Termites and Juvenile Hormone Analogues: A Review of Methodology and Observed Effects

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ABSTRACT

A review of the published literature on research involving juvenile hormone analogues and termites is presented. Specific aspects covered include experimental chemicals, bioassays, observed effects, and future research directions.

INTRODUCTION

Mechanisms of caste determination and regulation in termite colonies remain one of the major enigmas of termite biology. In recent years juvenile hormones (JH) have been increasingly implicated as central mediators of this process (13). Concomitant with this discovery has been the finding that a wide variety of synthetic organic chemicals can mimic the action of the three juvenile hormones known to occur naturally, and hence have important implications as biological control agents (17). Although most research on juvenile hormone analogues (JHA's) has been directed at agriculturally and medically important pest species, much also has been done with termites. Enough knowledge has now accumulated to warrant a review of the state-of-the-art regarding chemicals and bioassays used, species investigated, and observed effects. The literature was surveyed through April, 1979.

CHEMICAL ASPECTS

Thousands of synthetic chemicals have been developed and bioassayed against a variety of insect species for juvenile hormone activity (17). Most of these chemicals are either close structural analogs of juvenile hormones or they are topochemically related to them. Only a few of these chemicals, however, have ever been tested against termites. The structures and common names of those JHA's that have been so tested are listed in Table

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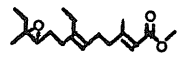
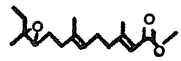
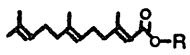
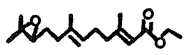
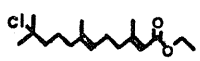
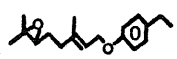
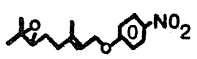
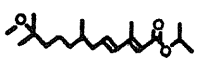
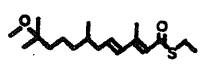
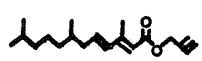
Table 1. Catalogue of known juvenile hormones (JH) and synthetic juvenile hormone analogues (JHA's) that have been used in termite research.

Chemical structure	Common name	Reference
	JH I[a]	23
	JH II[a]	23
	JH III	16
	Ester and alkoxy deriv. of farnesol and farnesenic acid	14, 18, 19 20, 22
		8, 10
		8, 10
	Stauffer R 20458	1, 3
		10
	Methoprene	1, 5, 6, 7, 11, 12
	Hydroprene	1, 2, 3, 4, 5, 11, 12, 15, 16, 21, 22
	Triprene	1, 3
	Kinoprene	1, 3

[a]Cecropia oil (Röller's JH) contains a mixture of JH I and JH II, as well as many other lipid components.

1. In no instance has any investigator used a JHA of high purity (>99%). Rather, all have used mixtures of isomers and/or technical grade materials.

TERMITES USED AS TEST ANIMALS

Termites from every family (except the Serritermitidae) have been used as test animals for JHA research (Table 2). More species of rhinotermitids have been tested than in any other family, but most of the pioneering JHA and caste differentiation research used the dampwood termites *Zootermopsis angusticollis* (Hagen) and *Z. nevadensis* (Hagen) (14, 15, 21, 22, 23) and the drywood termite *Kalotermes flavicollis* (Fabr.) (11, 12, 18, 19).

Table 2. Catalogue of termite species that has been used in JHA research.

Family	Species	Reference
Kalotermitidae	*Kalotermes flavicollis*	11,12,18,19
	Cryptotermes brevis	9
	Neotermes castaneum	9
	N. jouteli	9
	Incisitermes schwarzi	9
	Postelectrotermes nayari	20
Mastotermitidae	*Mastotermes darwiniensis*	3,4
Hodotermitidae	*Zootermopsis angusticollis*	9,14,15,16, 22,23
	Z. nevadensis	9,15,21,22
Rhinotermitidae	*Coptotermes amani*	11,12
	C. formosanus	5,9
	C. niger	12
	Heterotermes convexinotatus	9
	H. indicola	12
	Prorhinotermes simplex	9
	Reticulitermes flaviceps	1
	R. flavipes	5,6,7,12
	R. lucifugus	9,12
	R. lucifugus santonensis	8,10
	R. virginicus	5,7
Termitidae	*Nasuititermes exitiosus*	2
	N. nigriceps	12

BIOASSAY METHODS

Many techniques have been used to expose termites to JHA's (Table 3). Lüscher and his colleagues (9, 14–16, 21–22) usually used a vapor bioassay in which the JHA was applied as an acetone solution to the surface of a petri-dish lid and the solvent allowed to evaporate. Termites were then placed on a paper pad in the bottom of the petri dish with a source of moisture and/or food. The treated lid was usually replaced with a freshly treated one every 2–3 days. The assumption made using this bioassay is that termites receive the JHA as a vapor. The JHA presumably enters their body through the spiracles, although they probably also receive an unknown quantity of JHA via grooming, absorption through the cuticle, and

Table 3. Bioassay methods used for evaluating the effects of JHA's on termites.

Bioassay	Reference
Evaporation from a glass surface	9,14,15,16,21,22
Incorporation into a cellulose substrate	
filter paper	1,8,10,11,12,17, 19,20,22
filter pads	5,6,7
powdered cellulose	7
sawdust (sound or decayed)	2,7,12
surface treated wood blocks	3,12
vacuum impregnated wood blocks	4,11
Topical application	20
Injection into termites	23

ingestion of cellulosic substrate. Because relatively large dosages of JHA (mg range) are required to produce the full spectrum of JHA effects found in other bioassays, this bioassay does not seem particularly sensitive.

A more popular bioassay method consists of holding the termites on a JHA-treated cellulosic substrate upon which they may then feed (Table 3). Either filter paper (1, 8, 10–12, 17, 19, 20, 22) or a thick filter pad (5–7) has been used as substrates, although powdered cellulose (7) and sawdust, both sound and decayed (2, 7, 12), have also been used. The basic assumption of this bioassay is that most of the JHA is acquired via ingestion. Some is certainly acquired, however, by absorption through the cuticle and during grooming.

In another bioassay, treated wood blocks, either surface dipped (3, 12) or vacuum impregnated (4, 11), are placed with the termites in a petri dish, or in a vermiculite matrix in a larger container. Ingestion is assumed to be the major mode of JHA acquisition. Because wood varies greatly in its ability to absorb chemicals, controlling concentration is difficult in this bioassay.

A few workers have used topical application of JHA's on termites (20). Although a precise quantity of JHA can be applied to each termite, the method is extremely tedious, except when done with larger termite species. Injecting JHA's into termites is also tedious (23). Topical applications and injections, however, are useful for determining precise dosage-response relationships.

EFFECTS PRODUCED BY JHA'S

A diversity of effects results when termites are exposed to JHA's (Table 4). The most common effect is the production of many presoldiers. Relatively low dosages of JHA's (μg to mg range/test unit) convert up to 70% of workers, pseudergates, nymphs, or larvae into presoldiers within 2-6 weeks. Existing soldiers in the experimental units have been reported to stimulate (12) or to hinder (2, 12) presoldier production in the presence of JHA's. These presoldiers subsequently molt into apparently normal soldiers. However, Lenz (11) reported that JHA-induced presoldiers of *K. flavicollis* and *Coptotermes amanii* (Sjostedt) did not molt into soldiers, even after 1 year.

Lüscher and Van Doorn (15) reported that the morphological features of hydroprene-induced presoldiers of *Z. angusticollis* and *Z. nevadensis* were related to how long pseudergates had been exposed to the JHA's. Short exposure periods produced presoldiers with shortened mandibles; longer exposure periods produced normal presoldiers. Lüscher and Van Doorn concluded that normal soldier development in *Zootermopsis* probably depends on the activity of the *Corpora allata* of individual termites rather than on juvenile hormone taken up from the social environment.

Many investigators have reported that JHA treatments produce intercastes (1, 5, 6, 8-12, 16, 19-21). Worker-soldier intercastes have been most frequently reported; occasionally nymph-soldier forms are found. Lenz (11, 12) reported that nymph-soldier intercastes in *Heterotermes indicola* (Wasmann) lost their wing buds when they molted to the soldier stage. Springhetti (18) noted that morphological features were not constant in *K. flavicollis* intercastes produced by the action of the ethyl ester of crude preparations of farnesenic acid. In particular, the shape of the head and mandibles varied widely. He suggested that this variation may also be related to the physiological condition of the treated pseudergates. Wanyoni (21) found that hydroprene at a dosage of 0.4 mg per test chamber caused last-instar nymphs of *Z. nevadensis* either to form nymph-soldier intercastes or to molt back to the form of a first-stage nymph. Lower dosage levels (0.3 - 0.04 mg per test chamber) either caused stationary molts or produced nymph-imago intercastes. At 0.04 mg per test chamber, the JHA may have decreased molting frequency of both nymphs and larvae.

Meyer and Lüscher (16) obtained nymph-soldier intercastes of *Z. angusticollis* in a novel manner. Fifteen fully grown larvae were exposed to the vapors of 1 mg of hydroprene for 6 days, then placed in a container with 6 untreated last-instar nymphs for 20 days. Three of the 6 untreated nymphs molted into nymph-soldier intercastes. Unfortunately, the experiment was not replicated, so some caution must be used in interpreting the results. If these results are confirmed, two questions merit further

Table 4. Catalogue of effects of JHA's on termites.

Effect	Reference
Production of presoldiers	1-3,5-12,14-15,18-23
Production of intercastes	1, 5-6. 8-12,16,19-21
Increased or decreased production of neotenic reproductives	18,19,22,23
Production of psuedoimagoes	20,22
Ecdysis inhibition	5,6,10-12,20
Feeding inhibition	3,4,18
Apparent overt toxicity	1,5-8,10-12,20
Defaunation of protozoan symbiotes	5,6
Partial defaunation of bacterial endosymbionts	4
Atrophication of prothoracic gland	22

investigation. Were the observed results a consequence of direct sequestration of the hydroprene with trophallactic transmission to the nymphs? Or did the hydroprene in some manner cause increased endogeneous JH production in the larvae, with subsequent trophallactic transmission of the endogeneous JH to the nymphs?

A few investigators have reported that JHA's influence the development of castes other than workers and nymphs. Springhetti (18, 19) reported that production of neotenic reproductives of *K. flavicollis* apparently increased when groups of pseudergates were held on filter paper treated with the ethyl ester of farnesenic acid. Wanyonyi (21), however, reported that exposing pseudergates of *Z. nevadensis* to certain levels of hydroprene completely suppressed or delayed neotenic production. Likewise, Wanyonyi and Lüscher (22) and Yin and Gillot (23) reported suppression of neotenic formation in *Z. angusticollis* exposed to hydroprene, farnesyl methyl ether, or Röller's JH mixture.

It has been reported twice that JHA's stimulate pseudoimago production. Wanyonyi and Lüscher (22) obtained this unusual caste form by exposing *Z. angusticollis* and *Z. nevadensis* to 0.4 mg/test chamber of hydroprene or 0.03 ml/test chamber of farnesyl methyl ether. Varma (20) obtained 2 pseudoimagoes from an unspecified number of *Postelec-*

trotermes nayari Roonwal and Verma after topical applications of 1.5 mg of farnesyl methyl ether per termite. Neither experiment however was replicated.

Besides the described morphological effects, many workers have reported a variety of JHA induced effects leading to death of the termites (1, 5-8, 10-12, 20). JHA's frequently inhibit ecdysis in other insects (17), and the same has been shown for termites (Table 4). In general, however, JHA-induced inhibition of ecdysis seems unimportant in termites. Only one group (10) has claimed substantial mortality from this factor. They did not report the concentrations of the JHA's they used in their experiments, so evaluating their results is difficult.

Two research groups have found that JHA's act as feeding inhibitors at some or all concentrations tested. French and Robinson (3) and French et al. (4) examined wood consumption by *Mastotermes darwiniensis* Froggatt on JHA-treated pine blocks or plywood. They reported that termites ate significantly less JHA-treated wood than control wood in each experiment. Springhetti (18) reported that at a concentration of 128 parts per thousand of the ethyl ester of farnesenic acid, pseudergates of *K. flavicollis* essentially stopped eating.

Apparent direct toxicity of JHA's to termites has frequently been observed (Table 4). Until recently, however, the evidence for such direct toxicity has been largely circumstantial. It has now been shown (5, 6) that although increasing dosages of methoprene causes increasing mortality to *Reticulitermes flavipes* (Kollar), methoprene itself is not directly toxic to this species. Rather, the observed mortality results from starvation induced by the methoprene-induced loss of their symbiotic protozoa. Interestingly, French et al. (4) have found that hydroprene induces partial defaunation of the bacterial endosymbiotes of *M. darwinensis*. Unfortunately, they did not report whether they observed concomitant protozoan defaunation. Wanyoni and Lüscher (22) reported that hydroprene and farnesyl methyl ether induce atrophy of the prothoracic gland of *Z. angusticollis* and *Z. nevadensis*. Howard and Haverty (7) and Lenz (12) reported that the quality of the food available to the test termites seemed to influence the effectiveness of the JHA's. Finally, at least 2 species of termites either failed to respond to JHA treatments (*Coptotermes formosanus* Shiraki [5]) or responded sporadically (*M. darwiniensis* [3, 4]).

FUTURE DIRECTIONS

Much information has now been gathered on how JHA's affect termites. Much of it is contradictory, and much remains to be learned. More attention should be paid to experimental design in this type of research. It is almost always necessary to work with groups of termites (composed of individuals of unknown ages), making it extremely important that

adequate replication be used. Equally important is the utilization of as many source colonies as possible. It is also imperative that researchers begin using pure JHA's, so the nagging problem of biologically active impurities can be eliminated. Considerably more detail needs to be reported concerning the test termites. How long were they held in the laboratory before experimentation began? What time of year were they collected, and from what habitat? What food source were the termites held on? Was it decayed, and if so, were the decay-fungi dead or alive when fed to the termites? There is an urgent need for a standardization of dosage/concentration terminology in S. I. units by all researchers. And finally, every experiment should be subjected to statistical analysis. Attention to such details would alleviate much ambiguity in the JHA-termite literature.

Many interesting problems remain to be investigated. Almost all researchers have utilized small, homogeneous laboratory groups of termites. How effective will JHA's be in larger, more heterogeneous populations? No consideration has been given to effects of either temperature or time of year. What roles do the endosymbionts of termites play in JHA effects? Do JHA's affect the endogenous levels of JH in termites? Do JHA's affect the reproductive capabilities of the termites? Could JHA's be combined with insecticides, antibiotics, or other growth regulators (i.e., chitin inhibitors) to produce synergistic effects? All of these factors (and probably several more) need investigating. Only with such information can we hope to unravel the ways in which juvenile hormones influence caste development and composition, and the ways in which JHA's might be utilized as selective termite control agents.

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