

REPRESSION OF MATING BEHAVIOUR BY NYMPHAL EXPOSURE TO SUBLETHAL DOSES OF JUVENILE HORMONE ANALOGUES IN *DYSDERCUS KOENIGII* (FABRICIUS) (HEMIPTERA : PYRRHOCORIDAE)

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ABSTRACT

Nymphal exposure of *Dysdercus koenigii* to sublethal doses of juvenile hormone analogues affected mating behaviour of the emergent, apparently normal adults. Repression of mating activity, in particular, was found to be a function of the degree of juvenile hormone activity of the analogue used.

INTRODUCTION

THE insect juvenile hormone (JH) is known to be involved in the development and maintenance of reproductive functions¹ in adult insects. This involvement apparently extends to the mating behaviour also. JH treatment of *Gomphocerus rufus*² has been shown to induce receptivity in refractile females, while in *Drosophila grimshawi*³ females, it induced precocious sexual behaviour. In *Nomadacris septemfasciata* and *Oedipoda miniata*⁴ corpora allata exert complete control over male sexual behaviour. JH treatment of the adults increases mating activity in *Oncopeltus fasciatus*⁵ while it suppresses the same in *Trypodendron lineatum*⁶. Nymphal exposure to JH causes morphological inhibitions (MI) and the emerging adults are usually reproductive failures, unable to contribute to future generations. Doses of JH/JHAs (Juvenile Hormone Analogues) not causing MI are therefore called "sublethal" doses and it is presumed that the emerging, apparently normal adults are 'unaffected',^{7,8}. However, larval exposure to sublethal doses of JHAs has been shown to influence mating in *Plodia interpunctella*⁹.

While establishing the ID₅₀ morph. of three well-known JHAs (Hydroprene, Kinoprene and Methoprene) against a laboratory reared colony of *Dysdercus koenigii* (Fabricius), we used a wide spectrum of doses ranging from 0.0025 µg to 0.1 µg against fifth instar nymphs. Adults emerging from such treatments were kept under observation till death, irrespective of the degree or absence of MI obtained. A distinct effect on mating activity was noted in the apparently 'unaffected' adults. As such, these observations were pursued further in more specifically and elaborately designed experiments.

MATERIALS AND METHODS

0-12 hrs old water fed fifth instar nymphs of *Dysdercus koenigii* drawn from a laboratory culture maintained at temperature, 27 ± 1° C, 60-65% R.H. and photoperiod LD 12:12 were used in all experiments.

The JHAs used were Hydroprene, Kinoprene and Methoprene, gifted by the Zoecon Corporation, USA.

In the case of Hydroprene two samples were used : (A) a 'fresh sample' not more than 15 days old and (B) an 'old sample', 4-5 years old. Both (A) and (B) were stored at -4° C since their receipt. Doses of both (A) and (B) ranging from 0.0025 to 0.1 µg were applied in 1 µl of acetone on the mesonota of the fifth instar nymphs. At least 100-200 of the latter were tested in suitably sized replicates at each dose level. The treated batches were held at the temperature, humidity and photoperiod mentioned earlier. The emerged adults were (i) scored for MI ; (ii) the unaffected adults (from sublethal doses) were sexed and the sexes were kept separately in different containers on water and soaked cotton seeds. These insects were used to study the effect of the JHAs on mating behaviour.

Mating behaviour was evaluated by recording variations in (a) mean per cent mating (pairs formed = % m.f.) over a given period of time (30 mins.) and (b) time taken for initiation of mating (precopulatory period 'T'). Both (a) and (b) tests were conducted simultaneously on the same batch of insects, by introducing a single female in 5 cms diameter glass petridishes already containing a single male each. The time (I) taken for establishment of a stable pair (copulating pair which remains intact for 1 min or more) and the number of such pairs formed out of the total assayed in 30 mins were recorded both for control and experimental, i.e., 'unaffected' adults obtained from sublethal treatments of JHAs. Two controls, viz., untreated and those treated as fifth instar nymphs with acetone only, were taken as control I and control II, respectively. The experiments were conducted under constant light intensity from day 1 to day 12 of adult life between 08.00-13.00 hrs daily. The mating pairs were separated after the tests and again held in separate containers till the next test 24 hr later. Data on precopulatory period (T) and mating frequency (% m.f.) for 12 days were pooled together and means calculated for both. Percentage repression of mating frequency was calculated by comparing the % m.f. in test with the mean of % m.f. of both control I and control II.

TABLE I

Comparison of mating frequency¹ and precopulatory period² in adults of *Dysdercus koenigii* obtained from JHA treated fifth instar nymphs

Chemical	Age of the sample	Dose $\mu\text{g}/\text{V}$ instar	% Morphological inhibition	Total Mean Mating Responses (/30 mins/day: 1-12 days)		% m.f. Repression ^c
				T ^b (mins.)	% m.f. ^a	
Nil Control I	0.0	3.0 \pm 0.5	62.9 \pm 6.5	0.0
Acetone Control II	..	0.0	0.0	3.2 \pm 0.5	65.1 \pm 8.3	0.0
Hydroprene (B)	4.5 years	0.1	0.0	4.0 \pm 0.9	42.4 \pm 7.7	34.2
		0.05	0.0	2.9 \pm 0.6	68.2 \pm 7.2	0.0
Hydroprene (A)	15 days	0.1	100.0
		0.05	0.0	4.2 \pm 0.6	40.0 \pm 6.9	38.0
		0.025	0.0	3.7 \pm 0.7	44.7 \pm 8.2	30.7
		0.0025	0.0	3.5 \pm 0.6	58.6 \pm 8.9	9.2
Kinoprene	15 days	0.0025	0.0	3.0 \pm 0.6	42.7 \pm 7.7	33.7
Methoprene	15 days	0.0025	0.0	3.2 \pm 0.5	33.3 \pm 5.0	48.3

a = % mating frequency (calculated as described in text). b = Precopulatory period.
c = % mating frequency repression, calculated as described in text.

RESULTS

The dose level of 0.0025 $\mu\text{g}/\text{V}$ instar in the case of Kinoprene and Methoprene and those upto 0.05 $\mu\text{g}/\text{V}$ instar in the case of Hydroprene caused no MI in the emerging adults (Table I). However, a dose of 0.1 $\mu\text{g}/\text{V}$ instar of sample A (15 days old Hydroprene) caused 100% MI but the same dose of the sample B (4.5 years old, Hydroprene) failed to cause any MI. At all the sublethal doses of all JHAs, there was a distinct repression of mating frequency which was highest for Methoprene and lowest for Hydroprene. However, increase in precopulatory time was maximum for Hydroprene. Interestingly too, while 0.1 $\mu\text{g}/\text{V}$ fifth instar dose of the 'old sample (B)' of Hydroprene did not cause any MI, it did influence both mating frequency as well as precopulatory period.

The basic rhythmicity of mating in a normal colony ascending to a peak of 7th/8th day was maintained in the JHA treated insects also, despite the repression in overall (average) mating frequency (Fig. 1).

DISCUSSION

Of the two components of mating activity studied, mating frequency is more consistently affected by

sublethal doses of JHAs. Precopulatory period, on the other hand, was increased only in case of Hydroprene. This increase was correlated with repression of mating evoked by the same JHA. The present results confirm the earlier indication of delayed^{10/} subtle⁹ effects of JHAs manifesting themselves later in the life cycle in functional aspects rather than apparent morphological ones. Repression of mating activity can in fact be employed as a parameter to evaluate JH activity using only sublethal doses.

On the basis of % repression of mating frequency obtained at the sublethal dose of 0.0025 $\mu\text{g}/\text{V}$ instar nymph the activity of the 3 analogues examined in the present work is Methoprene > Kinoprene > Hydroprene. This order synchronizes with the ranking obtained by us for these analogues on the basis of the morphological inhibition observed at higher doses. Further, it is significant that the 4.5 years old sample (B) of Hydroprene at the high dose of 0.1 $\mu\text{g}/\text{V}$ instar did not cause any MI and therefore ranked as 'inactive'. However, by the criterion of % repression of mating activity it still ranked as 'active' at level comparable to 0.05 $\mu\text{g}/\text{V}$ instar dose of the fresh (A) sample of the same analogue.

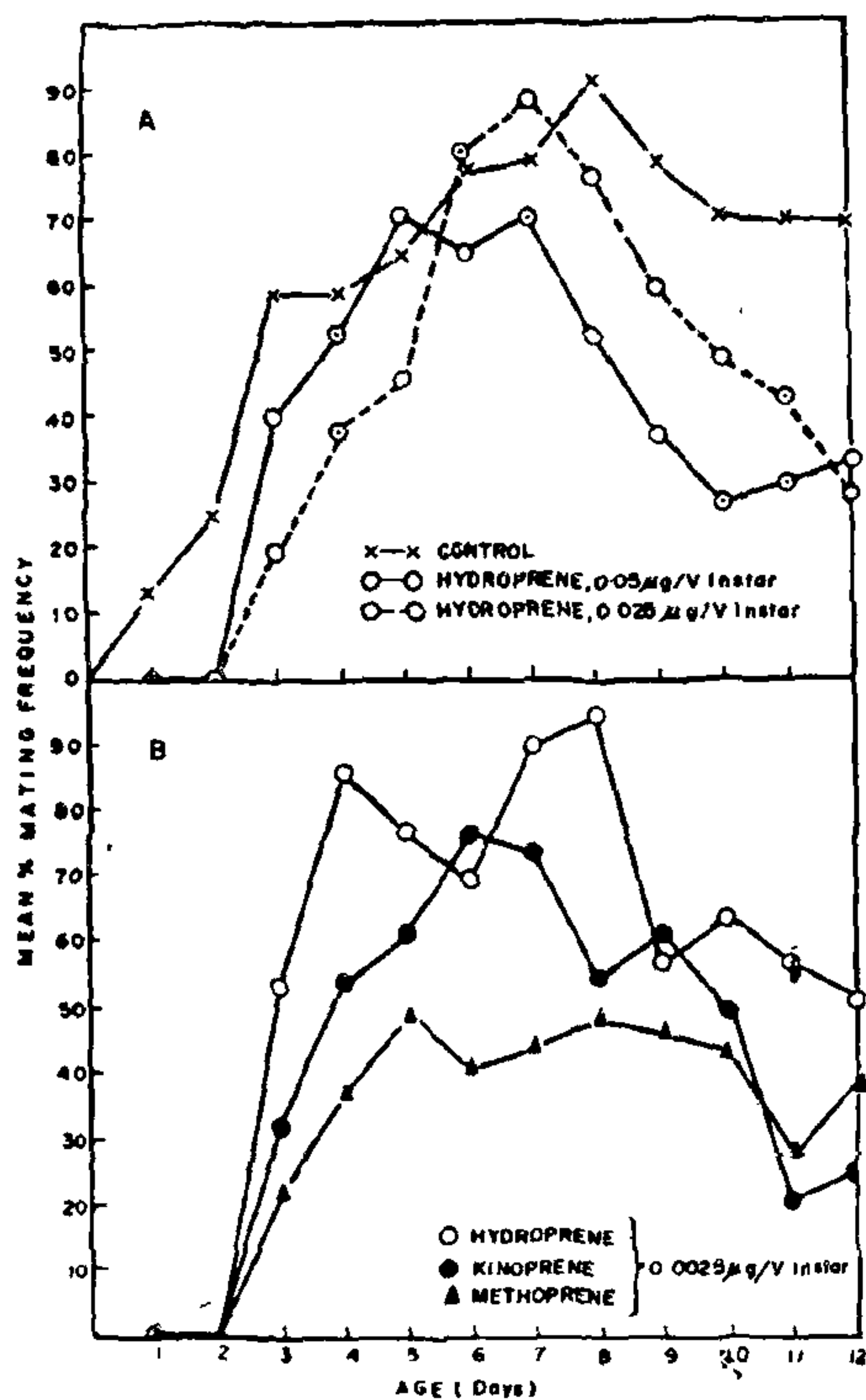


FIG. 1. Mating activity rhythms of control (A) and treated (B) in *D. koenigii* at different doses of different JHAs (at LD 12 : 12, Temp. $27 \pm 1^\circ \text{C}$, 60-65% R.H.).

There is some evidence⁹ that such functional effects are probably corollaries of subtle morphological distortions caused by JH activity. However, further elaborate investigations are needed to determine (i) the precise causal factors, (ii) whether these effects need to be taken into consideration in assessments of JH activity at the less than 100% MI doses and (iii) whether they can be exploited *per se* for control purposes, especially in view of some indications¹¹ of such a likelihood.

1. Engelmann, F., *The Physiology of Insect Reproduction*, Pergamon Press, New York, 1969.
2. Loher, W. and Huber, F., *Soc. Exp. Biol. Symp.*, 1966, 20, 381.
3. Ringo, J. M. and Pratt, N. R., *Ann. ent. Soc. Am.*, 1978, 71 (2), 264.
4. Pener, M. P., in *Experimental Analysis of Insect Behaviour* (Ed. Browne, L. B.), Springer Verlag, Berlin-Heidelberg, New York, 1974, p. 264.
5. Walker, W. F., *Physiological Entomology*, 1978, 3, 147.
6. Fockler, C. F. and Borden, J. H., *Ann. ent. Soc. Amer.*, 1973, 66 (3), 509.
7. Staal, G. B., in *Insect Juvenile Hormones Chemistry and Action* (Ed. Menn, J. J. Morton Beroza), Academic Press, New York, London, 1972, p. 69.
8. Slama, K. and Williams, C. M., *Biol. Bull.*, 1966, 130, 235.
9. Oberlander, H., Sower, L. and Silhacek, D. L., *J. Insect Physiol.*, 1975, 21, 681.
10. Riddiford, L. M. and Truman, J. W., *Nature (Lond.)*, 1972, 237, 458.
11. Ralph, C. P., *Oecologia*, 1976, 26, 157.

PALLADIUM(II) COMPLEXES OF SCHIFF BASES DERIVED FROM HETEROCYCLIC ALDEHYDES

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ABSTRACT

The Schiff bases $\text{R}-\text{CH}=\text{N}-\text{N}=\text{CH}-\text{R}$ (where R = furan or thiophene) have been prepared by the condensation of aldehyde with hydrazine in the ratio of 2 : 1. Their palladium(II) complexes $[\text{PdL}_2\text{Cl}_2]$ have also been synthesized and characterized by various physico-chemical studies. The complexes are diamagnetic and appear to have a square planar geometry.

INTRODUCTION

SCHIFF bases continue to find wide application in coordination chemistry, but the nitrogen/oxygen donor groups are, getting superseded by ligands containing other donors.

We are currently investigating the general donor properties of these ligands (I) and wish to report here, their preparation and characterization. Synthesis and structural studies on the palladium(II) complexes of these Schiff bases have also been described in the present communication.