TSETSE CONTROL

The Role of Pathogens, Parasites, and Predators

(Report of a Scientific Advisory Group
Convened at The Memorial University of Newfoundland, St. John's, Canada, —
25-29 March, 1974)
Abstract

Tsetse control is required in Africa to reduce the incidence of trypanosomiases in man, and domestic animals which provide human food. Control of tsetse has so far been achieved through the use of insecticides but problems of resistance and environmental non-selectivity have stimulated the search for both alternative compounds and biological methods of control. Numerous species of parasites, pathogens and predators are being considered as suitable control agents of tsetse. Research is recommended towards the recognition and use of biological agents, their health and environmental safety as well as relevant aspects of tsetse ecology, physiology and behaviour, and mass production. It is recommended that the development of integrated control (pest management), training programs be treated as a priority.

Résumé

Il est nécessaire, en Afrique, de lutter contre la tsé-tsé afin de réduire la fréquence de la trypanosomiase tant chez l’homme que chez les animaux domestiques servant à son alimentation. Jusqu’à présent, cette lutte a revêtu la forme de l’emploi d’insecticides, mais l’apparition de problèmes de résistance et leur non-sélectivité écologique ont stimulé les recherches en faveur d’autres composés et de méthodes de lutte biologique. Il semble qu’un grand nombre de parasites, de pathogènes et de prédateurs puissent être des agents efficaces de lutte contre la tsé-tsé. L’auteur recommande l’orientation des recherches vers l’identification et l’utilisation des agents biologiques, leur sécurité pour la santé et le milieu, ainsi que vers les aspects connexes de l’écologie, de la physiologie et du comportement de la tsé-tsé et de leur production en grand. Il recommande également que l’on accorde la priorité à la mise au point d’un programme de formation à la pratique de la lutte anti-parasitaire intégrée.

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Tsetse Control: the role of pathogens, parasites and predators

(Report of a Scientific Advisory Group Convened at The Memorial University of Newfoundland, St. John's, Canada, 25–29 March 1974)
INTRODUCTION

A scientific advisory group met at the Memorial University of Newfoundland (MUN), St. John's, Canada, from 25-29 March 1974, to consider the role of pathogens, parasites and predators in future integrated control methodologies for the control of tsetse flies. These vectors, all referable to the genus *Glossina* and restricted to the "fly-belt" of Africa, transmit sleeping sickness to man and other trypanosomiases to domestic and wild animals. Their consequences to human health and economic development in that continent have been, and remain, incalculable.

Seventeen participants including representatives of specialized agencies of the United Nations (the World Health Organization, and the Joint Division of the Food and Agriculture Organization/International Atomic Energy Agency) were invited by the International Development Research Centre (IDRC). There were also three observers from the National Research Council of Canada, the Canadian government Department of Industry, Trade and Commerce, and from industry.

This report was prepared for consideration for possible support by funding agencies in order to foster and accelerate research activities. These activities would be designed to improve the capacity to implement such measures of tsetse control as are required to interrupt transmission without posing needless hazards to man and his general environment.

*Adult tsetse (G. palpalis) trapped near Yaoundé, Cameroun*
THE PROBLEM

Several species of Glossina, each of them responsible for distinctive disease problems and differing on behavioural and other grounds from locality to locality, have been the subject of naturalistic control programs since the early years of this century. In the years since World War II, synthetic organic pesticides have generally proved the most effective single means of combating these insects. More than in some other vector-borne disease situations, though, tsetse control programs have involved what is nowadays termed a "pest management" or "integrated control" approach — one calling for the conjoint use of chemical, biological, mechanical and other measures, applied against a background of adequate ecological understanding of the particular problem under attack.

Lately, there have been some encouraging developments towards more effective and environmentally selective procedures for tsetse control by means of chemical pesticides. There have also been advances in other aspects of relevant integrated control procedures. Modern autocidal techniques have attracted and are attracting much attention and support in this context. Biological control (in the "classical" sense of using natural enemies against Glossina) has been much talked of, and experimented with occasionally. Also, there is growing recognition of the importance of safeguarding such natural regulatory factors bearing upon tsetse populations, as predators and insect parasites (or parasitoids).

The latter, it should be noted, have sometimes proved valuable elements in the control of certain insects of economic importance, in which (as in tsetse and other muscid flies) they are commonly present in nature. This fact certainly extends the range of candidate biological control agents which can be considered for possible field use against tsetse, by comparison with, for example, mosquitoes from which no insect parasitoids have ever been isolated (the small size, brief life-history and aquatic phase of this most important of all vector groups, presumably militate against such parasitization).

Through the use of known methods, endoparasitic Diptera and Hymenoptera can be mass-produced in the laboratory for field release. Verification of the apparent absence of one whole group of these parasitoids (the mutillid wasps) from West Africa would certainly make a case for adapting this technology to enable introductions of Mutilla spp. into the region from Glossina elsewhere.

In contrast to parasitoids, a great deal less is known of pathogens of tsetse than of such other vectors as mosquitoes and blackflies. This may partly reflect behavioural traits predisposing against infection (for example, by microsporidan Protozoa, the usual route of entry being oral). There has not yet been a general and intensive search for pathogens and non-insect parasites of tsetse. For example, specific inquiry has doubled the meagre number of mermithid nematode records from these hosts over the past two or three years. These facts suggest that a coordinated survey of tsetse populations for viral, bacterial, fungal, protozoal
and nematode agents should add materially to the present obviously inadequate knowledge of these pathogens and parasites in *Glossina*.

Representatives of all the groups of microbial pathogens and nematode parasites just named can be mass-cultivated *in vivo*, and *in vitro* methods suitable for industrial exploitation are already available in some instances and are being explored in others. Applied entomology may therefore be expected to contribute practical biological control procedures for use together with chemical and other measures in future integrated tsetse control methodologies.

**CURRENT STATUS OF TSETSE CONTROL**

The meeting of the Joint FAO/WHO Expert Committee on African Trypanosomiases in 1968 reviewed the status of the associated human and domestic animal diseases as well as the known presence of the various *Glossina* species-complexes in the countries concerned. Subsequently, degrees of short-term expansion and/or contraction of the *Glossina* fly-belts have been observed. However, these are difficult to quantify because of the inequity in reporting and the sparseness of regular surveys in certain areas.

The purpose of tsetse control is to reduce the incidence of trypanosomiases in man, and in domestic animals which serve as a major human food source. At the present time, this can best be accomplished not on a continental scale but by tsetse control in those areas of infection where the highest risk occurs. In each such area, the cost of tsetse control must be weighed against potential socio-economic developments as well as against the existing disease problems. Inevitably, the solution will differ in the various regions of Africa.

The cost of development, testing and application of any new tsetse control agents must be considered against the background of the improvement of diagnostic techniques and therapeutic agents to control trypanosomiasis, and the field availability of these procedures and substances. Local potentials for land management and properly-planned economic programs for the exploitation of agricultural and other natural resources must also be taken into consideration. It is noted, too, that control programs require adequate monitoring and that certain past failures have been due to logistic and economic problems and poor planning rather than to the techniques employed.

Tsetse control through the application of persistent organochlorine insecticides (notably DDT) has been achieved in various parts of Africa. Recently, though, widespread problems of resistance and environmental non-selectivity associated with the employment of some chemical pesticides for insect control, have stimulated the search for alternative compounds. A number of these have already been tested against *Glossina* in both the laboratory and the field. Synthetic pyrethroids are highly toxic to tsetse when used in topical applications. As these compounds are not residual, they may have future use as space sprays. Several organophosphates offer good possibilities for use as either space or residual sprays. All these compounds have low mammalian toxicity. Most carbamates tested have been only moderately effective against tsetse.

The number of new candidate chemical pesticides for vector control has lessened in recent years. Over the past two years, insecticides available for *Simulium* control in West Africa have been tested for degradability and effect on non-target organisms, resulting in the rejection of several compounds. The experience gained will be of value in planning subsequent campaigns against riverine *Glossina* in these areas.

Advances have recently been made in the development and testing of insecticide application equipment on fixed-wing aircraft and helicopters, allowing usage of smaller quantities of insecticides, more precisely and effectively placed, to achieve the desired levels of control. Tests with ultra low vol-
ume application show insecticide penetration through forest and woodland canopy, and effectiveness against Glossina. The available equipment and insecticides ensure that chemical control of tsetse, properly applied will continue to be effective in areas where humans are at risk.

Indirect control of tsetse by altering the habitat is no longer as widely employed as formerly, and where used, bush clearing is generally integrated with the application of insecticides. In recent years, the destruction of wildlife has seldom been practised as a routine control measure. Preliminary results of studies on the possible use of genetic control of tsetse are promising, and field experiments on the use of the sterile male technique as part of the campaign against certain Glossina are currently being implemented or prepared in East and West Africa.

TSETSE ECOLOGY, PHYSIOLOGY, AND BEHAVIOUR

Tsetse species occupy an area of 10 million square kilometres in tropical Africa, representing one-third of the continent and one-half of its inhabited area.

The distribution of the 30 described species and sub-species is now quite well known due to various surveys which have resulted in the preparation of maps on national, regional and continental scales. Certain of these maps are revised periodically. The close association of tsetse taxa with well-defined types of vegetation, even to particular phytocenoses, permits differentiation between riparian, savanna, savanna/forest and forest examples.

Studies of the environment and its action on individuals and populations has enabled the definition of the ecological requirements of the main vectors of trypanosomiases and development of the concept of permanent and temporary habitats (not to mention clarification of the ecological consequences of favourable and unfavourable seasons).

The essential behavioural characteristics and the variations of these under the influence of physical and biological factors have already been described. At present, there are some data for the major species (G. palpalis, G. fuscipes, G. tachinooides, G. moritans, G. pallidipes, G. longipalpis, and G. swynnertonii). These concern activity, host preferences, feeding grounds, resting places (diurnal and nocturnal), breeding sites and emergence times.

Extended observations have identified the chief elements of Glossina's biological cycle (duration of pupal stage and of the reproductive cycle, insemination rate, frequency of feeding), and of relevant seasonal factors.

The population composition (sex-ratio, age groups, and various physiological categories) has been analyzed. A survival curve calculation has been attempted using a method for determining physiological age.

Fluctuations in the apparent density of populations have been observed over periods of several years—for some species, for more than 15 years. These have been related to climatic factors and in some circumstances to biotic ones (e.g. predators, and bovine epidemics). Nevertheless, there are still some unknown features. These could be clarified by taking into consideration density-dependent factors which to date have been little studied.

Population sampling methods now make it possible to establish only relative values. Very low densities are hardly detectable. There have been attempts to evaluate true densities, but it is evident that fully satisfactory results must await the availability of a really adequate sampling method.

Dispersal patterns of tsetse (linear distribution for riparian species, and wide dispersion for savanna species) have been studied, although for a few species only. Throughout their entire life, they live within a very restricted area (the "ambit"). Tsetse are not "migrant" insects in the ecological sense of the word, although they sometimes cover
long distances very rapidly. In the dry season, populations are concentrated in favourable habitats, which individuals rarely leave. During the rainy season, the flies disperse, entering more open country.

Locality learning has not been demonstrated in Diptera—but the tsetse is an unusual fly; it is long-lived, it must find hosts consistently throughout its life, and it must care for and deposit larvae which represent an enormous biological investment. It could be of selective value for the fly to be able to return to frequently used resting places of hosts, such as warthogs, and to good sites for larviposition. The diurnal activity of preferred hosts may influence its population fluctuations. Consequently, an understanding of the adjustment of *Glossina* activity to any reduction in availability of preferred hosts is an important ecological parameter.

Knowledge of tsetse ecology has long provided a basis for the designing of control methods effective against the main vectors of human and animal trypanosomiases. The continuing improvement of research methods and techniques will facilitate further studies prerequisite to the implementation of practical biological control methods.

While our knowledge of pheromones has rapidly increased in recent years, deeper understanding of this topic as regards tsetse flies would be very relevant to the development of new approaches to control. Thus pheromones are of potential application to mass trapping, population assessment, and confusion by disorientation. Analysis of sound emission by *Glossina* and their responses to it is also important in this context. Unlike sight, sound is omni-directional, and perhaps less subject to hindrance by obvious, natural, limiting factors (e.g. wind, bush) than are olfactory and visual stimuli. Active males and teneral females emit the “alerting” and “prefeeding” calls whenever a prospective host is perceived, thereby announcing to other tsetse the fact of their having been attracted. If this intercommunicating system could be used to attract *Glossina* to decoys or traps, it would not only improve trapping technology but might lead to new control methods.

With regard to larvae, there are three distinct types of chemoreceptor (possibly serving as entry points for pathogens), for which only a response to humidity has yet been demonstrated. At least two other behavioural responses can thus be predicted, although their nature remains completely unknown.

**PREDATORS OF GLOSSINA**

Many predators from a broad spectrum of zoological groups prey on immature tsetse (larvae and especially pupae) as well as the adult flies. Among them, some stand apart in terms of the frequency of relevant references from widely separated parts of Africa. Nevertheless, it must be remembered that present knowledge indicates the predatory activities of these animals are generally accidental or seasonal rather than specifically directed.

Notable among the predators attacking tsetse pupae are Hymenoptera (especially Formicidae) and carnivorous Coleoptera. Diptera (e.g. Asilidae) and spiders deserve particular attention because of their occasional remarkable reduction of adult tsetse populations.

Spiders of the family Hersiliidae appear to play a major role in this regard, readily attacking *Glossina* resting on tree trunks and large branches. The intensity of such predation is much greater where climatic conditions favour the concentration of predator and prey species together in the same places. Thus, these conditions have been noted in the hot season in the northernmost areas of the distribution of *G. tachinoides*.

Asilid flies observed as predators of adult tsetse, or considered likely to be such, are permanent inhabitants of the range of these vectors. Behaving like birds of prey, these Diptera capture in flight all insects passing through their field of vision. Asilidae of numerous species station themselves close to
resting sites where Glossina are in danger of being caught as they take flight.

Quantitative studies on the effect of these two groups of predators have not been precise and pose a considerable task. The effects of predation by herisillid spiders are undoubtedly important, although less evident than those of the asilids whose prey is more varied by comparison with those organisms susceptible to capture by spiders.

Although all invertebrates or vertebrates recorded in Jenkins' compilation must be considered in field studies of predation upon Glossina, it is urgently necessary to foster fresh investigations on all carnivorous arthropods whose habitats coincide at one time or another in the year with that of tsetse flies.

PARASITES OF GLOSSINA

(1) ARTHROPOD PARASITES (PARASITOIDs)

Some 33 species of Hymenoptera, representing 10 families, have been recorded as parasitoids of Glossina. Most of them, being but rarely recorded, are probably only facultative parasites of tsetse puparia. Nevertheless, the common occurrence of insect parasitoids in Glossina poses biological control possibilities entirely lacking in the major group of arthropod disease vectors; for mosquitoes, presumably through their small size, generally short life history and the aquatic nature of the immature stages, appear to lack endoparasitic insect enemies altogether. Among the parasitoids of tsetse, Syntomosphyrum and Mutilla have been studied in some detail.

The species formerly known as Syntomosphyrum glossinae is now sub-divided into two—S. glossinae (with an east-west distribution across Africa from Senegal to the Indian Ocean) and S. albiclavus (with a north-south distribution along the eastern side of the continent). The two species coincide in Kenya, Tanzania and Malawi. The life cycle lasts from 20-40 days, about 30 adult parasitoids being produced from each invaded puparium. At emergence, the male: female ratio is about 1:6. Because of the ease with which these little wasps may be bred in captivity in puparia of diverse groups of Diptera, some attention has been directed to the possibility of using them in the control of Glossina populations.

Field experiments in Malawi and Tanzania, between 1922 and 1932, indicated that no marked increase in the rate of parasitization of puparia of G. morsitans occurred after the release of laboratory-bred Syntomosphyrum; despite the fact that in the latter case, 13,750,000 parasites were released over 14 months. In retrospect, these biological control field trials can be criticized on the grounds that too much attention was paid to the ease with which Syntomosphyrum can be bred in captivity, and too little to the fact that rates of natural parasitism above 3% have not been recorded.

Three species of Mutilla have been recorded from Glossina, and the biology of M. glossinae has been investigated in some detail. Natural rates of parasitism of Glossina puparia by Mutilla can be much higher than those involving Syntomosphyrum, despite a longer life cycle (2-4 months) and lower fecundity (one parasite per puparium). While rates of parasitism vary with locality and season, values in excess of 50% have been reported. The male:female ratio on emergence is again 1:6, the females being apterous. Some success with breeding Mutilla in the laboratory has been achieved, although the problems of large scale rearing have not been resolved. Mutilllidae are common parasites of Glossina in East, East/Central and Southern Africa; but they have not been recorded from Glossina puparia in West Africa.

Apart from a single record of a species of Conopidae, all known dipterous parasites of Glossina puparia belong to the Bombyliidae. At least 12 species of Thyridanthrax have been recorded from this group of hosts. Life

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cycles within the genus Thyridanthrax (which is difficult to maintain in the laboratory) have not been completely elucidated. The time spent within the tsetse puparium can vary from about one month to more than eight months. It is likely that some individuals go through a larval diapause, perhaps related to season. Only one parasite is produced per puparium. Rates of parasitism vary according to locality and season. Exceptionally, rates in excess of 50% occur. Males and females emerge in approximately equal numbers. Some 10 of the species of Thyridanthrax recorded from tsetse have been found in East and Central Africa, by comparison with only two from West Africa.

Care should be exercised when interpreting published data for percentage parasitism. Some authorities base such values on the percentage of parasites in all puparia collected, and others on the incidence of parasitoids deduced from the total number of parasites and tsetse that emerge. Neither method can give an exact estimate of the percentage of a tsetse population that is parasitized. Whereas the second one has the advantage of excluding dead puparia, both have a major drawback; namely, that as many parasitoids (including Mutilla and Thyridanthrax) can remain enclosed within the puparium for much longer periods than the developing tsetse fly itself, there will always be a bias in favour of parasitized whole puparia in any one sample.

Lack of a prolonged free-living larval stage and the normally well-protected puparium render Glossina relatively free from attack by insect parasitoids. In many areas, the incidence of parasitism is negligible. However, in some areas (e.g. parts of East, Central and Southern Africa) high rates of parasitism have been recorded. Under such circumstances, parasites probably play a part in regulating the numbers of Glossina. It is considered very possible that the incidence of natural parasitism might be enhanced by the introduction of further parasites.

(2) PHORETIC MITES

There are only a few records of mites from Glossina. Their role is unknown, and in all cases the levels of infestation have been low. Fifteen infestations in 9,000 observations of G. fuscipes were reported from Uganda early in the century. Larvae of Trombidium have been reported on G. palpalis and G. tachinoides in northern Nigeria, and larvae of Erythraeidea on G. fuscipes from Kenya.

(3) NEMATODE PARASITES

Probably, all the nematodes which have been found in tsetse flies can be assigned to the family Mermithidae. Reports of the occurrence of these nematodes in tsetse flies date back to 1910, when a specimen was reported from Glossina fuscipes in Uganda.

Since then, there have been a few published records of nematode occurrence. These reports, though, have concerned various species of tsetse in various African countries. In Uganda, two species of tsetse (namely, G. fuscipes and G. brevipalpis) have been shown to harbour nematodes. In Katanga (Zaire), Zambia and Tanzania, nematodes have been discovered in specimens of G. morsitans.

In West Africa, nematodes have been reported as parasites of G. palpalis in Liberia, Nigeria, and Upper Volta, and of G. morsitans and G. longipalpis in Nigeria.

The published records generally indicate that the incidence of nematodes as parasites of tsetse flies is low. In most cases the reports have been based on the discovery of only a few nematodes, often after dissection of many thousands of tsetse flies.

Only in Liberia and Nigeria have the rates of incidence been rather higher. In the first instance, dissection of 4,000 flies revealed that fifteen harboured nematodes. All these tsetse were G. palpalis. In the second, 43 infected flies were found during the course of dissecting approximately 11,000 tsetse. Of these 43 flies, 38 had originated from a single locality. Two species (G. palpalis and G. longipalpis) were present in this locality and both contributed some of the infected specimens.

Opinions differ concerning the effect of nematode parasitism on the health of the host
tsetse. There is little doubt, though, that the latter is killed by the departure from it of the fully developed nematode larva, for exit is by means of penetration of the body wall of the host. The resulting hole would allow leakage of body fluids from the host and also, perhaps, entry of other infective agents.

The limited amount of information available indicates that, in the case of female tsetse flies, reproductive cycles are not seriously affected by the presence of a nematode.

Parasitic worms found in tsetse have been widely variable in size, up to a maximum of about eleven centimetres. Studies in Nigeria have led to the conclusion that there is no correlation between the age of parasitized female Glossina and the size of the contained nematode. However, the possibility must always be kept in mind that individual nematodes vary greatly in both size and rate of development.

While there is no strong evidence relating to the ecological site where infection of Glossina by nematodes takes place, it should be noted that in other hosts, it is usually the immatures that are invaded by mermithids.

**PATHOGENS OF GLOSSINA**

(1) **Fungal pathogens**

Fungal infections of Glossina were first noted many years ago, but cultures of these strains were not maintained. The organisms were reported as belonging to the genus Cicadomyces (from G. morsitans and G. palpalis) and the Phycomycetes group (from G. morsitans). Their pathogenic effects were not elucidated. While rates of infection were sometimes recorded, this information is of little use in the absence of valid identification of the fungi.

More recently, Absidia repens and Penicillium lilacinum have been reported from Glossina. Mortality of puparia in nature has been attributed to A. repens. However, related fungi (A. ramosa and A. corynbfera) are vertebrate pathogens. Thorough studies of the stability of A. repens and its pathogenicity to invertebrates and vertebrates, including man, are therefore indicated.

Fungi found in other dipterans should be examined as possible pathogens of Glossina. Examples are the entomopathogens of the genus Coelomomyces. Once thought to be restricted to mosquitoes, these are not only now known from other Nematocera, but C. milkoi was recently described from Tabanidae in the USSR.

(2) **Other microbial pathogens**

Bacteria, Protozoa, and microsporidan-like organisms have occasionally been isolated from tsetse.

In 1935, Bacterium mathisi was isolated from G. morsitans in Tanzania. The organism was easily maintained in culture, and proved non-pathogenic to a variety of small mammals and to mosquitoes. However, examples of both G. morsitans and G. palpalis died within three days of being infected by this bacterium. Unfortunately, the original cultures of this bacterium were lost. Efforts to re-isolate the organism are considered highly desirable.

More recently, two further bacteria have been reported from G. tachinoides puparia. Also, Treponema glossinae has been described from the gut of G. palpalis, and similar organisms have been found in G. brevipes.

Haemogregarines have been obtained from specimens of G. palpalis originating from various areas, including Senegal and Ghana (crocodiles are the vertebrate hosts of the tsetse-borne Hepatozoon petiti).

*Myxosporidium heibergi* is an organism of uncertain standing, described nearly 70 years ago. It is mentioned here as it is the only parasite possibly referable to the protozoan order Microsporida yet recorded from tsetse. The interest of this lies in the fact that microsporidans are currently considered as candidate biological control agents for eventual use against mosquitoes and blackflies, in both of which groups they occur commonly.
Among the above organisms, only Bac-
terium multni has been clearly associated
with host mortality.

There are no published accounts of entomo-
pathogenic viruses isolated from Glossina.
Their close association with other groups
of Diptera suggests that they might well be found
once searched for. In this context, very recent
work has revealed virus-like particles in
G. morsitans centralis from both a British
laboratory stock and Uganda materials: and
in G. fuscipes fuscipes from Uganda (per-
sonal communication to the Scientific Secre-
tary from Dr. L. Jenni, Swiss Tropical Insti-
tute, Basle).

POTENTIALS FOR MASS
PRODUCTION

(1) GLOSSINA

The effective breeding of Glossina at pro-
duction levels of several thousand individuals
is only being carried out in a few laboratories.
All of these are located in Europe (e.g.
Tsetse Research Laboratory, Langford, UK; IEMVT 2, Maisons-Alfort, France; Joint
FAO/IAEA Laboratory, Vienna, Austria; Labo-
ratoire du Centre Universitaire de l'Etat, An-
vers, Belgium).

The species being bred all belong to the
morsitans and palpalis groups:
— Maisons-Alfort, 5 species (G. m.
morsitans, G. austeni, G. tachinoides,
G. f. fuscipes, G. p. gambiensis)
— Langford, 2 species (G. m. morsitans,
G. austeni)
— Vienna, 2 species (G. m. morsitans,
G. tachinoides)
— Anvers, 2 species (G. p. palpalis, G. f.
quanzensis).

There is no important colony of the species
of the fusca group.

Some other laboratories located in Europe
(Basle, Lisbon), Canada (Edmonton, Mon-

treal) and Africa (Nairobi, Kaduna, Tanga,
Bobo-Dioulasso) breed smaller numbers of
tse. Certain of these, though (particularly
African ones) are planning early increases
in their output.

All existing colonies use living animals
(rabbits, goats, guinea-pigs) for blood meals.
However, progress is being made towards the
feeding of Glossina through artificial mem-
branes. Although this technique has not yet
reached the stage of complete independence
from living host animals, results to date are
very encouraging. In the near future, indeed,
it is anticipated that it will become feasible
to maintain colonies fed exclusively on arti-
ficial diet.

Tsetse trap, Nasso, near Bobo-Dioulasso, Upper
Volta
While the low reproductive rate of tsetse flies curtails their mass-production, the main rearing laboratories in Europe can (without reducing the size of their colonies) divert more than half the production to other uses. Thus, both Langford and Maisons-Alfort are able to make available over 100,000 puparia per year.

(2) Parasitoids

One of the advantages of this aspect of biological control of Glossina is that many of their insect parasites (parasitoids) are already known and identified. Moreover, for several of the more common species, their general distribution is known and their life-cycles are understood. In a few instances, there is some knowledge of methods of breeding based upon experience with actual parasitoids of tsetse, and there is wider experience from parallel work in economic entomology to be drawn upon.

Therefore, there are certainly possibilities for breeding a number of these parasitoids which could be exploited with little delay, using currently available facilities in both Africa and Europe. Glossina breeding units could be utilized, for example. Various African stations could become sources of Glossina parasitoids for mass production units elsewhere, and Commonwealth Institute of Biological Control (CIBC) stations could cooperate in the development of rearing techniques up to the level required.

It must be borne in mind, too, that a number of parasitoids are known from other Diptera, particularly muscoid flies elsewhere than in Africa. One or more of these, if artificially established there, might possibly become important parasites of Glossina in some areas. Relevant laboratory experimentation and field trials are necessary. Preliminary exploration of this field would require the availability of limited quantities (of the order of hundreds a week) of puparia representing a range of tsetse species.

The ultimate aim of any such work is to make liberations of parasitoids in the field. For this, the numbers required for adequate introductions into a number of ecologically different areas, and at different seasons, would necessarily be large. To produce Glossina puparia in the required numbers would be difficult, and certainly very expensive.

It would thus be preferable, in connection with all such parasitoid breeding, to develop methods based upon the use of alternative hosts (e.g. Sarcophaga, Chrysomyia, Lucilia, Musca). Representatives of these genera are easily produced in any numbers required, either on inexpensive naturally-occurring substances, or on artificial diets. The basic facilities for small initial production exist at CIBC stations. They could be expanded very easily, as and when required.

The necessary numbers of the different parasitoids in question would vary with such factors as their biological characteristics, the number and diversity of sites for field liberations, the manner of use (e.g. inoculative, inundative). The last-mentioned point is of prime importance. Simple inoculative releases into small selected areas require few parasitoids (several hundred on each occasion, with perhaps 20 releases at each introduction site over a year, or concentrated within the most favourable seasons). The required numbers of parasitoids would be small enough for production in Glossina puparia at present supply levels, if alternative hosts could not be found. Similar releases over wider regions less densely populated by Glossina (e.g. savannas) would require far greater numbers of parasitoids, the production of which would be impractical with Glossina puparia as hosts (failing radical advances in production technology). Inundative releases designed to suppress Glossina numbers following each release, rather than to lead to permanent establishment of the parasitoids in the new locality, would have to be repeated periodically. Such an approach would require extremely large numbers of parasitoids and hence of hosts. Therefore, it is less practical than the previous two methods.
Actual breeding techniques and output would naturally vary from one parasitoid species to another. Among the parasitoids of *Glossina*, the mutillids and bombyliids should be the first groups to be tackled; in full realization that the latter would prove difficult to breed, and that the operation might not be feasible outside of Africa. At least three known species of mutillids and at least ten bombyliids are available for consideration, and there are a number of other parasitoids (chalcidoids, etc.) that should certainly be looked into as well.

On the basis of present knowledge, a dozen parasitoids from other hosts are available for testing against *Glossina*. The relevant breeding techniques have already been developed, and material is readily available for laboratory and field trials against tsetse. It is confidently predicted that investigation will rapidly produce a substantially wider range of candidates. Preliminary trials could indeed be undertaken prior to the development of suitable mass-breeding techniques, the work being centred upon existing facilities having the necessary expertise and near to existing sources of *Glossina* puparia (e.g. cnc stations — the station at Delemont, Switzerland, has the required staff and facilities, and could — via short and reliable air links — very easily obtain material from Maisons-Alfort, Langford, Anvers, and Vienna).

(3) Nematode parasites

There is no current capability for mass-producing mermithid nematode parasites of tsetse. As yet, too, the necessary preliminary field and laboratory studies remain to be undertaken. Not one species of mermithid has yet been described from tsetse flies. Indeed, it cannot yet be stated with certainty that more than one mermithid species is involved. Host-parasite relationships require exploration as well. All of our relevant knowledge may be summed up in the observations that;

i) several species of tsetse from a wide geographical swathe of Africa harbour mermithids;

ii) mermithids may infect approximately 1% of the adult flies.

It is not known if larvae and puparia are infected, or whether significant mortality really does occur. A detailed discussion pertaining to the mass-cultivation of tsetse mermithids would therefore be premature, since their potential impact on the host populations has not been established.

(4) Microbial pathogens

Superficially, it could be argued that the tsetse fly is not an ideal candidate for microbial control. After all;

i) several species of *Glossina* are important vectors and must be controlled with the means now available;

ii) the insects have widely varying distributions and behaviour patterns;

iii) their developmental stages have yielded few microbial pathogens and might be considered as unusually unavailable to such organisms.

On the other hand, it is self-evident that success in tsetse control by means of microbial control agents depends upon a number of factors — and, notably, upon the production and distribution of these organisms in adequate quantities.

Past experience with insects other than tsetse has indicated that multiple or periodic introductions of micro-organisms are the rule rather than the exception in ensuring the continuation of desired levels of control. Means for the mass production of microbial pathogens of tsetse are therefore prerequisite to the attainment of meaningful degrees of tsetse suppression.

Although *in vivo* and *in vitro* techniques are now being successfully employed in the production of insect microbials, the latter is preferred as it opens the way to adequate regulation of product standardization.

Assuming that some form of culture can be realized for the production of candidate
micrrobials for tsetse control, the following points need careful consideration in exploring the potential for mass production:

i) costs, including both operational costs and capital investment;

ii) the amounts of microbial needed and the frequency of production;

iii) product purity;

iv) product virulence;

v) product — including formulation — stability;

vi) production location; and

vii) method of distribution.

Few, if any, immediate research concerns impede the development of mass-cultivation techniques toward tsetse control. The most obvious potential candidates (i.e. microbial agents with high virulence to several species of Glossina, notably Bacterium mathisi and fungi of the genus Absidia) have already been cultured in vitro. Once one of these pathogens (or another such candidate for tsetse control) has been characterized sufficiently for full appreciation of its potential as a significant control agent, it will be possible to increase production toward mass cultivation.

Pathologies, pathogens, parasites and predators of Glossina

(a) Inventories of biological agents and non-infectious pathologies affecting the success of Glossina in specific localities should be implemented without further delay. The objectives for such inventories would be:

i) to establish basic information on the distribution of biological agents and non-infectious pathologies in advance of manipulation of indigenous biological agents or introduction of exotic ones;

ii) to detect the existence of potential agents for biological control, and the incidence of non-infectious pathologies, for example, genetically-determined reproductive anomalies;

iii) to elucidate infectious and non-infectious causes of abortion in tsetse.

(b) Existing studies of the widest possible range of individual biological control agents merit acceleration, and new ventures in this field should be fostered and adequately supported. These studies should include:

i) research within Africa toward the adequate characterization of such organisms, with further attention to their natural habitat, host preferences, behaviour, development, distribution, and the construc-

Research Needs

Research toward the recognition and use of candidate biological agents for development of additional control methods against Glossina, is recommended in four categories:

(1) pathologies, pathogens, parasites and predators of Glossina;

(2) relevant aspects of tsetse ecology, physiology and behaviour;

(3) relevant aspects of mass production; and

(4) health and environmental safety of biological control agents.

An existing global inventory system for biological agents affecting vector species is operated by the World Health Organization through the International Reference Centre for the Diagnosis of Diseases of Vectors, at the Ohio State University, Columbus, Ohio, U.S.A. For this Centre’s purposes, the term “diseases” embraces pathogens, parasites, predators and non-infectious diseases. The Centre has been largely concerned with the isolation of pathogens and parasites of the mosquito vectors of malaria, yellow fever, and other human diseases to date. Such a Centre might conceivably play a role in a network or program related to the recommendation cited above in implementation of an inventory of biological agents associated with Glossina and of potential value for biological control.
tion of relevant life-tables (from the results of this work, priorities could be established for trial inoculative introductions of candidate biological control agents from one region of Africa to another, and/or their propagation in very large amounts for inundative field releases);

ii) a comprehensive review of parasites and pathogens of muscoid flies from elsewhere than Africa should be directed towards exposure of the puparia of various species of *Glossina* to a variety of candidate agents, in the interests of selecting particularly promising organisms for trial against *Glossina* — first under simulated field conditions wherever facilities were available, but eventually through actual pilot projects in Africa;

iii) further studies of short-listed candidate parasitoids brought to light under i) and ii) above, towards their propagation in very large quantities — such studies to involve alternate hosts in place of actual tsetse flies wherever desirable, and to be scaled up to a fully operational level either in Africa or elsewhere, as dictated by circumstance; and

iv) the building up of a panel of collaborating specialists with the requisite skills for taxonomic identification of non-target invertebrates associated with *Glossina*, and of those predacious organisms (vertebrates as well as invertebrates) recognized to affect *Glossina*; and the coordination of these experts' services within the context of this report.

(c) Viewed as no less promising than the invertebrate parasites affecting *Glossina* (e.g. parasitoid Hymenoptera, Diptera) and these insects' invertebrate and vertebrate predators (e.g. spiders, rodents) are the non-insect parasites such as mermithid nematodes, and the microbial pathogens (viruses, bacteria, fungi, protozoa, and possibly the rickettsiae). According to published reports, the nematodes and fungi appear to be the predominant groups harming all stages of *Glossina* (which also, however, have been reported at times to suffer from bacterial attack). The detection of pathogens (and mutualistic symbionts) associated with tsetse must of course provide for systematic examination of the various life-history stages (with particular attention to immature forms) in both field and laboratory. The provision of tentative identifications and the referring of pathogens to specialists should be the responsibility of an enlarged network of laboratories serving formally in an inventory system.

The examination, characterization and identification of candidate biological control agents, and the screening of pathogens, once isolated, should be pursued by specialists (e.g. virologists, bacteriologists, protozoologists) collaborating with public health and veterinary entomologists handling tsetse in the laboratory and field.

Furthermore, the isolation of particularly promising strains of identified pathogens; the development of methods for the formulation of materials to be used in pilot programs and eventual field applications; together with proper monitoring of effectiveness and safety; must be the result of collaboration between entomologists specializing in tsetse problems, biological control workers, invertebrate pathologists and mass production experts. Also, there must be continuing liaison between representatives of all interested groups from the governmental, university and industrial sectors, and at both national and international levels.

Where invertebrate pathogens already in use and commercially available are known to affect Diptera, their effects on *Glossina* should be ascertained. It is also specifically recommended that such studies be conducted with respect to the activity of already-isolated and available metabolic products resulting from the fermentation-production of strains of *Bacillus thuringiensis* (a pathogen now widely-employed for the manufacture of first-generation "microbial pesticides").
RELEVANT ASPECTS OF TSETSE ECOLOGY, PHYSIOLOGY AND BEHAVIOUR

All behavioural activities which will help in predicting the location of tsetse puparia and adults are germane to biological control research. Through such studies, methods will be devised for attracting tsetse to locations where adults or immature forms can be infected with parasites or pathogens. The emphasis in such investigations should be ethological, pertinent observation and experiment being combined in both the laboratory and nature. Subjects for investigation under this heading should include:

i) factors determining selection of larviposition sites by pregnant tsetse;

ii) factors determining selection of resting sites by males and females at various stages of their activity and reproductive cycles;

iii) the isolation and testing of potential attractants from host animals;

iv) the basis of interspecific and intergeneric communication;

v) distribution of adults in the field (Is distribution random in both sexes? Do females return to larviposit at the same site as used previously?);

vi) the development of adequate techniques (e.g. reliable procedures permitting the accurate estimation of low densities of tsetse) for sampling Glossina populations (studies of tsetse population composition by age group: determination of the population structure in relation to research on the effects of the introduction of sterile males, natural enemies and diseases among Glossina adult populations and assemblages of larval and pupal forms);

vii) elucidation of life-tables to detect and rank the mortality factors for Glossina; and

viii) improved understanding of the sensory mechanisms of adults and immatures, towards the prediction of orientation behaviour patterns, notably studies of:

vision (pattern, colour movement);

hearing (communication);

chemoreception (attraction, repellence, resting, larviposition);

mechanoreception (resting, larviposition).

RELEVANT ASPECTS OF MASS PRODUCTION

There is a need for improved mass-production technology to ensure the availability for field application of adequate amounts and continuing supplies of selected insect and other parasites, and pathogens, of Glossina. Pertinent field application procedures will require attention too. Improved mass-production capabilities within Africa itself are viewed as fundamental to the above requirements; as are further diversification of the number of tsetse species being reared in the laboratory (with special reference to forest species of economic importance), and the early perfection of artificial feeding methods.

It is also recommended that whenever pathogenic microorganisms accidentally appear in tsetse colonies, the obvious urgency of correcting the problem should not be an excuse for neglecting the opportunity for isolating, identifying and evaluating what might prove to be additional candidate biological control agents.

HEALTH AND ENVIRONMENTAL SAFETY OF BIOLOGICAL CONTROL AGENTS

All candidate biological agents being considered for tsetse control must be assessed from the standpoint of their safety to non-target organisms.

The evaluation of e.g. microbial control agents has centred upon their manipulation as active ingredients in formulated preparations for area application or inoculative introductions. At the same time, there has been increasing recognition of the need to follow sound quarantine practice and to ensure the safe practice of operational biological control procedures. It cannot be too strongly emphasized that the use of microorganisms
for tsetse control must be evolved in accordance with an orderly sequence. At each step, research and assessment are necessary to furnish the proper safeguards covering the proposed use. Thus, following the detection, isolation, and characterization of a promising pathogen, the active principle must be identified and an original isolate must be maintained as a reference culture. In order to assure the capability to control or destroy a microbial pathogen, in case a dangerous situation should occur despite careful safety testing, there must be prior knowledge of appropriate anti-microbial agents.

A further stage towards the assessment of hazards involves investigation of the possibilities of mammalian, and general vertebrate and invertebrate, response to the pathogen. This phase can be divided into empirical studies, the testing of selected vertebrate and invertebrate species, and basic research designed to elucidate the reasons for apparent non-infectivity of insect pathogens to vertebrates. On reaching this point in the development of candidate microbial control agents, the results should be reviewed by an impartial panel of experts, charged by the responsible agency with recommending whether or not a limited field use of the candidate is acceptable. Such limited use should be designed to serve two purposes: firstly, to gather data on the pathogen's effectiveness; and secondly, to obtain data on its short-term environmental consequences, if any.

Provision should be made at this point for the detection of any long-term consequences of field applications of pathogens, if findings from limited use suggest such an eventuality. Results of the laboratory and limited-use field studies would of course be basic to any decision on the approval of relevant applications concerning operational field use.

The monitoring of any human effects of exposure to entomopathogens demands programs specifically devised for persons occupationally or incidentally exposed to such organisms under operational conditions. The possible effects on man must also be considered in a presumptive sense when reviewing the status of a pathogen in advance of limited use.

The totality of safety of entomopathogens and related research needs, thus rests on three issues:

(i) the identification of the active principle;

(ii) vertebrate and invertebrate infectivity studies emphasizing why a pathogen affecting an invertebrate is non-pathogenic for vertebrates; and

(iii) studies of short-term and long-term consequences to the environment.

**TRAINING NEEDS**

This section of the report refers to training needs for both professional and expert technical personnel.

Some problems of the present situation in Africa are:

(1) scientists working with the tsetse problem are seldom trained in the theory and practice of integrated control (pest management), and many of them are self-taught in vector control;

(2) information on educational opportunities potentially available to those concerned does not always reach their level;

(3) in some research centres indigenous personnel are still a minority among the scientific staff; and

(4) there is often inadequate communication between tsetse research centres in different countries, especially between the francophone and anglophone states.

Integrated control (pest management) programs are given at some universities in North America (in Canada, the Master of
Pest Management – MPM – program at Simon Fraser University, British Columbia). The development of a similar program oriented towards tropical integrated control (pest management) would be most timely. There is an urgent requirement for short-term intensive courses in practical management of specific kinds of pests and vectors (including tsetse). Such courses should include techniques for survey, rearing, etc., given by experts in the field, in Africa. Finally, it is desirable to encourage African students and scientists to take integrated control (pest management) training, and for the relevant administrations to facilitate their doing so.

RECOMMENDATIONS:

(1) that scientists working or planning to work on the tsetse problem in Africa be encouraged and financed to take training in integrated control (pest management) where it is now available, and that steps be initiated to set up a major integrated control (pest management) training centre oriented towards tropical problems at an appropriate location, perhaps with initial guidance and cooperation from the faculty of an existing centre in North America;

(2) that a system be organized to provide short-term (two weeks to two months) integrated control (pest management) courses in the field, in Africa;

(3) that for reasons of language and geography such courses be organized in different parts of Africa in different years;

(4) that the instruction be given by experts on the various aspects of integrated control (pest management) of which scientists of the CIBC represent one of a number of potentially-available groups, and that these provide refresher courses for working scientists as well as training for students entering the profession;

(5) that financial support be provided for the above programs and for the participation in them of students and scientists from all relevant countries; and

(6) that communications dealing with the availability of all such educational opportunities be improved (e.g. via the Liaison Officer of the International Scientific Council for Trypanosomiasis Research and Control at Nairobi, Kenya).

SUMMARY

Measures against pests and vectors in future will clearly involve the increasing application of integrated control (pest management) approaches. Equally clearly, biological control will comprise an essential component of integrated methodologies used against tsetse, as against other harmful insects. Therefore, funding agencies interested in early solutions to the pressing problem of the African trypanosomiases would do well to give the most serious consideration to supporting relevant research as detailed in this report.

As indicated, there are inequalities in our present understanding of the different fields that will contribute towards eventual biological control methods for use against Glossina. It would be presumptuous at this early stage to attempt the identification of an extended list of research and training priorities; a few of which will nevertheless be cited, purely as examples.

The first three are self-evidently prerequisite to many other needs individually indicated in the report; to inventory and characterize pathologies, pathogens and parasites of tsetse through the promotion of collaboration within a network of suitably-equipped and staffed research laboratories; by a similar approach, to facilitate the identification of other organisms associated with Glossina (particularly predatory invertebrates) through fostering the provision of skilled systematic advice; and to further integrated
control (pest management) training courses for African students and scientists concerned with tsetse control.

Finally, because of the relatively advanced state of knowledge of this particular issue, it is emphasized that confirmation of the apparent absence of mutillid wasp parasitoids from West African *Glossina* would open the way to early westward field introductions of already-known species of *Mutilla* from elsewhere in the continent.

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