TROPICAL PEST MANAGEMENT, 1992, 38(2) 176–180

Behavioural effects of a neem insecticide on *Coptotermes formosanus* (Isoptera: Rhinotermitidae)

(*Keywords:* Termite control, *Coptotermes formosanus*, neem, azadirachtin)

J. K. GRACE and J. R. YATES

Department of Entomology, University of Hawaii, 3050 Maile Way, Honolulu, Hawaii 96822, USA

Abstract. The neem tree, *Azadirachta indica*, is the source of azadirachtin and other compounds with potent insecticidal, feeding deterrent, and insect growth regulator activity. This study investigated the effects of a commercial insecticide formulation (Margosan-O) containing 0·3% azadirachtin and 14% neem oil on orientation, tunnelling, and feeding behaviour of the Formosan subterranean termite, *Coptotermes formosanus*. In short-term orientation assays, termite workers did not avoid papers treated with a 1000 ppm solution of azadirachtin. Termite workers also readily penetrated sand containing 20, 100, or 500 ppm azadirachtin, although significant mortality occurred in the 100 ppm treatment. However, subsequent observations indicated that termites avoided long-term contact with the treated sand. In two multiple-choice feeding assays, *C. formosanus* workers fed significantly less on papers containing azadirachtin concentrations $\geq$100 ppm. Azadirachtin, and possibly other neem oil components, thus show some toxicity, long-term repellency, and feeding deterrent activity towards *C. formosanus*, although the threshold for antifeedant effects is relatively high in comparison to thresholds for other insect species.

1. Introduction

The Formosan subterranean termite, *Coptotermes formosanus* Shiraki (Isoptera: Rhinotermitidae), is widely distributed throughout the tropics and subtropics, to approximately 35° north and south of the equator (Su and Tamashiro, 1987). This termite is an extremely serious pest of structures, and occasionally a pest in trees and agricultural crops. In Hawaii the annual cost attributable to *C. formosanus* damage and control are conservatively estimated to exceed US $60 million (Yates and Tamashiro, 1990). Cyclodiene soil insecticides are no longer available for use in the United States, and the insecticides currently applied to control or prevent subterranean termite infestations are less uniformly efficacious and less persistent (Tamashiro *et al.*, 1990). Thus, current research is focusing on alternative and environmentally acceptable methods and materials for termite control.

The neem tree, *Azadirachta indica* A. Juss (Meliaceae), is the source of the tetranortriterpenoid azadirachtin and other extractives of potential value in insect control (Schmutterer, 1988, 1990). This study was initiated to determine the potential for use of neem constituents in *C. formosanus* control. Published reports of the effects of azadirachtin and neem oil on termites are surprisingly few in number, and contradictory. Preliminary work in our laboratory with crude neem oil preparations suggested antifeedant effects on *C. formosanus*. Therefore, in order to quantify effects on termite orientation behaviour, tunnelling, and feeding, the present study employed a formulated commercial neem product, Margosan-O (Larsen, 1990), of known azadirachtin content.

2. Materials and methods

Formosan subterranean termites, *C. formosanus*, were collected as needed on the Manoa (Honolulu) campus of the University of Hawaii, using a trapping technique described by Tamashiro *et al.* (1973). In brief, foraging termites are collected in Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco) lumber placed on the soil surface and protected by a 5-gallon metal can. Termite workers (pseudergates, or undifferentiated individuals older than the third instar as determined by size) were used in laboratory bioassays within 6 h of collection from the field.

Bioassays were performed with dilutions of Margosan-O (W.R. Grace & Co., Cambridge, MA), an insecticide concentrate registered for use on non-food crops and trees by the United States Environmental Protection Agency, containing 3000 ppm (0·3% by weight) azadirachtin and 14% neem oil. During the assays, termites were maintained in crushed coral sand (pH 9·6) sieved to pass a US 20-mesh screen, in an unlighted temperature cabinet (29°C, ca. 80% RH).

2.1. Orientation behaviour

Orientation assays with individual *C. formosanus* workers were designed after those described by Grace (1989, 1990). In each assay, 40 μl of a 1000 ppm azadirachtin (a.i.) solution of Margosan-O in acetone were applied by pipette to 2·3 cm Whatman No. 3 filter paper disc. This disc was paired with a solvent-treated disc on either side of a 5 cm diameter glass Petri dish, and aired 15 min to evaporate the solvent. A single termite worker was then carefully placed in the centre of the Petri dish, and its position relative to the two discs recorded every 30 s for 20 min. Fifty workers were tested independently in separate Petri dishes. The number of individuals in contact with an azadirachtin-treated disc and a control disc at each 30-s observation were compared for each 5 min interval (total of ten observations per 5 min interval) by paired comparisons *t* tests at the 5% significance level (SAS Institute, 1987).

0143-6147/92 $3.00

2.2. Tunnelling behaviour

Termite workers were exposed to sand containing different levels of azadirachtin in an assay that mimicked the exposure of termite foragers to pesticide-treated soil under field conditions (Grace, 1991). The assay apparatus (Figure 1) had three compartments connected serially by 1 cm lengths of Tygon tubing: (1) a plastic vial containing *ca* 20 g untreated sand, 3 ml deionized water, 50 termites and a 1·5 × 2·5 cm length of wooden tongue depressor (Puritan No. 25-705, Hardwood Products Co., Guilford, ME) as food; (2) a glass sandwich-type tunnelling arena containing the treated sand; and (3) a second vial containing the same amounts of damp sand and cellulosic food as the first. The tunnelling arena consisted of two glass microscopic slides (2·5 × 7·5 cm) spaced 3–4 mm apart and secured in a horizontal upright position on one long edge by silicone rubber sealant (General Purpose Clear Sealant, Dow Corning Corp., Midland, MI) to a third flat glass slide as a base. The ends of the tunnelling arena were sealed with plastic spacers and silicone caulking, with a 1 cm long Tygon tube at the base of each end of the sandwich leading into the base of one of the two 55 ml (15 dram) polystyrene vials (60 × 35 mm diameter).

Concentrated Margosan-O was diluted in acetone and thoroughly mixed in oven-dry sand to achieve azadirachtin concentrations of 0 (acetone control), 20, 100, or 500 ppm (weight of azadirachtin/weight of sand). After evaporation of the acetone carrier, *ca.* 7 g of treated sand was poured into the tunnelling arena, and 1 ml water added by pipette along the open top edge. The addition of water visibly moistened the sand to the base of the tunnelling arena. The top edge of each tunnelling arena was sealed with plaster of paris to retain moisture, and 50 termite workers placed in one of the adjacent vials. The vials were capped (a heated insect pin was used to pierce air holes in the caps), and the three-chamber apparatus placed in the incubator. Each treatment was replicated four times. Ater 10 days, total tunnelling distance in the arena and termite mortality were recorded and subjected to analysis of variance (ANOVA), and means differing at the 5% level were separated by the Ryan–Einot–Gabriel–Welch (REGW) multiple *F* test (SAS Institute, 1987). Percentage mortality data were transformed by the arcsine of the square root prior to analysis.

2.3. Feeding deterrence

Two complementary two-choice feeding experiments were performed to evaluate feeding by *C. formosanus* workers on Whatman No. 2 filter papers treated with aqueous dilutions of Margosan-O. The dilute solutions were applied to achieve azadirachtin concentrations of 0 (control), 30, 100, 300, or 1000 ppm (weight of azadirachtin/weight of paper). In each assay a treated paper was paired with an untreated (water only) control paper, with three replicates per treatment.

In one-arena assay, a treated 4·25 cm diameter paper (*ca.* 130 mg) and a control paper were placed vertically (*ca.* 50% buried) along opposite sides of a 55 ml polystyrene vial (60 × 35 mm diameter) containing *ca.* 20 g sand, 3 ml water, and 50 *C. formosanus* workers. A three-arena assay without a tunnelling substrate was also developed to control for possible leaching of neem oil components into the damp sand. In this three arena assay, three disposable 9 cm diameter plastic Petri dishes were connected serially by 5 cm lengths of glass tubing inserted through the sides of each dish. A treated paper was placed in one end dish and a control paper in the other end dish, and 200 termite workers were added to the empty centre dish. In both experiments, termite mortality and mass loss from the papers were evaluated after 10 days of exposure in an unlighted temperature cabinet. Transformed (arcsine of the square root) mean percentage mortalities were subjected to ANOVA and the REGW multiple *F* test at the 5% level, and the difference in mass loss of treated and untreated papers in each treatment analysed by paired comparisons *t* test (SAS Institute, 1987).

3. Results and discussion

3.1. Orientation behaviour

Termite workers did not demonstrate either an initial negative orientation response to the papers treated with Margosan-O solution or any consistent avoidance behaviour over the 20-min assay period (Figure 2). No significant difference was noted in the number of termites in contact with treated and untreated papers (Table 1). Thus, azadirachtin and other neem oil components did not act as volatile or contact repellents at the relatively high concentration assayed (1000 ppm azadirachtin in solution).

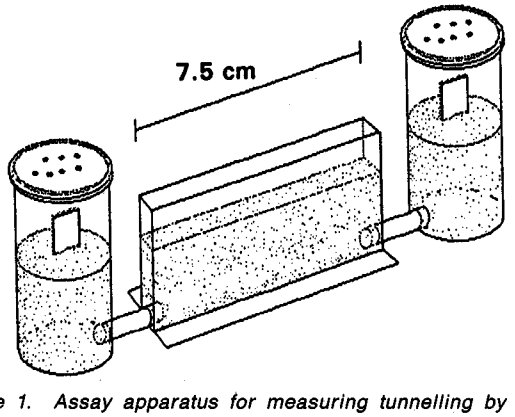

Figure 1. Assay apparatus for measuring tunnelling by termite workers through sand treated with formulated azadirachtin.

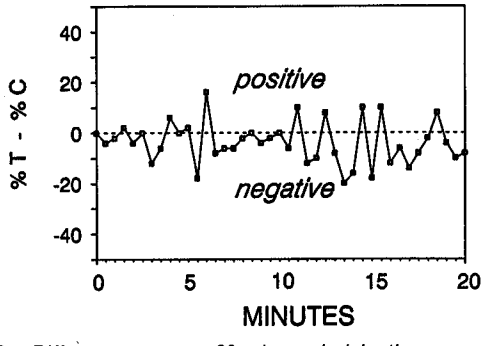

Figure 2. Difference over a 20-min period in the percentage of termite workers in contact with azadirachtin-treated (%T) and control papers (%C) in a two-choice orientation assay. Each 30-s observation represents fifty individual assays.

Table 1. Mean (± SE) numbers of C. formosanus *workers in contact with paper discs treated with a 1000 ppm azadirachtin solution during successive 5-min intervals (each disc paired with a control disc; positions of 50 individual workers recorded every 30 s, with each mean representing ten 30-s observations)*

Time interval (min)	Number of termites in contact with disc[1]	
	Treatment	Control
0–5	9·6 ± 0·4	10·5 ± 0·6
5.5–10	8·4 ± 0·8	9·9 ± 1·0
10·5–15	8·3 ± 0·9	11·4 ± 1·3
15·5–20	7·9 ± 0·6	10·2 ± 0·9

[1] Differences between treatment and control means for each time interval are not significant (paired comparisons *t* test, 5% level).

In contrast, neem oil and extractives have been reported to deter landing of plant-hoppers and oviposition by lepidopterous pests (reviewed by Schmutterer, 1990), and as repellents for a variety of other insect species (Warthen, 1989).

3.2. Tunnelling behaviour

Tunnelling was noted in each Margosan-O treatment. In each replicate where tunnelling occurred, *C. formosanus* workers completely penetrated the tunnelling arena. Total tunnelling distances in the treated sand did not differ significantly with azadirachtin concentration, nor did any of the Margosan-O treatments differ from the control (Table 2). As in the orientation assays, termites were not repelled by contact with the treated sand.

Extended observations of termite responses to the treated sand at the conclusion of the tunnelling assays, however, strongly suggest avoidance of long-term contact. The remaining termite workers exposed to each azadirachtin concentration were placed on damp filter paper in separate Petri dishes, along with a small amount of the sand from the tunnelling arena, to evaluate delayed mortality. After 7 days no significant mortality was noted. However, the workers had completely covered the sand containing 500 ppm azadirachtin with masticated filter paper. The 100 ppm sand was partially covered, and the 20 ppm sand was also partially but less thoroughly covered, while the control sand had not been covered with any masticated filter paper. Subterranean termites commonly bury diseased or infirm individuals and seal off portions of their foraging area to avoid contact with the undesirable stimuli (Su *et al.*, 1982).

Table 2. Tunnelling and mortality (mean ± SE) of C. formosanus *workers in sand treated with different dilutions of Margosan-O (ten days; four replicates of 50 workers per treatment)*

Azadirachtin concentration (ppm)[1]	Tunnel length (cm)[2]	Percentage mortality[3]
500	3·6 ± 1·6	7·5 ± 3·6b
100	4·9 ± 2·1	17·5 ± 3·0a
20	5·7 ± 1·9	9·0 ± 1·0ab
0	3·8 ± 2·2	6·0 ± 1·4b

[1] PPM based on weight of compound to weight of dry sand.
[2] Means are not significantly different (ANOVA, 5% level).
[3] Means followed by different letters are significantly different (ANOVA, REGW multiple *F* test, 5% level).

Although little termite mortality occurred during the 10-day assay period, mortality from exposure to 100 ppm azadirachtin was significantly greater than that observed in either the controls or the 500 ppm treatment (Table 2). The subsequent observations of long-term avoidance behaviour indicated that the rate with which *C. formosanus* covered the treated sand was concentration-dependent, which suggests that the declining mortality noted at 500 ppm may have resulted from rapid importation by the workers of untreated sand into the arena to line their tunnels, thus minimizing contact with the azadirachtin. A slower avoidance, or tunnel-lining, response to 100 ppm azadirachtin could lead to greater exposure to the treated sand, and thus a greater rate of mortality.

3.3. Feeding deterrence

In both the one-arena and three-arena feeding assays, *C. formosanus* workers fed significantly less on filter papers containing ≥100 ppm azadirachtin than on the corresponding control papers (Table 3). In the three-arena assay, with a greater number of workers per replicate, exposure to 300 ppm azadirachtin also elicited significant mortality, although no such trend was apparent in the one-arena assay.

Although these results demonstrate that azadirachtin and/or other neem oil components deter feeding by *C. formosanus*, the 100 ppm azadirachtin threshold greatly exceeds the antifeedant thresholds of 1 ppm or less reported for locusts (Butterworth and Morgan, 1968) and lepidopterous pests (Blaney *et al.*, 1990). Further, some limited feeding by *C. formosanus* was detected even with 1000 ppm azadirachtin, offering some explanation for the contradictory evidence in past reports. In India, *Coptotermes kishori* Roonwal & Chhotani has been found attacking trees, logs and dry stumps of *A. indica* (Sen-Sarma *et al.*, 1975). Butterworth and Morgan (1971), citing a personal communication from A. J. Flux, reported that wood impregnated with azadirachtin had no effect on *Reticulitermes santonensis* Feytaud feeding or survival; while Zanno *et al.* (1975) cited a personal communication from H. Roller indicating that azadirachtin was a potent antifeedant for the termite *Teticuli termis* (*sic*, probably a misspelling of *Reticulitermes* sp.).

4. Conclusions

Although *C. formosanus* readily tunnelled through sand containing up to 500 ppm azadirachtin, chronic exposure appeared to elicit both avoidance behaviour and gradual mortality. It is unlikely that these subtle effects would prove useful in soil applications for termite control. Neither was Margosan-O active as a contact repellant to *C. formosanus*.

Table 3. Feeding and mortality (mean ± SE) of C. formosanus *workers after 10 days on filter papers treated with Margosan-O in a one arena assay (three replicates of 50 workers per treatment) and a three-arena assay (three replicates of 200 workers per treatment)*

Azadirachtin concentration (ppm)[1]	One-arena assay				Three-Arena Assay			
	Percentage mortality[2]	Paper mass loss (mg)			Percentage mortality[2]	Paper mass loss (mg)		
		Treated	Untreated	$P > t$		Treated	Untreated	$P > t$
1000	10·0 ± 2·0a	12·8 ± 2·2	61·2 ± 7·0	0·034*	20·5 ± 2·7ab	12·9 ± 0·8	67·6 ± 3·3	0·003*
300	14·0 ± 1·2a	17·3 ± 2·0	47·7 ± 2·6	0·009*	37·0 ± 6·2a	11·2 ± 2·5	78·0 ± 14·3	0·033*
100	6·0 ± 2·3a	23·3 ± 2·9	45·8 ± 3·8	0·002*	22·0 ± 2·3ab	28·1 ± 3·8	89·6 ± 5·0	0·005*
30	9·3 ± 1·3a	33·7 ± 3·6	40·1 ± 2·3	0·300	15·2 ± 8·0ab	28·2 ± 4·2	50·7 ± 0·7	0·172
10	14·0 ± 8·3a	39·9 ± 3·8	36·8 ± 8·5	0·813	8·2 ± 2·6b	56·7 ± 10·7	51·6 ± 11·7	0·813
0	6·0 ± 4·2a	30·1 ± 4·4	45·0 ± 9·6	0·370	7·0 ± 0·9b	66·2 ± 4·7	46·7 ± 11·2	0·335

[1] PPM based on weight of compound to weight of paper.
[2] Means within a column followed by the same letter are not significantly different (ANOVA, 5% level).
* Difference in mean mass loss of treated and untreated papers is significant at the 5% level (paired comparisons *t* test).

However, the antifeedant activity of this material suggests its possibile utility in wood preservation, although feeding deterrence was only apparent with azadirachtin concentrations ≥100 ppm, and some feeding occurred even with 1000 ppm. Exploitation of this antifeedant activity would necessitate either a breakthrough in formulation to chemically protect the azadirachtin molecule from degradation or frequent reapplication of the insecticide, since the field life of the current azadirachtin formulation is less than 1 month (Larson, 1990; Walter and Knauss, 1990). In addition to its azadirachtin content, Margosan-O contains 14% neem oil, and other neem oil constituents (Schmutterer, 1990) may also be involved in eliciting termite feeding deterrence.

A variety of slow-acting toxicants are currently of interest as potential bait toxicants for subterranean termite control (Grace *et al.*, 1990; Su and Scheffrahn, 1991), and the relatively high antifeedant threshold of azadirachtin for *C. formosanus* suggests that examination of the long-term toxicity and growth regulator effects of neem constituents on termites could be of value. Such growth regulator effects are well known with other insect species (Subrahmanyam, 1990), and would be compatible with a baiting approach to termite control.

Acknowledgements

Journal Series No. 3630 of the Hawaii Institute of Tropical Agriculture and Human Resources. We thank R. Ebesu and C. Lai for technical assistance, and D. Ullman for providing the Margosan-O sample. Tunnelling and orientation assays were performed by M.-L. Hokama as a participant in the National Science Foundation Young Scholars Program. Financial support was provided by McIntire-Stennis funds and a University Research Council Seed Grant to J.K.G. Mention of trade names is for informational purposes only and does not constitute an endorsement by funding agencies or the University of Hawaii.

References

BLANEY, W. M., SIMMONDS, M. S. J., LEY, S. V., ANDERSON, J. C. and TOOGOOD, P. L., 1990. Antifeedant effects of azadirachtin and structurally related compounds on lepidopterous larvae. *Entomologia Experimentalis et Applicata*, **55**, 149–160.

BUTTERWORTH, J. H. and MORGAN, E. D., 1968. Isolation of a substance that suppresses feeding locusts. *Journal of the Chemical Society, Chemical Communications*, pp. 23–24.

BUTTERWORTH, J. H. and MORGAN, E. D., 1971. Investigation of the locust feeding inhibition of seeds of the neem tree, *Azadirachta indica*. *Journal of Insect Physiology*, **17**, 969–977.

GRACE, J. K., 1989. Habituation in termite orientation response to fungal semiochemicals. *Sociobiology*, **16**, 165–182.

GRACE, J. K., 1990. Effect of antioxidants on eastern subterranean termite (Isoptera: Rhinotermitidae) orientation to fungal extract. *Proceedings of the Entomological Society of Washington*, **92**, 773–777.

GRACE, J. K., 1991. Comparative response of *Reticulitermes flavipes* and *Coptotermes formosanus* to borate soil treatments. Document No. IRG/WP/1486. International Research Group on Wood Preservation; IRG Secretariat; Stockholm.

GRACE, J. K., ABDALLAY, A., and SISSON, J. M., 1990. Preliminary evaluation of borate baits and dusts for eastern subterranean termite control. Document No. IRG/WP/1433. International Research Group on Wood Preservation; IRG Secretariat; Stockholm.

LARSON, R. O., 1990. Commercialization of the neem extract Margosan-O in a USDA collaboration. In *Neem's Potential in Pest Management Programs, Proceedings of the USDA Neem Workshop* (J. C. Locke and R. H. Lawson, Eds) (United States Department of Agriculture, Agricultural Research Service), ARS-86, pp. 23–28.

SAS INSTITUTE, 1987. *SAS/STAT Guide for Personal Computers, Version 6 Edition* (Cary, North Carolina: SAS Institute Inc.).

SCHMUTTERER, H., 1988. Potential of azadirachtin-containing pesticides for integrated pest control in developing and industrialized countries. *Journal of Insect Physiology*, **34**, 713–719.

SCHMUTTERER, H., 1990. Properties and potential of natural pesticides from the neem tree, *Azadirachta indica*. *Annual Review of Entomology*, **35**, 271–297.

SEN-SARMA, P. K., THAKUR, M. L., MISRA, S. C. and GUPTA, B. K., 1975. *Wood Destroying Termites of India* (Dehra Dun: Forest Research Institute & Colleges).

SUN, N.-Y. and SCHEFFRAHN, R. H., 1991. Laboratory evaluation of two slow-acting toxicants against Formosan and eastern subterranean termites (Isoptera: Rhinotermitidae). *Journal of Economic Entomology*, **84**, 170–175.

SU, N.-Y. and TAMASHIRO, M., 1987. An overview of the Formosan subterranean termite (Isoptera: Rhinotermitidae) in the world. In *Biology and Control of the Formosan Subterranean Termite* (M. Tamashiro and N.-Y. Su, Eds). Hawaii Institute of Tropical Agriculture and Human Resources Research Extension Series 083; University of Hawaii; Honolulu, pp. 3–15.

SU. N.-Y., TAMASHIRO, M., YATES, R. J. and HAVERTY, M. I., 1982. Effect of behavior on the evaluation of insecticides for prevention of or remedial control of the Formosan subterranean termite. *Journal of Economic Entomology*, **75**, 188–193.

SUBRAHMANYAM, B., 1990. Azadirachtin—a naturally occurring insect growth regulator. *Proceedings of the Indian Academy of Sciences (Animal Sciences)*, **99**, 277–288.

TAMASHIRO, M., FUJII, J. K. and LAI, P.-Y., 1973. A simple method to observe, trap, and prepare large numbers of subterranean termites for laboratory and field experiments. *Environmental Entomology*, **2**, 721–722.

TAMASHIRO, M., YATES, J. R., EBESU, R. H. and YAMAMOTO, R. T., 1990. Effectiveness and longevity of termiticides in Hawaii. Hawaii Institute of Tropical Agriculture and Human Resources Research Extension Series 119; University of Hawaii; Honolulu.

WALTERS, J. F. and KNAUSS, J. F., 1990. Developing a neem-based pest management product. In *Neem's Potential in Pest Management Programs, Proceedings of the USDA Neem Workshop* (J. C. Locke and R. H. Lawson, Eds). (United States Department of Agriculture, Agricultural Research Service). ARS-86, pp. 23–28.

WARTHEN, J. D., Jr, 1989. Neem (*Azadirachta indica* A. Juss): Organisms affected and reference list update. *Proceedings of the Entomological Society of Washington*, **91**, 367–388.

YATES, J. R. and TAMASHIRO, M., 1990. The Formosan subterranean termite in Hawaii. Hawaii Institute of Tropical Agriculture and Human Resources Research Extension Series 117; University of Hawaii; Honolulu.

ZANNO, P. R., MIURA, I. NAKANISHI, K. and ELDER, D. L., 1975. Structure of the insect phagorepellent azadirachtin. Application of PRFTCWD carbon-13 nuclear magnetic resonance. *Journal of the American Chemical Society*, **97**, 1975–1977.