Defaunation, Mortality and Soldier Differentiation: Concentration Effects of Methoprene in a Termite

Ralph W. Howard[1]
and
Michael I. Haverty[2]

ABSTRACT

A juvenile hormone analog triggers various concentration-dependent, lethal effects in *Reticulitermes flavipes* (Kollar). Mortality after feeding on paper pads with high concentrations of Methoprene resulted from toxicity to the termites and/or their symbiotic protozoa or from incomplete ecdysis. At lower concentrations mortality was due to starvation from defaunation or production of superfluous soldiers.

INTRODUCTION

Effects of juvenile hormone analogs (JHA's) on caste proportion in laboratory colonies of termites have been the subject of several recent investigations(*1*). Among the Rhinotermitidae JHA's have been tested against *Reticulitermes flaviceps* (Oshima), *R. lucifugus* (Rossi), *R. lucifugus santonensis* (Rossi), *R. flavipes* (Kollar), *Heterotermes indicola* (Wasmann), *Coptotermes amanii* (Sjostedt), and *C. niger* (Snyder). Concentration responses were only briefly examined with these species and in no case was an effect on symbiotic protozoa determined. We report here our findings regarding these relationships for the eastern subterranean termite *R. flavipes*.

METHODS AND MATERIALS

Concentration effects of Methoprene on soldier differentiation and mortality were assessed by holding groups of 30 externally undifferentiated larvae of at least the third instar (*2*) on treated filter pads for 28 days (*3*). Each concentration was replicated 5 times (*3*). Live and dead insects were recorded by caste at 3-day intervals. Dead bodies were removed to avoid excessive bacterial and fungal contamination.

RESULTS

We assume most observed effects of Methoprene resulted from the observed ingestion of treated pads rather than from contact or vapor action, although the latter cannot be discounted entirely. Differentiation and mortality are apparently concentration dependent (Table I). An equal proportion of presoldiers is produced at concentrations below 0.063 mg/pad. At higher concentrations (0.125-0.500 mg/pad) presoldier production decreased as larvae died before differentiation. At the lower concentrations, a small proportion of the presoldiers successfully molted to

[1]Southern Forest Experiment Station, Forest Service, USDA, Gulfport, Mississippi.
[2]Pacific Southwest Forest and Range Experiment Station, Forest Service, USDA, Berkeley, California.

Table I. Differentiation and mortality of Reticulitermes flavipes (Kollar) exposed to various concentrations of Methoprene for 28 days.

Concentration mg/pad	Maximum percentage* produced as			Days to total mortality	$\bar{x}$ % mortality as*			Total mortality
	Presoldier	Soldier	Inter-caste		Larva	Presoldier	Incomplete molt	
0.000	0	0	0	-	12.0	0	0	12
0.016	57.3	4.0	3.3	-	13.3	12.0	0.7	26
0.032	56.0	2.7	0.7	-	28.7	43.3	0	72
0.063	54.7	0.7	0.7	-	34.0	56.0	0	90
0.125	5.3	0	0	23	81.3	11.4	7.3	100
0.250	1.3	0	0	23	84.7	3.3	12.0	100
0.500	0	0	0	20	95.3	0	4.7	100
1.250	0	0	0	15	100.0	0	0	100
2.500	0	0	0	15	100.0	0	0	100

*Mean of 5 replicates with 30 insects each.

the soldier caste or to a worker-soldier intercaste. Above 0.063 mg/pad, all termites died before 28 days. At lower concentrations (0.016-0.063 mg/pad) mortality was partitioned fairly evenly between larvae and presoldiers. For concentrations between 0.125 and 0.500 mg/pad, 80% of the mortality occurred in larvae. The remaining deaths were either ecdysis failures or presoldier deaths (*4*). No termites survived long enough to differentiate at concentrations greater than 0.500 mg/pad.

Since most observed mortality was not associated with molting failures (Table I) (*4*), it is possible that Methoprene kills the termites' intestinal protozoan symbionts. To test that hypothesis, groups of 30 larvae were exposed to concentrations of 0.016, 0.033, and 0.063 mg/pad of Methoprene in the same manner as above, with three replications of each concentration for each of the seven observation days (*5*). Total populations of 4 protozoan genera in *R. flavipes* were measured (*6*). Five termites were randomly selected by a tumbling process from each container. Thus the estimated protozoan population for each concentration-day combination is the mean of protozoan populations from 15 termites. A summary of the results obtained is presented in Figure 1.

Protozoan populations of termites exposed to 0.016 mg/pad were not significantly different from controls. Numbers of protozoans in termites exposed to the two highest treatment levels, however, decreased significantly with time (α= 0.05), and the extent of the decrease was related to concentration. This loss of protozoan numbers strongly suggests a true toxicity to the protozoa because for both 0.032 and 0.063 mg/pad concentrations at days 7 and 10, never more than 2 of the 15 termites examined per treatment were devoid of protozoa. If the lower protozoan numbers were a result of several individuals in the replicate having voided their protozoa in preparation for a molt, then one would expect the nonvoided individuals to have protozoan populations equivalent to that of the controls. Such was not the case. In addition, treated termites usually contained more dead protozoans than control termites. It is significant that at concentrations of 0.032 and 0.063 mg/pad, protozoan loss was essentially complete by the time presoldiers began to appear, approximately 1 week before termite mortality began to increase rapidly. In contrast, after 23 days, a concentration of 0.016 mg/pad failed to reduce the protozoan numbers to less than 60-70% of normally faunated termites. A corresponding low termite mortality was found, even though over half of the larvae molted to the presoldier form. Apparently at this treatment level the termites are able to recover from the effects of Methoprene.

Methoprene has been shown to kill protozoans other than the four termite symbionts studied here (*7*). Miura and Takahashi report an LC_{50} of 1.25 ppm against *Paramecium* spp., a value which we believe crudely approximates the actual quantity of Methoprene in the gut tract of single termites in our experiments.

The results of this study suggest at least three concentration-dependent modes of action of Methoprene which can cause mortality in *R. flavipes*. First, larvae can starve because their symbiotic protozoans die. Second, the production of a highly abnormal proportion of presoldiers and soldiers severely strains the capacity of the remaining larvae to feed them. In addition, because the remaining larvae are themselves partially or totally defaunated, they are unable to feed the presoldiers and soldiers adequately. Third, mortality can result from incomplete ecdysis between the larval and soldier caste. Further work will be necessary to evaluate the relative importance of each of these modes of action in a normal termite colony.

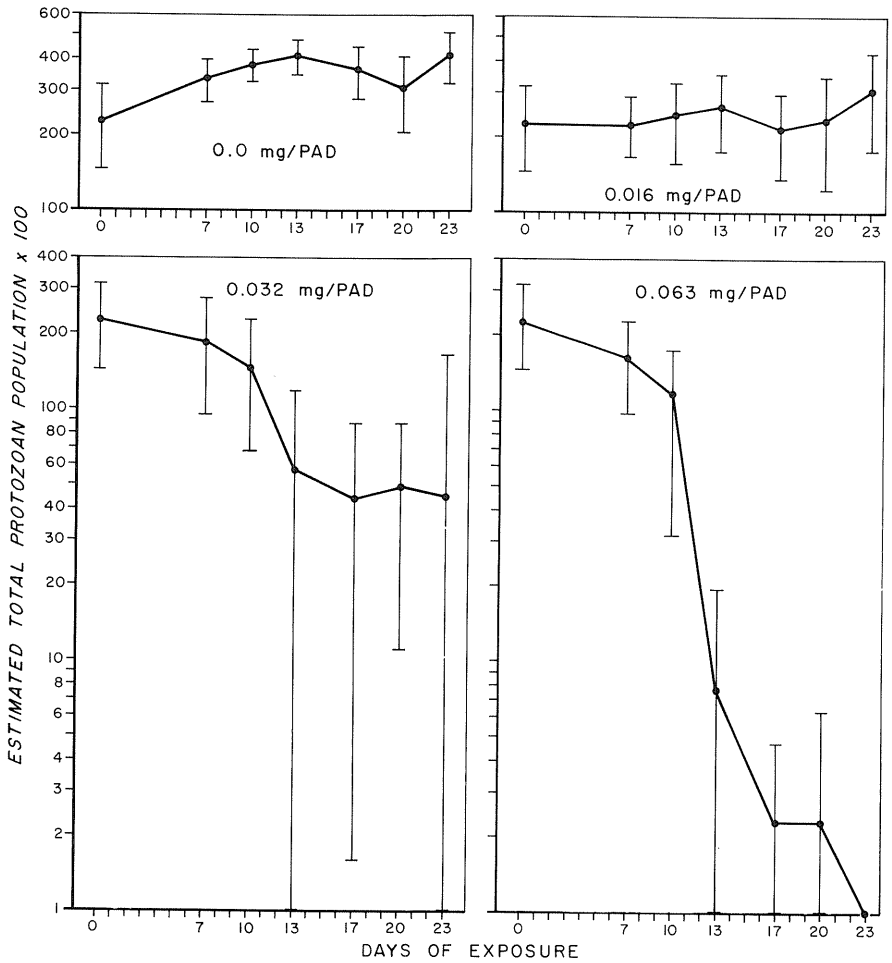


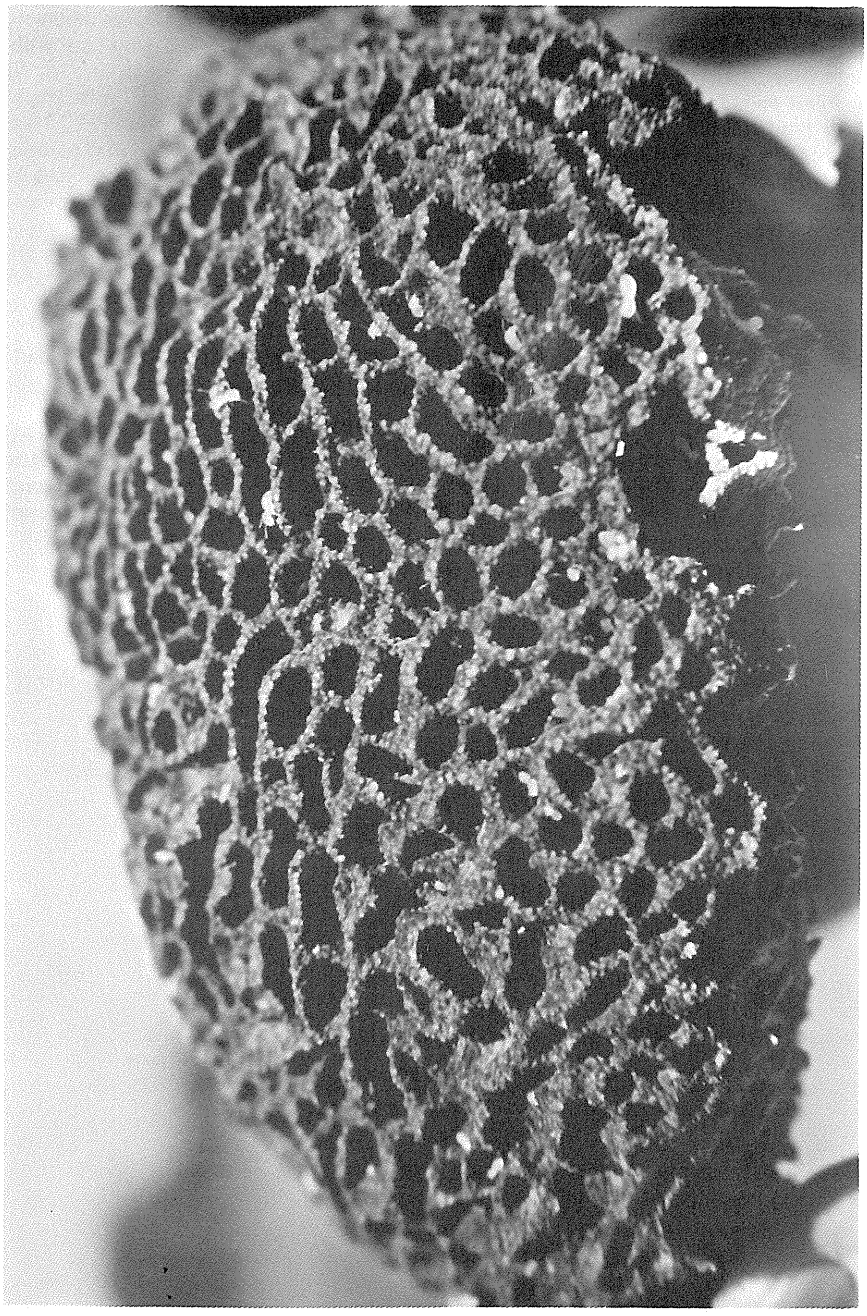
Fig. 1. Total protozoan populations ($\overline{X} \pm 95\%$ C.I.) in hindguts of *Reticulitermes flavipes* (Kollar) exposed to various concentrations of Methoprene for 23 days.

REFERENCES AND NOTES

1. H. H. Chu, T. D. Tai, T. F. Chen, and M. W. King, Acta Entomol. Sin. *17*(2), 161 (1974); J. R. J. French, J. Aust. Entomol. Soc. *13*, 353 (1974); I. Hrdý, Z. Ang. Entomol. *72*, 129 (1972); I. Hrdý and J. Křeček, Insectes Sociaux *19*, 105 (1972); M. Lenz, Mater. und Org. *3*, 377 (1976); M. Lenz, in *Phase and Caste Determination in Insects Endocrine Aspects*, Martin Lüscher, Ed. (Pergamon Press, New York, 1976), p. 73 [XV Int. Congr. Entomol., Washington, D. C.]; A. Springhetti, Experientia *30*(10), 1197 (1974); K. Wanyonyi, Insectes Sociaux *21*, 35 (1974); K. Wanyonyi and M. Lüscher, VIIth Int. Congr. IUSSI Proc., p. 392. [University of Southampton, England, 1973.]

2. Termites used in this study were collected from infested logs on the De Soto National Forest, ca. 32 km North of Gulfport, MS: Experimental units consisted of pads moistened with 1-ml deionized water placed in containers 5.0 cm in diameter by 3.5 cm high with a tight fitting lid.
3. Filter pads (ca. 0.5 g, 47 mm diameter, Gelman Instrument Company), were treated with 1-ml solutions of technical Methoprene, Isopropyl (2E,4E)-11-methoxy-3,7,11-trimethyl,2,4-dodecadienoate (Zoecon), in acetone and air dried in a fume hood. Treatment levels used were 0.0, 0.016, 0.032, 0.063, 0.125, 0.250, 0.500, 1.250, and 2.500 mg/pad A.I. Sources of laboratory materials are named only to identify materials and no endorsement is implied by the United States Department of Agriculture.
4. Other workers have found that JHA's frequently cause mortality by inducing premature ecdysis, wherein the insect is unable to successfully complete the molt. See for example, K. Sláma, M. Romanuk, and F. Sorm, *Insect Hormones and Bioanalogues*, (Springer-Verlag, New York, 1974). 477 p.
5. Observations were made on days 7, 10, 13, 17, 20, and 23. To count protozoa, we had to destroy some of the insects in each experimental unit. Thus, different experimental units were observed on each date.
6. R. Mannesman, Z. Angew. Zool. *57*, 1 (1970). A modification to the methodology described involved mincing the termite hindgut on a shallow well microscope slide rather than in the apparatus used by Mannesmann. The protozoan species counted were: *Dinenympha* spp., *Pyrsonympha vertens* Leidy, *Spirotrichonympha* spp., and *Trichonympha agilis* Leidy.
7. T. Miura and R. M. Takahashi, J. Econ. Entomol. *66*(4), 917 (1973).

FEATURE PHOTO



One of the central fungus gardens of Odontotermes culturarum Sjoestedt. From a nest at Amani, Tanzania. Photo by David H. Kistner