Radiation sterilization of *Glossina tachinoides* Westw. pupae. I. The effect of dose fractionation and nitrogen during irradiation in the mid-pupal phase

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VREYSEN (M.J.B.), VAN DER VLOEDT (A.M.V.). Stérilisation par irradiation de *Glossina tachinoides* Westw. pupae. I. Effet des doses fractionnées et de l'azote pendant l'irradiation à mi-course de la phase pupale. *Revue Élev. Méd. vét. Pays trop.*, 1995, **48** (1) : 45-51

L'effet de l'azote pendant l'irradiation de pupes de *Glossina tachinoides* ainsi que le fractionnement des doses d'irradiation, ont été étudiés au milieu de la phase pupale (15e au 20e j suivant la larviposition). L'effet protecteur de l'azote contre des irradiations de 10 à 80 Gy de pupes âgées de 15 à 20 jours a été démontré par l'accroissement du taux global d'éclosion, par des niveaux plus élevés de fertilité résiduelle chez les mâles et par des durées de vie plus longues. La proportion des anomalies de reproduction observées chez les femelles accouplées a augmenté avec la dose d'irradiation, chez les pupes les plus jeunes et lorsque le traitement était fait dans l'air. Après traitement de pupes âgées de 15 j, sous azote et à la dose de 10 Gy, la fertilité a atteint 0,068 pupe par femelle mature par jour, contre 0,035 pupe lorsque l'irradiation a eu lieu dans l'air. Cette augmentation n'a pas été observée lors du traitement de pupes âgées de 20 jours. Une dose de 60 à 80 Gy a été nécessaire pour obtenir une stérilité de 95 p. 100 des pupes femelles âgées de 20 jours. Le partage de la dose d'irradiation sous azote en 2 fractions à intervalle de 1, 2 ou 5 jours n'a pas eu d'influence sur le taux global d'éclosion, le taux d'accouplement et la capacité d'insémination des mâles. La stérilité des mâles traités par doses fractionnées séparées par 1 ou 2 jours a été identique à celle des mâles traités par une dose unique au 15e j après larviposition ; mais le taux de mutations létales induites était diminué pour des doses fractionnées séparées par un intervalle de 5 jours. La survie des mâles traités par doses fractionnées était similaire à celle des mâles traités avec une seule dose au 20e j suivant la larviposition ; mais elle était supérieure à celle des mâles traités au jour 15. La fécondité des femelles a été réduite lors du fractionnement de la dose d'irradiation à intervalle de 1 et 2 jours. Une stérilité complète a été obtenue chez les pupes femelles lorsque l'intervalle entre les doses fractionnées était de 5 jours, indépendamment de la dose utilisée. L'irradiation de pupes de *G. tachinoides* au milieu de la phase pupale sous atmosphère d'azote, par des doses fractionnées séparées par 1 ou 2 j (dose totale 40 Gy) ou 5 j (dose totale 60 à 80 Gy), a entraîné une meilleure stérilisation des mâles (longévité supérieure à 20 jours en moyenne et fertilité résiduelle inférieure à 5 p. 100).

Mots clés : Glossina tachinoides - Pupe - Stérilisation - Irradiation - Azote - Fractionnement - Lutte anti-insecte - Eclosion - Fertilité - Longévité.

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Reçu le 9.2.1995 , accepté le 13.3.1995.

INTRODUCTION

Both laboratory studies and operational field programmes using the Sterile Insect Technique (SIT), have indicated the feasibility of deploying male tsetse flies (*Glossinidae*) of the *morsitans* and *palpalis* group, sterilized by ionizing radiation in the pupal stage (1, 14, 11). A prerequisite for the successful application of the sterile insect technique is the release of highly competitive sterile insects with a behaviour similar to that of wild insects. Previous research on the effect of gamma irradiation on *Glossina tachinoides* pupae has shown that radiation treatment in air is limited to the last third of the pupal development (Vreysen, Van Der Vloedt, unpublished data). Attempts to obtain viable high- quality sterile males by treating pupae during the mid-pupal phase with doses of 10 - 120 Gy failed due to an increased rate of early pupal death because of radiation-induced somatic damage; reduced survival of adult males in the first week after emergence; inferior insemination capacity of the males or sub-optimal sterility levels with low dose radiation treatment. These results made us wonder whether the conditions under which the flies were irradiated led to the observed effects.

Curtis and Langley (1) and Langley *et al.* (8) have demonstrated that irradiation under low oxygen tension e.g. in a nitrogen atmosphere, reduced not only the amount of induced dominant lethals in *G. m. morsitans* males, treated in the late pupal phase, but also the level of somatic damage. The effects of dose fractionation on *G. m. morsitans* pupae of unknown age have in addition been described (2). This prompted us to examine the effect of nitrogen and dose fractionation on the radiation susceptibility of female and male *G. tachinoides* pupae during their mid-pupal phase of development. Reproduction and survival of both sexes was studied in relation to treatment atmosphere, treatment age and interval between fractions.

MATERIAL AND METHODS

Pupae and fly material

All pupae used for the experiments were derived from the *Glossina tachinoides* colony, originating from the Central African Republic, and maintained on a membrane feeding system at the Entomology Unit of the IAEA's labora-

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tory in Seibersdorf, Austria. Pupae and adult flies were kept under normal colony conditions *i.e.* 23° ± 1°C and 75 ± 5 % Relative Humidity. All flies were fed daily (except on Sundays) on equal proportions of frozen and thawed bovine and porcine blood (13).

Radiation procedures and experimental design

Batches of 100 pupae, collected on the same day and kept in Petri-dishes (diameter 5.5 cm, height 1.5 cm), were exposed in a ^{60}Co source (dose rate of 6 Gy/min) to irradiation doses ranging between 10 and 80 Gy in air and nitrogen on days 15 and 20 following larviposition. When nitrogen was used, the batches of pupae were transferred from the plastic Petri-dishes to the bottom of a glass container as described by Economopolous (3). For 15 - 20 min, nitrogen flowed through the inlet leading to the bottom of the device, allowing the oxygen to escape through the outlet. Pupae were irradiated in the device and after irradiation transferred to plastic Petri-dishes for completion of development. Maximum exposure of the pupae to the nitrogen atmosphere was 30 min.

All dose fractionation experiments were carried out under nitrogen atmosphere. Doses were split into 2 fractions separated by 1, 2 or 5 days, whereby the first dose of 10 Gy was administered on day 15 post larviposition (PL). Dose rates ranging between 10 and 70 Gy were applied for the second fraction.

Together with each experimental group, a batch of untreated control pupae was kept under standard colony conditions. Emerging flies were collected daily and transferred to standard fly holding cages. Mating procedures and experimental designs to assess male and female reproductive parameters were used as described in a previous paper (10). Male survival was assessed by recording daily mortality but the observations ended after 80 days.

RESULTS

Effect of nitrogen

Eclosion rates of male and female pupae irradiated on day 15 and 20 PL in air and nitrogen atmosphere with doses ranging from 10 to 80 Gy are presented in figure 1. The percentage adult eclosion of pupae irradiated on day 15 in air was significantly reduced as compared with pupae irradiated in nitrogen and control pupae (χ^2, $p < 0.01$). No such atmosphere-related differences in eclosion rates were found with 20-day-old pupae (χ^2, $p > 0.05$) except when the irradiation dose was increased to 80 Gy ($\chi^2 = 27.70$, $p < 0.01$). While the majority of the male flies were killed when treated in air as 15-day-old pupae with a dose of 80 Gy ($\chi^2 = 38.88$, $p < 0.01$) no such adverse effect was observed when an irradiation treatment of 60 Gy was administered in nitrogen ($\chi^2 = 0.015$, $p > 0.05$).

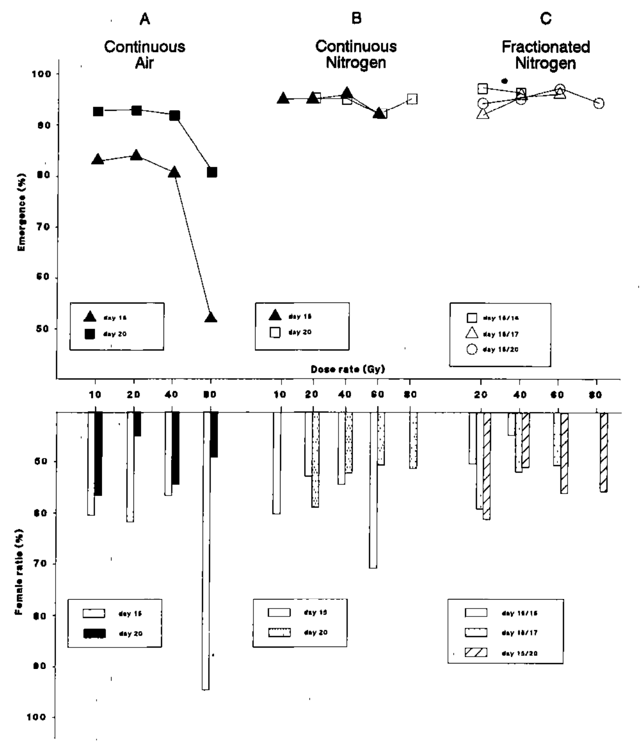

Figure 1 : Emergence rate and female ratio of G. tachinoides *pupae irradiated during the mid-pupal phase in air (A) and nitrogen (B) on day 15 and 20 following larviposition and in doses split into 2 fractions (C). (1st dose of 10 Gy given on day 15 PL, 2nd dose 1, 2 or 5 days interval).*

Fecundity of untreated females mated with males irradiated as 15 - 20-day-old pupae is presented in table I. Irradiation in nitrogen resulted in a substantial increase in male residual fertility as compared to irradiation in air. In addition, this increase in male fertility was related to the pupal age when treatment was given i.e. irradiation in nitrogen of 15-day-old pupae resulted in a twofold increase of male fertility versus an eightfold fertility increase for males irradiated as 20-day-old pupae. Fertility was decreased for the same radiation dose given at younger stages. Insemination capacity of the irradiated males was not affected, irrespective of the age of the male pupae during treatment, dose rate or irradiation atmosphere. This was evidenced by 90 to 100 % of the females, mated with treated males, displaying spermathecae impregnated with motile sperm versus 85.7 % of the control females. The average weight of all experimental pupae was significantly reduced as compared with pupae fathered by control males (Student's t-test, $p < 0.01$). Viability of the offspring was comparable with the controls (χ^2, $p > 0.05$) but whereas irradiation in air gave almost equal proportions of males and females, offspring of the pupae irradiated in nitrogen on day 20 were biased in favour of males (significant only for 20 Gy treatment group, (χ^2 6.88, $p < 0.01$). Remarkably, no such bias was observed in offspring fathered by males irradiated in nitrogen on day 15 PL. On the contrary, offspring fathered by males irradiated with 10 Gy were mostly females (91.7 %) ($\chi^2 = 14.37$, $p < 0.01$).

TABLE I
Fertility of *G. tachinoides* males, irradiated as pupae in air, nitrogen, in single and in fractionated doses and mated with untreated colony females

Irradiation 1 day/atm./dose (1)	Irradiation 2 day/atm./dose	Puparia produced (no.)	Mean puparial weight (mg) ± SD	Fecundity [3]	Production relative to control	Emergence/ females %
Control		83	18.7 ± 2.8	0.091	100	90.0 / 44.4
15 / A /10	— —	9	15.2 ± 3.2	0.017	19.1	88.9 / 3/8
20 / A /20	— —	4	12.6 ± 1.7	0.005	5.9	3/4 / 1/3
15 / N /10	— —	26	14.5 ± 2.9	0.032	34.6	88.5 / 91.7
/20	— —	18	16.1 ± 2.6	0.024	26.6	77.8 / 50.0
/40	— —	1	—	0.000	2.2	1/1 / 1/1
20 / N /10	— —	25	15.8 ± 1.6	0.041	44.6	84.0 / 19.0
/20	— —	16	16.1 ± 2.1	0.025	27.9	87.5 / 31.3
/60	— —	6	15.2 ± 1.3	0.009	9.3	5/6 / 1/5
/80	— —	6	16.2 ± 2.2	0.009	10.0	5/6 / 1/5
15 / N /10	16 / N /10	19	15.6 ± 2.5	0.022	23.6	68.4 / 30.8
	/30	3	17.3 ± 0.6	0.005	5.4	2/3 / 1/2
15 / N /10	17 / N /10	9	15.6 ± 2.4	0.025	27.1	8/9 / 2/8
	/30	4	15.2 ± 2.2	0.006	6.7	4/4 / 1/4
15 / N /10	17 / N /10	19	16.5 ± 2.5	0.032	34.6	84.2 / 37.5
	/30	9	15.1 ± 0.6	0.013	13.7	7/9 / 3/7
	/50	2	11.6 ± 2.4	0.003	3.6	1/2 / 1/1
	/70	1	—	0.002	1.9	1/1 / 0/1

1. Day: Days post larviposition, Atm. : Irradiation atmosphere (A = Air, N = Nitrogen), Dose: Gy.
2. Survival relative to mature female days.
3. No. pupae per mature female day.

Analysis of the reproductive status of the female mates revealed data confirming the fertility results *i.e.* the percentage of observed reproductive abnormalities due to radiation-induced sterility (and consequently the number of expelled dead embryos) was correlated with: pupal age when treatment was given; irradiation atmosphere and radiation dose (fig. 2). About 70 % of females mated with males irradiated in air as 15 and 20-day-old pupae with doses of 10 and 20 Gy respectively showed an empty uterus due to expulsion of the degenerating egg, or a degenerating egg *in utero*, whereas in a nitrogen atmosphere, a dose of 40 Gy was required to obtain the same amount of aberrations in the ovarian configuration and the uterus content.

Survival curves of untreated males and males treated as 15 - 20-day-old pupae in air and nitrogen are presented in figure 3. Survival of males irradiated in nitrogen was dramatically increased as compared with males irradiated in air. Whereas after 20 days, 50 and 58.5 % of the males, irradiated with 10 - 20 Gy in air as 15 - 20-day-old pupae respectively had already died, mortality was only 21.1 and 10.8 % when the same treatment was given in nitrogen. Survival curves of control males resembled those of males irradiated in nitrogen on day 20 PL with doses up to 60 Gy (> 50 % of the males surviving day 70). When the dose was increased to 80 Gy, 50 % of the

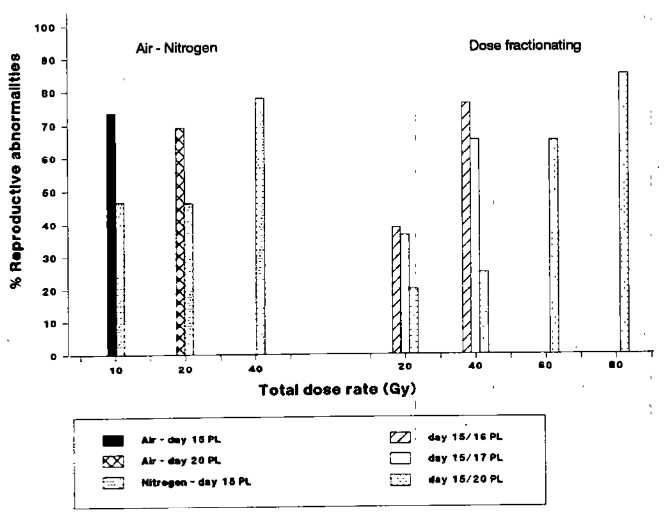

Figure 2 : Frequency distribution of reproductive abnormalities in female G. tachinoides *mated with males irradiated as pupae in air and nitrogen atmosphere and in doses split into 2 fractions 1,2 and 5 days apart.*

males were still alive on day 58 following emergence. Moreover, males treated on day 20 PL with a dose of 20 Gy in nitrogen survived better than control males (LT_{50} of 81 days *vs* 75 for control males).

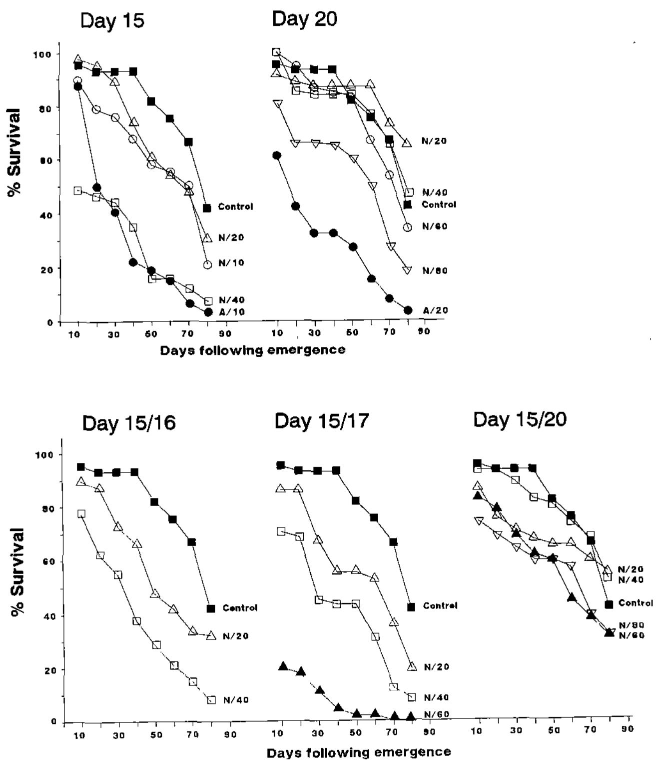

Figure 3 : Survival curves of male G. tachinoides *irradiated with continuous doses in air (A) and nitrogen (N) atmosphere on day 15 and 20 following larviposition (top graphs) and with doses split into two fractions (bottom graphs) (1st dose of 10 Gy was administered on day 15 post larviposition, 2nd dose was given 1, 2 and 5 days apart). Figures on graph indicate total dose received (in Gy).*

Fecundity of females, irradiated as 15-day-old pupae, increased from 0.035 pupa per mature female day (37.5 % residual fertility) when a 10 Gy treatment was given in air to 0.068 pupa/mature female day (74.8 % residual fertility) when the same irradiation dose was given in nitrogen (table II). No such nitrogen-related increase in fertility occurred when treated on day 20 PL (residual fertility decreased from 15.6 to 8.3 % for females irradiated with 20 Gy in air and nitrogen respectively). A high degree of sterility (> 95 %) was only observed in females treated in nitrogen with 60 and 80 Gy as 20-day-old pupae. Mating receptivity of females in all experimental groups was normal (> 95 % mating scars) except for the females irradiated in nitrogen with 40 Gy as 15-day-old pupae. The number of expelled eggs per mature female decreased from 1.5 for females treated in air with 10 Gy as 15-day-old pupae to 0.7 for treatments in nitrogen, but increased from 0.6 to 0.9 for a treatment with 20 Gy given to 20-day-old pupae. The number of expelled immature larvae, however, increased from 0.1/mature female for females treated on day 15 PL in air to 0.4/mature female for females treated in nitrogen.

Effect of dose fractionating

Splitting the radiation dose into 2 fractions separated by 1, 2 or 5 days had no significant effect on the eclosion rate of both male and female flies (fig. 1). More than 92 % of all the experimental pupae emerged which was comparable with the eclosion rate of the untreated pupae (χ^2, $p > 0.05$).

Female reproduction rates were similar when doses were split into fractions separated by 1 or 2 days as compared with reproduction of females mated with males who had received the same dose rate in a single dose on day 15 PL (table I). Increasing the time interval between the first and second dose to 5 days resulted in a higher residual male fertility as compared with fertility of males given a continuous dose on day 15 PL, but was slightly less as compared with males irradiated in a single dose on day 20 PL. Dissections revealed that males displayed normal mating vigour (> 93 % of females with mating scars) and insemination capacity with more than 90 % of their female mates being inseminated (with the exception of males irradiated in split doses (20 Gy) 2 days apart). Average weight of produced pupae was comparable with the weight of pupae fathered by males receiving a continuous dose ($p > 0.05$). Although the sex ratio of offspring was in general biased in favour of males, the differences were not significant (χ^2, $p > 0.05$).

For a given radiation dose, the proportion of reproductive abnormalities was comparable for females mated with males irradiated in a single dose on day 15 PL or with doses split 1 day apart. The percentage of reproductive abnormalities decreased when doses were separated by 2 and 5 days (fig. 2).

Survival of males treated with doses split in 2 fractions 1 and 2 days apart was slightly better (40 Gy treatment) or resembled (20 Gy treatment) the survival curve of males irradiated on day 15 with a single dose (fig. 3). Males irradiated in fractions on day 15 and 20 PL survived better for the same radiation dose as compared with males irradiated in one continuous dose on day 15 but had similar survival rates as males treated in one dose on day 20.

Females irradiated with 20 Gy in doses split into fractions had comparable survival rates as females treated with a continuous dose. However, when doses were increased to 40 Gy and administered in one dose on day 15 PL, more than 80 % of the females had died on day 40 following emergence. Survival rates increased to 66.8, 87.5 and 93.3 % when the fractions were separated by 1, 2 and 5 days. Receptivity to mating of females treated with 40 Gy in doses 1 and 2 days apart remained below the control level (< 75 % inseminated) but all females were found with motile sperm in the spermathecae when doses were separated by 5 days. Splitting the

TABLE II

Fertility of *G. tachinoides* females, irradiated as pupae in air, nitrogen, in single and in fractionated doses and mated with untreated colony males

Irradiation 1 day/atm./dose (1)	Irradiation 2 day/atm./dose	Female survival day 40 % % (2)	No. puparia produced	Mean puparial weight (mg) ± SD	Fecundity (3)	Production relative to control	No. of aborted eggs/ female	No. of aborted larvae/ female	Emergence/ females %
Control		93.8	83	18.7 ± 2.8	0.091	100	0.6	0.1	90.0 / 44.4
15 / A /10	— / — /—	100	16	15.1 ± 2.3	0.035	37.5	1.5	0.1	93.8 / 33.3
20 / A /20	— / — /—	100	5	16.0 ± 2.5	0.014	15.6	0.6	0.1	3/5 / 2/3
15 / N /10	— / — /—	86.6	70	16.8 ± 2.1	0.068	74.8	0.7	0.4	94.1 / 53.1
20	— / — /—	91.6	68	13.9 ± 3.6	0.065	71.4	1.0	0.2	73.8 / 41.7
40	— / — /—	18.3	1	—	0.007	8.1	1.1	0.3	0.0 / 0.0
20 / N /20	— / — /—	97.5	6	15.9 ± 1.5	0.008	8.3	0.9	0.0	5/6 / 4/5
40	— / — /—	93.1	7	16.4 ± 1.9	0.010	11.4	0.2	0.0	5/7 / 4/5
60	— / — /—	92.1	3	16.0 ± 1.4	0.005	4.9	0.2	0.0	2/3 / 1/2
80	— / — /—	96.1	0	—	0.000	0.0	0.1	0.0	—
15 / N /10	16 / N /10	91.2	47	14.9 ± 2.9	0.056	61.3	1.2	0.1	71.7 / 57.6
	30	66.8	9	11.5 ± 3.7	0.014	14.9	1.0	0.3	3/9 / 2/3
15 / N /10	17 / N /10	93.7	72	14.7 ± 3.4	0.060	65.8	0.8	0.2	63.6 / 42.9
	30	87.5	30	14.1 ± 3.0	0.038	41.4	1.1	0.2	55.2 / 75.0
15 / N /10	20 / N /10	96.4	2	—	0.003	2.9	1.0	0.0	2/2 / 0/2
	30	93.3	0	—	0.000	0.0	0.2	0.0	—
	50	97.8	2	—	0.003	2.7	0.0	0.0	2/2 / 1/2
	70	89.1	0	—	0.000	0.0	0.0	0.0	—

1. Day: Days post larviposition, Atm. : Irradiation atmosphere (A = Air, N = Nitrogen), Dose: Gy.
2. Survival relative to mature female days.
3. No. pupae per mature female day.

radiation dose in two fractions reduced fecundity of the females slightly (61.2 and 65.8 % residual fertility for fractions given 1 and 2 days apart *vs* 71.4 % when the dose was given on day 15 PL). When fractions were separated by 5 days, residual fertility remained below 3 %, irrespective of the irradiation dose.

Optimal radiation procedures

Figure 4 presents the relationship between male fertility (expressed as the number of pupae produced (per mature female day) of their female mates) and survival rate expressed as LT_{50} (time elapsing to obtain 50 % mortality). The top graph shows that irradiation on day 15 PL in nitrogen resulted in a 25 % loss in sterility as compared with irradiation in air, but survival was increased threefold (from LT_{50} of 20 to 68 days). The level of sterility was even more reduced (40 % loss) when the irradiation in nitrogen treatment was administered on day 20 PL, but average longevity was increased 4 times. The bottom graph indicates that fractionating of the doses was not accompanied with a loss in sterility except when fractions were separated by 5 days. Viability was however considerably increased. High-quality (LT_{50} > 20 days) sterile (residual fertility < 5 %) males could be obtained by irradiating pupae in nitrogen during the mid-pupal phase in 2 fractions separated by 1 and 2 days (total dose 40 Gy) and 5 days apart (total dose of 60 and 80 Gy).

DISCUSSION

The effect of irradiation in the absence of oxygen on the amount of induced lethal mutations and of somatic injury is very well documented for various insects. The protective effect of nitrogen is related to the absence of hydrogen peroxide, a mutation-inducing molecule which is produced as oxygen reacts with hydrogen atoms originating from water ionized by radiation (3). The use of nitrogen during irradiation of *G. tachinoides* pupae in the mid-pupal phase (day 15 - 20 following larviposition) reduced sterility levels of adult males by 20 to 40 % as compared to irradiation in air and increased their average longevity 3 to 4 times. These results are in agreement with the findings of Curtis and Langley (1) on *G. m. morsitans* pupae, irradiated in the late pupal phase.

The effect of dose fractionation on male fertility seems dependent on the insect species and the development stage when the fractions are applied. Similar levels of sterility were obtained after exposing male pupae of *Spodoptera littoralis* Boisd. (cotton leaf worm) to X-rays in one continuous dose or in various fractions (12). Likewise, exposure of *Ceratitis capitata* (Mediterranean fruit fly) and

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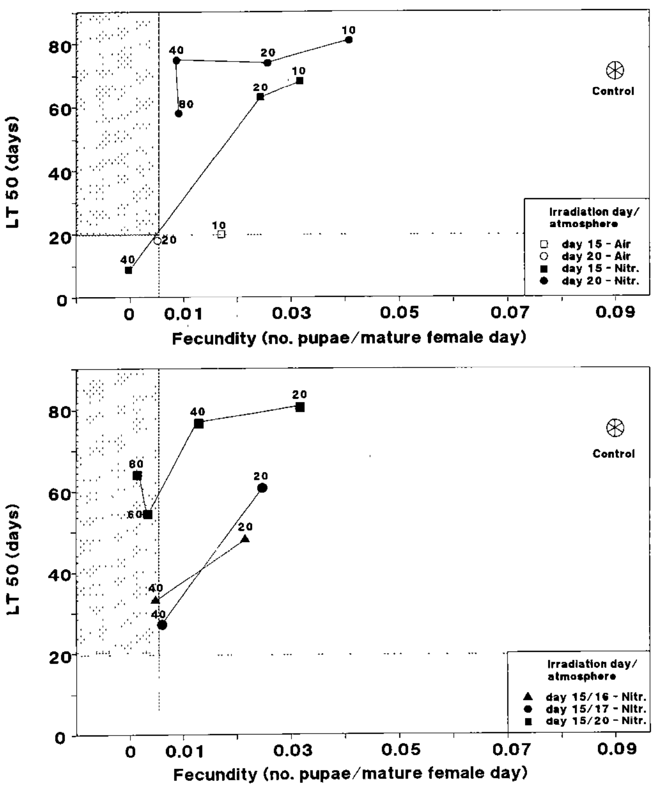

Figure 4 : Fertility and LT50 of male G. tachinoides *irradiated as pupae in air and nitrogen atmosphere (top graph) on day 15 and 20 following larviposition and in doses split into two fractions (bottom graph) (1st dose of 10 Gy was given on day 15 PL, 2nd dose on day 16, 17 or 20 PL). Figures on graph indicate the irradiation dose in Gy. Shaded area indicates males with an LT50 above 20 days and a residual fertility < 5 % of untreated controls.*

Sitophilus granarius pupae to fractionated doses of gamma radiation resulted in the same levels of induced lethal mutations as those obtained after continuous exposure of the same total dose (6, 9). Slightly lower levels of induced sterility were obtained with sperm of male *G. m. morsitans* treated in fractionated doses (2). These experiments with *G. tachinoides* pupae treated during the mid-pupal phase indicate that sterility levels are significantly influenced by the timing of the radiation treatment *i.e.* a treatment given in a single dose on day 15 or split into fractions on day 15, 16 or 17 resulted in similar levels of sterility. All these treatments coincide with the process of spermiogenesis occurring between day 12 and 18 (5). Spermatids have been formed and irradiation in a single dose or in fractions seems not to influence the degree of chromosomal damage. However, when the treatment interval is increased to 5 days, the second dose is administered to sperm reaching maturity. A higher second fraction is then required to obtain the same level of sterility. Moreover, these males showed lower fertility levels as compared with males treated in one continuous dose on day 20 PL, indicating a differential radiosensitivity of spermatids and spermatozoa related to age.

Even more significant is the increase in average male longevity when doses are split into 2 or more fractions, although some insect species e.g. the adult boll weevil (*Anthonomus grandis*) are exceptional in this respect (4). The reduction in somatic injury by dose fractionation is generally attributed to repair mechanisms at the molecular level or replacement of damaged cells during the interval after the first radiation dose (6). Our data with *G. tachinoides* pupae indicate that cell recovery and consequently male survival are significantly influenced by the timing of treatment, the interval between fractions and the total dose received. Dose fractionation had no significant impact on fly survival with low doses (20 Gy) administered at 1 or 2 day intervals. The increase in survival became more apparent with higher radiation doses and longer intervals. However, cytological studies, analyzing the impact of gamma radiation on spermatids, spermatozoa and on the mechanism of cell damage and repair, would be required to clarify some of our observations.

From a practical point of view, the most meaningful result from our experiments is the observation that *G. tachinoides* pupae can be irradiated during the mid-pupal phase to obtain sterile males with survival rates comparable to those of untreated males. For this, however, the pupae must be irradiated in a nitrogen atmosphere and the dose must be split into at least 2 fractions. (The effect of more fractions has not been studied as Jefferies (6) observed that the most significant somatic recovery occurs between the first and second fraction.) Although these procedures require certain logistic prerequisites and are more cumbersome than simple radiation treatments in air, more flexibility is allowed in the management of long-distance shipment of pupal material. Moreover, various reports have indicated the superior competitiveness of insects treated in nitrogen (1) and with doses split into fractions (9). More in-depth laboratory and field studies on the behaviour and competitiveness of *G. tachinoides* males when treated in the mid-pupal phase could consolidate our observations of their high biological quality and could prove the procedure to be a valid option for application in the field.

ACKNOWLEDGEMENTS

The authors 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, Vienna for reading the manuscript. In addition, our thanks are due to the technical staff of the Entomology Unit, Agency's laboratory, Seibersdorf for the pupal collection, feeding and maintaining of the experimental flies. The assistance of Dr. H. Barnor during some of the fly dissection work is highly appreciated.

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VREYSEN (M.J.B.), VAN DER VLOEDT (A.M.V.). Radiation sterilization of *Glossina tachinoides* Westw. pupae. I. The effect of dose fractionation and nitrogen during irradiation in the mid-pupal phase. *Revue Élev. Méd. vét. Pays trop.*, 1995, **48** (1): 45-51

A study was carried out to analyze the effect of nitrogen during radiation and dose fractionation on *Glossina tachinoides* pupae during the mid-pupal phase (day 15 - 20 following larviposition (PL)). The radiation protective effect of nitrogen during treatments of 10 - 80 Gy of 15 - 20-day-old pupae was demonstrated by an increased total eclosion rate (for 15-day-old pupae), higher residual male fertility levels (34.6 and 44.6 % for 15 and 20-day-old pupae treated in nitrogen respectively versus 19.1 and 5.9 % for treatments in air) and distinctive longer life spans. The proportion of reproductive abnormalities observed in their female mates increased with increased radiation dose, when treated at younger pupal stages and following treatment in air. After treatment of 15-day-old pupae with 10 Gy in nitrogen, female fertility was 0.068 pupae per mature female day as compared to 0.035 pupae/m.f.d. in air. No such increase was observed when treated as 20-day-old pupae. A dose of 60 - 80 Gy in nitrogen administered to 20-day-old female pupae was required to obtain 95 % sterility. Splitting the radiation dose in nitrogen atmosphere in 2 fractions 1, 2 and 5 days apart (first dose of 10 Gy given on day 15 PL) did not influence the total eclosion rate, mating response or insemination capacity of the male flies. Sterility of males treated in fractions separated by 1 and 2 days was similar to the level in those given a continuous dose on day 15 PL but the level of induced lethal mutations decreased with fractions separated by 5 days. Survival of the males treated in fractionated doses was similar as compared with males treated with one continuous dose on day 20 PL but better when compared with males treated with one continuous dose on day 15 PL. Female fecundity was reduced by splitting the radiation dose in fractions 1 and 2 days apart. Complete sterility was induced in female pupae when fractions were separated by 5 days, irrespective of the radiation dose used in this study. Irradiation of *G. tachinoides* pupae in the mid-pupal phase in nitrogen with doses split in 2 fractions separated by 1 or 2 days (total dose of 40 Gy) or 5 days (total dose of 60 - 80 Gy) resulted in high quality (average longevity > 20 days), sterile (residual fertility < 5 %) male flies.

Key words : *Glossina tachinoides* - Pupa - Sterilization - Irradiation - Nitrogen - Fractionation - Insect control - Hatching - Fertility - Longevity.

VREYSEN (M.J.B.), VAN DER VLOEDT (A.M.V.). Esterilización por radiación de *Glossina tachinoides* Westw. pupas. I. Efecto de una dosis fraccionada y del nitrógeno durante la irradiación en la fase pupal media. *Revue Élev. Méd. vét. Pays trop.*, 1995, **48** (1) : 45-51

Se estudió el efecto del nitrógeno durante la irradiación de las pupas de *Glossina tachinoides*, asi como el fraccionamiento de las dosis de irradiación en el medio de la fase pupal (15 a 20 d después de la postura de las larvas). Se demostró el efecto protector del nitrógeno contra las irradiaciones de 10 a 80 Gy, en pupas de 15 a 20 días, mediante el aumento de la tasa global de eclosión, por el aumento de los niveles de fertilidad residual en los machos, así como por una prolongación de la visa media. La proporción de anomalías de la reproducción observadas en las hembras acopladas, aumentó con la dosis de irradiación, en las pupas jóvenes, así como cuando el tratamiento se administró en el aire. Después del tratamiento, las pupas de 15 d, bajo nitrógeno y a dosis de 10 Gy, alcanzaron una fertilidad de 0,068 pupa por hembra adulta por día, contra 0,035 pupa en caso de irradiación en el aire. Este aumento no fue observado durante el tratamiento de las pupas de 20 d. Una dosis de 60 a 80 Gy fue necesaria para alcanzar una esterilidad del 95 p. 100 de las pupas hembras de 20 días. La division de la dosis de irradiación bajo nitrógeno, en 2 fracciones a intervalos de 1, 2 o 5 días no influyó sobre la tasa global de eclosión, la tasa de copulación o la capacidad de inseminación de los machos. La esterilidad de los machos tratados con dosis fraccionadas, separadas por 1 o 2 d, fue idéntica a la de los machos tratados con una dosis única al día 15 post-postura, pero la tasa de mutaciones letales inducidas fue reducida con las dosis fraccionadas, separadas por intervalos de 5 d. La sobrevida de los machos tratados con dosis fraccionadas fue similar a la de los machos tratados con una sola dosis al día 20, pero fue superior a la de los machos tratados al día 15. La fecundidad de las hembras se redujo con el fraccionamiento de la dosis de irradiación a intervalos de 1 y 2 días. La esterilidad completa en las pupas hembras fue obtenida con un intervalo entre las dosis fraccionadas de 5 d, independientemente de la dosis utilizada. La irradiación de las pupas de *G. tachinoides* en medio de la fase pupal, bajo atmósfera nitrogenada, con dosis fraccionadas separadas por 1 o 2 d (dosis total de 40 Gy) o 5 días (dosis total de 60 a 80 Gy), condujo a una major esterilización de los machos (longevidad superior a 20 días en promedio y fertilidad residual inferior a 5 p. 100).

Palabras clave : *Glossina tachinoides* - Pupa - Esterilización - Irradiación - Nitrógeno - Fraccionamiento - Lucha contra los insectos - Eclosión - Fertilidad - Longevidad.