Laboratory bioassays of deltamethrin, topically applied, during the hunger cycle of male *Glossina tachinoides*

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Key words

Glossina tachinoides - Deltamethrin - Nutrition - Pest control method - Topical application - Laboratory experimentation - Insecticide.

Summary

The susceptibility of mature and immature male *Glossina tachinoides* to topically applied deltamethrin was tested on successive days of their hunger cycle. Both in mature and immature flies, significant changes in tolerance were observed correlated to the digestion of the bloodmeal. In mature tsetse, the fly's fat level is also correlated to its susceptibility.

■ INTRODUCTION

The majority of tsetse control measures rely on the use of insecticides. The success of such control campaigns partly depends upon the tsetse's susceptibility to the insecticide used. One well known factor, affecting the susceptibility of female tsetse to insecticides, is associated with pregnancy (2, 17).

In male and female *Glossina palpalis palpalis*, the presence of undigested blood in the abdomen also affects their tolerance to several insecticides (18). Moreover, other studies (22) have indicated that replete male *G. tachinoides*, when brought into contact with a deltamethrin dipped guinea-pig, were also more susceptible than hungry ones. Similar observations were made in mosquitoes (10). Although the mechanism is not well understood, these findings suggest that susceptibility to deltamethrin is influenced by the feeding status of the insect.

Tsetse flies feed, at regular intervals, on the blood of vertebrates. The period between two consecutive blood meals, during which the blood meal is metabolized, is called the hunger cycle (5, 12). Clear differences, however, exist between blood meal metabolism in mature and immature male tsetse flies. In young or immature tsetse, the energy obtained from each blood meal is used for growth or maturation. This early growth phase is associated with the maturation of the flight system, the incorporation of new material in the cuticle and the gradual accumulation of a body fat reserve. In mature male tsetse, on the other hand, the body fat reserve undergoes significant changes during each hunger cycle. The net gain of fat from each blood meal is spent for resting metabolism, flight activity or spermatophore production (19).

This paper reports the toxicity of topically applied deltamethrin, a synthetic pyrethroid widely used in tsetse control, to mature and immature male *G. tachinoides* and tries to relate changes in the susceptibility to physiological changes during the male tsetse fly hunger cycle.

■ MATERIALS AND METHODS

Tsetse flies

Immature male *G. tachinoides* (at most 24 hours old) and mature male *G. tachinoides* (20 days old) were used in the experiments. They were maintained in the routine, guinea-pig fed, colony at the Veterinary Department of the Institute of Tropical Medicine in Antwerp, Belgium. They were kept in standard oblong cages (160 x 70 x 80 mm) at a maximum density of 30 flies per cage at 25 ± 0.5°C and 76 ± 5 % RH (21).

Mature male *G. tachinoides* were fed daily (except during weekends) on guinea-pigs, and received a last blood meal on day 20. Only fully engorged flies were retained and used for testing.

Immature flies had a feeding chance 24 h after emergence. Here also, only the fully engorged flies were used for testing.

An artificial hunger cycle was created by starving both mature and immature flies for 1 to maximum 5 days following the last blood meal.

Insecticide treatment

Technical deltamethrin (Roussel Uclaf) dissolved in 2–ethoxyethanol (Cellosolve®, BDH Ltd.) was administered to the flies, after immobilization with nitrogen gas, by topical application to the dorsal surface of the thorax. Each fly received 0.5 µl 2-ethoxyethanol containing 0.08 ng of deltamethrin by means of an Arnold Hand Applicator (Burkard Manufacturing Co.

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Ltd.). Different batches of 40 mature male *G. tachinoides* were topically treated once on one of the successive days of their hunger cycle: day 0, 1, 2, 3, 4 or 5. Day 0 being the day of the last blood meal. Treatment on day 0 was done immediately after the blood meal uptake. Groups of at least 54 immature males *G. tachinoides* were also topically treated on one of the days of their hunger cycle (day 0, 1, 2, 3 or 4). The test was repeated for immature flies, starved during 1 or 4 days but fed just before treatment.

Batches of mature and immature male control flies were handled similarly as described above receiving the solvent only. Mortality was recorded 24 and 48 h after treatment. During this period flies were not fed. Mortality among treated flies was corrected in comparison with the mortality observed in the control group according to the formula of Abbott (1).

Determination of changes in fat level during the hunger cycle

The technique described by Bursell (3) was used. Fat was extracted from dry flies using chloroform. Groups of 40 mature and immature male *G. tachinoides* were offered a blood meal on a guinea-pig. The fully engorged flies were retained and starved as described above. Their fat level was determined on the successive days of the hunger cycle.

■ RESULTS

Mortality of immature flies

The mortality and corrected mortality of immature male *G. tachinoides* treated topically with deltamethrin (0.08 ng/fly) on day 0, 1, 2, 3 or 4 of the hunger cycle is presented in table I.

Mortality when immature male *G. tachinoides*, starved as described above, were fed just before topical treatment with deltamethrin (0.08 ng/fly) on day 1 or 4 of their hunger cycle is shown in table II.

Table I clearly shows that mortality of immature flies increased with increasing starvation. If, however, flies were fed just before treatment mortality was significantly lower (table II).

Mortality of mature flies

The mortality and corrected mortality of mature male *G. tachinoides*, treated topically with deltamethrin (0.08 ng/fly), on one of the days of the hunger cycle are presented in table III.

Similarly to immature male flies, mortality in mature male flies increased when treatment was done later in the hunger cycle. For each of the days of the hunger cycle mortality of mature flies was lower than mortality of immature flies.

Fat level of mature and immature male G. tachinoides *during the hunger cycle*

The fat level of mature male *G. tachinoides* on successive days of the hunger cycle is presented in table IV.

The fat level of immature male *G. tachinoides* changed little during the first hunger cycle and had an average value of 0.48 ± 0.12 mg.

The fat level of mature male *G. tachinoides* on consecutive days of the hunger cycle, expressed as a percentage of the body weight, is presented in figure 1.

Fat level dynamics during the hunger cycle (figure 1) consisted of a short phase of lipogenesis, followed by a phase of lipolysis. A similar pattern was observed by Bursell *et al.* (5).

Table I

Mortality and corrected mortality of immature male *G. tachinoides* after a single treatment with deltamethrin (0.08 ng/fly) on one of the days of their hunger cycle

Day of hunger cycle	Flies		Mortality after 24 h (%)		Total mortality after 48 h (%)		%-Corrected mortality	
	Control	Treated	Control	Treated	Control	Treated	24 h	48 h
0	60	60	0 (0)	12 (20.0)	4 (6.7)	16 (26.7)	20.0	21.4
1	54	54	0 (0)	0 (0)	0 (0)	9 (16.7)	0	16.7
2	54	60	6 (11.1)	8 (13.3)	6 (11.1)	16 (26.7)	2.5	17.4
3	60	60	0 (0)	36 (60.0)	0 (0)	40 (66.7)	60.0	66.7
4	70	60	20 (28.6)	56 (93.3)	20 (28.6)	60 (100)	90.6	100

Table II

Mortality and corrected mortality of immature male *G. tachinoides* starved as described above and fed just before topical treatment with deltamethrin (0.08 ng/fly)

Day of hunger cycle	Flies		Mortality after 24 h (%)		Total mortality after 48 h (%)		%-Corrected mortality	
	Control	Treated	Control	Treated	Control	Treated	24 h	48 h
1	45	53	0 (0)	4 (7.5)	0 (0)	4 (7.5)	7.5	7.5
4	60	51	0 (0)	24 (47.0)	0 (0)	27 (52.9)	47.0	52.9

Table III

Mortality and corrected mortality of mature male *G. tachinoides* after a single treatment with deltamethrin (0.08 ng/fly) on one of the days of their hunger cycle

Day of hunger cycle	Flies		Mortality after 24 h (%)		Total mortality after 48 h (%)		%-Corrected mortality	
	Control	Treated	Control	Treated	Control	Treated	24 h	48 h
0	40	40	0 (0)	0 (0)	0 (0)	0 (0)	0	0
1	40	40	0 (0)	0 (0)	0 (0)	0 (0)	0	0
2	40	40	0 (0)	4 (10.0)	2 (5.0)	8 (20.0)	10.0	15.8
3	40	40	0 (0)	8 (20.0)	0 (0)	12 (30.0)	20.0	30.0
4	40	40	0 (0)	10 (25.0)	0 (0)	16 (40.0)	25.0	40.0
5	40	40	4 (10.0)	10 (25.0)	8 (20.0)	30 (75.0)	16.7	68.7

Table IV

Mean body weight, mean dry weight, mean residual dry weight and mean fat level of mature male *G. tachinoides* on successive days of the hunger cycle

Day of hunger cycle	Number of flies	Mean body weight (mg)	Mean dry weight (mg)	Mean residual dry weight (mg)	Mean fat level (mg)
0	40	19.20 ± 2.34	6.84 ± 1.52	3.67 ± 0.58	2.64 ± 0.59
1	40	15.60 ± 2.10	6.15 ± 1.14	3.61 ± 0.55	2.44 ± 0.87
2	40	13.95 ± 1.85	5.27 ± 1.06	3.56 ± 0.45	1.71 ± 1.04
3	40	11.38 ± 1.63	4.88 ± 0.96	3.56 ± 0.34	1.32 ± 0.78
4	40	12.11 ± 1.84	4.30 ± 0.97	3.31 ± 0.49	0.99 ± 0.72
5	40	12.03 ± 2.16	4.21 ± 1.34	3.15 ± 0.54	0.84 ± 1.03

***Figure 1**: Changes in the fat reserve during the hunger cycle of mature male* G. tachinoides.

The correlation between fat level and mature male mortality, 48 h after the application of deltamethrin (table III), was highly significant ($r = -0.95$, $p < 0.01$). A similar negative correlation ($r = -0.995$, $p < 0.01$) was obtained between mature male mortality, 48 h after the application of deltamethrin (0.08 ng/fly) and their average residual dry weight on the corresponding days.

■ DISCUSSION

Mortality of immature male *G. tachinoides* (table I) shows the high level of tolerance to topically applied deltamethrin when treatment is done shortly after feeding. Once the blood meal is being digested and excreted, 4 days after the blood meal uptake (12), mortality increases up to 100 % (table I). Moreover, independently of the degree of starvation, the uptake of a blood meal prior to treatment decreases mortality by about 50 % (table II). These observations clearly show the protective role of the blood meal which is attributed to the high amount of non-target tissue diverting the insecticide to sites of ‘non lethal action’ (18).

Increasing susceptibility to deltamethrin, on successive days of the hunger cycle, in mature male *G. tachinoides* (table III) could partly be explained in a similar way. Mortality is significantly negatively correlated to the average corrected residual dry weight, reflecting the gradual excretion of the bloodmeal on the successive days of the hunger cycle. However, this correlation cannot explain the general lower susceptibility of mature flies compared to immature flies. The latter is attributed to a supplementary non-target tissue in mature male tsetse, i.e. the body fat reserve. Compared to immature tsetse flies, fat level in mature flies

Revue Élev. Méd. vét. Pays trop., 1996, 49 (4) 329-333

undergoes considerable changes during the hunger cycle (figure I) and is significantly negatively correlated to the mature male fly susceptibility to topically applied deltamethrin on the respective days. This observation gives supportive evidence of the supplementary role of the fat body in the mature male tsetse fly susceptibility to topically applied deltamethrin. It is assumed that the high tolerance of flies with a higher fat body level is due to the accumulation of deltamethrin in the fat. Equally in the cockroach, *Periplaneta americana*, the progressive accumulation of pyrethroids in the fat body with simultaneous decreasing efficacy of the insecticide has been observed (6). Whether this observation is applicable to other insecticides is not yet known and merits further investigation. Although, it will probably be restricted to highly liposoluble insecticides such as deltamethrin.

These observations might have practical implications.

Both Golder (8, 9) and Nitcheman (15, 16) observed that male and female *G. morsitans morsitans* harboring metacyclic trypanosomes were more susceptible to topically applied endosulfan, pyrethrum and deltamethrin than non-infected flies. This could be related to our observations. It is known that metacyclic trypanosomes interfere with the normal function of the labrial mechanoreceptors (11, 13, 14) causing a higher probing frequency without increase in feeding frequency. Moreover, an infected tsetse may experience a significant daily energy loss as a result of the competition between parasites and vectors for metabolic substrates (4). The infected tsetse impaired feeding ability and the high energy requirements of the trypanosomes may result in a poor nutritional status and thus lower fat body level. This was observed by Ryan (20) in *G. longipalpis* and could be the reason for the increased susceptibility to deltamethrin of infected tsetse. Deltamethrin is extensively used in tsetse control. According to the results in this study the efficacy of such control operations could be largely affected by the average fat level of the tsetse population or the fat level of the flies attracted to the deltamethrin-treated baits. Moreover, since the fat level in tsetse changes seasonally (7), mortality caused by deltamethrin application could be significantly higher in seasons when fat level is lowest.

Acknowledgements

The author wishes to thank Drs. R. De Deken and A. Van der Vloedt for their helpful discussion and comments on the manuscript. Professor J. Mortelmans is thanked for his help in various ways.

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Reçu le 18.9.96, accepté le 25.2.97

Résumé

Van den Bossche P. Etude en laboratoire de l'application topique de la deltaméthrine pendant le cycle nutritionnel des mâles de *Glossina tachinoides*

La sensibilité des mâles matures et immatures de *Glossina tachinoides* aux applications topiques de deltaméthrine a été testée sur plusieurs jours successifs de leur cycle nutritionnel. Des variations significatives à la tolérance de la deltaméthrine chez les mâles matures et immatures ont été observées en corrélation avec la digestion du repas sanguin. Chez les mouches tsé-tsé matures, le pourcentage de graisse est également corrélé à cette sensibilité.

Mots-clés : *Glossina tachinoides* - Deltaméthrine - Nutrition - Méthode de lutte antiparasite - Application locale - Expérimentation en laboratoire - Insecticide.

Resumen

Van den Bossche P. Bioanálisis de laboratorio de la deltametrina, aplicada en forma tópica, durante el «ciclo de hambre» del macho de la *Glossina tachinoides*

Se analizó la susceptibilidad de machos maduros e inmaduros de *Glossina tachinoides*, a la deltametrina aplicada en forma tópica, en días sucesivos del «ciclo de hambre». En el caso de ambas moscas, maduras e inmaduras, se observaron cambios significativos en la tolerancia, correlacionados con la digestión de la sangre utilizada para la alimentación. En las tsetse maduras, el nivel de materias grasas de la mosca se encuentra igualmente correlacionado con la susceptibilidad.

Palabras clave : *Glossina tachinoides* - Deltametrina - Nutrición - Método de control de plagas - Applicación local - Experimentación en laboratorio - Insecticida.