Rapid Elimination of Field Colonies of Subterranean Termites (Isoptera: Rhinotermitidae) Using Bistrifluron Solid Bait Pellets

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ABSTRACT   The efficacy of bistrifluron, a chitin synthesis inhibitor, in cellulose bait pellets was evaluated on the mound-building subterranean termite, *Coptotermes acinaciformis* (Froggatt). Three concentrations of the bistrifluron were used: 0 (untreated control), 0.5, and 1.0% over an 8 wk period. Both doses of bistrifluron bait eliminated (viz. termites absent from nest or mound) termite colonies: 83% of colonies (10 of 12) were either eliminated or moribund (viz. colony had no reproductive capacity and decreased workforce) after 8 wk, compared with none of the control colonies. The remaining two treated colonies were deemed to be in decline. Early signs that bistrifluron was affecting the colonies included: 3 wk after baiting mound temperatures showed a loss of metabolic heat, 4 wk after baiting foraging activity in feeding stations was reduced or absent, and dissection of two mounds at 4 wk showed they were moribund. Colony elimination was achieved in around half or less the time, and with less bait toxicant, than other bait products tested under similar conditions in the field, because of either the active ingredient, the high surface area of the pellets, or a combination of both. This suggests the sometimes long times reported for control using baits may be reduced significantly. The use of a mound building species demonstrated clearly colony level effects before and after termites stopped foraging in bait stations.

KEY WORDS   baiting, *Coptotermes*, chitin synthesis inhibitor, nest temperature, peritrophic membrane

Baiting, including trap and treat with dusting, was one form of pest termite control in China and south east Asian countries before the introduction of in-soil organochlorine insecticides in the 1940s. The soil based methods were considered so effective they replaced the other forms of termite control. Baiting was reintroduced in the 1990s as a relatively environmentally benign control method as: baiting uses small amounts of toxicant that are relatively specific to insects and generally of low mammalian toxicity; toxicants are contained in a food matrix, typically cellulose, of interest only to termites; and bait is usually confined within an impervious bait station (Su 1994, Su et al. 1995, Forschler and Ryder 1996).

Clearly these are desirable benefits; however, baiting has one clear drawback compared with other termite control options: it has proved to be comparatively slow. "The biggest complaint, common to all the current baiting systems, is that it is slow, time-consuming and tedious" (Potter 1999); an attitude still voiced a decade later (Anon. 2008). Published field studies of termite baiting using hexaflumuron, chlorfluazuron, noviflumuron, ivermectin, and fipronil report control times of up to half a year, sometimes over a year: 12–23 wk in Australia (Peters and Fitzgerald 1999, Peters and Fitzgerald 2003), 15–39 wk in Asia (Tsunoda et al. 1998, Su and Hsu 2003, Huang et al. 2006, Wang et al. 2007), 10–43 wk in the United States (Su 1994, Forschler and Ryder 1996, Su et al. 2000, 2002, Rojas and Morales-Ramos 2003, Cabrera and Thomas 2006), and 17–64 wk in Latin America (Su et al. 2000, Ripa et al. 2007). Although these studies report on treatments against different termite species under variable conditions, all are long compared with soil based insecticides, which may stop infestations in structures in very short periods, days or less. Bait toxicants must be slow acting, for the toxicant to be spread by the foragers to the entire colony. However, perhaps faster acting bait products will reduce the biggest criticism of bait systems in termite pest management.

In addition to these environmental benefits, baiting promised, or at least aimed for, colony elimination; that is, death to all members of the colony. Most of the published studies (e.g., Su 1994, Forschler and Ryder 1996, Tsunoda et al. 1998, Su et al. 2000, 2002, Rojas and Morales-Ramos 2003, Su and Hsu 2003, Cabrera and Thomas 2006; Huang et al. 2006, Wang et al. 2007, Ripa et al. 2007) demonstrated control by an absence of termites in active bait or monitoring stations or structures. Although it is possible, even probable, that a long absence of termites from bait stations is because of the elimination of the colony (e.g., Vargo 2003), it

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is not the only interpretation. For example, termites are known to abandon bait stations that are inspected too frequently as they avoid disturbance (Evans and Gleeson 2006, Woodrow et al. 2008), or for no apparent reason (Tamashiro et al. 1973, Forschler 1996, Forschler and Ryder 1996, Evans 2001). Clear demonstration of colony elimination by using unambiguous metrics is also desirable.

Termite species that build obvious nests, such as epigeal mounds or arboreal nests, are useful for examining colony level effects. Almost all such species belong to the higher termites, the family Termitidae. However, the majority of termites that are pests of wood in service belong to the lower termite family Rhinotermitidae. There are biological differences between these families, including development and growth (such as number of molts to adulthood, capacity for individuals to move between the wingless or worker developmental line and the winged or nymphal line) and presence or absence of symbiotic protozoa in the gut; consequently, studies using termitids may not be predictive of results for rhinotermitids. However, there are four species of the rhinotermitid *Coptotermes* found in Australia that build mounds, the only lower termites to do so (Hill 1942, Grassé 1984). These species offer an excellent opportunity to examine colony level effects in pestiferous rhinotermitid species in field experiments.

This study aims to test the efficacy of bistrifluron in a solid, pelletized alpha-cellulose bait at the rates of 0.5 and 1.0% using a Commonwealth Scientific and Industrial Research Organization developed method based on the mound-building form of the subterranean termite *Coptotermes acinaciformis* (Froggatt) in tropical northern Australia. The bistrifluron bait pellets are a new method of bait matrix presentation: importantly the pellets have gaps between them that allow a much greater surface area of the bait to be chewed immediately upon contact by termites, compared with other bait matrices. Greater surface area has been shown to be an important component of rapid bait consumption (Evans and Gleeson 2006). The study aimed to use standard monitoring methods, such as present or absence of termites in bait stations and monitoring stations, and condition of termites in these stations, but also effects on the colony and mound, including presence or absence of reproductives, eggs and larvae, building activity, and mound temperature, to identify more precisely the level and timing of the effect of the bait toxicant on the termite colony.

Materials and Method

Field Site. The field site was in wet-dry tropical northern Australia (12°23.S 131°10.E) ≈50 km north east of Darwin, Northern Territory. The area receives an annual rainfall of 1,702 mm, which falls predominantly between October and April. The average annual daily temperature ranges between 23.2 and 31.9°C. The vegetation is open forest dominated by *Eucalyptus tetradonta* (F. Muell.), *E. miniata* (ex

Schauer), and *E. bleeseri* (Blakely). Termite diversity is high, as expected in a tropical location, >50 species of termite are found in the area (Abensperg-Traun and Steven 1997).

Test Species. *C. acinaciformis* is the most wide spread member of the genus in Australia (Hill 1942, Watson and Abbey 1993). It is the most destructive pest termite species in the country; capacity to damage wood and plastic materials is equal to or higher than other *Coptotermes* species and other pest species (Hill 1942, Peters et al. 1997). This central-site nesting, subterranean species has several above and below ground nesting habits; in areas close to the tropical coast (≈300 km), *C. acinaciformis* builds mounds (Hill 1942). The 'nest' consists of the well-defined royal chambers, occupied by the queen and king, and the nearby well-defined nursery, occupied by eggs, larvae, and any molting individual. A mass of partially digested wood called 'carton material' with a trabecular structure surrounds and covers the nest, which is covered by a clay wall (see Hill 1942 for a complete description).

Field Installation. On the 26 June 2007, 16 healthy (i.e., mound without structural damage, with recent building and no ants) *C. acinaciformis* colonies were selected for the experiment. Four feeding stations were installed in holes dug ≈1 m from each mound at the cardinal compass points. Feeding stations consisted of 11 liters steel drums, pierced on the base, containing 14 pieces (240 × 100 × 10 mm; ≈3 kg in total) of *E. regnans*, a susceptible, preferred timber, with a plastic lid. Feeding stations were large for three reasons: first, larger food resources attract more termites; second, they persist for longer; and third, larger food sources are less susceptible to abandonment because of disturbance during inspections (Evans and Gleeson 2006). To ensure that termites in the mound-colonies would explore, detect and infest the feeding stations rapidly, a trench was dug between the mound and the feeding station, with strips of *Pinus radiata* veneer (≈1,000 × 5 × 1 mm) placed in the trench, with water, and backfilled with soil. After the trenches were completed, the feeding stations watered and closed, a 300 × 300 mm sheet of plastic (construction grade moisture membrane) was placed over each feeding station and covered with soil to provide protection from the weather and animals, and also to contain moisture around the feeding stations (apparatus following Evans et al. 1998, 1999; Evans 2001).

The temperature in the termite mounds was monitored to track colony health. Healthy termite colonies maintain relatively constant temperature in their mound nests (Holdaway and Gay 1948, Lüscher 1961, Korb and Linsenmair 1998), whereas a decrease in population size results in a more variable mound temperature, more similar to the daily fluctuations in air temperature (Greaves 1964). HOBO data loggers (Onset Computer Corporation, Bourne, MA) were used to monitor mound temperatures. The temperature was recorded each hour from installation to the dissection of the mound at the end of the experiment. Twelve mounds had 'external' HOBO data loggers

(model numbers H12–003, H08–003-02, and H8–006-04), which were located within weatherproof PVC containers buried adjacent to the mound with a steel probe inserted into the mound. Four mounds had 'internal' HOBO data loggers (model number H08–032-08), which were protected by a stainless steel mesh bag.

Placement of the data loggers required damaging the mound; this process demonstrated the health of the colony. An opening was made in the clay wall of the mound (200×200 mm), the carton material was excavated to ≈300 mm depth where either the probe from the external data logger or the internal data logger was placed, with the excavated carton material and pieces of clay wall replaced into position. Termites in healthy colonies repair the damage their mounds within 24 h, to maintain the internal environment constant and keep a defense against predators (Evans 2006). All colonies repaired the damage to their mounds sustained during installing the data loggers (and trenching) completely overnight, with the building of new clay covering double the damaged area.

The control and bistrifluron bait pellets were placed on the 21 August 2007, 12 wk after the installation of the feeding stations and data loggers. Mound-colonies were allocated to treatments randomly (four controls, 1, 5, 9, and 13; six 0.5% bistrifluron, 2, 4, 7, 10, 12, 15; and six 1.0% bistrifluron, 3, 6, 8, 11, 14, 16). After feeding stations were inspected, 2×200 g sachets of bait pellets (=383.2 g dry weight) were placed in two plastic containers ($200 \times 60 \times 60$ mm) between the wooden slats in each of two termite infested feeding stations on opposite sides of each mound (i.e., a total of 800 g of bait per colony); these were now bait stations whereas the two without bait pellets were now monitoring stations. The holes (4 mm in base, 8 mm in sides) in the plastic containers were small enough to hold the bait pellets, but large enough to allow termite access.

Assessment. Assessment was over two inspections. The first inspection took place on 18 September 2007, exactly 4 wk after the placement of the bait. All feeding stations were inspected, remaining bait was estimated by eye, and termite activity and health were noted. Healthy *C. acinaciformis* workers are pale beige and walk fast at temperatures around 32°C (typical day temperatures in Darwin), whereas sickly workers are a marbled white from a build up of uric acid, and walk slowly. Colony health was tested with a mound-damage-and-repair manipulation, in which the clay walls of the mounds were damaged by creating an opening similar to that made for the trench or data logger installation. An opening (≈120×120 mm) was made through the clay wall to the carton material. The broken pieces of clay wall were replaced into the opening to allow for easier repair (following Evans 2006), as for the data logger installation. The mounds were examined 1 d later and the repairs scored. Repairs were placed into three categories: full repair, clay wall seal, and carton material seal. A full repair was the complete rebuilding of the clay wall, including a newly built clay layer that covered the damage completely so

that no broken clay pieces were visible. A clay wall seal was a less complete repair, which involved building with clay between the broken clay pieces, but with little or no covering layer of newly built clay; consequently, the broken clay pieces were visible but fixed into position. A carton material seal was a minimal repair, which involved sealing only the carton material usually with carton material; building with clay was absent and the broken clay wall pieces were untouched, completely visible and able to be moved.

The second inspection occurred on 16 October 2007, exactly 8 wk after bait placement. All feeding stations were inspected and termite activity noted as for the first inspection. All remaining bait was collected and dry weighed to determine bait removal. All remaining mounds were dissected and colony status determined using 10 assessment criteria (Table 1). Colony status was assessed as: (1) healthy, that is, a normal functioning colony; (2) declining, viz. colony had reproductive capacity in some form yet with fewer and less healthy termites; (3) moribund, meaning near death, viz. colony had no reproductive capacity and few, sick termites; or (4) eliminated, viz. all termites in colony dead. Note that workers and soldiers are sterile in *Coptotermes* spp., and only nymphs and alates can form secondary reproductives (Lenz and Barrett 1982, Lenz et al. 1988, Lenz and Runko 1993, Costas-Leonardo et al. 2004). Consequently, nymphs, alates, or larvae (that could grow into nymphs) had to be present for a colony to retain some form of reproductive capacity. The HOBO data loggers were retrieved and temperature data were analyzed.

Data recorded from feeding stations were analyzed using Kruskal-Wallis test (nonparametric equivalent of a one-way ANOVA because of the low maxima; i.e., four per colony; following Sokal and Rolf 1998). Data analyzed were the number of feeding stations per colony infested with *Coptotermes;* the number of feeding stations per colony infested with termites other than *Coptotermes;* and the presence of protective mudding built by *Coptotermes* termites over the wood inside the feeding stations. The dry weight of bait removed by *Coptotermes* was analyzed by ANOVA and *t*-tests. Data from the mound damage-and-repair manipulation were analyzed with χ^2 tests. Data were analyzed with Systat nine (Chicago, IL).

Results

Bait Placement. On 21 August 2007, the date of bait placement 12 wk after installation, *C. acinaciformis* had infested 62 of the 64 feeding stations; a 97% infestation rate. One colony with only three drums contacted was from the control bait treatment group; the other from the 1.0% bistrifluron treatment group. There were no significant differences between treatments in *C. acinaciformis* infestation rate (Kruskal-Wallis = 1.429; df = 2; $P = 0.490$). The termites had built protective mudding over the inner top surface in almost all feeding stations, an average of 3.6 per mound-colony across the 16 mounds used in the trial;

Table 1. The 10 criteria used to assess colony condition

Observation	Healthy	Declining	Moribund	Eliminated
Termites				
No. live[a]	Many	Few-many	Few	None
No. dead[a]	None	Few	Many	Many
Appearance[b]	Beige, quick	White, slow	White, slow	N/A
Queen	Present	Present	Absent	Absent
Eggs[a]	Many	Few	None	None
Larvae[a]	Many	Few	None	None
Mound				
Royal chamber	Present	Absent or present	Absent	Absent
Nursery	Present	Absent or present	Absent	Absent
Carton material[c]	Dry	Dry-wet	Wet	Wet
Fungus[d]	Absent	Absent or present	Present	Present
Other species[e]	Absent	Absent	Present	Present

'Healthy' is a normal colony; 'declining' is less healthy but the colony has reproductive capacity in some form; 'moribund' is a colony with no reproductive capacity 'eliminated', viz. all termites in colony dead.

[a] Many, thousands of individuals; few, dozens to hundreds.

[b] Healthy *C. acinaciformis* workers are pale beige, whereas sickly workers are white from a build up of uric acid.

[c] Although the relative humidity in a healthy mound is 100%, the carton material is dry to the touch. In unhealthy colonies the moisture pools and wet patches occur.

[d] Fungi can be either entomopathogenic or growing on termite cadavers, or a wood rot growing on the carton material.

[e] Healthy *C. acinaciformis* colonies do not have other termite species in the carton material.

this is normal behavior and is a sign of a healthy colony, and there were no significant differences between treatments (Kruskal-Wallis = 0.397; df = 2; P = 0.840). This mudding layer was broken to inspect the feeding stations and to place the bait.

First Inspection. On 18 September 2007, the date of first inspection 4 wk after bait placement, all four colonies in the control treatment appeared to be healthy, whereas, all colonies treated with bistrifluron bait showed signs of declining colony health. All four feeding stations around control mound-colonies were infested by *C. acinaciformis* (including the single feeding station that was unoccupied at the time of bait placement); this was not significantly different from the average of 3.8 feeding stations around mound-colonies fed both 0.5 and 1.0% Bistrifluron bait (Kruskal-Wallis = 0.714; df = 2; P = 0.700 The termites in the feeding stations around control mound-colonies appeared healthy with a beige color and walked quickly, whereas those from bistrifluron baited colonies were not numerous, were marbled white and moved slowly. Correlated with this, almost all feeding stations around control mound-colonies had the protective mudding layer inside the feeding stations, where as fewer than half the feeding stations around mound-colonies fed 0.5% bistrifluron bait (average of 1.7 per mound colony) and 1.0% bistrifluron bait (average of 1.8) did a significant difference (Kruskal-Wallis = 7.673; df = 2; P = 0.022). Termites had removed the majority ($\approx$85%) of the untreated control bait, which was significantly more than either of the bistrifluron baits (3.8% of 0.5% bistrifluron and 1.3% of 1.0% bistrifluron) (Kruskal-Wallis = 13.699; df = 2; P = 0.001). It was clear the termites had explored the bait pellets evidenced by the many marks left by chewing on the bait pellets, cementing together of and fecal spotting on the pellets. There were no other termite species observed in the control feeding stations, whereas other termite species had occupied

some bistrifluron bait feeding stations; particularly so for 0.5% bistrifluron bait (an average of 0.8 feeding station per mound colony), however, this difference was not significant (F = 3.494; df = 2; P = 0.174) (Table 2).

One colony (#4) fed 0.5% bistrifluron bait appeared to be very unhealthy. The four feeding stations had neither *C. acinaciformis* termites nor protective mudding, and three were occupied by other termite species. Clearly, the usually competitively dominant *C. acinaciformis* was unable to protect its food resources against subordinate species. A strong smell of decay was immediately apparent after a small hole was made in the clay wall of the mound. Therefore, this mound was dissected and found to be moribund (Table 1); with few unhealthy workers inside, no queen, nymphs, eggs, or larvae, neither the royal chamber nor the nursery was found. Other species were found inside the mound feeding on the carton material: two species of *Macrognathotermes* and patches of fungus were found also. Carton material is desirable food for cellulose eating species, termite and fungus, as it has higher nitrogen content; the presence of such species in the mound demonstrated clearly the morbidity of the *C. acinaciformis* colony.

The mound damage-and-repair manipulation showed stark differences between colonies in the different treatments. All of the colonies given control bait built full repairs, whereas not one of those fed bistrifluron did so; a significant difference (χ^2 = 16.067; df = 4; P = 0.003). Instead, carton material seals were common in the colonies treated 0.5% bistrifluron; these and clay wall seals were observed in the colonies fed 1.0% bistrifluron (Table 3). It was decided to dissect a second mound-colony (#10, also fed 0.5% bistrifluron) that showed the poorest repair with the fewest and sickest looking *C. acinaciformis* in the feeding stations. The colony in this dissected mound was as-

Table 2.   Observations on the feeding stations around *C. acinaciformis* mound-colonies during the exp

Activity date	Feeding stations with	Treatment		
		Control (4, 4, 4)	0.5% Bistrifluron (6, 6, 4)	1.0% Bistrifluron (6, 6, 6)
Bait placement	*Coptotermes*	3.8 ± 0.2a	4.0 ± 0.0a	3.8 ± 0.2a
21 Aug. 2008	Other sp.	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a
	Protective mud	3.5 ± 0.3a	3.5 ± 0.2a	3.7 ± 0.2a
First inspection	*Coptotermes*	4.0 ± 0.0a	3.8 ± 0.2a	3.8 ± 0.2a
18 Sept. 2008	Other sp.	0.0 ± 0.0a	0.8 ± 0.5a	0.2 ± 0.2a
	Protective mud	4.0 ± 0.0a	1.7 ± 0.6b	1.8 ± 0.6b
	Bait removed	86.2 ± 10.7a	3.8 ± 0.2a	1.3 ± 0.1b
Second inspection	*Coptotermes*	4.0 ± 0.0a	0.0 ± 0.0b	1.5 ± 0.8b
16 Oct. 2008	Other sp.	0.0 ± 0.0a	1.8 ± 0.8b	1.3 ± 0.7a,b
	Protective mud	4.0 ± 0.0a	0.0 ± 0.0b	0.7 ± 0.4b
	Bait removed	97.5 ± 2.5a	3.8 ± 0.2b	1.3 ± 0.1b

NB numbers are the avg ± SEM for each treatment based on four feeding stations per replicate; the no. of colonies assessed for each date is in brackets after treatment column headings. NB. Two colonies from the 0.5% treatment were dissected after the first inspection and were not available for the second inspection. Letters following averages indicate significant differences along rows.

sessed as moribund, with observations similar for those of mound-colony number four (Table 1).

Second Inspection. On 16 October 2007, the date of the second inspection 8 wk after bait placement, all four colonies in the control treatment appeared to be healthy, whereas all colonies treated with bistrifluron bait showed increased signs of failing or failed colony health. All four feeding stations around control mound-colonies were still infested by healthy, beige, fast walking *C. acinaciformis* workers; this was in complete contrast to zero around mound-colonies fed 0.5% bistrifluron bait, or less than half (an average of 1.5 feeding stations per mound-colony) for 1.0% bistrifluron bait, a significant difference (Kruskal-Wallis = 8.119; df = 2; $P = 0.017$). All feeding stations around control mound-colonies had the protective mudding layer inside the feeding stations, whereas they had built protective mudding in none of the feeding stations around mound-colonies fed 0.5% bistrifluron, and fewer than one feeding station (average of 0.7 feeding stations per mound colony) fed 1.0% bistrifluron bait, a significant difference (Kruskal-Wallis = 10.593; df = 2; $P = 0.005$). Termites had removed almost all ($\approx$95%) of the untreated control bait, but there appeared to be no further removal of either of the bistrifluron baits, still significantly different (Kruskal-Wallis = 12.093; df = 2; $P = 0.002$). There were still no other termite species observed in the control feeding stations, whereas other termite species had occupied nearly half of all feeding stations around the mound-colonies feed 0.5% bistrifluron bait (average of 1.8 per mound-colony): and over one for those fed 1.0% bistrifluron bait (1.3 feeding stations per mound colony) (Table 2), this difference was not

quite significant at the 5% level (Kruskal-Wallis = 5.741; df = 2; $P = 0.057$).

All mounds were dissected and condition recorded (Table 4). The mounds of all four colonies fed control bait were assessed as healthy using assessment criteria in Table 1. The control colonies contained many healthy live termites including queens, many eggs, and larvae; no dead termites were found. The carton material inside the mounds was dry to touch, contained royal chambers and nurseries, and had no presence of fungus (Table 5).

The four remaining colonies treated with 0.5% bistrifluron baits and three of the six colonies (mounds 3, 6, and 14) treated with 1.0% bistrifluron baits were

Table 3.   Observations on the repair to mounds in the damage-and-repair manipulation conducted during the first inspection, 4 wk after bait placement

Treatment (n)	Full repair	Clay wall seal	Carton material seal
Control (4)	4	0	0
0.5% Bistrifluron (5)	0	2	3
1.0% Bistrifluron (6)	0	4	2

Table 4.   Observations from the dissection of the *C. acinaciformis* mounds at the end of the experiment

Item	Treatment			χ^2, P value df = 2
	Control	0.5% Bistrifluron	1.0% Bistrifluron	
(No. mounds)	(4)	(4)	(6)	
Coptotermes				
Live	4	0	3	8.00, 0.018
Dead	0	4	6	14.00, 0.001
Queen[a]	4	0	2	8.56, 0.014
Nymphs	4	0	0	14.00, 0.001
Eggs	4	0	2	8.56, 0.014
Larvae	4	1	0	8.56, 0.014
Royal chamber[b]	4	0	2	10.73, 0.005
Nursery	4	1	0	10.73, 0.005
Other species				
Termite[c]	0	4	3	8.00, 0.018
Fungus[d]	0	4	5	10.37, 0.006

Numbers indicate the no. of colonies with the presence of *C. acinaciformis* or other species of termite, castes with the potential to reproduce (queen, nymphs, eggs, larvae), or mound section (royal chamber, nursery).

[a] Queens are often killed when the mound is dissected. In some cases, queen presence was inferred from presence of royal chamber and large quantity of eggs.

[b] The royal chamber and nursery were found in one mound in the 0.5% bistrifluron treatment. However, these were not functional as they were both filled with dead and decaying termite cadavers.

[c] Eight other species were recorded in the genera: *Heterotermes*, *Macrognathotermes*, *Nasutitermes*, and *Schedorhinotermes*.

[d] The fungi were either growing on termite cadavers on the carton material.

Table 5. The assessment of the *C. acinaciformis* colonies 8 wk after bait placement

Treatment	Healthy	Declining	Moribund	Eliminated
Control	4			
0.5% Bistrifluron			(2)	4
1.0% Bistrifluron		2	1	3

NB definitions of colony status listed in Table 2; the two moribund colonies fed 0.5% bistrifluron bait were dissected four weeks after bait placement, signified by brackets.

assessed as completely eliminated (Table 5). No live *C. acinaciformis* of any of the castes were found in any of these mounds, but dead and decaying corpses were found. The carton material inside the mounds was usually moist to touch or had wet patches. Not one royal chamber was located and only one mound had an identifiable nursery, but this was filled with the cadavers of rotting termites. Fungus was found in all mounds. Other species of termites had invaded the *C. acinaciformis* mounds and were consuming the carton material.

One colony fed 1.0% bistrifluron bait (#16) was assessed as moribund (Table 5). As observed for the two mounds dissected after 4 wk (mounds 4 and 10), royal cells, nursery, queen, eggs, and larvae were absent, and only a few workers and soldiers were found. Two colonies fed 1.0% bistrifluron bait were assessed as declining (mounds 8 and 11) (Table 5). The state of the mounds showed both colonies under severe stress. The royal chambers, nurseries, and the carton material at the bottom of the mounds (as for all eliminated and moribund colonies) were filled with decaying cadavers of other termites. Fungus was often found in the wet carton material. The queens had relocated from the now uninhabitable bottom of the mounds, along with the few sickly but surviving workers, to the upper parts of the mound, and had produced small numbers of eggs and some larvae.

Termites had removed an average of 727.4 ± 16.0 g (dry weight) of untreated control bait, 43.8 ± 10.6 g of the 0.5% bistrifluron bait and 23.3 ± 1.8 g of the 1.0% bistrifluron bait, corresponding with visual estimates. These differences were significant ($F = 718.02$; df = 2, 13; $P < 0.001$); but were due entirely to control versus both treated (Bonferonni corrected post hoc pairwise comparisons $P < 0.001$) as there was no significant difference found between 0.5 and 1.0% bistrifluron baits (Bonferonni corrected post hoc pairwise comparisons $P > 0.05$). The amount of the active ingredient bistrifluron in the removed bait was 218.8 ± 52.9 mg in the 0.5% treatment, and 233.2 ± 18.2 mg in the 1.0% treatment, which was not significantly different (*t*-test, $t = 0.183$; df = 10; $P = 0.859$). The amount of the active ingredient in the removed bait from eliminated colonies was 179.9 ± 28.3 mg, and from moribund colonies was 281.3 ± 139.9 mg, which was not significantly different (*t*-test $t = 0.634$; df = 7; $P = 0.546$) because of the very large quantity removed by the moribund colony #10.

Temperature Data. Four data loggers failed during the experiment: one in a control baited mound and

three in 1.0% bistrifluron baited mounds. The daily 3 a.m. and 3 p.m. temperatures were chosen for comparison as these were typically the coolest and warmest temperatures recorded. Data from 4 wk before bait placement (24 July-20 August) was included for comparison with the 8 wk of postbaiting temperature data (21 August-16 October 2007). There were four broad patterns evident (Fig. 1).

(1) Air temperatures increased during the experimental period: the 3 p.m. maxima increased $\approx 3°C$, and the 3 a.m. minima increased $\approx 7°C$. Consequently, the daily range in air temperature decreased. This pattern is normal for tropical climes during season change from the winter 'dry' season to the monsoonal summer 'wet' season.

(2) Minima and maxima mound temperatures from control, 0.5% bistrifluron and 1.0% bistrifluron mounds were similar during the prebaiting period. Air temperatures did influence the mound temperatures, but it is clear the mound minima are $\approx 10°C$ warmer than air minima, demonstrating the metabolic heating from the termites.

(3) The temperatures in the bistrifluron baited mounds began to differ from those of control mounds about 3 wk after bait placement. The minima from the treated mounds decreased and the daily range increased; eventually mimicking air temperatures. This suggested the loss of metabolic heating from the termites.

(4) The daily range in mound temperatures was high in the bistrifluron treated mounds (mean $\approx 7°C$) compared with control mounds (mean $\approx 3°C$), especially from 3 to 5 wk after bait placement. This was due primarily to the drop in minima in these mounds; this phenomenon may indicate the effect of the bistrifluron. The decrease in daily range in bistrifluron treated mounds 6 wk and later after bait placement may indicate an increase in metabolic heat coming from the other termite and fungal species that had entered the mounds.

Discussion

The results from this study show that the bistrifluron bait was effective at eliminating *C. acinaciformis* colonies. In the 8 wk of the baiting period of this study, 7 of the 12 colonies were eliminated. Three colonies were moribund, which means these colonies had lost all capacity to reproduce and so were just a short time from elimination. Therefore, the bistrifluron bait achieved 83% success. The remaining two colonies were deemed to be in decline, because the colony population had been reduced and the nest in the mound had been abandoned; however, the two colonies did appear to retain reproductive capacity. It is not possible to predict whether these two colonies would have survived and returned to fully healthy status or succumbed (a real possibility considering the nest and lower areas of the mounds were filled with rotting cadavers and entomopathogenic fungi), had the experiment continued for a longer period.
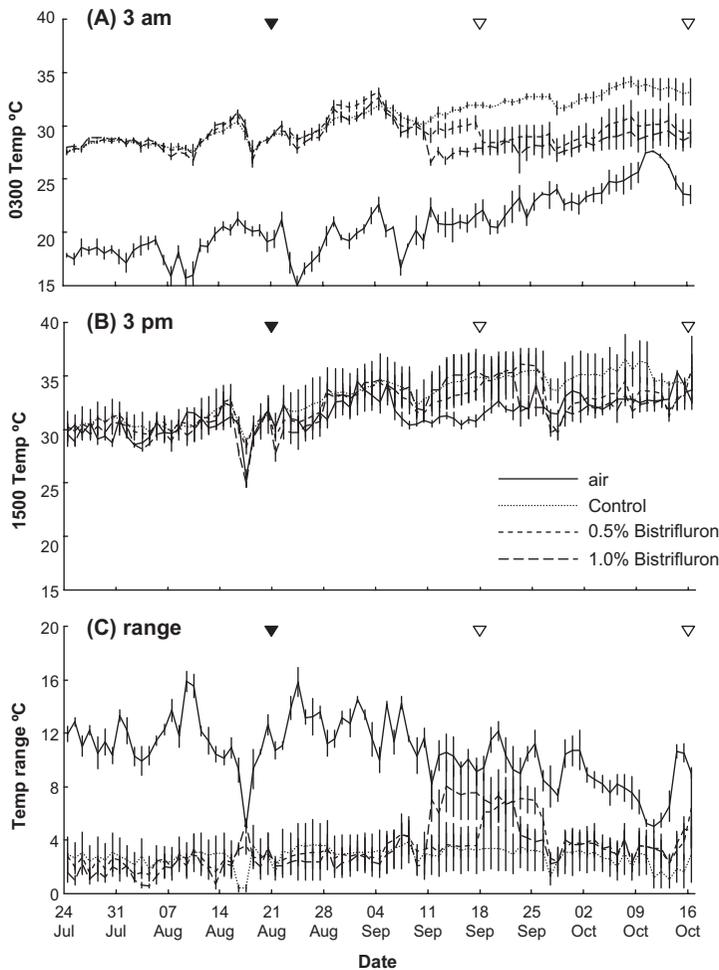
Fig. 1. The means (±SEM) temperatures recorded in the bistrifluron baiting trial against *Coptotermes acinaciformis* near Darwin, Australia. (A) minima recorded at 3 a.m., (B) maxima recorded at 3 p.m., (C) daily temperature range. The solid black triangle on 21 August indicates bait placement, the two open triangles on 18 September and 16 October indicate the first and second inspection.

All six of the colonies fed 0.5% bistrifluron bait succumbed; whereas four of the six colonies fed 1.0% bait did so. This may be because of reduced palatability: the amount of 0.5% bait removed was double the amount of the 1.0% bait; similarly Kubota et al. (2006) found that higher doses of bistrifluron were less palatable to *C. formosanus* in the laboratory. Bait removed from stations may not be eaten; the decision to eat, store or discard collected food occurs in the nest, so bait removed may not have been eaten because of reduced palatability (Duncan 1997). It is also possible that the higher dose of bistrifluron acted too quickly, killing foraging termites before they could return sufficient toxicant to the nest. Alternatively, some colony populations may have been larger or more vigorous in a fashion that was not apparent during the prebaiting period. This third explanation has support from comparing two colonies baited with 0.5% bistrifluron bait. Colony #10 was moribund yet it removed the most bait (≈112 g containing 560 mg bistrifluron), whereas colony #2 was eliminated, yet it removed the least bait (≈25 g containing 127 mg bistrifluron).

The mound dissections demonstrated that the bistrifluron bait can eliminate colonies in 8 wk from bait placement. The temperature data suggested that elimination occurred earlier; around 5 wk from bait placement. The exact time until elimination is difficult to determine, because of the variation in the temperature data and variation in the effect of the bistrifluron (i.e., declining versus moribund versus eliminated status). It is clear moribund status was achieved 3 to 4 wk after placement, using both temperature data and the physical 4 wk inspections.

It is difficult to compare previous baiting studies as these normally had the aim of demonstrating that elimination was possible, whereas the current study aimed to determine the time to elimination. The high variability in time to control (10–64 wk) reported in previous studies would depend on toxicants, concentrations, termite species, colony sizes, environment,

geography, season, and so forth; for example, wood-eating *Reticulitermes* or *Coptotermes* species (family Rhinotermitidae) in single structures or smaller areas (e.g., Su 1994, Forschler and Ryder 1996, Tsunoda et al. 1998, Su et al. 2000, Cabrera and Thomas 2006), to multiple structures in larger areas (Su et al. 2002, Su and Hsu 2003, Rojas and Morales-Ramos 2003, Ripa et al. 2007) to fungus culturing *Odontotermes* and *Macrotermes* species (family Termitidae, subfamily Macrotermitinae) termites in dams (Huang et al. 2006, Wang et al. 2007).

Yet, comparison is possible to some extent as mound-building *C. acinaciformis* have been baited in tropical Australia using similar methods with two other active ingredients. Peters and Fitzgerald (1999) baited with hexaflumuron, and Peters and Fitzgerald (2003) baited with chlorfluazuron, using five baits placed along a 1 meter plank of wood connected to mound nests of *C. acinaciformis.* A total of 62.5% of colonies baited with hexaflumuron were eliminated or moribund (as defined in Table 2) after 23 wk (NB mounds were inspected regularly and were dissected when foraging activity stopped, and only three were considered to have been eliminated "for some time" (Peters, personal communication), whereas 85% of colonies baited with chlorfluazuron were eliminated or moribund after 12–17 wk. Therefore, colony elimination successes were similar for chlorfluazuron and bistrifluron, both of which had higher success than hexaflumuron. However, chlorfluazuron required about two times as long and hexaflumuron required about four times as long as bistrifluron to achieve colony elimination or moribund status.

The study of Peters and Fitzgerald (1999) compared two doses of hexaflumuron and was monitored less frequently than in the current study, as the authors were concerned with demonstrating that elimination was possible, rather than the time to elimination. Nevertheless, it is noteworthy that proportionally more *C. acinaciformis* colonies were found to be moribund instead of eliminated at 23 wk for hexaflumuron (Peters and Fitzgerald 1999) and 17 wk for chlorfluazuron (Peters and Fitzgerald 2003), than colonies fed bistrifluron at 8 wk in the current study. Further, the pattern of the results from these field experiments corresponds with those of Kubota et al. (2006) from laboratory experiments. Quantity of toxicants differed also: around 180 mg of bistrifluron was required for colony elimination. This was ≈10–15% less than the 200–220 mg of chlorfluazuron required for colony elimination (Peters and Fitzgerald 2003), and about one-third the 670 mg of hexaflumuron required for colony elimination (Peters and Fitzgerald 1999).

All three bait toxicants (bistrifluron, chlorfluazuron, and hexaflumuron) were slow-acting enough to be distributed throughout *C. acinaciformis* colonies and so eliminate them. However, bistrifluron was effective in a shorter time. This raises the question of just how slow acting bait toxicants need to be. For example, Huang et al. (2006) used fipronil, which is a neurotoxin and normally considered to be fast acting, in baits against *Odontotermes formosanus* in Wuhan,

China. The baits 'suppressed' termite populations in 3–4 mo, which is not particularly fast (NB 'suppressed' meant no active termites in their stations for 10–12 mo in two of three sites, which other authors consider to be 'eliminated'). The longer time to control *O. formosanus* is probably because it is a fungus growing termite, and delivery of toxicants in food to these termites is more complex than for the wood-feeding Rhinotermitidae. Such examples further highlight the variability in baiting different termite species in different regions. In any case, if bistrifluron bait pellets have the time required to control termites relative to other bait toxicant products, then the long control times of half to more than one year required for control by baiting (e.g., Tsunoda et al. 1998, Su et al. 2002, Rojas and Morales-Ramos 2003, Su and Hsu 2003) may be reduced by months; or may produce control where it was rare (e.g., Glenn et al. 2008). Investigation into the optimal speed for bait toxicants may be warranted, and may differ between termite species and regions.

There are two nonmutually exclusive reasons as to why the bistrifluron bait acted so rapidly compared with chlorfluazuron and hexaflumuron: (1) greater surface area of the pellets and (2) greater toxicity of bistrifluron. The greater surface area of the pellets did allow faster exploration of the bait matrix as was evidenced by the chewing marks and faucal spotting on all pellets. The rapid consumption of the control pellets (≈ 800 g in 4 wk) demonstrates this effect clearly, and confirms the observation of Evans and Gleeson (2006) that baits with greater surface area are consumed rapidly. Unfortunately, it is not possible to separate this effect from that of toxicity because they are confounded; the hexaflumuron and chlorfluazuron baits used by Peters and Fitzgerald (1999, 2003) had low surface area.

It is possible to speculate about the reasons why toxicity may differ between chitin synthesis inhibitors. These toxicants are known to work by disrupting α-chitin synthesis during molting. This mode of action may be too slow to explain the rapid elimination of *C. acinaciformis* colonies using bistrifluron bait; it seems unlikely that all ca. one million individuals in a colony (Evans et al. 1999) molted during the same month. Disruption of γ-chitin synthesis for the peritrophic membrane in the midgut is likely to cause death faster than disruption of molting because this will interrupt digestion and lead to starvation. This possibility was suggested (Morales-Ramos et al. 2006a) and tested (Morales-Ramos et al. 2006b) for *C. formosanus.*

The current study using the mound building *C. acinaciformis* allowed the measurement of colony level effects, such as loss of reproductive capacity, loss of breeding sites, loss of temperature control in the nest, and the invasion of other termites and fungi. Such colony level effects are difficult to demonstrate using termites without obvious nests. Cabrera and Thomas (2006) used acoustic emission detectors and a microwave detector to confirm their results from feeding stations. These methods are useful additional metrics on foraging termites, but do not measure colony level effects. The results for *C. acinaciformis* in the current

study showed that metrics from feeding stations were correlated with colony level effects; however, final elimination of the colony lagged the disappearance of termites in the feeding stations. Therefore, the time to actual elimination of termite colonies in previous studies is probably longer than those times reported. In addition, previous studies reported reappearance in feeding/bait stations during the period of baiting, showing that absence is not necessarily permanent and so a less than perfect metric (e.g., Su 1994, Forschler and Ryder 1996, Pawson and Gold 1996, Su and Hse 2003, Glenn et al. 2008). This study confirms the advantages of using termites with obvious nests for assessing methods and colony level effects (see also Evans et al. 1998, 1999, Evans 2002).

Acknowledgments

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