 2010), and noviflumuron (*C. formosanus*, Cabrera and Thoms 2006). Kubota et al. (2008) reported that almost all *C. formosanus* workers were killed at lethal dose of 400 ng per termite and that the LT_{50} of bistrifluron against *C. formosanus* workers were 2.9 wk at 0.5% bistrifluron.

It is possible that bistrifluron cellulose pellet bait also can be used to manage other termite pest species in the subfamilies Termitinae and Nasutitermitinae, in which the worker termites undergo several successive moltings (Roisin 2000). In fact, we have eliminated colonies of *Microcerotermes* sp. with the same bait used in this study (K.-B.N. and C.-Y.L., unpublished data). We tried to bait *Macrotermes gilvus* (Hagen) by using CSI-based baits and, even though enormous amount of baits were removed by the termites, no detrimental effect on the treated colonies was observed. Although Peters et al. (2008) reported successful CSI baiting against many termitids (including *Macrotermes*), their results were based on onsite inspection without any direct evidence via mound dissection.

We propose two hypotheses to explain why CSI-based baits are ineffective against pest species of the subfamily Macrotermitinae. First, the developmental pathway of the Macrotermitinae differs from that of the Termitinae. Only a single stage worker caste exists in macrotermitines. Thus, unlike its counterpart in termitines, macrotermitine workers do not molt and thus are not affected by CSIs that only serve as larvicides (Peppuy et al. 1998).

Second, macrotermitines are known to cultivate basidiomycete fungus of the genus *Termitomyces* (or white rot fungi) in the fungus combs within their nests. Increasing evidence suggests that white rot fungi may potentially degrade a diverse array of environmental pollutant (Ohkuma 2003, D'Annibale et al. 2005, Gao et al. 2010). Much remains to be explored about the detoxifying spectrum of the basidiomycete fungus in macrotermitines, and we cannot exclude the possibility that the bistrifluron that was removed from the bait station by the termites was being incorporated into the fungus comb and then degraded by the fungus after a period of time. These hypotheses are currently under investigation.

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