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Field cage evaluation of the competitiveness of male *Glossina morsitans orientalis* Vanderplank sterilised with tepa or gamma irradiation

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Introduction

The use of sterile males was suggested as a possible method of eradicating tsetse flies (*Glossina* spp.) by Simpson (1958) and Knipling (1963). The effect of chemosterilisation and irradiation on the reproduction and survival of *G. morsitans orientalis* Vanderplank in the laboratory was described by Dame & Ford (1966) and Dean & Wortham (1969): in small cages and with controlled conditions, sterile males competed successfully with untreated males for normal females. This paper presents results of further investigations of the competitiveness of sterile males in the laboratory and of tests made in field cages in the natural habitat.

Methods

The insect material used in the tests emerged from puparia of mixed age collected in the Kariba area of the Zambezi valley, Rhodesia, and flown to Salisbury where they were maintained at 25±2°C and about 70% r.h. Laboratory procedures for collecting, feeding and maintaining male and female flies separately to ensure virginity, and methods of sterilising the males (either with tepa or with gamma irradiation from a ^{60}Co source) were as described by Dame & Ford (1966) and Dean & Wortham (1969). In all tests in the laboratory and in the field, the numbers of treated and untreated flies of different ages were always proportional to insure that the results of competitive trials would not be biased by age differences.

Eight treatments were used: puparia were exposed to (1) 8 000 or (2) 15 000 rad of gamma irradiation delivered at a rate of about 110 rad/min (only male flies emerging during the first week after treatment were used since later emergents survived poorly); untreated one-day-old male flies were irradiated with (3) 8 000 or (4) 12 000 rad delivered at a rate of about 90 rad/min; adult male flies (5) 0 or (6) 2 days old were treated with tepa by exposure for 60 min to a residual deposit of 10 mg tepa/ft^2 of glass surface; or adult male flies (7) 0 or (8) 2 days old were exposed to a spray of 0·25 ml of 5% tepa in methanol atomised in a wind tunnel at speeds of 3–4 miles/h. Laboratory tests were made with samples of virgin flies from all batches prepared for field trials.

Laboratory small cage tests

Small cage tests to determine the sterility obtained by the eight treatments were made in the laboratory by pairing 25 males (at least 6 days old) with 25 2-day-old

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females in 8×8×11-in. (0·6 ft²) cages. Also, competitiveness of treated males was evaluated in the small cages by releasing treated and untreated males of similar ages into the mating cage at ratios of 4:1:5 or 3:1:3 (treated males:untreated males: untreated females). The males were introduced 30 min before the 25 females. For both tests, the resulting puparia were collected after 28 days and placed in 3×1-in. glass vials; eclosion of adult flies was checked after a further six weeks. Also, the number of females surviving the 28 days was recorded, and all were checked for insemination by dissection and microscopic examination of the spermathecae. Percentage sterility and competitiveness of the treated flies was based on the number of viable puparia produced in cages containing treated males compared with the number produced in control cages containing 25 pairs of untreated flies.

Field tests

Before the field tests, groups of 50–80 treated or untreated flies were placed separately in hinged 8×8×9·5-in. holding cages and fed for an hour on a guinea-pig. Then the cages were placed in pairs in polystyrene boxes containing ice packs (to reduce fly activity) and transported by automobile 230 miles from the Salisbury laboratory to the field station near Chirundu in the Zambezi valley for release in the field cages. Mortality by this method of transport was less than 2%.

The small 8×12×24-ft (288 ft²) cage constructed of galvanised mosquito mesh on 2×2-in. wooden framing and with a concrete floor was used for the majority of the field trials. Half the cage was covered with black cloth to provide shade, and strips of cloth were hung from the roof or from the wooden frames to provide additional resting sites. A calf was tethered at the lighter end of the cage. The second cage used in the field tests measured 10×90×90 ft (8 100 ft²) and was made of mosquito mesh on a metal frame. It enclosed a lower bush canopy of *Combretum*, *Boscia* and *Cleistochlamys* spp. and was overshadowed by taller *Colophospermum mopane* and *Kirkia acuminata*. No special provisions were made for the flies except that a single ox was provided as a host animal.

Treated and untreated males were released into the small cage by 07.30 h on the morning after arrival at the field station and 30 min before the females were released. The irradiated males were allowed two minutes to escape from the opened holding cage; those that did not leave were considered sick or damaged and were removed from the field cage and subsequently counted. No such selection for fly quality was attempted in the tests with chemosterilised males. Tests with flies from each treatment were replicated two to three times. In each test with irradiated males, between 141 and 304 treated males were released with untreated males and females to give ratios of treated males to untreated males to females ranging from 9:2:10 to 5:2:5; in each test with chemosterilised males, between 147 and 264 treated males were used with enough untreated males and females to provide a ratio of treated males to untreated males to females of 3:1:3. Similar numbers of untreated flies were released in separate (control) trials. After two days, the flies were recaptured manually in 3×1-in. vials. The males were discarded and the females were placed in the small holding cages and transported to the Salisbury laboratory the same day. The females were divided into groups of 25 and placed in standard mating cages. Survival, reproduction and insemination were checked after a further 26 days. Since the final evaluations of field tests with chemosterilised and irradiated flies were made in different laboratories in Salisbury, the females used as controls (caged with untreated males only) were also divided between the two laboratories and assessed separately.

The tests in the large cage were similar but not replicated. Treated and untreated males were released at the same time in the corner of the cage diagonally opposite the corner where the females were released to ensure that they would have to cover a maximum distance to find the females. The flies were recaptured the third and fourth

days after the start of the trial, the males were discarded, and the females were placed in holding cages, fed on an ox, and held in an insectary before their return to Salisbury the fifth day. Reproduction, insemination and survival of groups of 25 females were assessed in the Salisbury laboratories after a further 23 days.

The data from the cages in the laboratory assessment of each replicate were averaged; the mean of these averages was then used to determine the percentage reduction in reproductive capacity. The expected reduction was dependent on the mean ratio of sterile to untreated males and was adjusted, when necessary, for lack of complete sterility.

TABLE I. *Sterility and competitive tests with male* G. m. orientalis *in laboratory cages*

Treatment	Age when exposed (days)	Number in cross			Mean no. females surviving	% insemination	Mean no. progeny	
		T♂	N♂	N♀			Pupae	Adults
Gamma irradiation								
8 krad	Pupae	24	8	25	10	100	4·5	4·0
		25	—	25	12	96	0·0	0·0
	1	20	5	25	18	100	8·0	7·0
		25	—	25	20	96	2·5	2·5
12 krad	1	20	5	25	18	100	4·5	4·5
		25	—	25	19	98	1·3	0·7
15 krad	Pupae	24	8	25	16	100	2·5	2·0
		25	—	25	17	84	0·7	0·7
Control*	—	—	25	25	19	94	22·2	19·3
Tepa								
Contact	2	20	5	25	20	100	12·0	11·0
		25	—	25	18	96	0·3	0·0
Spray	0	20	5	25	19	98	8·0	7·0
		25	—	25	21	100	1·0	0·5
	2	20	5	25	18	99	6·3	6·3
		25	—	25	20	97	0·7	0·0
Control†	—	—	25	25	19	100	26·2	24·0

Means of 2–3 replicates except * 8 replicates, † 18 replicates.
T=treated, N=normal.

TABLE II. *Competitive tests with sterile male* G. m. orientalis *in field cages*

Treatment	Age when exposed (days)	Cage size (ft²)	Mean number in cross			Mean no. females surviving laboratory evaluation	% insemination	Mean no. progeny	
			T♂	N♂	N♀			Pupae	Adults
Gamma irradiation									
8 krad	Pupae	288	208	90	221	12	76	4·2	2·8
	1	288	194	56	224	20	89	6·4	5·1
12 krad	1	288	176	47	200	19	88	5·0	4·3
	1	8100*	404	159	550	19	85	10·8	9·6
15 krad	Pupae	288	141	48	137	13	89	3·5	3·2
Control	—	288	—	242	188	16	81	12·3	10·0
		8100*	—	603	451	16	98	22·3	21·0
Tepa									
Contact	2	288	228	76	228	16	83	5·0	4·7
		8100*	405	135	405	18	89	13·4	11·4
Spray	0	288	195	65	195	20	75	7·8	7·5
	2	288	216	72	216	20	81	8·0	7·5
Control	—	288	—	242	204	18	84	25·9	23·6
		8100*	—	603	451	21	83	28·7	26·3

Means of 2–4 replicates except * 1 replicate.
T=treated, N=normal.

TABLE III. *Percentage reduction in progeny in competitive tests with sterile male* G. m. orientalis

Treatment	Age when exposed (days)	Mating location	Cage area (ft²)	Mean % reduction in viable progeny: Expected	Actual
Gamma irradiation					
8 krads	Pupae	Laboratory	0·6	75	79
		Field	288	70	72
	1	Laboratory	0·6	70	64
		Field	288	68	49
12 krads	1	Laboratory	0·6	77	77
		Field	288	76	57
		Field	8100*	69	54
15 krads	Pupae	Laboratory	0·6	72	90
		Field	288	72	68
Tepa					
Contact	2	Laboratory	0·6	80	54
		Field	288	75	80
		Field	8100*	75	57
Spray	0	Laboratory	0·6	78	71
		Field	288	73	68
	2	Laboratory	0·6	80	74
		Field	288	75	68

Means of 2–3 replicates except * 1 replicate.

Results

Irradiated insects

Males irradiated in the pupal stage and tested in the laboratory had a mean sterility of 100% and 96% when exposed to 8 000 and 15 000 rad, respectively (Table I). In the competitive tests in the laboratory, the mean reduction in viable progeny was equal to that expected when the dose was 8 000 rad and somewhat better than expected when the dose was 15 000 rad (Tables I & III). In the smaller field cage, a mean reduction of 72% was achieved in the number of viable progeny when males were exposed to 8 000 rad compared with the expected 70% reduction (Tables II & III); when males were exposed to 15 000 rad, the mean reduction was 68% and the expected result 72%.

Males irradiated with 8 000 rad in the adult stage and mated with normal females in the laboratory had 87% fewer adult progeny; those treated with 12 000 rad had a 96% reduction. When males irradiated with 8 000 and 12 000 rad competed with untreated males in the laboratory the reductions in the number of progeny were 64 and 77%, respectively, compared with expected reductions of 70 and 77%. In the small field cage with males irradiated at 8 000 and 12 000 rad, the reduction was only 49% (expected 68%) and 57% (expected 76%), respectively. In the unreplicated trial in the large cage with males treated as adults with 12 000 rad, the reduction was 54% compared with an expected reduction of 69% (Tables II & III). The rates of insemination by treated males were generally high in all tests made in the laboratory, but they were somewhat lower in the field cages. Survival of female flies was satisfactory and similar for females mating in the laboratory and in the field cages.

Chemosterilised insects

In the laboratory tests, 2-day-old males exposed for 60 min to residual deposits of 10 mg tepa/ft² were 100% sterile (Table I) but the reduction in number of progeny (54%) in the competitive tests was lower than expected (80%) (Tables I & III). However, in the small field cage, these males produced an 80% reduction compared with the expected 75% (Tables II & III). In the large field cage, the reduction was 57% compared with the expected 75%. In the laboratory tests, 0-day-old males exposed to tepa spray were 98% sterile, and the reduction in adult progeny was 71%

(78% expected) (Tables I & III). In the small field cage, the mean reduction in viable progeny was 68% compared with the 73% expected. In the laboratory tests, 2-day-old males exposed to the spray were completely sterile, and the mean reduction in progeny was 74% (80% expected); in the small field cage, these males reduced adult progeny 68% compared with the expected 75%.

The rates of insemination were high in the laboratory but somewhat lower in the field cages. Survival of female flies was good for females mating in either the field cages or the laboratory.

Discussion

Previous laboratory tests had shown that longevity decreased when male flies were treated with gamma irradiation as pupae but was not affected by treatments given after eclosion (Dean & Wortham, 1969); Dame & Ford (1966) demonstrated that contact treatment with tepa did not seriously reduce longevity. During the present trials, male survival before introduction into the field cages was not particularly affected by the type of sterilisation; however, the field tests did not allow a reliable comparison to be made of the survival of treated and untreated males.

The percentages of male sterility obtained with either treatment with tepa and with irradiation of puparia and young adult males were similar to those obtained in previous laboratory tests (Dame & Ford, 1966; Dean & Wortham, 1969). Irradiation produced between 87 and 100% sterility (mean, 95%); tepa usually produced complete sterility (mean, 99%). Laboratory tests of competitiveness generally produced percentage reductions in reproduction close to or better than the expected values except for males treated by contact with tepa. However, in these tests, reproduction varied with female survival and rates of insemination, and though both were generally acceptable, mean survival did vary as much as 16% from that of the controls. We therefore considered that treated males were competitive with untreated males when the reduction in reproduction fell within 10% of that expected. Where the deficit between the experimental and the expected reduction exceeded 10% it is possible that the competitiveness of the sterile males was much less than indicated by the percentage reduction values. For example, a 3:1, 2:1, or 1:1 ratio of sterile to untreated males should produce a reduction in reproduction of 75, 67 and 50%, respectively. Thus, a reduction in progeny of 50% obtained when 67% is expected suggests that the effective ratio was 1:1 instead of 2:1. From this viewpoint the reduction in competitiveness would be 50%, rather than the 17% which represents the difference between the expected and the actual reduction in reproduction; in this hypothetical case two sterile males would be required to equal the mating prowess of one untreated male.

Then, with these frames of reference, only males irradiated as adults were deficient in the small field cage, and the deficiency was of a magnitude which suggests that two to three males irradiated as adults would be required to equal the mating potential of one untreated male. In comparison, in the large cage, males irradiated or chemosterilised in the adult stage were deficient performers. Thus, the competitiveness of the chemosterilised males compared with that of untreated males appeared to decrease with an increase in the volume of the cage. Unfortunately, the large cage tests could not be replicated, and this suggestion of reduced competitiveness with increased volume could not be confirmed. Therefore, though the competitiveness of males irradiated in the pupal stage or chemosterilised as adults was demonstrated in small field cages, their effectiveness in free flight in the natural habitat cannot be assumed.

Summary

Laboratory and field cage trials were made with male *Glossina morsitans orientalis* Vanderplank treated with tepa or gamma irradiation to assess the ability of the sterile

males to compete with untreated males for normal females when the ratios of treated males to untreated males to untreated females ranged from 4:1:5 to 5:2:5. Irradiation of the pupal and adult stages with 8 000 and 15 000 rad or 8 000 and 12 000 rad, respectively, reduced reproduction by 87–100% (mean, 95%). Contact for 60 min on a glass surface coated with 10 mg tepa/ft² or exposure to 0·25 ml of 5% tepa in a wind tunnel usually produced complete sterility (mean, 99%) in 0- or 2-day-old male flies. Trials in the laboratory and in a small field cage (288 ft²) with chemosterilised flies generally reduced reproduction to near the expected values. Similar results were obtained with male flies emerging from irradiated puparia, but males treated as adults produced somewhat smaller reductions than expected. Unreplicated competitive trials with chemosterilised and irradiated males in a large field cage (8 100 ft²) produced considerably smaller reductions in reproduction than expected, suggesting that treated males released in nature might not compete for normal females as readily as untreated males.

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