Evaluation of a Two-Step Management Strategy Using Fipronil-Treated Wood and Bistrifluron Bait for Rapid Feeding Suppression and Colony Elimination of *Reticulitermes speratus* in a Simulated Wooden Cultural Heritage System

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A two-step management strategy was evaluated for rapid feeding suppression and colony elimination of termites (*Reticulitermes speratus*) infesting wooden cultural heritage structures (WCHS). A large-scale (15-m foraging distance) arena system with a colony size of 10,000 termites — consisting of a main nest, a shelter, and fipronil-treated wood units connected by foraging tunnels — was utilized to compare an untreated control group, a fipronil-treated group, a bistrifluron-treated group, and a combined treatment (F+B) group. In the fipronil-treated group, feeding on the treated wood rapidly decreased, demonstrating rapid reduction in wood consumption on treated wood; however, colony survival persisted and feeding activity was redistributed toward the untreated shelter zone. In contrast, the bistrifluron-treated group ultimately achieved complete colony collapse, although feeding on both the treated wood and shelter wood continued during the elimination process. The combined treatment rapidly suppressed feeding on the treated wood and completely eliminated the colony within 10 weeks through subsequent bait consumption. Furthermore, the combined treatment significantly reduced the feeding redistribution toward untreated wood sources that were observed in the fipronil-only group. These findings suggest that the combined application of fipronil-treated wood and CSI bait can provide both immediate protection for WCHS members and long-term colony elimination, offering a promising approach that warrants further field validation as an efficient termite management strategy for WCHS.

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INTRODUCTION

Reticulitermes spp., a prominent group of subterranean termites, feed primarily on wood and are major wood-destroying pests that continuously damage building structures and wooden materials (Vargo and Husseneder 2009). They break down cellulose, causing critical damage to structural components such as columns, beams, and flooring; if accumulated over a long period, this can compromise overall structural stability (Rust and Su 2012). In particular, termite infestations tend to progress covertly within the wood,

making immediate detection from the outside difficult. Consequently, by the time the damage becomes visible, significant internal decay has often already occurred (Hassan and Nanda 2024). Non-destructive detection methods such as visual inspection and acoustic monitoring have been explored to identify termite activity in concealed structures prior to visible damage.

In Korea, termite damage to wooden buildings is continuously reported, primarily identified in wooden cultural heritage structures (WCHS) such as temples, palaces, and pavilions (Im *et al.* 2021). Since most of these historical structures have been preserved over centuries, termite infestations risk not only structural integrity but also the irreversible deterioration of their cultural value. Notably, because internal damage to the WCHS members is rarely visible from the exterior, detection is frequently delayed, emphasizing the critical importance of continuous monitoring and preventive management (Kim and Chung 2022).

To mitigate these threats, various control technologies—such as soil treatment (liquid termiticide), wood surface treatment, fumigation, and baiting systems—have been applied on-site (Rust and Su 2012). However, unlike modern buildings, WCHS are large in scale, and active control measures involving dismantling and repair are heavily restricted due to their historical value and the necessity of preserving their original form. Furthermore, since the infested areas are often located deep within large structural timbers, direct chemical injection faces technical limitations. For these reasons, soil treatment—which applies termiticides to the surrounding soil to block termite access—and baiting systems aimed at complete colony elimination, remain the primary control methods utilized in actual field settings (Su and Scheffrahn 1998; Osbrink et al. 2005; Rust and Su 2012; Kim and Chung 2017).

In Korea, fipronil, a non-repellent termiticide, has been widely applied since the 2010s for colony suppression and elimination. Fipronil targets γ-aminobutyric acid (GABA) receptors to paralyze the insect nervous system (Moffat 1993; Hainzl and Casida 1996). Owing to its non-repellent nature, which allows termites to contact or ingest the agent without avoidance, it has been extensively utilized for colony elimination (Kim and Chung 2017). However, field applications have repeatedly shown that the expected level of colony elimination is not consistently achieved. This inconsistency is attributed to fipronil's relatively rapid lethal effect on individual termites; exposed individuals often die before effectively transmitting the toxicant to other colony members through trophallaxis, thereby preventing the spread of toxicity throughout the entire colony (Im and Han 2023).

Existing efficacy evaluations have primarily focused on individual-level laboratory exposure tests, failing to adequately reflect the colony-level processes of pesticide transmission and colony collapse observed in the field. In actual subterranean termite control, key evaluation metrics should encompass pesticide transmissibility, intra-colony diffusion, and ultimate colony elimination, rather than simply individual mortality. Nevertheless, individual-level evaluation results have frequently been applied to Korean WCHS sites without verifying colony-level efficacy (Kim *et al.* 2010; Kim and Chung 2017; Im and Chung 2019; Yoon *et al.* 2021). Consequently, failures to achieve the expected level of colony elimination in actual field settings have been repeatedly reported. Meanwhile, chitin synthesis inhibitor (CSI)-based baits for subterranean termite colony elimination have been actively researched since the 1990s (Su 1993; Su and Scheffrahn 1996) and have since been established as one of the most effective methods for this purpose. CSI baits inhibit molting, causing individuals within the colony to die off gradually; furthermore, their slow-acting nature facilitates the widespread transmission of

the active ingredient among colony members (Chouvenc 2018; Su 2019). However, bait-based colony elimination has the disadvantage of requiring substantial time for termites to discover and enter the bait stations, generally taking several weeks to several months for complete colony collapse.

In WCHS, termite infestations are often first detected by the naked eye during the swarming season in April and May. However, swarming indicates that significant internal damage has already occurred, characterized by the formation of tunnels and cavities within the WCHS members that facilitate free termite movement. Therefore, once an infestation is confirmed, immediate action is required not only to eliminate the swarming individuals but also to rapidly suppress the active colony and prevent further spread.

Effective termite management at WCHS requires a comprehensive approach that combines fast-acting chemicals for rapid contact control with slow-acting agents for long-term colony elimination. While fipronil provides immediate localized exclusion and CSI baits offer complete colony elimination, the mechanistic rationale for combining these two contrasting agents remains insufficiently explored. A clear knowledge gap exists regarding how a fast-acting non-repellent termiticide interacts with a slow-acting CSI bait at the colony level without suppressing overall bait transmission. In this study, a dual-treatment strategy utilizing the fast-acting agent fipronil alongside a slow-acting agent was evaluated within a controlled simulated arena system, serving to evaluate this conceptual approach under laboratory conditions before practical field application. Based on these findings, an efficient and practical termite management plan applicable to WCHS is proposed.

EXPERIMENTAL

Termite Collection

Infested wood samples were collected from hilly areas in Cheongju, North Chungcheong Province, and transported to the laboratory. Three independent field colonies were collected from separate locations in Cheongju. Each experimental replicate consisted of termites obtained from a different field colony. Specimens were maintained in ventilated plastic enclosures at 20 ± 4 °C and >90% relative humidity. Termite species were identified as *Reticulitermes speratus* (Im and Han 2024). To extract termites without causing damage to the wood, individuals were transferred to wet corrugated cardboard rolls using the non-destructive method (Im and Han 2021).

Experimental Arena System and Treatment Design

An arena system was constructed to evaluate the effects of fipronil-treated wood, bistrifluron bait, and their combined application on the foraging behavior and colony elimination of *Reticulitermes speratus* (Su 2005; Chouvenc 2018; Im and Han 2021; 2023). All soil substrates were prepared with the same composition following the method of Lee and Forschler (2016). The experimental setup consisted of a central main nest connected to two planar arenas through transparent acrylic tubing, with a total foraging distance of 15 m (Im and Han 2021; 2022). Each planar arena measured 150 × 150 mm in area and 2 mm in thickness (Im and Han 2023). A total of 10,000 *R. speratus* individuals were introduced into the main nest in divided portions over a one-week acclimation period. Each treatment was conducted in triplicate.

A shelter (S) was installed in the left arena. Soil and matrix were layered inside to support termite activity and feeding, and a cover was fitted to retain moisture during the

experimental period (Im and Han 2021). A small wood specimen (10 × 10 × 20 mm) was placed inside the shelter as a feeding source. All wood specimens were air-conditioned under the same laboratory conditions prior to the experiment.

The right planar arena contained a bait chamber (C) and a wood installation zone (W). In zone C, paper baits treated with bistrifluron mixed with 0.2% Nile blue A were installed to confirm bait consumption and colony elimination (Im and Han 2023). In zone W, a pine wood specimen (40 × 40 × 100 mm) simulating WCHS members was placed 5 cm above the soil surface. A hole (2 mm diameter, 30 mm depth) was drilled into the center of each specimen at a height of 20 mm from the base, and 2 mL of a 10 ppm fipronil solution mixed with 0.2% Neutral red was injected through five repeated injection–drying cycles to establish localized internal treatment. This localized injection method was designed to simulate the remedial internal treatment commonly applied to wooden cultural heritage structures in Korea. In the control group, no bistrifluron bait was installed in zone C, and distilled water was injected into the wood specimens in zone W using the same procedure. Both dyes were previously reported to have no effect on the feeding behavior of *R. speratus* while allowing visual identification of stained individuals (Im and Han 2021). The paper bait contained 5,000 ppm bistrifluron, and the preparation and treatment procedures followed those described by Im and Han (2023).

The experiment consisted of four treatment groups: untreated control, fipronil-treated, bistrifluron-treated, and combined treatment with fipronil-treated wood and bistrifluron bait (F+B). All experiments were conducted for 10 weeks. During the experiment, wood consumption in both the shelter and zone W was measured. Colony survival, changes in foraging location, and colony elimination were recorded photographically throughout the experiment. At the end of testing, the remaining workers, soldiers, and neotenics in each treatment were counted to evaluate colony survival.

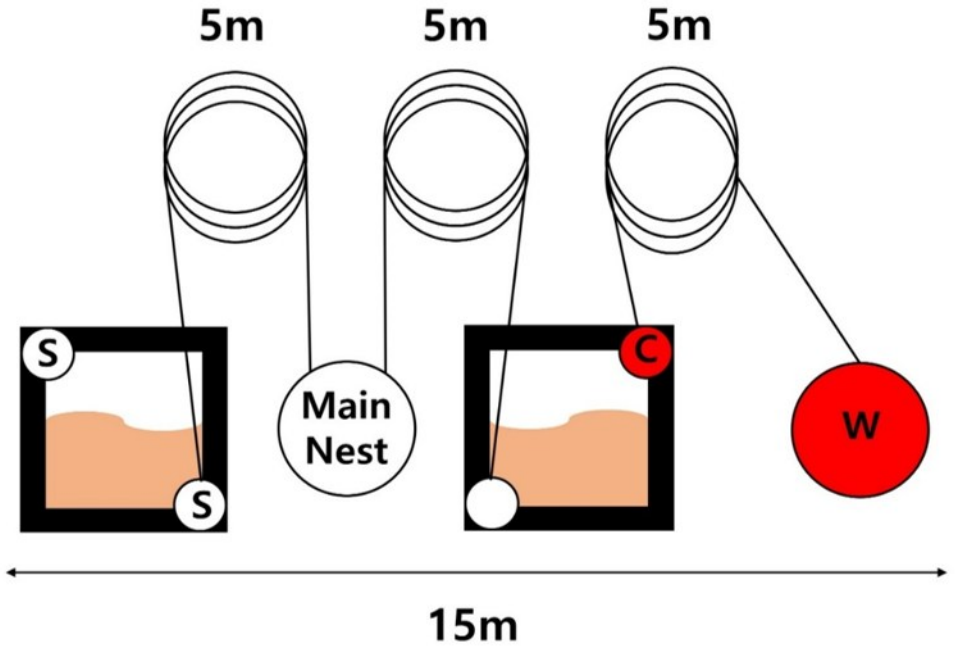


Fig. 1. Experimental arena system constructed for colony elimination and feeding redistribution assays in *R. speratus*. The system consisted of a central main nest connected to shelter and wood-feeding zones through 15 m-long foraging tunnels. Bistrifluron bait was placed in the bait chamber (C). The W unit simulated treated members of WCHS.

Wood Consumption Measurement and Colony Assessment

Wood consumption was measured during the 10-week experimental period and at the end of the experiment. Because the wood specimens placed inside the shelters were relatively small and rapidly consumed by termites, they were checked every 3 days. When a specimen was completely consumed, a new specimen of the same size was added as a replacement food source. Replacement wood specimens were oven-dried at 60 °C for 24 h to obtain initial dry weight and then immersed in distilled water for 24 h before use to restore moisture levels. Total wood consumption in each shelter was calculated by summing the mass loss of all wood specimens used during the experiment. At the end of the 10-week period, all remaining wood specimens from both the shelters and zone W were collected and oven-dried at 60 °C until constant weight was reached, and wood consumption was calculated from the difference between the initial and final dry weights.

Surviving termites were collected at the end of the experiment and separated into workers, soldiers, and neotenics. For each caste, 10 individuals were randomly selected and weighed to obtain the average individual mass. The total biomass of each caste was then measured, and the number of surviving individuals was estimated by dividing the total biomass by the average individual mass of each caste. Colony condition, egg and nymph occurrence, corpse accumulation, and feeding location were recorded photographically throughout the experiment.

Statistical Analysis

All statistical analyses were performed using IBM SPSS Statistics 26.0 (IBM Corp., Armonk, NY, USA). One-way analysis of variance (ANOVA) was used to compare wood consumption and surviving termite populations among treatment groups. Prior to ANOVA, the assumptions of normality and homogeneity of variance were assessed. When significant differences were detected, Tukey's honestly significant difference (HSD) test was used for multiple comparisons among treatments at a significance level of $P < 0.05$.

RESULTS AND DISCUSSION

Colony Survival under Different Treatment Conditions

Colony survival differed markedly among treatments after 10 weeks (Figs. 2 and 3). Large numbers of workers and soldiers remained alive in both the untreated control group and the fipronil-treated group, and neotenic reproductives were continuously observed throughout the experimental period. In contrast, the bistrifluron-treated group and combined treatment (F+B) group showed a sharp decline in all castes, and no surviving neotenics were detected at the end of the experiment. Worker populations in the untreated control group and the fipronil-treated group remained above 11,000 individuals, whereas nearly complete colony collapse occurred in both the bistrifluron-treated group and the combined treatment (F+B) group (Fig. 2). Soldier numbers also remained high in the untreated control group and the fipronil-treated group but were significantly reduced in treatments containing bistrifluron. Neotenic reproductives persisted in the fipronil-treated group, although at lower numbers than in the untreated control group, while none survived in either the bistrifluron-treated group or the combined treatment (F+B) group (Fig. 3).

The results indicate that fipronil treatment rapidly reduced feeding activity on the treated wood but did not eliminate the colony within the experimental period. Colonies exposed to fipronil maintained substantial worker populations and continued reproductive

activity through the presence of neotenic reproductives. Previous studies have shown that fipronil can suppress subterranean termite activity through delayed toxicity and horizontal transfer among nestmates (Vargo and Parman 2012; Itakura *et al.* 2021). However, under field and semi-field conditions, surviving termites may avoid treated areas or redistribute foraging activity toward untreated sites, resulting in incomplete colony elimination (Su 2005; Chouvenc 2018; Chouvenc 2024; Im and Han 2023). A similar pattern was observed in the present study, where termites in the fipronil-treated group shifted feeding activity toward the shelter zone and continued colony maintenance. The present study indicates that localized wood treatment alone was insufficient for complete colony elimination.

Colonies exposed to bistrifluron, however, showed marked reductions in all castes and eventually collapsed. Feeding activity continued for several weeks after bait exposure, but colony elimination was confirmed by the end of the experiment. This pattern is consistent with previous studies reporting that CSI (chitin synthesis inhibitor)-based baits induce delayed colony mortality because toxic effects appear during molting rather than immediately after ingestion (Su and Scheffrahn 1993; Chouvenc 2018; Im and Han 2023; Su *et al.* 2025). CSI termiticides generally produce little feeding deterrence or repellency, allowing active ingredients to spread throughout the colony through continued feeding and trophallaxis (Kakkar *et al*. 2018; Kakkar and Su 2018; Chouvenc 2024). In the present study, sustained feeding during the early exposure period likely facilitated the transfer of bistrifluron among colony members, which led to mortality of workers, soldiers, and neotenic reproductives, followed by complete colony collapse.

The combined treatment (F+B) produced the strongest suppression effect among all treatments. Feeding on wood in zone W declined rapidly following fipronil exposure, and subsequent consumption of bistrifluron bait resulted in complete colony elimination within 10 weeks. Unlike the fipronil-treated group, surviving termites in the F+B group did not maintain long-term feeding activity in alternative locations, and no reproductives remained at the end of the experiment. The combined treatment compensated for limitations associated with each individual treatment. Fipronil treatment provided rapid protection of wooden materials but failed to eliminate the colony, whereas bistrifluron bait ultimately achieved colony elimination but allowed continued wood consumption during the elimination process. While the slow-acting nature of chitin synthesis inhibitors (CSIs) is essential for thorough distribution of the active ingredient throughout the colony, the approximately 10-day fasting period prior to ecdysis (which comprises at least 22% of a worker's molting cycle) can create a temporary window where termites escape bait ingestion, thereby prolonging the overall time required for complete colony elimination and potentially extending the period of timber damage (Kakkar and Su 2018). Similar tendencies have been reported previously, where non-repellent liquid termiticides effectively reduced localized feeding activity but often failed to eliminate entire colonies, while CSI baits achieved colony elimination through sustained feeding over extended periods (Chouvenc 2018; Kakkar *et al.* 2018; Chouvenc 2024). In WCHS, where even limited feeding damage can result in irreversible loss of structural and historical value, additional feeding during the colony elimination period represents a critical management concern. The results indicate that rapid suppression of feeding damage by fipronil, followed by colony elimination through bistrifluron baiting, successfully counteracts this defense window and may serve as a practical management strategy for the protection of WCHS.

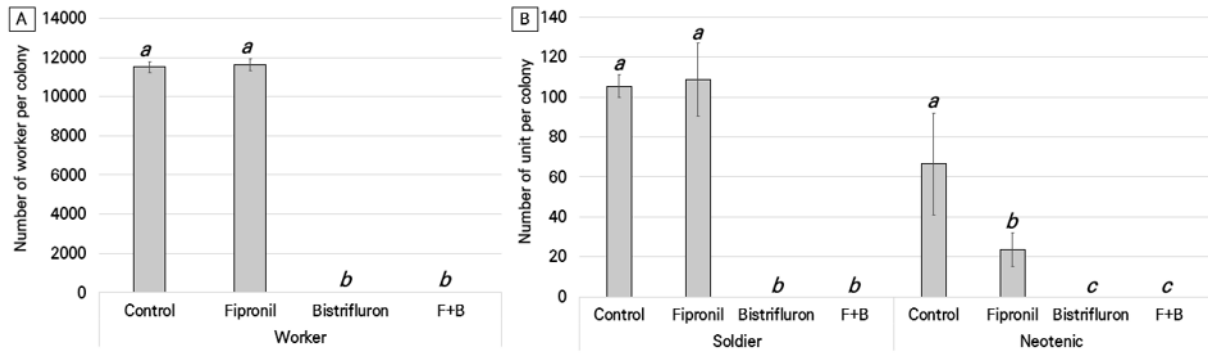


Fig. 2. Survival of different castes of *R. speratus* after 10 weeks under different treatment conditions. (A) Worker populations remaining in each treatment group. (B) Numbers of surviving soldiers and neotenic reproductives. Different letters above bars indicate significant differences among treatments according to Tukey's HSD test ($P < 0.05$).

Analysis of Wood Feeding Patterns and Total Consumption under Different Treatment Conditions

Wood consumption over the 10-week experimental period differed among treatment conditions and between feeding locations (shelter and Zone W) (Figs. 4 and 5). In the untreated control, both shelter and Zone W showed consistently high levels of wood consumption throughout the observation period. *R. speratus* colonies maintained active foraging across the arena without restriction. In the fipronil treatment, consumption in Zone W decreased sharply after exposure to treated wood. In contrast, consumption in the shelter increased relative to the control. Feeding activity was redistributed from the treated area to untreated locations. Similar shifts in feeding location following exposure to non-repellent insecticides have been reported in previous studies (Su 2005; Chouvenc 2018; Im and Han 2023). These results reflect localized suppression of feeding at the treated site, while activity persisted in untreated areas.

In the bistrifluron treatment, shelter consumption remained low throughout the experiment, while feeding continued in Zone W where bait was installed. Feeding activity did not cease immediately after exposure. Chitin synthesis inhibitor (CSI)-based baits act during molting rather than at the point of ingestion, resulting in delayed mortality (Su and Scheffrahn 1993; Evans and Iqbal 2015). During this period, feeding behavior continued, and transfer of active ingredients among nestmates occurred through trophallaxis (Kakkar *et al.* 2018; Chouvenc 2024). Colony decline proceeded gradually under bait exposure conditions (Grace and Su 2001).

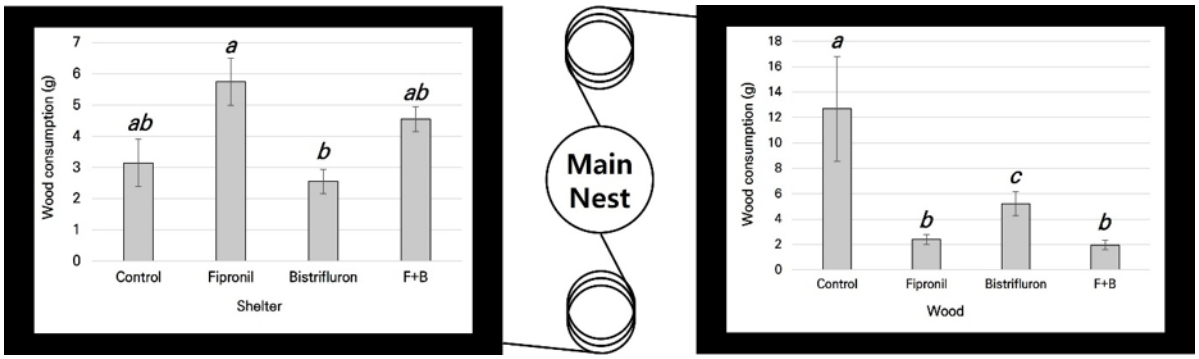


Fig. 3. Wood consumption by *R. speratus* in the shelter and wood installation zone (W) after 10 weeks under different treatment conditions

In the combined fipronil and bistrifluron treatment (F+B), wood consumption remained low in both shelter and Zone W. Feeding on treated wood ceased shortly after exposure to fipronil, followed by colony collapse within 6 to 7 weeks after bait ingestion, consistent with previous observations (Im and Han 2023). The sequence of responses reflected an initial reduction in feeding activity followed by delayed colony-level effects.

The results show different temporal responses between the two treatments. Fipronil affected feeding activity at the treated site shortly after exposure. Bistrifluron acted at the colony level after a delay associated with molting. In WCHS, where limited feeding activity may lead to structural damage, the combined application of both approaches provides a more stable control outcome than either treatment alone (Forschler and Jenkins 2000; Grace and Su 2001; Su and Scheffrahn 1998).

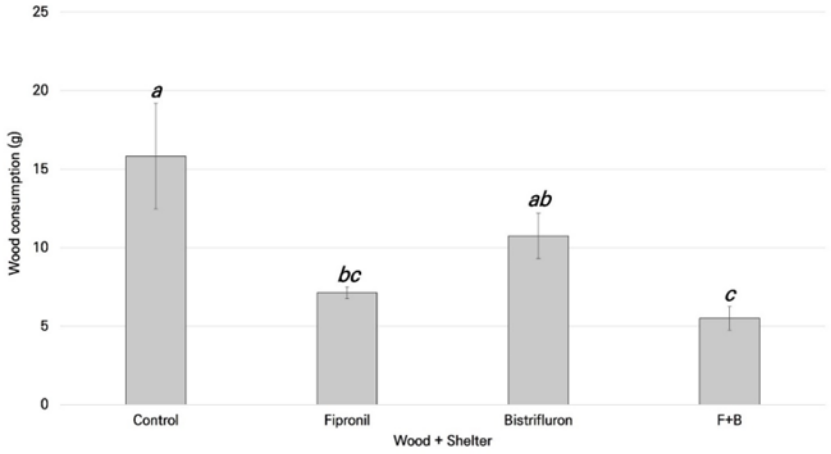


Fig. 4. Total wood consumption (structural wood + shelter wood) by *R. speratus* colonies during the 10-week experimental period. The combined treatment (F+B) showed the lowest total wood consumption. Values are means ± SD. Different letters indicate significant differences according to Tukey's HSD test ($P < 0.05$).

Behavioral Observations and Colony Conditions

Colony condition and mortality patterns of *R. speratus* were visually examined across treatment conditions (Fig. 5). In both the control and fipronil-treated groups, termites remained active within untreated shelter areas throughout the 10-week period. Continuous oviposition occurred, with workers tending eggs and soldiers defending the brood area (Fig. 5A, 5B). These observations indicate stable colony conditions with sustained reproductive activity and brood care, consistent with previously reported egg recognition and protection behaviors in *R. speratus* (Matsuura *et al.* 2007). Notably, colonies exposed to localized fipronil treatment sustained normal development after relocating to untreated refuge areas.

Species of the genus *Reticulitermes* rapidly differentiate neotenic reproductives following primary reproductive loss or physical colony fragmentation (Vargo and Husseneder 2009; Matsuura *et al.* 2010). Here, neotenic reproductives continuously appeared in the fipronil-treated group, resulting in numerous secondary reproductives in the untreated shelter zone at week 10 (Fig. 5C). This indicates that a portion of the colony survived fipronil exposure by relocating to untreated zones to maintain reproduction. Thus, while localized treatment with fast-acting contact toxicants temporarily suppresses feeding at treated sites, it rarely achieves complete colony elimination (Vargo and Husseneder 2009; Im and Han 2023).



Fig. 5. Colony conditions and mortality patterns of *R. speratus* across treatment groups. (A) Worker activity and egg/larva care within the main nest and (B) shelter area following fipronil treatment; (C) differentiation of numerous secondary reproductives in the untreated main nest and shelter zone 10 weeks post-fipronil exposure; (D) accumulation of carcasses and subsequent white fungal growth inside a fipronil-treated tunnel; (E) neotenics and workers with blue-stained abdomens from Nile blue A consumption in the bistrifluron group; (F) progressive accumulation of dead termites within the main nest during bistrifluron-induced colony collapse.

In contrast, rapid mortality occurred inside fipronil-treated tunnels shortly after exposure, followed by white fungal growth where termite activity had completely ceased (Fig. 5D). Subterranean termites typically maintain nest hygiene through grooming and corpse removal behaviors that limit pathogen spread (Chouvenc *et al.* 2009; Chouvenc and Su 2009, 2012). In the present study, however, the rapid toxicity of fipronil caused simultaneous mortality within the narrow tunnels and likely exceeded the colony's capacity for corpse removal. As carcasses accumulated, fungal growth increased and tunnel activity ceased. Surviving termites subsequently concentrated feeding activity in the untreated shelter area, where wood consumption increased during the later stages of the experiment (Fig. 4).

The bistrifluron-treated group showed a mortality pattern typical of chitin synthesis inhibitor (CSI) termiticides. Workers that consumed the bait were identified by blue-stained abdomens caused by Nile blue A (Fig. 5E) (Im and Han 2021). Because CSIs do not produce rapid mortality, exposed termites continued feeding and trophallactic behavior for an extended period after bait consumption (Su and Scheffrahn 1993, 1996; Gordon *et al.* 2022). As termites entered subsequent molting stages, mortality gradually increased and dead individuals accumulated inside the main nest (Fig. 5F). These mechanisms have been previously described in detail by Im and Han (2023), where colony collapse was associated with disruption of molting processes and colony-level physiological imbalance induced by CSI exposure. This pattern differed clearly from the localized mortality observed near the fipronil-treated tunnels. Previous studies have shown that CSI baits allow toxicants to spread through trophallaxis and social interactions before lethal effects appear during molting (Chouvenc 2018; Kakkar *et al.* 2018). The accumulation of dead individuals inside the main nest suggests that bistrifluron spread widely throughout the colony before colony collapse occurred.

In the combination group, fipronil exposure rapidly decreased feeding damage on treated wood, followed by complete colony elimination driven by ongoing bistrifluron bait consumption. These findings suggest that combining fipronil with bistrifluron baiting is effective for termite management in WCHS demanding both immediate damage mitigation and long-term colony elimination.

CONCLUSIONS

1. This study evaluated the combined application of fipronil-treated wood and bistrifluron baiting for suppressing feeding activity and eliminating *Reticulitermes speratus* termite colonies associated with WCHS. Localized fipronil treatment rapidly reduced feeding on treated wood and limited initial damage. However, surviving termites maintained colony activity in untreated areas, indicating that localized treatment alone was insufficient for complete colony elimination. In contrast, bistrifluron baiting caused gradual colony decline and eventual colony collapse despite continued feeding during the early treatment period.
2. The combined treatment reduced feeding damage shortly after exposure and resulted in complete colony elimination within 10 weeks after bait consumption. Continued feeding in untreated areas and persistent neotenic reproductive differentiation, which were observed in the fipronil-treated group, were not detected in the combined treatment group. These findings indicate that the combined treatment was effective for both rapid feeding suppression and colony-level elimination.
3. Even limited termite feeding may cause irreversible structural deterioration in wood cultural heritage structures (WCHS). Under such conditions, reducing feeding damage during the colony elimination process is important. The present results show that combining localized fipronil treatment with subsequent bistrifluron baiting reduced early feeding damage while allowing colony elimination to proceed concurrently. This treatment approach may be applicable to termite management programs for the preservation of WCHS.

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