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The sterile-male technique against tsetse flies, *Glossina spp.*

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The Sterile-Male Technique Against Tsetse Flies,

Glossina spp.1

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Simpson (1958) discussed the relationship between the biological characteristics of the genus Glossina and the use of the sterile-male technique in the control of this vector of trypanosomiasis, as did Knipling in an informal report in 1963 on Practical Role of the Sterility Principle for Tsetse Fly Eradication in WHO/Vector Control/27. Knipling estimated that an initial overflooding ratio of sterile to wild males of 3:1 with successively smaller releases could eradicate a low-density population of flies in 12 months (Table 1) at a cost of about $125/mile², even if the sterilized males cost as much as 5 cents each. With larger populations, other methods would have to be used to reduce the density before the releases of sterile males. Also, Knipling (1964) emphasized the economic advantage that would be gained by reduction in the total number of sterile males required if their release were preceded by a single application of a nonpersistent insecticide which would eliminate most of the adult population. When populations cover a wide area, simultaneous treatment of the entire infested area might not be feasible. A more realistic approach (Table 2) could be to systemically expand small control areas along a common front (Dame 1968).

A research program was therefore begun in 1964 in Rhodesia to develop methods of rearing and sterilizing Glossina spp. and to evaluate the sterile-male technique in the field. This program was sponsored by the Agency for International Development and was conducted by the Entomology Research Division, Agr. Res. Serv., USDA; the Agricultural Research Council of Central Africa; and the University College of Rhodesia. Headquarters were at Salisbury, and field stations were situated in the natural habitat of Glossina morsitans Westwood in the valley of the Zambezi River near Chirundu and Kariba. G. morsitans was chosen as the primary test species because it is the most important and widespread vector of trypanosomiasis among the 22 species of Glossina found in Africa. Therefore, any success achieved with this species would encourage similar attempts with other important vectors which, because of behavioral and ecological characteristics, might prove to be even more suitable targets for the sterile-male technique.

The major source of live flies for the testing done by the Tsetse Fly Investigations Laboratory has been the collection of live pupae in the bush near the Kariba field station. When conditions were optimum, as many as 30,000 pupae were gathered weekly by 15 searchers (Fig. 1) at a cost of 3–5 cents/pupa, an expenditure within the limits given by Knipling (1964) as being economically reasonable for the control of low-density populations. However, this method of obtaining insects for sterilization may not be suitable for control programs because of known seasonal variations in the availability and quality of the pupae. Large-scale rearing would provide the most reliable source of flies for sterile-male-release programs.

1Diptera: Muscidae.
2 Mention of a pesticide in the paper does not constitute a recommendation by the USDA.
3Salisbury, Rhodesia. Present address: Gainesville, Fla. 32601.
4Baltimore, Md. 20295.

METHODS OF REARING

When our studies in Rhodesia were being planned, there had been little success in breeding Glossina in the laboratory, and only a few workers had managed to maintain flies in captivity for more than 2 or 3 generations (Nash 1963). Moreover, the available data suggested that reproduction of the flies was inhibited in the laboratory because Glossina were refractory to close confinement. We therefore directed our rearing effort toward maintaining the fly in its natural habitat.

A field station was established in a relatively undisturbed segment of the natural habitat along the southern bank of the Zambezi River near Chirundu, and many attempts were made to rear G. morsitans in screen cages covering as much as 10,000 ft² of suitable habitat and in smaller cages that were specially designed to take advantage of behavioral traits of the species (Dean et al. 1968). Both types of cages were stocked with pupae collected in the field, with flies that had emerged from such pupae, or with adult flies captured in the nearby bush, but regardless of season, cage design, or experimental variations, the survival of the introduced flies was inadequate, and breeding on a self-sustaining basis was unsuccessful (Phelps and Dean, personal communication).

This result was similar to that of Harley (personal communication) who was attempting to rear G. fuscipes Newstead, G. pallidipes Austen, and G. brevipalpis Newstead in a large outdoor cage on the shores of Lake Victoria at Lugala, Uganda. However, the flies held in our small (8X8X11-in.) cages within the larger cages invariably survived and reproduced, while their semiconfined counterparts, which could roam freely and feed at will on their natural host (cattle and bush-pig) failed to survive and to reproduce efficiently. Also, attempts to concentrate natural populations by increasing the host density (cattle) in suitable habitats were also unsuccessful, and the density of the fly populations remained similar to those in the control areas over the 2 years of testing (Dean et al. 1969c). After more than 3 years of work, the field rearing studies were discontinued.

Meanwhile, Nash et al. (1968) had developed methods of rearing G. austeni Newstead in the laboratory which were singularly effective, so much so that the flies had a reproductive capacity which undoubtedly exceeds that in nature. We therefore solicited Dr. Nash’s assistance in attempting to rear G. morsitans in 1967, and when he agreed we sent him pupae collected in the bush near Kariba. By maintaining the emerging adults in geigy-type cages and feeding them on either goats or lap-eared rabbits, Dr. Nash and his colleagues were able to achieve a high reproductive efficiency. Other methods are now being used in Salisbury where our colony of G. morsitans is now in its 4th year. Also Ward (1970) showed other colonies of Glossina are now being maintained with varying degrees of success. Thus, the essential techniques for efficient rearing of G. morsitans (in small cages) are now available for adaptation to large-scale rearing methods, though several problems remain.

One such problem relates to the need to provide a blood meal with a live host. The proved rearing technique relies exclusively on the use of live host animals. Studies are therefore under way to find a possible artificial feeding...
Fig. 1.—Collecting tsetse fly pupae in the bush near the Kariba field station.

system (Southon and Cockings 1963, Azevedo et al. 1968, Galun and Margalit 1969, and Langley 1970). In our laboratory (R. C. Heaversedge, personal communication) flies feed as readily on blood offered through such natural membranes as rabbit skin or chamois leather in combination with Baudruche as on live hosts. However, investigators agree that a blood meal obtained in this way is not adequate for normal reproduction and survival. Extensive investigation is therefore needed on the relationship between the blood or artificial meal and the metabolism and physiology of Glossina spp. before we can hope to replace the live host with artificial feeding techniques.

Another problem arises because the difficulties in establishing healthy, self-sustaining colonies of Glossina suggest that adaptation may occur before a strain of flies collected in nature can perform adequately in the laboratory, and such inbred colonies may have lost behavioral characteristics that are necessary for survival in nature. For example, the Lisbon colony went through a prolonged adaptation before a stabilized reproductive capacity was achieved (Azevedo and Pinhao 1968). We therefore investigated the adaptation of the Lisbon colony (Dame et al. 1970): in mating tests, the males competed successfully for native females against native males that had emerged from pupae collected in the field. However, excessive mortality resulted, because the colony males had diminished ability to locate and feed on the host animal when conditions were such that the native males thrived. In another test, we successfully colonized a native strain by using the standard techniques of maintenance used with the Lisbon colony. However, in this test, genetic selection and adaptation proved to be as important in the establishment of the new colony as it had been in establishing the Lisbon colony. Similar mating studies conducted in the laboratory revealed no differences between native and Bristol colony males. When males of the Bristol colony were released into their original field habitat 2 years after colonization, they survived and dispersed as well as the native flies which served as controls. Thus, any adaptation that may have taken place in this colony had no determinable effect on survival or behavior in nature. These different results indicate that the

Table 1.—Theoretical trend of a low-density population of tsetse flies (average 100 males and 100 females/mile²) subjected to sterile male releases at the rates and periods indicated (after Knipling 1964).

<table>
<thead>
<tr>
<th>Period (months)</th>
<th>Natural density at beginning of each period (males + females)</th>
<th>No. sterile males released each month</th>
<th>Estimated avg. ratio of sterile to fertile males</th>
<th>Assumed natural density at the end of each period</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-3</td>
<td>200</td>
<td>900 (300/month)</td>
<td>3:1</td>
<td>75</td>
</tr>
<tr>
<td>4-6</td>
<td>75</td>
<td>450 (150/month)</td>
<td>4:1</td>
<td>22</td>
</tr>
<tr>
<td>7-9</td>
<td>22</td>
<td>225 (75/month)</td>
<td>7:1</td>
<td>4</td>
</tr>
<tr>
<td>10-12</td>
<td>4</td>
<td>112 (37/month)</td>
<td>16.5:1</td>
<td>1</td>
</tr>
</tbody>
</table>

*In calculating the theoretical effect of the sterile male releases, it is assumed that the released males are fully competitive with native males and exert full effect in reducing the reproductive potential of the total population. The assumption is also made that in the absence of control, the natural population would increase by 50% from a low level each 3 months (each generation). Thus, in the absence of sterile male releases, the population trend would be as follows: 1st period 200 flies/mile²; 2nd period 300 flies/mile²; 3rd period 450 flies/mile²; 4th period 675 flies/mile².
treated plastic strips while they were en route to an exit from was complete when freshly emerged flies walked ad libitum over a glass surface treated with 10 mg tepa/ft² or through (1958), performance of a colony-reared fly in nature may depend on the degree of selection which occurs during the colonization. With the Bristol colony, colonization did not reduce field viability.

**METHOD OF STERILIZATION**

Radiosterilization of *Glossina* was first attempted by Potts (1958), who achieved a high degree of sterility in *G. morsitans* pupae exposed to 6000 or 12,000 r of gamma irradiation from a cobalt-60 source. At our laboratory, we subsequently obtained completely sterile males by exposing pupae less than 14 days old to doses above 4000 r. With older pupae, 8000–15000 r produced over 95% sterility, and female pupae of all ages were completely sterilized by exposure to 1000 r (Dean and Wortham 1968). Moreover, in our tests, irradiation caused pupal mortality ranging from 23 to 64%, and the younger pupae suffered the highest mortality. Survival among adults emerging from these pupae was reduced less severely at the lower doses. In contrast, adult males exposed to 8000–16000 r shortly after emergence were 95% sterile and survived as well as untreated flies. Moreover, males radio-sterilized as pupae or adults were sexually competitive with untreated laboratory-emerged males in small cages in the laboratory, in larger field cages in the natural habitat (Dean et al. 1969b). Similar results were obtained in the laboratory with pupae and adults of *G. austeni* exposed to cobalt-60 (Dean and Clements 1969), and adult *G. morsitans* and adult *G. tachinodes* Westwood irradiated with Cesium-137 (Itard 1968a,b), pupae of *G. pallidipes* irradiated with cobalt-60 (Dean and Clements 1969), and adult *G. austeni* exposed to cobalt-60 (Curits 1968).

Chemosterilization of *Glossina* was first attempted by Chadwick (1964), who reported that treatment with apholate and metepa caused excessive mortality of *G. morsitans* at sterilizing doses. In our laboratory, we found that metepa- and tepa-sterilized male *G. morsitans* without undue mortality when they were injected at rates of 5 and 1 μg/fly, respectively. Also, in subsequent tests, adult males were permanently sterilized by contact with 10 mg/ft² of tepa or metepa on glass surfaces for 30 or 60 min, and length of life was not seriously affected. However, flies exposed for a shorter time to 10 mg tepa/ft² recovered some fertility after an initial sterile period, and exposures of 120 min or longer to either tepa or metepa reduced longevity. Auto-sterilization was complete when freshly emerged flies walked ad libitum over a glass surface treated with 10 mg tepa/ft² or through treated plastic strips while they were en route to an exit from the holding chamber. Adult males sprayed in a wind tunnel with 0.25 ml of a 5% solution of tepa or metepa solution in methanol were sterilized. Finally, flies emerging during the first 2 weeks after pupae were treated by a 60-sec immersion in a 5% solution of tepa or metepa were sterilized by contact with their own puparium, but flies emerging after that period were not completely sterile. In general, freshly emerged adults are less tolerant of the chemicals than older flies, but it is usually possible to minimize the toxic effect by reducing the time of exposure of the young flies. Adults sterilized by contact or by exposure to sprays were competitive with untreated laboratory-emerged flies in small cages in the laboratory, in larger field cages in the natural habitat, and in free flight field releases. Thus, radio-sterilization of pupae at doses sufficient to cause a high percentage of sterility and reduced emergence often results in some residual fertility unless the pupae are young when they are treated. (This degree of fertility, as much as 5% might not seriously affect a control program because of the low innate reproductive capacity of the genus.) On the other hand, with radio-sterilization or chemosterilization of adults, which involves extensive handling during sexing, treating, and feeding (the flies can be immobilized with carbon dioxide or nitrogen gas (Birkmeyer, personal communication) or by using lowered temperatures) complete sterility can be obtained with chemosterilants and 95% sterility with irradiation. However, the physiological and behavioral effects of holding adult *Glossina* in captivity, though not thoroughly understood, are known to be detrimental. Those that emerged in our laboratory failed to develop normal flight musculature and survived poorly in the field compared with native flies, even when they were fed before release.

Then, since adults that actually emerge in the field have survival equal to that of the native fly (Dame et al. 1969), we are currently seeking a more reliable and satisfactory treatment for pupae, perhaps one in which low-level irradiation and chemosterilization are combined to obtain the advantages of both and to avoid the disadvantages of each. One possibility would be to treat the pupae with irradiation in the laboratory and then expose them to the chemosterilant as they emerge in the field.

Sterilization of native populations of *Glossina* would avoid some problems associated with treating reared flies and would eliminate the necessity of releasing large numbers of sterile males into the environment; also, there is no reason to believe that sterilization of native males of unknown ages would result in undesirable side effects, since males and females as much as 3 weeks old have been sterilized in the laboratory. However, effective chemosterilants are rapidly degraded in the field and would have to be reapplied every few days. In addition, a prolonged program of this kind might cause the development of resistance to the sterilants, so any field sterilization must insure exposure of *Glossina* to adequate levels. And, finally, the main obstacle to treating native populations is the mutagenic activity of the available chemicals, which are potentially capable of affecting any living organism. Any field application must therefore be selective to insure that only the target species would contact the sterilant. Selectivity could probably be achieved by using an attractant for *Glossina* in combination with a sterilant, but the most satisfactory attractant for *Glossina* currently available is the live host animal. We have been unable to demonstrate the existence of pheromones in the laboratory or in the field with either *G. pallidipes* or *G. morsitans* (Dean et al. 1969a). However, Langridge (1960) and Persoons (1967) reported some success in field trials with a crude extract from the skin of natural hosts of *G. pallidipes* and *G. fusipes*. The exact nature of the response they obtained, i.e., whether the material is actually an attractant or merely an antarrestant, has not been elucidated. Also, both *G. morsitans* and *G. pallidipes* responded positively to UV light in the laboratory but it did not act as an attractant in the field (Dean et al. 1970).

It is plain that the development of effective chemical or physical attractants for *Glossina* is essential if native populations are to be sterilized directly. However, even with a
to sterilize males to obtain complete eradication. Certainly, sterile males will be needed if behavioral selection during such a program results in an insufficient proportion of flies in the population being responsive to the attractant. Thus, the need for mass production of Glossina cannot be dismissed.

**POPULATION DYNAMICS**

Information about the seasonal fluctuations in feeding, dispersal, mating behavior, reproductive status, birth and death rates, and length of life of the target species and about the interaction of these and other factors with density of population is particularly important when sterilization is to be used to control populations. Such knowledge is necessary to evaluate the fluctuations in density and reproductive viability of the target population throughout the year and to estimate the effect of environmental stresses on the viability of the released sterile males. The released fly is likely to be at a disadvantage because it must rapidly find both a suitable habitat and a host animal; resident populations already inhabit suitable niches. Those sterile males that are unable to find a host animal would be unlikely to survive long enough to mature sexually and thus would not affect the reproductive capacity of the native population.

Our investigations in the Zambezi valley demonstrated that densities of native Glossina of 8000–16000 flies/mile² are probably not uncommon. Moreover, by using marking and recapture data (Fig. 2, 3), we found that the seasonal changes in death rate in these native populations are sufficiently balanced by changes in the birth rate to insure that the density does not vary by more than a factor of 2 during most years. In the winter months (May through July) when the birth rate is very low, longevity is at a maximum; in October, when the population has reached its maximum, the death and birth rates are also maximum. Mating occurs at all seasons, and rates of insemination in samples of native female G. morsitans invariably exceed 95% though their mean reproductively age (determined by ovarian dissection) varied seasonally. In the laboratory, female Glossina mated more than once but only 1 of 3 normally did so, even when mating pressure was heavy (Dame and Ford 1968, Curtis 1968). Such findings cannot be translated into estimates of multiple mating in the field, but it is more than likely that the incidence would be even less than that observed in the laboratory. Females mated by both sterile and fertile males in the laboratory sometimes produce viable offspring. Sterile
sperm were found to be competitive with untreated sperm; the only apparent difference was that dominant lethality had been induced in the treated sperm (Dame and Ford 1966, 1968). Actually, the multiple mating of Glossina should not seriously affect control in the field as long as the sterile sperm are competitive with sperm from the native population (von Borstel 1960) and the sterile male behaves normally; in a native population overflown by sterile males, females would have the same mathematical chance of encountering sterile males for 2nd matings as they had for 1st matings. Moreover, multiple mating by males would be detrimental to the technique only if the sterile male behaved abnormally or failed to inseminate as many females as its native counterpart; laboratory studies indicate that the sterile male mates normally.

FIELD RELEASES OF STERILE FLIES

Our first release of sterile flies was made in 1967 on an island in Lake Kariba with males that had emerged from pupae collected in the field and had been sterilized by exposing them for 30 or 60 min to deposits of 10 mg of tepa/ft² of glass surface. Adult sterilization was selected for this test because (1) complete sterility could be assured whereas the pupal treatment was known not to be reliable; (2) the release could be limited to 1 sex, a factor that would be important during the initial stages of a practical control program when large numbers of flies must be released (we have demonstrated that the sterilized flies are capable of trypanosome transmission (Dame and MacKenzie 1968)); (3) the adult release required fewer pupae (only pupae of unknown age were available); and (4) distinctive markings for each release group were possible with adults but not with pupae, so the means of obtaining data on the survival of the released flies was simplified. The releases were begun at the beginning of the winter season when the birth rate was low, the longevity was high, and the population was about 4,000 G/mile². Two aerial applications of lindane made 30 days apart accomplished an initial reduction of about 50% in the native population. The marked sterile males were released the day after the 1st application, and similar releases were continued for 180 days (Fig. 4). Bioassays showed that the released males were completely sterile. Their overall rate of survival in the laboratory was only 10% lower than the untreated controls. However, their survival in the field was only 17% that of the native males. Also, although the rate of release during most of the trial was such that the number of sterile males introduced exceeded the number of native males, the maximum ratio of sterile to native males actually achieved in the field was only 12:100. After 6 months, the program was terminated without achieving control.

A 2nd test was then made on an island populated with 600-1200 G. morsitans to determine whether control could be achieved by sufficient overflooding to compensate for poor survival of the released sterile males, and whether the marking techniques used in the earlier test had influenced survival. This program was terminated in the 20th month, 3 months after the last native female had been sighted. A total of 26,000 unmarked sterile males had been released. Subsequent surveys confirmed that eradication of G. morsitans had been accomplished, though the other resident species, G. pallidipes, persisted. However, eradication had been assisted in part by a natural decline in the population of host animals. The results confirmed that sterile males released as adults were inefficient.

As noted, the investigation of male adults held in the laboratory and released in the field demonstrated that a high percentage failed to develop normal flight musculature, and that both our laboratory and field trials suggest that this inhibition of muscle development is related to poor survival in the field (Dame et al. 1969). Apparently, the physiological inhibition is triggered early in the adult life and is irreversible. Whether the phenomenon is caused by specific handling methods or merely results from close confinement during the early hours of adult life is unknown. However, released flies which actually emerged in the natural habitat did not suffer this inhibition and survived as well as the native males.

With this information at our disposal, we returned to Lake Kariba in 1968 to the island where we had made our 1st release of sterile males. First, the population which had returned to a density of about 3000 flies/mile² was reduced by 2 aerial applications of dieldrin. Then we made releases by placing pupae which had been dipped in a solution of 5% tepa in protected trays on the island. Recent tests had indicated that the chemosterilant might remain effective in the field if the pupae were protected from direct exposure to sunlight and rain; irradiation was not used because only pupae of unknown ages were available and because the losses expected from direct pupal mortality and from emergence over a long period were considered prohibitive.

Complete sterility was achieved during the first 3 months of the program, but thereafter the chemosterilant was less effective, and fertile males and females were occasionally released into the population. Nevertheless, 98% control was obtained in 9 months. At this writing, the 14-month program is in the final week of release (14th month), and eradication has probably not been achieved, though further observations will be required to confirm this tentative conclusion. The available data do suggest that the competitiveness of these released flies was much higher than that of the adults released after emergence in the laboratory.

The major hindrance to complete eradication lay in the fact that only a limited supply of field-collected pupae of unknown ages was available for the releases. Therefore, only 1.2% of any batch of treated pupae emerged as sterile males each day, and it was necessary to leave the pupae in the field as long as possible to obtain an adequate number of released males. As noted, the stability of the chemosterilant during such a prolonged period was not always adequate to assure complete sterility. Thus, though complete sterility was achieved in preliminary trials and during the first 90 days of
the test, the technique did not stand up to all field conditions. With enough laboratory-bred pupae of known age, it would be possible to program the dispersal to all flies emerge within a day or so, and chemical degradation in the field would be minimized because of this short emergence period. Then, lower doses of sterilant could be used and the competitiveness of the release flies would be increased. However, in large-scale programs, it may be impossible to prevent environmental conditions from immediately reducing the effectiveness of the sterilant on the pupal case. Thus, we must devise a scheme that will produce almost complete sterility of males and complete sterility of females even if all the sterilant is removed before the flies emerge. A combination of a low dose of irradiation and prerelease chemosterilization is therefore being sought which will provide reliable sterility without excessive mortality or loss of competitiveness.

EXPECTATIONS

The results obtained in the studies to date reveal that further research is required to improve the technique of sterilization if pupae are to be treated and to improve the viability of flies if adult treatments are anticipated. However, promising techniques have progressed sufficiently for an attempt to establish a large rearing facility for G. morsitans. This plant, which would provide adequate numbers of pupae of known age could assist us to perfect the techniques of sterilization and ultimately to conduct a demonstration trial to test both the efficacy and the economics of the sterile male technique. The planning for the 2nd phase of this study therefore includes both the establishment of a rearing plant and a large-scale field operation.

Also, a similar project has recently been initiated in the Central African Republic under the auspices of the European Common Market Overseas Development Program. The target species is G. fuscipes, which inhabits the dense thicket bordering rivers and stream beds. In the research area, the fly is restricted to the stream bed. Therefore, it would be possible to pinpoint the releases of sterile males, the efficiency of the program may be very high. Studies of the field populations, rearing, and sterilization started in 1969.

Further work on attractants is being conducted by various groups that wish to sterilize natural populations of Glossina directly and also that wish to improve existing detection methods. Also, control of natural populations by genetic manipulation is being investigated in the laboratory where suitable transdominant homozygote strains of G. austeni are being sought and developed (Curtis 1969).

The existence of 22 Glossina species, many of which coexist over large areas, indicates the nature of the problem of controlling the vectors of trypanosomiasis. The sterile-male technique will probably not be the ultimate panacea, but if the sterile-male technique proves efficient and economical, it will add a very potent weapon to the control armory.
reference to combining this method with conventional methods. USDA ARS 33-98, 54 p.


