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Laboratory Evaluation of Two Chitin Synthesis Inhibitors, Hexaflumuron and Diflubenzuron, as Bait Toxicants Against Formosan and Eastern Subterranean Termites (Isoptera: Rhinotermitidae)

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ABSTRACT Two chitin synthesis inhibitors, hexaflumuron and diflubenzuron, were evaluated in a laboratory choice test for their potential as bait toxicants against the Formosan subterranean termite, *Coptotermes formosanus* Shiraki, and the eastern subterranean termite, *Reticulitermes flavipes* (Kollar). Concentrations of hexaflumuron that elicited feeding deterrence were >125 ppm for *C. formosanus* and >62.5 ppm for *R. flavipes*, whereas concentrations required to cause >90% mortality at 9 wk were >15.6 ppm and >2 ppm for *C. formosanus* and *R. flavipes*, respectively. Diflubenzuron deterred feeding of *C. formosanus* at such low concentrations (>2 ppm) that the highest recorded mortality was only 50% and is not likely to be effective in a bait against this termite species. More than 80% of *R. flavipes* workers died after feeding on diflubenzuron at >7.8 ppm, whereas feeding deterrence was recorded at >31.3 ppm. We conclude that hexaflumuron is superior to diflubenzuron as a bait toxicant because it is effective over a concentration range of 15.6–62.5 ppm. This concentration range is lethal and nondeterrent for both *C. formosanus* and *R. flavipes*. Diflubenzuron is efficacious against *R. flavipes* over a fairly narrow range of concentrations (7.8–31.3 ppm) and does not appear to be useful as a bait toxicant against *C. formosanus*.

KEY WORDS *Coptotermes formosanus*, *Reticulitermes flavipes*, insect growth regulators

POPULATIONS OF SUBTERRANEAN TERMITES can be suppressed using a baiting program to deliver an active ingredient that is both slow-acting and nondeterrent (Su et al. 1982). Insect growth regulators (IGRs) are promising candidates for bait control of termites because of their gradual and cumulative mode of action (Jones 1984, Su et al. 1985, Haverty et al. 1989, Su & Scheffrahn 1989). A literature review (Su & Scheffrahn 1990) showed that juvenile hormone analogs (JHAs) were more likely to induce significant presoldier formation for termite species that contained lower soldier proportions (e.g., 1–2% for *Reticulitermes* spp.) than for termite species with higher soldier proportions (e.g., 10–20% for *Coptotermes* spp.).

Another common class of IGRs includes derivatives of benzoylphenyl ureas (BPUs) that inhibit chitin synthesis (Hajjar & Casida 1978). These chitin synthesis inhibitors (CSIs) may be effective against many termite species. To date, only one CSI, diflubenzuron (Dimilin), has been tested against a limited number of termite species. A laboratory study (Doppelreiter & Korioth 1981) demonstrated ecdysis inhibition by diflubenzuron against *Heterotermes indicola* (Wasmann) and *Reticulitermes flavipes* (Kollar). Subsequent testing with diflubenzuron on field colonies of *Microcerotermes* species, however, provided inconclusive results (Faragalla et al. 1985). Hexaflumuron is one of the CSIs that has been tested against a variety of insect pests but not against termites.

In this study, we evaluated the potential of two CSIs, hexaflumuron and diflubenzuron, as bait toxicants against the Formosan subterranean termite, *Coptotermes formosanus* Shiraki, and the eastern subterranean termite, *R. flavipes*.

Materials and Methods

Technical grade hexaflumuron (98.7%, DowElanco, Indianapolis, IN) and diflubenzuron (95.0%, Duphar B.V., Amsterdam, Netherlands) were used in this study. Feeding deterrence and lethal effects of these CSIs were determined using a choice test similar to that described by Su & Scheffrahn (1989). Wooden cubes (8 cm^3, *Pinus* spp.) were dried at 80°C for 48 h and weighed (± 0.1 mg). The wooden cubes were then

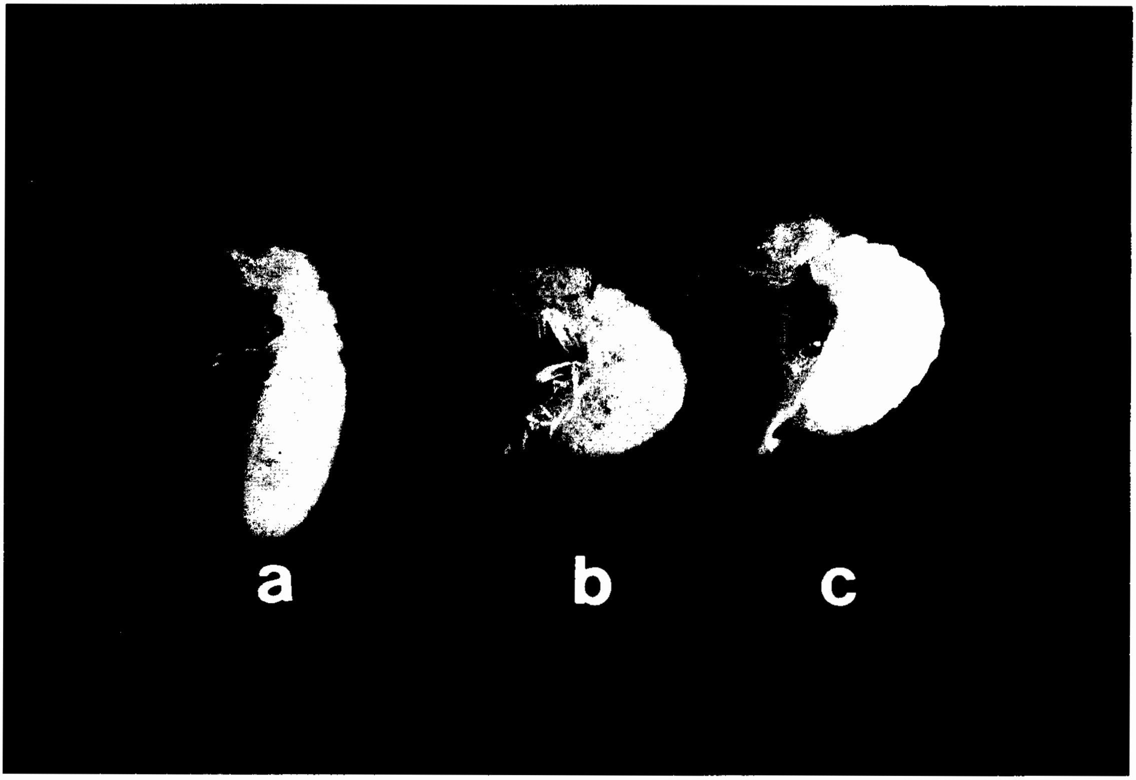

Fig. 1. Symptoms of ecdysis inhibition exhibited by workers of *C. formosanus* when exposed to one of the two CSIs hexaflumuron or diflubenzuron. Some affected workers were unable to shed exuviae fully, resulting in a "jackknife" pose (c), whereas others had their antennae (b) or appendages (a) cannibalized by the nestmates.

vacuum-impregnated (ASTM 1976) with acetone solutions of either CSI at desired concentrations. Concentrations of hexaflumuron used were 0, 0.25, 0.5, 1.0, 2.0, 3.9, 7.8, 15.6, 31.3, 62.5, 125, and 250 ppm (wt [AI]/cc acetone) for *R. flavipes*, and 0, 1.0, 2.0, 3.9, 7.8, 15.6, 31.3, 62.5, 125, 250, 500, and 1,000 ppm for *C. formosanus*. The latter concentration range was also used for diflubenzuron solutions in tests with both termite species. Acetone absorption in the wooden cube was approximately 1.48 (cc acetone/g wood) after the vacuum impregnation. The resultant CSI concentrations in wood ([AI] weight/wood weight), therefore, were 1.48 times the acetone solution concentrations. An experimental unit comprised a screw-top glass jar (6.0 cm diameter, 6.5 cm high) in which two feeding cubes (one treated, the other untreated) were placed 1.5 cm apart and covered with 75 ml sand moistened with 18 ml deionized water.

Termites of each species were collected from three field colonies by the method of Su & Scheffrahn (1986). One hundred workers (undifferentiated larvae of at least the third instar) plus five soldiers for *C. formosanus* or one soldier for *R. flavipes* were placed on the moistened sand in each unit. Six experimental units were prepared for each concentration (12) by species (2) by colony (3) by CSI (2) combination for a total of 864 units. Experimental units were held at 28 ± 1°C and in total darkness. At 3, 6, and 9 wk, six units (two subsamples from each of three colonies) were selected at random from each treatment combination (species by concentration by CSI) and were disassembled to count the number of surviving termites. Termites that exhibited molting inhibition were considered moribund and were excluded from the survivor count. Wood block remnants were rinsed under running water, oven-dried, and weighed as above. Mean differences in wood weight loss (mg) between treated and untreated blocks at 3 wk were compared for each compound by concentration by termite species combination using paired t tests (SAS Institute 1987). Significantly less feeding ($P < 0.05$) on treated cubes indicated feeding deterrence. Feeding data for 6 and 9 wk were not analyzed because termites were so severely affected by CSIs after 3 wk that their normal feeding behavior could not be adequately measured.

Results and Discussion

Three wk after exposure to hexaflumuron, symptoms of ecdysis inhibition were observed among workers of both termite species (Fig. 1).

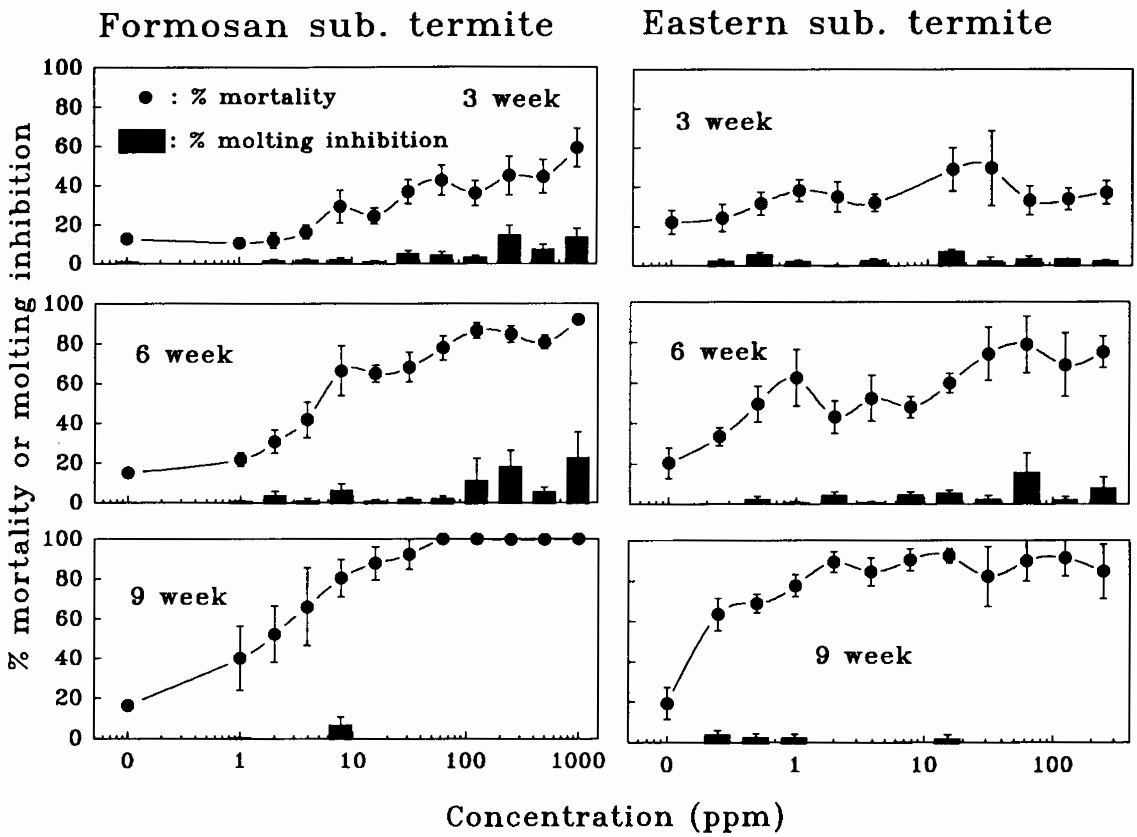

Fig. 2. Mean (± SE) percentage mortality and molting inhibition of the Formosan subterranean termite and eastern subterranean termite after exposure to hexaflumuron in a choice test for 3, 6, and 9 wk.

Some affected workers were typically enveloped by exuviae, resulting in a "jackknife" pose (Fig. 1c), whereas in some affected termites, antennae (Fig. 1b) or appendages (Fig. 1a) were apparently cannibalized by their nestmates. Termites often groom off and consume exuviae of molting nestmates (La Fage & Nutting 1978). Because the newly formed cuticle and exuviae were not completely separated, injuries incurred during the grooming process may lead to cannibalism by the groomers (Castle 1934, Williams 1959). Individuals enclosed in exuviae that escaped cannibalism eventually died after developing dark necrotic lesions.

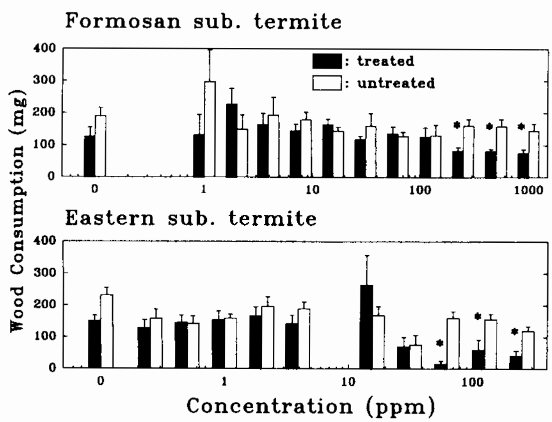

Fig. 3. Mean wood consumption (± SE) of untreated cubes and those treated with hexaflumuron after 3 wk. Asterisks denote a significant difference between untreated and treated cubes at $P < 0.05$ (paired t test), which implies feeding deterrence.

Mortality and wood consumption data of *R. flavipes* exposed to 7.8 ppm hexaflumuron for 3 wk were lost because of handling errors and are not presented in Figs. 2 and 3. Concentrations of hexaflumuron at thresholds that caused >90% mortality were 15.6 ppm and 2 ppm for *C. formosanus* and *R. flavipes*, respectively. As shown in Fig. 3, concentrations of hexaflumuron that elicited feeding deterrence were considerably higher (>125 ppm for *C. formosanus* and >62.5 ppm for *R. flavipes*) than concentrations required to cause 90% mortality. If impregnated in wood blocks as baits, a relatively wide range of concentrations can be used effectively for both *C. formosanus* (15.6–125 ppm) and *R. flavipes* (2–62.5 ppm).

The highest mortality of *C. formosanus* exposed to diflubenzuron was ≈50% at the end of the experiment (Fig. 4). Feeding by *C. formosa-*

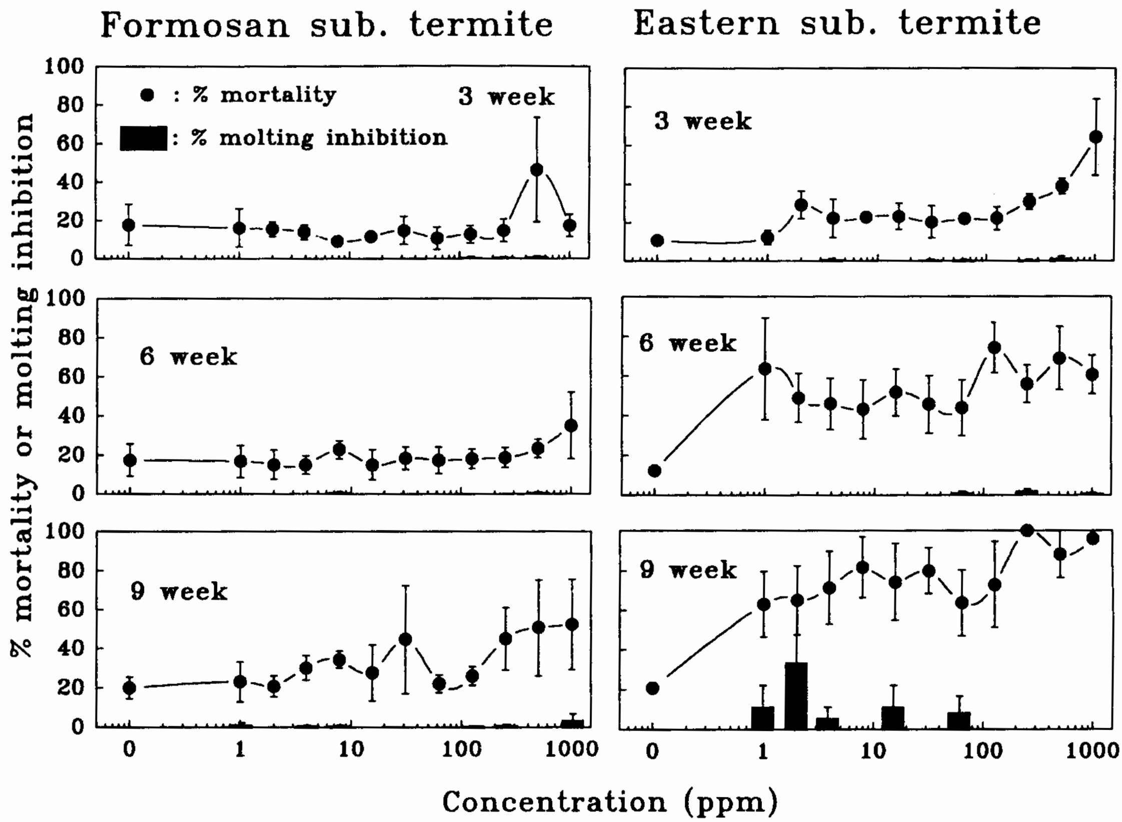

Fig. 4. Mean (± SE) percentage mortality and molting inhibition of the Formosan subterranean termite and eastern subterranean termite after exposure to diflubenzuron in a choice test for 3, 6, and 9 wk.

nus workers was deterred by diflubenzuron at concentrations >2 ppm (Fig. 5); thus, the majority probably did not ingest a lethal dose. At concentrations of 500 and 1,000 ppm diflubenzuron, differences in consumption by *C. formosanus*

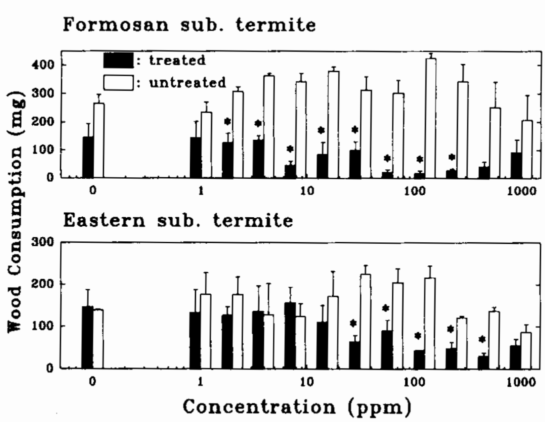

Fig. 5. Mean wood consumption (± SE) of untreated cubes and those treated with diflubenzuron after 3 wk. Asterisks denote a significant difference between untreated and treated cubes at $P < 0.05$ (paired t test), which implies feeding deterrence.

between treated and untreated cubes were significant only at $P < 0.10$. The overall low consumption of both treated and untreated cubes at 500 and 1,000 ppm probably contributed to the lower significance level. Diflubenzuron would not be an effective material in baits for *C. formosanus* because it inhibited feeding. This may explain the inconclusive results of field trials using diflubenzuron baits against *Microcerotermes* spp. (Faragalla et al. 1985), although its feeding deterrence against these species was not studied.

Feeding deterrence of diflubenzuron was also observed with *R. flavipes* at concentrations >31.3 ppm (Fig. 5). As with *C. formosanus,* differences in consumption between untreated wood and cubes treated with 1,000 ppm diflubenzuron were significant at $P < 0.10$, possibly related to the overall low consumption in these units. Because of the relatively high mortality (>80%) recorded among *R. flavipes* groups exposed to diflubenzuron at >2 ppm, a concentration range of 2–31.3 ppm may be most effective in baits.

This study showed that hexaflumuron is preferable to diflubenzuron for use in baits to control subterranean termites because of the wide range of efficacious concentrations. Mirex (dechlorane), a bait–toxicant that was used in field tests to suppress populations of *Reticulitermes* spp. (Esenther & Beal 1974, 1978), has an effective concentration range of 4–10 ppm for *C. formosanus* and 10–90 ppm for *R. flavipes* (Su & Scheffrahn 1991). Hexaflumuron may be superior to Mirex because its effective concentration range is wider for both termite species (15.6–125 ppm for *C. formosanus* and 2–62.6 ppm for *R. flavipes*). Another advantage of hexaflumuron is its apparent delayed toxicity (3–6 wk). Workers of *C. formosanus* select foraging sites at random; thus, within 8 wk, the majority of foragers may visit a given foraging site (Su et al. 1984). The extended lethal time of hexaflumuron may allow the majority of foragers to ingest a lethal dose before the onset of death.

Acknowledgments

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