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EFFECT OF CHEMOSTERILANT TEPA ON *GLOSSINA MORSITANS* WESTWOOD.

BY

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(Received)

In recent years the sterile male technique for the control of a broad spectrum of economically important insects has rapidly become promising. With this technique practical control can probably be achieved with many species which have previously presented severe physical or economic limitations. Tsetse flies, *Glossina* spp., have been the object of entomological, medical and veterinary research for several decades and have proved to be exceedingly difficult vectors of trypanosomiasis to control. Measures that have proved most effective against the more important of the 23 *Glossina* spp. which infest approximately 4 million square miles of Africa include insecticide application, bush clearing, and game control (Hocking & others, 1963). However, such measures, though effective, are expensive and usually must either be repeated at regular intervals or followed by extensive human settlement of the areas to prevent reinvasion. The campaign against the screw-worm fly [*Cochliomyia hominivorax* (Coquerel)] (Knipling, 1960) in Florida demonstrated that large land areas could be economically cleared of a pest insect by repeated release of sterile males.

The technique appeared adaptable to *Glossina* if male sterilization could be accomplished without severe side effects and if efficient methods of mass-rearing tsetse flies or safe treatment of natural populations could be devised. Potts (1958) and Chadwick (1964) showed that the reproductive capacity of *G. morsitans* can be reduced by radio-sterilization and chemo-sterilization. Simpson (1958) and Knipling (1963) outlined

the theoretical potential and the limitations of tsetse fly control or eradication with sterile males. A program to examine the feasibility of both sterilization and mass rearing of G. morsitans to be conducted under the joint auspices of the United States Department of Agriculture and the Agricultural Research Council of Central Africa was outlined by Smith & Dame (1963). A preliminary report (Dame & others, 1964) described the initial progress of this research. The present report outlines the effects of the chemosterilant tepa on G. morsitans.

Methods.

Standard conditions.

The G. morsitans orientalis pupae used in our experiments were hand-collected in the Zambezi River valley near Kariba, Southern Rhodesia. After the parasitized pupae were removed by flotation in methanol (Phelps, 1964) the remaining pupae were maintained in the laboratory at a temperature of 79 $\pm$ 2°F. and a relative humidity of 70 $\pm$ 2 per cent. Newly emerged adult males and females were separated daily to insure the virginity of both sexes. Test cages containing 25 pairs of flies were set up when the males were 6 - 7 days old and the females 2 - 3 days old, the ages at which sexual maturity was achieved. The 8 x 8 x 11 in. plywood cages (fig. 1) were covered on two sides with plastic or cloth screen and on the third side with a wide-mesh cotton mosquito netting. A stockinette sleeve was mounted on the front of the cage. A wire mesh enclosure that extended into the interior was placed in the floor of the cage. Thus when the cage was placed over a guinea pig for one hour daily, the flies could obtain a blood meal through the wire screen. Between feedings the cages were kept on a 9 x 12 in. flat pan containing dry sand, with the wide-mesh mosquito netting adjacent to the sand. Larvae deposited within the cage crawled through the netting into the sand where they soon pupated. The standard test duration was 28 days, adequate time for the caged females to produce one or more larvae each. At the end of the test a record was made of male and female survival, and the rate of insemination was often determined by dissection and microscopic examination of the spermathecae. The pupae were sifted from the trays and stored in glass jars covered with netting; and the emergence of adults was determined and recorded after six weeks.

Treatment by injection.

The chemosterilant was injected into adult males to determine the sterility and toxicity caused by known dosages. Solutions of tepa (0.01 - 1.0 µg. sterilant in 1 µl. ∓ 8 per cent. of distilled water) were injected through the cervical region into the pronotum with a calibrated hand-operated micrometer syringe equipped with number 27 gauge hypodermic needles. Before injection the males were individually immobilized by a 5 - to 10 - sec. exposure to carbon dioxide which allowed sufficient time to inject the sterilant and put the male into a holding cage. Approximately 25 sec. elapsed between the time the male was exposed and its recovery to flight activity inside the cage. In each replicate test 30 - 50 recently emerged males (0 - 24-hrs. old) were injected. When they were six days old, 25 of the survivors were caged with 25 virgin females for evaluation of fertility.

Treatment of pupae.

The effect of tepa on pupae was determined by dipping pupae into tap water or into 50 per cent. aqueous methanol solutions containing 0.5, 1.0, or 5.0 per cent. of tepa. Groups of 500 - 1500 pupae of mixed ages were placed in a small wire basket and immersed for one minute in the solution. Then the excess liquid was blotted from the outside of the wire basket with absorbent paper, and the wet pupae were placed en masse in a clean petri plate in a holding cage. In one test the pupae were rinsed in 50 per cent. aqueous methanol solution for one minute the day after they were dipped in the 5 per cent. tepa solution. Adults emerging during the first, second, and third weeks after treatment with 5 per cent. tepa were evaluated for sterility, but in the other series only the emergence of the first post-treatment week were evaluated. Because of the need for pupae, only the 5 per cent. treatment was replicated.

Treatment by contact.

The contact effect of tepa was determined by exposing adult flies of both sexes to residual deposits on glass. Even distribution of the sterilant on the surface was achieved by pipetting 5 ml. of the tepa-methanol solution into a quart jar and then rotating the jar until it appeared dry. During the rotation period a small amount of the solution was dropped from the jar into a petri dish, which was also rotated to dryness. The treated petri dish served as a cover for the jar during the treatment period. The glassware was air-dried overnight and used the next day. Males, which have a strong positive phototropism, were rapidly transferred into the treated jar by inserting the jar in a mount built in the plywood end of the holding cage. When the cage was covered with a black cloth, the majority of the 2-day-old males immediately migrated into the underkened treatment jar. The females, which have little phototropism at that age, and the occasional unmoved male, were captured manually in vials in groups of 4 or 5. When sufficient females were accumulated, they were rapidly released into the treated jar through a hole in a temporary cardboard cover. The jar was then covered with the treated petri dish and placed in a quiet corner for the prescribed time. Groups of 40 - 60 flies of one sex were treated at one time. At the end of the exposure period a rapid transfer from the treatment jar to a holding cage was accomplished by using the phototrophic response in reverse, in the case of the males or by agitating the jar, in the case of the females. In one series of tests 0-, 1-, or 2-day old flies were exposed to tepa deposits of 10 or 50 mg./ft.^{2} for 15 - 240 min. The controls were exposed for 240 min. in jars treated with 100 per cent. methanol. In another series, emerging flies were treated by placing a container of pupae inside a treated jar and allowing the emergees to make their way ad libitum out of the jar through a funnel exit into a holding cage.

Evaluation of effect of treatment.

Two-day-old males exposed 240 min. or less to 10 mg./ft.^{2} deposits of tepa on glass were tested for permanency of sterility, effects on longevity, and sexual vigor or competitiveness.

Permanence of sterility was evaluated by using the standard testing procedures described previously, except that after 7 days with one group of 25 females the surviving males were transferred to a new group of 25 females for a second, third, fourth, and fifth series of matings. Each group of females was then maintained separately for the standard evaluation of reproductive capacity. A similar series was conducted with untreated males.

Effect on longevity was determined by placing 42 - 51 six-day-old treated males in test cages without females. The cages were observed weekly, and the dead were removed. Similar observations on survival were made during the permanency test described previously to obtain information on the longevity of mated males over a 5-week period.

The competitiveness of treated males was evaluated by allowing treated and untreated males to compete for untreated virgin females. Six-day-old males were placed in a test cage and allowed to orient themselves for an hour. Females were then introduced into the cage, and standard test procedures were used to determine the reproductive capacity of the females. The ratios of treated males - untreated males - untreated females in these tests were 4:1:5, 1:1:5, and 1:1:2. Twenty-five females were used in all except the 1:1:2 ratio test, in which only 24 females were used. The total number of males ranged from 10 to 25, depending on the competitive ratio. One series of competitive tests was conducted with the males that emerged the first week after being dipped as pupae in 5 per cent. tepa in 50 per cent. aqueous methanol solution.

Results and discussion.

Reproductive behaviour under standard test conditions.

The reproductive capacity of untreated G. morsitans under standard test conditions is shown in Table I, and is a useful guide in evaluating the effects of the various treatments. Insemination rates ranged from 94 to 100 per cent., and an average of 28 pupae was produced per cage, with 26 adults (93%) emerging successfully. Mortality of either sex was negligible during the holding period before the test cages were set up, and survival over the 28-day test period was 72 and 77 per cent. for males and females, respectively. The test period was sufficiently long for some females to deposit two larvae; others deposited one or none. The first larval deposition began when the females were 15-day-old, though most females did not larviposit this early.

Treatment by injection.

Injection of males with either tepa solutions or distilled water caused moderate mortality during the 6-day holding period and abnormally high male mortality over the 28-day test period (Table II). Despite this effect the insemination rates were invariably normal, i.e., 94 per cent. or higher, an indication that the toxicity of the technique did not interfere with our evaluation of the resulting male sterility. Although pupal production was subnormal in the control groups, apparently because of low female survival, normal pupal production occurred when the smaller doses of tepa were injected. High sterility resulted at doses of 0.3 - 0.5 µg., and complete sterility occurred at a dose of 1 µg. The response of C. morsitans to tepa was very similar to that of the house fly, Musca domestica (L.), which had 50 per cent. sterility after a dose of approximately 0.1 µg. of tepa given in a similar manner (Chang & Borkovec, 1964); in G. morsitans 50 per cent. sterility occurred at a dose between 0.1 and 0.2 µg. Based on the relative sterilizing effectiveness of tepa apholate, and metepa (12.5:4:1) as determined by Chang and Borkovec with the house fly (1964), we expect that doses of apholate or metepa exceeding 0.5 - 1.0 µg. would be necessary to sterilize G. morsitans males. However, Chadwick (1964) found that apholate and metepa were detrimental to G. morsitans survival at doses of 0.5 µg. or less (applied topically in methyl ethyl ketone) though they did not cause complete male sterility.

Treatment of pupae.

The results of pupal treatments are given in Table III. The 5 per cent. tepa dip produced complete sterility in males and females emerging during the first 2 post-treatment weeks and partial sterility in males emerging during the third week. However, neither sex was completely sterilized at concentrations of 1 and 0.5 per cent. tepa, and the insemination rate in females was normal in all these tests. In the unreplicated test in which pupae were washed one day after treatment with 5 per cent. tepa, the males and females eclosing during the first post-treatment week showed normal fertility. This result, coupled with the lack of full sterility in males emerging during the third week after treatment with 5 per cent. tepa, suggests that sterilization occurs primarily as a result of the direct contact of the emerging adult with the sterilant deposit on the exterior of the puparium rather than as a result of sterilization of the pupa per se. Eclosion from treated pupae was normal, and the longevity of males emerging during the first two post-treatment weeks did not appear to be seriously affected though male survival was below normal for those emerging during the third week.

Treatment by contact.

The results of exposure to residual deposits of tepa on glass are given in Table IV. Complete sterility resulted from all treatments (except for one escapee in the 30-min. exposure of 2-day-old males) to 10 mg./ft.^{2} though insemination rates were normal. The long-term survival of flies exposed to 10 mg./ft.^{2} for less than 120 min. was normal, but longer exposure or exposure to 50 mg./ft.^{2} usually resulted in abnormally short survival. An unreplicated 24-hr. exposure of 2-day-old males to 10 mg./ft.^{2} (not included in Table IV) caused complete mortality within 48 hours after treatment.

Thus, though it is possible to overexpose the flies to the sterilant and to produce undesirable side effects, a fairly wide range of exposure times (15 - 60 min.) is available for contact sterilization that will not reduce the lifespan of the male.

Permanency of sterility.

Male sterility caused by a 240-min. exposure to 10 mg./ft.^{2} of tepa was permanent (Table V), and none of the treated males recovered fertility during the 5-week test period. The successful emergence of one adult from the first mating series indicated that one male had not been completely sterilized, but escapeeism did not reoccur. At the end of the test period 54 per cent. of the untreated 6-week-old males and 42 per cent. of the treated 6-week-old males survived. Exposures of 30 and 60 min. produced permanent sterility (with the exception of an escapee in the 30 min. series) without reducing longevity, but the 15 min. exposure allowed some recovery of fertility. The insemination rate was 94 per cent. or higher in the first three mating groups of all series, but ranged from 97 - 34 per cent. in the fourth and fifth matings. In spite of an insemination rate of 73 per cent., a distinct decrease in fertility was observed in females mated to untreated males in the fifth matings. The subnormal amounts of sperm detected in the spermathecae of females from these last matings with untreated males, indicated that the untreated male gradually loses its capacity to fertilize the female. A similar decrease in amount of sperm was also seen in the spermathecae of females mated with treated males.

Longevity of treated males.

The 240-min. exposure to 10 mg./ft.^{2} of tepa reduced the longevity of virgin males by approximately 25 per cent. and of mated males by 33 per cent. (Table VI). Treated males reached or exceeded 50 per cent. mortality in 4 weeks when mated and in 6 weeks when virgin compared with 6 and 8 weeks for untreated mated and untreated virgin males, respectively. For practical control programs this reduction in longevity would be quite significant but could be overcome by releasing sterile males more frequently than would be necessary if the treated males had normal life expectancy. Fortunately, however, shorter exposure periods seem to reduce this detrimental effect on longevity while still producing permanent sterility (see Table IV and V).

Competitiveness of treated males.

The results of competitive tests with males sterilized by contact as adults or by pupal dipping are given in Table VII. At ratios of 4:1:5 (treated males - untreated males - untreated females) an 80 per cent. reduction of female reproductive capacity is theoretically expected; at ratios of 1:1:5 and 1:1:2, a 50 per cent. reduction is expected. In actual fact, after sterilization each ratio tested produced a reduction equal to or greater than that anticipated. Since pairs normally remain in copula for periods of one hour or longer, small cage tests with equal numbers of males and females allow the less aggressive males an extra hour to find their mates before the more aggressive males begin to seek their second mate. Thus a sexually unaggressive male is not severely handicapped. Where there is a surplus of females, however, true competition does exist because an aggressive male may have an opportunity to mate with several females while a male less disposed to perform its reproductive responsibilities is not likely to mate with as many females. The results of the 1:1:5 ratio test showed the sterile males to be highly effective with a surplus of females.

While tests of this nature are indicative of the potential ability of treated males to compete with males in natural populations, only large cage and free-flight field trials can confirm the results. Also the effect of multiple mating by the female on the effectiveness of sperm obtained from a first mating is not yet known, though Potts (1958) found that multiple matings do occur. However, opportunities for multiple mating were available in our competitive tests but this did not prevent the expected reduction in fertility recorded in Table VII.

In all instances the resulting sperm were motile and behaved normally in the spermathecae of the female. On some occasions inviable pupae were obtained from females mated to sterile males, an indication that the dominant lethality induced by the sterilant occasionally expressed itself late in the life of the developing progeny. More frequently, however, the zygote formed by the union of the treated sperm and the normal ovum failed to develop beyond the embryonic stages, and most females examined showed no evidence of larval development. In the contact sterilization tests less than 0.3 per cent. of the zygotes formed with sperm from treated males actually eclosed as adults. It is unlikely that this degree of fertility in treated males would seriously jeopardize a release program with Glossina spp., which have such a low natural reproductive capacity. It could, of course, seriously affect a program with an insect of high reproductive capacity, e.g. the house fly, M. domestica. The sterility achieved in our tests exceeds that thus far demonstrated with radiosterilization (Potts, (1958) and Dame & others).

SUMMARY

Glossina morsitans pupae were collected in the Zambezi River valley near Kariba, Southern Rhodesia, and sterilization techniques were applied to these pupae or to the resulting adults. Under standard test conditions 25 pairs of untreated flies held in a common cage produced an average of 28 pupae in a 28-day test period; insemination rates were 94 per cent. or higher, and 72 and 77 per cent. of the untreated males and females, respectively, survived the 28-day test period. Injection of 1 µg. of tepa into the pronotum of young males produced complete sterility. Dipping the pupae in 5 per cent. tepa solution for 1 minute caused complete sterility of adults emerging during the first two post-treatment weeks and partial sterility of males emerging during the third week. Adults emerging from dipped pupae washed 1 day after treatment were fertile. Exposure of pupae to solutions containing 1 and 0.5 per cent. of tepa failed to sterilize completely. Contact exposure of adult males and females of various known ages usually resulted in complete sterility (99.7%) after exposures ranging from 15 to 240 min. to deposits of 10 and 50 mg./ft.^{2} of tepa on glass. Males exposed for 240 min. to 10 mg./ft.^{2} of tepa retained their sterility throughout a 42-day test period and were competitive with untreated males, but their longevity was reduced 25 per cent. when unmated and 33 per cent. when mated. Males exposed for 60 min. or less survived as well over a 42-day test period as untreated males, but those exposed for 15 min. recovered an undesirable degree of fertility. No treatment affected the ability of the male to inseminate the females. Sperm from treated males were motile and appeared normal in their behaviour in the female spermathecae. Dominant lethality was usually expressed during the embryonic stages, but occasionally was delayed until the pupal stage.

Footnotes

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Fig. 1. -- Test cage showing enclosure for guinea pig and pupal collection tray.

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TABLE I.

Reproductive capacity of 10 groups of 25 pairs of Glossina morsitans under standard test conditions

	Percentage survival after 28 days		Number of progeny	
	Male	Female	Pupae	Adults
	72	64	23	23
	80	76	25	23
	40	68	15	14
	96	76	20	18
	72	84	30	26
	72	96	33	31
	72	76	36	35
			29	26
			37	33
			34	33
Mean	72	77	28	26

TABLE II.

Reproductive capacity of groups of 25 Glossina morsitans females mated with males injected with 1 µl. of tepa solution (2 replicates)

Dose (µg.)	Percentage of male survival		Mean number of progeny	
	6 days	28 days	Pupae	Adults
1.0	86	26	0	0
0.5	84	36	0.5	0.5
0.4	88	46	1.5	1.5
0.3	76	42	1.5	1.5
0.2	87	44	5.5	4.0
0.1	90	36	24.5	23.0
0.05	99	26	26.5	24.0
0.01	69	26	23.5	22.0
0.00	91*	29+	16.0+	13.8+

* 3 replicates.

\+ 4 replicates.

TABLE III.

Reproductive capacity of groups of 25 pairs of Glossina morsitans after one sex was dipped as pupae in tsetse solutions (2 replicates)

Concentration (%)	Emergence period (weeks after treatment)	Treated sex	Percentage survival after 4 weeks		Mean number of progeny	
			Male	Female	Pupae	Adults
5	1st	Male	78	78	0	0
	2nd	Male	72	76	0.5	0
	3rd*	Male	42	70	5.5	4.0
	1st	Female	74	80	0	0
	2nd	Female	76	78	0	0
5 ‡	1st	Male	56	76	28.0	26.0
	1st	Female	60	80	28.0	26.0
1 †	1st	Male	40	80	8.0	8.0
	1st	Female	68	64	3.0	3.0
0.5 †	1st	Male	52	80	22.0	21.0
	1st	Female	72	68	9.0	7.0
0.0 †	1st	Male	60	76	29.0	27.0
	1st	Female	80	48	13.0	11.0

* 19 Males in second replicate.

† 1 replicate only.

‡ Pupae washed one day after treatment.

Table III.

Reproductive capacity of groups of 25 pairs of Glossina morsitans after exposure of one sex to residual deposits of tabs on glass (2 replicates)

Dose (mg./ft.^{2})	Age (hrs.)	Exposure time (Min.)	Treated sex	Percentage of treated sex surviving: Initially	Percentage of treated sex surviving: 4 weeks	Mean number of progeny: Pupae	Mean number of progeny: Adults
50	48-72	60	Male	16	0	0.5	0
		30	Male	70*	22	0	0
		15	Male	70*	30	0	0
	0-24	ad. lib.†	Male	81	26	0.5	0
			Female	77	52	0	0
10	48-72	240	Male	69	36	0.5	0
			Female	100	72	0	0
		120	Male	81	46	1.5	0
		60	Male	92	64	0	0
		30	Male	94*	76	0.5	0.5
		15	Male	96	72	0.5	0
	24-48	240	Male	96	44	0	0
			Female	100	64	0	0
		15	Male	99	42	0.5	0
			Female	100	80	0	0
	0-24	240	Male	67*	42	0	0
			Female	72*	34	0	0
		15	Male	95	60	0	0
			Female	98	66	0	0
		ad. lib.†	Male	96	66	0	0
			Female	94*	82	0	0
0	48-72	240	Male	90	80	24.0	23.0

* Data available from 1 replicate only.

† Emergers allowed to pass from treated surface to holding cage *ad libitum*.

Permanency of sterility of Glossina morsitans males exposed to residual deposits of 10 mg./ft.^{2} of tera on glass (groups of 25 females. 2 replicates)

Exposure time (min.)	Male age (days)	Mean number of males	Mean number of progeny	
			Pupae	Adults
240	6-14	25.0	0.5	0.5
	13-21	19.5	1.0	0
	20-28	13.0	0.5	0
	27-35	11.5	0	0
	34-42	10.5	0	0
60	6-14	25.0	0.5	0
	13-21	24.0	0	0
	20-28	20.0	0	0
	27-35	17.5	0.5	0
	34-42	15.0	0	0
30	6-14	25.0	0.5	0.5
	13-21	24.0	0	0
	20-28	22.0	0	0
	27-35	20.0	1.5	0.5
	34-42	17.0	0	0
15	6-14	25.0	0.5	0
	13-21	21.0	0.5	0.5
	20-28	19.0	0.0	0.0
	27-35	15.5	1.0	1.0
	34-42	11.0	1.0	1.0
0*	6-14	25.0	29.8	26.8
	13-21	22.8	25.8	23.2
	20-28	21.2	27.8	25.2
	27-35	17.2	23.[illegible]	20.0
	34-42	14.5	8.5	7.2

* 4 Replicates.

TABLE VI.

Survival of Glossina morsitans males exposed for 4 hours to residual deposits of 10 mg./ft.^{2} of tepa on glass (2 replicates)

Treatment	Mean percentage survival at indicated age (in weeks)								
	2	3	4	5	6	7	8	9	10
Virgin males									
Treated	94	86	68	57	48	38	24	20	16
Untreated	95	84	77	65	60	56	50	50*	39
Mated males									
Treated	78	52	46	42	34				
Untreated	86	80	68	54	50				

* 1 Replicate.