# Mortality Patterns in *Coptotermes gestroi* (Blattodea: Rhinotermitidae) Following Horizontal Transfer of Nonrepellent and Repellent Insecticides: Effects of Donor:Recipient Ratio and Exposure Time

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**ABSTRACT** The donor: recipient ratio and the time of donor exposure to termiticide required for maximal toxicant transfer among termites are crucial information for the development of termite management plans. Most of the available information on termiticide toxicity came from temperate zonal termite species, whereas little is known about tropical Asian species. In this study, mortality patterns of recipient termites, *Coptotermes gestroi* (Wasmann) subjected to seven formulated insecticide exposures under different donor exposure times and donor: recipient ratios were examined. For fipronil, lethal transfer was not affected by donor exposure time but was affected by the mixing ratio. The moderate-to-less toxic termiticides (imidacloprid, indoxacarb, bifenthrin, chlorfenapyr, and chlorantraniliprole) required long exposure time and a high mixing ratio to ensure maximal uptake by recipient workers compared with fipronil. For chlorantraniliprole and chlorfenapyr, donors must constitute >30% of the donor–recipient mixture to achieve 100% mortality of the recipient workers. Among the termiticides tested, cyantraniliprole was the most fast-killing insecticide against *C. gestroi.* The potential of lethal transfer among recipient termites does not necessarily require both high donor exposure time and a high mixing ratio, but the toxicity of a given termiticide against termites must be factored in to achieve colony elimination.

**KEY WORDS** phenylpyrazole, neonicotinoid, oxadiazine, pyrethroid, pyrrole

Conventional termite control involves creating a chemical barrier in the soil to kill or repel termites from building structures. This method has dominated the termite control industry for the past 90 yr (Su and Scheffrahn 1998, Peterson 2010). The conventional method, however, can provide limited protection, if poor treatment regimens result in gaps that allow termites free access into the building structure. More recently, nonrepellent termiticides have been gaining popularity. In soil treated with these termiticides, the chemicals are undetectable by termites, thus providing constant exposure as long as the termites remain in the treated areas. This method allows termites to acquire a lethal dose of toxicant and transfer it to healthy termites within the colony, thereby leading to colony decline or elimination (Parman and Vargo 2010, Hu 2011, Vargo and Parman 2012). These lethal effects largely depend on mutual grooming between recipient and insecticide-exposed termites. However, if the recipients die or become impaired too fast, the transfer of toxicant from one termite to another would be reduced.

Pest control personnel have long wondered about the optimal donor exposure time to toxicant and the donor: recipient mixing ratios that would ensure maximal successive toxicant transfer. To date, several published studies document termiticide toxicity against termites (Tsunoda 2006, Haagsma and Rust 2007, Sheikh et al. 2008, Mulrooney and Gerard 2009). However, currently available data likely are not universally applicable. For example, most existing data are from temperate zonal termite species that, to a certain extent, differ physiologically and biologically from tropical species such as *Coptotermes gestroi* (Wasmann). This premise is supported by the fact that the susceptibility of *C. gestroi* to termiticides differs from that of temperate termites (Neoh et al. 2012a). For example, chlorantraniliprole applied topically was found to be more toxic to *Reticulitermes flavipes* (Kollar) workers than indoxacarb (Spomer et al. 2009), but this was not the case for *C. gestroi* (Neoh et al. 2012a). In addition, the amount of fipronil needed to kill 50% of *Coptotermes formosanus* Shiraki was threefold higher than that required to kill 50% of *C. gestroi* (Ibrahim et al. 2003). This difference might be because of size variation and the capability of certain termite species to detoxify the toxicants (Neoh et al. 2012a).

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*C. gestroi* was selected for this study because of its notoriety for infesting building structures in tropical regions (Kirton and Azmi 2005, Lee et al. 2007). In addition, anthropogenic activities have resulted in the introduction of *C. gestroi* to locales beyond its native range of the Indo–Malayan region. For example, it is now found in Polynesia, Madagascar, Mauritius, the Caribbean Islands, South America, Taiwan (Scheffrahn et al. 1994, Su et al. 1997, Tsai and Chen 2003, Scheffrahn and Su 2005), and even Sicily, Italy (Ghesini et al. 2011). This species requires special attention for several reasons. First, *C. gestroi* reportedly is highly adaptable to a wide range of weather conditions during swarming events, and it can disperse throughout the year (Neoh and Lee 2009). Second, the reproductive strategy of *C. gestroi* is highly flexible, as neotenics may develop a new colony if a pest management program fails to eliminate the entire colony (Costa-Leonardo and Arab 2004). The interplay of these two factors makes *C. gestroi* a difficult target for pest management programs.

To date, few studies have been designed primarily to examine the effects of termiticides on *C. gestroi* (Yeoh and Lee 2007, Neoh et al. 2012a). Thus, information about this Asian subterranean species is needed for development of better management programs. The objectives of this study were twofold: 1) to examine the time effect of toxicant exposure to donor termites and the effect of the termite mixing ratio on horizontal transfer of termiticides, and 2) to evaluate the mortality patterns of toxicant recipient termites after exposure to seven formulated termiticides of different toxicities.

## Materials and Methods

**Termites.** *C. gestroi* was obtained from Universiti Sains Malaysia, Minden Campus, Penang, Malaysia (5° 21′ N, 100° 18′ E). Termites were collected from different in-ground monitoring stations located adjacent to buildings that are actively infected by termites. The in-ground monitoring stations consisted of a plastic container measuring 30 by 24 by 10.5 cm, baited with rubber wood (*Hevea brasiliensis* Müller Argoviensis). Collected termites were brought back to the laboratory and separated from soil debris. Each termiticide was tested against individuals from two different colonies.

**Insecticides.** The following seven formulated insecticides were used in this study: fipronil 2.5% EC (BASF Corp., Research Triangle Park, NC), imidacloprid 20% suspension concentrate (SC) (Bayer Environmental Service, Montvale, NJ), indoxacarb 14.5% SC (DuPont Crop Protection, Wilmington, DE), chlorfenapyr 24% SC (BASF Corp., Research Triangle Park, NC), chlorantraniliprole 18.5% SC (DuPont Crop Protection, Wilmington, DE), cyantraniliprole10% SC (DuPont Crop Protection, Wilmington, DE), and bifenthrin pyrethroid 24% emulsifiable concentrate (EC) (FMC Corporation, Philadelphia, PA).

**Experimental Design.** Workers (recipients) were dyed with Nile blue A (Aldrich, Milwaukee, WI) for 2 d using a fast-marking method; conversely, the donors were not dyed. The recipients were exposed in the open under laboratory conditions (26.4 ± 0.2°C and 63.2 ± 0.63% relative humidity) until they lost ≈20% of their body weight. They were then transferred into a plastic container (15.5 by 10 by 6.5 cm) provisioned with Nile blue A-dyed filter papers (0.05% wt:wt).

For each of the seven insecticide sat a selected concentration (Table 1), workers (donors) were allowed to forage freely in a petri dish (15 cm in diameter) containing 100 g of termiticide-treated sand at three different exposure times (5, 10, and 30 min). The termites were then transferred to a clean petri dish to dislodge any soil debris from their body and then were mixed with recipients at five different donor:recipient ratios: 1:10 (10% donor), 1:5 (20%), 3:10 (30%), 2:5 (40%), and 1:2 (50%) by standardizing the number of recipients at 20 workers per replicate (i.e., seven insecticides × three exposure times × five mixing ratios). Each combination was replicated five times. For control, 20 workers per replicate (five replicates for each colony) were allowed to forage on nontermiticide-treated sand for 5 min. The experimental setup was maintained in the dark and moist filter papers were provided as a food source during the experiment. The number of surviving recipients was recorded every 12 h until all the recipient termites died. Dead termites were retained in the arena throughout the experiment.

**Statistical Analysis.** The data from the two colonies were pooled. Mean survival time of recipients was generated using Kaplan–Meier analysis. Percentage mortality was subjected to arcsine square-root transformation before statistical analysis. Two-way between-groups analysis of variance (ANOVA) was used to determine the effects of time of donor exposure to treated sand, donor: recipient ratios, and their interaction effects on recipient mortality. All analyses were performed using SPSS, version 11.0 (SPSS Inc., Chicago, IL), with statistical significance set at $\alpha = 0.05$.

## Results

Mean survival time of recipient workers in a control replicate ranged from 597 to 714 h, which was significantly higher than those in the mixed donor:recipient treatments, as indicated by the overlapping confidence limits (Table 1).

**Fipronil.** Exposure of recipient workers to fipronil-treated donor workers resulted in average survival times ranging from 32 to 239 h (Table 1). Relative to other combinations tested, survival time of recipient workers was significantly lower in the 10-min exposure and 40 and 50% mixing ratio treatments and for all mixing ratios at 30-min exposure (except 10%). Generally, the survival rate of recipient workers declined to 50% of the total test insects by 6–10 d. However, ≈1–2.5 d were required to kill 50% of the total test insects in the 10-min exposure group and 40 and 50% mixing ratio treatments and for all mixing ratios at 30 min of exposure (except 10%; Fig. 1a). The mixing ratio significantly affected the mortality of

**Table 1. Mean survival time of recipient *C. gestroi* workers after being exposed to termiticide-treated donor workers at various exposure times and donor:recipient ratios**

| Termiticides (wt:wt) donor:recipient ratio | Mean survival time, hours (95% CL) | | |
|---|---|---|---|
| | 5-min exposure | 10-min exposure | 30-min exposure |
| Control | 655.800 (597.130–714.470) | | |
| Fipronil (11 mg $kg^{-1}$) | | | |
| 1:10 | 215.520 (191.176–239.864)a | 185.400 (161.249–209.551)a | 103.200 (88.224–118.176)a |
| 1:5 | 180.600 (165.437–195.763)a | 167.880 (149.913–185.847)a | 69.960 (59.770–80.150)b |
| 3:10 | 151.560 (136.017–167.103)a | 117.120 (103.405–130.835)a | 51.720 (44.255–59.185)b |
| 2:5 | 155.520 (140.179–170.861)a | 64.080 (55.863–72.297)b | 38.520 (31.773–45.267)b |
| 1:2 | 142.530 (124.941–160.119)a | 76.440 (65.344–87.536)b | 38.640 (31.991–45.289)b |
| Imidacloprid (25 mg $kg^{-1}$) | | | |
| 1:10 | 569.040 (527.835–610.245)a | 450.600 (414.853–486.347)a | 310.800 (276.323–345.277)a |
| 1:5 | 534.240 (482.314–586.166)a | 449.640 (407.574–491.706)a | 153.120 (120.693–185.547)b |
| 3:10 | 385.340 (343.331–427.349)a | 487.920 (446.673–529.167)a | 87.360 (61.554–113.166)b |
| 2:5 | 455.640 (405.534–505.746)a | 434.880 (386.427–483.333)a | 112.200 (81.959–142.441)b |
| 1:2 | 454.600 (403.426–505.774)a | 221.880 (182.805–260.955)b | 29.520 (19.800–39.420)c |
| Indoxacarb (100 mg $kg^{-1}$) | | | |
| 1:10 | 200.400 (163.951–236.849)a | 181.320 (159.668–202.972)a | 101.040 (88.881–117.199)a |
| 1:5 | 201.600 (163.758–239.442)a | 147.000 (125.177–168.823)a | 104.400 (88.683–120.117)a |
| 3:10 | 239.280 (204.917–273.643)a | 159.960 (139.727–180.193)a | 103.200 (88.856–117.544)a |
| 2:5 | 249.120 (208.868–289.372)a | 137.520 (117.300–157.740)a | 159.120 (131.855–186.385)a |
| 1:2 | 227.760 (196.776–258.744)a | 173.100 (149.986–196.214)a | 75.120 (62.458–87.782)b |
| Chlorfenapyr (30 mg $kg^{-1}$) | | | |
| 1:10 | 486.150 (453.792–518.508)a | 477.600 (430.187–525.013)a | 435.600 (403.948–467.252)a |
| 1:5 | 501.480 (455.053–547.907)a | 535.550 (490.494–580.606)a | 481.860 (439.226–524.494)a |
| 3:10 | 487.320 (444.984–529.656)a | 477.800 (434.422–521.178)a | 414.720 (375.400–454.040)a |
| 2:5 | 508.150 (454.444–561.456)a | 473.000 (419.043–526.957)a | 385.080 (358.829–411.331)a |
| 1:2 | 539.760 (481.614–597.906)a | 438.200 (391.255–485.145)a | 307.800 (276.230–339.370)b |
| Chlorantraniliprole (100 mg $kg^{-1}$) | | | |
| 1:10 | 387.720 (351.343–424.097)a | 349.320 (321.861–376.779)a | 300.480 (299.884–361.076)a |
| 1:5 | 405.840 (364.462–447.218)a | 390.000 (355.189–424.811)a | 307.320 (282.257–332.383)a |
| 3:10 | 430.680 (397.275–464.085)a | 349.500 (314.969–384.031)a | 217.800 (183.349–252.251)b |
| 2:5 | 350.280 (307.282–393.278)a | 378.120 (335.066–421.174)a | 253.320 (232.997–273.643)b |
| 1:2 | 433.920 (384.614–483.226)a | 345.240 (343.939–440.861)a | 226.200 (205.947–246.453)b |
| Cyantraniliprole (100 mg $kg^{-1}$) | | | |
| 1:10 | 147.120 (104.873–189.367)a | 90.000 (60.435–119.565)a | 30.240 (18.774–41.706)b |
| 1:5 | 31.200 (20.052–24.348)b | 38.400 (21.152–55.648)b | 21.720 (13.432–30.008)b |
| 3:10 | 13.440 (12.478–14.402)b | 13.680 (12.622–14.738)b | 13.320 (11.686–14.954)b |
| 2:5 | 12.960 (12.163–13.757)b | 12.840 (12.150–13.530)b | 13.440 (12.368–14.512)b |
| 1:2 | 14.040 (12.238–15.842)b | 14.640 (12.853–16.427)b | 14.280 (12.619–15.941)b |
| Bifenthrin (30 mg $kg^{-1}$) | | | |
| 1:10 | 513.480 (450.765–576.195)a | 160.320 (118.501–202.139)a | 68.160 (53.117–83.203)b |
| 1:5 | 406.080 (340.615–471.545)a | 175.680 (136.287–215.073)a | 56.280 (42.559–70.001)b |
| 3:10 | 308.880 (259.706–358.054)a | 198.960 (155.531–242.389)a | 81.120 (64.179–98.061)b |
| 2:5 | 388.680 (312.951–464.409)a | 74.280 (57.147–91.413)b | NA |
| 1:2 | 215.280 (157.280–273.280)a | NA | 75.480 (51.389–99.571)b |

Values of 95% CL followed by similar letters overlap between treated combinations within a test insecticide.
NA, not available.

recipient workers ($F = 13326.866$; df = 2, 27; $P < 0.001$; Table 2).

**Imidacloprid.** Exposure of recipient workers to imidacloprid-treated donor workers resulted in average survival times of 20–610 h (Table 1). Relative to other combinations tested, the survival times of the recipient workers were greatly reduced in the 10-min exposure and 50% mixing ratio treatment, and in the 30-min exposure and all mixing ratios (except 10%). For the 30 -in exposure, only 1–3 d were required to kill >50% of recipient workers as the mixing ratio increased (e.g., from 30 to 50% donor; Fig. 1b). Exposure time ($F = 59.103$; df = 2, 27; $P < 0.001$), mixing ratio ($F = 14.365$; df = 2, 27; $P < 0.001$), and their interaction ($F = 3.215$; df = 2, 27; $P < 0.001$) significantly affected the mortality of recipient workers.

**Indoxacarb.** Survival time of recipient termites ranged from 62 to 289 h after exposure to indoxacarb-treated donor workers (Table 1). Relative to the combination tested, the mean survival time of recipient workers decreased significantly after being mixed with donor termites exposed to indoxacarb for 50% mixing ratio and 30-min exposure; in this treatment, 50% of the total test insects died from day 2 to 3 (Fig. 1c). Exposure time significantly influenced the mortality of recipient workers ($F = 13.914$; df = 2, 27; $P < 0.001$; Table 2). Although mixing ratio was found to have no effect on the mortality ($F = 2.126$; df = 2, 27; $P > 0.001$), the interaction between the effects significantly affected the survival probability of recipient workers ($F = 2.361$; df = 2, 27; $P < 0.001$).

**Chlorfenapyr.** Among the tested termiticides, transfer of chlorfenapyr from donor to recipient termites was least effective. The mean survival time of the recipient workers remained high (mean survival time ranged from 276 to 598 h) for all tested combinations

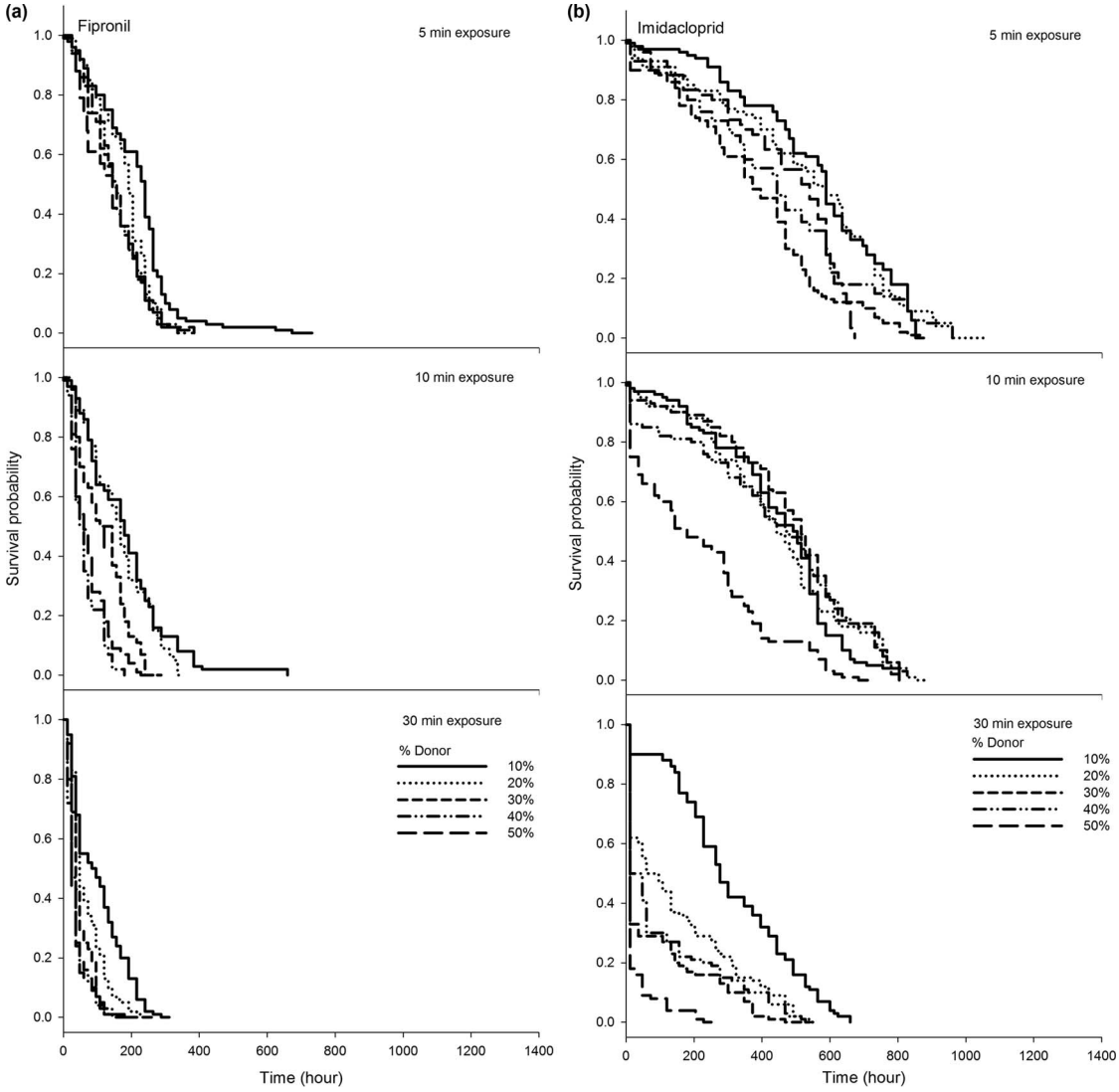


**Fig. 1.** (a–g) Survival patterns of recipient termites after confinement with donor termites treated with various conventional and novel termiticides under the effect of donor exposure time and donor:recipient ratio.

(Table 1). Statistical analysis revealed that exposure time and mixing ratio were the main effects affecting the mortality of recipient workers (exposure time: $F = 6.296$, df = 2, 27, $P < 0.001$; mixing ratio: $F = 6.387$, df = 2, 27, $P < 0.001$; Table 2). At least 17 d of exposure were needed to kill 50% of recipient workers, with the exception of the 30-min exposure and 50% mixing ratio treatment, which required 13 d (Fig. 1d).

**Chlorantraniliprole.** Unlike the second generation of anthranilic amide insecticides (cyantraniliprole), recipient workers exposed to chlorantraniliprole-treated donors had remarkably longer mean survival times (183–483 h; Table 1). The life spans of recipient workers exposed to chlorantraniliprole-treated donors for 30 min at the 30% (mean survival time, 218 h), 40% (253 h), and 50% (226 h) mixing ratios were significantly shorter than those for the other treatment combinations. Fifty percent mortality of recipient worker was recorded by day 13 (Fig. 1e). As exposure time ($F = 21.988$; df = 2, 27; $P < 0.001$) and mixing ratio ($F = 12.028$; df = 2, 27; $P < 0.001$) increased, significant mortalities in recipient workers were observed (Table 2).

**Cyantraniliprole.** Exposure of recipient workers to cyantraniliprole-treated donor workers resulted in average survival times of 12–189 h (Table 1). Among the termiticides tested, the mean survival time of recipient workers exposed to cyantraniliprole was shortest. This was true for all treatment combinations (except for the 10% mixing ratio at 10- and 20-min exposures; Table 1), in which most recipient workers were dead by 12–24 h (Fig. 1f). Mixing ratio significantly influenced the mortality of recipient workers ($F = 6.625$; df = 2, 27; $P < 0.001$; Table 2). However, exposure time

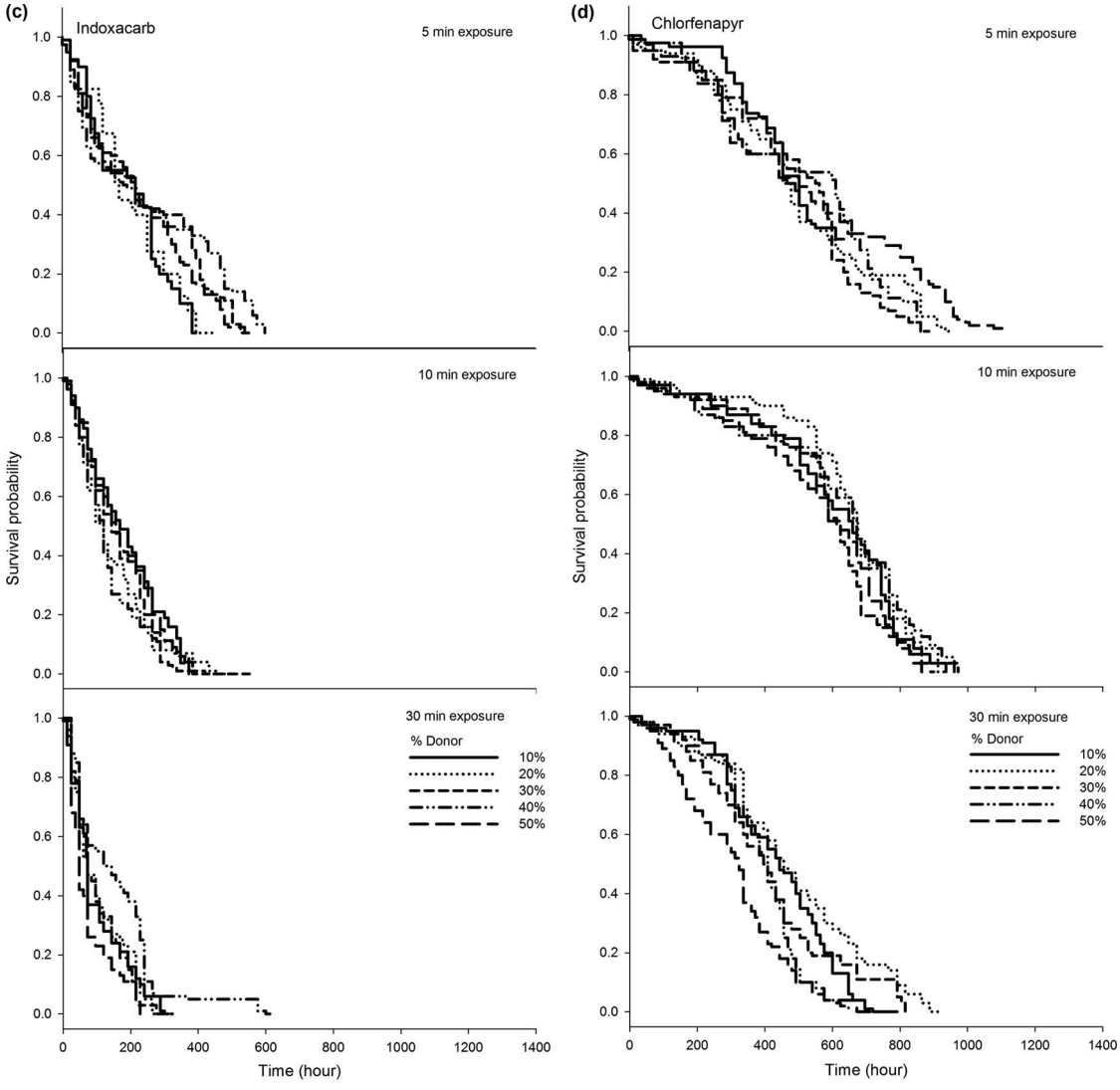


**Fig. 1.** (Continued).

($F = 2.291$; df = 2, 27; $P > 0.05$) and the interaction between mixing ratio and exposure time ($F = 1.790$; df = 2, 27; $P > 0.05$) did not have a significant effect on mortality.

**Bifenthrin.** Survival time of recipient termites exposed to bifenthrin-treated donor workers ranged from 43 to 576 h (Table 1). Compared with other combinations tested, survival probabilities of recipient termites decreased significantly when they were subjected to 40% mixing ratio and 10-min exposure, and the 30-min exposure for all mixing ratios (Table 1), and >50% mortality of recipient workers was recorded at 12 h postmixing (Fig. 1g). Result from two-way ANOVA demonstrated that exposure time ($F = 46.307$; df = 2, 27; $P < 0.001$) and mixing ratio ($F = 5.726$; df = 2, 27; $P < 0.001$) significantly affected the mortality of recipient workers (Table 2).

## Discussion

A successful termiticide treatment to soil requires that a large proportion of the target population (i.e., donors and recipients) picks up a lethal dose and dies after a sufficient period to allow for horizontal transfer of the toxicant. This process ensures that healthy workers receive a lethal dose and can lead to elimination of the entire colony. It is generally accepted that recipient mortality increases in parallel with the time of donor exposure to insecticide (Buczkowski et al. 2012) and the donor:recipient ratio (Ibrahim et al. 2003). However, this premise is not true for all cases, and the toxicity of an insecticide toward a particular pest must be taken into account. Comparatively, the degree of toxicity of chlorantraniliprole toward *C. gestroi* was fourfold lower than those of *C. formosanus* and *R. flavipes;* but the toxicities of bifenthrin, chlo-

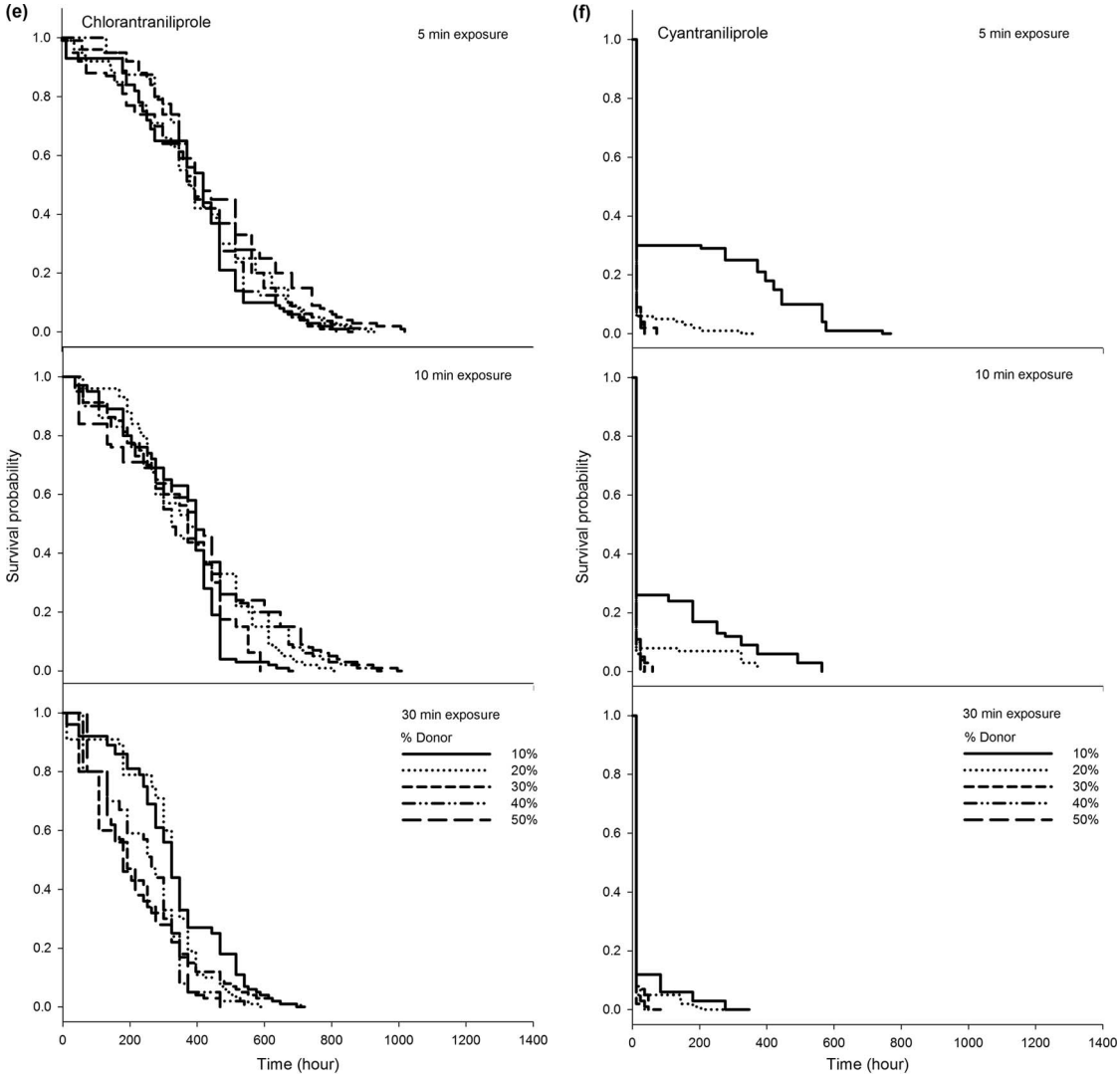


**Fig. 1.** (Continued).

rfenapyr, fipronil, and indoxacarb toward the insects (except for *C. formosanus*) were reportedly comparable (Mao et al. 2011, Neoh et al. 2012a). Nevertheless, it is worth noting that the current topical toxicity data for *C. gestroi* are based on measurements taken 24 h postexposure, the amount of termiticide required for an insect to cause 50% mortality of *C. gestroi* is expected to decrease proportionally as exposure time increases (Neoh et al. 2012a). Thus, the time of donor exposure to insecticide and the donor:recipient ratio to achieve maximal toxicant transfer in *C. gestroi* might differ from those of other well-studied termite species.

**Fipronil.** Fipronil is claimed to exhibit delayed toxic effects. In the current study, this premise holds true for recipient termites in the 5- and 10-min exposure treatments with mixing ratios <30%. Conversely, the mean survival time decreased to <72 h when the recipient workers were subjected to >40% mixing ratios at 10-min exposure and >20% mixing ratios at 30-min exposure to donors. However, statistically, the current study revealed that lethal transfer was not affected by donor exposure time but was affected by mixing ratio. Using a lower concentration of fipronil (1 mg $kg^{-1}$), Shelton and Grace (2003) found that exposure to fipronil for several selected time intervals up to 24 h did not have a significant effect on mortality among the recipient workers of *C. formosanus*, although the amount of toxicant uptake was significantly related to the amount of time donor workers were exposed to the toxicant. Saran and Rust (2007) reported that recipient *C. formosanus* exposed to donor workers treated with 5 mg $kg^{-1}$ fipronil for 24 h exhibited 100% mortality by Day 7. In a previous study, a topical bioassay revealed that a mere 0.9 ng of fipronil was sufficient to cause 50% mortality by 24 h, and it is assumed that the amount of toxicant required to cause

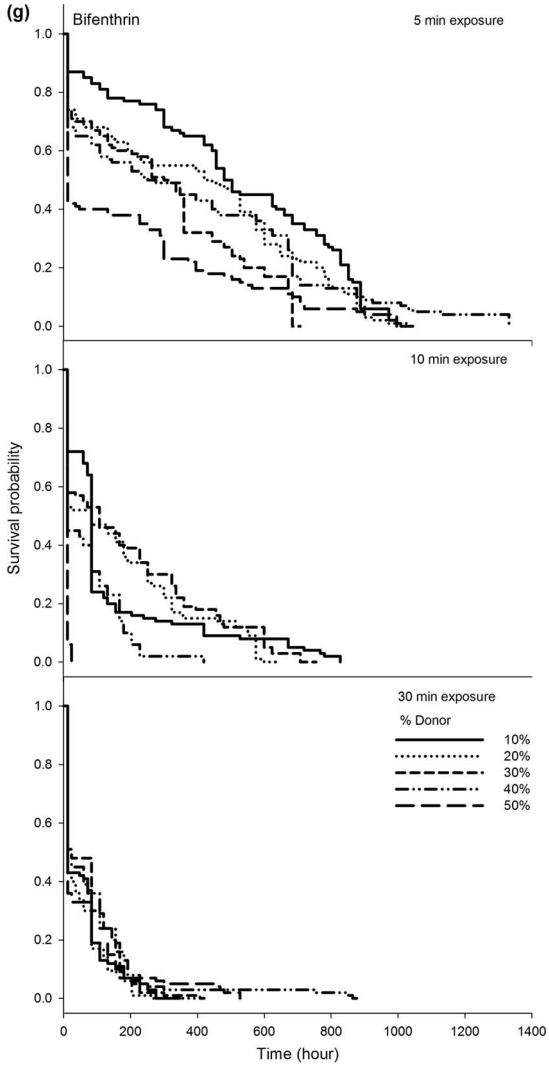


**Fig. 1.** (Continued).

50% mortality of *C. gestroi* is proportionally less over a longer period of time (Neoh et al. 2012a). This also explains why the at least 10% mixing ratio was lethal to *C. gestroi* recipient workers within 10 d irrespective of donor exposure time. Gautam et al. (2012) used 0.5% fipronil dust (trade name: Termidor Dry, a Microllose-based formulation with 0.5% fipronil; BASF Corp., St. Louis, MO) to manage *C. formosanus* infestations. They showed that a single donor exposed to the dust could readily transfer a lethal dose to at least nine recipients.

**Imidacloprid.** Recovery of insects after imidacloprid treatment was reported for the tropical bed bug (*Cimex hemipterus* F.; How and Lee 2011), German cockroach (*Blattella germanica* L.; Kaakeh et al. 1997), and house fly (*Musca domestica* L.; Quintela and McCoy 1997). Similarly, recipient termites exposed to imidacloprid-treated donors were observed to recover from the poisoning symptoms in this study. Knockdown was temporary if the termites were exposed to a sublethal dose when exposure time was short or when the mixing ratio was low. This occurs because the termites can detoxify the parent toxic compound (imidacloprid) into less toxic secondary metabolites (Tomalski et al. 2010). This matter should be given adequate attention, as behavioral aversion to a subsequent exposure was shown in *Reticulitermes virginicus* Banks when the workers were sublethally intoxicated (Thorne and Breisch 2001). This might indirectly impact the success of pest management programs. Our results show that the mean survival probability declined significantly within 7 d after the recipient termites were confined with donors treated with imidacloprid for at least 30 min at donor:recipient ratios >20%. Shelton and Grace (2003) noted in their study that donor termites exposed to a minimum of 100 mg $kg^{-1}$ imidacloprid needed to constitute 5% of the total to achieve ≈60% mortality of *C. formosanus* recipient workers. They also reported that when donor workers exposed to 1 mg $kg^{-1}$ imidacloprid for up to 24 h made up 5% of the mix, they were unable to deliver a lethal dose to the recipients (Shelton and Grace 2003). Conversely, a study of *Reticulitermes hesperus* (Banks) demonstrated that 7.5–16.5% of the imidacloprid was transferred within 2 h, irrespective of the concentration tested (Haagsma and Rust 2007). These examples illustrate the variability in susceptibility of different termite species to imidacloprid.

**Table 2. Effects of exposure time and mixing ratio on mortality of untreated recipient worker termites**

| Termiticides | Factors | df | *F* | *P* |
|---|---|---|---|---|
| Fipronil | time | 2 | 0.391 | 0.678 |
| | ratio | 5 | 13,326.866 | 0.000 |
| | time × ratio | 8 | 0.160 | 0.995 |
| Imidacloprid | time | 2 | 59.103 | 0.000 |
| | ratio | 5 | 14.365 | 0.000 |
| | time × ratio | 8 | 3.215 | 0.004 |
| Indoxacarb | time | 2 | 13.914 | 0.000 |
| | ratio | 5 | 2.126 | 0.074 |
| | time × ratio | 8 | 2.361 | 0.027 |
| Chlorfenapyr | time | 2 | 6.296 | 0.003 |
| | ratio | 5 | 6.387 | 0.000 |
| | time × ratio | 8 | 0.943 | 0.488 |
| Chlorantraniliprole | time | 2 | 21.988 | 0.000 |
| | ratio | 5 | 12.028 | 0.000 |
| | time × ratio | 8 | 1.265 | 0.278 |
| Cyantraniliprole | time | 2 | 2.291 | 0.109 |
| | ratio | 5 | 6.625 | 0.000 |
| | time × ratio | 8 | 1.790 | 0.096 |
| Bifenthrin | time | 2 | 46.307 | 0.000 |
| | ratio | 5 | 5.726 | 0.000 |
| | time × ratio | 8 | 1.915 | 0.073 |

**Indoxacarb.** Generally, confinement with donor termites treated with 100 mg $kg^{-1}$ indoxacarb for at least 10 min significantly decreased the survival time of *C. gestroi* to 7 d or less irrespective of the mixing ratio (except for 10% and 10-min exposure time). Moreover, exposure time of the donors and the interaction between exposure time and mixing ratio contributed significantly to recipients' survival rate. In another study of the effects of 100 ng indoxacarb, the mortality of recipient *C. formosanus* workers was sig-

nificantly affected by mixing ratio, in that, at least 20% of the termite mix needed to be donors to cause 94% mortality of recipient workers by day 24 (Hu et al. 2005). Mortality onset of *R. flavipes* occurred faster as the exposure time increased (Quarcoo et al. 2010).

**Chlorfenapyr.** Overall, chlorfenapyr did not transfer well among recipient workers in the current study. In another study by Rust and Saran (2006), for *R. hesperus*, the uptake of chlorfenapyr by donor termites increased with time of donor exposure, and the transfer efficiency to recipients ranged from 13.3 to 38.4%; however, the amount transferred to recipients was not enough to have a secondary killing effect. Data from topical bioassays suggested that chlorfenapyr is less toxic to termites compared with other test termiticides and that high doses are required to achieve 50% mortality in most termite species tested (Rust and Saran 2006, Pan et al. 2011, Neoh et al. 2012a). Although adequate horizontal transmission of chlorfenapyr was demonstrated among *R. flavipes* workers, the dose of chlorfenapyr needed for donor exposure was relatively high (>250 mg $kg^{-1}$; Shelton et al. 2006). This might explain the inefficient transfer among the healthy *C. gestroi* in the current study, as the dose used was only 30 mg $kg^{-1}$ chlorfenapyr.

**Chlorantraniliprole.** Chlorantraniliprole acts on insect ryanodine receptors, causing impairment of normal muscle contraction, paralysis, and death (Lahm et al. 2009). This impairment results in feeding cessation followed by starvation (Yeoh and Lee 2007, Neoh et al. 2012a) and leads to subsequent intensive social interaction from healthy termites (Neoh et al. 2014). Thus, chlorantraniliprole is a promising candidate for use in termite management programs. In *R. flavipes*, the efficiency of horizontal transfer of chlorantraniliprole depends on the concentration used and the time of donor exposure to the toxicant (Buczkowski et al. 2012). Chlorantraniliprole is comparatively less toxic against *C. gestroi* compared with other test termiticides, which means that longer donor exposure time and a higher mixing ratio are required to ensure maximal uptake by donors followed by lethal transfer among recipients. In this study, we found that donors must be exposed to the termiticide for 30 min and constitute >30% of the mixture to significantly shorten the survival time of recipients to 10 d compared with other test combinations. In another study in which *C. formosanus* was exposed to 25 and 50 mg $kg^{-1}$ chlorantraniliprole for 1 h, up to 80% mortality was recorded in recipients at day 5 postexposure when the donor:recipient mix was 50:50 (Gautam and Henderson 2011). However, in nature, it is questionable whether the large number of effective donors needed can be achieved (at least 30% target population in *C. gestroi* case) as millions of termite individuals may populate a colony.

**Cyantraniliprole.** Cyantraniliprole is a second generation anthranilic diamide insecticide, and it shares a similar mode of action with chlorantraniliprole. It is a novel insecticide that is showing promise as a control agent against agricultural and horticultural pests (Fettig et al. 2011, Hardke et al. 2011, Jacobson and Kennedy 2011, Li et al. 2011), but it is rarely used for termite management. In the current study, we found that cyantraniliprole is toxic to *C. gestroi.* On average it caused 50% mortality of *C. gestroi* recipient workers within 48 h (except for those confined with the lowest mixing ratio with donors exposed to toxicant for 5 and 10 min). Because of its relatively fast action, in a field setting recipients may not have sufficient time to receive lethal transfer from donor workers before the onset of donor mortality.

**Bifenthrin.** Bifenthrin seemed to cause unexpectedly lethal transfer to healthy termites in the current study. In contrast, Shelton et al. (2005) observed a lack of transfer of permethrin among nestmates of *R. flavipes* in laboratory trials, as the repellent properties of pyrethroid on donor termites inhibited social interactions from healthy termites. The mortality of recipient workers in the current study might be associated with secondary contamination caused by the accumulation of donor carcasses and their associated fungus growth, carcass handling by healthy termites, or a crowding effect, as found for *Reticulitermes* (Neoh et al. 2012b, Shelton 2012).

In conclusion, results of this study emphasize that the potential for lethal transfer of toxicant among recipient termites depends on donor exposure time and mixing ratio as well as the toxicity of a given termiticide toward a particular termite species. For example, unlike fipronil, both donor exposure time and mixing ratio significantly affected the uptake of imidacloprid, indoxacarb, chlorfenapyr, and chlorantraniliprole by recipient workers. The current study gives a better understanding of how the mortality pattern of *C. gestroi* varies with different termiticides. Overall, fipronil and indoxacarb showed satisfactory lethal transfer among nestmates, as mortality occurred in 7–10 d. For bifenthrin, lethal insecticide transfer can occur via contaminated corpse handling and crowding effect. However, those insecticides could cause premature mortality as donor exposure time to insecticide as well as mixing ratio increased. As highlighted by Quarcoo et al. (2010), the length of time until the onset of termite mortality after exposure is crucial to ensure that inoculated termites have sufficient time to transfer the lethal amount of toxicant among healthy termites. Ideally, intoxicated termites can walk back to their own nest before the toxic effects (i.e., abnormal behavior and reduced walking speed) are fully expressed, because at that time, the transfer of toxicant among healthy termites ceases. Similarly, it is doubtful that cyantraniliprole would give colony impact to treated termite colony due to its rapid mortality onset. Imidacloprid caused inconsistent mortality as exposure time and mixing ratio increased partly due to the capability of the test insects to detoxify the toxicant as well as behavioral aversion to the intoxicated nestmates. For chlorantraniliprole and chlorfenapyr, a significantly higher proportion of donor was required to achieve the expected outcome. However, this outcome is unlikely achievable in nature, as a termite colony can be populated by millions of members. We propose that additional insecticide evaluations should

be conducted using *C. gestroi* instead of using currently available data for temperate species. *C. gestroi* shows considerable variation in susceptibility toward different termiticides when compared with the published data for temperate species.

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## References Cited

**Buczkowski, G., C. W. Scherer, and G. W. Bennett. 2012.** Toxicity and horizontal transfer of chlorantraniliprole in the eastern subterranean termite. J. Econ. Entomol. 105: 1736–1745.

**Costa-Leonardo, A. M., and A. Arab. 2004.** Reproductive strategy of *Coptotermes gestroi* (Isoptera: Rhinotermitidae) in Brazil. Sociobiology 44: 123–125.

**Fettig, C. J., C. J. Hayes, S. R. McKelvey, and S. R. Mori. 2011.** Laboratory assays of select candidate insecticides for control of *Dendroctonus ponderosae.* Pest Manage. Sci. 67: 548–555.

**Gautam, B. K., and G. Henderson. 2011.** Effect of soil type and exposure duration on mortality and transfer of chlorantraniliprole and fipronil on formosan subterranean termites (Isoptera: Rhinotermitidae). J. Econ. Entomol. 104: 2025–2030.

**Gautam, B. K., G. Henderson, and R. W. Davis. 2012.** Toxicity and horizontal transfer of 0.5% fipronil dust against formosan subterranean termites. J. Econ. Entomol. 105: 1766–1772.

**Ghesini, S., G. Puglia, and M. Marini. 2011.** First report of *Coptotermes gestroi* in Italy and Europe. Bull. Insect. 64: 53–54.

**Haagsma, K. A., and M. K. Rust. 2007.** The effect of imidacloprid on mortality, activity, and horizontal transfer in the western subterranean termite (Isoptera: Rhinotermitidae). Sociobiology 50: 1127–1148.

**Hardke, J. T., J. H. Temple, B. R. Leonard, and R. E. Jackson. 2011.** Laboratory toxicity and field efficacy of selected insecticides against fall armyworm (Lepidoptera: Noctuidae). Fla. Entomol. 94: 272–278.

**How, Y. F., and C. Y. Lee. 2011.** Surface contact toxicity and synergism of several insecticides against different stages of the tropical bed bug, *Cimex hemipterus* (Hemiptera: Cimicidae). Pest Manage. Sci. 67: 734–740.

**Hu, X. P. 2011.** Liquid termiticides: their role in subterranean termite management, pp. 114–132. *In* P. Dhang (ed.), Urban Pest Management: An Environmental Perspective. CAB International, Oxon, United Kingdom.

**Hu, X. P., D. Song, and C. W. Scherer. 2005.** Transfer of indoxacarb among workers of *Coptotermes formosanus* (Isoptera: Rhinotermitidae): Effects of dose, donor:recipient ratio and post-exposure time. Pest Manage. Sci. 61: 1209–1214.

**Ibrahim, S. A., G. Henderson, and H. Fei. 2003.** Toxicity, repellency, and horizontal transmission of fipronil in the formosan subterranean termite (Isoptera: Rhinotermitidae). J. Econ. Entomol. 96: 461–467.

**Jacobson, A. L., and G. G. Kennedy. 2011.** The effect of three rates of cyantraniliprole on the transmission of tomato spotted wilt virus by *Frankliniella occidentalis* and *Frankliniella fusca* (Thysanoptera: Thripidae) to *Capsicum annuum.* Crop Prot. 30: 512–515.

**Kaakeh, W., B. L. Reid, T. J. Bohnert, and G. W. Bennett. 1997.** Toxicity of imidacloprid in the german cockroach (Dictyoptera: Blattellidae), and the synergism between imidacloprid and *Metarhizium anisopliae* (Imperfect Fungi: Hyphomycetes). J. Econ. Entomol. 90: 473–482.

**Kirton, L. G., and M. Azmi. 2005.** Patterns in the relative incidence of subterranean termite species infesting buildings in Peninsular Malaysia. Sociobiology 46: 1–15.

**Lahm, G., D. Cordova, and J. Barry. 2009.** New and selective ryanodine receptor activators for insect control. Bioorg. Med. Chem. 17: 4127–4133.

**Lee, C. Y., C. Vongkaluang, and M. Lenz. 2007.** Challenges to subterranean termite management of multi-genera faunas in Southeast Asia and Australia. Sociobiology 50: 213–221.

**Li, X., B. A. Degain, V. S. Harpold, P. G. Marcon, R. L. Nichols, A. J. Fournier, S. E. Naranjo, J. C. Palumbo, and P. C. Ellsworth. 2012.** Baseline susceptibilities of B- and Q-biotype *Bemisia tabaci* to anthranilic diamides in Arizona. Pest Manage. Sci. 68: 83–91.

**Mao, L., G. Henderson, and C. W. Scherer. 2011.** Toxicity of seven termiticides on the formosan and eastern subterranean termites. J. Econ. Entomol. 104: 1002–1008.

**Mulrooney, J. E., and P. D. Gerard. 2009.** Tunneling and activity of *Reticulitermes flavipes* (Isoptera: Rhinotermitidae) exposed to low concentrations of nonrepellent termiticides. Sociobiology 53: 695–706.

**Neoh, K. -B., and C. -Y. Lee. 2009.** Flight activity and flight phenology of the Asian subterranean termite, *Coptotermes gestroi* (Blattodea: Rhinotermitidae). Sociobiology 54: 521–530.

**Neoh, K.-B., J. Hu, B.-H. Yeoh, and C.-Y. Lee. 2012a.** Toxicity and horizontal transfer of chlorantraniliprole against the Asian subterranean termite *Coptotermes gestroi* (Wasmann): effects of donor:recipient ratio, exposure duration and soil type. Pest Manage. Sci. 68: 749–756.

**Neoh, K.-B., B.-K. Yeap, K. Tsunoda, T. Yoshimura, and C.-Y. Lee. 2012b.** Do termites avoid carcasses? behavioral responses depend on the nature of the carcasses. PLoS ONE 7: e36375.

**Neoh, K.-B., C.-C. Lee, and C.-Y. Lee. 2014.** Effects of termiticide exposure on mutual interactions between the treated and untreated workers of the Asian subterranean termite *Coptotermes gestroi.* Pest Manage. Sci. 70: 240–244.

**Pan, C., Q. Dai, and Y. Zhu. 2011.** Barrier and transfer properties of termiticides against the subterranean termite *Reticulitermes flaviceps* (Isoptera: Rhinotermitidae). Sociobiology 57: 659–668.

**Parman, V., and E. L. Vargo. 2010.** Colony-level effects of imidacloprid in subterranean termites (Isoptera: Rhinotermitidae). J. Econ. Entomol. 103: 791–798.

**Peterson, C. 2010.** Considerations of soil-applied insecticides for termite control. Outlooks Pest Manage. 21: 89–93.

**Quarcoo, F. Y., A. G. Appel, and X. P. Hu. 2010.** Effects of indoxacarb concentration and exposure time on onset of abnormal behaviors, morbundity, and death in eastern subterranean termite (Isoptera: Rhinotermitidae). J. Econ. Entomol. 103: 762–769.

**Quintela, E. D., and C. W. McCoy. 1997.** Pathogenicity enchancement of *Metarhizium anisopliae* and *Beuvaria bassiana* to first instar of *Diaprepes abbreviatus* (Coleoptera: Cucurlionidae) with sublethal doses of imidacloprid. Environ. Entomol. 26: 1173–1182.

**Rust, M. K., and R. K. Saran. 2006.** Toxicity, repellency, and transfer of chlorfenapyr against western subterranean termites (Isoptera: Rhinotermitidae). J. Econ. Entomol. 99: 864–872.

**Saran, R. K., and M. K. Rust. 2007.** Toxicity, uptake, and transfer efficiency of fipronil in western subterranean termite (Isoptera: Rhinotermitidae). J. Econ. Entomol. 100: 495–508.

**Scheffrahn, R. H., and N. Y. Su. 2005.** Distribution of the termite genus *Coptotermes* (Isoptera: Rhinotermitidae) in Florida. Fla. Entomol. 88: 201–203.

**Scheffrahn, R. H., J.P.E.C. Darlington, M. S. Collins, J. Krecěk, and N. Y. Su. 1994.** Termites (Isoptera: Kalotermitidae, Rhinotermitidae, Termitidae) of West Indies. Sociobiology 24: 213–238.

**Sheikh, N., F. Manzoor, R. Ahmed, N. Naz, and S. A. Malik. 2008.** Laboratory study of repellency and toxicity of three insecticides (Tenekil, Termidor and Terminus) against the subterranean termite *Heterotermes indicola* in Pakistan. Sociobiology 51: 749–764.

**Shelton, T. G. 2012.** Assessment of donor/recipient ratios in permethrin transfer studies with *Reticulitermes virginicus* Banks. J. Entomol. Sci. 47: 139–149.

**Shelton, T. G., and J. K. Grace. 2003.** Effects of exposure duration on transfer of nonrepellent termiticides among workers of *Coptotermes formosanus* Shiraki (Isoptera: Rhinotermitidae). J. Econ. Entomol. 96: 456–460.

**Shelton, T. G., C. D. Bell, and T. L. Wagner. 2005.** Lack of transfer of permethrin among nestmates of *Reticulitermes flavipes* in laboratory trials (Isoptera: Rhinotermitidae). Sociobiology 45: 69–75.

**Shelton, T. G., J. E. Mulrooney, and T. L. Wagner. 2006.** Transfer of chlorfenapyr among workers of *Reticulitermes flavipes* (Isoptera: Rhinotermitidae) in the laboratory. J. Econ. Entomol. 99: 886–892.

**Spomer, N. A., S. T. Kamble, and B. D. Siegfried. 2009.** Bioavailability of chlorantraniliprole and indoxacarb to eastern subterranean termites (Isoptera: Rhinotermitidae) in various soils. J. Econ. Entomol. 102: 1922–1927.

**Su, N.-Y., R. H. Scheffrahn, and T. Weissling. 1997.** A new introduction of a subterranean termite, Coptotermes havilandi Holmgren (Isoptera: Rhinotermitidae) in Miami, Florida. Fla. Entomol. 80: 408–411.

**Su, N. Y., and R. H. Scheffrahn. 1998.** A review of subterranean termite control practices and prospects for integrated pest management programmes. Integr. Pest Manage. Rev. 3: 1–13.

**Thorne, B. L., and N. L. Breisch. 2001.** Effects of sublethal exposure to imidacloprid on subsequent behavior of subterranean termite *Reticulitermes virginicus* (Isoptera: Rhinotermitidae). J. Econ. Entomol. 94: 492–498.

**Tomalski, M., W. Leimkuehler, C. Schal, and E. L. Vargo. 2010.** Metabolism of imidacloprid in workers of *Reticulitermes flavipes* (Isoptera: Rhinotermitidae). Ann. Entomol. Soc. Am. 103: 84–95.

**Tsai, C. C., and C. S. Chen. 2003.** First record of *Coptotermes gestroi* (Isoptera: Rhinotermitidae) from Taiwan. Formosan Entomol. 23: 157–161.

**Tsunoda, K. 2006.** Transfer of fipronil, a nonrepellent termiticide, from exposed workers of *Coptotermes formosanus* (Isoptera: Rhinotermitidae) to unexposed workers. Sociobiology 47: 563–575.

**Vargo, E. L., and V. Parman. 2012.** Effect of fipronil on subterranean termite colonies (Isoptera: Rhinotermitidae) in the field. J. Econ. Entomol. 105: 523–532.

**Yeoh, B. H., and C. Y. Lee. 2007.** Tunneling responses of the Asian subterranean termite, *Coptotermes gestroi* in termiticide-treated sand (Isoptera: Rhinotermitidae). Sociobiology 50: 457–468.

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