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EFFECT OF GAMMA-RADIATION ON THE TSETSE FLY *GLOSSINA MORSITANS* WESTW.

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Twenty-three species of *Glossina* infest about three million square miles of Africa south of the Sahara. They limit or endanger human occupation and agriculture within this area as they are the primary vector of trypanosomiasis infecting both man and domestic animals. The problem of eradicating tsetse flies has occupied numerous workers for several decades, and aerial and ground spraying of insecticides, bush clearing and shooting certain game species have been found to be effective to some extent (Hocking, Lamerton & Lewis, 1963). However, these methods are usually expensive in relation to the value of the cleared land, and they must either be repeated at regular intervals or need immediate, intensive, and well-planned African settlement to prevent re-infestation.

Knipling (1960) and others have described how sterile male screw-worm flies (*Callitroga hominivorax* (Coquerel)) were released to eradicate this insect from the southern states of North America. This technique seemed applicable to tsetse flies if sterilisation could be achieved without harmful side effects. Simpson (1958) and Knipling (1963) indicated the theoretical possibilities of using sterile male tsetse as a control technique. Both Potts (1958) and Chadwick (1964) concluded from preliminary trials that the reproductive potential of *G. morsitans* Westw. could be reduced by gamma-radiation and chemosterilants, respectively. Investigations into the possibilities of gamma-radiation were begun under a co-operative agreement between the International Atomic Energy Agency and the Agricultural Research Council of Central Africa. Preliminary results on sterilisation and associated mass rearing are given by Dame, Dean & Ford (1965).

Methods

The reproduction rate of tsetse flies is very slow compared with other insects. The female fly is larviparous and produces, about every two weeks, a single fully grown larva which soon pupates in the soil. In the present paper, the effects of gamma-radiation on the developmental stages within the puparium, which last for about 28 days at 25°C. (Glasgow, 1963), and on the adult fly are considered. This involved studying the permanency and degree of sterility obtained from different treatments and any harmful side effects, reduced life span and competitiveness compared to untreated flies, that might occur.

The puparial stages concerned in these experiments were those between the final phase of the third-instar larva and the fully developed adult fly ready to emerge and will be referred to hereafter as 'pupae,' using the term in a broad sense. They were collected by teams of Africans in the Kariba area of the Zambesi drainage system during a study of tsetse breeding sites (Phelps,

* The investigation reported in this paper was carried out under the auspices of the Agricultural Research Council of Central Africa.

1964). They were flown to Salisbury and maintained in the laboratory at 25 ± 2°C. and about 70 per cent. relative humidity after flotation in methylated spirits to remove dead and parasitised pupae (Potts, 1958; Phelps, unpublished). The number of pupae collected was relatively low during the wet season (November–April) and tests were attempted only during the remaining months. The pupae were stored in 8×8×11-in. wooden cages with transparent plastic walls and a mutton cloth sleeve at one end. The emerging male and female flies were separated every morning into 8×8×11-in. cages with fine wire mesh walls, cotton mesh top and mutton cloth sleeve, and with a wire mesh enclosure inserted into the cage from the floor (Dame & Ford, 1966), thus ensuring that they were virgin for all tests (Dame & Dean, unpublished). A guinea-pig was placed in the enclosure for an hour every day so that the flies could obtain a blood meal through the wire mesh.

Cages for standard reproduction tests contained 25 pairs of mature flies and were made up when the males were at least six, and the females at least two, days old (Dame & Dean, unpublished). Between the daily feeds, the cages were placed upside down over 9×12-in. pans containing sand. Larvae deposited within the cage crawled through the cotton mesh and pupated in the sand. These tests lasted for 28 days during which time individual females could potentially have deposited two pupae. Records were kept of the number of pupae sifted from the sand and the survival of male and female flies, while the insemination rate of the remaining females was determined by dissection and microscopic examination of the spermathecae. The pupae were stored in 3×1-in. glass vials capped with mosquito netting, and eclosion was checked after six weeks.

An Eldorado G Cobalt 60 Teletherapy Unit at Salisbury Central Hospital was used for the gamma-radiation and gave dosages of between 54 and 122 rads per min. during these trials. The pupae were held in fine cotton mesh bags and exposed for varying periods on a 15-sq. cm. surface at a distance of 55 cm. beneath the source. The adult flies were enclosed in a 4×12×12-cm. wooden-framed box covered with fine cotton mesh. The Cobalt room was maintained at about 26°C., a fan was used to assist aeration during the irradiation, and the material for treatment was conveyed in a Kaylite box to and from the laboratory.

The effect of radiation on the reproduction rate was evaluated by the use of standard mating procedures, and the comparison of the number of pupae produced by, and the percentage survival and insemination of, treated and untreated flies. Tests on the permanency of sterility involved the same technique except that surviving males were transferred after shorter intervals to new groups of two-day-old virgin females for up to nine series of matings. The successive groups of females were tested for insemination and pupal production after 28 days and compared with untreated controls.

The life span of virgin males and females in the laboratory was ascertained by placing 20 to 65 flies of one sex into a cage immediately after emergence or treatment and recording the dead flies at weekly intervals. The survival of mated flies was obtained at similar intervals from the tests on reproduction.

The competitiveness of irradiated male flies was tested by placing different ratios of treated and untreated males into cages to compete for equal or smaller numbers of two-day-old virgin untreated females introduced about an hour afterwards. Standard mating test procedures were used to evaluate reproduction from ratios of 1:1:2, 2:1:2, 3:1:3, 4:1:2 (treated males:untreated males: untreated females), and 1:1:1:1 where irradiated females were included. Twenty-five females were used in these tests except for the 1:1:1:1 and 1:1:2 ratios when there were 24.

Results and conclusions

Emergence of adult flies from pupae

Mortality was measured as the percentage of pupae held for 40 days and failing to produce flies. Allowance was made for the natural mortality exhibited by parallel batches of untreated pupae by applying Abbots correction (Finney, 1952) to the observed mortality in each batch of irradiated pupae. The per cent. effective mortality due to irradiation was calculated from $\frac{P_{obs} - C_{obs}}{100 - C_{obs}} \times 100$, where P = mortality from irradiated pupae and C = mortality from untreated pupae, and the results were checked from tables compiled by Ayers (pers. comm.).

The percentage effective mortality due to irradiation for pupae collected during the spring months (August–October), and the winter months (May–July) is given in fig. 1. Mortality increases with dosage in both series. Calculations

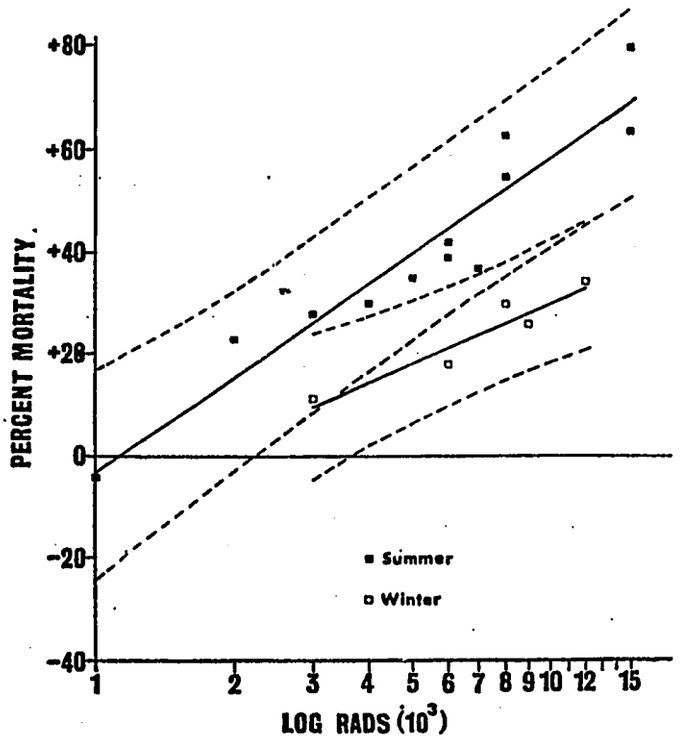

Fig. 1.—Per cent. effective mortality of pupae collected during the winter (May–July) and spring (August–October) related to radiation doses. The broken line indicates the 95% confidence limits.

from the effective mortalities gave a regression coefficient of 61·8 $\log_{10}$ (rads) − 189·1 and a correlation coefficient of P = +0·946 (which is significant at the 99·9 per cent. level) in spring; whereas in winter the regression coefficient was 38·5 $\log_{10}$ − 124·1 with a correlation coefficient of P = +0·951 (significant at the 99·0 per cent. level). The individual effective mortalities lay relatively close to their respective regression lines and those from one period, spring or winter, lay outside the estimated confidence limits of the other period.

Thus, mortality increased with dosage and there were seasonal differences in sensitivity to gamma-radiation. Dean, Wilson & Wortham (1968) have shown seasonal variations in natural mortality among "wild" pupae maintained under insectary conditions, and have shown that radiation effects increase with a decrease in pupal age. Potts (1958) concluded that pupal mortality did not increase with doses up to 12,000 rads if pupae were more than a third of their way through development, and blow-fly pupae were found to be more susceptible to gamma-radiation during the first third of their life (MacLeod, 1957). This evidence of the effects of radiation on pupae strongly supports the necessity for developing a breeding technique that will produce viable pupae of a known age.

Reproductive capacity of untreated flies

Reproductive capacity was measured in 25 pairs of untreated *G. morsitans* under standard laboratory conditions (Table I). Dissection showed that insemination rates of the surviving females varied from 80 to 100 per cent. with a mean of 95 per cent. and developing larvae were often found in the uterus. The number of pupae produced during the 28-day test period ranged from 14 to

TABLE I. *The number of progeny obtained from 12 groups of 25 pairs of untreated* G. morsitans *under standard laboratory conditions*

	Survival after 28 days (%)		Insemination of surviving females (%)	Number of	
	Male	Female		Pupae	Adults
	72	76	93	23	20
	80	76	86	24	20
	40	52	100	16	11
	60	68	100	23	23
	40	64	100	17	17
	68	64	100	22	13
	64	60	93	14	12
	20	56	93	19	17
	60	68	100	25	24
	52	56	95	26	23
	76	60	80	19	19
	44	44	100	24	22
Mean	56·3	62·0	95·0	21·0	18·4

26, and averaged 21, while 18·4 flies (88%) emerged successfully. This eclosion rate compares favourably with the 87 and 89 per cent. obtained by Potts (1933, 1958) with *G. morsitans* and the 83 per cent. for *G. palpalis* (R.-D.) (Geigy, 1948). Survival prior to the fly's sexual maturity and setting up these cages was good, and 56·3 and 62·0 per cent. of the males and females, respectively, survived the test period. The first larvae were deposited on the twelfth day after the start of the test, when the females were approximately 15 days old, and 50 per cent. of the pupae were deposited by the twentieth day. Phelps (unpublished) obtained a 21-day larval development period with *G. morsitans* kept at 25°C. and Jackson (1949) estimated that it lasted for 19·2 days at 24°C. Potts (1958) found a period of 31 ± 4 days at 25°C. and considered that a 60-day test period was necessary to evaluate reproduction. Female flies kept individually, and mated with males for 48 hours, in 3 × 1-in. glass vials and fed on sheep showed that 20·7 per cent. failed to larviposit and were naturally barren (Dean, unpublished) compared to the 22 per cent. reported by Potts (1958).

Reproductive capacity of male flies emerging from irradiated pupae

The pupae used in these tests contained different stages of spermatogenesis because of their variable age, and it was expected that irradiation of these pupae might produce different degrees of sterility. The reproduction rate of males given doses between 1,000 and 15,000 rads and emerging during the first, second and third weeks after irradiation was obtained from standard mating tests. Irradiation did not appear to affect greatly the rate of pupal development (Dean, Wilson & Wortham, 1968) and these periods may be directly related to the 28–30 days of pupal life expected at 25°C.

The reproduction obtained from males emerging during the first and second week after treatment with dosages up to 8,000 rads were analysed by the model $\log_e Ni_jR = A + b_i + w_j + kR$ where

Ni_jR = the number of pupae from the ith batch in the jth week at radiation level R
A = the number of pupae expected from the control group
b_i = the effect of the ith batch of pupae
w_j = the effect of the jth week after treatment
k = a biological constant
R = the radiation level.

The data from the third week after treatment, and dosages above 8,000 rads for males emerging during the first and second week, were omitted from the analysis because it was mathematically impossible to analyse the zero values (log 0 is $-\infty$) without using a transformation of the type $\log_e (N + Z)$ which was not done (Ayers, unpublished). Parameters due to time of emergence after treatment and differences between batches of pupae had no effect on R^2, the per cent. of variability explained by the model (Table II), and were non-significant compared to their standard errors. A seasonal effect, which was

TABLE II. *Effects due to the time of emergence after treatment* (w_j) *and differences between batches of pupae* (b_i) *on the percentage of variability* (R^2) *explained by the model (46 units)*

R^2	Model $\log_e Ni_jR =$
90·42	$A + kR$
91·04	$A + b_i + kR$
91·19	$A + b_i + w_j + kR$

shown with the analysis of pupal mortality due to irradiation, was not detected with an analysis of the type $\frac{T}{C} = e^{-kR}$ where T = number of pupae in the test group and C = number of pupae in the control group (Ayers, unpublished).

Thus, all data from the first and second weeks and for radiation levels up to 8,000 rads were combined and analysed by the model $N_R = e^{A}e^{kR}$. A Chi-square analysis between the observed and predicted reproduction gave a value of 3·411 which is non-significant at the 95 per cent. level. An extrapolation for higher dosages gave a value of 4·496, also non-significant at the 95 per cent. level, which indicated that the model might be valid for dosages above 8,000 rads.

The predicted reproduction for the first two weeks after treatment, and the observed values for the first three weeks are shown in fig. 2. The percentage sterility of males emerging during the first two weeks increased following treatments with 1,000 to 8,000 rads and thereafter remained relatively constant at between 95 and 100 per cent. The fertility of males emerging during the third week was reduced to zero between 4,000 and 9,000 rads.

The insemination rates in these matings between treated males and untreated female flies were normal, and larvae were rarely found in the uterus. Eclosion from the few pupae deposited varied between zero and 100 per cent. compared with the 80 per cent. (from 10 pupae) obtained by Potts (1958). The single pupa obtained in some tests with treated males was probably due to the presence of a few, normal sperm within a single male that are quickly expended, rather than to a male fly that has completely escaped the effects of gamma-radiation. These results indicate that the dominant lethality due to irradiation which prevents the production of offspring occurs in the early embryonic stages.

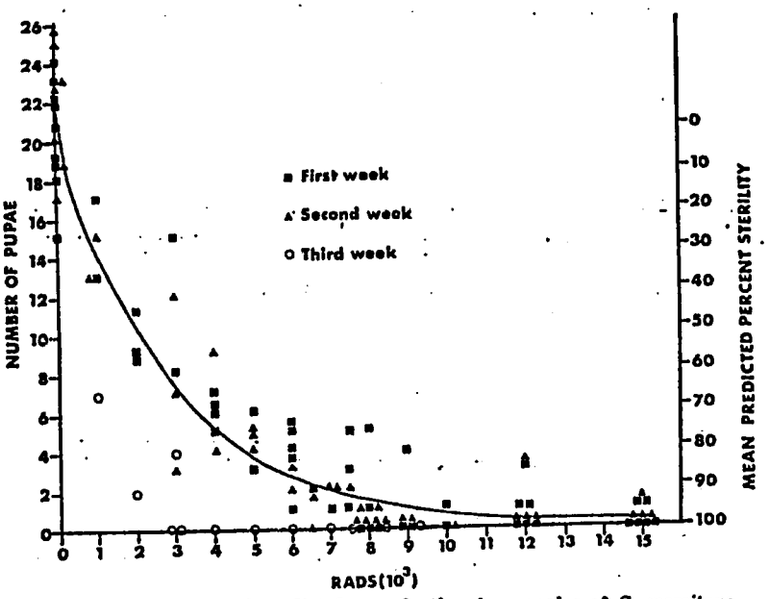

Fig. 2.—Observed and predicted reproduction from males of *G. morsitans* emerging from pupae during the first and second weeks after irradiation with 1,000 to 15,000 rads and mated with virgin untreated females. Observed reproduction from treated males emerging during the third week is indicated.

Potts (1958) estimated that 21 per cent. of the matings with males irradiated with 6,000 rep (5,760 rads) would be fertile, but he obtained a 51 per cent. reduction in the number of pupae produced. He found also that 22 per cent. of the treated males failed to inseminate a female, which is higher than the values obtained in the present trials, and considered that irradiation increased the length of the gestation period. Dissection of the surviving females at the end of the 28-day mating period very rarely ($< 0{\cdot}1\%$) yielded larvae. Thus, a longer gestation period was unlikely to have contributed to the level of sterility obtained in these tests.

The reduction in life span after treatment (Table VII) prompted a trial in which the radiation given to batches of mixed-age pupae was split into fractions one or four days apart. The reproduction rates obtained after these treatments were generally very slightly higher than those obtained after a continuous exposure to the same total dosage (Table III). The mean number of progeny produced in mating trials after these fractionated treatments was

TABLE III. *The mean number of progeny obtained from 25 pairs of* G. morsitans *after exposure of males to continuous or fractionated doses of gamma rays during the pupal stage*

Total dosage (rads)	Treatment	Mean survival after four weeks (%)		Mean number of progeny	
		Male	Female	Pupae	Adults
8000	(3)* Continuous	22	65	0·7	0·3
	(3) 4,000 × 2, one day apart	12	38	1·7	1·7
	(3) 4,000 × 2, four days apart	10	38	3·0	3·0
	(3) 2,000 × 4, one day apart	4	40	1·3	0·3
12000	(4) Continuous	13	60	0·5	0·5
	(5) 4,000 × 3, one day apart	22	62	0·8	0·6
15000	(5) Continuous	12	68	0·2	0·2
	(4) 5,000 × 3, one day apart	23	53	1·0	1·0
	(2) 8,000 + 7,000, one day apart	—	—	0·5	0·0
Control	(6) None	34	64	17·9	16·2

* Numbers in brackets refer to replication

much lower than that obtained with males given a continuous exposure to a dose equal to a single fraction, except for the 8,000 and 7,000 rad fractions (= 15,000 rads).

Jefferies (1962) found that the reproductive capacity of *Sitophilus granarius* (L.) recovered when eggs, larvae and pupae were given fractionated dosages, but that this did not occur with the adults; and divided doses of X-rays given to *Bracon hebetor* Say provided only temporary sterility (Cork, 1957). The 28-day period used in these tests on tsetse did not indicate a recovery in fertility, but this will be investigated further.

TABLE IV. *The number of progeny obtained from 25 pairs of* G. morsitans *where the female has emerged from irradiated pupae (two replicates)*

Treatment	Survival after 28 days (%)		Mean insemination (%)	Mean number of progeny	
	Male	Female		Pupae	Adults
1000	58	58	92	0	0
2000	38	68	92	0	0
3000	44	44	100	0	0
4000	26	52	88	0	0
5000	52	40	100	0	0
6000	36	36	87	0	0
8000	48	52	97	0	0

Reproduction rate of female flies emerging from irradiated pupae

The reproduction obtained from matings between normal males and irradiated females showed that complete sterility was obtained with all treatments (Table IV). The age of the pupae at the time of irradiation was not found to affect this sterility. Dissection revealed that the insemination rate was normal, and the failure of the ovaries to develop indicated that dominant lethals were expressed in the suppression of oogenesis. Potts (1958) concluded from his work that 16 per cent. of irradiated females could be expected to show some residual fertility, and he found that development of the ovaries ranged from 25 per cent. at 3,000 rep (2,880 rads) to seven per cent. at 12,000 rep (11,520 rads).

Reproduction rate of male and female flies irradiated after emergence

Male flies were irradiated either within 24 hours of emergence from normal pupae or after maturity (> 6 days), and females were treated during the first day while they were still immature. The number of pupae obtained from untreated females mated to irradiated male flies showed that age at the time of irradiation did not affect the level of sterility (Table V). Mean reproduction in terms of pupae was reduced by 19·5, 91·2, 96·3, 91·2, 95·1 and 97·6 per cent.

TABLE V. *The mean number of progeny obtained from 25 pairs of* G. morsitans *where one of the sexes was irradiated after emergence (two replicates)*

Treatment (rads)	Age at irradiation (days)	Survival after 28 days (%)		Number of	
		Male	Female	Pupae	Adults
Control*	—	39	56	20·5	20·0
		Treated male			
4000	1	57	60	16·5	15·0
6000	1	16	28	1·5	1·5
	8–13**	8	40	2·0	1·5
8000	1	35	43	1·0	1·0
	6–11	18	56	0·5	0·5
12000	1	18	46	3·0	1·5
	6–13	22	42	0·5	0·5
15000	1	28	48	1·5	0·5
	6–8	14	28	0·5	0·5
18000	1	24	43	0·5	0·0
		Treated female			
2000	1	24	42	0·0	0·0
3000	1**	31	28	0·0	0·0
4000	1	33	54	0·0	0·0

* Four replicates. ** One treatment only.

in the six treatments from 4,000 to 18,000 rads, respectively. The irradiated females were completely sterilised and oogenesis was apparently inhibited. The insemination rates obtained were normal in all of these tests. These results were comparable with those obtained from irradiating mixed-age pupae.

Permanence of sterility

Males irradiated with 8,000 and 12,000 rads and mated with two-day-old virgin females were permanently sterile throughout 39- to 45-day mating periods, though reproduction was not completely inhibited initially as a single pupa was produced in each of the first two matings with both treatments (Table VI). The absence of pupae in subsequent matings tended to confirm that incomplete sterility was due to a few, normal sperm in a single male fly that were quickly expendable. The insemination rate of females was 88·5 per cent. or higher in the first three mating groups. There was a distinct reduction in reproduction in the untreated series in the third and fourth matings in spite of insemination rates of 92 and 85·5 per cent., respectively. The amount of sperm visible in the spermathecae also was reduced in these later mating groups with untreated flies and this showed that the males gradually lose their capacity to fertilise a succession of females. A similar decrease in the amount of sperm transferred to the female was also observed in the tests with irradiated males. Dame & Ford (1966) reported a similar decrease in insemination rates and subnormal amounts of sperm in the spermathecae in the fourth and fifth mating groups during their tests on the permanence of sterility after treating *G. morsitans* with tepa.

TABLE VI. *Permanence of sterility in males of* G. morsitans *irradiated as pupae with 8,000 and 12,000 rads and mated to successive groups of 25 untreated female flies (two replicates)*

Treatment (rads)	Male age (days)	Mean number of males	Insemination (%)	Mean number of Pupae	Mean number of Adults
8000	6–10	25·0	97·5	1	0·5
	13–17	18·5	91·0	1	0·5
	20–24	13·5	95·5	0	0
	27–31	11·0	61·0	0	0
	35–38	4·5	61·0	0	0
	42–45	3·0	12·5	0	0
12000	6– 9	25·0	100·0	1	1
	13–16	16·5	100·0	0·5	0
	20–23	14·5	88·5	0	0
	29–32	12·0	64·5	0	0
	36–39	7·0	42·0	0	0
Control	6– 9	25·0	95·0	23·0	21·0
	13–16	19·5	97·0	23·5	22·0
	20–23	14·5	92·0	19·5	17·5
	28–30	14·5	85·5	17·5	15·5
	38–40	7·5	62·5	10·5	10·0
	45–47	6·0	57·0	5·0	4·5
	50–57	4·0	24·5	3·5	3·0

TABLE VII. *Survival of adult males and females of* G. morsitans *exposed as pupae to continuous gamma-radiation (two replicates)*

Treatment (rads)	Mean survival (%) at indicated age (weeks)									
	1	2	3	4	5	6	7	8	9	10
Virgin males										
4000	75	52	27	23	18	16	10	8	5	1
8000	86	52	35	20	14	7	3	1	0	0
12000	72	48	28	16	11	4	2	0	0	0
15000	76	42	18	7	6	3	0	0	0	0
Untreated	81	67	54	43	30	25	22	14	6	3
Mated males										
1000	—	76	72	52	48	—	—	—	—	—
2000	—	80	72	48	48	—	—	—	—	—
4000	—	72	64	44	32	—	—	—	—	—
8000	—	44	40	36	24	—	—	—	—	—
12000	—	60	56	48	44	—	—	—	—	—
15000	—	80	76	50	36	—	—	—	—	—
Untreated	—	72	60	52	44	—	—	—	—	—
Virgin females										
4000	66	52	40	34	19	17	8	—	—	—
8000	83	50	36	33	30	15	10	8	—	5
12000	63	45	42	40	38	34	31	26	15	—
15000	74	64	48	42	37	32	25	20	15	—
Untreated	81	65	52	46	40	35	33	29	27	20

Length of life of irradiated flies

Irradiation during pupal life with 4,000, 8,000, 12,000 and 15,000 rads reduced the life span of virgin males (Table VII). Survival rates were similar, though slightly lower than for the controls, for the first two weeks with all dosages. The effects due to the different treatments separated out from the fourth week onwards, and 100 per cent. mortality occurred in the eleventh, ninth, eighth and seventh weeks after emergence for the four dosages, respectively, as compared with the thirteenth week with the untreated flies. Potts (1958)

concluded from his tests that irradiated males lived only half as long as untreated males.

The effect on the length of male life when pupae of different ages were given 8,000 rads was evaluated by holding flies emerging during the first to third weeks after irradiation (fig. 8). Survival rates were similar in all groups for the first three weeks of adult life and thereafter decreased with a decrease in pupal age at the time of irradiation.

The survival of virgin female flies given a continuous exposure to radiation during pupal life was also reduced (Table VII), but less than with the males given similar dosages. The 4,000- and 8,000-rad treatments apparently reduced

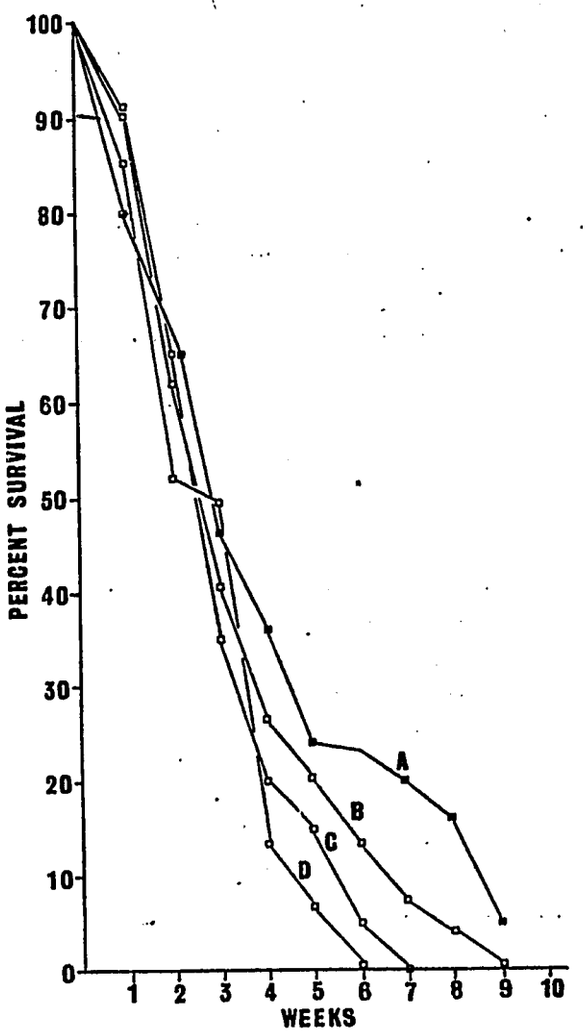

Fig. 8.—Survival of untreated male *G. morsitans* (A) and male flies exposed as pupae to 8,000 rads and emerging during the first (B), second (C), and third (D), weeks after irradiation.

length of life more than the two higher dosages (12,000 and 15,000 rads). Female flies emerging during the first, second and third weeks after irradiation also showed a reduction in survival with a decrease in the age of the female pupa at the time of treatment (*cf.* fig. 3).

The male flies irradiated during the pupal stage and mated with untreated female flies for four weeks tended to live longer than the virgin flies over a five-week period (Table VII). Irradiation with 1,000 and 2,000 rads apparently reduced mortality compared to the controls, but survival decreased between 4,000 and 8,000 rads. There was an apparent increase in survival when the males were given 12,000 and 15,000 rads. It is not known yet why flies irradiated and mated tended to live longer than virgin flies, while the reason for the apparent increases in length of life with 1,000 and 2,000, 12,000 and 15,000 rads is also unknown.

Jefferies (1962) and others reported that chromosomal damage was not reduced by fractionation of the irradiation dosage, but when somatic injury occurred the effect was reduced or prevented by dividing the total dose into fractions separated by intervals for recovery. In the present test, pupae were

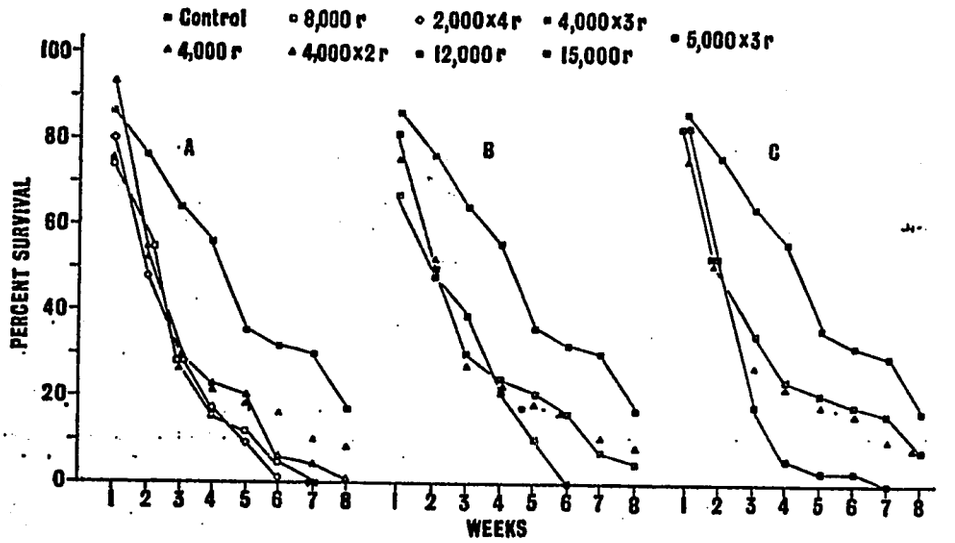

Fig. 4.—Survival of virgin males of *G. morsitans* emerging from pupae given continuous and fractionated dosages totalling 8,000 (A), 12,000 (B), and 15,000 (C) rads.

given fractions of 2,000 × 4, 4,000 × 2, 4,000 × 3 and 5,000 × 3 rads, each fraction given one day apart, and survival of the adults was compared with controls and adult flies from pupae receiving similar but continuous, total dosages (fig. 4). Survival rates from all treatments with 8,000 rads did not appear to differ substantially and were lower than the controls. Fractions of 4,000 × 3 rads tended to increase the life span after the fourth week, while 5,000 × 3 rads definitely increased length of life after the second week compared to continuous exposures of 12,000 and 15,000 rads, respectively.

The survival obtained with a continuous exposure to 4,000 rads is indicated in fig. 4 and tends to lie close to the curves associated with 4,000 × 2, 4,000 × 3

and 5,000 × 3 rads. This suggests that somatic damage was reduced to about the level of the first fraction even though the total dosage was much higher. Jefferies (1962) working on *Sitophilus granarius* found that recovery and final survival depended on the time intervals between fractions, the number of fractions and the temperature between fractions, and that, given a sufficiently long interval, the insect was able to withstand a further dose of the same magnitude with no further increase in mortality.

Virgin male flies treated after emergence showed that their life span was increased compared to untreated flies for the first seven weeks with dosages between 4,000 and 18,000 rads (Table VIII). The virgin female flies were given dosages of 2,000 and 4,000 rads, and they lived longer than the controls and the

TABLE VIII. *Survival of males and females of* G. morsitans *exposed as adults to continuous gamma-radiation (two replicates)*

Treatment (rads)	Mean survival (%) at indicated age (weeks)							
	1	2	3	4	5	6	7	8
Virgin males								
4000	97	85	80	57	35	35	27	12
8000	87	73	67	60	40	40	20	7
12000	83	67	50	45	38	31	21	6
15000	91	77	70	59	42	36	19	5
18000	84	73	66	43	28	22	11	3
Untreated	90	68	55	30	23	18	10	10
Virgin females								
2000	78	64	58	53	45	42	41	35
4000	93	88	76	64	60	55	51	51
Untreated	73	59	49	45	36	22	18	13

males. These results reversed the trend obtained from flies irradiated during the pupal stage where treatment increased mortality. Jefferies (1962) and others have reported that cells are most susceptible to radiation damage during periods of rapid division. There is extensive development and cell division during pupal life compared to the adult stage, and this may explain the observed differences in length of life between irradiated pupae and irradiated adults. In addition, it is possible that the tsetse irradiated as adults are less active and that this helps them to live longer. However, casual observations have not confirmed this hypothesis.

In field programmes, a reduction in the life span of male flies after irradiating the pupae could have a harmful effect on the efficiency of the control technique if it were not recognised. This effect of radiation could be overcome by more frequent releases of sterile males. However, the present results indicate that irradiating the male fly shortly after emergence might be more satisfactory as length of life is not affected and sterility is substantially comparable with that obtained from irradiating the pupae.

Competitiveness of treated males

The results of competitive tests with males emerging from pupae during the first week after irradiation are given in Table IX. Males from the second and third weeks were not used because of their relatively poor survival rate. At the ratios tested (treated males: untreated males: untreated females) it was expected that the reproduction rate would be reduced by 50 to 80 per cent. The results obtained showed that, in all but three tests, the actual reduction was equal to, or exceeded, the expected value. Surprisingly, neither increasing

TABLE IX. *Competitiveness of males of* G. morsitans *exposed as pupae to 4,000, 6,000, 8,000, 12,000 and 15,000 rads (two replicates)*

Treatment	Ratio+	Mean insemination of surviving females (%)	Mean number of		Mean reduction in adult progeny (%)	
			Pupae	Adults	Expected	Actual
4000	1:1:2	100·0	6·5	5·0	50·0	67·7
	1:0:1	88·0	8·0	5·0	100·0	67·7
6000	1:1:2	100·0	5·0	4·5	50·0	71·1
	1:0:1*	95·5	2·8	2·0	100·0	87·1
8000	1:1:2	90·0	2·0	1·5	50·0	90·3
	2:1:2	81·5	6·5	5·5	66·7	64·5
	3:1:3++	100·0	5·0	4·0	75·0	74·2
	4:1:2	94·5	6·5	6·5	80·0	58·1
	1:1:1:1+++	98·0	4·5	3·0	50·0	80·7
	1:0:1	95·0	1·0	0·5	100·0	96·8
12000	1:1:2	100·0	6·5	5·0	50·0	67·7
	1:0:1	100·0	1·0	1·0	100·0	94·8
15000	3:1:3	100·0	2·5	2·0	75·0	87·1
	1:0:1	96·0	0·5	0·5	100·0	98·7
Untreated	0:1:1*	89·0	18·5	15·5	—	—

+ Treated males: untreated males: untreated females.
++ 3 replicates.
+++ Including irradiated females.
* 10 replicates.

the dosage nor the proportion of treated males affected the reproduction obtained, and this has not yet been explained. Competitive tests involving irradiated females (1:1:1:1) also produced fewer pupae than were expected. These reductions in the number of pupae produced were similar to those obtained by Dame & Ford (1966) using tepa. The level of sterility obtained with irradiated males mated to normal female flies (1:0:1) was 94·8 to 98·7 per cent. for the 8,000-, 12,000- and 15,000-rad treatments compared to the 100 per cent. sterility obtained with tepa. However, the results from the competitive tests indicate that the failure to obtain complete sterility might not adversely affect a release programme with an insect having such a low reproductive capacity.

Copulation normally lasts for periods of one to four hours (Dean, unpublished). Thus, in tests with equal numbers of males and females it might be expected that the less aggressive males would not be severely handicapped since they would have to find a female before the more aggressive males begin a second mating. Where there is a surplus of males true competition does exist because the less aggressive males have difficulty in securing an unattached female. However, there was an excess of males in three of the series with 8,000 rads and there was true competition for the virgin females. The reproduction rates obtained indicated that there were no differences in aggressiveness between treated and untreated males.

Summary

Puparia of *Glossina morsitans* Westw. were collected in the Zambesi valley and contained developmental stages from final phases of third-instar larvae to pupae near to eclosion. These puparia or the emerging adults were exposed to gamma-radiation from a Co_{60} source at rates between 54 and 122 rad/min. Effective mortality in the pupal stage increased from 23 per cent. at 2,000 rads to 64 per cent. at 15,000 rads, while those deposited in spring (August–October) were more sensitive to gamma-rays than those collected in winter (May–July).

Under described test conditions, 25 pairs of untreated flies produced a mean of 21 pupae in a 28-day test period, insemination averaged 95 per cent., and 56 and 62 per cent. of the males and females survived, respectively. Continuous irradiation reduced reproduction by over 95 per cent. between 8,000 and 15,000 rads with males emerging within the first two weeks after treatment; while males emerging during the third post-treatment week were completely sterilised with dosages above 4,000 rads. Treatments given as fractions one and four days apart gave a slightly higher level of residual fertility than that obtained with similar but continuous total dosages. Irradiation of adult males reduced reproduction to an extent comparable with that observed in males emerging from pupae within two weeks of treatment (> 90 per cent. from 8,000 rads). Females treated either as adults or as pupae were completely sterilised with doses of 1,000 to 8,000 rads. Males exposed to 8,000 and 12,000 rads retained their sterility throughout 39- and 45-day test periods, and were fully competitive with untreated males.

Survival of virgin flies emerging from treated pupae was reduced both by increasing dosage from 4,000 to 15,000 rads, and decreasing pupal age at the time of treatment. Mortality during the 28-day mating period decreased with 1,000 and 2,000 rads, increased up to 8,000 rads, and apparently decreased with 12,000 and 15,000 rads. When the doses were fractionated, survival was longer than when treatment was continuous, and mortality was reduced to the level expected from the size of the first fraction. Virgin adult males given doses between 4,000 and 18,000 rads, and females given 2,000 and 4,000 rads, lived longer than the corresponding controls.

The ability of the males to inseminate females was not affected by any of the treatments, and sperm from irradiated males were mobile and apparently behaved normally. Dominant lethality in the males was expressed by the failure to produce pupae in matings with untreated females, and in the treated female fly by the complete failure of oogenesis.

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