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RESERPINE INDUCED EFFECTS ON GROWTH AND REPRODUCTION OF *CULEX QUINQUEFASCIATUS* MOSQUITOES (DIPTERA: CULICIDAE)

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Abstract: Reserpine, a plant alkaloid, which acts by depleting stores of monoamines in the nervous system, was used to probe neuroendocrine regulation in *Culex quinquefasciatus* mosquitoes. Larval development was slightly delayed and, the resulting adults were small but their gonads were not affected by reserpine. Reserpine treatment of adults had a profound effect on their ability to feed on a vertebrate host and significantly reduced mosquito fecundity.

Key words: Bloodmeal, *Culex quinquefasciatus*, fecundity, reserpine.

INTRODUCTION

In anautogenous mosquitoes, events starting from the intake of a bloodmeal to oviposition are controlled by neuroendocrine mechanisms involving the release of peptide and other hormones.[1] The digestion of a bloodmeal is followed by the synthesis of yolk proteins in the fatbody. These proteins accumulate inside the oocytes which are eventually deposited as eggs during oviposition. As in vertebrates, the monoamines present in the insect nervous system appear to serve important roles as neurotransmitters and neurohormones.[2] Monoamine neurotransmitters of the neuroendocrine system in insects can be depleted from their storage sites by the plant alkaloid reserpine.[3] Reserpine, isolated from *Rauwolfia serpentina* L. alkaloid=serpentine (S:Ekaweriya; T:Chivan-ampelpodi) has been reported to induce developmental defects in insects. These include a delay in moulting in Lepidoptera,[4] interference with egg maturation in Diptera, Coleoptera and Lepidoptera,[5-7] and the blockage of ovulation and insemination in Diptera.[8] Though not conclusively demonstrated, these effects on development and reproduction could probably be correlated with the disruption of monoaminergic transmission.

Here we examine the effect of reserpine on larval growth and reproduction in *Culex quinquefasciatus* Say, the vector of *Wuchereria bancrofti* Cobbold, the causative agent of bancroftian filariasis.

METHODS AND MATERIALS

First instar larvae from *Cx. quinquefasciatus* eggs obtained from the laboratory colony were reared in stainless steel trays (40x29.5x5cm) at a density of 200 larvae in 2 l of tap water per tray and held at 27 ±1°C, 70% relative humidity and natural day - night photoperiod. Larvae were fed powdered soyameat and the adults provided 10% glucose supplemented with multivitamins. The colony was blood fed overnight on Balb/c mice.

Two hundred and fifty first instar larvae were treated with 200 μg of reserpine (Sigma, U.S.A) which was dissolved in 20 μl of acetone and added to larval trays containing 2 l water (i.e 0.1 μg/ml) immediately after hatching of eggs and thereafter at 48h intervals throughout the entire larval period until pupation. Trays serving as controls received only acetone at 48h intervals. Reserpine treated and control larval trays were maintained in triplicates. Powdered soyameat (375 mg) was added to each tray daily. The water level in the trays was maintained constant by the daily addition of water. Larvae were observed daily to determine growth, ecdysis and then pupation. Adults emerging from these pupae were held in cages, provided glucose and vitamins and blood fed on mice overnight. A surface for oviposition (dish containing water from larval tray) was provided in the adult rearing cage and the egg rafts collected. The viability of these eggs was assessed by counting the larvae hatched and the adults that subsequently resulted from them.

In another series of experiments, adult mosquitoes from the colony were treated with reserpine. Approximately 300 newly emerged adults (equal numbers of males and females) were fed reserpine which was introduced into the glucose solution at a concentration of either 10 μg/ml or 30 μg/ml. Control mosquitoes had acetone added to the glucose solution. Immediately after adult eclosion and prior to reserpine treatment, 5-6 female and 5 male mosquitoes were removed from each cage to determine body weight (Mettler M3 balance) and wing length (measured under the microscope using a stage micrometer); the total carbohydrate content in individual mosquitoes was determined using freshly prepared anthrone reagent and the optical density determined at 625 nm.[9] On day 4 after emergence, reserpine fed mosquitoes were blood fed overnight on a restrained mouse and the proportion of bloodfed mosquitoes and the body weight of the bloodfed females were determined. Due to logistical reasons the same mosquito could not be weighed immediately before and after a bloodmeal. Therefore, mosquitoes of similar size (wing length used as indicator of size) were removed from the cage and weighed before the vertebrate host was presented, and another group of mosquitoes removed and weighed and wing measurements taken after blood feeding.

Groups of mosquitoes were removed at 12, 36 and 72h after the bloodmeal and dissected to determine the extent of ovarian development.[10] The remaining bloodfed mosquitoes were held in cages with access to glucose without reserpine and provided with a surface for oviposition. Fecundity of the mosquitoes was

determined by counting the numbers of eggs in the egg rafts produced. Statistics of data analysis is indicated in the results section.

RESULTS

Larvae maintained in the presence of 0.1 μg/ml reserpine in the rearing medium were sluggish and poor feeders with larval food accumulating in the rearing trays. This effect of reserpine was pronounced in instars 1 and 2, but not later in instars 3 and 4; they pupated in 8 days compared to the control larvae which were more active and pupated in 7 days. There was no difference in mortality observed between the two groups of larvae. The adults emerging from these reserpine treated larvae/ pupae were smaller in size (as indexed by wing length) when compared to controls (Table 1). Body weights of treated unfed female mosquitoes were significantly lower than unfed controls (Table 1). Male mosquitoes resulting from reserpine treated larvae weighed 1.25 ± 0.19 mg while control males had a weight of 1.43 ± 0.13 mg.

Table 1: Effect of reserpine treatment of larvae on mosquito size

		Control (mean ± SE)	Reserpine (mean ± SE)	t*	p
Wing length (mm)	Non bloodfed	4.06 ± 0.04	3.80 ± 0.08	2.63	<0.05
	Bloodfed	4.10 ± 0.11	3.70 ± 0.14	2.21	>0.05
Body weight (mg)	Non bloodfed	2.34 ± 0.07	1.79 ± 0.06	5.78	<0.001

* Students 't' test

Reserpine treatment during the larval stages did not appear to influence the development and function of gonads in the adults. Mosquitoes that emerged after this treatment produced viable eggs that hatched similarly to those of control mosquitoes and produced adults (results not presented). The total carbohydrate content in females was not significantly influenced by the intake of reserpine. Mosquitoes fed either 10 or 30 μg/ml reserpine had a mean carbohydrate content of 20.36 ± 5.77 and 34.6 ± 4.1 mg compared to 38.99 ± 9.26 and 37.89 ± 10.4 mg of control mosquitoes. There was no significant difference in body weights of blood ingested mosquitoes when control and reserpinised mosquitoes of similar size were permitted to feed on a host (Table 2). The body weights of newly emerged mosquitoes in Table 2, are lower than those observed in Table 1. This could be attributed to size variations between different batches of insects in the colony. Feeding 30 μg/ml reserpine for 4 days to the adults prior

to being presented with a restrained vertebrate host, had a profound influence on blood feeding. Reserpinized mosquitoes were sluggish and not readily attracted to the host (Table 3) and a significant reduction in feeding was observed. On dissection of bloodfeds, oocytes in stages II, IIIb and IVa[10] were observed at 12, 36 and 72h after bloodmeals in both control and reserpine (30 μg/ ml) fed mosquitoes. Feeding reserpine to adults reduced the number of eggs in the rafts of mosquitoes fed reserpine. This reduction was statistically significant at a dosage of 30 μg/ ml of reserpine (Table 4).

Table 2: Effect of reserpine on the size of the bloodmeal

Dose of reserpine		Body weight mg (mean ±SE) Control	Reserpine
10 μg/μl	Non bloodfed (10)	1.46 ±0.12	1.21±0.10
	Bloodfed (10)	2.53 ±0.16	2.56 ±0.15
30 μ/μl	Non bloodfed (10)	1.53 ±0.14	1.44 ±0.15
	Bloodfed (10)	3.69 ±0.44	3.58 ±0.27

Numbers in parentheses indicate number of observations.

Table 3: Effect of reserpine (30 μg/ml) on the ability of *Cx. quinque - fasciatus* to feed on a restrained host.

	Total no. of mosquitoes	No. of blood feds	% engorged
Control	151	136	90*
Reserpine	123	26	21

*$p < 0.0001$ ($\chi^2 = 133.26$)

DISCUSSION

Although the dosage of reserpine used in our investigations was lower than the amounts used in previous studies, the major effect of reserpine on the larval stage of *Cx. quinquefasciatus* was to produce smaller adults. Reserpine at a dosage of 0.1 μg/ml was most effective on larvae of stages 1 and 2, the effect declining later, probably due to increased body size and possible changes in metabolism of the larvae. A large dose of reserpine at this stage may be required to prolong the larval stages beyond 8 days. The smaller sized adults that were

produced from reserpine treated larvae, may be attributed to poor larval feeding in the early instars. Since the size of the bloodmeal was not affected by reserpine treatment of adults, the reduction in fecundity (number of eggs) may be a consequence of reduced synthesis of yolk protein and/ or its accumulation in the oocytes.

Reserpine treatment resulted in the accumulation of neurosecretory material in tenebrionid beetles[6] and in *Galleria mellonella*[11] suggesting a breakdown in the pathway of release of such material, probably due to the depletion of monoamine neurotransmitters. The role of monoamines in regulating the release of neurohormones in *Cx. quinquefasciatus* is not known. A reduction in the ability of reserpine treated mosquitoes to bloodfeed and the observed decline in fecundity of this important medical vector have implications for the dynamics of the vector and disease transmission. Due to its effects on man and other animals there is little potential for using reserpine for controlling mosquitoes at present. However, this study provides insight into neuroendocrine control mechanisms that exist in mosquitoes, which can be exploited in the development of new vector control strategies.

Table 4: Effect of reserpine on the fecundity of *Cx. quinquefasciatus*

	Dose of reserpine	No. of eggs (mean ±S.E.)
Experiment 1	10 μg/ml	68.9 ±3.9[a] (13)
	Control	82.0 ±8.3[a] (10)
Experiment 2	30 μ/ml	95.5 ±16.1[b] (39)
	Control	148.6 ±10.1[b] (9)

[a] $p>0.05$, $z = 1.30$ and [b] $p<0.05$, $z = 2.33$ by Wilcoxon sum of ranks scores.

*Numbers in parentheses indicate number of observations.

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