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Colony Elimination of Subterranean Termites by Bait Application Using Benzoylphenylurea Compounds, with Special Reference to Bistrifluron

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1. Introduction

1.1 Termite as pest insect

Termites are insects that belong to Termitidae and from systematic point of view cockroaches are most closely-related to them. Although termites had been formed the independent order, Isoptera, recently they became a part of Blattodea (Inward et al., 2007). While cockroach is one of the most important household and hygiene pest insects all over the world, termite is also occupying a high position as a household pest insect. However their position is bit different from their closest relatives and is a structural pest which attacks and gives serious damage to wooden structures such as residences [c.f., in developing countries they are pests to trees and agricultural products (Constantino, 2002)]. In Japan, several hundred million dollars are spent annually for the prevention and control of termites (Yoshimura, 2001; Tsunoda, 2003). One thing to be addressed here is that termites are very important decomposers of cellulose in the ecosystem. They become pest insects when their habitats overlap those of humans.

Fig. 1. Left: workers and soldiers of *Coptotermes formosanus* in their nest. Right: Damage on a residential structure caused by foraging termites of *C. formosanus*

Termites are eusocial insects similarly as Hymenopterous insects, like ants and wasps, which are taxonomically distantly-related groups. They usually build up queen-and-king-centered

colonies. Thousands to millions of worker termites are usually deployed in each colony. They are systematically mobilized to attack and feed wooden materials, therefore sometimes give critical damages to wooden structures. Among them, economically the most important group in temperate and tropical regions is Rhinotermitidae which includes genera *Coptotermes* and *Reticulitermes*. Termites belonging to Rhinotermitidae are 'subterranean termites' as they build their nests in the ground. Since they come on underground tunnel and often invade through underfloor space of structures, few people are aware of them until their houses are assaulted. *Coptotermes* and *Reticulitermes* termites widely occur in human habitat and are seriously harmful to structures. *Coptotermes* termites, like *Coptotermes formosanus* Shiraki, *Coptotermes gestroi* (Wasmann), *Coptotermes acinaciformis* (Froggatt) and so on, build huge colonies and extremely problematic species around the Pacific countries (Fig. 1).

1.2 Chemical control of termites and colony elimination by insecticidal baiting

In general, methods to control termites using insecticides are preventive: typically liquid formulations are applied to perimeter or underfloor of a premise or to wooden materials directly. However, their unique characteristics of eusocial nature had been drawn large interests of researchers to control their whole colonies by application of toxic baits. Actually such an approach has been examined for its feasibility for decades and various kinds of insecticide have been evaluated as active ingredients (table 1). Although some insecticides succeeded in reducing population of a colony for the meanwhile, they could seldom bring about elimination of it. In the mid of 1990's benzoylphenylurea (BPU) compounds such as hexaflumuron were proven to be effective as bait toxicants for eliminating rhinotermitid colonies (Su 1994; Su et al., 1997). Since then many studies have documented that BPUs can successfully eliminate subterranean termite colonies in the field (Tsunoda et al., 1998; Peters & Fitzgerald, 1999, 2003; Getty et al., 2000; Sajap et al., 2000; Su et al., 2000; Lee, 2002; Tsunoda et al., 2005; Husseneder et al., 2007; Ripa et al., 2007).

Chemical class or mode of action	Active ingredient	Source
Juvenile hormone analogue	Methoprene	e.g., Su et al., (1985)
	Fenoxycarb	e.g., Jones, (1989)
Metabolic inhibitor	A-9248	e.g., Su & Sheffrahn, (1996)
	Sulfluramid	e.g., Su & Sheffrahn, (1996)
	Hydramethylnon	e.g., Powson & Gold (1996)
Phenylpyrazol	Fipronil	e.g., Huang et al., (2006)
Benzoylphenylurea	Hexaflumuron	e.g., Su, (1994)
	Diflubenzuron	e.g., Rojas & Morales-Ramos (2003)
	Lufenouron	e.g., Haverty et al., (2010)
	Chlorfluazuron	e.g., Peters & Fitzgerald (2003)
	Noviflumuron	e.g., Cabrera & Thoms (2006)

Table 1. Active ingredients that have been evaluated as bait toxicant for termite control in the field

Up to now, only BPUs have been successful as bait toxicants in terms of elimination of subterranean termites' colonies. As BPUs are chitin synthesis inhibitor, it is presumed that their success as bait toxicant should be attributed to 1) their extreme slow action, 2) less dose dependency and 3) less feeding deterrency (Su & Scheffrahn 1993, 1996). However, there have been few reports that tried to examine such properties of BPUs.

1.3 Bistrifluron

Bistrifluron (Fig. 2) is one of BPUs (Kim et al., 2000), therefore it was expected to show good efficacy in terms of colony elimination of subterranean termites. Author conducted several laboratory studies to evaluate inseciticidal efficacy of the compound against subterranean termites in order to examine how baiting with BPUs bring about colony elimination of termites. *C. formosanus* and *Reticulitermes speratus* (Kolbe) which are the most important pest species in Japan were used for the studies.

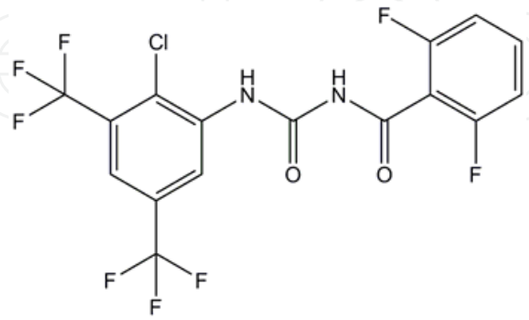

Fig. 2. Chemical structure of bistrifluron

2. Termiticidal activity of bistrifluron and its speed of action

In order to quantitatively evaluate feeding toxicity of bistrifluron against termite, no-choice and two-choice feeding tests with filter paper baits were conducted with *C. formosanus* workers in the laboratory (Kubota et al., 2006).

2.1 Evaluation methods

In the no-choice feeding test, filter paper disks treated with 0.005, 0.05, 0.5 and 5.0% (w/w) of bistrifluron were air-dried and weighed. Each disk was placed in a small plastic cup (ca. 14 ml) with small entry holes to allow termite access. Plastic cups were then put into separate plastic containers (200 ml) and each container retaining 100 of *C. formosanus* workers. The bottom of this larger container was covered with 2-3 mm of plaster and had several small holes made in the base. Assembled units were placed on a damp cotton pad in an incubation chamber so that termites could uptake water through the plaster. Five units were prepared for toxic baits and untreated controls. The units were maintained under appropriate condition for 12 weeks. Dead or moribund termites were counted at given interval to determine the change in mortality over time.

In the two-choice feeding tests, Filter paper disks were treated with 0.5% (w/w) of bistrifluron. The test container was a plastic Petri dish (140 mm in diameter) with approximately 5 mm-thick agar [4% (w/w)] on the bottom. Four wells (35 mm in diameter) were made through the agar and plugged by two treated and two untreated disks. One hundred termites were introduced before the dish was covered with a lid and sealed with parafilm. Units were kept under appropriate condition. Mortality and weight of disks consumed were checked at given intervals after termites were released. Five replications were made.

2.2 Speed of action

In the no-choice test, mortality increases significantly faster as bistrifluron concentration were higher (Fig. 3). When termites were exposed to 0.5% bistrifluron (possible concentration in a commercial product), there had been no significant increase in mortality

until the 4th week. Even when exposed to overdosing 5.0% bistrifluron, 10 times higher concentration, there was no significant increase in mortality at the 1st week. These results indicate that bistrifluron shows very slow action on termites. In comparison to sulfluramid which is often used for ant bait, *C. formosanus* workers exposed to 0.01% (w/w) sulfluramid would die off within a week (Grace et al., 2000). On the other hand, dose dependency of bistrifluron's speed of action suggests that faster or better effect will be obtained by larger dosing. The no-choice feeding test with various concentrations of bistrifluron baits was also conducted against Japanese *R. speratus* workers and similar dose dependency and very slow action at even high doses were also shown (Kubota et al., 2007).

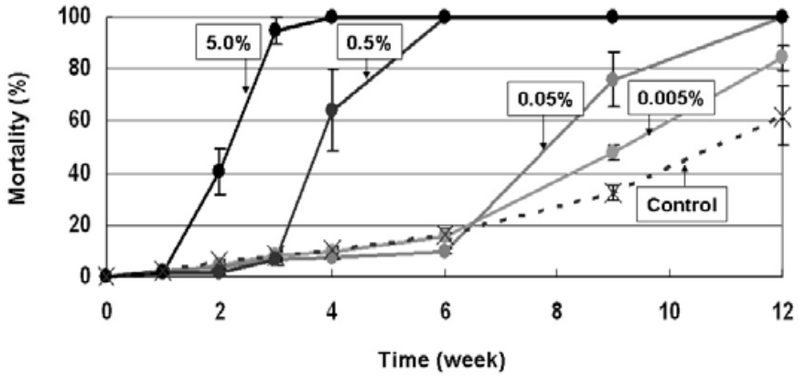

Fig. 3. Time-course change of mortality of *C. formosanus* workers exposed to 0.005, 0.05, 0.5 and 5.0% bistrifluron baits and blank bait in the no-choice feeding test (data from Kubota et al., 2006)

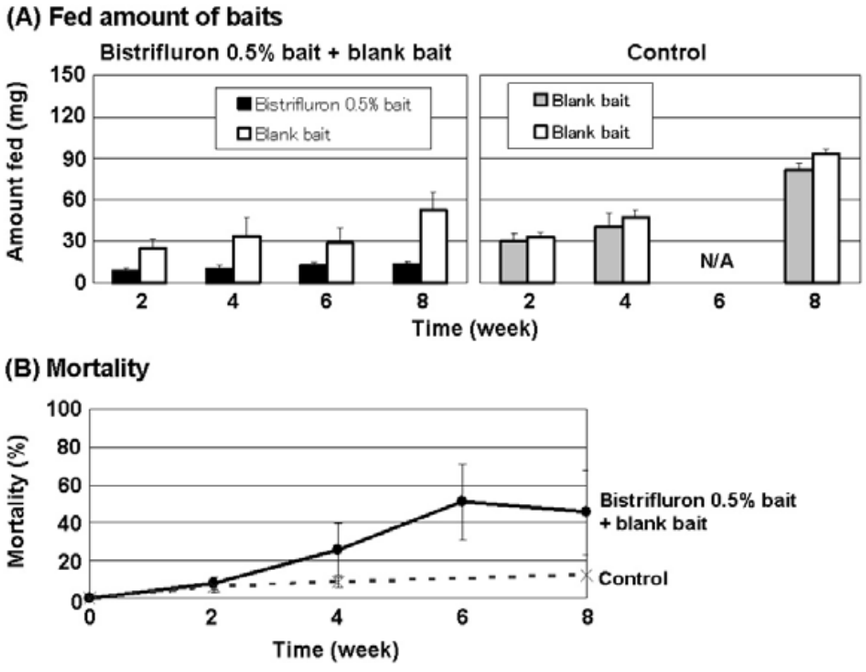

Fig. 4. The results of two-choice feeding test: A) fed mounts of bistrifluron 0.5% baits vs. blank baits, and also blank baits vs. blank baits in controls; B) time-coarse change of mortality of *C. formosanus* workers exposed to 0.5% bistrifluron baits and blank baits (data from Kubota et al., 2006)

In the two-choice test, fed amounts of baits treated with 0.5% bistrifluron seemed less than untreated baits and significant effect on termite survival was not shown during 8-week test period. These results also indicate that feeding preference of termites will greatly influence on performance of a bait product.

2.3 Allogrooming inhibition effect

Some subterranean termites build their nest in the ground, form tunnel networks and extend their territories. Workers and soldiers are patrolling every time and everywhere in their territories because they are always under threat of attacks from various kinds of enemies. Various microbes are living very next to termites' territory in the ground and trying to take every opportunity to invade it. Some of them are pathogenetic to termites. It is considered that termites have developed behaviours to protect their territories from attacks of many invaders. Allogrooming, the behaviour that a worker grooms other colony member's body, is one of the most important behaviour since microbes attached onto termite's body are effectively removed through the behaviour (Thorne & Traniello, 2003).

It is considered natural that incompetence of the above described behaviour should be involved in the mechanism of colony elimination by bait application. That is, once large part of workers are intoxicated with bait toxicant and fail in colony protecting behaviours like allogrooming their colony will collapse by intruding enemies shortly. To examine such a effect of bait toxicant, allogrooming inhibition effect of bistrifluron was evaluated by the no-choice feeding test similarly designed as the test described in the section 2.1 (Kubota et al., 2006).

In the no-choice feeding test *C. frmosanus* workers were exposed to filter paper baits treated with 0.5% (w/w) bistrifluron during the test period (5 weeks). At every week termites fed on the bait were stained with red dye, erythrosin (0.5 g/l), on their tergites. Five red-stained workers were left together on a moistened filter paper in a petri dish for 3 hours then the intensity of the red dye that remained on each termite was rated as follows: (1) red dye unchanged, (2) faded or partly disappeared, and (3) no dye remaining at all. Five replications were made for each evaluation time.

When termites were exposed to untreated filter paper no red dye were remaining on all the termites throughout the study period. On the other hand, when they were exposed to toxic bait there were two termites with red dye partly remaining even at 1st week and 24 termites with dye partly remaining or intact at the 2nd week. These results indicate that workers uptaking more than a critical dose of bistrifluron soon become unable to do their regular works to maintain colony health and that it will then result in acceleration of the collapse of a colony.

3. Colony elimination in the field cases

Colony elimination performance of bistrifluron has been examined in the field. In this section the results of the Japanese and Malaysian cases with *Coptotermes* are described. Aside from these studies, Colony elimination performance of bistrifluron was demonstrated against Australian *C. acinaciformis* (Evans 2010) and Malaysian *Globitermes sulphreus* (Neoh et al. 2011).

3.1 Field trial against *Coptotermes formosanus* in Japan

A bait system using bistrifluron as an active ingredient was applied to a *C. formosanus* colony which naturally occurred in Okayama city, Okayama prefecture in Japan (Aki, 2005). The bait system worked in the following procedure: bait stations containing wood blocks

were installed in the ground. Every station was investigated as to whether wood blocks were infested with termites at given timings. If any station was infested with termites, infested blocks were replaced with paper baits impregnated with 0.5% (w/w) bistrifluron. The location of their nest was identified in this case: their nest had been established within a tree trunk in the vicinity of the premise being attacked. Therefore a couple of bait stations were installed in the ground around the trunk.

The progress and results were as follows: bait stations containing wood blocks were installed on June 29, 2002. Toxic baits were applied on July 18, 2002 as some wood blocks were infested. After some inspections of bait stations there was no live termite and were a lot of dead soldiers on September 3, 2002 at any place where they had been seen before. Inspection within the nest with a microfiber scope revealed that there was also no living termite in their nest. Therefore it can be concluded that the colony had been eliminated by the 1.5-month bait application.

3.2 Field trial against *Coptotermes gestroi* in Malaysia

Efficacy of bistrifluron as a bait toxicant was evaluated against *C. gestroi* which is the most dominant species in Malaysia (Lee, 2007). The study was performed using premises in Penang Island, which had been suffered from their heavy infestation. Blank paper baits stored in plastic cases (the side to which an infested site was attached was open) were applied onto several infested sites of each premise. Since blank baits had been infested at all the premises, 30-50% of them were replaced with tablet bait made of powder cellulose incorporated with 0.5 or 1.0% (w/w) bistrifluron for each premise. All the bait stations were monitored on a weekly basis. Baits with less than 20% remaining matrix were replaced with new ones. The replaced bait matrix were dried and weighed to determine consumed amount. At the time point when there was no termite activity in all the stations applied to each premise it was concluded that the colony was eliminated.

In the four cases applied with 0.5% bistrifluron baits every colony has been eliminated 6-8 weeks after application of toxic baits. Consumed amounts of toxic baits were 211.4-645.2 g per colony, which were equivalent to 1.06-3.23 g of the active ingredient. In the other four cases applied with 1.0% bistrifluron baits every colony has been eliminated 4 or 5 weeks after application of toxic baits. Consumed mounts of toxic baits were 172.9-833.9 g, which were equivalent to 1.73–8.34 g of the active ingredient. Although how much amount of and how intensively baits are consumed would depend on some factors like colony population and activity, faster elimination was obtained with 1.0% bistrifluron baits than 0.5% baits in this study.

3.3 Discussion on the field studies

As in most cases location of each nest will be out of reach and unidentified, judgement of colony elimination by absence of termite in their nest is difficult and unrealistic. Whether colony elimination has been achieved should be determined by monitoring all the bait stations for a certain period after no termite activity was observed in all the stations by baiting, like the Malaysian case described above. If status of no termite activity continues for the given period (EPA guideline mentions that it should be more than 12 months) then it should be concluded that the colony applied with bait has been eliminated (US EPA, 2004). On the other hand, in the Japanese case colony elimination was confirmed by direct inspection of inside of the nest. Separately from this case, there were many Japanese cases that colony elimination seemed to

be achieved by application of bistrifluron bait based on the above described procedure (unpublished data). These data indicate that application of bistrifluron bait will successfully eliminate colonies of *Coptotermes* termites within 1–2 months. Although numbers of foraging termites varies from ten thousands to over a million, it will take weeks that single colony fall into the collapse after large part of foraging termites of single colony take more than a critical amount of bistrifluron and become unable to take part in maintaining colony's health. The laboratory study described above also showed that individual workers will become incompetent and die within a couple of weeks. Combining such laboratory results with the fact that colonies having hundreds of thousands to a million of foragers were eliminated in 1–2 months, it is suggested that large part of foragers took a critical amount of bait toxicant in very short time and it is an interesting fact for discussion of termite's feeding behaviour. A manner in which termites will take up bait toxicant will be discussed in the next section.

4. Feeding behaviour of termites and kinetics of bistrifluron in the termite body

To discuss how termites will take up a critical amount of bistrifluron through their feeding acitivities, some laboratory studies were conducted to determine its lethal dose and kinetics in their bodies (Kubota et al., 2008). Firstly analytical method had to be established to perform these tests.

4.1 Analytical method

Method to chemically analyze bistrifluron amount in termite body was examined using high performance liquid chromatography (HPLC). The outline of analytical method established for the study is as follows.

The termites were analyzed by HPLC immediately after they were collected. The termites were kept chilled in a vial on ice until they were crushed and homogenized in a mortar with a pestle after being rinsed with solvent. A preliminary chemical assay demonstrated that there was no peak of bistrifluron in HPLC analysis with termites that had been exposed to blank bait. When a given amount of bistrifluron was mixed well with homogenized termites in solvent, 100% bistrifluron was recovered by the above-described procedure. The amount of bistrifluron recovered from solvent that was used to clean termites that had been exposed to 0.5% bistrifluron bait for 1 or 2 weeks was much smaller than 5% of that recovered from whole bodies. The amount of bistrifluron that remained on the external surface of termites was considered to be negligibly small. Homogenized termites were washed into a flask with acetonitrile and then subjected to ultrasonication (42 kHz ± 6%) for more than 1 hour in an ultrasonic device to extract bistrifluron from termites. The acetonitrile suspension was filtered with a filter, PTFE of 0.45 µm. HPLC analysis was performed by a Shimadzu LC-10Avp (Shimadzu Corporation, Kyoto, Japan), with a column of SUMIPAX ODS A-217 (4.6 mm in internal diameter, length 150 mm, Sumika Chemical Analysis Service, Ltd., Tokyo, Japan). Flow rate, injection volume and wave length were 0.5 ml/min, 20 µl and 254 nm, respectively. Mobile phase was acetonitrile/water = 80/20. Analytical-grade 2, 4, 6–anilinetrichloride was used as an internal standard. HPLC-grade acetonitrile was used as a solvent.

4.2 Lethal dose of bistrifluron to workers of *Coptotermes formosanus*

How much amount of bait should be fed by individual foragers was investigated to examine how much amount of bistrifluron should be necessary to be taken to give a critical effect on them. The similar no-choice test with *C. formosanus* workers as described in the 2nd section was designed to examine how long termites should be exposed to 0.5% (w/w) bistrifluron

bait to feed enough amounts. Time-course changes of mortality for 3-, 7- and 14-day exposures were recorded for 12 weeks and compared to that in the untreated control. In each case of 3-, 7- or 14-day exposure toxic baits were replaced with blank baits just after exposure to toxic for given periods. Five replications were made for each exposure period.
While 3-day exposure did not cause significant increase in termite mortality in comparison to untreated control, 7- and 14-day exposures resulted in mortality increase at the 6th week and 4th week, respectively, and for both cases all the termites had been dead by the end of test period. It is indicated that 7-day exposure should be enough for most of workers to take up a lethal dose under the test condition. LT_{50} (50% lethal time) was ca. 6 weeks.

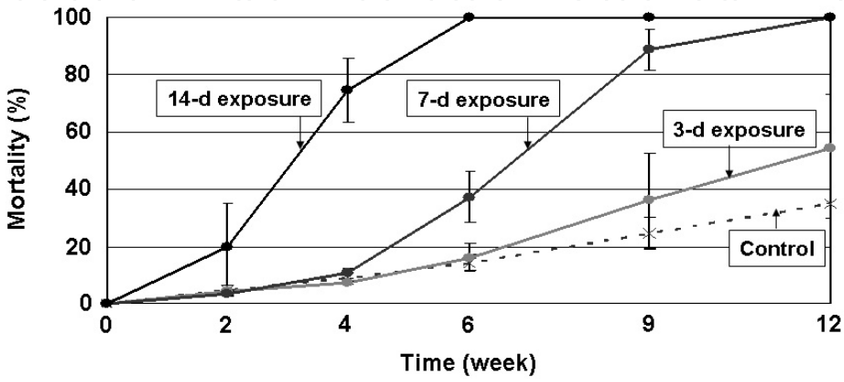

Fig. 5. Time-coarse change of mortality of *C. formosanus* workers exposed to 0.5% bistrifluron bait for 3, 7 or 14 days (data from Kubota et al., 2008)

Based on these results, *C. formosanus* workers exposed to 0.5% bistrifluron baits for 1 week and those intoxicated were analyzed at the 6th week after the test initiation in order to determine how much amount of bistrifluron would remain and how bistrifluron moves in a termite body. The amount of bistrifluron that was detected from moribund termites at the 6th week was 397.7 ng/termite, which indicates that the uptake and accumulation of ≥400 ng bistrifluron by an individual termite could possibly provide slow-acting insecticidal efficacy.

4.3 Distribution and kinetics of bistrifluron in the termite body

Distribution of bistrifluron in the termite body was examined. *C. formosanus* workers were exposed to 0.5% bistrifluron bait for 7 days in the above-described no-choice feeding test. The termites were analyzed right after 1-week exposure to toxic bait and also after subsequent 2-week exposure to blank bait. They were dissected into heads, legs, alimentary tracts, and remaining bodies under a stereoscopic microscope. Each body part from ten termites was analyzed collectively and four replications were made.
The amounts of bistrifluron recovered from heads, legs and other body parts of termites were 90.5, 4.5 and 559.1 ng/termite, respectively, just after *C. formosanus* workers were exposed to toxic bait for 1 week (Fig. 6). These amounts were 95.8, 6.4 and 385.7 ng/termite, respectively, after termites were then exposed to blank bait for 2 weeks (Fig .6). There was no significant difference in the amount of bistrifluron between the post-exposure periods for any of the body parts. The amounts of bistrifluron recovered from alimentary tracts were 60.8 and 48.8 ng/termite, respectively, whose levels were approximately equivalent to 10% of that from a whole termite body. These results indicate that bistrifluron molecules should move quickly from inside the alimentary tract toward each body part and then they would exist in each body part stably.

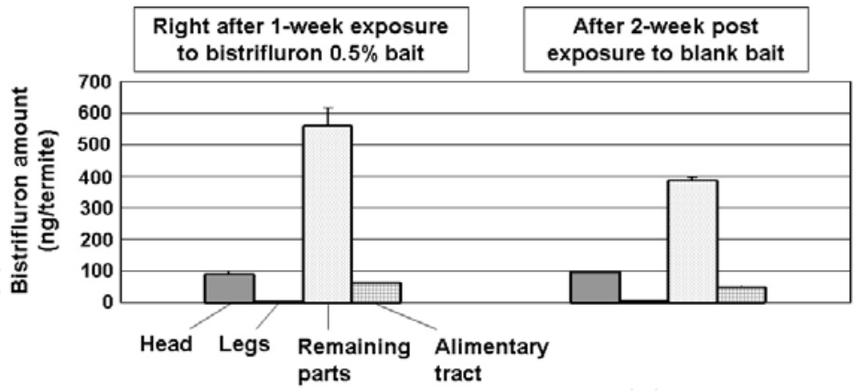

Fig. 6. Amounts of Bistrifluron recovered from each body part of *C. formosanus* workers exposed to 0.5% bistrifluron bait for 1 week (data from Kubota et al., 2008)

Finally how much amount of bistrifluron would be transferred and/or lost from an individual termite which fed toxic bait to their nestmates by trophallaxis. As termites transfer materials which they foraged to their nestmates from their mouths, bait toxicant may be also transferred from foragers to other nestmates. It is also possible that a large proportion of bistrifluron is not present in a termite body in a stable form, but just circulates in a termite body and among individual termites by trophallaxis. Similarly as the previous tests *C. fromosanus* workers were exposed to 0.5% (w/w) bistrifluron bait for 1 week. Right after 1-week exposure to toxic bait 10 exposed termites (donors) were left together with 10 intact workers (recipients) and blank bait. Recipients were replaced with new intact workers every week. Donors and recipients were analyzed every week to monitor transfer/lost of bistrifluron between termites. Ten termites were collectively analyzed and four replications were made for both donors and recipients at each time point.

The amount of bistrifluron detected in the donors was 546.0 ng/termite just after the exposure for 1 week, and this significantly decreased to 250–300 ng/termite after contact with recipients for 1-3 weeks (Fig. 7). However, the loss of bistrifluron from donors was not significant during the 1-3 weeks of contact with recipient. The amount of bistrifluron recovered from the recipients was 32.6 ng/termite after 1 week of contact with donors, and the amounts were very small after an additional period of contact for 1 and 2 weeks (2.2 and 1.6 ng/termite, respectively).

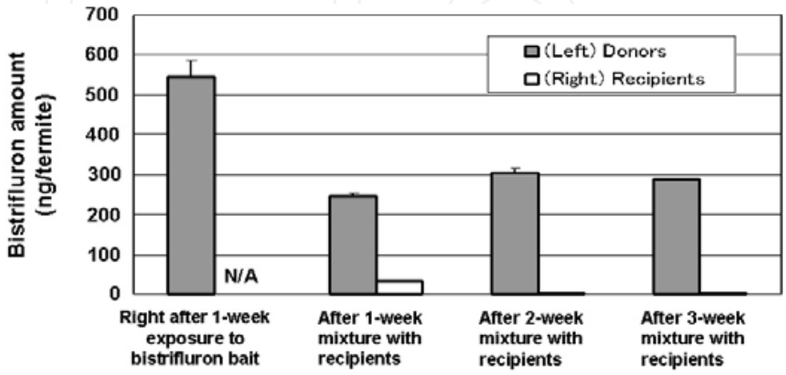

Fig. 7. Changes in the amount of bistrifluron regularly recovered from *C. formosanus* worker donors and recipients after the 1-week exposure to 0.5% bistrifluron bait (data from Kubota et al., 2008)

When termites were first exposed to 0.5% bistrifluron bait for 1 week and then mixed with the same number of unexposed nestmates, 6% of the bistrifluron taken by donors was transferred to recipients, and some bistrifluron was lost from the donors during the 1-week period of mixing. However, bistrifluron appeared to remain unchanged inside the termite body because there was no significant difference in the bistrifluron amount in the donors at the 2nd, 3rd and 4th weeks and very little bistrifluron was detected in recipients at the 2nd and 3rd weeks.
These results support the notion that once a large amount of bistrifluron is taken by a *C. formosanus* worker, it stably exists in a termite body for several weeks. Unfortunately, there is no evidence regarding how much bistrifluron can be transferred among nestmates in the field to compare the results further. However, it is possible that some proportion of bistrifluron is transferred to nestmates by frequent trophallaxis within a short period of time. For example, the largest proportion of hexaflumuron taken by *Reticulitermes hesperus* Banks in California was transferred from donors to recipients after exposure to hexaflumuron bait for a day (Haagsma & Rust, 2005). In addition, the materials transferred from donors to recipients seem to be retransferred to other nestmates by cascade events (Sùarez & Thorne 2000).

5. Process of colony elimination by the uptake of bait toxicant through termites' foraging activity

The results reported in section 4 were all from laboratory evaluation and possibly based on termites' feeding behaviours under controlled conditions. They should be examined by comparing them with outcomes obtained in a simulated study under the field condition. Threfore, bistrifluron baits were applied to a colony of *C. formosanus* and foraging termites were collected and analyzed for bistrifluron amount at given timing, which would give an insight in terms of how bistrifluron would be taken up by foragers and distributed among colony members (Kubota et al., 2009).

5.1 Experimental setup

A colony of *C. formosanus* obtained from the field was used for the study. The nest of the colony originally infested residence in Wakayama Prefecture, Japan, and collected in December 2006, and then placed in a Styrofoam box (52 cm wide x 35 cm long x 44 cm high) to form an artificial nest.
Experimental baits were prepared by folding three sheets of paper towels (ca. 20 g). Bistrifluron-treated baits were prepared by pretreatment of core paper towels with an acetone solution of bistrifluron to give 0.1 g of bistrifluron per bait [ca. 0.5% (w/w)].
The laboratory arena was set up with a plastic container (77 cm wide x 122 cm long x 21 cm deep) containing four concrete blocks (39 cm wide x 19 cm long x 10 cm high) aligned so that adjacent blocks were in contact. The Styrofoam box, the artificial nest, was placed over two of the concrete blocks. Water was added to the plastic container to supply termites with water and to create a moat around the blocks to prevent termite from escaping. A wood block of *Pinus thunbergii* Parl. was placed on the concrete block in the opposite side from the nest. Alignment of the materials is roughly illustrated in Fig. 8. The laboratory arena, with nest and wood block, was set up in December 2006. By the end of January 2007, the termites had constructed a mud tunnel between the nest and the block. On 10 September 2007, one blank bait each was placed on the mud tunnel at three sites (site 1, 2, and 3), and each bait was covered with an aluminum cup (ca. 200 ml). Because foraging termites started consuming blank baits immediately after they were installed, the bait at site 1 was replaced with a bistrifluron bait on 25 September 2007. Allocation of bait (toxic or blank) at each baiting site is shown in Fig. 9.

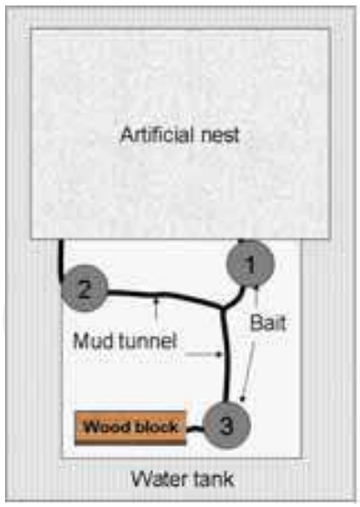

Fig. 8. Illustration of the experimental arena and alignment of the artificial nest, wood block, tunnels and baits (copied from Kubota et al., 2009) This is a copy from Sociobiology 2009.

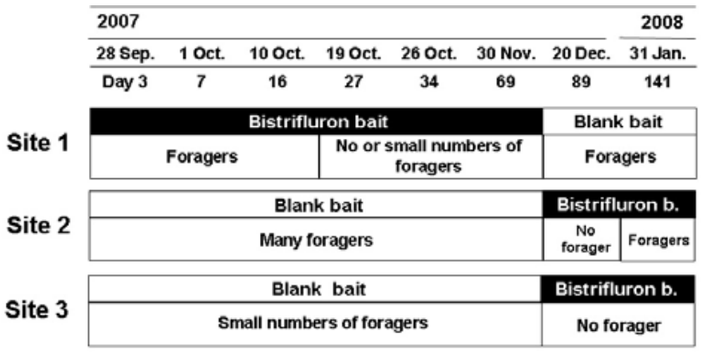

Fig. 9. Allocation of bistrifluron/blank bait and status of termite activity at given dates at each baiting site (site 1, 2 and 3)

5.2 Collection of foraging workers and analysis

Termites were separately collected from each site (~5 termites/bait) on 28 September 2007 (day 3 after installation of a bistrifluron bait), and individual termites were subjected to chemical analysis of bistrifluron in the same way with HPLC as section 4.1. Termites were also collected in the same manner on five other dates: 1 October (day 7), 10 October (day 16), 19 October (day 27), 26 October (day 34), and 30 November (day 69) before baits were renewed. Because termite foraging always decreased shortly after toxic baits were added (termites appeared to stop feeding on baits for about 1 month after placement of a toxic bait), all three baits were renewed on 30 November 2007 (a blank bait was placed at site 1 and a toxic bait was placed at sites 2 and 3, see Fig. 9). Additional termites were collected for bistrifluron analysis on 20 December 2007 (day 89) and 31 January 2008 (day 141).

5.3 Confirmation of colony elimination

The baits were more heavily consumed by termites at site 2 than at sites 1 and 3, regardless of treatment with bistrifluron. Foraging activity appeared to decline with time after toxic baits were added as evidenced by the decreased number of foragers at all baiting sites (both

treated and untreated). Because no forager was present at site 1 (treated bait added on 25 September) on sampling dates from 26 October to 30 November, all baits were replaced with new ones on 20 November as previously noted: toxic baits were added at sites 2 and 3 and a blank bait was added at site 1. Foraging termites were seen at site 1 on 30 December, and foraging termites were observed at site 2. One possible explanation for the decreased foraging population and disappearance of foragers from site 1 is feeding deterrence by bistrifluron, as previously suggested in the two-choice test described in section 2.2. Although it is unclear why foragers came back to the toxic bait at site 2, the shuffle of treated bait sites might lead to reinfestation of the treated bait by foragers at site 2 where termites previously fed on untreated bait. Dead workers and soldiers were found at site 1 and site 2 several days after the last collection (31 January 2008), and no live termite was present at these sites. Author concluded that the colony was eliminated because no live termites were found over half a year after application of toxic baits. Although the current study demonstrated that *C. formosanus* colonies could be eliminated by application of bistrifluron, the results clearly supported the importance of placement of baits.

5.4 Manner of the uptake of bait toxicant by foraging termites

Bistrifluron amounts recovered from each termites collected from each site are shown in Fig. 10. The recovered amounts of bistrifluron ranged from 101 to 1026 ng/termite when five foragers were collected from site 1 (with toxic bait) on 28 September (day 3) and 1 October (day 7). On the other hand, when blank baits were present at sites 2 and 3, only two of 17 foragers contained bistrifluron, and the quantities detected were small (45 and 57 ng/termite). Between 10 October and 30 November 2007 (toxic bait at site 1 and blank baits at sites 2 and 3), bistrifluron was detected from seven foragers collected from site 1, and the amounts ranged from 31 – 477 ng/termite. During this same period, analyses of 28 foraging workers from sites 2 and 3 indicated that 17 termites contained bistrifluron (22 – 196 ng/termite) and 11 termites did not. These results appear to demonstrate that up to a few hundred milligrams of bistrifluron was taken up by an individual worker through foraging activities.

Because much smaller amounts of bistrifluron were recovered from workers collected at sites 2 and 3 than site 1, it is uncertain whether those workers were donors or recipients of bistrifluron among colony members. If they were recipients, they received some bistrifluron from donors that originally took bistrifluron at site 1. This was quite possible because only a small portion of materials taken up by donors would be transferred to recipients (Sùarez & Thorne 2000; Haagsma & Rust 2005; Buczkowski et al., 2007). If they were donors, they consumed bistrifluron at site 1 and were caught at sites 2 or 3 after losing some portion of the bistrifluron through trophallaxis and metabolism. Unfortunately, distinguishing between donors and recipients will be difficult until foraging behaviour of termites is better understood: after feeding at one site, whether do foragers feed at other sites on their way back to the nest? And how frequently do they go the rounds of their feeding sites?

Analyses of eight termites collected on 31 January 2008 (several days before colony elimination) revealed that these termites consumed sufficient amounts of bistrifluron (483 – 1380 ng/termite) to cause death by 31 January 2008. Because of the lack of data describing the temporal change in the quantity of bistrifluron taken up by termites during this period, we were unable to delineate how bistrifluron spread within the colony, although the results clearly showed that almost all foragers acquired a lethal dose of bistrifluron. Slow mortality will allow the foragers to move too far to identify source of toxin and is therefore essential if the bait is to eliminate colonies of subterranean termites like *C. formosanus*. Workers of *C. formosanus* exposed to 0.5% (w/w) noviflumuron bait could move as far as 50 m before they were killed by the insecticidal effect of noviflumuron (Su, 2005).

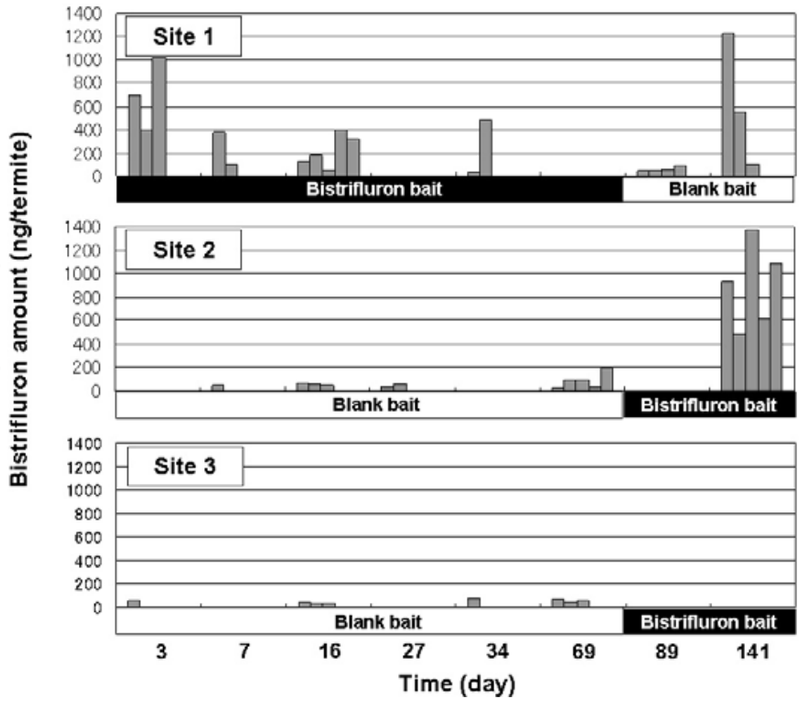

Fig. 10. Bistrifluron amounts recovered from foraging *C. formosanus* at each site from 28 September 2007 to 31 January 2008

It is quite reasonable that the colony was eliminated soon after colony mates acquired sufficient amounts of bistrifluron. They could not maintain the health of the nest by allogrooming or by fighting against invading microbes, even if almost of them were still live as discussed in section 3. Many dead soldiers were found at the bait sites, indicating that they came there for feeding or for escaping from a microorganism-contaminated nest. The present study showed that colony elimination by bistrifluron involves acquisition of lethal quantities of bistrifluron by large portion of foragers. Further extensive studies on artificial or natural colonies are needed to more completely understand the mechanisms and processes of colony elimination by baits.

6. Conclusions

It can be concluded as below by a series of experiments:

- Bistrifluron acts on termites extremely slowly, however, its efficacy is significantly dose-dependent.
- Bistrifluron inhibits colony-maintaining activities of termites like allogrooming behavior before the colony is eliminated.
- A lethal dose of bistrifluron against *C. formosanus* is ≥400 ng per termite and some portion of bistrifluron once taken up by foraging termites would remain in termite body for weeks, while the rest of bistrifluron is discharged.
- Sufficient amount of bistrifluron can be taken up by foraging termites owing to its slow action and subsequently bistrifluron would be transferred their colony mates.
- Feeding deterrency of bistrifluron as a reflection of its dose-dependence is not always an unfavorable characteristics since the improvement of feeding preference by termites could lead to the faster colony elimination.

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