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Toxicity and horizontal transfer of chitin synthesis inhibitors in the western drywood termite (Blattodea: Kalotermitidae)

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Three chitin synthesis inhibitors (CSIs), bistrifluron, chlorfluazuron, and noviflumuron, were evaluated for their toxicity and horizontal transfer against the western drywood termite, *Incisitermes minor* (Hagen), when used to treat wood. In a no-choice bioassay, bistrifluron provided significantly faster kill than chlorfluazuron or noviflumuron treatments at 0.1 and 0.5% (wt/wt) rates over a 60-d period, providing 99% mortality. In a choice bioassay using 0.1% rate, bistrifluron provided a significantly faster kill than chlorfluazuron or noviflumuron treatments over a 60-d period, resulting in 96% mortality. In a transfer bioassay, a group of bistrifluron-fed termites, donors (D), was placed with a group of unexposed nestmates, recipients (R). Based on the visual marking, the food material of the donor termites was readily transferred to the recipients within 24 to 48 h. Overall, survival curves were similar between 1:19 (5% donor) and 10:10 (50% donor) D:R ratios, resulting in 100% mortality by day 90. This result indicated that lethal doses of bistrifluron were retained and effectively transferred, even from limited numbers of termites that originally ingested the compound. Implications for drywood termite management and future development are discussed.

Keywords: Incisitermes minor, bistrifluron, chlorfluazuron, noviflumuron, bait.

Introduction

The western drywood termite, *Incisitermes minor* (Hagen) (Blattodea: Kalotermitidae), causes significant economic damage in its native range of the southwestern United States and northwestern Mexico, especially California (Harvey 1934, Cabrera and Scheffrahn 2001). Beyond its native range, *I. minor* has been introduced to other parts of the United States such as Hawaii, New York, and Florida, as well as abroad in Canada, China, Japan, Korea, and Australia (Evans et al. 2013, Scheffrahn 2019, Horwood and Lo 2022, Lee et al. 2024). Increased urbanization and globalization involving the movement of wood and wood-containing products worldwide, along with the cryptic lifestyle of *I. minor*, contribute to its status as an important structural pest in several parts of the world (Lewis et al. 2014). Western drywood termites typically nest and forage inside 1 piece of wood (or pieces that contact), with only winged reproductive (alates) leaving the nest for dispersal (Harvey 1934), making their detection and management challenging.

Insecticides are widely used for the remedial control of drywood termite infestations. Structural fumigation with sulfuryl fluoride is the most effective method for whole-structure treatment of drywood termite infestations (Lewis et al. 2014, Zilberman and Lewis 2024). However, structural fumigation for drywood termite control has several drawbacks, including its high cost, the requirement of residents to vacate the structure for several days, and lack of residual protection (Cabrera and Scheffrahn 2001). In addition, sulfuryl fluoride is classified as a significant greenhouse gas (Papadimitriou et al. 2008, Mühle et al. 2009) and its use for structural fumigations may undergo more regulatory scrutiny in the future (Barreau et al. 2019, Gaeta et al. 2021).

Another common treatment method for drywood termite infestations is localized insecticide injections, also known as spot treatments or drill-and-treat. In this method, subsurface injections of insecticides are made into the galleries via drill or kickout holes (Cabrera and Scheffrahn 2001, Lewis et al. 2014). Inherently, spot

treatments are for relatively smaller termite infestations that are accessible for drilling and treatment. The success of spot treatments largely depends upon the ability to locate active infestations and inject effective toxicants (Cabrera and Scheffrahn 2001, Lewis et al. 2014). For active ingredients (AIs) to be effective in controlling drywood termite infestations via spot treatment, they must be nonrepellent, slow-acting, and transferred from exposed termites to unexposed nestmates (Woodrow et al. 2006, Lewis et al. 2009). Residues from commercial formulations containing fipronil (aqueous suspension concentrate and foam), thiamethoxam (foam), and disodium octaborate tetrahydrate (dust) are nonrepellent to drywood termites and readily transferred from the treated individuals to untreated nestmates (Rust and Venturina 2009, Hassan et al. 2023). While spot treatment is a useful tool to control existing drywood termite infestations (Cabrera and Scheffrahn 2001, Rust and Su 2012, Lewis et al. 2014), its treatment efficacy is highly variable and complete colony elimination is hard to achieve (Ebeling and Wagner 1964, Woodrow et al. 2006, Lewis et al. 2009).

The presence of some surviving termites does not necessarily mean that the treatment is a total failure since the treatment may greatly reduce the infestation and future damage (Ebeling 1975). Nevertheless, potential "incomplete" coverage remains a concern when applying localized insecticide injections for the remedial control of drywood termites (Woodrow et al. 2006, Hickman and Forschler 2012). Aside from the potential difficulties in locating and accessing infestations for treatment, the complex gallery system architecture of mature drywood termite colonies presents a challenge in reaching all active galleries (Woodrow et al. 2006, Hickman and Forschler 2012). Infestations of *I. minor* consist of many larger chambers interconnected by tunnels or galleries, often so narrow that only an individual termite can move through (Himmi et al. 2016). These narrow galleries can be blocked by fecal pellets or individual termites, preventing rapid penetration and dispersion of the injected insecticides in liquid, foam, or dust (Hickman and Forschler 2012). Furthermore, drywood termites wall off the treated areas within their galleries by creating "blockades" with their fecal material, limiting the colony's further exposure to the injected insecticide (Indrayani et al. 2008).

Considering the factors mentioned above, one of the important questions is whether the products and AIs currently available for localized treatment would provide effective control when only a small number of the termites (eg 5% to 10%) are exposed to the injected insecticides. Hassan et al. (2023) showed that when only 10% of West Indian drywood termites, *Cryptotermes brevis* (Walker), are exposed to fipronil (via either topical or residual exposure) in a group of 20 termites, 7% to 47% of the recipient termites were still surviving on day 26. Furthermore, based on a laboratory study, Ferster et al. (2001) showed with *Incisitermes snyderi* (Light), that when 1 termite exposed to the residue of disodium octaborate tetrahydrate (as either dust or liquid) was placed with 10 unexposed termites, 46% to 96% of the recipient termites were still alive by day 28. These observations lead to an important question: are there any other AIs that can substantially impact the drywood termite colony even with a limited amount of exposure?

Lee and Neoh (2023) described benzoylphenylurea CSIs as type IV termiticides that possess 3 important characteristics: slow action, nonrepellency, and a dose-independent lethal time (Su and Scheffrahn 1996b, Su 2003). The dose-independent lethal time implies that the CSIs, at effective concentrations, will not cause the insects' mortality until their next molting event (Su and Scheffrahn 1996b, Xing et al. 2014), which allows enough time for effective transfer of CSIs among individuals within a colony (Su 1994, 2005). Many different chitin synthesis inhibitors (CSIs) have been tested with subterranean termites, with several being incorporated into commercial bait products targeting them (Rust and Su 2012, Evans and Iqbal 2015, Su 2019). These commercial bait products with CSIs can provide cost-effective and sustainable control of subterranean termites (Rust and Su 2012, Evans and Iqbal 2015, Chouvenc 2018, Su 2019). However, CSI baits are not currently available as a treatment option for drywood termite control. Neither have they been tested on important drywood termite species such as *C. brevis* and *I. minor*.

To examine the potential of CSIs to be incorporated in drywood termite control as a localized treatment option, the current study tested the toxicity of 3 selected CSIs (bistrifluron, chlorfluazuron, and noviflumuron) against the western drywood termite, *I. minor*. Laboratory no-choice and choice bioassays were conducted to compare efficacies between the CSIs. Based on findings from these initial experiments, bistrifluron was chosen to further test its transfer potential among termites at 3 different donor to recipient (D:R) ratios.

Materials and Methods

Termites

Western drywood termites, *I. minor*, were extracted from different pieces of infested wood collected in Riverside, California. Each colony was stored in a plastic container (30.5 × 21.6 × 6.4 cm) with small pieces of basswood (150 × 100 × 3.2 mm; Midwest Products Co., Inc., Darien, Illinois) as a food source. A couple of small openings were made on the sides of the containers for ventilation, and the containers were placed within a larger plastic box (40.6 × 22.9 × 27.9 cm) containing a saturated NaCl solution to maintain 75% relative humidity (RH) within the larger box (Winston and Bates 1960). The stock colony boxes were kept in a growth chamber (Thermo Fisher Scientific, Waltham, Massachusetts) at 26 °C without light. To minimize the chance of using termites that were damaged during the collection process, the collected termites were left undisturbed for at least 7 d, and the surviving termites were used in the bioassays at the end of this period. One or 2 different populations were randomly chosen to set up each bioassay where a random mixture of pseudergates and nymphs, ≈5 mm in length, were used.

Chemicals

Three different technical grade CSIs were used in this study: bistrifluron (98.4%; FarmHannong Company., Ltd, Republic of Korea), chlorfluazuron (99%; Chem Service Inc., West Chester, Pennsylvania), and noviflumuron (98%; Dow AgroSciences LLC, Indianapolis, Indiana).

No-Choice Bioassay

Stock acetone solutions of bistrifluron, chlorfluazuron, and noviflumuron were prepared so that a piece of 4 × 2.7 cm balsa wood (≈0.64 g) (Fig. 1A) treated with 200 µl of a CSI solution would provide 0.1% or 0.5% (wt/wt) of the CSI in the wood. Acetone-only was used for the control. The treated wood pieces were left in the fume hood for 3 h to allow for solvent evaporation. One piece of the treated wood was placed in a plastic Petri dish (55 mm diameter, Fisher Scientific, Waltham, Massachusetts), and 20 randomly selected termites were introduced. The Petri dish was covered with its lid and placed in a humidity chamber at 75% RH. The treatment and control groups were kept in separate humidity chambers to prevent any cross-contamination. The bioassay ran at 26 °C in complete

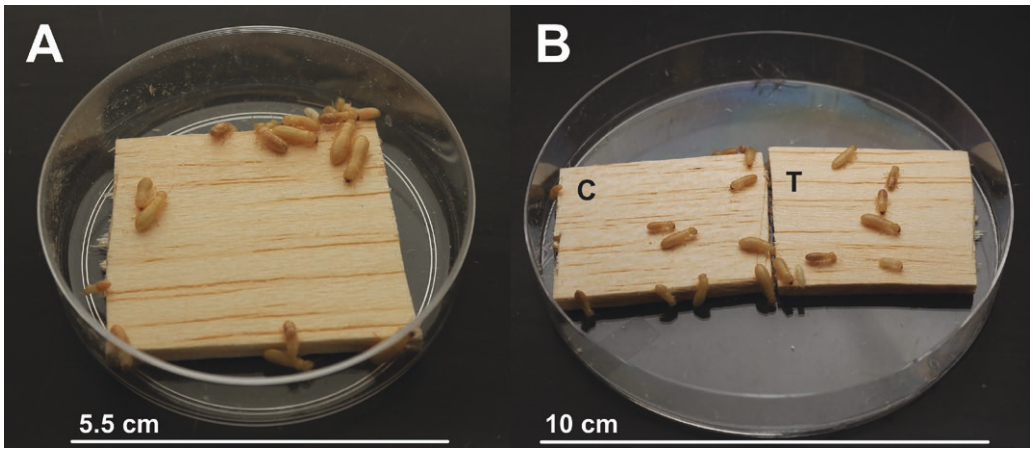

Fig. 1. No-choice (A) and choice (B) bioassay designs. For the choice bioassay, 1 piece was the control wood (C), and the other was the treated wood (T).

darkness except during observation. Observations were made daily for the first 3 wk, and every 3 d thereafter. Petri dishes were gently tapped and termites without any movement for≈5 s were recorded as dead. The dead termites were left in the arena for the duration of the experiment. Treatments and control were replicated 10 times.

Choice Bioassay

The choice bioassay used a similar experimental protocol as the no-choice bioassay. However, the choice bioassay provided the treatment (CSI-treated) and control (acetone-only) wood pieces side by side in a plastic Petri dish (100 mm diameter) (Fig. 1B). Twenty randomly selected termites were introduced where the 2 wood pieces met. Due to the lack of significant differences between 0.1% and 0.5% treatment rates in the no-choice bioassay (see Results), 0.1% was used in the choice bioassay. Petri dishes were gently tapped and termites without movement for ≥5 s were recorded as dead. The dead termites were left in the arena for the duration of the experiment. Treatments and control were replicated 10 times.

Transfer Bioassay

Bistrifluron was chosen for the transfer bioassay based on its highest relative performance in the no-choice and choice bioassays (see Results). Whatman No.1 filter paper (Cytiva, Marlborough, Massachusetts) was cut into discs that fit flush at the bottom of 100 mm plastic Petri dishes. The discs were dipped in a 1% Brilliant Blue G-250 (Fisher Scientific, Fair Lawn, New Jersey) dye solution (water) for blue coloration. G-250 has an affinity for attaching to proteins (Bradford 1976) and was used to qualitatively track the transfer of alimentary material between termites. For treated discs, 700 µl of bistrifluron in acetone was applied to the colored discs to achieve a final concentration of 0.5% (wt/wt) bistrifluron. For the control, 700 µl of clean acetone was applied to the colored discs. The discs were left in the fume hood to dry for 24 h before being placed in soda-lime glass Petri dishes (100 mm diameter, Steriplan, Czech Republic). Termites were then introduced to the prepared discs and kept for 3 d (Supplementary Fig. S1 and S2). Termites were randomly selected, marked on the dorsal side of the abdomen with permanent marker (Quill Corporation, Lincolnshire, Illinois), and placed onto new untreated discs in Petri dishes (100 mm diameter). These termites (fed on the CSI-treated or control discs, and subsequently marked) were referred to as donors (D). Untreated termites from the same stock colony were introduced into the Petri dish containing the donors. These newly introduced termites were referred to as recipients (R). Three different D:R ratios, 20:0, 10:10, and 1:19, were tested. Treatments and the 20:0 control were replicated 5 times each. Donors and recipients remained together in the same Petri dish for the duration of the bioassay. The treatment and control groups were kept in separate humidity chambers to prevent cross-contamination. The bioassay ran at 26 °C in 75% RH in complete darkness except during observation. Observations were made daily for the first 3 wk, and every 3 d thereafter until all bistrifluron treatments achieved 100% mortality. To determine mortality, Petri dishes were gently tapped and termites without any movement for ≈5 s were recorded as dead. The dead termites were left in the arena for the duration of the experiment. Termites with blue coloration were identified based on visual inspection under standard laboratory-lit conditions by 1 observer (NAP). Due to a vibrant blue hue of Brilliant Blue G-250, and translucent body of the termites, it was possible to record the presence/absence of blue coloration within the alimentary tract of a termite (binary data). Even slight blue coloration was clearly distinguishable from the natural coloration of *I. minor* (Supplementary Fig. S3).

Statistical Analysis

The data were analyzed using Kaplan–Meier survival analysis. The distribution of survival times of termites from each group was described using the survivorship function $S(t)$, the probability of an individual termite surviving past a given time point, t (in days). Log-rank tests (Peto and Peto 1972) were used for overall comparisons among survival curves and subsequent multiple comparisons using the Holm–Sidak correction (Šidák 1967).

For each CSI, cumulative mortality levels (% dead) were compared among groups at 5 time points (ie 1, 15, 30, 45, and 60 d after treatment). The no-choice and choice bioassay data were analyzed with a Kruskal–Wallis test followed by Dunn's post hoc test for pairwise comparisons. Additionally, to understand the mortality caused by insecticides, the average final cumulative mortality data were corrected using Abbott's formula (Abbott 1925).

The cumulative mortality levels (% dead) were compared among groups at 60 d after treatment for the transfer bioassay. The data were analyzed with a 1-way ANOVA followed by Tukey's post hoc test for pairwise comparisons. Additionally, to understand the mortality caused by insecticides, the average 60-d cumulative mortality data were corrected using Abbott's formula. All statistical analyses were performed using Sigma Plot ver.14.5 (Systat Software, San Jose, California).

Results

No-Choice Bioassay

Bistrifluron killed significantly faster than chlorfluazuron or noviflumuron at 0.1% and 0.5% (wt/wt) rates over a 60-d period, providing 99% mortality. Overall, a significant difference among the survival curves was found (log-rank test: $\chi^2 = 677.473$; df = 6; $P < 0.001$) (Fig. 2). Survival curves from the 2 different treatment rates (0.1% or 0.5%) were not significantly different from each other for bistrifluron and chlorfluazuron ($P = 0.639$ and $P = 0.199$). The 0.1% noviflumuron survival curve was not significantly different from 0.1% chlorfluazuron ($P = 0.0556$) or 0.5% chlorfluazuron ($P = 0.732$). All other pairwise comparisons were significantly different (Supplementary Table S1).

Total cumulative mortality values were compared between treatments and control. On day 15, both 0.1% bistrifluron (33.5 ± 0.7%) (mean ± SEM) and 0.5% bistrifluron (37.0 ± 0.6%) treatments and 0.5% noviflumuron (26.5 ± 3.2%) were significantly different from the control (4.5 ± 2.5%) (Dunn's test for multiple comparisons: $P < 0.001$, $P < 0.001$, $P = 0.01$, for 0.1% bistrifluron, 0.5% bistrifluron, and 0.5% noviflumuron, respectively). On day 30, 0.1% noviflumuron (42.0 ± 4.3%) was significantly different from both 0.1% bistrifluron (88.5 ± 0.3%) and 0.5 % bistrifluron (85.0 ± 0.4%) ($P = 0.01$, $P = 0.033$, for 0.1% bistrifluron and 0.5% bistrifluron, respectively). All other pairwise comparison relationships remained the same for the rest of the bioassay (Table 1). When corrected by Abbott's formula, the final cumulative mortalities on day 60 were 99.4%, 98.8%, 64.0%, 71.5%, 74.4%, and 88.4% for 0.1% bistrifluron, 0.5% bistrifluron, 0.1% chlorfluazuron, 0.5% chlorfluazuron, 0.1% noviflumuron, and 0.5% noviflumuron, respectively.

Choice Bioassay

Bistrifluron provided a significantly faster kill than chlorfluazuron or noviflumuron at 0.1% rates over a 60-d period, resulting in 96% mortality. Overall, a significant difference among the survival curves was found (log-rank test: $\chi^2 = 497.83$; df = 3; $P < 0.001$) (Fig. 3). All CSI treatments were significantly different from each other and the control (Supplementary Table S2).

Total cumulative mortality values were compared between treatments and control. Both bistrifluron (29.5 ± 0.7%) and noviflumuron (16.5 ± 2.9%) treatments were significantly different from the control (2.0 ± 1.3%) on day 15 and the differences stayed significant throughout the entire experimental period (60 d) (Table 2). The chlorfluazuron treatment was never significantly different from the control at any time point (Table 2). When corrected by Abbott's formula, the final cumulative mortalities were 95.7%, 22.6%, and 81.7% for bistrifluron, chlorfluazuron, and noviflumuron, respectively.

Transfer Bioassay

The mortality data from the 20:0 ratio (all donors) served as the standard for efficacy given that all exposed termites acquired bistrifluron by directly consuming the treated filter paper during the 3-d period. Overall, a significant difference among the survival

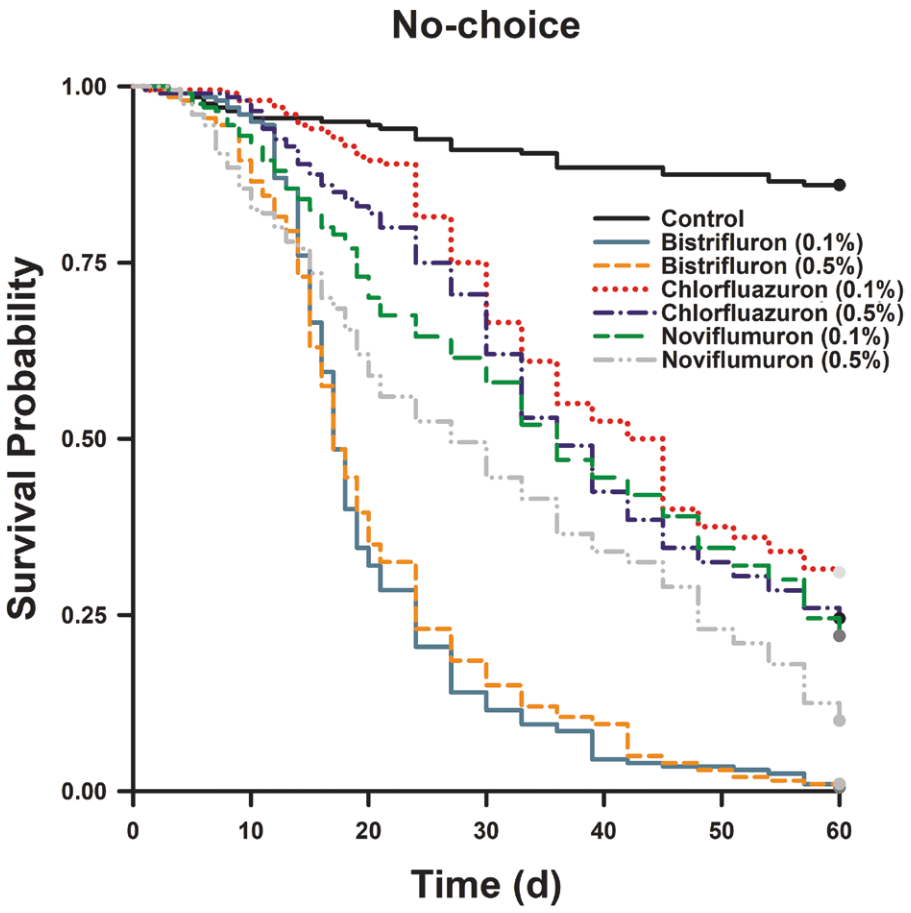

Fig. 2. Kaplan–Meier survival curves of *I. minor* when treated with 0.1% or 0.5% bistrifluron, chlorfluazuron, and noviflumuron in the no-choice bioassay.

Table 1. Percent (mean ± SE) mortality of *I. minor* after exposure to 0.1% or 0.5% of bistrifluron, chlorfluazuron, or noviflumuron in the no-choice bioasssay

No-choice[a]							
		Bistrifluron		Chlorfluazuron		Noviflumuron	
Time	Control	0.1%	0.5%	0.1%	0.5%	0.1%	0.5%
Day 1	0.0 ± 0.00a	0.0 ± 0.00a	0.5 ± 0.10a	0.0 ± 0.00a	0.5 ± 0.10a	0.0 ± 0.00a	0.0 ± 0.00a
Day 15	4.5 ± 2.54a	33.5 ± 0.68b	37.0 ± 0.62b	6.0 ± 0.33a	12.0 ± 0.43ac	17.5 ± 3.01ab	26.5 ± 3.16bc
Day 30	9.0 ± 2.46a	88.5 ± 0.37b	85.0 ± 0.42b	33.5 ± 2.36ac	38.0 ± 2.50ac	42.0 ± 4.36ac	55.5 ± 1.74bc
Day 45	12.5 ± 1.96a	96.5 ± 0.21b	96.0 ± 0.20b	60.0 ± 2.24ac	65.5 ± 2.41ac	61.0 ± 3.71ac	71.0 ± 3.40bc
Day 60	14.0 ± 2.58a	99.5 ± 0.10b	99.0 ± 0.13b	69.0 ± 1.94ac	75.5 ± 2.73ac	78.0 ± 3.51ac	90.0 ± 2.69bc

[a]Data within a row followed by the same lowercase letter are not significantly different (α = 0.05; Dunn's all-pairwise comparisons).

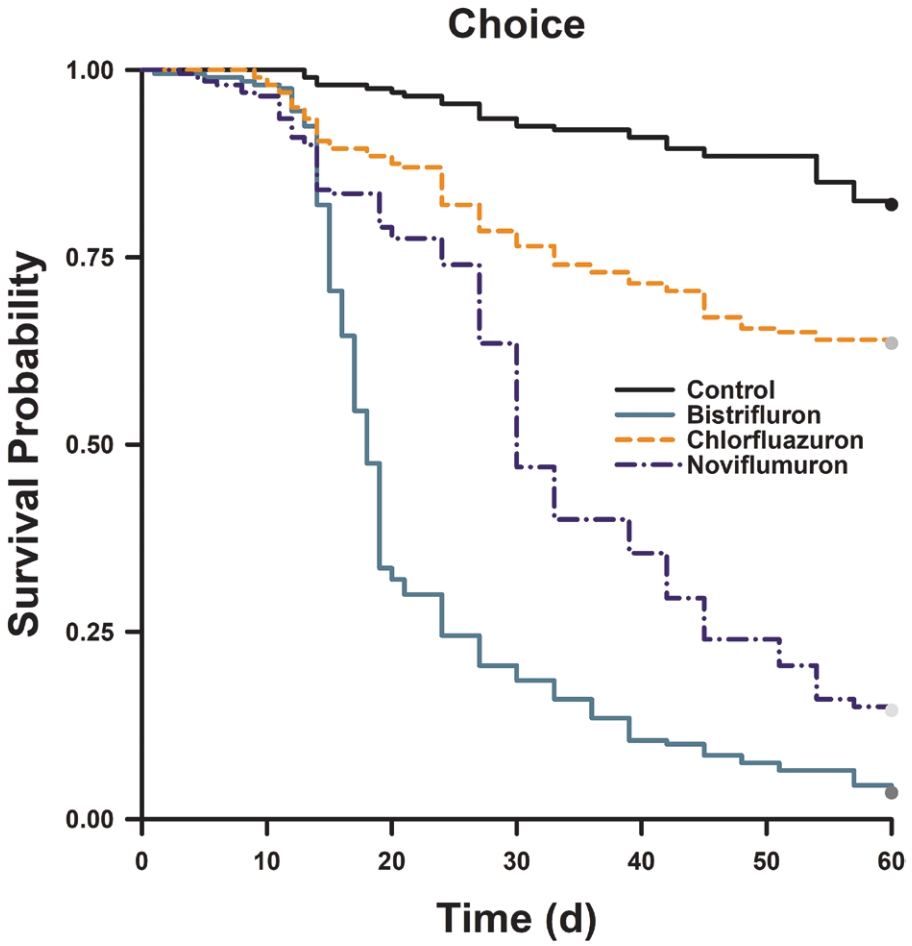

Fig. 3. Kaplan–Meier survival curves of *I. minor* when treated with 0.1% bistrifluron, chlorfluazuron, and noviflumuron in the choice bioassay.

curves was found (log-rank test: $\chi^2 = 290.28$; df = 3; $P < 0.001$) (Fig. 4). The survival curve from the 20:0 treatment was significantly different from the survival curves of the other 2 treatment ratios and the control (Supplementary Table S3). The survival curve from the 10:10 treatment was significantly different from the survival curve of the control (Holm–Sidak: $P < 0.0001$) (Supplementary Table S3). The survival curve from the 1:19 treatment was significantly different from the survival curve of the control (Holm–Sidak: $P < 0.0001$) (Supplementary Table S3). The survival curve from the 10:10 treatment was not significantly different from that of the 1:19 treatment (Holm–Sidak: $P = 0.287$) (Supplementary Table S3).

Total cumulative mortality values were compared between the different treatment D:R ratios and control on day 60. The 20:0 treatment ratio (96.0 ± 2.9%) was not significantly different from the 10:10 treatment ratio (89.0 ± 3.3%) but was significantly different from the 1:19 treatment ratio (83.0 ± 3.4%) and control (8.0 ± 2.6%) (Supplementary Table S4). When corrected by Abbott's formula, the day 60 cumulative mortalities were 95.7%, 88.0%, and 81.5% for the 20:0, 10:10, and 1:19 bistrifluron treatments, respectively.

For the 10:10 D:R ratio (both control and treatment), 90 ± 3.2% (mean ± SEM) of recipient termites displayed visible blue coloration

Table 2. Percent (mean ± SE) mortality of *I. minor* after exposure to 0.1% of bistrifluron, chlorfluazuron, or noviflumuron in the choice bioassay

Choice test[a]				
Time	Control	Bistrifluron	Chlorfluazuron	Noviflumuron
Day 1	0.0 ± 0.00a	0.5 ± 0.10a	0.0 ± 0.00a	0.0 ± 0.00a
Day 15	2.0 ± 1.33a	29.5 ± 0.69c	10.0 ± 0.49ab	16.5 ± 2.89bc
Day 30	7.5 ± 2.01a	81.5 ± 0.58c	23.5 ± 2.12ab	53.0 ± 3.00bc
Day 45	11.5 ± 1.67a	91.5 ± 0.33c	33.0 ± 3.96ab	76.0 ± 2.45bc
Day 60	18.0 ± 1.70a	96.5 ± 0.26c	36.5 ± 3.80ab	85.0 ± 1.50bc

[a]Data within a row followed by the same lowercase letter are not significantly different (α = 0.05; Dunn's all-pairwise comparisons).

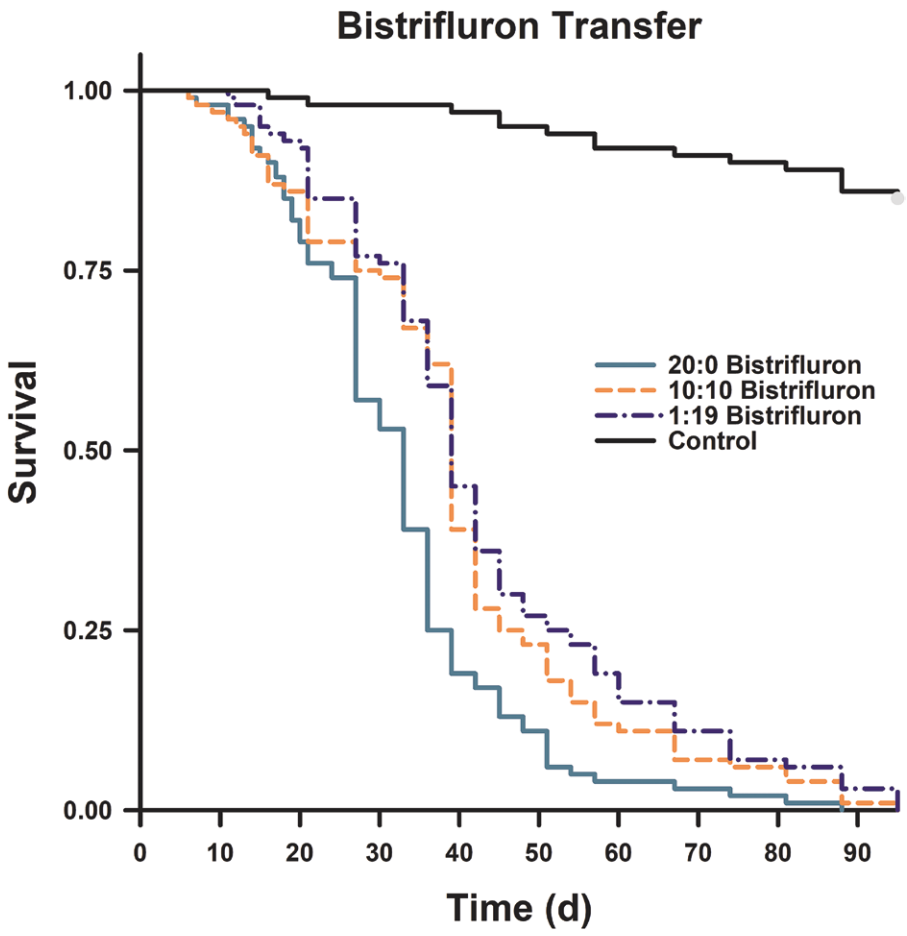

Fig. 4. Kaplan–Meier survival curves of *I. minor* for different D:R ratios in the transfer bioassay.

at 24 h (Supplementary Table S5). For the 1:19 D:R ratio, we observed 47.4 ± 6.2% and 31.6 ± 3.7% of recipients displaying blue coloration after 24 h in the control and treatment, respectively. After 48 h, for the 1:19 D:R ratio, the percentage of recipients showing blue coloration increased to 68.4 ± 5.6% and 59.0 ± 5.1% in the control and treatment, respectively (Supplementary Table S5).

Discussion

The current study investigated the lethal effects of bistrifluron, chlorfluazuron, and noviflumuron on the western drywood termite, *I. minor*. Results from the no-choice and choice bioassays suggested that bistrifluron was the most effective against *I. minor* compared to chlorfluazuron and noviflumuron. The reason for varying levels of efficacies between different CSIs is unclear but may be due to the differences in intrinsic activity and clearance time from the termites between respective CSIs (Karr et al. 2004). The slower time to kill in chlorfluazuron treatments might be due to ineffective concentrations of chlorfluazuron. Although CSIs can be effective across a relatively broad range of concentrations, concentrations that are too high can have dose-dependent effects (ie higher dose leads to a faster kill, negating the slow action characteristic of CSIs) or cause repellency preventing adequate toxicant uptake while too low concentrations can result in unsatisfactory mortality rates (Su and Scheffrahn 1993, 1996a, Karr et al. 2004, Kubota et al. 2006, Vahabzadeh et al. 2007). Perhaps a higher concentration of chlorfluazuron (eg ≥1.0%)

would have yielded results similar to those observed in bistrifluron or noviflumuron treatments. The concentrations (0.1% and 0.5%) chosen for the current research were based on the typical rates of CSIs found in commercial bait products in the United States. Future research could test these CSIs at broader concentration ranges to better understand the time to kill of CSIs on *I. minor*.

Treatment with 0.1% and 0.5% bistrifluron caused ≥99% mortality by day 60 in the no-choice bioassay. Day 45 mortality of *I. minor* colonies was ≥96% mortality for both concentrations of bistrifluron in the no-choice bioassay. Previous studies reported a similar level of efficacy (speed of kill) of bistrifluron for subterranean termite species. For example, 0.5% bistrifluron caused 100% mortality for *Coptotermes formosanus* Shiraki workers by 42 d in a no-choice bioassay (Kubota et al. 2006). Shamsuri and Ab Majid (2024) reported that 1.0% bistrifluron caused 94% mortality in *Coptotermes gestroi* (Wasmann) by 28 d in a no-choice bioassay. We observed a comparable level of day 30 mortality (89%) in *I. minor* using a much lower concentration (0.1%) of bistrifluron. Our findings and other previous reports indicate a similar timeline for *I. minor* and subterranean termites regarding the effect of bistrifluron (ie ≥95% to 99% mortality by day 45 to 60 after treatment). However, a CSI can show considerable variation in its efficacy when tested on different termite species (Su and Scheffrahn 1993, 1996a, Karr et al. 2004). For example, Shamsuri and Ab Majid (2024) reported 89% mortality of *C. gestroi* workers with a 0.1% chlorfluazuron bait by day 28, while the current study observed only 34% mortality in *I. minor* for 0.1% chlorfluazuron by day 30.

Trophallaxis, the stomodeal (oral) or proctodeal (anal) exchange of digestive fluids between nestmates within a colony (McMahan 1969, Wilson 1971), is the main mechanism by which bait toxicants are spread throughout a termite colony (Sheets et al. 2000, Karr et al. 2004, Haagsma and Rust 2005, Lewis and Forschler 2017). There is a limited amount of information available on trophallaxis in drywood termites. Cabrera and Rust (1999) examined trophallaxis in *I. minor* using non-radiolabeled rubidium (Rb) as a tracer. A group of 10 termites was allowed to feed on Rb-treated filter paper for 3 d (donors) before being placed with 10 untreated termites (recipients). After 72 h, up to 16.6% of the total Rb from the donor termites was found in the recipient termites. The degree of transfer of ingested Rb varied depending on the caste composition of donors and recipients, with nymphs (wing buds present) and pseudergates (wing buds absent) functioning as the primary donors and recipients, respectively. In the current study, the filter paper provided to the donor termites was dyed blue to help visually track the movement of ingested material from donors to recipients. Although we did not quantify the amount of transferred compounds (dye and bistrifluron) between the donors and recipients, the presence of blue coloration in the recipient termites provided a piece of information supporting the transfer of ingested materials within *I. minor* colonies. The blue coloration acquired by donor termites was internal (heavily concentrated in the gut, Supplementary Fig. S4), eliminating contact or allogrooming as the main mode of transfer. Since a portion of fecal pellets produced by the donor termites did display light blue coloration (Supplementary Fig. S5), fecal pellets' possible contribution to the dye (and bistrifluron) transfer cannot be completely ruled out. However, considering what has been reported for the transfer of CSIs in subterranean termites, it is reasonable to consider trophallaxis as one of the primary modes of CSI transfer in drywood termites. Details of trophallaxis (eg rate, amount, distribution pattern, etc.) and other possible modes of transfer of CSIs in drywood termite colonies warrants further investigation.

In the transfer study with bistrifluron, the survival curves for 10:10 and 1:19 D:R ratio treatments were not significantly different. Furthermore, the day 60 cumulative mortality rates were similar between 10:10 and 1:19 D:R ratio treatments, indicating the retention and spread of lethal doses of bistrifluron. Although the current study used small groups of 20 termites, this observation supports the potential of bistrifluron as an active bait ingredient for drywood termite control. Similar results have been reported for subterranean termites. In a laboratory setting, Lewis and Forschler (2017) showed that 5% of a group of 20 *Reticulitermes flavipes* (Kollar) workers exposed to noviflumuron bait was sufficient to kill 90% by day 47. Gordon et al. (2022) made a similar observation in larger colonies of *C. gestroi* (Wasmann), where a small proportion (~5%) of workers that were exposed to noviflumuron baits was sufficient to cause ~62,500 worker colonies to collapse by day 90. However, it remains to be seen if similar transfer levels and final mortality could be obtained when bistrifluron is used for larger groups of *I. minor*.

In the current study, the CSIs dissolved in a solvent were directly applied to the wood which was used by the termites for feeding and excavation activities. However, to develop practical drywood management tools based on the current findings, the development of optimal formulations will be necessary. For example, the CSIs dissolved or suspended in a solvent carrier could be directly injected into the wood to turn portions of the termite galleries into a toxic substrate. Alternatively, some bait media containing CSIs could be injected through drill holes into the infested wood, serving as bait that drywood termites can consume. Although CSIs were not used, Indrayani et al. (2008) tested a gel bait containing hydramethylnon against *I. minor* within wooden experimental arenas. After 2 wk, the average cumulative mortality response to the gel bait ranged from 60.0% to 86.7%. We believe that an "in-wood baiting" system with CSIs as the AI could be possible, and it might provide a novel tool for drywood termite control. Unlike some subterranean termites where the foragers tunnel in the soil to find a piece of wood or a bait station, the drywood termites reside inside their food (wood). For this reason, to make any "in-wood baiting" idea for drywood termites work, it is crucial to make the termite colony within the wood quickly discover the injected bait or the treated portion of their gallery. Future development could include testing the potential inclusion of an adjuvant in the form of an attractant or feeding stimulant to increase the efficacy of a CSI-based treatment. Poulos et al. (2024) demonstrated that the inclusion of β-pinene increased the effectiveness of residual fipronil deposits against *I. minor*. A ready-to-use product that contains effective CSIs and other attractants/feeding stimulants would be a valuable tool for drywood termite control.

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Author contributions

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[supporting], Writing—review & editing [equal]), and Dong-Hwan Choe (Conceptualization [equal], Formal analysis [supporting], Funding acquisition [lead], Methodology [equal], Project administration [lead], Resources [lead], Supervision [lead], Writing—original draft [supporting], Writing—review & editing [equal])

Supplementary material

Supplementary material is available at *Journal of Economic Entomology* online.

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