Toxicity, Repellency, and Horizontal Transfer of Foam Insecticides for Remedial Control of an Invasive Drywood Termite, *Cryptotermes brevis* (Blattodea: Kalotermitidae)

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Laboratory tests were conducted to compare imidacloprid and fipronil foams against various criteria to determine the effect of the deposit conditions, exposure method, and exposure time on the toxicity, repellency, and horizontal transfer of selected foam insecticides. Results of toxicity assays showed that imidacloprid and fipronil foams caused significantly higher mortality than control treatments; however, fipronil foam in fresh or old deposits killed *Cryptotermes brevis* pseudergates more quickly in the short and continuous exposure tests than foam containing imidacloprid. In brief exposure tests, imidacloprid failed to kill all termites when exposed to fresh deposits and delayed total mortality when exposed to dry residues. The mortality of *C. brevis* pseudergates was also significantly quicker when the fipronil foam was applied topically compared with the imidacloprid foam. In the repellency test, termites were not repelled from the surface treated with fipronil foam, but more than 90% repellency was observed after 24 h of exposure to imidacloprid-treated surfaces. Moreover, the non-repellent mortality of *C. brevis* with fipronil was significantly higher than imidacloprid in avoidance tests. Results showed that fipronil was effectively transferred to untreated termites from live or dead donors exposed *via* residual and topical spray.

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INTRODUCTION

Cryptotermes brevis (Walker 1853), the West Indian drywood termite, is a significant pest and the most destructive drywood termite in various countries worldwide (Scheffrahn *et al.* 2009). It feeds on timber-in-service inside buildings, such as framing, cladding, flooring, and furniture. It is an invasive pest that humans have introduced to several countries, including Australia (Evans *et al.* 2013). *Cryptotermes brevis* was initially discovered in Australia in the 1940s in a small infestation of imported furniture in Sydney, New South Wales, which was eradicated quickly. Later an established infestation of *C. brevis* was discovered in Maryborough in Southeast Queensland in the late 1960s. This termite species has been prevalent in Queensland for decades, and a prevention and control program has been in place since the late 1960s under the Diseases in Timber Act 1975 until 2016 and then until early 2021 under the Biosecurity Act 2014 (Peters 1990a; McDonald *et al.* 2022). Control of *Cryptotermes brevis* in Queensland was reported to have cost more

than AUD 4.2 million in 2007 (Peters *et al*. 2007). When combined with an ongoing annual cost of around AUD 500,000 (Horwood 2008), more than AUD10 million has been spent on its control (Haigh *et al.* 2022).

Cryptotermes brevis is a tramp insect pest that lives in small colonies and is exceedingly cryptic. The worker caste can reproduce, and all caste members exist entirely in the infested timber except for dispersing alates. It is challenging to discern termite nest architecture outside a piece of dimensional timber. Because of these characteristics, detecting *C. brevis* in timber and controlling it is challenging, especially at an early stage of infestation (Scheffrahn *et al.* 2009; Evans *et al.* 2013).

Existing drywood termite infestations are eradicated using a variety of control measures, categorized as whole-structure or localised treatments. In whole structure treatment, the entire building is either fumigated with a gaseous toxicant or heated to a specific temperature. Although fumigation completely eradicates an infestation, the process can be expensive and disruptive. Whole-of-house heat treatment requires a considerable amount of energy to kill termites present in the wood (Scheffrahn *et al.* 1997) and has not been used in Australia to eradicate this pest. In Queensland, whole-of-house fumigation at government expense was used to eradicate confirmed *C. brevis* infestations under the WIDT Prevention and Control Program (Peters 1990 b). This program was managed by the Department of Agriculture and Fisheries (DAF) on behalf of the Queensland Government. However, the program ceased on the 15th of January 2021, which means no more treatment at government expense and no more monitoring and surveillance for *C. brevis* by DAF. Whole-of-house fumigation is no longer mandatory, and it is envisaged that homeowners and pest control technicians will look for alternative ways to treat an infestation of *C. brevis* (Queensland Government 2021).

Until January 2021, the only option available to the pest management industry in Queensland to treat infestations of *C. brevis* was whole of structure fumigation because fumigation was mandatory. Various research and development initiatives are now underway to better prepare the Queensland pest control industry to identify, detect, and control this pest in the future. An alternative to whole-of-house fumigation can be localized treatments with registered termiticides against drywood termites. These will be much less expensive and less disruptive to the homeowner (Woodrow *et al.* 2007). In localized treatments on an insecticide application, drywood termites in the wood could be exposed to the insecticide through topical application or contact with freshly treated or previously treated wood surfaces. Termites may encounter a treatment for only a short time, after which they move to an untreated area. Some insecticides are registered for localized remedial control of drywood termites; however, there is no history of their use to control *C. brevis* in Australia. Currently, at least three active ingredients (a.i.) are available in Australia for drywood termite localized treatments. These include permethrin, imidacloprid, and fipronil in liquid formulations and the latter two also in foam formulations. The effectiveness of localized remedial treatments for drywood termites, which are applied directly to infested wood, has not been adequately evaluated partly because of the cryptic nature of drywood termites and an absence of meaningful laboratory colonies. In the current study, two foam formulations containing fipronil and imidacloprid as active ingredients were selected. This was because of the perceived accuracy and precision of product placement of foams in active termite galleries. The aim of this study was to determine the insecticidal activity and potential efficacy of two ready-to-use foam insecticides registered for localized treatment of existing infestations of *C. brevis* in Australia.

EXPERIMENTAL

Termites

Hoop pine floorboards, a small portion of plywood, and door panels infested with *C. brevis* were retrieved in 2020 from a structure in Maryborough, Queensland. Termite presence in the wood material was confirmed using a Termatrac™T3i termite detection device (Termatrac Australia Pty Ltd., Ormeau, Queensland, Australia) before harvesting from the site. The infested material was cut into short lengths (300 to 400 mm) and transported in sealed containers to a laboratory at the EcoSciences Precinct at Dutton Park, Queensland, and stored at 27 °C and 70% relative humidity (RH) until testing began. *C. brevis* was confirmed by the fecal pellet morphology under a microscope. The infested timber was dissected with a chisel and hammer, and the termites were gently tapped from the wood into Petri plates (9-mm diameter), or a camel-hair brush was used to gently remove exposed termites from the wood. The termites belonging to multiple colonies were kept in glass Petri plates containing 2 to 3 layers of black filter paper for one week. This time-lapse allowed termites injured during the collection process to die, thereby excluding them from the experiment. As indicated by the absence of dark-colored paper in the gut, termites that were not feeding were also not included in the trials. Several groups of 50 healthy termites were transferred to Petri plates containing 2 to 3 hoop pine veneers (1.5 to 3 mm thick) and conditioned (27 °C and 70% RH) for at least two months before the trial commenced. In all experiments, approximately 2900 pseudergates were used belonging to 35 different colonies. The wood was heavily infested, and the termite galleries were interconnected. Therefore, it was challenging to determine the exact number of termite colonies, with most having no clear boundary. Thus, the number of colonies is approximate and based on the number of reproductives retrieved from the infested wood. Only the 3rd or 4th instar pseudergates with no evidence of wing buds were included in all studies.

Insecticides

Two ready-to-use foam insecticides were used in the trials. Termidor® foam containing fipronil (0.05 g/kg a.i.) manufactured by BASF (Badische Anilin und Soda Fabrik) Australia Ltd. (Brisbane, Australia) and Shieldrite foam containing imidacloprid (0.5 g/kg a.i.) developed by Sherwood Chemicals Australia Pty. Ltd. (Brisbane, Australia) were selected for the current study. Termidor® and Shieldrite foams are registered for drywood termites in Australia under APVMA (The Australian Pesticides and Veterinary Medicines Authority) Approval Number 70346/63237 and 82820/106857, respectively. Both insecticides were purchased from Garrards Pty. Ltd. at Brendale, Queensland. Termidor® foam was supplied in a pressurized container. When the foam was dispensed, it expanded rapidly, generating a dry foam with an expansion ratio of approximately 30:1, with 30 g of the product being dispensed in approximately 5 s producing about 1 L of foam. Shieldrite self-pressurised container dispensed foam at a rate of approximately 0.2 L of expanded foam per second. Both insecticides contained fast-breaking foam that rapidly broke down when applied and left a residual coating on the exposed surface. Foam containers were shaken well for approximately 5 s as per label directions before use. Foams were applied for approximately 10 s or until foam back flows per injection point. After application, the injector tip was held in place for approximately 5 s to allow the foam within the injector tube to dispense into the treatment area.

In further text, both foams are designated based on their active ingredients, *i.e.*, fipronil or imidacloprid.

Preparation of Termite Exposure Wood Units

A three-dimensional exposure, such as a groove in a piece of wood, results in a larger toxicant transfer in drywood termites than two-dimensional exposure, such as a flat wood surface or filter paper in a Petri plate (Scheffrahn *et al.* 1997). Therefore, to evaluate the two insecticide treatments in the laboratory while simulating drywood termite damage in wood, a simple bioassay was designed to expose termites in a gallery-within-wood scenario. The method is a modified version of the form developed by Scheffrahn *et al.* (1997). Briefly, hoop pine boards ($20 \times 4 \times 4$ cm^3) were cut longitudinally in half with a band saw. On the sawn surface of one board, a groove that was 4.5 mm deep, 4.5 mm wide and 180 mm long was routed starting at the 1 cm point and finishing at the 19 cm point. A small hole was drilled at one end on the top of the non-grooved board to apply the insecticides. The entire groove was uniformly treated with an insecticide when required for testing. A total of 170 units were prepared, out of which 160 units were used (81 for toxicity assays, 19 for repellency assays, and 60 for horizontal transfer studies). All units were stored in the conditioning chamber for several weeks to achieve the desired moisture content (~12 to 13%) before testing.

Exposure of Termites to Insecticides

Termites were exposed to the insecticides for 4 and 8 h or extended duration, either through topical application or contact with the treated surface (Rust *et al.* 2008). All tests were performed at 27 °C, 70% RH. See Fig. 1 for a schematic summary of experimental methods.

Residual exposure through wood-treated surfaces

Cryptotermes brevis pseudergates were continuously confined to the treated wood surface or exposed for brief periods to determine the insecticidal transfer activity of treated surfaces. The groove in the wooden units was treated with each insecticide by clamping two halves (G clamp at each end of the wooden unit and one in the middle) in the absence of termites. The units were placed in the conditioning room after treatment. Continuous or short exposure tests were conducted when the treated surfaces of wooden units were 1 day old (fresh deposits) or 30 days old (aged deposits) (Fig. 1a). For the continuous exposure test, treated units were dismantled, and 15 pseudergates were released into the groove of each unit. The units were re-clamped and stored in the conditioning room at 27 °C and 70% RH. There were six treatments (two fipronil, two imidacloprid, and two controls) and each treatment was replicated four times. For control treatments, termites were continuously confined in the testing units without any treatment. Termite mortality was recorded after 1, 2, 5, 6, 7, 8, 9, 16, 23, 30, 37, 45, and 52 days post-exposure. For the short exposure tests, 15 pseudergates in each unit were exposed to treated surfaces for 4 and 8 h in separate tests described above. Then, termites were removed and held on untreated surfaces, *i.e.*, filter paper in the Petri plates and placed in the conditioning room. For short exposure tests, the mortality of termites was recorded before transferring them to Petri plates (day 0) and then after 1, 2, 5, 6, 7, 8, 9, 16, 23, 30, 37, 45, and 52 days post-exposure. For control treatments, termites were continuously confined in the testing units without any treatment for 4 or 8 h, then transferred to Petri plates. There were six treatments for each type of treated surface (30 days or 1 day old) and each treatment was replicated four times.

Topical exposure test

In the topical exposure test, 20 pseudergates were released into a rounded groove of the testing unit. Both halves were clamped together using three G clamps. Each insecticide was injected into the testing unit until it flowed back through the injection point. There was no application of insecticides in the control treatment units. There were three treatments (fipronil, imidacloprid, and control), and each treatment was repeated three times. In this way a total of nine wooden units were used each containing 20 termites. Termite mortality was recorded after 1, 2, 4, 7, 24, and 48 h of fipronil application. After applying imidacloprid, the authors assessed mortality at 24, 48, 72, 96, and 168 h of application.

Termite Repellency or Avoidance Test

This test was conducted to determine whether the termites would contact, avoid, or tunnel through surfaces treated with either fipronil or imidacloprid to determine non-repellent kill. For the repellency test, five compartments (2 cm in diameter, 8 mm in depth) were carved out of a hoop pine board ($2 \times 4 \times 20$ cm^3). A 4-mm simulated gallery that was level with the floor of each chamber served as the longitudinal connection between the chambers. Two chambers on the far left were treated with the insecticide and allowed to soak into the wood for 24 h in the conditioning chamber. The remaining three chambers were not treated (Fig. 1 b). In this way, two chambers on the far left served as the treated surface and the two on the far right as the untreated surface, while the middle chamber housed the live termites (Fig. 1b). A total of 20 termites were released into the middle chamber, and the remaining half of the board was clamped on the top. Termite locations were recorded after 1, 3, 5, and 24 h by carefully dismantling the unit. Termites present in the middle chamber were excluded from the data analysis. There were two treatments (fipronil and imidacloprid), and each treatment was replicated five times for which 10 wooden units were used. The repellency of each chemical was calculated using the following formula (Kadir *et al*. 2014; Hassan *et al.* 2018) or Eq. 1,

$$\text{Repellency (\%)} = (Nc - Nt) / (Nc + Nt) \times 100 \quad (1)$$

where *Nc* and *Nt* are the numbers of termites present on untreated and treated chambers, respectively.

In another test, five chambers were excavated similarly to the above in a hoop pine board. However, only one chamber on the far-left side was treated with the insecticide (imidacloprid or fipronil), and the remainder were untreated. Two groups of termites color-coded green or black with a permanent marker were prepared. Ten termites marked with black marker (permanent) were placed into the treated chamber, and ten termites marked with green marker were placed into the untreated chamber on the far right. The other board half was clamped on top. Termite mortality and locations were recorded after 1, 3, 5, 24, 48, 96, 120, and 224 h. There were two treatments (imidacloprid, fipronil, and the control), and nine wooden units were used as each treatment was replicated thrice. There was no application of insecticides in all chambers to serve as a control treatment (Myles *et al.* 2007).

Horizontal Transfer of Fipronil Among Nestmates

The closed gallery system of drywood termites and their grooming habits generate a high probability of insecticide transfer among nestmates. The horizontal transfer trial aimed to determine insecticide transfer from primary donor termites to recipient termites

(Ferster *et al.* 2001). Based on previous results and the limited availability of termites, only fipronil was selected for this test. Approximately 200 termites were held on disks of Whatman No.1 filter paper (9 cm diameter) that had been dyed with 1 mL of aqueous Nile blue (0.008% w/w) (Rust *et al.* 2008). Termites were allowed to feed on the filter paper for at least two weeks before exposure to an insecticide. Hoop pine exposure units (20 × 4 × 4 cm^3) were prepared as described above in the toxicity bioassays. In naturally infested wood, drywood termites can acquire toxicants topically during the application and or when in contact with the treated surface. Therefore, two different methods of exposure were used. The aim was to compare the survival level in recipient termites when the donor termites were either topically treated with fipronil or had received residual exposure.

Residual exposures

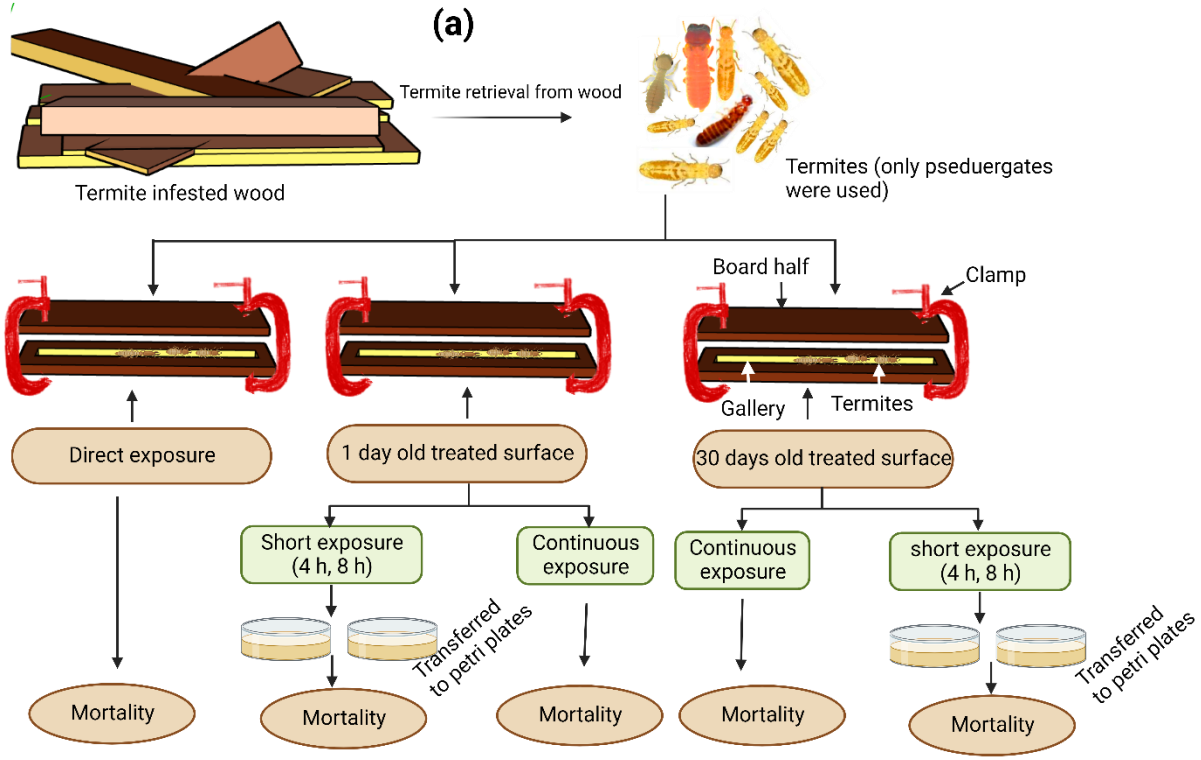
Dyed pseudergates (donors) were placed in fipronil-treated exposure units for 1 or 4 h. After each of these times, the termites were removed from the exposure units and placed in Petri dishes with recipient undyed, untreated pseudergates on untreated filter paper in a ratio of 1:1 and 1:9 (Fig. 1c). Based on these two ratios, a total of 20 termites (treated and untreated) were placed in each plate. The number of alive and dead termites on each plate was counted after 1, 3, 5, 12, 19, and 26 days. Dead termites were not removed from the Petri plates. Based on two ratios, there were eight treatments (including controls). There was no application of fipronil to the exposure units for the control treatment, but the remainder of the procedure was the same as for treated termites. Each treatment was replicated four times.

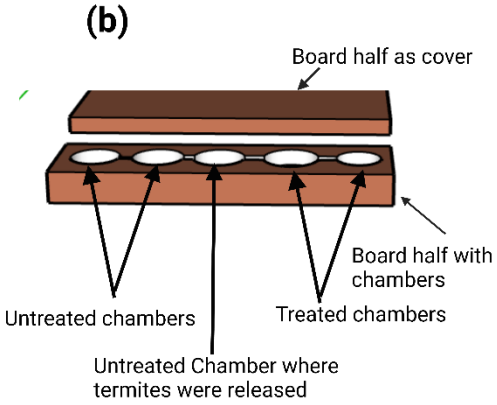
In the second part of the trial, the horizontal transfer of insecticide from treated dead termites to live untreated termites (recipients) was investigated (Fig. 1c). Dyed termites were housed in the fipronil-treated exposure units until they died. The dead termites were removed from the units and frozen at −20 °C for 15 min in the freezer. These dead-treated and frozen donor termites were placed in the Petri plates among live untreated recipient termites in a 1:1 and 1:9 ratio. The number of alive untreated and undyed termites on each plate was counted after 1, 3, 5, 12, 19, and 26 days. Dead termites were not removed from the Petri plates. There were four treatments and for the control treatment, untreated termites were frozen at −20 °C for 15 min to prepare the control corpses. Each treatment was replicated four times.

Topical exposures

Dyed termites were exposed directly by topical application of fipronil, as described above in the topical exposure test. Once the foam was broken and the liquid was absorbed or evaporated and no longer visible on the termites, the dyed alive termites (donors) were removed from the exposure units and placed in a Petri plate with undyed, untreated recipient termites in 1:1 and 1:9 ratio as described above and there were four treatments (fipronil, control) (Fig. 1c). After placement, the survival of undyed, untreated termites was recorded after 1, 3, 5, 12, 19, and 26 days. There was no application of fipronil in the control treatment.

A similar method as described above in residual exposure was adopted to determine the transfer of fipronil from the dead donors to live recipient termites, except the termite corpses were prepared by topical application of fipronil followed by freezing.


(a)
Termite retrieval from wood
Termite infested wood
Termites (only pseduergates were used)
Board half
Clamp
Gallery
Termites
Direct exposure
1 day old treated surface
30 days old treated surface
Short exposure (4 h, 8 h)
Continuous exposure
Continuous exposure
short exposure (4 h, 8 h)
Transferred to petri plates
Transferred to petri plates
Mortality
Mortality
Mortality
Mortality
Mortality


(b)
Board half as cover
Board half with chambers
Untreated chambers
Treated chambers
Untreated Chamber where termites were released

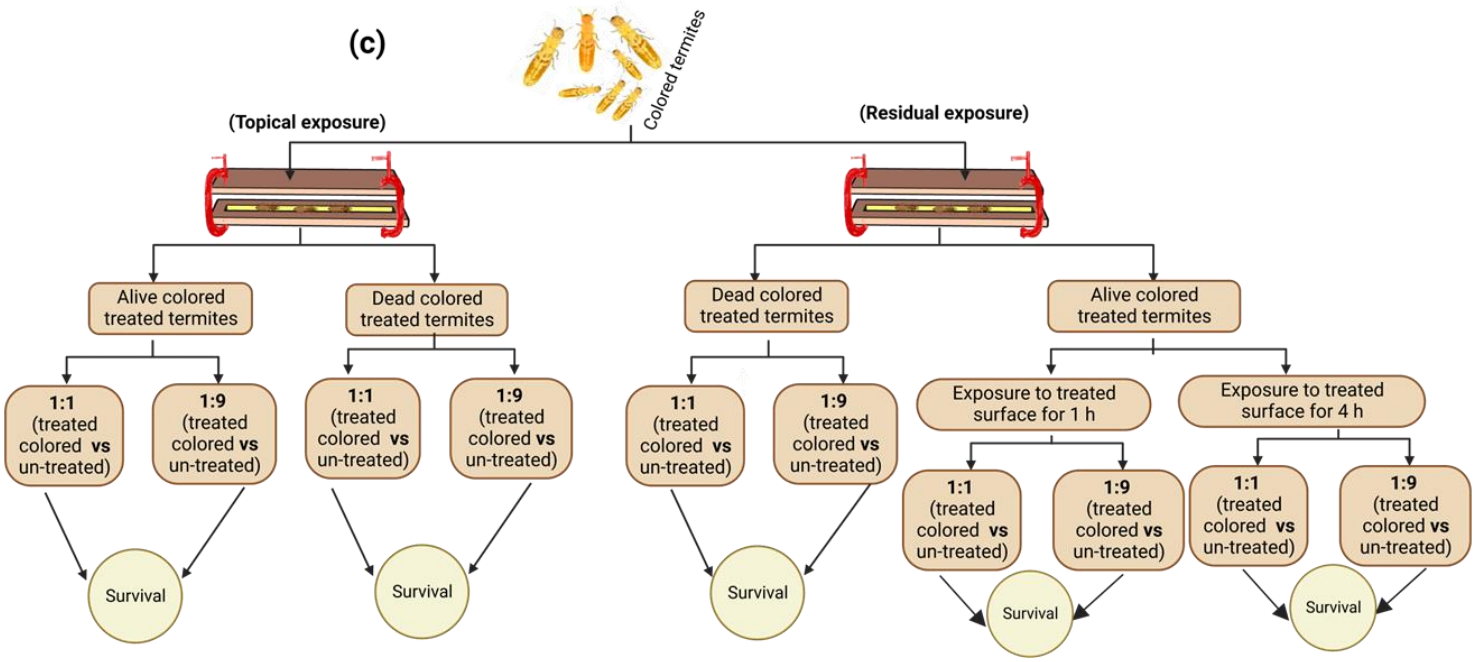


Fig. 1. Schematic summary of the experimental procedures: Toxicity tests (a), repellency test (b), and horizontal transfer bioassays (c). In all experiments, only pseudergates of *C. brevis* ≥ 3rd instar were used.

Data Analysis

GraphPad Prism 7 statistical software (GraphPad, San Diego, CA, USA) and Jamovi 16.9 (Jamovi, Sydney, Australia) under AGPL3 license were used for all data analysis. Data from toxicity studies were subjected to multivariate repeated measure analysis to investigate the effects of treatments, exposure times, and age of the treated surface. Only treatments were considered as factors in topical exposure and repellency tests. In horizontal transfer bioassays, multivariate repeated measure analysis tests were performed to test the influence of treatment, time of exposure to acquire toxicant and donor: recipient ratio on recipient survival in interactions with donor termites. Each analysis of variance (ANOVA) was followed by Tukey's Honest Significant Difference (HSD) test for significant differences between means. The level of significance was set at $\alpha = 0.05$.

RESULTS AND DISCUSSION

If applied correctly, localised treatments using registered insecticides are the most common and economical method for treating drywood termite infestations. The infestation is directly treated (drilled and injected) to kill termites confined in the wood (Lewis 2003). A few active ingredients are registered for localized drywood termite treatment in Australia. These are predominantly imidacloprid, permethrin, or fipronil. Most of the products containing these active ingredients are available as liquid formulations and a few as foams. Pest management technicians may prefer foam formulations because of their immediate effect, ease of use, and quick and effective placement in termite galleries, especially during vertical application. Liquid formulations may be difficult to deliver to the colony's core and may not fully penetrate the termite galleries, especially during vertical application.

Short-time Residual Exposure Tests

Foam insecticides containing either imidacloprid or fipronil caused significantly higher mortality than control treatments in 4 and 8 h of residual exposure. A maximum of 22% of termites died in the untreated control tests. (Fig. 2 a, b). Fipronil, however, killed *C. brevis* pseudergates more quickly in a 4 and 8-h exposure test than imidacloprid. In brief exposure tests, the effect of treatment ($F_{2,30} = 241.2$; $p < 0.01$), the age of treated surface ($F_{1,30} = 17.9$; $p < 0.01$), and treatments × surface age ($F_{2,30} = 23.3$; $p < 0.01$) were highly significant. However, exposure time ($F_{1,30} = 2.36$; $p = 0.13$) and treatment × exposure time ($F_{2,30} = 1.29$; $p = 0.29$) or interaction of all three factors ($F_{4,30} = 129.45$; $p = 0.059$) were not significantly different. All termites died within two days when placed on the wood coated with fresh residues (1 day old) of fipronil for 4 or 8 h. However, mortality was slightly delayed when termites were exposed to 30-day-old fipronil residues on the wooden surface, and termites all perished seven days after exposure (Fig. 2a, b). Results of short exposure tests showed considerably lower and delayed mortality of *C. brevis* pseudergates when exposed to fresh and aged imidacloprid residues compared to fipronil deposits. Termite mortality was less than 40% for 16 days following exposure to treated surfaces for 4 and 8 h (Fig. 2 a, b). However, irrespective of time exposure, considerably higher *C. brevis* pseudergates died when exposed to aged imidacloprid residues than fresh residues on wood surfaces. In contrast to fipronil, a considerable impact of exposure time on termites was also noted. In the 4-h exposure test, termite mortality was 85% at the completion of the trial when exposed to old-treated surfaces and significantly lower

mortality (42%) when termites were exposed to fresh imidacloprid treated surfaces. In 8-h exposure tests, all termites died when exposed to 30-day-old deposits in 30 days of exposure. However, the fresh imidacloprid treated surfaced failed to provide complete mortality of termites, and the mortality of termites was 64% at the end of the test (Fig. 2 a, b).

Continuous Exposure and Topical Exposure Tests

Similarly, with the short residual exposure tests, fipronil foam provided a faster kill of *C. brevis* pseudergates when termites were confined to 1-day and 30 days old-treated surfaces for 52 days. However, the mortality of termites caused by the fipronil, and imidacloprid-treated surfaces was significantly higher than for the control treatment. The maximum mortality of termites in the control treatment was 24.5% at the end of the tests (Fig. 3a). Effects of treatments ($F_{2,12} = 439.7$; $p < 0.01$) and treated surface age ($F_{1,12} = 8.5$; $p = 0.013$) were highly significant in continuous exposure tests; however, the interaction of treatments and treated surface age (treatments × surface age) was non-significant ($F_{2,12} = 2.2$; $p = 0.146$). After 1-day of exposure, where termites were exposed to either fresh or aged fipronil residues, the mortality of termites was 69 and 85%, respectively. However, all of the termites died, irrespective of the age of the treated surface after two days of exposure to fipronil residues. Delayed and lower mortality of termites exposed to imidacloprid fresh and aged residues were observed compared to fipronil. Aged imidacloprid residues provided a slightly faster kill of *C. brevis* than fresh. Aged imidacloprid residues on the wood surface killed all termites in 37 days compared to fresh residues, where termite mortality was 65% at the same time. However, all termites died at the completion of the test (Fig. 3a). In topical tests, after applying imidacloprid, the termites showed signs of immobility and narcosis but then recovered. These termites eventually died but sometime later. As a result, data from the first 1, 2, 4, and 7 h were excluded. However, the mortality of *C. brevis* pseudergates was considerably faster when the fipronil foam was applied topically than imidacloprid foam. However, the mortality of termites was significantly higher compared to control treatments, and no mortality of termites was observed in control treatments. Mortality of pseudergates was 84 and 63%, respectively, within the first 24 h of exposure following topical treatment with fipronil and imidacloprid foam. All fipronil-treated termites were dead within 48 h of exposure ($F_{1,24} = 73.7$; $p = 0.003$); however, it was 168 h before all imidacloprid-treated termites died ($F_{1,24} = 61.5$; $p < 0.001$) (Fig. 3b).

Overall, both insecticides were effective by contact and ingestion but differed in mode of action. Imidacloprid acted on post-synaptic nicotinic acetylcholine receptors in the nervous system of insects (Mullins 1993). By contrast, fipronil blocked GABAA-gated chloride channels in the central nervous system (Gant *et al*. 1998). In both tests, among these two insecticides, fipronil caused termite mortality at a faster rate than imidacloprid. These results agreed with a previous study reporting 100% mortality of fipronil-exposed drywood termites within seven days of brief exposure; however, the exposure time was more (24 h) than in the current study (Rust *et al.* 2008). Imidacloprid foam residues may not readily be available to termites and only killed the termites after long exposure. This is consistent with previous studies in which Premise foam (a.i. imidacloprid) failed to kill 100% of the termites within 26 days (Rust *et al.* 2008). Moreover, fresh fipronil foam residues caused mortality at a greater rate than aged residues. In contrast, aged imidacloprid residues were more lethal to *C. brevis* pseudergates at the tested concentration than fresh ones.

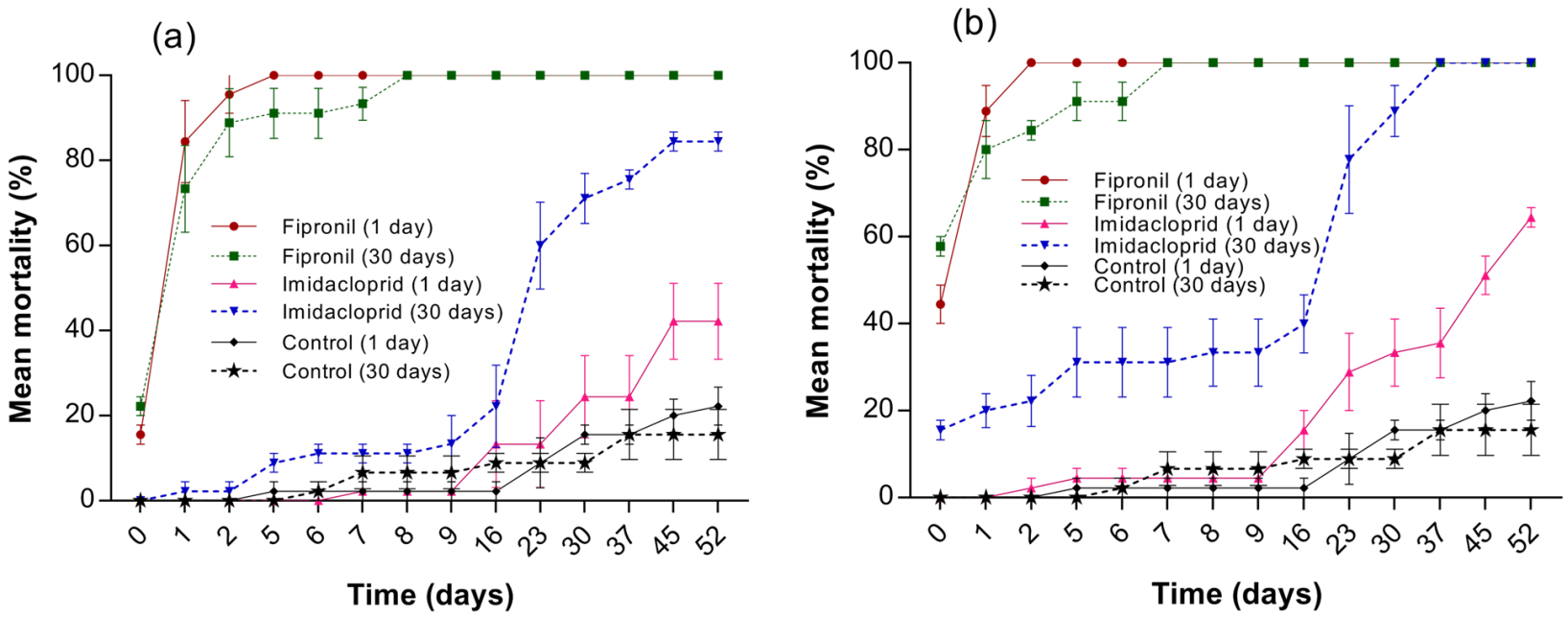


Fig. 2. Mean mortality (%) of *C. brevis* pseudergates in short exposure tests. Termites were housed for 4 h (a) and 8 h (b) on the one-day and thirty-day-old insecticide-treated surfaces in the wooden test units before being transferred to Petri plates containing filter papers.

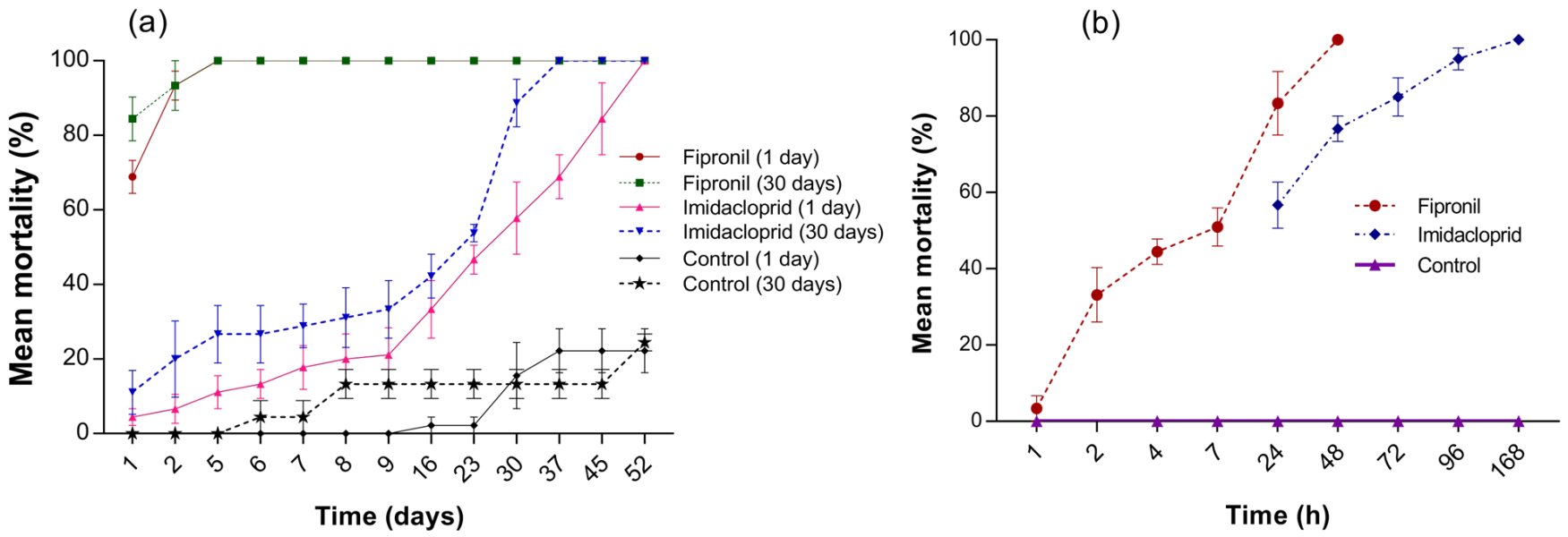


Fig. 3. Mean mortality (%) of *C. brevis* pseudergates in continuous exposure (a) and topical exposure (b) tests. In continuous exposures, termites were kept for 52 days on the 1-day, and 30-day-old insecticide-treated surfaces in the wooden test units. In topical exposure tests, pseudergates were kept on untreated wood surfaces, but the termites were topically treated with fipronil and imidacloprid.

Differences in how readily termites can retrieve these residues from treated surfaces may account for the difference in termite mortality between wet and dry residues of imidacloprid or fipronil. Insecticide residues that restrict feeding and are not easily accessible to termites do not give rapid kill because drywood termites can live for more than two months without food (Sajap and Sahri 1983). Previous studies have reported feeding inhibition in subterranean and drywood termites after exposure to imidacloprid (Ramakrishnan *et al.* 2000; Rust *et al.* 2008). Like residual exposure tests, delayed mortality of *C. brevis* was observed after imidacloprid topical exposure. Delayed mortality of termites after imidacloprid exposure has been demonstrated in previous studies (Ramakrishnan *et al.* 2000; Gahlhoff, Jr. and Koehler 2001; Woodrow *et al.* 2005). The results of rapid mortality caused by fresh fipronil residues are consistent with previous studies (Myles *et al.* 2007; Rust *et al.* 2008) and slow mortality caused by aged fipronil residues could be due to its penetration into wood surfaces, making it less available to termites.

Repellency and Avoidance Tests

Results of repellency tests showed a significant effect of insecticides on repellent activities against *C. brevis* ($F_{2,12} = 179$; $p < 0.01$). Except for the first hour of exposure, surfaces treated with fipronil had less than 5% repellency. The surfaces treated with fipronil had a greater number of termites. After 24 h of exposure, all termites were present in fipronil-treated chambers, which suggests that this product is non-repellent. In contrast, most termites avoided the imidacloprid-treated surfaces at each observation period. More than 90% repellency was observed after 24 h with chambers treated with imidacloprid (Fig. 4a). In a subsequent test, ten black-marked termites entered the test units on the treated chamber, but they had the choice to move or not towards the untreated side. Ten green-marked termites that entered the untreated chamber were able to either stay safely on the untreated side or if not repelled, wander onto the treated side. Thus, this test was used to measure criteria called escapability, nonrepellent kill, or avoidance. The test showed that fipronil foam caused significantly higher mortality of *C. brevis* pseudergates than imidacloprid and control treatments ($F_{2,12} = 97.4$; $p < 0.01$). For fipronil treatment, initially, slightly lower and delayed mortality of green termites was observed; however, after 224 h, mortality of green (untreated) termites was 65%. The mortality of black-treated termites was 82%. In contrast, the mortality of termites in imidacloprid tests was similar to the control treatment (< 5%) (Fig. 4b).

Insecticide repellency may influence the amount of contact termites must have with a treated surface and the horizontal transfer of insecticides (Rust *et al.* 2006). Termites may not acquire a lethal dose of a toxicant if they avoid contacting the surfaces treated with repellent insecticides. However, only a few studies have evaluated the repellency of insecticides against *C. brevis* (Myles *et al.* 2007). The results of this trial showed that, unlike fipronil-treated surfaces, imidacloprid-treated surfaces were highly repellent to *C. brevis* pseudergates, and significantly low non-repellent kill was observed in repellency tests. Fipronil-treated surfaces did not repel termites. Consequently, imidacloprid foam as a localized treatment of *C. brevis* in naturally infested wood may not cause high mortality of termites because of its repellency; termites elsewhere in the gallery system or those not immediately exposed to the insecticide may not be affected (Woodrow *et al.* 2007). Fipronil may require fewer injection points in the infested timber than imidacloprid and only treating a portion of the infested area with fipronil may be sufficient to kill most termites. Termites were foraging across the gallery system and treated with fipronil, leading

to higher termite mortality and greater efficacy than imidacloprid foam. These results contradict previous studies that have claimed that imidacloprid foam is a non-repellent to subterranean and drywood termites and kills termites when they forage on treated surfaces (Reid *et al.* 2002; Woodrow *et al.* 2005; Luo 2010; Oi 2022). This could be because of the different inert ingredients in the imidacloprid foam formulation. A previous study reported that when imidacloprid foam was used as a localized treatment of Western drywood termite, there was a 90% reduction in acoustic emission (AE) counts post-treatment (Lewis *et al.* 2009). However, the repellency of an insecticide depends on the concentration tested, the formulation, the substrate, the insect species, and the environmental conditions (Gould 1984; Rust *et al.* 1995: Manzoor *et al.* 2012).

Horizontal Transfer of Fipronil

Regardless of the donor exposure method (topical *vs.* residual) and recipient-donor ratio, horizontal transfer was highly effective. Minimal recipient *C. brevis* pseudergates survival was observed compared to control treatments (Table 1). Even 1-h exposures were sufficient for donor termites to acquire a lethal dose of the toxicant. In tests involving a live donor and live recipient termites, the effect of treatment ($F_{1,36}$ = 439.5; $p < 0.01$), donor exposure time ($F_{2,36}$ = 9.6; $p < 0.01$), ratio ($F_{1,36}$ = 42.3; $p < 0.01$), treatment × exposure time ($F_{2,36}$= 9.7; $p < 0.01$), treatment × ratio ($F_{1,36}$ = 27.2; $p < 0.01$) were highly significant. However, exposure time × ratio ($F_{2,36}$ = 0.12; $p = 0.81$) and interaction of all factors (treatments × exposure time × ratio) were non-significant ($F_{2,36}$ = 0.23; $p < 0.88$). The rate of recipient termite survival in tests involving a 1:1 donor-recipient ratio was significantly less than recipient survival in tests with a 1:9 ratio ($F_{1,24}$ = 13.2; $p < 0.01$). No recipient termites survived after 19 days of exposure to donor termites when donors were topically treated and present in a 1:1 ratio. Similar results were observed when donors were treated using fipronil residues for 4 h. However, 1 h of donor exposure to fipronil residues did not produce 100% mortality in recipient termites in a 1:1 ratio. When the donor-to-recipient termites were 1:9, however, about 7 to 47% of termites were still alive at the end of the experiment. The lowest survival of recipient pseudergates (6.9%) was observed when donors were topically treated, followed by 25% recipient survival when donor termites were treated through 4-h residual exposures. Significantly, higher termite survival (47%) was observed at 26 days of exposure in the 1:9 donor-recipient ratio when donor termites were treated through 1-h residual exposures. More than 85% of recipient termites survived in control treatments (Table 1).

Results of tests involving dead donor and live recipient termites are presented in Table 2. As with live donor termites, dead donors were also effective in transferring fipronil to recipient termites. The effect of treatment ($F_{1,24}$ = 189.9; $p < 0.01$), donor-recipient ratio ($F_{1,24}$ = 42; $p < 0.01$), recipient exposure method ($F_{1,24}$ = 4.7; $p = 0.04$), and ratio × treatment ($F_{1,24}$ = 27.7; $p < 0.01$), exposure method × treatments ($F_{1,24}$ = 4.69; $p = 0.04$) were significant, while the remaining interactions were non-significant. No recipient termite survived after 12 days of exposure when dead donors were topically treated but were in a 1:1 donor-recipient ratio. All termites died after 19 days of exposure to dead donors when the recipients were treated through residual exposure. At the completion of the 1:9 donor-recipient ratio test, only 2.7% of recipient termites survived when dead donors were treated topically. However, there was 19.4% survival when donor termites were treated through residual exposure (Table 2).

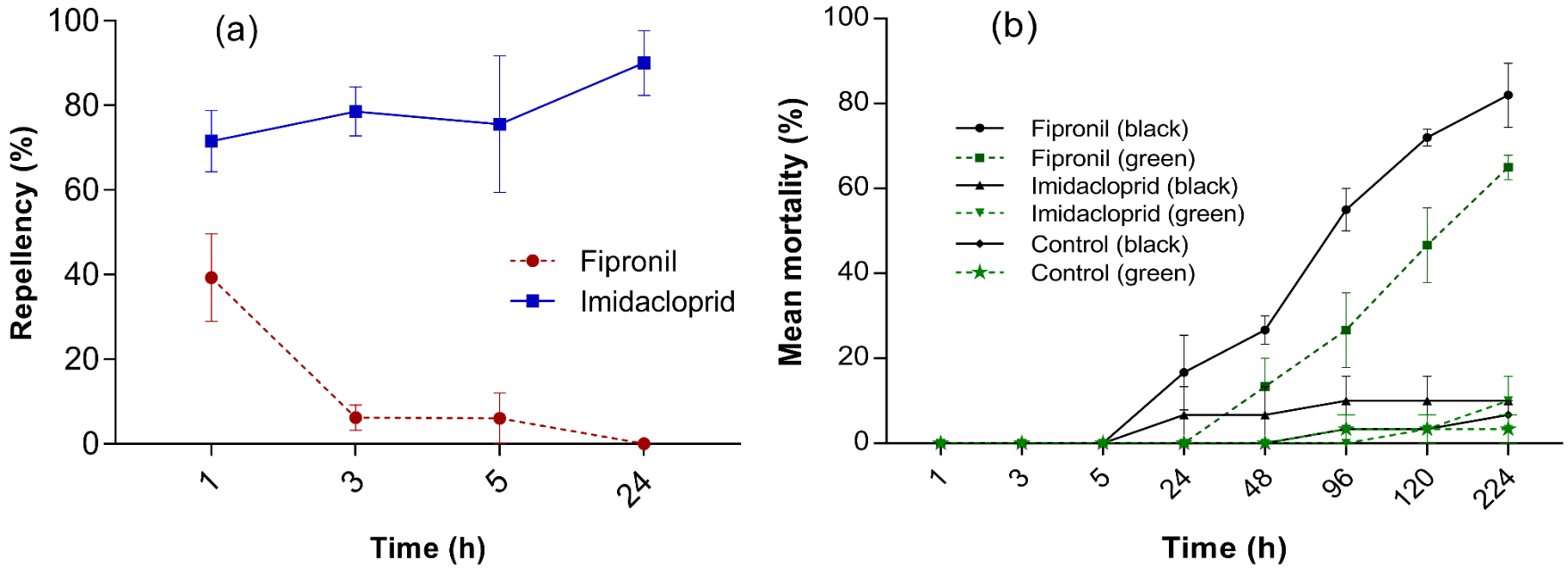


Fig. 4. Mean repellency (%) of imidacloprid and fipronil on 1-day-old treated wood surfaces to *C. brevis* pseudergates after 1, 3, 5, and 24 h of exposure in five chamber test (a) and mean mortality (%) of black (entered from treated chamber) and green (entered from untreated chambers) pseudergates in five chamber tests at different time intervals (b)

Table 1. Mean (± SEM) Percent Survival in *C. brevis* Pseudergates Exposed to Alive Donor Nestmates Treated with Fipronil in Residual and Topical Exposure Tests

Exposure Method	Treatments	Donor: Recipient	Donors' Exposure Time (h)	Survival of Recipient Termites (%)					
				Time After Exposure (days)					
				1	3	5	12	19	26
Residual	Fipronil	1:1	1	97.5 ± 2.50a	65.0 ± 8.66c	52.5 ± 11.08d	32.5 ± 6.00d	20.0 ± 10.00c	10.0 ± 7.07d
		1:9	1	100.0 ± 0.00a	86.1 ± 7.34b	83.3 ± 8.78b	68.0 ± 7.02b	50.0 ± 8.17b	47.2 ± 8.33b
	Fipronil	1:1	4	95.0 ± 2.88a	35.0 ± 2.88d	22.5 ± 2.50e	7.5 ± 4.78e	0.0d	-
		1:9	4	98.6 ± 1.38a	77.7 ± 6.80b	70.8 ± 3.49c	44.4 ± 3.92c	36.1 ± 4.81c	25.0 ± 5.31c
	Control	1:1	4	100.0 ± 0.00a	95.0 ± 5.00a	95.0 ± 5.00a	95.0 ± 5.00a	92.50 ± 4.78a	92.5 ± 4.78a
		1:9	4	100.00a	98.6 ± 1.38a	98.6 ± 1.38a	97.2 ± 1.60a	97.2 ± 1.60a	97.2 ± 1.60a
Topical	Fipronil	1:1	-	80.0 ± 4.08b	25.0 ± 6.45e	7.5 ± 4.78f	2.5 ± 2.50f	0.0d	-
		1:9	-	95.8 ± 1.38a	68.0 ± 7.30c	59.7 ± 4.74d	34.7 ± 6.15d	18.0 ± 4.74c	6.9 ± 3.49d
	Control	1:1	-	100.0 ± 0.00a	96.0 ± 4.50a	95.0 ± 5.00a	95.0 ± 5.00a	92.5 ± 4.18a	87.5 ± 4.78a
		1:9	-	100.0 ± 0.00a	98.6 ± 1.38a	97.2 ± 1.38a	97.2 ± 1.60a	93.2 ± 1.62a	85.2 ± 1.23a

Values show the mean survival (± SEM). Means in the same column followed by the same letter are not significantly different by Tukey's HSD test ($P \leq 0.05$).

Table 2. Mean (± SEM) Percent Survival in *C. brevis* Pseudergates Exposed to Dead Donor Nestmates Treated with Fipronil in Residual and Topical Exposure Tests

Exposure Method	Treatments	Donor: Recipient	Survival of Recipient Termites (%)					
			Time After Exposure (Days)					
			1	3	5	12	19	26
Residual	Fipronil	1:1	75.0 ± 8.66c	30.0 ± 5.77d	15.0 ± 6.45c	5.0 ± 2.88c	0d	-
		1:9	93.0 ± 2.65b	57.2 ± 5.31b	38.8 ± 8.17b	25.0 ± 4.81b	22.2 ± 6.24b	19.4 ± 7.34c
Topical	Fipronil	1:1	67.5 ± 7.50d	20.0 ± 4.08e	7.5 ± 4.70d	0.0d	-	-
		1:9	79.1 ± 4.74c	47.2 ± 3.58c	37.5 ± 2.62b	19.4 ± 3.58b	8.3 ± 3.56c	2.7 ± 1.6d
-	Control	1:1	100.0 ± 0.00a	100.0 ± 0.00a	100.0 ± 0.00a	92.5 ± 4.78a	92.5 ± 4.78a	80.0 ± 4.08b
		1:9	100.0 ± 0.00a	100.0 ± 0.00a	100.0 ± 0.00a	100.0 ± 0.00a	97.5 ± 2.50a	88.8 ± 2.26a

Values show the mean survival (± SEM). Means in the same column followed by the same letter are not significantly different by Tukey's HSD test ($P \leq 0.05$).

The horizontal transfer of insecticides is an important mode of action to control social insect pests, such as drywood termites, with closed gallery systems, which could facilitate the transfer of toxicants (Kofoid and Williams 1934). Several potential pathways for termites to contact with toxicants have been introduced to their foraging environment, including foraging in treated galleries, grooming, trophallaxis, cannibalism, and incidental contact with live-treated termites (Ferster *et al.* 2001). Untreated termites may also consume a lethal quantity of toxicants when eating, touching, or moving the termite corpses killed by insecticides. Horizontal transfer of fipronil and other insecticides has been extensively investigated against subterranean termites (Rust *et al.* 2006; Green 2008; Gentz *et al*. 2009; Buczkowski *et al.* 2012; Neoh *et al.* 2012; Zhang *et al.* 2022). However, only a few studies have reported horizontal transfer of insecticides in drywood termites (Ferster *et al.* 2001; Rust *et al.* 2008).

Although fipronil produced rapid mortality in the foraging or topically treated termites (donors), it was effectively transferred from treated donor pseudergates to untreated recipients and caused considerable mortality in recipients. Fipronil was effectively transferred to untreated termites from donors exposed *via* residual and topical exposures, and 100% mortality was achieved with both exposure routes at a 1:1 recipient-to-donor ratio. However, topical exposures resulted in faster mortality in both donors and recipients. A much higher survival of recipient termites was observed when the donor to recipient ratio was 1:9. In residual exposures, donor termites were exposed to fipronil deposits for 1 and 4 h and found no effect of the donor exposure period on the mortality of recipients. A previous study reported that exposures of 15, 30, and 60 min of donor drywood termites to fipronil deposits produced 100% mortality of the recipients in a 1:1 ratio. Five-minute exposures produced 94.4% mortality of recipients at week 3 (Rust *et al.* 2008). Necrophoresis is an essential characteristic of adaptation to social living, and it has been observed in subterranean and drywood termites (Neoh *et al.* 2012a,b; da Silva *et al.* 2018). The previous study reported that mainly the nymphs and pseudergates of *C. brevis* are involved in corpse management, and they can eat intercolonial and interspecific nestmates. Soldiers participate through inspection and agonism (da Silva *et al.* 2018). Transfer of insecticides through necrophagy may increase the efficacy of control of drywood termites. The results showed a faster kill of recipients when there were dead donor termites compared to live donor termites. Still, mortality depended on the recipient-to-donor ratio and the type of exposure. Previous studies showed that corpses killed by fast-acting insecticides caused higher horizontal toxicity in recipient ants than live donors (Wiltz *et al.* 2009). The recipient termites ate all dead donor termites in all control treatments within five days of exposure. However, as reported in a previous study, dead-treated donor termites were not eaten by live recipient termites in any treatment (Rust *et al.* 2008). *Cryptotermes brevis* start corpse management by inspecting cadavers through antennation, followed by the individuals exhibiting alarm behaviour and then ingesting corpses (da Silva *et al.* 2018). Mortality of recipient termites from dead-treated donor termites can be because of contact with contaminated corpses, or recipients might have eaten a minor part of the donor's body, leading to mortality of recipients, as corpses were not observed under the microscope

Although high mortality of termites was observed after exposure to fipronil-treated surfaces and topical treatment, fipronil also produces high secondary mortality in horizontal transfer bioassays. However, efficacy under laboratory conditions does not necessarily ensure the quality of a given drywood termite treatment under natural conditions. The success of a localized treatment depends on locating the termite infestation

in the wood to ensure the best possible efficacy. In addition to visual searches, locating a drywood termite infestation using microwave or acoustic-based tools will hopefully make it easier to discern the termite gallery system with active termites hidden in the wood. A Termatrac™ T3i microwave-based device was successfully tested under laboratory conditions to detect *C. brevis* in infested timber and factors affecting its detection ability are also discussed (paper under review). Currently, further studies examining how far and deep foam insecticides can travel in drywood termite galleries and field studies using insecticides as localized treatment and the use of Termatrac™ to discern termite gallery systems are planned.

CONCLUSIONS

1. The current study demonstrated that the fipronil-containing foam insecticide produced mortality in *C. brevis* at a faster rate than the imidacloprid-containing foam. This was the case with fresh and dry deposits even when termites were exposed to toxicants for as little as 4 h.
2. Prolonged exposures of drywood termites on deposits of imidacloprid were necessary to achieve higher termite mortality. Though dry imidacloprid residues produced greater mortality of *C. brevis* than wet residues, imidacloprid was repellent to *C. brevis* at the test concentration, and it might not provide a more non-repellent kill than the surface treated with fipronil foam.
3. Fipronil was transferred efficiently among the termites, and the rate and the level of secondary mortality in the recipient termites depended on toxicant exposure routes to donors, recipient and donor ratio, and exposure time to recipient termites.

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