COMPARISON OF FEEDING SUBSTRATES FOR EVALUATING EFFECTS OF INSECT GROWTH REGULATORS ON SUBTERRANEAN TERMITES[1]

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ABSTRACT

Of four feeding substrates (decayed sawdust, α-cellulose, artificial diet, and absorbent paper pads) evaluated in bioassays of Methoprene against *Reticulitermes flavipes* (Kollar) and *R. virginicus* (Banks), paper pads appeared to be the best substrate examined. They provided maximum soldier differentiation, ease of handling, and easy observation of test insects.

Key Words: *Reticulitermes flavipes* (Kollar), *Reticulitermes virginicus* (Banks), methoprene, soldier differentiation, juvenile hormone analogues, bioassay

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INTRODUCTION

Recent research evaluating effects of insect growth regulators (IGRs) on juvenile hormone activity of subterranean termites has shown that these chemicals induce dosage-dependent superfluous soldier differentiation and loss of symbiotic intestinal protozoa in both soldiers and workers (Howard and Haverty [in press]). Comparison of our results with earlier results from other laboratories is difficult, however, because investigators seldom used the same bioassay techniques. Thus far, four major techniques have been used: (1) injection of the test chemical or implantation of *corpora allata* into the haemolymph (Lüscher 1976; Yin and Gillott 1975); (2) topical application (Varma 1977); (3) evaporation from a smooth suspended surface (Lüscher 1974; Lüscher and van Doorn 1976; Wanyonyi 1974); and (4) ingestion of substrates treated with IGRs (French 1974; Howard and Haverty [in press]); Hrdý 1972, 1976; Hrdý and Křeček 1972; Chu *et al.* 1974; Lenz 1976a, b; Lüscher 1972; and Springhetti 1974).

Further research into the evaluation of IGRs would be expedited if a common bioassay could be developed. We feel that for subterranean termites, coelomic injections and topical applications are impractical because termites

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are too small and many of them must be treated for each test. We also believe that exposing termites to evaporation of IGRs fails to sufficiently approximate a normal termite milieu. IGR-treated feeding substrates, however, allow for acquisition of the chemicals via contact, vapor action, or ingestion, as well as by trophallaxis. Accordingly, we have worked exclusively with feeding substrates and report here on a comparative evaluation of four substrates routinely used for termite experiments in our laboratory.

METHODS AND MATERIALS

Termites. – Externally undifferentiated larvae of at least the third instar (hereafter called workers) from two colonies each of *Reticulitermes flavipes* (Kollar) and *R. virginicus* (Banks) were collected from fallen logs or snags of southern pine in southern Mississippi. Colonies were held in the laboratory in 20-gallon (75.7 ℓ) galvanized metal garbage cans for less than 10 days before use.

Substrates. – (1) Paper pad: absorbent paper pad, approximately 0.5 g, 47-mm diam. (Gelman Instrument Company[4], Ann Arbor, Michigan); (2) α-cellulose (National Biochemical Corporation, Cleveland, Ohio); (3) artificial diet: distilled water (65.87%), α-cellulose (34.06%), β-sitosterol (0.06%), and methyl p-hydroxy-benzoate (0.01%) (Mauldin and Rich 1975); and (4) decayed pine sawdust: boards cut from one tree (*Pinus elliottii* Engelm. var. *elliottii*) at the Harrison Experimental Forest, Saucier, Mississippi, kiln-dried, decayed by *Gloeophyllum trabeum* (Pers. ex Fr.) Murr. to about 12% weight loss, and then ground in a Wiley mill to pass a 20-mesh screen.

Technical Methoprene (isopropyl (2E,4E)-11-methoxy-3,7,11-trimethyl-2,4-dodecadienoate), 95% pure (Zoecon Chemical Company, Palo Alto, California) was incorporated into α-cellulose, artificial diet, and decayed sawdust by adding 2 ml of the appropriate acetone-Methoprene solution for every gram of substrate, mixing thoroughly, then evaporating the acetone. Methoprene was incorporated into pads by evenly applying 1 ml of acetone containing either 0.00, 0.16, 0.32, or 0.63 mg Methoprene, then allowing the acetone to evaporate. The resulting concentrations were approximately 0, 32, 62, and 125 ppm for each substrate.

Experimental units. – One treated pad of ½g of one of the treated ground substrates was placed in the bottom of a cylindrical plastic, snap-top container (5.0-cm diam. by 3.5-cm high). One ml of deionized water was then added as a moisture source. Thirty workers of *R. flavipes* or *R. virginicus* were added to each container, and containers were placed in an incubator at 24 ± 1 C. After 21 days, experimental units were disassembled and live and dead termites were counted and identified as to caste.

Experimental design and analysis. – There were 16 substrate X Methoprene concentration combinations. Each treatment combination (substrate X Methoprene X concentration X termite species X colony) was replicated five times. Resulting data were subjected to analysis of variance for a randomized

[4]Mention of a company or trade name is simply to identify a material and does not imply endorsement by the U.S. Department of Agriculture.

complete-block design. Colony origin was used as the block effect, and percent total differentiation (sum of presoldier, soldier, and worker-soldier intercaste) was the response variable. Each species was analyzed separately. The response variable was transformed to arc sine of the square root of the percentage before analysis. Significantly different treatment means were statistically separated at the $\alpha = 0.05$ level by Tukey's procedure.

RESULTS AND DISCUSSION

There were highly significant differences ($\alpha = 0.01$) in soldier differentiation between the 16-treatment combinations for both *R. flavipes* and *R. virginicus* (Tables 1 and 2). In none of the acetone controls for either species were many presoldiers produced, nor did number of presoldiers differ significantly. Differentiation by termites of both species feeding on Methoprene treated paper pads and α-cellulose, it was generally higher at all Methoprene levels than those feeding on the other two substrates. While mean differentiation was consistently greater on the pads than on the α-cellulose, it was not always significantly so. The unusually low differentiation observed for *R. virginicus* feeding on pads treated with 125 ppm Methoprene (Table 2) probably resulted from high worker mortality (41.4%) in this one treatment combination; other Methoprene treatments had 15% or less worker mortality over the 21-day test period. Reasons for this high mortality are not clear, but could be a result of defaunation-induced starvation (Howard and Haverty [in press]).

The mean differentiation by termites of both species feeding on the artificial diet was always less than that observed for termites feeding on α-cellulose, but not significantly so. Differentiation by termites held on the decayed sawdust, however, was significantly below that for groups fed all other substrates at all Methoprene concentrations but the control. As noted in Table 2, decayed sawdust treatments for one colony of *R. virginicus* were lost because of mortality from fungal contamination.

Several factors must be considered in seeking a standard bioassay method

Table 1. – Percent total soldier differentiation of groups of 30 workers of *Reticulitermes flavipes* after exposure for 21 days to various concentrations of Methoprene in four substrates[1, 2]

	Methoprene Concentration (ppm)			
Substrate	**0**	**31**	**62**	**125**
Paper pad	0.00g	22.66de	72.04a	73.35a
α-cellulose	0.00g	12.67ef	51.99bc	62.10ab
Artificial diet	0.00g	9.34f	37.67cd	48.41bc
Decayed sawdust	0.00g	0.66g	1.33g	21.67de

[1] Mean of five replications from each of two source colonies.

[2] Means which are followed by the same letter are not significantly different at the $\alpha = 0.05$ level by Tukey's procedure.

Table 2. – Percent total soldier differentiation of groups of 30 workers of *Reticulitermes virginicus* after exposure for 21 days to various concentrations of Methoprene in four substrates[1, 2]

Substrate	Methoprene Concentration (ppm)			
	0	31	62	125
Paper pad	0.00f	42.98b	61.33a	44.00b
a-cellulose	0.67ef	11.34cd	52.00ab	63.34a
Artificial diet	0.33ef	4.01de	41.00b	60.67a
Decayed sawdust[3]	0.67ef	0.00f	2.65ef	21.37c

[1] Mean of five replications from each of two colonies.

[2] Means which are followed by the same letter are not significantly different at the $a = 0.05$ level by Tukey's procedure.

[3] The values for the decayed sawdust substrate are means of five replications from one colony only. The replications from the second colony suffered heavy mortality from fungal contamination.

for comparing effects of insect growth regulators on subterranean termites. Of primary importance is the sensitivity of response in terms of percent differentiation. The paper pads and *a*-cellulose were nearly equivalent in this respect, and clearly superior to the other two substrates. Another important factor is ability of the substrate to remain relatively free of microbial contamination for up to 2 months. Our laboratory has had extensive experience with all four substrates tested in this study, and the paper pads, *a*-cellulose, and artificial diet have proved superior to decayed sawdust in this respect. A third factor, vitally important for large scale experiments, is ease of handling and visual observation of test units. In this respect, paper pads are far superior to the other three substrates, since on the pads termites are always visible and may be observed without mechanical disturbance. Furthermore, if it is necessary to physically manipulate the termites, the operation is much faster with the paper pads than with the other three substrates. Considering all these factors, we feel that paper pads offer the most advantages and thus appear to us to be an excellent candidate as a standard substrate for bioassays of IGRs against subterranean termites.

LITERATURE CITED

Chu, Hsang-Hsoung, T.-D. Tai, Tse-Fu Chen, and Mei-wen King. 1974. Induction of soldier differentiation in the termite *Reticulitermes flaviceps* Oshima with juvenile hormone analogues [In Chinese, English summary]. Acta Entomol. Sin. 17(2): 161-4.

French, J. R. J. 1974. A juvenile hormone analogue inducing caste differentiation in the Australian termite, *Nasutitermes exitiosus* (Hill) (Isoptera: Termitidae). J. Aust. Entomol. Soc. 13: 353-5.

Haverty, Michael I. 1977. The proportion of soldiers in termite colonies: a list and a bibliography (Isoptera). Sociobiology 2(3): 199-216.

Howard, Ralph W. and Michael I. Haverty. [In press]. Defaunation, mortality

and soldier differentiation: concentration effects of Methoprene in a termite. Sociobiology.

Hrdý, I. 1972. Der Einfluss von zwei Juvenilhormonanalogen auf die Differenzierung der Soldaten bei *Reticulitermes lucifugus santonensis* Feyt (Isopt.: Rhinotermitidae). Z. ang. Entomol. 72: 129-34.

Hrdý, Ivan. 1976. The influence of juvenile hormone analogues on caste development in termites. Page 71 *in* Martin Lüscher, ed. Phase and caste determination in insects: Endocrine aspects. Pergamon Press, New York (Abstr.)

Hrdý, I. and J. Křeček. 1972. Development of superfluous soldiers induced by juvenile hormone analogues in the termite, *Reticulitermes lucifugus santonensis.* Insectes Sociaux, Paris. 19(2): 105-9.

Lenz, Michael. 1976a. Die Wirkung von Juvenilhormon-Analoga (JHA) auf *Kalotermes flavicollis* und *Coptotermes amanii* (Isoptera: Kalotermitidae, Rhinotermitidae) bei unterschiedlicher Ernährung der Tiergruppen. Mater. und Org. 3: 377-92.

Lenz, Michael. 1976b. The dependence of hormone effects in termite caste determination on external factors. Pages 73-89 *in* Martin Lüscher, ed. Phase and caste determination in insects: Endocrine aspects. Pergamon Press, New York.

Lüscher, M. 1972. Environmental control of juvenile hormone (JH) secretion and caste differentiation in termites. Gen. Comp. Endocrinol. Suppl. 3: 509-14.

Lüscher, M. 1974. Die Kompetenz zur Soldatenbildung bei Larven (Pseudergaten) der Termite *Zootermopsis angusticollis.* Rev. Suisse de Zool. 81(3): 710-14.

Lüscher, Martin. 1976. Evidence for an endocrine control of caste determination in higher termites. Pages 91-103 *in* Martin Lüscher, ed. Phase and caste determination in insects: Endocrine aspects. Pergamon Press, New York.

Lüscher, M. and J. van Doorn. 1976. Die Abhängigkeit der Soldatenbildung bei der Termite *Zootermopsis* von der Dauer der Einwirkung des Juvenilhormon-Analogons Altozar. Rev. Suisse de Zool. 83(4): 939-42.

Mauldin, Joe K. and Nely M. Rich. 1975. Rearing two subterranean termites, *Reticulitermes flavipes* and *Coptotermes formosanus* on artificial diets. Ann. Entomol. Soc. Am. 68(3): 454-6.

Springhetti, A. 1974. The influence of Farnesenic Acid Ethyl Ester on the differentiation of *Kalotermes flavicollis* Fabr. (Isoptera) soldiers. Experientia 30(10): 1197-8.

Varma, R. V. 1977. Influence of juvenile hormone analogue, Farnesyl Methyl Ether on caste differentiation in termite *Postelectrotermes nayari* Roonwal and Verma, 1971. Indian J. Exp. Biol. 15: 564-5.

Wanyonyi, Kizito. 1974. The influence of the juvenile hormone analogue ZR512 (Zoecon) on caste development in *Zootermopsis nevadensis* (Hagen) (Isoptera). Insectes Sociaux, Paris. 21(1): 35-44.

Yin, Chih-Ming and Cedric Gillott. 1975. Endocrine control of caste differentiation in *Zootermopsis angusticollis* Hagen (Isoptera). Can. J. Zool. 53(11): 1701-8.
