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The Juvenile Hormone Analogue W-328 Affects Adult Development and Emergence in the Tsetse Fly, *Glossina fuscipes fuscipes* (Diptera: Glossinidae)

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Abstract

The tsetse fly *Glossina fuscipes fuscipes* Newstead (Diptera: Glossinidae) transmits protozoan parasites of the genus *Trypanosoma*, which cause human trypanosomosis. The disease leads to morbidity and eventually mortality if not treated. Biological compounds such as the juvenile hormone mimics methoprene, precocene and pyriproxifen have been shown to disrupt reproduction and development in some species of tsetse flies. There is need to identify other such compounds that may be used in control programmes. This study evaluated the effects of the juvenile hormone (JH) analogue W-328 on reproduction, development and emergence in *Glossina fuscipes fuscipes*. Topical treatment of adult female flies with the juvenile hormone analogue W-328, ranging from 0.001 to 100 mg showed no effects on number of larvae deposited, pupariation and pupal weight. However, adult emergence was inhibited in most pupae. Physical examination of the pharate adults showed that tergites were unclerotized in about 67.4 % (n= 181) of the flies. The analogue also interfered with pigmentation of the abdominal integument, mid-dorsum of the thorax and eyes. Treatment of puparia of varying ages with the analogues inhibited emergence only in puparia less than five days old. Those aged five days and above showed high emergence rates irrespective of dose of analogue used. The results indicate that the analogue may be used to inhibit development of *G. f. fuscipes*.

Keywords: *Glossina fuscipes fuscipes*; juvenile hormone analogue; W-328; reproduction; development; emergence.

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1. Introduction

The tsetse fly *Glossina fuscipes fuscipes* Newstead transmits protozoan parasites of the genus *Trypanosoma*, which cause African human trypanosomosis. The disease leads to morbidity and eventually mortality if not treated. An estimated four million people are at risk of acquiring the disease in Africa [1]. The survival of the tsetse fly is determined by its fecundity and offspring viability. Thus, compounds that affect such parameters may be used to suppress the tsetse fly population in control programmes. Some of the compounds that have been shown to disrupt reproduction and development in *Glossina morsitans morsitans* include juvenile hormone (JH) mimics [2, 3, 4], precocene [5], diflubenzuron [6, 7], pyriproxifen [8] and benzyl-1, 3-benzodioxoles [9], among others. These biological compounds act on tsetse flies by interfering with development of the adult within the puparium, and are easily applied topically on the integument. Langley and others topically treated female *G. m. morsitans* flies with several JH mimics and found that the females larviposited normally, but adult development within the puparium and emergence were inhibited [2]. Male tsetse flies may also transfer sterilizing effects of JHAs during mating [8]. It is important to formulate and screen other compounds for their efficacy in inhibiting development in tsetse flies. In the present study, the effects of the juvenile hormone analogue W-328 on reproduction, development and emergence of *Glossina fuscipes fuscipes* was evaluated. Our hypothesis was that the analogue has no effect on reproduction and adult development.

2. Limitations of the Study

The study was limited by the small sample sizes, due to low rate of laboratory establishment of the species.

3. Materials and Methods

3.1 Effect of W-328 on adult Glossina fuscipes fuscipes

The juvenile hormone analogue W-328 (2-(4[(1, 4-dioxaspirol [4, 5] dec-6-yl) methyl]phenoxy) ethyl carbamate) (Institute of Organic Chemistry and Biochemistry, Prague) [10] was used in this study. The analogue was dissolved in acetone and serially diluted into 0.001, 0.01, 0.1, 1, 10, and 100 µg\ µl dosages. Female tsetse flies were mated when two to three days old and placed in each of the treatment or control groups. Treatment was effected 24 h post-mating. During treatment, a fly was held between the thumb and index finger and the analogue topically applied on the abdomen ventrally. In control experiments, adults were treated with acetone only. Experimental flies were maintained under insectary conditions at 24 ± 1°C and 80-85% RH, and observed daily. Fecundity was evaluated over a 45-day period, and was calculated as the actual number of puparia produced per surviving female divided by the theoretical maximum number of puparia that each surviving female could produce in the given time interval. Only females that survived through at least one pregnancy cycle were taken into consideration. Puparia formed from larvae deposited by the females were kept in vials under similar conditions as adults. Following emergence of adults from control groups, puparia from experimental groups were observed for a further 10 days. They were then dissected [11] and assessed for morphological effects of the treatment.

3.2 Effect of W-328 on puparia

Puparia deposited by female *Glossina fuscipes fuscipes* were collected daily, between 0800 and 0900 h, at which time they were considered to be one day-old. Puparia aged between one and 10 days were treated with one µl containing between 0.001 to 100 µg W-328. Control puparia were exposed to acetone only. The puparia were kept under similar conditions to adult flies until the expected date of emergence. The emergence rate and any abnormalities in the emerged flies were then determined and recorded. Those that failed to emerge were dissected for morphological assessment.

3.3 Classification of morphological effects

Following dissections of puparia that failed to emerge, the morphological effects of the analogue were classified and scored depending on degree of the effects (Table 1). A zero score represents no effect while score nine shows most severe effect. Mean scores for each dose of W-328 were then calculated by multiplying scores by the proportion of individuals and summing them up as follows: $S\emptyset = \sum(Si \times pa)$ Where, Sø is the mean score at a given dose, Si are the individual scores (0-9) and pa are the proportions of affected individuals.

Table 1: Classification of the morphological effects of W-328 on *Glossina fuscipes fuscipes.* Puparia that failed to eclose were dissected and assessed for morphological effects of the analogue. Scores 0-3 represent adults which emerged (+) while in scores 4-9, emergence was inhibited (-)

Score	Description of effects	Ability to emerge
0	Normal adults	+
1	Wings crumpled in both males and females, reflecting failure in expansion of wings	+
2	Male genitalia straight, or pointing sideways, reflecting incomplete rotation	+
3	Lack of expansion in cuticle of pre- and/or post-abdomen. Small weak adults with reduced number of abdominal segments	+
4	Epidermis well formed, moulting fluid resorbed.	-
5	All tergites formed as narrow bands; no hairs on segments; moulting fluid resorbed	-
	Whole abdomen pigmented brown, with long hairs on tergal areas. Head and thorax normally formed, but mid-dorsum with scanty hair; only the last one to three tergites sclerotized	-
7	Abdomen, mid-dorsum and proboscis not pigmented; lack of resorption of moulting fluid; abdomen flabby, with scanty hairs; tergites not joined in mid-dorsal line	-
8	Abdominal cuticle of pupal type, with no hairs; moulting fluid not resorbed; head and thorax formed, and both eyes pigmented;	-
9	Dead at pupal stage	-

4. Results

4.1 Effect of W-328 treatment on adult tsetse flies

General Linear Models procedure (SAS version 9.0) showed that when topically applied to female flies, W-328 had no effect on puparial weights and fecundity, irrespective of the dose tested ($p > 0.05$) (Table 2).

Table 2: Effect of W-328 on puparial weight and fecundity of female *G. f. fuscipes*. Two to three day-old female *G. f. fuscipes* were mated with males then topically treated with doses of W-328 ranging from 0.001 to 100 µg in 1 µl, 24 hours later

Dose (µg/µl)	n	(Mean ± S.E.) Puparial weight (mg)	Fecundity
0.001	20	31.6 ± 1.53 a	0.90 ± 0.10 a
0.01	20	31.9 ± 1.24 a	0.90 ± 0.05 a
0.1	27	31.0 ± 0.53 a	0.80 ± 0.09 a
1.0	20	30.6 ± 0.91 a	0.91 ± 0.10 a
10	15	31.1 ± 0.76 a	0.73 ± 0.13 a
100	20	30.4 ± 0.53 a	0.77 ± 0.08 a
Acetone	19	32.3 ± 0.49 a	0.92 ± 0.03 a

Means followed by the same letter along a column are not significantly different ($P > 0.05$).

The puparia produced appeared physically normal, with high mean weights at all doses tested. Although larviposition was high, most adults of treatment groups failed to eclose (Table 3). Puparia from acetone treated females showed 86.4% (n= 66) emergence rate.

Following dissection of puparia that failed to eclose, it was observed that the most common morphological effect of the analogue on pharate adults was on abdominal tergite formation. The tergites were unsclerotized and not joined in the mid-dorsal line in about 67.4% (n= 181) of the offspring.

The abdominal integuments were flabby, moist and transparent. Adults had hairs and bristles pointing in various directions instead of posteriorly, and tergal areas and the mid-dorsum of the thorax were covered with short, sparsely distributed hairs.

The abdomen, proboscis and mid-dorsum of the thorax were unpigmented. The compound eyes were unevenly pigmented, with sections or whole of an eye having yellow pigments. This contrasted with flies from control groups that showed 86.4% emergence, had well developed integuments, with the whole body pigmented and eyes pigmented reddish brown.

Table 3: Emergence rates in pupae of *G. f. fuscipes* following topical treatment of two to three day-old females with doses of W-328 ranging from 0.001 to 100 µg in 1 µl.

Dose (µg/µl)	No. of puparia	Emergence rate (%)
0.001	64	1.6
0.01	75	2.6
0.1	84	0
1.0	66	0
10	36	2.8
100	56	0
Acetone	66	86.4

The females were mated with males 24 hours later and allowed to larviposit.

4.2 Effects of W-328 treatment on puparia

There was a generally slight increase in mean morphological scores with concentration of W-328 (Fig. 1). Morphological effects of the analogue on pharate adults were age-dependent, with higher emergence rates being recorded in older puparia (Fig. 2). Emergence was inhibited in puparia aged between one to four days post larviposition. Emergence rate in puparia treated at five days was about 26.7% (n= 60). However, 53 % (n = 16) of these emergents were deformed (Fig. 2). Acetone treated puparia showed high rates of emergence, with a mean of 86.4% (n= 66). Other effects of the analogue included a reduction in width, length, or both width and length of either the pre-abdomen, post-abdomen or the whole abdomen. This abnormality affected both male and female tsetse flies.

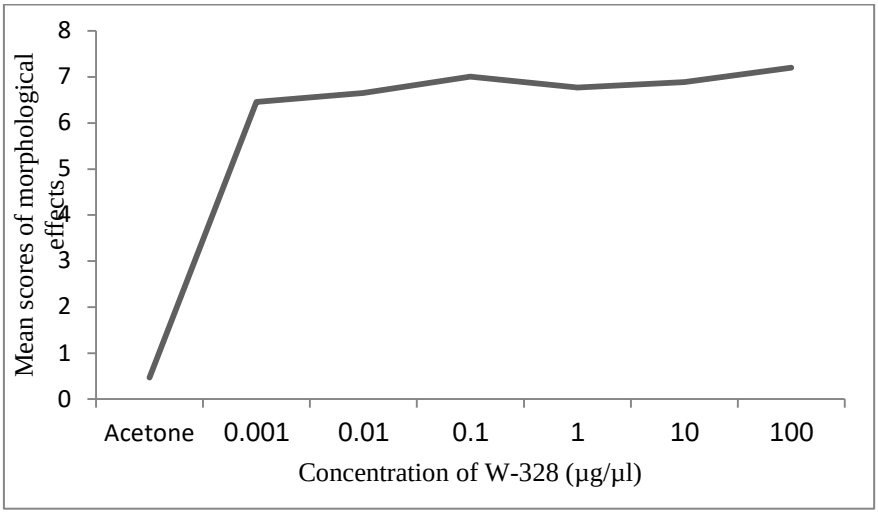

Figure 1: Relationship between concentrations of W-328 and mean scores of morphological effects. Puparia that failed to eclose were dissected and morphological effects of W-328 assessed.

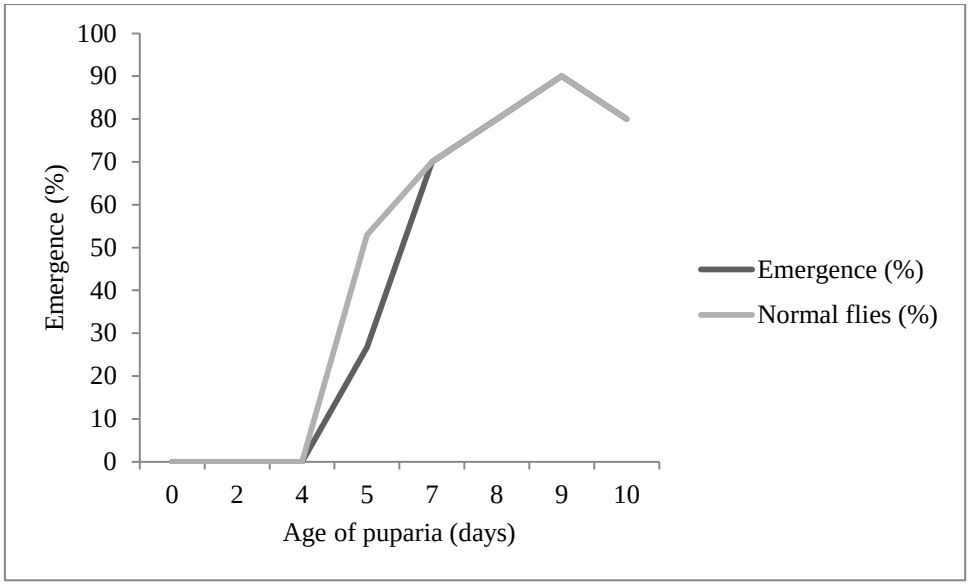

Figure 2: Relationship between age of puparia, % flies emerging and % normal flies following treatment of puparia with 0.1 µg/µl of W-328.

5. Discussion

The fact that the juvenile hormone analogue W-328 had no effect on larviposition shows that vitellogenesis and embryogenesis are not sensitive to the compound. This has been reported for *Drosophila*, where tissues that derive from imaginal discs are insensitive to exogenous juvenile hormone [12]. However, it blocks differentiation of abdominal histoblasts, thereby inhibiting tergite formation and emergence of adults from puparia [13,14]. This concurs with observations by Langley & Pimley [15] and [2]. The analogue also interfered with resorption of moulting fluid [16], as evidenced by the moist, flabby integuments. In control experiments, adults had dry, pigmented integuments, meaning that differentiation of abdominal histoblasts and resorption of moulting fluid proceeded successfully. The unpigmented abdomens in adults that failed to emerge had sparsely distributed microchaetae on tergal areas, pointing to failure of proper formation of adult epidermis. This is consistent with observations by Gnatzy and Romer [17] that JH analogues affect the ability of histoblasts to differentiate fully to adult epidermis, hence affecting growth of microchaetae and pigmentation. Adult cuticle is made by the epidermis, which is also responsible for pigmentation [18]. The untimely presence of juvenile hormones or its analogues suppresses the synthesis of adult cuticular proteins [17] which normally interact with chitin to produce the adult cuticle [18]. The JH analogue inhibited emergence in young puparia but not in older ones. In young puparia, the pupa is still closely adhered to the puparial wall and may be easily reached by the analogues. However, when pupal apolysis occurs around day five post-larviposition, the pupa withdraws from its wall and the wall probably becomes tougher and impermeable to liquids [19]. Field applications of the analogues should therefore be done at critical stages when puparia are still young. For the few flies that emerged, there were abdominal deformities and mortality was high, probably due to inability to digest the blood meal as a result of metamorphic defects in the digestive tract [20]. The observed morphological effects could be due to inability of degradative enzymes to metabolize exogenous JH [21]. The analogues apparently have no effects on mating ability and pupariation [2, 22]. This is a contrast to investigations on the cockroach *Blaberus*

craniifer which showed that W-328 reduces mating performance and oviposition, with high doses leading to sterilization [23].

6. Conclusion

This study has demonstrated that W-328 disrupts normal adult development in *G. f. fuscipes* and inhibits emergence of adult flies.

7. Recommendation

The findings of the study indicate that the juvenile hormone analogue W-328 can be used to reduce populations of *G. f. fuscipes*. The first step should be to carry out field trials of the analogue to assess its efficacy under natural conditions, particularly in islands such as Rusinga Island, Kenya.

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