Radiation sterilization of *Glossina tachinoides* Westw. pupae. II. The combined effects of chilling and gamma irradiation

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VREYSEN (M.J.B.), VAN DER VLOEDT (A.M.V.). Stérilisation par irradiation de *Glossina tachinoides* Westw. pupae. II. Effets combinés du froid et des rayons gamma. *Revue Élev. Méd. vét. Pays trop.*, 1995, **48** (1) : 53-61

Des femelles et des mâles de *Glossina tachinoides* Westwood ont été exposés, à l'état de pupes âgées de 5 jours, à une température de 15°C pendant des périodes allant de 9 à 21 jours. Le développement pupal des femelles a été retardé de 10,4 et 18,4 j et celui des mâles de 9,9 et 18,4 j, pour les pupes dont la durée d'incubation était respectivement de 9 et 21 j. L'éclosion pupale n'a été troublée que pour les périodes de refroidissement excédant 15 jours. Une durée de refroidissement de 9 j n'a pas affecté la réponse à l'accouplement, l'aptitude à l'insémination et la fertilité des mâles exposés à l'état de pupes, mais leur durée de survie a été réduite de façon significative de 52,1 ± 26,2 j à 35,3 ± 18,8 j. La survie des femelles adultes a été réduite, après exposition à l'état de pupes à des périodes de refroidissement dépassant 12 jours. Après 9 j d'incubation à 15°C, les femelles ont cependant produit 11 p. 100 de pupes en moins par rapport aux non traitées. Les pupes mises en incubation à 15°C pendant 9 j, à l'âge de 5 ou 10 jours, ont été irradiées avec des doses de 10 et 20 Gy sous air ou sous azote pendant 1 h, 7 h, 1, 3, et 5 j après traitement par incubation. En général, le taux d'éclosion, la fertilité des mâles et leur survie moyenne ont augmenté quand le traitement aux rayons a été effectué sous azote et lorsque le refroidissement et l'irradiation ont été appliqués tardivement dans la vie des pupes. Seuls les mâles soumis à refroidissement pendant 9 j à l'état de pupes à l'âge de 5 j, et irradiés avec une dose de 10 Gy sous air au 20e j PL (post-larviposition), ont montré une fertilité inférieure à 5 p. 100 et ont survécu en moyenne au-delà de 20 jours. La survie de toutes les femelles de l'expérience s'est trouvée réduite si on la compare à celle des animaux témoins. Leur réceptivité à l'accouplement est restée cependant normale dans la plupart des cas. La stérilité complète a été obtenue chez les femelles en pupaison incubées à 15°C pendant 9 j à l'âge de 5 j, et irradiées avec 10 Gy en atmosphère d'air entre le 15e et le 20e j après la larviposition. Il en est de même chez les femelles incubées à 15°C pendant 9 jours à l'état de pupes âgées de 10 j et traitées avec 10 Gy sous air aux 20e et 21e j de leur vie pupale.

Mots clés : Glossina tachinoides - Pupe - Stérilisation - Irradiation gamma - Résistance à la température - Azote - Lutte anti-insecte - Éclosion - Fertilité - Longévité.

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Reçu le 9.2.1995, accepté le 13.3.1995.

INTRODUCTION

Previous radiation research carried out on *Glossina tachinoides* pupae has acknowledged the feasibility of irradiating pupae in air during the late pupal phase without a significant reduction in male fly quality (Vreysen and Van Der Vloedt, unpublished data). The brief interval between irradiation and eclosion of the flies remains a constraint when pupae have to be dispatched for long-distance transport. It is worthwhile to evaluate manipulation techniques to increase the total time for preparing and transporting pupae i.e. pupae can be irradiated at earlier stages or efforts can be made to arrest pupal development. Vreysen and Van Der Vloedt (13) have shown that high-quality sterile *G. tachinoides* males can be obtained by treating pupae with ionizing radiation during the mid-pupal phase (day 15-20 post larviposition) (PL). This, however, requires the use of nitrogen during the radiation treatments and the splitting of the radiation dose into at least two fractions.

The major factor influencing the duration of pupal development is the ambient temperature (3). The total development period from pupation to emergence can be substantially extended by exposing pupae to low temperatures. Cooling of pupae to inhibit male eclosion after the female eclosion flush has been used successfully during the SIT pilot trial with *G. m. morsitans* in Tanzania. Storing the male pupae during their late pupal phase at 10 ± 1°C for 4 days combined with an irradiation treatment in nitrogen did not affect male eclosion (16) or male quality after release in the field (17).

This paper presents the results of a study analyzing the effects of prolonged periods (9-21 days) of low temperature incubation (15°C) of *G. tachinoides* pupae and the combined effects of gamma irradiation treatments at ambient temperatures in air and nitrogen. The parameters examined were the pupal development period, eclosion, fertility and survival of both male and female flies.

MATERIAL AND METHODS

All *G. tachinoides* pupae used for the experiments were derived from the mass-rearing colony maintained at the Entomology Unit of the IAEA laboratories in Vienna (Austria). Experimental adult male and female flies were maintained under standard holding conditions (23 ± 1°C and 75 ± 5 % R.H.) together with untreated controls as described earlier (13).

The effects of long-term incubation at low temperatures was assessed by exposing batches (n = 100) of 5-day-old pupae at 15 ± 1°C in an incubator for 9, 12, 15, 18 or 21 days and a batch (n = 100) of 10-day-old pupae for 9 days. Relative humidity was maintained at 75 ± 5 %. Afterwards, all experimental pupae were transferred to the insectary to complete their development under standard holding conditions.

For the assessment of the combined effects of chilling and irradiation, batches (n = 100) of 5-day-old (incubation group I) and 10-day-old (incubation group II) pupae were incubated for 9 days at 15 ±1°C prior to the irradiation treatments. Pupae were irradiated with doses of 10 and 20 Gy in a ^{60}Co. source (dose rate 6 Gy/min) 1 h, 7-8 h, 1, 3 and 5 days respectively after incubation. Irradiation in nitrogen atmosphere was carried out as described in a previous paper (13), while collection of emerging flies and experimental procedures to assess reproductive parameters were performed as described by Vreysen *et al.* (14).

RESULTS

Effects of low temperature (15°C) incubation

The eclosion rate and average pupal period of female and male *G. tachinoides*, incubated at 15°C as 5-day-old pupae for 9 to 21 days and as 10-day-old pupae for 9 days, are presented in figure 1. An average pupal period of 34.2 ± 0.5 and 36.9 ± 0.6 days was recorded for female and male pupae respectively under the prevailing standard holding conditions. The 9-day chilling period delayed female and male development, with 10.4 and 9.9 days respectively, whereas female and male pupae exposed to 15°C for 21 days required 52.6 ± 0.6 days and 55.3 ± 0.7 days for completion of their development. A difference of 1.04 days in average development period was recorded between female and male pupae incubated at 15°C for 9-15 days. This sex-related difference was comparable to the one observed for untreated pupae (1.07 days) but was increased to 2.7 and 3.1 days with longer chilling periods. Adult eclosion was not adversely affected by cooling periods up to 12 days (88 - 94 % eclosion versus 92 % for control pupae (χ^2, $p > 0.05$), but longer cooling periods reduced fly eclosion significantly to 82 - 86 % (χ^2, $p < 0.01$).

The mating response of all experimental adult males was normal with > 81 % of the female mates showing mating scars. Male insemination capacity, however, dropped from 97.4 to 89 % and 66.6 - 30 % for males exposed to 15°C as 5-day-old pupae for 9, 12 and 15 - 21 days respectively. Sperm quality of males exposed to 15°C for 9 days as 5- and 10-day-old pupae was not affected as evidenced by a fertility of 0.083 and 0.098 pupae per mature female day respectively (control females produced 0.081 pupae/mature female day) (table I). Cooling male pupae

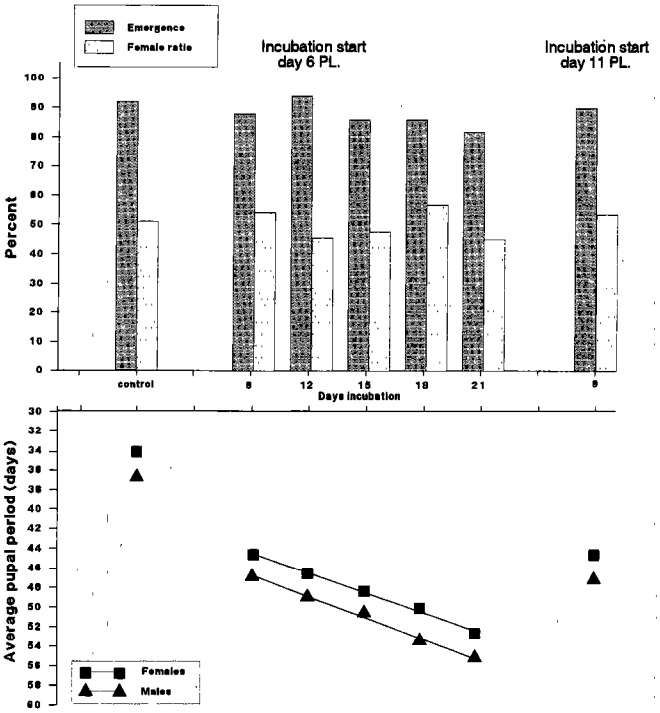

Figure 1 : Eclosion rate and average pupal period of G. tachinoides *pupae incubated for various periods at 15°C.*

for 12 days reduced their fertility with 11 % as compared with untreated males. Longer cooling periods reduced male fertility proportionally with the length of incubation. These data were corroborated by the dissection data of the female flies. The proportion of inseminated females showing aberrations in the ovarian configuration and uterus content did not exceed 3 % for females mated with males incubated for 9 days. This percentage of females displaying reproductive abnormalities increased to 4.8 - 13.8 % for females mated with males incubated at 15°C for longer periods. Viability of offspring was normal, however (eclosion rate > 87 %), with sex ratios slightly biased in favour of females (only significant for 15 day cooling group ($\chi^2 = 4.22$, $p < 0.05$).

Unchilled control males lived on average for 52.1 ± 26.2 days (fig. 2). Incubation at 15°C of 5-and 10-day-old male pupae for 9 days reduced the average longevity of the adult males significantly ($p < 0.01$) with 16.8 and 19.2 days respectively. Average life spans of adult males were proportionally decreased when cooled as pupae for longer periods. An average life span of 16.5 ± 10.2 days was recorded for males incubated at 15°C as pupae for 21 days.

Untreated female flies and females cooled as 5-and 10-day-old pupae for 9 days and 5-day-old pupae for 12 days showed comparable survival rates during the 45-day experimental period (table I). Increasing the cooling period to 15, 18 and 21 days reduced female survival significantly (38.8, 28.2 and 12.6 % survivors respectively on day 45). Although more than 83 % of all experimental females

TABLE I
Fertility of female and male *G. tachinoides* incubated as pupae at 15 °C for 9 to 21 days and mated with untreated colony flies (Experimental groups of 40 flies, control = 80 flies)

Incubation period (1)	Female survival day 45 % (2)	No. puparia produced	Mean puparial weight (mg) ± SD	Fecundity [3]	Emergence/ females %
Control	80.8	145	17.3 ± 2.8	0.081	83.2 / 48.2
Males treated - Females untreated					
6-15	88.8	82	16.5 ± 2.7	0.083	91.3 / 50.0
6-18	86.6	70	16.1 ± 2.7	0.072	91.4 / 54.7
6-21	88.9	42	17.9 ± 2.0	0.048	95.2 / 67.5
6-24	83.9	16	16.5 ± 4.1	0.023	87.5 / 78.5
6-27	82.9	15	16.6 ± 2.1	0.022	93.3 / 55.5
11-20	84.0	92	17.8 ± 3.1	0.098	92.1 / 54.8
Females treated - Males untreated					
6-15	78.7	63	17.1 ± 2.6	0.072	95.2 / 55.9
6-18	78.8	48	15.9 ± 2.8	0.054	83.3 / 45.0
6-21	38.8	21	15.5 ± 2.9	0.048	76.2 / 62.0
6-24	28.2	7	13.3 ± 1.8	0.022	4/7 / 2/4
6-27	12.6	0	—	0.000	—
11-20	80.1	64	15.7 ± 2.3	0.072	95.2 / 44.1

1. Days post larviposition.
2. Survival relative to mature female days.
3. No. pupae per mature female day.

were receptive to mating, the insemination rate dropped below 72 % for females incubated at 15°C for more than 18 days. The reproduction rate of all experimental females was inferior to the reproduction of untreated controls. No pupae were produced by females cooled as pupae for 21 days.

Combined effects of low temperature (15°C) incubation and gamma irradiation in air and nitrogen

Pupal development and eclosion

Eclosion data of pupae incubated at 15°C from day 6 to day 15 PL (incubation group I) and from day 11 to day 20 PL (incubation group II) and irradiated with 10 and 20 Gy in air and nitrogen atmosphere are presented in figure 3. Irradiating pupae of incubation group I with 10 Gy in air on day 15 and 16 PL reduced eclosion significantly to < 34 % (χ^2, $p < 0.01$). Examination of the content of the not yet hatched pupae revealed that both males and females completed their development but failed to emerge. Eclosion rate was increased to the control level of > 92 % when the same irradiating treatment was given on day 18 - 20 PL. All pupae were killed with a treatment of 20 Gy in air. The use of nitrogen during the 10- and 20-Gy radiation treatment resulted in optimal eclosion rates (> 90 %) except for pupae treated with 20 Gy on day 15 - 16 PL (81 % (χ^2, $p < 0.01$) and 88 % (χ^2, $p < 0.05$).

More than 87 % of the pupae emerged when incubated at 15°C from day 11 to 20 PL prior to an irradiation treatment of 10 Gy in air and 10 - 20 Gy in nitrogen (χ^2, $p > 0.05$). An irradiation dose of 20 Gy applied in air on day 20 reduced the eclosion rate to < 36 % (χ^2, $p < 0.01$). Female and male pupae were not killed by the irradiation treatment and continued their development to completion but failed to rupture the puparium. The same radiation treatment applied 3 to 5 days after the incubation resulted in normal pupal development and 90 % eclosion.

Adult male fertility and survival

All experimental adult males exposed as pupae to 9 days of cooling followed by an irradiation treatment showed a normal mating response (> 82 % of the female mates showed mating scars), spermatophore formation and sperm transfer (> 80 % of the females inseminated). Aberrant results were observed for males of the first incubation group, treated with 20 Gy in nitrogen on day 20 PL, who failed to inseminate 32 % of their female mates.

All emerged males from the first incubation group treated as 15 - 16-day-old pupae with 10 Gy in air died before reaching sexual maturity (table II.). Similarly, not enough sexually-mature males were available for assessment of fertility parameters, among those emerged from pupae treated with 20 Gy in nitrogen on day 15 - 18 PL. The use of nitrogen during irradiation increased adult male fertility significantly (threefold) as compared to treatments given

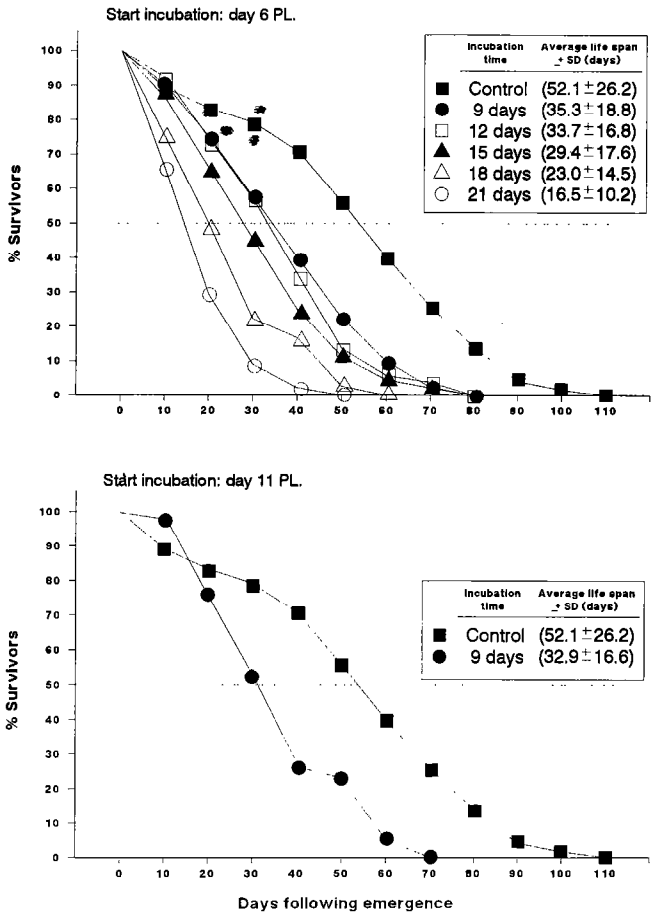

Figure 2 : Survival curves of G. tachinoides *males, incubated at 15°C for various periods starting on day 6 (top) and day 11 (bottom) PL.*

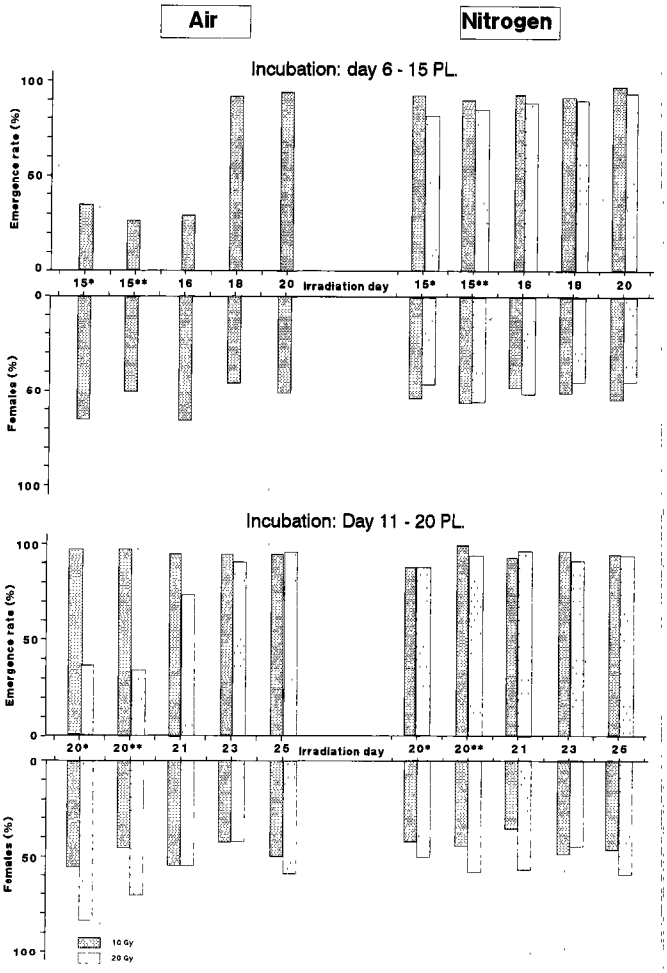

Figure 3 : Eclosion rate and female ratio of G. tachinoides *pupae incubated from day 6-15 PL (top) and from day 11-20 PL (bottom) and irradiated with 10 and 20 Gy in air or in nitrogen atmosphere. (*irradiation treatment 1 h and ** 7-8 h after incubation).*

in air. Adult male fertility was dependent on the age of the pupae when treated with 10 Gy in nitrogen i.e. fertility decreased from 0.041 - 0.056 to 0.027 pupae/mature female for males treated as 15 - 16-day-old pupae and 18 - 20-day-old pupae respectively. More than 95 % sterility was induced in the sperm of adult males when treated on day 20 PL with 10 Gy in air and 20 Gy in nitrogen. Although all pupae produced by females mated with irradiated treated males weighed significantly less than pupae fathered by control males ($p < 0.05$), their viability was found to be normal ($p > 0.05$).

Fertility of males of the second incubation group, irradiated in air, increased with increasing pupal treatment age. No such age-dependent relation was found when pupae were irradiated in nitrogen. The protective effect of nitrogen on the amount of induced lethal mutations was more pronounced with younger pupae i.e. residual fertility increased 12 - 48 times with a dose of 10 Gy on day 20 PL *vs* the same treatment in air, but resulted in a three-to-four fold fertility increase only when administered on day 23 - 25 PL. 95 % sterility was induced only in males treated with 10 Gy in air on the 20 th day of their pupal life. All treatments in nitrogen resulted in male residual fertility levels exceeding 20 % of that of the controls.

The proportion of females displaying reproductive abnormalities i.e. uterus empty due to expulsion of a dead embryo or an egg *in utero* in embryonic arrest, increased with increasing radiation dose but decreased when nitrogen was used during irradiation. 25 to 52 % of the females mated with males belonging to the first incubation group and treated with 10 Gy in air revealed reproductive abnormalities. This proportion of females decreased to 8.3 - 23 % when the same dose was administered in nitrogen, but increased to > 88 % when the treatment dose was 20 Gy. The same trend was observed in females mated with males belonging to the second incubation group.

Table III presents data on survival of untreated and experimental males. Cooling of pupae followed by irradiation reduced the mean longevity of adult males significantly ($p < 0.05$) compared to exposure to low temperature alone. In general, average male longevity was less severely affected when the radiation treatment was given in nitrogen and when the chilling and irradiation treatments occurred later in pupal life. Only males (incubation group I)

TABLE II
Fertility of male *G. tachinoides*, incubated as pupae at 15 °C for 9 days (day 6-15 PL and day 11-20 PL) and irradiated with 10 and 20 Gy in air or nitrogen atmosphere and mated with untreated colony females

Irradiation atmosphere/dose/day (A-N) (Gy) (PL) 1/	No. puparia produced	Mean puparial weight (mg) ± SD	Production relative to control	No. of aborted eggs	No. of aborted immature larvae	Emergence/ females %
Control	145	17.3 ± 2.8	100	20	5	83.2 / 48.2
Group I (incubation day 6-15 PL)						
A / 10 /18	2	—	9.6	36	2	2/2 1/2
/20	0	—	0.0	97	1	—
N / 10 /15*	19	14.0 ± 3.0	50.6	42	6	100 / 52.4
/15**	31	13.9 ± 2.6	69.5	30	4	96.9 / 59.4
/16	38	13.6 ± 2.3	61.7	43	3	92.8 / 51.3
/18	12	13.1 ± 2.5	33.3	45	0	100 / 41.2
/20	13	15.3 ± 2.8	32.9	45	5	88.2 / 33.3
N / 20 /20	1	—	2.7	63	0	1/1 / 1/1
Group II (incubation day 11-20 PL)						
A / 10 /20*	2	—	4.9	74	0	0/2 / 0/0
/20**	1	—	1.5	120	0	1/1 / 1/1
/21	6	16.0 ± 3.3	9.9	104	0	6/6 / 3/6
/23	10	16.1 ± 3.1	15.2	110	0	6/10 / 3/6
/25	16	16.5 ± 2.7	21.3	114	1	92.9 / 30.8
N / 10 /20*	36	16.3 ± 2.6	60.3	50	7	82.8 / 37.9
/20**	36	16.4 ± 3.0	59.6	50	4	88.8 / 62.5
/21	26	17.8 ± 2.4	39.2	72	9	80.7 / 47.6
/23	37	17.0 ± 2.7	59.2	58	7	86.1 / 41.9
/25	51	16.8 ± 2.9	62.8	75	8	84.0 / 76.2
N / 20 /20*	12	17.1 ± 2.4	25.8	59	1	83.3 / 50.0
/20**	10	16.2 ± 3.0	20.2	71	1	90.0 / 44.4
/21	19	15.6 ± 2.6	31.8	69	2	78.9 / 20.0
/23	18	16.8 ± 3.0	27.1	94	0	82.4 / 35.7
/25	18	17.0 ± 2.5	30.9	76	1	77.8 / 57.1

1. PL : days post larviposition, A : Air, N : Nitrogen.
(*) Irradiation 1 hour and (**) 7-8 hours after incubation.

treated with 10 Gy in air and nitrogen on day 20 PL and (incubation group II) treated with 10 Gy in air on day 21 and 25 PL and with 10 Gy in nitrogen on day 23 - 25 PL lived on average > 20 days.

Consequently, combined survival and fertility data show that only males incubated for 9 days at 15°C as 5-day-old pupae and irradiated with 10 Gy in air on day 20 PL had a residual fertility < 5 % of the control and an average longevity above 20 days. None of the males irradiated as pupae in nitrogen could meet these criteria.

Adult female fertility and survival

Survival of all experimental females was reduced (< 67 and < 71 % survivors on day 45 for females of incubation groups I and II respectively) compared with survival of untreated females (table IV). Female survival increased in general with a lower irradiation dose and when irradiation took place in nitrogen and during later pupal stages. Their receptivity to mating, however, remained unaffected except for females of incubation group I treated with 10 Gy in air or with 20 Gy in nitrogen (75 - 84 % spermathecae impregnated with sperm).

Females treated as 15 - 20-day-old pupae (incubation group I) with 10 Gy in air failed to produce any offspring. Treating female pupae with 10 Gy in nitrogen revealed reproduction rates of 69 < 84 % compared with that of the control but the residual fertility decreased to 15 - 51 % with a radiation dose of 20 Gy.

Complete sterility was induced in females of incubation group II treated with 10 Gy in air on days 20 or 21 of pupal life. The same treatment given on day 23 - 25 PL resulted in residual fertility of 30 - 55.5 %. The use of nitrogen during irradiation increased residual fertility to 64 - 84 %. A dose of 20 Gy in nitrogen resulted in residual fertility of 19 - 75 %, depending on the age of the pupae during treatment.

All pupae produced by the experimental females weighed significantly less in comparison with pupae produced by untreated females ($p < 0.01$). Viability however was unaffected except for offspring produced by females of incubation group I treated with 20 Gy in nitrogen on day 15 - 18 (χ^2, $p < 0.01$) and by females of incubation group II treated with 20 Gy in nitrogen on day 20 - 23 (eclosion rates < 54.1 %, $p < 0.01$).

TABLE III
Average longevity of *G tachinoides* males incubated for 9 days at 15 °C and irradiated in air and nitrogen with 10 and 20 Gy during various moments of their pupal development

Incubation period	Irradiation atmosphere/dose/day (A/N) (Gy) (PL)	Males no.	Average life span ± SD (days)
Control	— — —	80	52.1 ± 26.2
Day 6-15 PL	— ——	40	35.3 ± 18.8
	A / 10 /15*	12	3.0 ± 0.3
	15**	13	3.2 ± 0.5
	16	10	3.7 ± 2.2
	18	50	8.8 ± 9.9
	20	46	24.4 ± 21.2
	N / 10 /15*	40	17.0 ± 17.2
	15**	40	14.4 ± 17.3
	16	49	24.4 ± 26.3
	18	43	17.0 ± 31.0
	20	39	26.7 ± 32.2
	N / 20 /15**	44	3.8 ± 2.0
	15*	38	5.2 ± 3.3
	16	43	5.0 ± 4.1
	18	50	5.9 ± 3.3
	20	51	11.0 ± 9.1
Day 11-20 PL	— — —	41	32.9 ± 16.6
	A / 10 /20*	42	18.4 ± 17.0
	20**	51	18.0 ± 14.7
	21	41	20.8 ± 16.2
	23	47	17.4 ± 10.0
	25	47	20.3 ± 16.5
	A / 20 /20*	6	4.0 ± 3.0
	20**	10	2.2 ± 0.8
	21	33	2.8 ± 1.1
	23	51	4.3 ± 1.6
	25	39	8.3 ± 6.3
	N / 10 /20*	50	19.8 ± 19.3
	20**	53	19.0 ± 14.9
	21	56	14.3 ± 12.2
	23	48	20.6 ± 18.0
	25	51	22.7 ± 18.8
	N / 20 /20*	43	14.9 ± 13.7
	20**	39	12.7 ± 10.6
	21	41	19.9 ± 18.4
	23	48	15.2 ± 14.1
	25	42	15.5 ± 16.2

* Irradiation 1 hour after incubation.
** Irradiation 7-8 hours after incubation.

DISCUSSION

Buxton and Lewis (4) and Buxton (3) have shown that the duration of the pupal development period of tsetse flies can be significantly shortened by exposing pupae to temperatures exceeding the optimum of 23-24°C. In addition, recent research has indicated the feasibility of extending the total pupal development period by cooling tsetse pupae for long periods at young stages. Exposing 4-day-old *G. austeni* pupae for 9 - 15 days at 15°C (7) and 5 - 20-day-old *G. p. palpalis* pupae for 5 days at 15°C (Feldmann and Vreysen, unpublished data) had no adverse effects on total pupal eclosion rate and male insemination capacity. During the entire pupal development period, fat constitutes the sole source of energy (4), with the rate of metabolism being greatest at the start and end of the pupal period (1). The importance of the fat metabolism in tsetse pupae is underlined by the fact that newly emerged teneral flies have to rely solely on their residual fat content for energy until their first blood meal. Moreover,

TABLE IV

Fertility of female *G. tachinoides*, incubated as pupae at 15 °C for 9 days (day 6-15 PL and day 11-20 PL) and irradiated with 10-20 Gy in air or nitrogen atmosphere and mated with untreated colony males

Irradiation atmosphere/dose/day (A-N) (Gy) (PL) [1]	Female survival day 45 % [2]	No. puparia produced	Mean puparial weight (mg) ± SD	Production relative to control	No. of aborted eggs/ female	No. of aborted larvae/ female	Emergence/ females %
Control	80.8	145	17.3 ± 2.8	100	0.29	0.07	83.2 / 48.2
Group I (incubation day 6-15 PL)							
A / 10 /15*	9.5	0	—	0.0	1.00	0.00	—
/15**	0.0	0	—	0.0	0.00	0.00	—
/16	15.8	0	—	0.0	1.00	0.00	—
/18	23.1	0	—	0.0	1.92	0.25	—
/20	31.8	0	—	0.0	1.19	0.63	—
N / 10 /15*	46.0	29	14.0 ± 3.0	69.4	0.78	0.26	87.5 / 50.0
/15**	45.4	35	13.9 ± 2.6	72.4	0.69	0.31	91.8 / 52.9
/16	41.4	29	13.6 ± 2.3	83.4	0.50	0.27	84.3 / 55.6
/18	36.4	22	13.1 ± 2.5	70.0	0.26	0.53	84.0 / 61.9
/20	67.9	69	15.3 ± 2.8	84.5	0.67	0.26	84.6 / 48.5
N / 20 /15*	21.4	5	8.9 ± 1.5	28.5	0.92	0.15	1/5 / 0/1
/15**	13.1	2	9.4 ± 3.8	14.9	0.45	0.18	1/2 / 1/1
/16	31.5	11	9.5 ± 1.6	34.2	1.06	0.17	4/11 / 2/4
/18	33.2	5	6.7 ± 0.9	17.5	1.47	0.47	0/5 / 0/0
/20	62.4	29	12.2 ± 2.9	51.2	1.52	0.11	75.0 / 61.9
Groupe II (incubation day 11-20 PL)							
A / 10 /20*	71.4	0	—	0.0	0.85	0.73	—
/20**	60.0	0	—	0.0	0.91	0.87	—
/21	71.3	0	—	0.0	1.24	0.63	—
/23	67.3	28	15.2 ± 2.7	55.5	0.92	0.46	80.0 / 40.0
/25	67.9	19	14.7 ± 3.9	30.8	1.42	0.19	76.5 / 46.2
N / 10 /20*	49.2	33	14.6 ± 3.5	84.5	0.36	0.14	78.1 / 72.0
/20**	39.2	23	12.9 ± 3.2	64.6	0.45	0.45	72.7 / 37.5
/21	17.1	9	14.2 ± 1.6	70.3	0.71	0.14	100 / 55.6
/23	45.2	39	16.0 ± 2.5	84.3	0.46	0.13	97.4 / 47.4
/25	41.2	26	14.9 ± 3.1	69.5	0.60	0.40	75.0 / 80.0
N / 20 /20*	21.8	4	10.9 ± 2.4	19.2	0.61	0.52	1/4 / 1/1
/20**	27.9	9	7.0 ± 2.8	31.6	0.89	0.47	1/9 / 0/1
/21	43.8	18	8.3 ± 1.7	34.8	0.74	0.58	4/18 / 3/4
/23	44.4	28	15.2 ± 3.7	75.1	0.74	0.05	54.1 / 53.8
/25	33.1	19	13.2 ± 3.1	46.9	0.81	0.15	66.6 / 41.6

1. PL : Days post larviposition, A : Air, N : Nitrogen.
2. Survival relative to mature female days.
(*) Irradiation 1 jour and (**) 7-8 hours after incubation.

fat consumption in tsetse pupae is greatly influenced by the ambient temperature. Bursell (2) showed that consumption of fat in *G. m. morsitans* pupae was most economic at 24°C and increased significantly above and below this optimum (12). Although inter-specific differences in the rate of fat consumption have been demonstrated (8), the total amount of fat reserves of a small tsetse species such as *G. tachinoides* is far less than that of larger species. Consequently, smaller individuals exposed to low temperatures during their pupal development will reach critical levels of their total reserves of fat sooner as compared with larger ones. Our data with *G. tachinoides* pupae indicate that chilling pupae for 9 - 12 days at 15°C did not deplete their total fat content, and male and female pupal development could be completed. Extending the incubation period beyond 15 days resulted in a 10 % increase in pupal death. However, a 9-day chilling period seemed to be the upper threshold in terms of male insemination capacity and fertility. Average male survival was, however, significantly reduced. Moreover, female flies exposed as pupae to the 9-day cooling treatment, had similar survival rates as untreated females but produced 11 % fewer offspring. These results strongly suggest that viability of adult male and female *G. tachinoides* flies is affected by factors other than pupal fat metabolism when exposed as pupae to extended periods of low temperatures. In addition, a study to reveal the mechanisms responsible for the reduced fertility in both male and female flies would certainly be a challenging research topic.

The feasibility of chilling late pupal stage *G. m. morsitans* pupae followed by a sterilizing radiation dose has been demonstrated by Curtis and Langley (5). A cooling period of 5 days at 10°C combined with a sterilizing treatment in nitrogen after the incubation could effectively control male emergence without a loss of adult male quality. Prolonging the cooling period to 7 - 10 days resulted, however, in 10 % pupal death (11). Likewise, viability of adult male *Ceratitis capitata*, exposed as pupae for 2 hours or 2 days to 5 or 15°C prior to irradiation, was not significantly affected. The chilling treatment however, reduced sterility compared with pupae incubated at 25°C (15). In addition, cooling *Ceratitis capitata* pupae during irradiation i.e. reducing the metabolic rate, did not give any protection due to the increased solubility of oxygen in the tissues (10). These experiments with *G. tachinoides* pupae have shown that combining chilling with low dose irradiation treatment at ambient temperatures increased the loss in viability of adult males already observed with a cooling treatment alone. The use of nitrogen during irradiation reduced the amount of somatic injury expressed by an increase in mean longevity but was accompanied by a reduction in the amount of induced lethal mutations. These data are in accordance with observations made by Curtis and Langley (5) and Vreysen and Van Der Vloedt (13).

In conclusion, exposing young *G. tachinoides* pupae to low temperatures followed by a sterilizing irradiation treatment seems to leave only limited possibilities for the manipulation required for an extended handling period. Sufficient sterility was induced in male adult tsetse flies exposed to a 9-day incubation period (15°C) starting on day 6 of their pupal development, followed by a 10 Gy irradiation treatment in air on day 20 PL. Although the treatment created a 25-day interval between irradiation and male eclosion, average survival of the adult males was reduced to 24 days. These survival data are comparable with those obtained by treating *G. tachinoides* pupae in air with the sterilizing dose of 20 Gy on day 20 PL (Vreysen and Van Der Vloedt, unpublished data). The interval between treatment and onset of male eclosion remained however limited to 12 - 14 days. Higher-quality sterile males in terms of survival (mean longevity > 30 days) were obtained by irradiating unchilled 15 - 20-day-old pupae in nitrogen in doses split into two fractions. An interval of 12 to 16 days between treatment and male eclosion was obtained, depending on the treatment option (13).

This series of experiments amply demonstrated the feasibility of producing high-quality sterile males by irradiation of *G. tachinoides* pupae aged between 15 to 20 days. In addition, the exposure of young pupae to low temperatures significantly prolonged the pupal period, but the viability of the adult males was negatively affected when combined with a sterilizing treatment. Further research should be carried out to improve the quality of the obtained males. Splitting the radiation dose into two fractions after the incubation period is certainly one of the options that should be investigated.

ACKNOWLEDGMENTS

The authors would like to thank Drs. J.C. van Lenteren and W. Takken (Wageningen Agricultural University) and Drs. D.A. Lindquist and H.U. Feldmann (Joint FAO/IAEA Division, IAEA) for reviewing the manuscript. The technical assistance of the staff of the Entomology Unit, IAEA Laboratory, Seibersdorf, Austria is highly appreciated.

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VREYSEN (M.J.B.), VAN DER VLOEDT (A.M.V.). Radiation sterilization of *Glossina tachinoides* Westw. pupae. II. The combined effects of chilling and gamma irradiation. *Revue Élev. Méd. vét. Pays trop.*, 1995, **48** (1): 53-61

Female and male *Glossina tachinoides* Westwood were exposed as 5-day-old pupae to 15 °C for 9 to 21 days. Female pupal development was delayed at 10.4 and 18.4 days and male pupal development at 9.9 and 18.4 days for pupae incubated for 9 and 21 days respectively. Pupal eclosion was only affected by chilling periods exceeding 15 days. Mating response, insemination capacity and fertility of males exposed as pupae to a 9-day chilling period were not affected, but their survival was significantly reduced from 52.1 ± 26.2 days to 35.3 ± 18.8 days. Survival of adult females was reduced when exposed as pupae to chilling periods exceeding 12 days. After 9 days at 15°C, however, females produced 11 % less offspring than untreated females. Pupae, incubated for 9 days at 15°C when 5 or 10 days old, were irradiated with 10 and 20 Gy in air or nitrogen 1 h, 7 h, 1, 3 and 5 days after the incubation treatment. In general, the eclosion rate, male fertility and average male survival were increased when the radiation treatment was given in nitrogen and when chilling and irradiation treatments occurred later in pupal life. Only males chilled for 9 days as 5-day-old pupae and irradiated with 10 Gy in air on day 20 PL (post larviposition) had a residual fertility below 5 % and lived on average longer than 20 days. Survival of all experimental female flies was reduced as compared with the control. Their receptivity to mating remained however normal in most cases. Complete sterility was induced in females, incubated at 15°C for 9 days as 5-day-old pupae and irradiated with 10 Gy in air on day 15 - 20 post larviposition and in females, incubated at 15°C for 9 days as 10-day-old pupae and treated with 10 Gy in air on days 20 or 21 of pupal life.

Key words: *Glossina tachinoides* - Pupa - Sterilization - Gamma irradiation - Temperature resistance - Nitrogen - Insect control - Hatching - Fertility - Longevity.

VREYSEN (M.J.B.), VAN DER VLOEDT (A.M.V.). Esterilización por radiación de *Glossina tachinoides* Westw. pupas. II. Efectos combinados del enfriamiento y de la irradiación gamma. *Revue Élev. Méd. vét. Pays trop.*, 1995, **48** (1) : 53-61

Pupas de 5 días de *Glossina tachinoides* Westwood, machos y hembras, fueron expuestas a 15°C durante un período 9 a 21 días. El desarrollo pupal de las hembras se retardó de 10,4 y 18,4 días y el de los machos de 9,9 y 18,4 días, para las pupas incubadas durante 9 y 21 días respectivamente. La eclosión de las pupas fue afectada unicamente por los períodos de enfriamiento superiores a 15 días. La respuesta al acoplamiento, la capacidad de inseminación y la fertilidad de las pupas machos, expuestas a un período de enfriamiento de 9 días, no fueron afectadas, sin embago, la sobrevida se redujo significativamente de 52,1 ± 26,2 días a 35,3 ± 18,8 días. La sobrevida de las hembras adultas se redujo cuando la exposición de las pupas al frío excedió 12 días. Las hembras expuestas a 9 días de incubación a 15°C, produjeron 11 p. 100 menos descendencia que las hembras no tratadas. Las pupas de 5 o 10 días, incubadas durante 9 días a 15°C, fueron irradiadas con 10 y 20 Gy en aire o nitrógeno, 1 hora, 7 horas, 1 día, 3 días y 5 días después del tratamiento de incubación. En general, la tasa de eclosión, la fertilidad del macho, así como el promedio de sobrevida del macho aumentaron cuando el tratamiento de radiación se administró en nitrógeno y cuando los tratamientos de enfriamiento e irradiación se administraron tardíamente en la vida pupal. Unicamente los machos sometidos a un enfriamiento de 9 días, a los 5 días de edad pupal, e irradiados con 10 Gy en aire al día 20 PL, presentaron una fertilidad inferior a 5 p. 100 y presentaron un promedio de 20 días más de vida. La sobrevida de todas las moscas hembras del experimento fue inferior a la de los controles. La receptividad al acoplamiento se mantuvo normal en la mayoría de los casos. La esterilidad completa fue inducida en las hembras incubadas a 15°C durante 9 días al día 5 de edad pupal e irradiadas con 10 Gy en aire al día 15-20 PL y en las hembras incubadas a 15°C durante 9 días al día 10 de edad pupal y tratadas con 10 Gy en aire al día 20 o 21 de la vida pupal.

Palabras clave : *Glossina tachinoides* - Pupa - Esterilización - Irradiación gamma - Resistencia a la temperatura - Nitrógeno - Lucha contra los insectos - Eclosión - Fertilidad - Longevidad.