Concentration-Dependent Presoldier Induction and Feeding Deterrency: Potential of Two Insect Growth Regulators for Remedial Control of the Formosan Subterranean Termite (Isoptera: Rhinotermitidae)

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ABSTRACT Laboratory experimental groups of 120 workers and 30 soldiers of *Coptotermes formosanus* Shiraki were given a choice of feeding on untreated pine blocks or pine blocks treated with one of five concentrations of methoprene (0, 4, 20, 100, and 500 ppm) or S-31183 (2-[1-methyl-2(4-phenoxy-phenoxy)ethoxy]pyridine) (0, 20, 100, 500, and 2,500 ppm) for 4, 8, or 12 wk. Soldier production and total mortality were significantly increased by 100 and 500 ppm methoprene at 8 and 12 wk. Biologically significant mortality (>50%) resulted from feeding on blocks treated with methoprene at 500 ppm after 8 wk. The response of *C. formosanus* to S-31183 was much reduced when compared with the response to methoprene. None of the concentrations of S-31183 caused biologically significant mortality to the *C. formosanus* groups. In addition, the highest concentration of S-31183 caused feeding deterrency that was apparently learned. Effective baits for remedial control of *C. formosanus* colonies should be evaluated under conditions that simulate actual use. These baits would require methoprene concentrations from 500 to 1,500 ppm, depending on the pattern of use.

KEY WORDS Insecta, caste differentiation, termite control, bait-block technique

INJECTION OF INSECTICIDES with a high residual toxicity is a control measure for structural infestations of subterranean termites, including the Formosan subterranean termite, *Coptotermes formosanus* Shiraki. This treatment creates an impenetrable, chemical barrier between the soil and the structure at risk. However, when a source of moisture is available aboveground, *C. formosanus* colonies are able to survive even though they no longer have contact with the soil. For such aerial colonies, soil treatments are ineffective.

Three approaches (injection of aerosol insecticides directly into galleries, fumigation of the entire structure, and placement of poison baits in foraging galleries) are possible for control of aerial colonies. Injection of insecticides into the galleries is a localized treatment and is not considered effective against large colonies with an extensive gallery system. Fumigation with sulfuryl fluoride can be used in some situations, however, the size of the structure may make fumigation physically and economically unacceptable. If effective, remedial control with baits is the most reasonable alternative. Control of colonies of *Reticulitermes* spp. in the soil has apparently been achieved with baits impregnated with mirex (Esenther & Beal 1974, 1978). Control of *C. formosanus* infestations in structures has also been successful with mirex baits (Gao 1987). However, use of mirex is now illegal in many countries including the United States.

Insect growth regulators (IGRs) have potential for use in baits for remedial control of subterranean termites. Their delayed action and apparent nonrepellency make them attractive candidates as termiticides (Howard & Haverty 1978, 1979b; Haverty & Howard 1979; Su et al. 1982, 1985, 1987; Howard 1983; Jones 1984, 1987a). IGRs have unusual, concentration-dependent modes of action: defaunation and starvation, induction of presoldier and intercaste development, and ecdysis inhibition (Howard & Haverty 1979a, Su & Scheffrahn in press a). Methoprene is among the few IGRs commercially available. Su et al. (1985) tested methoprene against *C. formosanus* in the laboratory. Over a 2-wk period, methoprene (500, 1,000, and 1,500 ppm) caused significant mortality of workers and soldiers and a significant increase in presoldier production. Jones (1987b) found similar results over a period of 17 wk. Neither Su et al. (1985) nor Jones (1987b), however, found statistically significant differences in mortality or soldier differentiation among methoprene concentrations.

Change in mortality or caste differentiation in *C. formosanus* as concentration is lowered or increased has not been reported for any IGR. Su et al. (1985) and Jones (1987b) found no statistically significant differences in response of *C. formosanus*

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to methoprene in concentrations from 500 to 2,000 ppm. Baits with an excessively high methoprene concentration may repel *C. formosanus* or cause this species to stop feeding on baits (Jones 1987b). Identification of the lowest effective concentration is critical to optimize efficacy of treatments with IGRs and baits. As baits age, IGRs will likely degrade and the remaining quantity may decrease below an effective level. Thus, our goal is to define a range of concentrations that will remain effective for the life of the bait, yet cause no initial antifeedant or repellent response.

Haverty (1979b) demonstrated that if percentages of *C. formosanus* soldiers are artificially doubled (from 24 to 48%), group survival is significantly reduced; however, groups can survive for 12 wk. If proportions of soldiers were tripled (from 24 to 72%), 80% of laboratory experimental groups succumbed. At these soldier proportions, the remaining workers are apparently unable to feed the soldiers or to cannibalize the excess soldiers. As a result, many of the soldiers die and decompose in the foraging galleries. Excessive soldier mortality then leads to microbial contamination and total mortality of the colony.

Here we report an evaluation of the effects of five concentrations each of two insect growth regulators against *C. formosanus*. Our objective was to identify acceptable IGR concentrations that cause excessive, biologically significant mortality (>50%, Haverty 1979b) by induction of superfluous presoldiers and to eliminate IGR concentrations that are high enough to elicit feeding deterrency.

Materials and Methods

Two IGRs—methoprene (Zoecon Industries, Dallas) and S-31183 (2-[1-methyl-2(4-phenoxyphenoxy)ethoxy]pyridine) (Sumitomo Chemical Company, Higashi-ku, Japan)—were tested. Both were technical grade. Termites were collected from three field colonies on the University of Hawaii campus, Honolulu, and from one field colony in Kaneohe, Hawaii. Termites were sorted in the laboratory as described by Tamashiro et al. (1973). Ponderosa pine (*Pinus ponderosa* Douglas ex Laws) blocks (1.8 by 1.8 by 1.8 cm) were cut from sapwood boards. Blocks were allowed to absorb moisture at ambient laboratory humidity for 48 h, then weighed. Blocks were vacuum impregnated with acetone or acetone solutions of one of the two IGRs (ASTM 1976). The estimated resulting concentrations in the blocks were 0, 4, 20, 100, and 500 ppm (AI) for methoprene and 0, 20, 100, 500, and 2,500 ppm (AI) for S-31183. To allow complete evaporation of the acetone, the treated blocks were air dried for at least 24 h before testing. Three subsamples were prepared for each treatment combination (five concentrations × three observation periods × four colonies).

A total of 150 termites (120 workers, undifferentiated larvae of at least the third instar, and 30 soldiers) was introduced into circular, plastic containers (9 cm diameter by 3 cm high, 200 ml volume) containing one treated and one untreated block covered with 70 cm^3 (approximately 100 g) of washed Monterey sand, moistened with 14 ml deionized water (Haverty 1979a). All blocks were numbered and treated blocks were identified by placing a mark on the outside of the container adjacent to the treated block. All experimental units were stored at 29 ± 1°C for 4, 8, or 12 wk and then disassembled. The numbers of surviving workers, soldiers, and presoldiers were recorded. At the end of each observation period, individual blocks were placed in a labeled, air-tight plastic film canister, shipped to Berkeley, Calif., and stored at −20°C. All blocks were cleaned of sand and termite fecal material, renumbered (if necessary), air dried to ambient laboratory humidity for at least 72 h, and then reweighed.

The experiment was evaluated as separate, one-way analyses of variance for each IGR and observation period (SAS Institute 1987), using a randomized complete block design with concentration × colony interaction as the error term. Concentration was the treatment effect of interest and colony was the blocking factor. Response variables were percentage of reduction in workers (dead workers plus those that molted into the presoldier caste), percentage of total mortality (worker + soldier), percentage of dependent castes (presoldier and soldier), and number of presoldiers remaining at the end of the observation period. Percentages were transformed to $\log(1 + y)$ before analysis. Significant differences among means were identified by Student–Newman–Keuls test ($\alpha = 0.05$, SAS Institute 1987). Differences in weight loss between treated and untreated blocks in the same container were compared by a paired t test ($\alpha = 0.05$) for each treatment combination (IGR × concentration × observation period) to ascertain feeding deterrency.

Results and Discussion

A statistically significant increase in the production of presoldiers was caused by the 100- and 500-ppm methoprene treatments at 8 wk (Table 1). This substantial increase in presoldier (and presumably soldier) production was responsible for the large proportion of dependent castes at the last two observation periods. Two methoprene treatments (4 and 20 ppm) did not cause significant increases in either the number of presoldiers or the percentage of dependent castes. In fact, the soldier percentage was 20% at the beginning of the study and never varied significantly from that rate in the 0-, 4-, or 20-ppm methoprene treatments during the study.

Percentage of worker reduction and percentage of total mortality ranged from 8.0% at 4 wk to 15.9% at the end of 12 wk for the control (0 ppm methoprene) (Table 2). With 4 and 20 ppm meth-

Table 1. Number of presoldiers and percentage of dependent castes (soldiers and presoldiers) present in groups of *C. formosanus* after exposure to pine blocks in a choice test

IGR	Concentration, ppm	4 Weeks		8 Weeks		12 Weeks	
		No. presoldier	% Dependent caste	No. presoldier	% Dependent caste	No. presoldier	% Dependent caste
Methoprene	0	0.0 ± 0.0a	19.8 ± 0.7a	0.3 ± 0.2a	20.5 ± 0.8a	0.0 ± 0.0a	19.3 ± 0.9a
	4	0.0 ± 0.0a	19.0 ± 0.7a	0.2 ± 0.1a	19.5 ± 1.0a	0.3 ± 0.2a	20.0 ± 0.7a
	20	0.0 ± 0.0a	18.9 ± 0.4a	0.0 ± 0.0a	19.8 ± 0.6a	0.2 ± 0.1a	20.8 ± 0.6a
	100	1.1 ± 0.5a	19.2 ± 1.0a	1.8 ± 1.5b	24.7 ± 0.9b	2.8 ± 1.0a	29.1 ± 2.3b
	500	2.5 ± 1.0a	21.3 ± 0.5a	4.7 ± 1.8b	29.1 ± 2.6b	6.3 ± 3.0a	47.3 ± 3.0c
S-31183	0	0.2 ± 0.1a	19.2 ± 0.7a	0.6 ± 0.4a	18.7 ± 0.8a	0.1 ± 0.1a	19.4 ± 1.1a
	20	0.1 ± 0.1a	19.0 ± 0.6a	0.2 ± 0.2a	19.8 ± 0.6ab	0.3 ± 0.1a	19.8 ± 0.5a
	100	0.0 ± 0.0a	19.3 ± 0.7a	0.1 ± 0.1a	20.9 ± 0.7ab	0.5 ± 0.2a	20.9 ± 1.8a
	500	0.5 ± 0.4a	19.8 ± 0.8a	1.9 ± 0.9a	22.9 ± 1.4b	3.5 ± 1.1b	23.9 ± 1.6a
	2,500	3.5 ± 3.1a	21.9 ± 2.3a	2.9 ± 1.2a	23.3 ± 1.8b	2.0 ± 0.6ab	24.6 ± 1.0a

$\bar{x}$ ± SEM. Means are based on 12 observations (four colonies with three subsamples each). Means in a column for each IGR × observation period followed by the same letter are not significantly different ($\alpha = 0.05$ level; Student–Newman–Keuls test [SAS Institute 1987]).

oprene, no statistically significant increase in percentage of worker reduction or percentage of total mortality occurred during any of the observation periods. For the 100- and 500-ppm methoprene treatments, reduction in the number of workers was significantly greater than the control after 8 and 12 wk. These significant decreases in worker numbers were a result of differentiation of workers into presoldiers and eventually soldiers, as well as mortality of workers caused by incomplete ecdysis and toxic effects of methoprene to the termites or their symbiotic protozoans (Haverty & Howard 1979). At 100 ppm, the percentage of total mortality caused by methoprene was significantly greater than the next lower concentration, but not high enough to threaten the survival of these laboratory groups.

For methoprene at 500 ppm, total mortality at 8 and 12 wk was substantial (72.6 and 73.7%, respectively) and 47.3% of the surviving individuals were soldiers at 12 wk. This level of mortality and increase in the proportion of dependent castes is statistically and biologically significant. Such a large proportion of soldiers would likely be eliminated from the colony's population (Haverty 1979b). We suspect that dependent caste proportion and percentage of total mortality for 500 ppm methoprene indicates an irreversible decline. Therefore, for methoprene 500 ppm is apparently an effective, threshold concentration for induction of increased proportions of soldiers and increased mortality. Lack of feeding deterrency observed from this treatment suggests its suitability for bait formulations.

S-31183 was much less effective in causing presoldier development in *C. formosanus*. At 4 and 8 wk, none of the S-31183 treatments resulted in a statistically significant increase in the production of presoldiers (Table 1). At 12 wk, however, 500 ppm resulted in a significantly greater number of presoldiers than the lower treatments, which were not statistically different from the untreated control. Increased presoldier induction in the 500 and 2,500 ppm treatments of S-31183 caused a statistically significant increase in the percentage of dependent castes during the 8-wk observation period, but not during the 12-wk period. These increases

Table 2. Percentage of worker reduction and percentage of total mortality in groups of *C. formosanus* after exposure to pine blocks in a choice test

IGR	Concentration, ppm	4 Weeks		8 Weeks		12 Weeks	
		Worker	Total	Worker	Total	Worker	Total
Methoprene	0	8.5 ± 1.6ab	8.8 ± 1.3ab	13.4 ± 1.8a	12.9 ± 1.5a	15.2 ± 1.6a	15.9 ± 1.7ab
	4	8.2 ± 1.4ab	9.3 ± 1.4ab	12.8 ± 1.9a	13.3 ± 1.6a	14.5 ± 1.8a	14.4 ± 1.8a
	20	5.2 ± 0.9b	6.4 ± 1.1b	10.5 ± 1.3a	10.7 ± 1.4a	14.0 ± 2.0a	13.2 ± 1.7a
	100	9.0 ± 1.2ab	9.8 ± 1.2a	33.2 ± 7.6b	29.1 ± 7.5b	44.4 ± 8.3b	37.2 ± 9.2b
	500	12.8 ± 1.4a	11.4 ± 1.1a	76.4 ± 8.7c	72.6 ± 10.2c	82.8 ± 6.6c	73.7 ± 10.0c
S-31183	0	8.0 ± 0.9a	8.8 ± 1.2a	10.5 ± 1.4a	11.8 ± 1.7a	14.0 ± 2.0a	14.7 ± 1.6a
	20	7.2 ± 1.1a	8.2 ± 1.1a	10.6 ± 1.8a	10.7 ± 1.8a	13.0 ± 1.3a	13.2 ± 1.4a
	100	8.8 ± 1.4a	9.6 ± 1.4a	14.3 ± 1.9b	13.4 ± 1.5a	20.1 ± 4.1a	19.6 ± 3.3a
	500	11.4 ± 1.3a	11.5 ± 1.5a	23.1 ± 2.8c	20.2 ± 2.5b	35.6 ± 9.3a	32.5 ± 9.7a
	2,500	15.6 ± 3.7a	13.1 ± 3.0a	28.5 ± 4.9c	25.4 ± 4.6b	32.7 ± 4.8a	28.9 ± 4.5a

$\bar{x}$ ± SEM. Means are based on 12 observations (four colonies with three subsamples each). Means in a column for each IGR × observation period followed by the same letter are not significantly different ($\alpha = 0.05$ level; Student–Newman–Keuls test [SAS Institute 1987]).

Table 3. Weight loss (mg) of treated pine blocks (T) or untreated pine blocks (U) after feeding by *C. formosanus* in a choice test

IGR	Concentration, ppm	Wood weight loss (mg) and t statistics								
		4 Weeks			8 Weeks			12 Weeks		
		T	U	t^a	T	U	t^a	T	U	t^a
Methoprene	0	482 ± 49	380 ± 41	−1.21	579 ± 64	604 ± 72	0.19	790 ± 49	447 ± 50	−0.95
	4	492 ± 45	447 ± 44	−0.75	690 ± 62	547 ± 57	−1.25	877 ± 61	670 ± 65	−1.66
	20	485 ± 62	426 ± 52	−0.54	620 ± 56	576 ± 69	−0.38	849 ± 63	712 ± 53	−1.28
	100	425 ± 34	309 ± 42	−1.69	486 ± 40	524 ± 74	0.38	624 ± 41	548 ± 80	−0.54
	500	287 ± 27	378 ± 54	1.17	385 ± 31	381 ± 76	−0.03	373 ± 19	538 ± 103	1.57
S-31183	0	404 ± 40	537 ± 43	1.68	667 ± 63	591 ± 67	−0.61	954 ± 73	738 ± 93	−1.59
	20	470 ± 54	442 ± 58	−0.26	644 ± 64	595 ± 68	−0.39	874 ± 65	767 ± 83	−0.78
	100	519 ± 53	409 ± 51	−1.10	568 ± 71	637 ± 86	0.46	759 ± 78	675 ± 78	−0.56
	500	370 ± 33	385 ± 50	0.39	442 ± 53	604 ± 90	1.17	475 ± 54	736 ± 112	1.57
	2,500	213 ± 14	614 ± 28	10.0	265 ± 19	735 ± 59	6.94	253 ± 11	1,109 ± 50	15.6

$\bar{x}$ ± SEM. Means are based on 12 observations (four colonies with three subsamples each).
[a] For 12 observations, the critical t value ($\alpha = 0.05$) is 2.201.

in the percentage of dependent castes were not large enough to be considered biologically significant (i.e., the increase in number of dependent castes from 20% to ≤25% would not likely cause mortality because of starvation of the soldiers). As was observed in methoprene treatments, the two lowest concentrations of S-31183 had percentages of dependent castes equivalent to the control (0 ppm), i.e., about 20%.

Except at the highest concentrations, the pattern of mortality caused by S-31183 was similar to that of the methoprene treatments (Table 2). At 4 wks, none of the treatments significantly increased mortality or reduction of the worker population. At 8 wk, the percentage of worker reduction and percentage of total mortality for the 500- and 2,500-ppm S-31183 treatments were equivalent, but significantly greater than the other S-31183 treatments ≤100 ppm. At 12 wk, total mortality resulting from 2,500-ppm S-31183 was not significantly different from any of the other S-31183 treatments including the control. Lack of significant differences was probably related to feeding deterrency caused by this high concentration of S-31183 (Table 3).

This experiment was designed as a choice-feeding test; *C. formosanus* workers could feed on either an untreated ponderosa pine block or a block treated with IGR. Only S-31183 at 2,500 ppm resulted in a significantly lower weight loss at all observation times than the untreated blocks in the same container (Table 3). Reduced feeding was not total; some of each treated block was consumed. The consumption of the treated block apparently did not increase over time; the amount of the treated blocks consumed at 4, 8, and 12 wk was about the same. The workers apparently shifted their feeding activities to the untreated blocks because a significantly greater amount of these untreated blocks was consumed at each observation period (Table 3). We speculate that the termites learned to associate this treatment with the physiological effect (Su & Scheffrahn 1988).

In summary, S-31183 did not appear as promising as methoprene for *C. formosanus* control. At 500 ppm, presoldier production with pine blocks treated with S-31183 is insufficient to cause biologically significant (>50%) mortality. At the highest concentration (2,500 ppm), feeding on treated wood was reduced compared with untreated blocks. An effective, nonrepellent concentration might be found between 500 and 2,500 ppm, but we did not test for that in this study. Comparison of the effects of S-31183 against two subterranean termite species also suggests effective concentrations may be between 300 to 1,500 ppm for *C. formosanus* (Su & Scheffrahn in press b).

In contrast, methoprene appears to have potential as a slow-acting, nonrepellent, termiticide for control of *C. formosanus* in bait formulations at concentrations of at least 500 ppm. Formulations as high as 1,500 ppm do not cause feeding deterrency or repellency (Su et al. 1985, Jones 1987b). In a slow-acting bait, incorporation of the appropriate concentration is important. We recommend further testing of methoprene under field conditions. Baits with methoprene at 500 ppm should be used if they are to be replaced frequently. Baits with methoprene at 1,000 to 1,500 ppm would be more appropriate if they are placed in soil or infrequently replaced.

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