Sublethal effects of insecticides on the reproductive success of the Tsetse species *Glossina pallidipes* and *G. m. morsitans*

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ABSTRACT

Female *Glossina pallidipes* Austen and *G. m. morsitans* Westwood (Diptera: Glossinidae) were treated with deltamethrin or pyrethrum extract through topical application or by exposure to cloth or glass treated with the same insecticides. Reproductive performance was assessed in terms of survival, pupae number and viability and reproductive deformities and abnormalities within the reproductive systems including abortions. Survival and fecundity of treated flies were significantly reduced ($P<0.001$). Pupae were small, mostly non-viable, and were arrested at various stages of development. Abortions of egg and all stages of larvae were observed in pregnancy cycles 1-7 following insecticide treatment. Various concentrations ranging from $1\text{-}10^{14}$ nanograms per micro litre (ng/µL) of insecticide in redistilled acetone topically applied (1µ/fly) on the dorsal thorax of the females, 24 hr after their previous blood meal, resulted in both insecticides in various concentrations causing reproductive abnormalities in both species, including egg re-absorption after ovulation. The sublethal effects were observed to prevent ovulation altogether in some of the flies, which implies effects on the reproductive performances and control strategies for tsetse flies. Sublethal doses could impact negatively on beneficial organisms in the environment where tsetse flies occur mainly riverine, woodlands and forest ecosystems.

INTRODUCTION

Insecticides applied in the field to control tsetse often do not reach their targets in sufficient quantities to kill all of them (Allsopp, 1984), mainly because tsetse resting sites are typically on the underside of leaves and twigs (Moisier, 1912; MacDonald, 1960; Carnevale & Adam, 1971; Challier, 1973; Baldry, 1980), where the flies are less exposed to aerosol droplets. Even when insecticides are applied by helicopters (Baldry et al., 1978; Lee *et al.*, 1978; Molyneaux, *et al.*, 1978; Van Wettere *et al.*, 1978), control measures do not reach 100% effectiveness. Lee *et. al.*, (1980) noted that applications by helicopter result in the deposition of insufficient droplets to kill *G. tachinoides* Westwood, hence effects on tsetse

would largely depend on nocturnal vertical air movements (MacLennan & Cook, 1972). Other authors also questioned the penetration of residual helicopter applications (Johnstone *et al.*, 1974). The use of helicopters to apply dieldrin into dense thickets in Kenya's Lambwe valley (Le roux & Plat, 1968) revealed similar problems.

Most of the droplets applied during aerial spraying are presumably intercepted by the canopy before reaching tsetse lies. Similarly, after ground spraying of residual insecticides, new flies quickly enter the area (Itard, 1988; Finelle, 1980). Pupae underground are also not directly affected. By the time these adults emerge, most of the insecticides application has been dilute by rain. Flies are exposed to low doses.

Laboratory assessments of toxicity to tsetse are necessary before an insecticide is put into operational use. The term "sublethal effects" has usually describes effects demonstrated in survivors of doses which kill a given proportion of the experimental insects within a defined period of time, for instance 24 hr or 48 hr after exposure. The pioneer work on sublethal effects of insecticides on female tsetse fly *G. palpalis* is by Baldry (1964). He reported effects on longevity and reproductive performance in the survivors of doses of DDT and dieldrin which killed 72.9% - 97.4% of the flies in the 48hrs after treatment. According to Baldry, insecticides which have deleterious effects on those insects surviving acute poisoning are likely to be more efficient than insecticides with comparable acute toxicity but no such after effects on survivors. Moriaty (1969) reported sublethal effects of insecticides on a wide range of insects, and suggested that, although they must often contribute to the impact of insecticides on pest populations in the field, they have largely been ignored in relation to control.

Kwan and Gatehouse (1978) showed that low doses of insecticides topically applied to *G.m.morsitans* affected its reproductive activities in several ways (for example, treated flies separated earlier than untreated flies during mating) and reduced the number of pupae surviving. Such doses also affected feeding activities of the tsetse flies. Later on, exposure to endosulfan during the first cycle resulted in F1 mortalities of 39-63%, while the offspring of females treated in the second cycles suffered 19-29% mortality (Kwan and Gatehouse, 1982).

Deltamethrin is particularly effective in killing tsetse (Barlow *et al.*, 1977; Spielberg *et al.*, 1979; Challier *et al.*, 1979; Lavéissiére *et al.*, 1980). It is commonly used to impregnate traps and targets. However, when these traps and targets are left in the field under the tropical sun and rain, the insecticides breakdown rapidly. In Zimbabwe, older techniques employing DDT-impregnated targets also failed to maintain toxicity under the tropical environment conditions (Allsopp, 1984). Hence, under field conditions, tsetse flies are often exposed to sublethal doses of insecticides in typical control; campaigns. Deltamethrin is potentially the most

valuable pyrethroid for tsetse control (Hadaway, 1977; Spielberger *et al.*, 1979; Opiyo e*t al.*, 1988). It is one of the photostable pyrethroids, its stability having been achieved by modifying the two photo-labile zones of the permethrin structure (Elliot *et al.*, 1978). Preliminary laboratory studies involving topical application techniques demonstrated that it had an intrinsic toxicity to *Glossina* species approximately 125 times higher than that of dieldrin (Barlow and Hadaway, 1974). A number of workers have demonstrated the potential of deltamethrin for future tsetse control programmes (Barlow *et al.*, 1977; Challier *et al.*, 1977; Hadaway, 1978; Spielberger *et al.*, 1979; Quinlan and Gatehouse, 1981). Trials on ground (Spielberger *et al.*, 1979), in mixtures with endosulfan (Andrews *et al.*, 1983), in traps and targets (Lavéissiére *et al.*, 1981), have revealed the possibility of using extremely low doses for residual and aerosol applications for tsetse control. More field trials and laboratory bioassays are required in order to firmly establish deltamethrin as an effective substitute of the well-established insecticides in tsetse control.

Field and laboratory observations of insecticides on *Glossina*

Accounts of the use of dieldrin and endosulfan in *Glossina* control have been given by Spielberger *et al.* (1977) and Davies (1978). Earlier on, Lee *et al.*, (1967) had assessed the effects and economics of aerial applications of pyrethrum as an aerosol spray against *G. pallidipes* in Zambia. The early ground-spraying operations were reviewed and successful campaigns have been described by Davies (1971) for Nigeria, while Lavéissiére *et al.*, (1981) observed a reduction in apparent density by trapping after trapping riverine tsetse flies with screens impregnated with insecticide.

Tsetse are adenotrophic viviparous insects, whose pregnancy cycle dynamics are often unknown. The reproductive abnormalities observed in the females are described in detail in this paper in relation to the insecticide impact on the reproductive potential and eventual effects on the population dynamics. The low fecundity of tsetse makes them particularly vulnerable targets for insecticides. Control methods should focus on disrupting physiological processes in insect vectors.

Reports on sublethal effects are few but include the observation that low doses may indirectly result in death for example by starvation (Quinlan & Gatehouse, 1981; Baldry, 1964; Sebitosi & Chaudhry, *et al.,* 1988). Despite these studies, sublethal effects of insecticides on the reproduction of *G. pallidipes* and *G.m.morsitans* remain unknown. The study presented here investigated the effects of sub-lethal doses of insecticides on the reproduction success of tsetse.

MATERIAL AND METHODS

Tsetse

Female mated tsetse flies of two species *Glossina pallidipes* and *Glossina morsitans morsitans* were used in these experiments. *G. pallidipes* adults were collected from Nguruman near Magadi town, 70 Km southwest of Nairobi, Kenya where the species inhabits a wooded bushland and open grass alluvial plains of semi-arid land. The Tsetse flies were collected in biconical traps (Challier and Lavéissiére, 1965) baited with acetone and cow urine. They were maintained in an insectary at 25^0C; 70-80% relative humidity. Flies were fed daily with rabbit ears and held for a period of one week before treatment o screen out mortalities due to handling and were allowed to acclimatize to laboratory conditions. Pregnant females recognized by the presence of third instar larvae *in utero* were separated out to synchronize larviposition and the next pregnancy cycles.

Glossina m. morsitans were obtained from the International Centre of insect Physiology and ecology (ICIPE) colony which originated from Langford, Bristol, U.K, in 1965. They were kept under similar conditions like *G.pallidipe*.

Insecticides

Deltamethrin (98%), pyrethrum extract (25%) from Wellcome Kenya Ltd were dissolved in acetone at doses between -10 and 15ng and applied in a volume of one micro litre to the dorsal thorax of 10-20 flies per replicate, without prior anaesthesia. Control tsetse flies were treated with 1μl acetone. All treated flies were maintained in the insectary kept at 25 0C and 70% R. The flies were observed for abnormalities.

Reproductive abnormalities &Tsetse fly dissections

The females were dissected in a drop of normal saline (0.9 NaCl) after they died. Within 24 hrs of the fly's death, the female's legs and wings were removed, and then the fly was put on a glass slide dorsal ventrally. In a drop of normal saline, the second abdominal segment was gently pulled out using fine forceps, while the thorax was firmly held on the slide. The ovaries could be seen as white oval structures. The Reproductive abnormalities were determined according to Saunders (1962) and Challier (1965) and suspected abortions recorded according to Madubunyi (1975). The ovaries of each fly were examined for configuration and evidence of ovulation through the presence of follicular relics and the number of eggs. The normal ages of death were established according to Saunders (1962) ovarian dissections.

Progeny from sublethal doses treated females

The pupae from all the females were maintained singly in plastic tubes until they emerged. A record of incubation periods was kept. The feeding frequency and the actual blood meal size by low dose treated females were also kept and results of feeding patterns and behaviour published else where.

RESULTS

Normal tsetse fly female reproductive system

The female reproductive system is comprised by a uterus and two fallopian tubes on either side of the uterus (Fig. 1). One egg develops to maturity, goes down the tube into the uterus where it develops into a larva. The larvae feeds on the uterine glands until it develops into a third instar stage when it is larviposited. The larvae pupates within 1 hr of larviposition.

Reproductive abnormalities

The females treated with pyrethrum extract and deltamethrin showed reproductive abnormalities in their reproductive systems. These included:

Abnormality 1: Egg retention

Egg retention in the ovary where by the

Table 1: Percentage distribution of reproductive abnormalities in uterine contents of female
G. pallidipes and _G.m.morsitans_ following treatment with pyrethrum extract

Treatment topical & cloth	Number dissected	Mean Surv (days)	S.E.	No. Normal (%)	No. Abno (%)	Non ovulation (%)	Egg Resorptio (%)	Blocked oviduct (%)	Empty (%)	Egg (%)	Larva (%)	Dry/ brown (%)
Untreated control	27	29.24	1.732	77.8	22.23	22.2	7.4	7.4	3.7	3.7	-	-
Acetone control	26	25.07	2.292	50	30.77	19.23	14.81	-	3.7	18.51	-	19.23
0.001 ng	10	40.8	9.89	70	30	20	20	-	10	10	-	-
0.1	13	40.08	7.414	30.77	61.53	30.769	7.692	-	15.38	15.38	15.38	7.69
1	69	22.97	2.477	15.943	73.913	18.84	5.797	-	-	2.89	-	10.144
3&4	2	56.16	-	100	100	-	-	-	-	-	-	-
5	8	30.75	10.826	25	75	37.5	12.5	-	12.5	-	-	37.5
8	19	17.42	2.11	36.84	52.63	10.53	5.26	-	1.53	5.26	-	10.526
10	10	14.1	2.94	30	70	30	-	10	10	10	-	20
G.m.morsitans												
Untreated control	10	26.75	4.26	90	10	10	-	-	-	100	-	-
Acetone control	29	30.25	5.198	68.96	31.04	20.68	17.24	-	20.68	6.8	3.448	13.793
0.001ng	2	65	1.524	50								

egg failed to go down into the uterus. The next egg in sequence therefore began to develop thus making 2 mature eggs appear in the ovaries on each side (Fig. 2 &3). These eggs afterwards started to undergo resorption, appearing brown in colour, with patches of yolk (Fig. 4). The abnormality is having 2 mature eggs on either side of the ovary.

Abnormality 2: larvae retardation

With the abnormality of having 2 mature eggs in the ovary, although these eggs are mature, there were a 1st instar larva in the uterus in some cases. These were abnormalities because, when the egg is mature and ready for ovulation, the larvae in the uterus should be 3rd instar, soon to be larviposited. This indicated retarded development of the larvae. In some cases, there were mature eggs at both sides of the ovary although the uterus was empty. This implied a recent larviposition or an abortion. In a normal situation, the egg on one side of the ovary is mature (1.5.mm) while the other side shows the small egg next in sequence, on top of which is usually a very small egg. In the uterus, a 3rd instar larva is normally present, which would be larviposited soon (1-2 days).

The percentage distribution of intra-uterine contents recorded from routine dissections of dead female *G. pallidipes* and *G. m. morsitans* treated with deltamethrin given in Table 1 shows high frequencies of abnormalities for both species. The bar graphs in Fig. 5 for *G. pallidipes* demonstrate an increase in abnormalities with insecticide dose. There was a high percentage of non-ovulation for *G. pallidipes* (33%), and egg reabsorption (17%) in *G. m. morsitnas*. However, no egg blocked oviduct or larvae were present in the uterus of *G. m. morsitans*. In *G. pallidipes* all types of reproductive abnormalities were observed (Fig. 5). Both tsetse flies revealed relationships of a positive correlation between the percentage of females with reproductive abnormalities and the increasing dose of insecticides. This increase was, however, only up to appoint (3 ng) marking the end of low doses. After that, the trend sharply dropped.

Egg development

Normally, the ovary of a fly ready to begin a new pregnancy cycle or to ovulate would have one mature egg next in sequence of ovulation on one side of the ovary (Fig. 1), while the other side has a small egg. Abnormal development in an ovary (reproductive abnormalities, Fig.2), included egg retention in one side of the ovary (32 %) so that the next egg in sequence began to develop. Two mature eggs then appeared in the ovaries. These eggs soon started undergoing resorption (11 %). They were brown in colour, with patches of yolk reabsorbed. The normal mature eggs was white and compact, with no signs of egg reabsorption as shown.

Other kinds of reproductive abnormalities included two mature eggs, one on each side of the ovary instead of having only on one side, but with an empty uterus (Fig.3). Also cases with two mature eggs one on either side of the ovary but with a 1st or 2nd instar larva were observed. This was an abnormality because when the egg is mature and ready for ovulation, the larva in the uterus should have been a 3rd instar, soon to be larviposited. This observation indicated retarded development of the larva. Some females had up to 6 eggs in the ovaries, which was a most abnormal situation (Fig.4).

Larval development and abortions

Treatment of females with either insecticide resulted in either rapid expulsion of egg within 24 hr of treatment or delayed abortion of larvae (1st, 2nd and 3rd instar, Figure 6).

Generally, higher percentage of non-ovulation egg resorption, and empty uteri were recorded for low doses than the control, however, there were slight differences between the treatments. While there were more flies with non ovulation with the low dose (1 ng), there was more empty uteri and egg resorption with higher doses (5-10ng). Flies appeared to be more affected by the higher doses inducing abortions and expelling eggs (hence the empty uteri). On the other hand, there was an accumulation of eggs in the ovaries with los doses treatment.

The empty uteri implies suspected abortions when the next egg in sequence is mature (Table 1). There was, however, no significant positive correlation between the percentage reproductive abnormality and the insecticide ($P<0.05$).
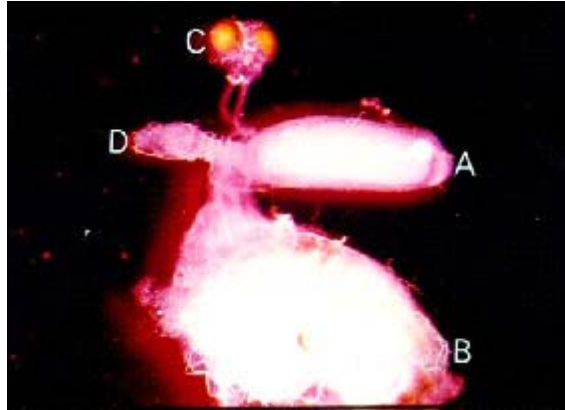
**Fig. 1: Female reproductive system in *G. pallidipes*. Explanation of abbreviations:
A. 1 mature egg next in sequence of ovulation on the right side of ovary; B. Larva in
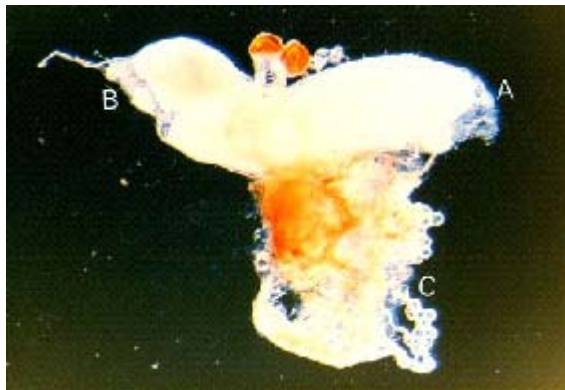the uterus; C. Spermathecae; D. 1 small egg on the left side of the ovary**

**Fig. 2: Ovary of *G. pallidipes* showing 2 mature eggs on both sides of the ovary together
with an empty uterus, following treatment with pyrethrum extract. A. mature egg on
right side of the ovary; B. mature egg on left side of the ovary; C. empty uterus**

**Fig. 3: Egg accumulation in the ovaries of *G. pallidipes* following
treatment with pyrethrum extract. A. mature egg; B. Spermathecae**
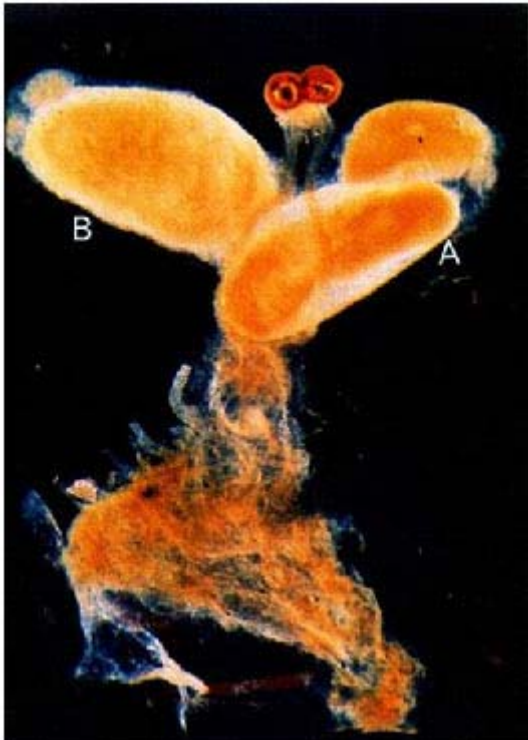
Fig. 4: Ovary of *G. pallidipes* showing resorption of eggs (brown in colour) in both the ovaries following treatment with pyrethrum extract. A. egg on right side of the ovary undergoing resorption; B egg on left side of the ovary undergoing resorption
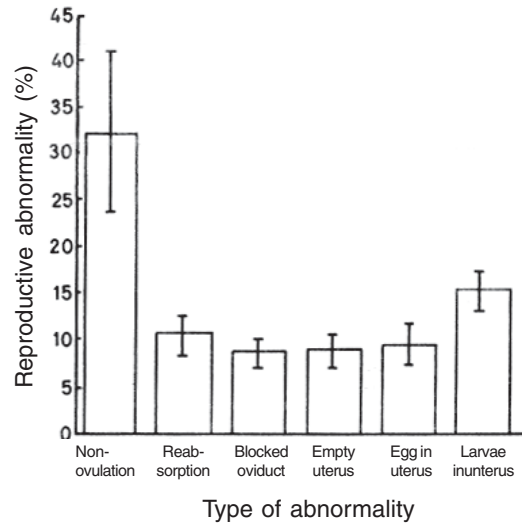
Fig. 5: Occurrence of reproductive abnormalities in *G. pallidipes* following treatment with insecticides (data pooled for the two insecticides)

Fig. 6: Abortion of larvae in *G. pallidipes* females following treatment with deltamethrin. A. female *G. pallidipes* in the process of aborting a 3rd instar larva Aborted 3rd instar larva showing black respiratory lobes marked D

On the frequencies of the different reproductive abnormalities, flies with non ovulation were most abundant followed by egg resorption. There was no consistency in the ranked frequencies of the egg, second or third instar larva bearing flies. The mean percentage for the two species of flies obtained for the reproductive abnormalities were as follows: *G. pallidipes, n*ormal females= 42.0%, abnormal females= 57.3 %, and for *G. m. morsitans,* normal females = 53.61%, abnormal females = 61.7%

DISCUSSION

The dynamics of tsetse fly pregnancy is given by Denlinger, (1978) and the susceptibility of topical application of dieldrin in tsetse in unsprayed and sprayed populations in Kenya (Turner and Golder, 1986) provide a background for the dynamics of tsetse and insecticide interactions. The fact that the percentage of flies with reproductive abnormalities increased with the increasing concentration of insecticide up to a certain point suggest that flies exposed to low doses are affected in their reproductive systems. This implies that flies can tolerate the low dose to a certain extent, but prevent ovulation to occur which subsequently are reabsorbed, where as the higher doses result in ovulation. The most common abnormality was non ovulation. The development of the next egg in sequence of the opposite side of the ovary and the gradual accumulation of the non ovulated eggs mean that the ovaries are stimulated to produce eggs. In other words, low doses of pyrethrum seem to be stimulating egg maturation in tsetse flies. Similar reports have been observed in the spotted lady beetle *Henosepilachna vigintioctopuncata* (Kono & Ozeki., 1987), who observed that pyrethroids (flucythriante, permethrin and cypermethrin) at 0.05 µl caused about half the females to complete egg maturation, and were also showed to have stimulating effects to vitellogenesis.

This fact further implies that probably low doses caused an increase in the reproductive abnormalities which the inset tolerates. The two species showed similar patterns of abnormalities, which reflect abnormalities due to treatments for both tsetse flies. The higher doses on the other hand, caused egg re-absorption and high numbers of empty uteri which signified abortions.

As to the influence of insecticides of insecticides on the egg maturation without ovulation (non ovulation category) it is logical to speculate that since the pyrethroids used are nerve stimulating, they must have caused a release of neurosecretory substances in the females. These in turn induced a hormone responsible for the vitellogenesis and egg maturation to be released, as may be supported by reports on a hyperglycaemic hormone by DDT in *Periplaneta Americana* (Grant and Leeling, 1972) and by á-BHC in *Schistocerca gregaria* (Samaranayaka, 1974) and by bioresmethrin (Sing and Orchard, 1983). These results were in accordance with the action of bioresmethrin (Sing and Orchard, 1983) on the electrical activity of neurosecretory cells of insects.

These results suggest that the stimulating action of deltamethrin and pyrethrum extract at low doses can be manifested in vivo and be used to induce reproductive abnormalities, which in future may be a form of control in tsetse flies. All in all, sublethal doses have an adverse effect on the progeny of the treated females. The question that arises in what proportion of the population was affected. In this study, it has been demonstrated that at least 40% emerged abnormally, but did not survive up to 10 days means that a total of 65% was affected. In the field situation, this is likely to increase thus contributing to mortality after spray application. The implication of these results to the environment and non-target beneficial organisms is fundamental. It implies that insecticides sprayed can interfere with the reproduction of beneficial insects for instance bees and butterflies with detrimental effects to the eco systems and the environment.

Application of pyrethroids to intact insects causes behavioural and physiological manifestations of poisoning superficially resembling those of DDT. Initially, there is a period of sensory hyperexcitation which leads to loss of coordination, ataxia, prostration, convulsions and eventual death. The pyrethroids effects however, are more rapid than DDT. Lowenstein (1942), while working on the nerve cord of cockroach *Blattela germanica*, and

using electrophysiology *in vitro*, observed an excitory phase induced by pyrethrins, and then a blockage of the nerve conduction mechanism. The author adduced evidence to show that the time interval between the excitory phase and the blockage phase depends on the dose used. The higher the dose, the shorter the time between the two phases. In contrast, low doses tended to increase the duration of the excitory phase, but always allow a return to the normal conduction rate. *In vitro* investigations have shown that pyrethrins and pyrethroids have a rapid stimulating action on the nervous system of insects. Various possible modes of action on the nervous system in relation to the visible intoxication symptoms show that motor unit discharges originate in peripheral motor nerve endings. Trains of such discharges may well be the cause of convulsions, suffered by the poisoned insects (Roussel, 1982).

Takken *et al*. (1986), using integrated control of biconical traps and targets impregnated with deltamethrin and the sterile insect technique in Nigeria, managed to reduce the *G. palpalis palpalis* population by more than 90%, but failed to eradicate it. An unsuccessful attempt to eradicate *G. pallidipes* from the Lambwe Valley using sequential aerial application of endosulfan was described by Turner and Brightwell (1986). Vale *et al*. (1988) used targets impregnated with dieldrin and deltamethrin and baited with 1-octen-3-ol attractants, to reduce the populations of *G. morsitans morsitans* and *G. pallidipes* in Zimbabwe. Several authors including Torr (1985) and Lavéissiére *et al*., (1986) described experiments with synthetic pyrethroids with *Glossina*.

In this study, sublethal doses of insecticides have been shown to affect the reproduction of tsetse flies through abnormalities. This phenomenon is not new to insects. Duncan (1963) had exposed female *Aedes aegypti* to Dieldrin at LD2 to LD5 24 hr and observed an inhibition of feeding. The minor effects of the solvent and the insecticides on blood meal size and the frequency of feeding when blood was offered daily was recommended for further investigation.

Tsetse show interspecific as well as intraspecific variation in susceptibility to an insecticide. Generally, teneral male and female flies, and older fed males are similar in susceptibility to organochlorine insecticides. Fed pregnant females are appreciably less susceptible by a factor of 4-9 (Burnett, 1962; Irving, 1968; Kwan *et al*., 1982). Pregnant females are also more tolerant of synthetic pyrethroids (Burnett, 1962; Hadaway, 1972; Kwan *et al*., 1982; Riordan, 1987; Turner and Golder, 1986). The results have confirmed earlier observation that pregnant females tolerate insecticide treatment through abortions.

The results suggest that the stimulating action of deltamethrin and pyrethrum extract at low doses can be manifested *in vivo* and be used to induce reproductive abnormalities. Results from the survival have shown that both deltamethrin and pyrethrum extract reduced the survival of *G. m. morsitans* but not of *G. pallidipes*. This difference may be genetic in origin. Nevertheless it implies that sublethal doses of these insecticides have an adverse effect on the survival of the flies. If such flies do not survive well, then these low doses do make a significant contribution to the fly mortality after spray application. All in all, sublethal doses have an adverse effect on the progeny of the treated females. The question that arises is at what stage and what proportion of the population could they be effective?

Irregular feeding was the main cause of abortions, an observation similar to that of Mellanby (1937). The same author noted that egg maturation and "milk" production are dependent upon the nutrients made available from the blood meal of the female, and that the pupal weight could be directly connected with the total amount of blood taken during the pregnancy cycle.

Burnett (1963), Irving (1968), Kwan *et al*. (1982) and Riordan (1987) all reported an increase in tolerance of pregnant flies to insecticide treatment. Burnett (1963) suggested that tolerance raised for a low dose of fenthion was due to diffusion of insecticide into the in utero embryo. Similar diversion of DDT into the in utero larva was termed "inert storage" by Irving (1968), and Kwan et al (1982) suggested that partitioning of endosulfan into lipids in the maternal fat body during early pregnancy and in uterine milk during late

pregnancy, explained increasing in tolerance through each pregnancy cycle.

Abortion by tsetse flies living in natural conditions has been recognised for many years and it was suggested by Buxton (1955) that this could be due to undernourishment of individual flies. Estimates of abortion rates in wild populations have varied considerably, ranging from 0.04% (Harley, 1966) to 0-12 % (Okiwelu (1977) for *G.brevipalpis* in Uganda and G.m.morsitans in Zambia respectively. Using a precise method of detecting abortions, Madubunyi (1975) recorded a rate of 8.72 % of parous flies of a population of *G.m.morsitans* in Zambia and later he recorded 7% in a large sample of the same population. Denlinger (1975) reported abortion rate of 6.9% for acetone treated flies which were fed daily.

The mechanism and mode of action of tsetse abortifacients are not very well understood. It has been suggested that they can act directly or indirectly. The direct action influences the parturition; indirectly, they may act by turning off the milk gland. Other possible modes of action include the disruption of the in utero larval morphogenesis and the desynhcronisation of the female neuro endocrine integration of normal pregnancy. Denlinger (1975) working on *G.m .morsitans o*bserved very rapid abortions 2-24 hr after Juvenile Hormone Analogue treatment. He suggested that an affect on the maternal milk gland was not the cause of abortion, because the resultant starvation of the in utero progeny would not have proceeded sufficiently far in such a short period. The author also reported that injection of the antichlolinesterase substance, physostigmine, caused rapid abortion in *G. morsitans* . He concluded that compounds acting on the neuromuscular processes could also act as abortifacients.

Langley (1977) recognized that starvation of the pregnant fly or larval death of the embryo in the uterus can result in abortion. There is evidence that brain neurosecretory cells are involved in the control of parturition (Foster, 1974) and that the release of neurosecretions reaches a peak at the time of parturition (Ejezie & Davey, 1974). Langley (1977) suggested that normal parturition is initiated by the maternal neuroendocrine system which

causes muscular relaxation of the vaginal sphincter and the fully developed larva escapes from the uterus by its own reverse peristaltic movements.

A significant effect on the larval development was observed for the two insecticides on the two species. The egg and immature larva would have little muscular activity. It is therefore likely that abortion resulted by inducing relaxation of the maternal muscles, opening of the vaginal sphincter and smoothering of the uterine wall.

Irving (1968) reported that the insecticides were channelled to the developing larvae through the milk. Both insecticides induced abortions in the egg stages as well as in all three larval instars. Although the insecticides were applied to individuals in early pregnancy, they affected the abortion of 2nd and 3rd instar larvae. The 3rd instars which were aborted were not fully grown, and they often failed to pupate. This observation tends to agree with Irving's findings that the developing larvae incorporate the insecticide via the milk hence they are the most frequently aborted, a direct action of the insecticides.

The time between insecticide application and abortion was much shorter with deltamethrin than with the pyrethrum extract. Deltamethrin is recommended for utilization in the tsetse control programmes (Barlow et al., 1977; Lavéissière *et al*., 1980; Spielberger *et al.,* 1979). It is used in impregnated traps and targets for tsetse control.

It is clear that low-dose insecticide effects on reproduction do cause a significant contribution to the overall mortality of the tsetse fly population. When insecticides are applied therefore, those flies that get sublethal doses, although they are not affected in terms of reducing their feeding behaviour, they are affected through induction of abortions, reproductive abnormalities inside their ovaries, and pupal development retardation.

It has been demonstrated that sublethal doses of pyrethrum extract and deltamethrin affect tsetse reproduction through: abortions of eggs and larvae, retarding pupal development and viability, reducing post-treatment survival, and reproductive abnormalities in the reproductive system. However

these studies have not shown the actual mode of action of these insecticides. Further studies are recommended using radio labelled deltamethrin *in vivo*, which would show where the sites of action are. Also, the effect of these insecticides on the F2 and F3 should be investigated to establish the long term effects on the progeny, if any.

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