Toxicity and Transmission of Thiamethoxam in the Asian Subterranean Termite *Coptotermes gestroi* (Isoptera: Rhinotermitidae)

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**ABSTRACT.** The toxicity and horizontal transmission of thiamethoxam was evaluated in the workers of the Asian subterranean termite *Coptotermes gestroi* Wasmann (Isoptera: Rhinotermitidae). Brief exposure to sand treated with thiamethoxam at concentration ranging from 0.25 to 50 \(\mu\)g/ml resulted in a dose-dependent mortality in *C. gestroi*. Sand treated with 50 \(\mu\)g/ml thiamethoxam resulted in very high mortality within 30–60 min of exposure. Termites exposed to sand treated with 0.25–25 \(\mu\)g/ml exhibited delayed toxicity and non-repellency in *C. gestroi*. A horizontal transmission study using 25 \(\mu\)g/ml of thiamethoxam at donor–recipient ratio of at least 2:5 (treated:untreated) indicated that thiamethoxam can be transferred between exposed and unexposed workers, resulting in significant termite mortality in unexposed termites within 1–3 d post exposure.

**Key Words:** termite, thiamethoxam, *Coptotermes gestroi*, nonrepellent, horizontal transmission

Termites are structural pests that cause significant structural damage to wood and timber structures in tropical and subtropical regions of the world (Rust and Su 2012). The traditional control method against termites is by injection of liquid termicide to the soil to establish a toxic or repellent chemical barrier and prevent termites from entering homes and wood structures (Su and Scheffrahn 1990, Grace et al. 1993, Gahlhoff and Koehler 2001). Treatment results in varying degrees of success depending on the skills of the applicator, type and dosage of chemical used, and degree of infestation (Potter 2011). To date, the use of nonrepellent termiticides (e.g., fipronil) is gaining popularity for termite management and control (Rust and Su 2012). Nonrepellent termiticides are slow acting insecticides that are not detected by termites when they forage through treated soil. Termites ingest and contaminate their body with the chemical which they inadvertently transfer to unexposed member of the colony through social grooming and trophallaxis (Thorne and Breisch 2001, Ibrahim et al. 2003, Saran and Rust 2007, Parman and Vargo 2010). Once a lethal dose had been transferred throughout the colony, nonrepellent termiticides can cause colony collapse (Potter and Hillery 2002). However, the transfer effects can be highly variable, depending on the physical and chemical properties of the soil and the termiticide used (Neoh et al. 2012).

Thiamethoxam is a new neonicotinoid insecticide with stomach and contact activity (Yamamoto 1996, Mason et al. 2000, Meienfisch et al. 2001). It interferes with the nicotinic acetylcholine receptor, thereby disrupting the activity of the central nervous system and causing death to the insect. Recent studies showed that thiamethoxam was toxic and provided an effective barrier against the Formosan subterranean termite (*Coptotermes formosanus* Shiraki) and the eastern subterranean termite (*Reticulitermes flavipes* Kollar) (Remmen and Su 2005a,b). It has also been reported to be nonrepellent and has anti-feeding action against higher species of African and Philippine termites (Delgarde and Lefèvre 2002, Acda 2007). However, limited studies have been reported on the potential for transmission of thiamethoxam in subterranean termites. Horizontal transfer of insecticides is a strategy currently being exploited in nonrepellent termiticides for termite control (Osbrink et al. 2005, Spomer et al. 2009). The Asian subterranean termite *Coptotermes gestroi* (Wasmann) is a major structural pest of wood structures in Southeast Asia. The species was formerly known as *Coptotermes vastator* in the Philippines until it was reclassified recently as a junior synonym of *C. gestroi* based on molecular and phylogenetic evidence (Yeap et al. 2007). Termite damage caused by *C. gestroi* was estimated to be around US$400 million annually (Lee et al. 2007). The present paper reports on the insecticidal activity and potential for horizontal transmission of thiamethoxam in the workers of Asian subterranean termite *C. gestroi*.

**Materials and Methods**

**Termites.** Secondary nests of three active colonies of *C. gestroi* were collected from infested buildings in the University of the Philippines Los Banos campus and placed in black garbage bags. The nests were immediately transported to the laboratory and placed inside 100-liter plastic containers with lids and kept in a room at 25°C for 3 d. Distilled water was sprayed on the sides of the container to keep the relative humidity above 80%. Mature worker (pseudergates beyond the third instar as determined by size) and soldier termites were separated from nest debris by breaking apart and sharply tapping materials into plastic trays containing moist paper towels. Termites were then sorted using a soft bird feather and used for bioassay within 1 h of extraction and segregation.

**Thiamethoxam.** Thiamethoxam, 3-(2-chloro-thiazol-5-ylmethyl)-5-methyl-\(N\)-(1,3,5)oxadiazinan-4-ylidine-N-nitroamine, was provided by Syngenta Philippines, Inc. (Makati City, Philippines). The formulation used (Optigard ZT) contains 21.6% thiamethoxam (AI). Stock solution containing 500 g/ml in distilled water was prepared and serial dilutions for contact toxicity and horizontal transmission studies were prepared accordingly.

**Exposure Test.** Brief exposure tests similar to that used by Saran and Rust (2007) were performed to determine concentration and time required to kill some but not all exposed termites. The protocol would also give an indication on the repellency and horizontal transmission of thiamethoxam in *C. gestroi*. One hundred workers plus ten soldiers of *C. gestroi* were placed in Petri dishes (9 by 1.5 cm—width by height) containing 100 g sifted sterilized sand (100°C for 24 h, 20 mesh) treated with varying concentration of thiamethoxam solution. Concentrations tested were 0, 0.25, 2.5, and 50 \(\mu\)g/ml thiamethoxam [wt AI/wt sand] based on a preliminary screening that allowed exposed termites...
to survive for a period of time and interact with unexposed individuals for transmission studies. The experimental units were placed in an unlit incubator at 28°C and 85% relative humidity for 1 h. Petri dishes containing treated sand were allowed to stand in a fume hood for 24 h before the introduction of termites. Surviving termites were transferred to similar Petri dishes containing moist sand after 1 h. Filter paper (Whatman #1, 2.5 by 2.5 cm) was added to serve as food. Petri dishes containing sterilized sand moistened with distilled water and filter paper were used as control. All Petri dishes containing the termites were stored in an incubator as described above. Dead and moribund termites was counted and removed daily to give an indication of the toxicity at each concentration used in this study. Workers were considered moribund when they could no longer walk or stand when probed with forceps. Mortalities were corrected by Abbott’s formula (1925) and subjected to an analysis of variance using a completely randomized design. Significant differences in mortality with varying concentration were separated by Tukey’s Honest Significant Difference (HSD) test at α = 0.05 (Statgraphics 1999). The test was replicated three times for each colony with a total of nine replicates for each concentration. Effective concentrations that showed delayed toxicity were used in the succeeding horizontal transmission experiment.

**Horizontal Transmission.** A simple donor–recipient transfer model was used to determine whether workers of *C. gestroi* contaminated with sand treated with thiamethoxam can transfer lethal amount of the insecticide to unexposed termites through grooming or trophallaxis (Thorne and Breisch 2001, Ibrahim et al. 2003, Saran and Rust 2007). Treatment experiment was performed by allowing worker termites (donor), which were stained with 0.5% Nile Blue A (Aldrich, Milwaukee, WI, USA), to walk on sand treated with thiamethoxam at various concentrations (0, 0.25, and 2.5 μg/ml) for 60 min. Donor termites were stained by feeding them with filter papers dyed with 0.5% Nile Blue A (Aldrich) for 1 w prior to the start of the experiment (Su and Scheffrahn 1991). Donor termites were gently transferred to a Petri dish lined with filter paper and allowed to walk for 30 min to allow residual sand on their bodies to fall off and not contact the recipient termites later. The donor termites were then transferred to Petri dishes (9 by 1.5 cm—width by height) containing moist sand and unexposed worker termites (recipient). Treated workers were placed with untreated workers at the proportion of 1:10, 1:5, 2:5, and 1:2 (treated:untreated) with a total of 50 termites in each dish. A rectangular filter paper (2.5 by 2.5 cm, Whatman #1) was placed on top of each dish to serve as food for the termites. The completed Petri dishes were placed in an unlit incubator at 28°C and 85% RH. Control dishes contained 50 untreated worker termites only with moist sand and filter paper. Three replicates were used for each treatment. Termite mortality for each concentration used in both donor and recipient termites was determined each day for 14 d as described above to determine whether lethal amount of thiamethoxam was transferred to kill unexposed termites.

**Results**

**Exposure Test.** Sand treated with 50 μg/ml thiamethoxam was lethal to *C. gestroi* killing 100% of the insect within 30–60 min of exposure (Table 1). The termites immediately experienced intoxication and lethargy followed by death upon exposure to treated sand. In contrast, mortality of termites placed in sand treated with 0.25–25 μg/ml was dose dependent (Table 1). Termites exposed to sand treated with thiamethoxam at 25 μg/ml killed >98% of the insect after 24 h post exposure. However, thiamethoxam exhibited delayed toxicity at 0.25 and 2.5 μg/ml killing about 70 and 94% of the termites, respectively, after 7 d of exposure. The latent activity of thiamethoxam also permitted *C. gestroi* to penetrate and tunnel through treated sand during the exposure period. However, thiamethoxam at 0.25 μg/ml showed relatively low to moderate mortalities of about 70–73% against *C. gestroi* after 7 and 14 d of exposure, respectively. Since the 2.5 and 25 μg/ml concentrations of thiamethoxam resulted in delayed toxicities and significant termite mortalities, these concentrations were tested for horizontal transmission to unexposed workers of *C. gestroi* to explore its potential for termite control.

**Horizontal Transmission.** Recipient mixed with donor termites exposed to 2.5 μg/ml thiamethoxam at 1:10 to 1:2 donor–recipient ratios experienced relatively low mortalities (about 12–63%) after 14 d post exposure (Table 2). However, mortality of recipient termites increased significantly (*P < 0.001*) with increasing donor–recipient ratio and longer exposure period when mixed with donor termites exposed to 25 μg/ml thiamethoxam (Table 3). Recipient termite mortality increased from about 87–94% with donor–recipient ratio of 1:5 to 1:2, respectively after 24 h of exposure. Surviving termites showed severe signs of intoxication and immobility after 24 h. Mortality of recipient termites mixed with donor termites exposed to 25 μg/ml thiamethoxam at 2:5 donor recipient ratio rose to 100% after 3 d exposure.

**Discussion**

The immediate lethal effect of thiamethoxam at 50 μg/ml on workers of *C. gestroi* was evident. Apparently, thiamethoxam acted as a fast acting poison against *C. gestroi* at 50 μg/ml limiting its potential transfer application for termite control at this concentration at label rates. Similar toxicity of thiamethoxam was reported against *C. formosanus* (Remmen and Su 2005a,b). However, the delayed toxicity and nonrepellency of thiamethoxam observed at 0.25–25 μg/ml can potentially facilitate horizontal transmission of the insecticide to unexposed termites. Such strategy had been demonstrated to be effective with nonrepellent termiticides, resulting in significant mortality to unexposed termites in the laboratory (Thorne and Breisch 2001, Ibrahim et al. 2003, Saran and Rust 2007, Parman and Vargo 2010). The tunneling and penetration of treated sand also indicated the nonrepellency of thiamethoxam at 0.25–25 μg/ml. Repellent termiticides (e.g., synthetic pyrethroids) are known to limit penetration or tunneling of termites in treated materials (Su and Scheffrahn 1990). However, the mortality levels observed in this study were higher compared to that reported by Remmen and Su (2005a) against *C. formosanus* at about the same concentration. The reason for this is not clear but the difference in size between *C. gestroi* (*C. vastator*) and *C. formosanus* may have affected their susceptibility to thiamethoxam. *C. formosanus* worker is relatively larger than workers of *C. gestroi* (Su and Scheffrahn 1991, Neoh et al. 2012). Differences in toxicity of insecticides are also known to vary considerably among termite species (Ibrahim et al. 2003, Rust and Saran 2008, Spomer et al. 2009).

**Horizontal Transmission.** Recipient termites mixed with donor termites exposed to 2.5 μg/ml thiamethoxam at 1:10 to 1:2 donor–recipient ratios experienced relatively low mortalities (about 12–63%) after 14 d post exposure (Table 2). The result would indicate that although there is a delayed toxicity against *C. gestroi*, the low mortality at 2.5 μg/ml would have limited application in termite control. Thorne and Breisch (2001) and Rust and Saran (2008) reported parallel results with 10 μg/ml imidacloprid and acetamiprid, both related neonicotinoid insecticide used for termite control. However, mortality of recipient termites increased significantly (*P < 0.001*) with increasing

### Table 1. Mean mortality ± SEM of *C. gestroi* exposed to sand treated with various concentrations of thiamethoxam

| Day | Concentration (μg/ml) | Mortality (%) |
|-----|-----------------------|---------------|
| 0.25| 70.43 ± 2.45a | 98.67 ± 3.54a |
| 2.5 | 70.43 ± 2.45a | 98.67 ± 3.54a |
| 25  | 70.43 ± 2.45a | 98.67 ± 3.54a |
| 50  | 70.43 ± 2.45a | 98.67 ± 3.54a |

Each value is the mean of three replicates each; numbers within a column followed by the same letter are not significantly different (Tukey’s HSD test, α = 0.05).
Table 2. Mean mortality ± SEM of donor and recipient C. gestroi treated with 2.5 μg/ml thiamethoxam at various exposure periods and donor-recipient ratio.

| Donor:Recipient ratio (n = 50) | Days | Donor | Recipient |
|--------------------------------|------|-------|-----------|
|                                | 1    | 3     | 7         | 14        |
| 1:10                           | 12.35 ± 3.46a | 3.02 ± 1.24a | 16.27 ± 2.86a | 4.57 ± 0.85a |
| 1:5                            | 22.16 ± 3.14b | 7.46 ± 3.12a | 18.64 ± 3.41a | 7.64 ± 1.53b |
| 2:5                            | 33.53 ± 1.25c | 18.43 ± 2.55b | 41.52 ± 1.76b | 35.12 ± 1.76c |
| 1:2                            | 41.75 ± 2.72d | 36.06 ± 1.86c | 48.87 ± 1.24c | 37.18 ± 2.54c |

Each value is the mean of three replicates; numbers within a column followed by the same letter are not significantly different (Tukey’s HSD test, α = 0.05).

Table 3. Mean mortality ± SEM of donor and recipient C. gestroi treated with 25 μg/ml thiamethoxam at various exposure periods and donor-recipient ratio.

| Donor:Recipient ratio (n = 50) | Days | Donor | Recipient |
|--------------------------------|------|-------|-----------|
|                                | 1    | 3     | 7         | 14        |
| 1:10                           | 34.12 ± 3.75a | 23.24 ± 1.45a | 43.18 ± 2.86a | 35.85 ± 1.79a |
| 1:5                            | 92.16 ± 3.15b | 87.56 ± 3.15b | 98.16 ± 2.58b | 98.52 ± 0.0b |
| 2:5                            | 98.42 ± 2.50c | 92.32 ± 2.50c | 100 ± 0b | 100 ± 0b |
| 1:2                            | 99.12 ± 0.50c | 94.56 ± 1.70c | 100 ± 0b | 100 ± 0b |

Each value is the mean of three replicates; numbers within a column followed by the same letter are not significantly different (Tukey’s HSD test, α = 0.05).

donor-recipient ratio and longer exposure period when mixed with donor termites exposed to 25 μg/ml thiamethoxam (Table 3). Recipient termite mortality increased to about 87–94% with donor-recipient ratio of 1:5 to 1:2, respectively after 24 h of exposure. Surviving termite showed severe signs of intoxication and immobility after 24 h. Mortality of recipient termites rose to 100% after 3 d exposure to donor termites for 1:5 to 1:2 donor-recipient ratio. This would indicate that the lethal effects occurred sooner at higher concentration. Similar time trend mortality for thiamethoxam was observed in C. formosanus by Remmen and Su (2005a). Apparently, 25 μg/ml thiamethoxam showed horizontal transmission and high toxicity within 3 d post exposure at donor-recipient ratio of at least 2:5. In comparison, similar level of termite mortality were reported using >5 μg/ml fipronil against Reticulitermes hesperus Banks (Saran and Rust 2007), >50 μg/ml chlorpenafyr and acetamiprid against R. hesperus (Rust and Saran 2006, 2008), >50 μg/ml against C. gestroi (Neoh et al. 2011).

In general, the study showed that sand treated with thiamethoxam at 0.25–50 μg/ml resulted in dose-dependent mortality in C. gestroi. Sand treated with 50 μg/ml thiamethoxam resulted in very high termite mortality within 30–60 min of exposure. However, termites exposed to sand treated with 0.25–25 μg/ml exhibited delayed toxicity in workers of C. gestroi. Termites were able to tunnel and penetrate treated sand, indicating nonrepellency and slow action of thiamethoxam at the said concentrations. Horizontal transmission study at 25 μg/ml using donor-recipient ratio from 1:10 to 1:2 indicated that thiamethoxam can be transferred from exposed to unexposed worker termites with significant mortality with at least 2:5 donor-recipient ratio (treated:untreated) within 1–3 d after exposure. Additional research is warranted to determine how these factors might influence toxicity, horizontal transmission and impant control under field conditions.

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