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Understanding tsetse flies*

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ABSTRACT

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The discovery that tsetse flies are the vectors of African trypanosomosis, causing sleeping sickness in man and nagana in cattle, occurred at the start of a rapidly expanding colonialism in sub-Saharan Africa. Hence, the first research on the fly was largely taxonomic, coupled with a painstaking ecological approach to determine the identities and distribution limits of the different species.

This was followed by closer attention to the physiology of the fly, both from the academic standpoint as related to its survival and reproduction in the field, and from the standpoint of its vectorial capacity. There are still conflicting hypotheses concerning the maturation of trypanosomes within the fly.

Increasing concern for the environment led to a ban in the developed nations on the use of DDT as an insecticide which had been used successfully for tsetse control in Africa. This was followed by a ban on the use of organochlorine insecticides in general, and no doubt the next restrictions will be on the use of organophosphates and upon synthetic pyrethroids which have already been banned in the UK for the control of houseflies.

Fortunately, research on the role of olfactory and visual stimuli of the tsetse, in the location of potential hosts, led to an improvement in methods for monitoring fly populations by means of traps and targets upon which the flies alight. So successful are such devices that, when treated with an insecticide, they can be used to sustain an increase in natural mortality in fly populations to such an extent that these populations decline to manageable levels. The techniques constitute an appropriate technology for the countries of Africa, and attention is now focused on replacing conventional insecticides with more environmentally acceptable compounds whose development is based on a sound knowledge of the physiology of the insect.

Perhaps the next major step will be to understand the physiological basis of the acquisition and maturation of trypanosome infections in tsetse. Modern genetic techniques may then permit the engineering of flies which cannot transmit trypanosomosis and are therefore reduced to the level of nuisance flies.

INTRODUCTION

The identification of the tsetse fly as the vector of pathogenic trypanosomes in sub-Saharan Africa came at a time when very little was known about insect biology in general, and insect physiology and behaviour in particular. Hence, the achievements of the early pioneers of tsetse study are greatly to be admired (e.g. Newstead 1924). Researchers adopted a detailed approach in studying the distribution and abundance of tsetse species. This, together with a painstaking evaluation of the environmental factors involved in the regulation of fly numbers, provided

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a sound ecological basis for further improvements in our understanding of this insect. Likewise, the efforts of Jack in Rhodesia in the 1930s provided a legacy of knowledge of the physiology and behaviour of the fly, and evoked amazement at the achievements of the man (Jack 1939).

The study of insect physiology received its impetus between the two World Wars when attention was drawn to the extent of crop losses caused by insect pests. However, the discovery of DDT was expected to solve this problem and it was not until the publication of "*Silent Spring*" (Carson 1962) that we were made aware of the damage caused by the indiscriminate use of insecticides.

Those who wished to study the physiology of tsetse in the laboratory were rewarded with the establishment of techniques to rear tsetse in captivity, firstly on living hosts (Azevedo & Pinhao 1964; Nash, Jordan & Boyle 1968) and later artificially, through membranes (Mews, Langley, Pimley & Flood 1977). The availability of laboratory colonies of tsetse also permitted the study of behavioural responses to stimuli in a variety of controlled situations.

It is generally recognized that the control of trypanosomosis is best achieved by removing tsetse flies from the environment. However, only a few African countries have ever practised tsetse control on a wide scale. Notable among those which have, are Nigeria and Zimbabwe.

Selective ground spraying and aerial spraying of insecticide have been practised, while techniques and compounds have improved, so that smaller amounts are required, thus reducing the risk to the environment. Nevertheless, risks remain and aerial spraying has been limited in its success. It is also expensive and often considered an inappropriate technology for Africa.

Although the development of resistance to insecticides has never been reported in tsetse populations, the insect possesses the capability (Maudlin, Green & Barlow 1981), and this may become a more serious problem as appropriate technologies such as the deployment of insecticide-treated targets or traps are used more widely in Africa.

In the present paper an attempt is made to outline the key steps in our understanding of tsetse and to indicate how such understanding can assist in the battle against trypanosomosis.

THE TSETSE LIFE CYCLE

Most flies direct their maternal investment towards producing eggs which hatch into larvae that exploit a different environment from that exploited by the adult. Survival rates are low, but are compensated for by the production of large numbers of eggs. Tsetse, on the other hand, have their ovaries reduced so that each contains only two ovarioles and only a single egg is matured and ovulated at a time. The egg is fertilized by spermatozoa stored in the body of the female from a single mating and the egg is retained in the oviduct. After hatching, the larva is nourished on a milky secretion produced by the female accessory glands. Within this sheltered environment the larva grows, moults and completes its development, so that when it is born (Fig. 1) it immediately burrows into the ground where metamorphosis occurs. Soon after giving birth, the next egg is ovulated and fertilized by stored spermatozoa and the cycle begins again.

In this way the tsetse female produces one fully grown offspring every 9 or 10 d (at 25 °C) for the whole of her life. Under laboratory conditions she can produce as many as ten, but in the field it is doubtful whether a female fly produces more than four offspring. Hence the survival of the larva depends entirely upon the survival of the mother, and both adult and larva are (indirectly) exploiting the same environment and food source. The pupa is protected underground. This strategy leads to a very high survival rate and as a rule tsetse populations do not fluctuate violently as do those of the egg-laying Diptera.

However, the accessory glands of the female appear not to be selective in their uptake of molecules from the haemolymph (Langley 1977) and thus provide a means of affecting the larva through treatment of the mother, a phenomenon which is only now being exploited for tsetse control.

REPRODUCTIVE AND DIGESTIVE PHYSIOLOGY

Adenotrophic viviparity in a blood-feeding insect poses considerable logistical problems. Vertebrate blood is a poor source of energy and the energy content of about half of each blood meal must be expended to turn the rest into utilizable substrate (Bursell, Billing, Hargrove, McCabe & Slack 1974). Hence, in a female tsetse, a single blood meal may be three times its own body mass (Fig. 2). This includes a large quantity of water which is rapidly voided via the Malpighian tubules under the influence of a diuretic hormone produced in response to the distention of the abdomen (Gee 1975). Not surprisingly, the timing of blood meals must be carefully regulated with respect to larval growth. Generally, the last blood meal ingested by the female before parturition is taken when the larva is still a second, or has just moulted to its third, instar and is still quite small. The larva then increases its size enormously during the last 2 or 3 d before parturition (Denlinger & Ma 1974; Langley & Pimley 1975). Although the fly has large stores of triglyceride derived from blood meals ingested early in the reproductive cycle, it has no means of storing significant amounts of protein. Hence the bulk

FIG. 1 Adult female *Glossina morsitans morsitans* giving birth to a fully grown larva (Courtesy Douglas Fisher FRPS)

FIG. 2 Adult female *Glossina morsitans morsitans* ingesting a blood meal through a silicon rubber membrane (Courtesy Douglas Fisher FRPS)

of the nutritive secretion (a mixture of equal amounts of protein and lipid) (Cmelik, Bursell & Slack 1969) produced by the accessory glands of the female is formulated from lipids stored in the body fat and proteins synthesized directly from the digestive products of the final blood meal ingested before parturition (Langley & Pimley 1979). As the volume of blood meal and lipid stores is reduced, so space becomes available for the larva to increase in size. In this way a bulk transfer of nutriment occurs within the female abdomen without its degree of distension being altered.

Randolph, Rogers & Kiilu (1991) suggested that *Glossina pallidipes* feeds only three times per reproductive cycle and that meals can be ingested on any day of the cycle. However, this is hardly credible, since it has been estimated that, in order to satisfy metabolic demands for flight and for larval nourishment, tsetse females must feed at least four times during a 9- or 10-d reproductive cycle, and the final meal is ingested before the seventh or eighth day (Langley & Stafford 1990).

EXPLOITATION OF REPRODUCTIVE AND FEEDING BEHAVIOUR FOR TSETSE CONTROL

The manner in which tsetse locate their hosts and their mates was unknown until the mid-1970s. It was always assumed that tsetse located their hosts by sight, and only when Vale (1974) reported his classical series of experiments, did it become clear that a strong element of attraction by olfactory stimuli was involved. The observation had such important implications for population-monitoring through the use of traps, that an all-out effort was made to identify the components responsible. Two decades of effort have resulted in the production of a cocktail of compounds which simulates the natural odour of animals to which the savanna species of tsetse are attracted (Vale & Hall 1985; Bursell, Gough, Beevor, Cork, Hall & Vale 1988). However, there is a missing component which, so far, has defied identification (Torr, Hall & Smith 1994, unpublished data).

Nevertheless, the technique of combining an olfactory stimulus with a visually attractive trap or electrified target for monitoring tsetse populations was extended when such targets, treated with insecticide, were shown to be effective in the control of an island population of tsetse on Lake Kariba in Zimbabwe (Vale, Lovemore, Flint & Cockbill 1988).

The identification of the sex pheromone of one species of tsetse, *Glossina morsitans morsitans* (Carlson, Langley & Huyton 1978) was thought to open the way for attraction of males to a position where they might be sterilized (Langley, Huyton, Carlson & Schwarz 1981). However, male tsetse hunt females by sight. It is only after physical contact has been made that sex recognition is achieved (Fig. 3). The placement of decoys treated with synthetic pheromone on targets in the field provided the means of intercepting males which might then be sterilized by the combination of pheromone and sterilant on the decoy (Langley *et al.* 1981; Hall 1987). However, theoretical considerations indicate that autosterilization of tsetse males may be ineffective. The more successful strategy is sterilization of both males and females (Langley & Weidhaas 1986) (Fig. 4).

FIG. 3 Laboratory-reared adult males of *Glossina morsitans morsitans* attempting to mate with a shoelace knot impregnated with the sex pheromone of the species

The hunt for effective sterilants for insects followed an unacceptable route during the 1970s because of the mutagenic, carcinogenic and teratogenic nature of the compounds involved. Ironically, the chitin synthesis inhibitor, diflubenzuron, had already been shown to disrupt reproduction in tsetse following topical application to females (Jordan & Trewern 1978; Jordan, Trewern, Borkovec & Demilo 1979). Cyromazine had a similar effect (Jordan 1980, unpublished data) but, in spite of their relatively low toxicity to other life forms, neither of these compounds was exploited as a potential sterilizing agent for tsetse.

More recently, the juvenile hormone mimic, pyriproxyfen, has been shown to sterilize tsetse females (Langley, Felton & Oouchi 1988). It is stable and can be formulated in oil. Two successful field trials have

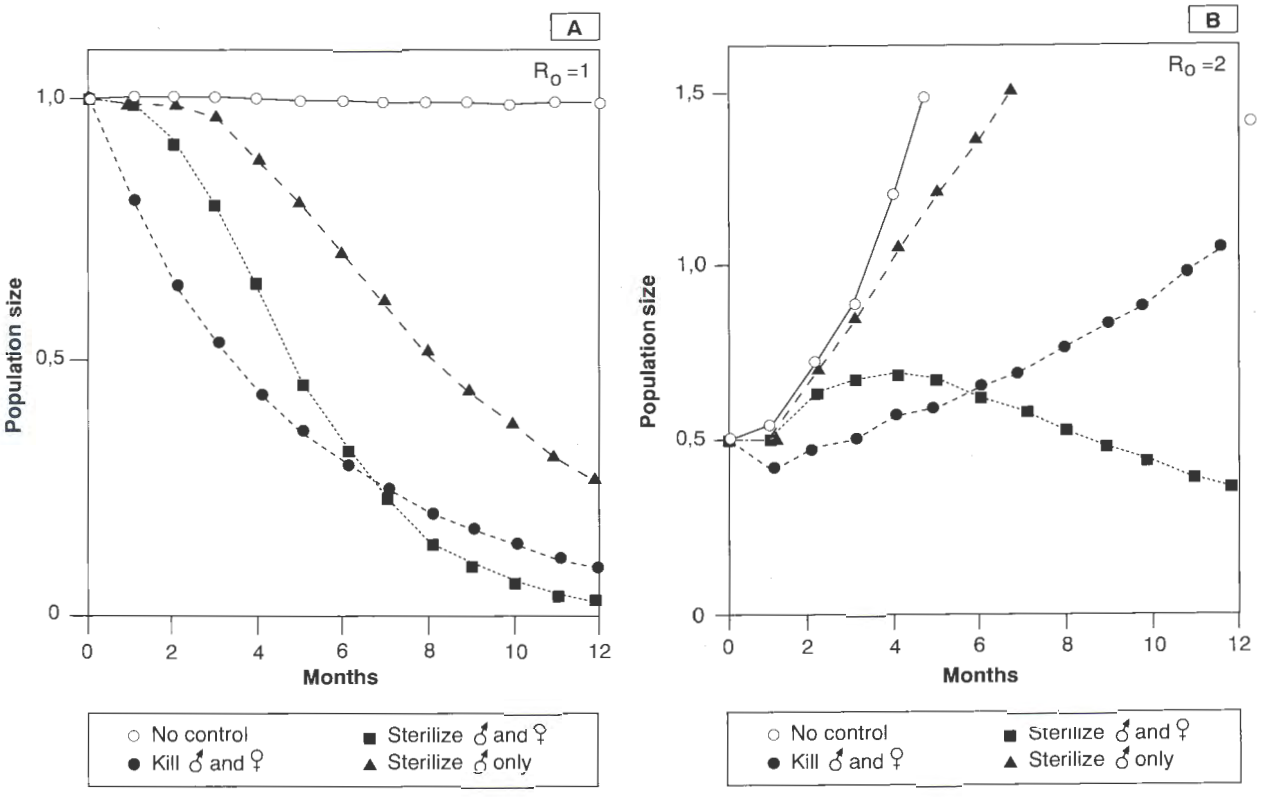

FIG. 4 Theoretical changes in numbers of tsetse in (a) a stable population in response to a trapping intensity of 1 % per day and (b) a population that is doubling per generation (i.e. approximately every 105 d) and the trapping intensity is 1 % per day. Trapped flies were either killed or sterilized and released. Where males only were sterilized, females were not involved at all. [Reproduced from Langley & Weidhaas (1986)]

been undertaken in which this compound was used in sterilizing traps (Hargrove & Langley 1990) and on sterilizing targets (Hargrove & Langley 1993) for the control of *G. pallidipes* and *G. m. morsitans* in Zimbabwe.

These successes have led to a re-evaluation of the chitin synthesis inhibitor, triflumuron, as a potential agent for tsetse control. Laboratory experiments (Langley 1994, unpublished data) have shown that brief tarsal contact with surfaces treated with a 3% suspension concentrate will sterilize females for life, in that they produce normal-looking offspring which are unable to form a puparium. Furthermore, males which become contaminated can pass on sterilizing doses to females when they mate. Hence such males may be considered to be at least temporarily sterilized themselves (Langley 1994, unpublished observations).

A field trial in Zimbabwe, involving triflumuron-treated targets is to begin in October 1994.

The potential for suppressing tsetse populations to manageable levels by use of sustainable techniques, with minimum impact on the environment, has never looked more promising.

TSETSE AND TRYPANOSOMES

During the last five years much has been learned about the mechanisms involved in the establishment of trypanosome infections in tsetse. Susceptibility to midgut trypanosome infections in *G. m. morsitans* is maternally inherited, but it is a condition expressed only in previously unfed "teneral" flies (Welburn & Maudlin 1992). Laboratory-colonized flies are more susceptible than wild flies, owing to the spread of flies carrying rickettsia-like organisms (RLO) through colonization (Maudlin & Welburn 1993). These organisms produce chitinases *in vitro* and could be responsible, in the teneral fly, for increasing the midgut content of either D-glucosamine or N-acetyl-D-glucosamine, which are inhibitors of lectin activity (Welburn, Arnold, Maudlin & Gooday 1993). Lectins in the midgut of the tsetse are known to be lethal to trypanosomes (Maudlin & Welburn 1987). Non-teneral flies, having fed, would secrete large amounts of lectin, rendering them refractory to trypanosome infection (Maudlin & Welburn 1993).

Claims that non-teneral tsetse can acquire trypanosome infections and hence are of epidemiological significance, may not be true, since experiments

have been conducted mainly on laboratory-colonized flies which are known to be more susceptible than wild flies (Maudlin & Welburn 1993). However, the fact that older flies can become superinfected by the addition of lectin-inhibiting sugars to the infected blood meal, suggests that lectins normally act as the barrier to infection and that there is no physical barrier such as the peritrophic membrane in the gut (Maudlin & Welburn 1993).

THE FUTURE

There is little doubt that in areas of high human population pressure on the land, the tsetse fly is forced out of existence. However, in areas where cattle are important, there is no doubt that tsetse control can bring benefits to animal health. The fear is that with the demise of the fly there will be an accelerated loss of wild life and natural vegetation, accompanied by degradation of the remaining land, owing to overstocking with cattle. Clearly, this is a matter for regulation by Government. In Zimbabwe, large areas of agricultural land are kept free of tsetse only by constantly operating control measures. The extermination of tsetse in the Zambezi Valley would remove the source of reinvasion and reduce the costs of tsetse-control operations. It is doubtful whether the human population in the Zambezi Valley would increase as a result of such control measures.

Interestingly, the relationship between chitinase production by RLOs, lectin inhibition by glucosamine and the lethal effect of lectins upon trypanosomes in the tsetse midgut, might be upset by the use of chitin synthesis inhibitors to sterilize tsetse in the field. The prospect that tsetse-control measures and a greater understanding of the mechanisms involved in the acquisition of trypanosome infections by tsetse might lead to development of techniques to eliminate trypanosomes from tsetse in the field, is exciting. Such possibilities, together with the identification in tsetse of genes for susceptibility and refractoriness, could result in the fly being relegated to the status of a nuisance rather than that of the carrier of a killer disease.

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