The use of gamma irradiated *Glossina austeni* females as sentinel insects for entomological monitoring in tsetse control programmes

M.J.B. Vreysen [1]*

A.M.V. Van der Vloedt [2]

VREYSEN (M.J.B.), VAN DER VLOEDT (A.M.V.). Utilisation de femelles *Glossina austeni* irradiées par rayons gamma comme insectes sentinelles dans les programmes de lutte contre les glossines. *Revue Élev. Méd. vét. Pays trop.*, 1992, **45** (3-4) : 303-309

Un traitement aux rayons gamma administré à des femelles de *Glossina austeni* à la dose de 60 Gy, le 2e ou le 9e jour après éclosion, et à la dose de 50 Gy à l'état pupaire, soit le 33e jour après larviposition, a induit une stérilité complète chez les mouches femelles sans changer leur comportement sexuel. Les femelles irradiées restent réceptives à l'accouplement avec des mâles non traités jusqu'au 15e jour après éclosion (84 p. 100). Le moment du traitement (33 jours après larviposition, 2 et 9 jours après éclosion) influence significativement la dynamique du développement des follicules. Toutes les femelles traitées montrent un développement normal des follicules en position A_1 et C_1, c'est-à-dire une vitellogenèse, maturation et ovulation normales. Les follicules en position B_1 et D_1 ne se développent pas quand les femelles sont traitées à l'état pupaire et, à partir du 15e jour, on observe des ovaires inactifs caractérisés par une atrophie des ovules et des cellules nourricières. L'irradiation des femelles âgées de 2 et 9 jours entraîne un degré de développement différent des follicules B_1 et D_1. Lors des expériences en laboratoire où des mâles non traités sont mis en présence de femelles vierges, traitées et non traitées en nombre égal, aucune tendance significative de copulation préférentielle n'a été mise en évidence. Des accouplements multiples sont observés quand des femelles traitées et non traitées sont mises en présence de mâles en plusieurs occasions. Agées de 9 jours, 24,0 p. 100 des femelles non traitées et 23,8 p. 100 des femelles traitées aux rayons gamma (60 Gy) le 2e jour après éclosion, s'accouplent pour la 4e fois. La réceptivité aux accouplements multiples diminue avec une dose d'irradiation plus forte (120 Gy) et quand le traitement a eu lieu sur des femelles plus âgées. Les résultats de ces expériences de laboratoire sont discutés en vue d'utiliser des femelles stériles de *G. austeni* comme insectes sentinelles dans les zones à faible densité de glossines, mais surtout, dans les régions ayant bénéficié d'un programme de lutte contre les tsé-tsé pour exposer les populations résiduelles éventuelles de glossines. *Mots clés* : *Glossina austeni* - Femelle - Stérilisation -Irradiation gamma - Lutte anti-insecte.

INTRODUCTION

Entomological monitoring using biological evaluation systems, provides essential information during tsetse control and eradication operations on the degree of control achieved at any given time. Monitoring and consequently the successful planning of a campaign depends primarily on the reliability and efficiency of the monitoring device. Serious problems are experienced by tsetse control officers when the wild fly population drops below the threshold level of detection of the used trapping device. Therefore, the release-recapture of gamma sterilized female tsetse flies was postulated (8) as an efficient method for the detection of low density fly populations and especially, at the end of the control programme, to confirm the status of eradication. Evidence for mating with a wild male is revealed by sperm impregnation of the spermathecae of the recaptured female and for *palpalis* species by the presence of mating scars. First laboratory observations with gamma treated *G.p. palpalis* females, revealed a normal receptivity to mating and insemination rate (8).

On the island of Unguja (Zanzibar), *Glossina austeni* is the only tsetse species present and solely responsible for the cyclical transmission of trypanosomosis (3). Control operations, undertaken by the Tsetse Unit of the Department of Livestock Development are assisted by the UNDP/FAO Animal Disease Control project and IAEA Technical Cooperation project "The eradication of *G. austeni* from Unguja by means of the Sterile Insect Technique". Monitoring operations however, are hampered by the elusiveness of the fly and the low apparent densities over large parts of the island. The use of virgin gamma sterilized female *G. austeni* is anticipated to increase the efficiency of the global monitoring activities on the island.

Therefore, laboratory studies were initiated to examine :

- the dynamics of follicle development in gamma treated females ;
- receptivity, insemination status and fecundity of females in relation to timing of treatment and female age at mating ;
- the rate of multiple mating of untreated and treated females ;
- the mating preference of untreated males in the presence of equal densities of treated and untreated females at different ages.

MATERIAL AND METHODS

Experimental flies

The flies used in the experiments originated from Unguja (Zanzibar) island, United Republic of Tanzania. They are maintained at the Entomology Unit of the IAEA Laboratories in Seibersdorf (Austria) on a membrane feeding system since 1986. The flies were kept under

1. Entomology Unit, Joint FAO/IAEA Programme, IAEA Laboratories, A-2444 Seibersdorf, Autriche.

2. Insect and Pest Control Section, Joint FAO/IAEA Division, Vienne, Autriche.

* Adresse actuelle : Department of Livestock Development, Ministry of Agriculture, POB 159, Zanzibar, Tanzanie.

Reçu le 12.11.1992, accepté le 12.1.1993.

constant climatic conditions of 24 ± 1 °C and at a relative humidity of 85 ± 5 % and fed 6 times a week on frozen and thawed bovine blood through a silicone rubber membrane.

Experimental procedures

Follicle development in gamma treated females

Virgin females were given a treatment (60 Gy in air) in a ^{60}Co source on day 2 or 9 following emergence and a batch of pupae was treated (50 Gy in air) on day 33 following larviposition. Treated females were mated when 2 days old with untreated sexually mature colony males. After separation, females were kept in standard colony cages and groups of at least 5 females were dissected every 5 days for examination of the insemination status and ovarian configuration. Follicle length was measured with an ocular micrometer in the eyepiece of a phase-contrast Leitz compound microscope at x 64 magnification.

Mating response and fecundity of untreated and irradiated females

Virgin females and a batch of pupae were exposed to gamma radiation in air at doses of 60 Gy on day 2 or 9 following emergence and 50 Gy on day 33 post larviposition respectively. Receptivity of 3 to 15-day old treated (n = 40) and untreated females (n = 40) was assessed by exposing them (20 females per cage) to the same number of sexually mature males in a cage with a diameter of 20 cm and 4.5 cm high. Immediate mating response was expressed as the number of females observed *in copula* within the first 30 min of confinement. Mating pairs were isolated in plastic tubes (2.5 cm diameter and 6 cm high) and copulation time recorded. After separation, females were pooled in standard colony cages and survival and pupae production monitored for 45 days. Larviposition receptacles were examined for aborted eggs and immature larvae every 5 days. At the end of the experimental period, all females were dissected and their reproductive system examined microscopically.

Multiple mating behaviour

Virgin females were given an irradiation treatment (60 and 120 Gy in air) on day 2 (group I) and day 5 (group II) post emergence. A first mating opportunity was offered on day 3 (group I) and day 5 (group II) and receptivity assessed as described above. After termination of the mating act however, males were immediately removed, females left singly in the plastic tubes and on day 4, 7, 9 and 15 for the first group and on day 6, 8, 11 and 14 for the second group, the females were offered a 2nd, 3rd, 4th and 5th mating opportunity.

Mating preference and receptivity tests

The mating preference of untreated males and the receptivity to mating of females with increasing age was assessed by exposing the males to equal numbers of treated and untreated females. Each of 5 containers (32 cm high, 20 cm diameter) was filled with differently colour-marked untreated (UT) (N=40) and 60 Gy treated (T) (N=40) females of the following ages : +2 days, +5 days, +8 days, +11 and +14 days. For each container, 4 x 10 males were introduced, the mating pairs were removed and the type of female determined.

RESULTS

Follicle development of irradiated females

Follicle development of females treated as pupae with 50 Gy on day 33 following larviposition and with 60 Gy on day 2 and 9 following emergence is illustrated in figure 1. In all females, the radiation treatment did not inhibit vitellogenesis supported by the nurse cells of the A_1 (first follicle in ovulation sequence A.C.B.D., internal right) and C_1 follicle (second follicle to ovulate, internal left) resulting in normal growth and maturation. Only in one 20 day-old female (treated in pupal stage) was maturation of the C_1 follicle interrupted. Mature oocytes in position A_1 and C_1 ovulated normally in all flies, but no development occurred *in utero* beyond the egg stage. All degenerating A_1 and C_1 oocytes were extruded or absorbed.

No differentiation between oocyte and nurse cells was observed for the B_1 and D_1 follicles (3rd and 4th in ovulation sequence, respectively) in 89.6 % of the females treated as pupae (fig. 1a). Evidence for an initial development of a B1 follicle was found in 3 females (10.3 %), but development was inhibited before reaching the maturation stage. From day 15 on, all flies displayed inactive ovaries characterized by atrophied oocytes and nurse cells.

In 85 % of 15-21 day old females, treated on day 2 following emergence (fig. 1b), the oocyte in position B_1 could be differentiated from its nurse cells but no advanced stage of maturation of the B_1 follicle was found. Consequently, no flies were observed with an ovulated B_1 *in utero*. Maturation of the B_1 follicle was inhibited in 38.5 % of 25-35 day old females, whereas, complete atrophy was observed in 61.5 % of the females. No evidence of development was found for a follicle in position D_1 (4th in ovulation sequence, external left).

A normal development of the B_1 follicle was observed in 55 % of 28-35 day-old females treated on day 9 following emergence (fig. 1c). However, in 35 % of the dissected females, the maturation process was inhibited. Young differentiated D_1 follicles were observed in 40 % of the dissected females from day 21 on, but none fully matured.

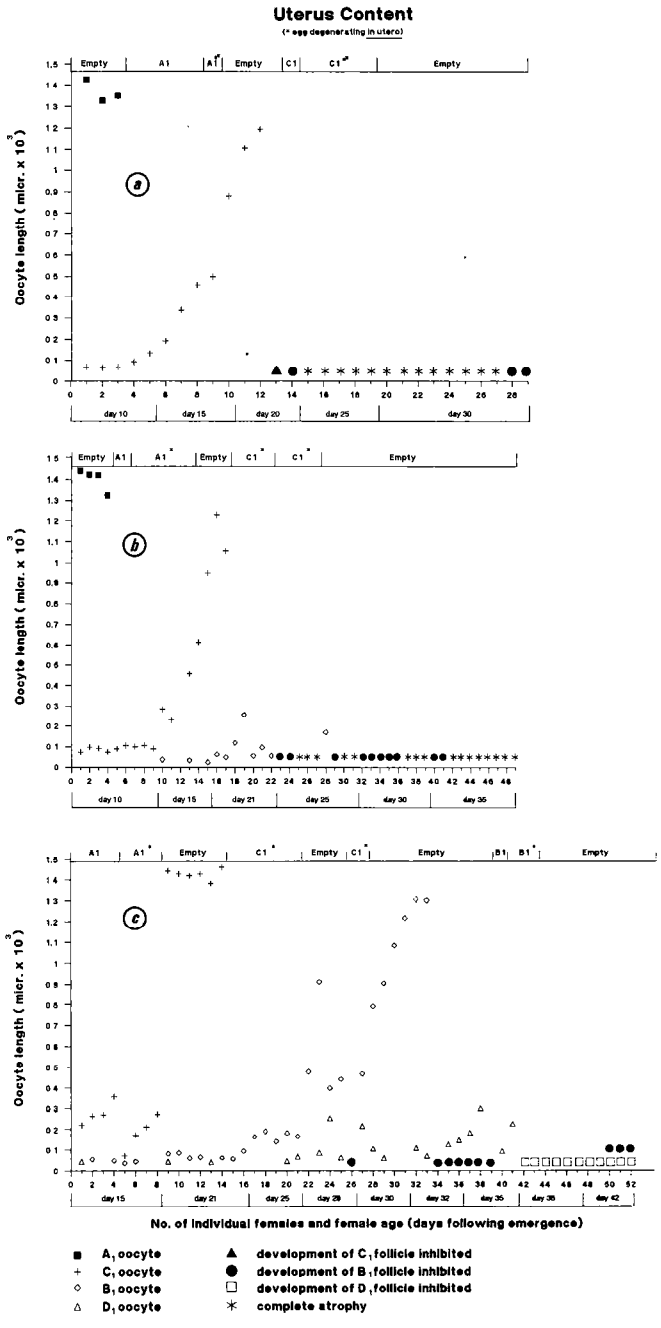

Fig. 1 : Follicle development and uterus content of G. austeni *females, irradiated with 50 Gy on day 33 post-larviposition (a), with 60 Gy on day 2 following emergence (b), and with 60 Gy on day 9 following emergence (c). All females were mated with untreated males.*

Receptivity and fecundity of untreated and irradiated females

Data on receptivity and performance of treated and untreated females are presented in table I. Untreated females displayed the highest receptivity on day 3 and 5 following emergence, with 91 and 86 % of the females observed *in copula* within 30 min. Mating of untreated females at older ages (7-15 days old) resulted in a mating response of at least 77 %. No major differences in receptivity were observed for treated females as compared to control females, with neither the moment of treatment nor the age at mating having any influence. The duration of copulation decreased in accordance with increasing age at mating *i.e.* 92 % of untreated females, mated on day 3, copulated longer than 120 min, whereas 59.5 and 91.5 % of untreated females mated on day 12 and 15 respectively, were observed *in copula* for less than 120 min. The same trend was found with irradiated females. The radiation treatment administered to pupae 3 days before emergence and to adult females had no deleterious effect on their survival (> 92 % by day 45 for all treatment groups) and insemination rate (> 95 %). Fecundity of the untreated control females decreased from 0.084 pupae/mature female day for females mated on day 3 to 0.068 and 0.052 pupae/mature female day for females mated on day 12 and 15, respectively. In addition, the number of extruded eggs/initial female increased from 0.02 (females mated on day 3) to 0.35 (females mated on day 15). No larvae were produced by the females of the different treatment groups and the number of recovered dead eggs varied from 0.9 to 1.7 per initial female.

Dissection of the females on day 45 revealed marked differences in the reproductive status of untreated and treated females. As expected, untreated females always displayed an egg undergoing normal embryogenesis or a developing larva *in utero* whereas treated females were always found with a degenerating egg *in utero* or an empty uterus due to recent expulsion of the egg. Dissection results of the treated females confirmed the above made observations on follicle dynamics, *i.e.* 97.1, 18.9 and 16.7 %, respectively of the females treated as pupae, as adults on day 2 and 9 after emergence were found with completely inactivated ovaries on day 45 (oocyte degeneration and atrophied nurse cells). Inhibition of the maturation process of the follicle in position B_1 and D_1 was found in 2.9, 81.1 and 83.3 %, respectively of the females treated as pupae, on day 2 and 9 following emergence.

Multiple mating rate

A high rate of multiple mating was observed when untreated females and females treated with 60 Gy on day 2 were offered several mating opportunities (fig. 2). It was observed that 74.2, 52.0 and 24.0 % of untreated females and 77.1, 38.9 and 23.8 % of treated females, respectively accepted a male on a second, third and fourth mating

M.J.B. Vreysen A.M.V. Van der Vloedt

TABLE I Receptivity and fecundity of irradiated **G. austeni** *females, treated as puparia and at various ages following emergence. Adult females were treated with 60 Gy, puparia with 50 Gy.*

Treatment	Mating	Mating[1] Response (%)	Duration of copulation (min)				Insemination rate (%)	Survival[2] rate (%) by day 45	Fecundity[3]	Extruded eggs recovered (Number/ female)
			< 60 %	60 – < 120 %	120 – < 180 %	> 180 %				
Control	day 3	91.1	0.0	7.3	46.3	46.3	100	99.0	0.084	0.02
Control	day 5	86.7	7.7	5.1	59.0	28.2	91.4	99.7	0.085	0.17
Control	day 7	77.8	5.7	34.3	51.4	8.6	97.0	97.1	0.076	0.24
Control	day 12	82.2	2.7	56.8	37.8	2.7	97.1	96.3	0.068	0.11
Control	day 15	77.8	22.9	68.6	8.6	0.0	93.8	93.2	0.052	0.35
day 33 PL*	day 3	84.4	2.6	7.9	34.2	55.3	97.3	92.8	0.000	0.97
day 33 PL*	day 9	64.7	20.0	20.0	50.0	10.0		no record		
day 33 PL*	day 14	85.0	5.9	52.9	41.2	0.0		no record		
day 2	day 3	86.7	0.0	7.9	39.5	52.6	97.3	94.9	0.000	0.95
day 2	day 7	77.8	14.7	26.5	52.9	5.9	96.9	97.0	0.000	1.09
day 2	day 9	68.9	10.0	66.7	23.3	0.0	96.4	99.5	0.000	1.46
day 2	day 15	84.4	23.7	63.2	10.5	2.6	95.0	100	0.000	1.28
day 9	day 10	80.0	5.6	30.6	44.4	19.4	100	97.3	0.000	1.73

(1) Observed pairs during the first 30 min of confinement.
(2) Survival relative to mature female days.
(3) No. puparia/mature female day.
(*) Irradiated as puparia.

occasion. None of the untreated females (n = 6) accepted a male on day 15 (5th mating occasion) but 2 treated females (n = 5) were willing to copulate for the fifth time. A higher radiation dose administered on day 2, gave a mating response of 83.8 % on a first mating occasion (day 3), but receptivity dropped to 56.1, 29.7 and 9.1 % on later mating opportunities. Receptivity to remating decreased when the treatment was given on day 5 following emergence.

An average mating time of 208 ± 54 min, 199 ± 58 min and 200 ± 58 min was recorded for the first mating of untreated, 60 Gy and 120 Gy treated females, respectively. Although variations occurred in average mating time for later matings, both untreated and treated females tended to mate less long with increasing age and number of matings (fig. 2).

Mating preference and receptivity

Table II indicates that untreated males, irrespective of the age of the females on mating day, had no obvious preference for untreated or treated females. Although treated females were more receptive to mating in most of the test series, no significant deviations from the 1:1 (UT:T) ratio were found ($p > 0.05$).

TABLE II Receptivity of untreated (UT) and treated (T) (60 Gy in air) **G. austeni** *females in equal population densities (2 x 40) and mating preference of untreated* **G. austeni** *males.*

	FEMALE AGE (days following emergence)									
	+ 2 days		+ 5 days		+ 8 days		+ 11 days		+ 14 days	
Number of males introduced	Type of female found in copula UT	T	Type of female found in copula UT	T	Type of female found in copula UT	T	Type of female found in copula UT	T	Type of female found in copula UT	T
10	4	6	4	6	5	5	4	6	5	5
10	4	6	5	5	2	8	5	5	5	5
10	4	6	5	5	6	4	7	3	6	4
10	5	5	5	5	3	7	6	4	4	6
Total	17	23	19	21	16	24	22	18	20	20

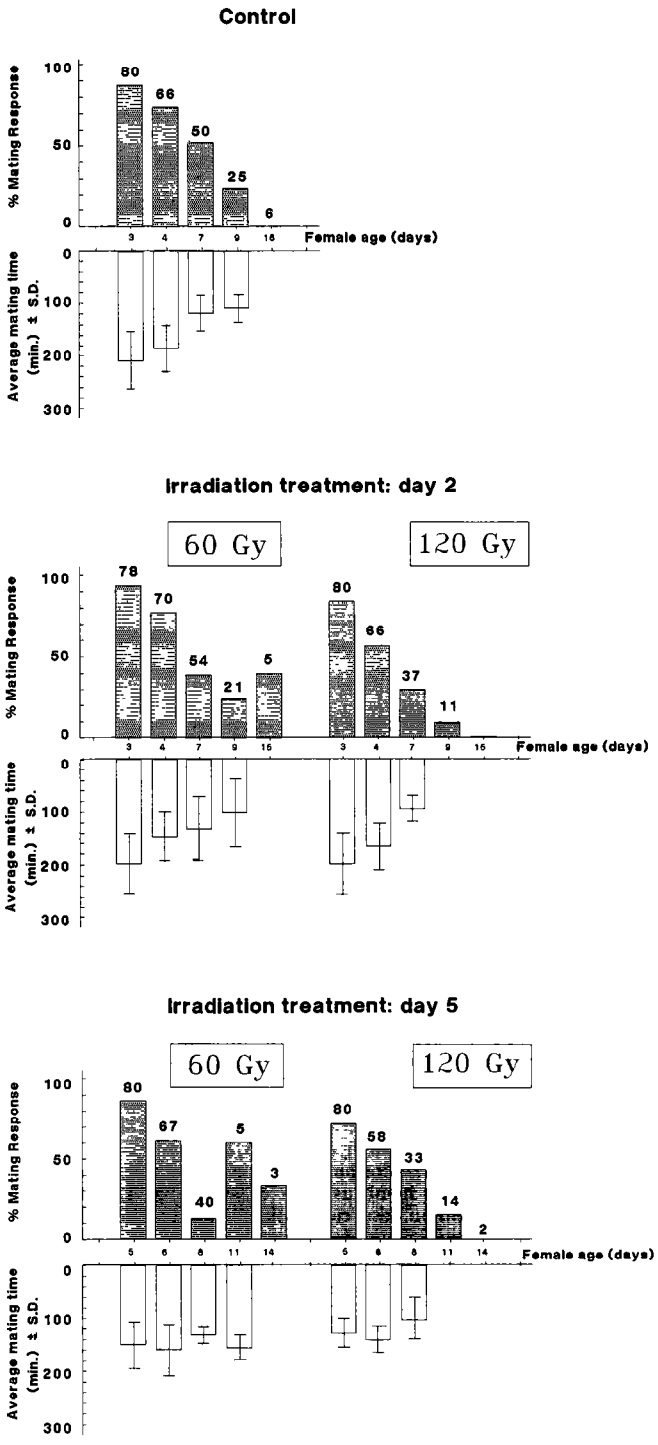

Fig. 2 : Mating response and average mating time of untreated and irradiated G. austeni *females, mated with untreated males, during 5 mating opportunities (on day 3, 4, 7, 9 and 15 for control and treatment group day 2 ; on day 5, 6, 8, 11 and 14 for treatment group day 5). (Figures on top of bars indicate number of experimental females)*

DISCUSSION

The findings of VAN DER VLOEDT *et al.* (8) that relatively low doses (50-60 Gy) of gamma radiation results in complete sterility in female *Glossina palpalis palpalis*, without altering their mating behaviour are corroborated by our study with female *G. austeni.* In addition, during laboratory cage tests with equal numbers of untreated and treated females, untreated males showed no significant preference to mating with either treated or untreated females.

Moreover, the present study revealed that the age of the female when the radiation treatment is administered influences significantly the dynamics of the follicle development. SAUNDERS (7) showed that the ovarian development in untreated tsetse females is characterized by a sequential maturation of the 4 egg follicles. Although ovaries are already present in the pupae seven days after larviposition (6), differentiation of oogonia into oocyte and nurse cells in the internal ovarioles and subsequently the onset of vitellogenesis supported by the nurse cells is initiated 5-10 days before emergence (9). In general, radiosensitivity of cells can be related to their proliferative activity and mitotic figure, and inversely to their degree of differentiation (2). The most radiosensitive stage encountered in *G.p. palpalis* is when the nurse cells undergo endomitotic replication of chromosomal material (9). From our observations on follicle dynamics in female *G. austeni*, endomitosis in follicles A_1 and C_1 is apparently already completed at the moment of irradiation, even when the treatment is given on day 33 post larviposition. In addition, nurse cell function was not adversely affected by the radiation treatment as vitellogenesis could proceed to completion resulting in fully matured follicles in position A_1 and C_1.

In females treated as pupae, the absence of any differentiation between oocyte and nurse cells of follicles in position B_1 and D_1 may be associated with the lethal treatment given to the germinal tissue. No development is observed beyond the maturation of follicles A_1 and C_1, and the ovaries enter the phase of inactivity with females displaying completely atrophied ovaries. Treatment given to adult females resulted, however, in different degrees of development of the follicles in position B_1 and D_1, depending on the timing of the treatment. The failure to form mature ova was more pronounced when treatment was given in early life. Treating 2-day old females apparently coincided with nurse cell endomitosis of follicle B_1 and consequently, normal nurse cell function was hampered resulting in cessation of the vitellogenesis process. In addition, the fact that follicles in position B_1 did not reach maturity in 35 % of the females treated on day 9, might be an indication of radiation-induced damage to the oocyte nucleus and its adverse effect on normal nurse cell function (5).

The willingness of female *G. austeni* to accept more than once a mating in captivity was demonstrated by CURTIS (1). The same observation was made for *Glossina palpalis palpalis* by JORDAN (4) and VAN DER VLOEDT *et al* (10). The present study not only revealed that irradiated female *G. austeni* exhibit an extensive multiple mating behaviour, but also that higher radiation doses (120 Gy) and treating flies later in life (day 5) tend to decrease their remating ability.

Our findings on receptivity, mating behaviour and mating preference strongly indicate the feasibility of using radiosterilized *G. austeni* females in the field in release-recapture exercises. After completion of control operations, it is of uttermost importance to have the assurance that no residual fly populations are present which can re-infest cleared areas. The method, however, will be limited to a yes/no determination whether relic fly pockets exist or not. Our observations on the extensive multiple mating capacity of irradiated females in captivity and the absence of mating scars (which can expose multiple matings in *palpalis* species) indicate that quantitative assessments of population densities might not be feasible. Nevertheless, this high rate of re-mating of irradiated females could be exploited in operational eradication campaigns, by releasing large quantities of sterile females in an area where prior suppression of the wild fly population has been achieved, and where releases of sterile males have not yet been initiated. The high frequency of mating of the released females might cause severe sperm depletion in wild males.

ACKNOWLEDGEMENTS

The authors would like to thank Drs R. GINGRICH and W. KLASSEN for reviewing the manuscript. We thank also the technical staff of the Tsetse Unit of the Seibersdorf Laboratory for their assistance.

VREYSEN (M.J.B.), VAN DER VLOEDT (A.M.V.). The use of gamma irradiated *Glossina austeni* females as sentinel insects for entomological monitoring in tsetse control programmes. *Revue Élev. Méd. vét. Pays trop.*, 1992, **45** (3-4) : 303-309

A 60 Gy gamma treatment administered to female *Glossina austeni* on day 2 or 9 following emergence, and likewise, a 50 Gy gamma treatment given to pupae on day 33 following larviposition, induced complete sterility in the female flies without altering their mating behaviour. Treated females remained receptive to mating with untreated males up to 15 days following emergence (mating response of 84 %). The timing of treatment (on day 33 post larviposition, on day 2 and 9 following emergence) influenced significantly the dynamics of the follicle development. Females, irrespective of their age when treatment was received, showed a normal development pattern of the follicles in position A_1 and C_1 *i.e.* normal vitellogenesis, maturation and ovulation. Females treated as pupae however, revealed no visible signs of a development of follicles in position B_1 and D_1. From day 15 on, females displayed inactive ovaries characterized by atrophied oocytes and nurse cells. Treating females on day 2 or 9 of their adult life, resulted in various degrees of development of the B_1 and D_1 follicles. During laboratory cage tests, untreated males exposed to equal numbers of virgin untreated and treated females, showed no significant preference to mating with either type of female. A high degree of multiple mating was observed when untreated and treated females were offered several mating opportunities. On day 9 following emergence, 24,0 % of untreated and 23.8 % of females treated with 60 Gy (on day 2), accepted a male during a fourth mating occasion. The receptivity to remating decreased with a higher radiation dose (120 Gy) and when treatment was given later in the female life (day 5). The results of the laboratory experiments are discussed in view of deploying gamma sterilized female *Glossina austeni* for entomological monitoring in those areas where low fly densities exist and especially, to expose potential relic fly pockets after control operations have been completed. *Key words* : *Glossina austeni* - Female - Sterilization - Gamma irradiation - Insect control.

VREYSEN (M.J.B.), VAN DER VLOEDT (A.M.V.). Uso de hembras de *Glossina austeni* irradiadas con rayos gamma como sentinelas en los programas de lucha contra las glosinas. *Revue Élev. Méd. vét. Pays trop.*, 1992, **45** (3-4) : 303-309

La administración de 60 Gy gamma a hembras de *Glossina austeni*, entre el segundo y el noveno día de vida adulta y de 50 Gy gamma a pupas en el día 33 (después de la postura de las larvas), indujo la esterilidad total, sin alteración del comportamiento copulatorio. Las hembras irradiadas aceptaron la copulación con machos normales hasta 15 días después de la emergencia del estadio larval (respuesta copulatoria de 84 p. 100). La duración del tratamiento (tanto en el día 33 después de la postura de las larvas, como entre el segundo y noveno día de vida adulta), influenció de manera significativa la dinámica del desarrollo folicular. Las hembras mostraron un patron de desarrollo normal de los folículos en posición A_1 y C_1, con vitelogenésis, maduración y ovulación normales, sin relación alguna con el estadio al momento de la irradiación. Sin embargo, las hembras tratadas durante el estadio pupal, no mostraron signos de desarrollo folicular en las posiciones B_1 y D_1. A partir del día 15, las hembras mostraron ovarios inactivos, caracterizados por oocitos atróficos y células nodrizas. El tratamiento de las hembras entre el segundo y el noveno día de vida adulta, permitió un desarrollo folicular gradual de los folículos en B_1 y D_1. Bajo condiciones de laboratorio, los machos normales no presentaron preferencia copulatoria alguna para las hembras irradiadas o para las no irradiadas. Se observó un alto grado de copulación multiple en los casos en los que se multiplicaron las oportunidades de copulación, tanto con las hembras irradiadas como con las no irradiadas. Al día 9 de la vida adulta, 24,0 p. 100 de las hembras no tratadas y 23,8 p. 100 de las tratadas con 60 Gy (al día 2 de vida), aceptaron al macho durante el cuarto episodio copulatorio. La receptividad a la copulación múltiple disminuyó conforme se aumentó la dosis de irradiación (120 Gy) y en los casos en los que el tratamiento fue administrado mas tardíamente (5 días de edad). Se discuten los resultados de laboratorio, con el fin de utilizar hembras de *Glossina austeni* esterilizadas con rayos gamma, para el seguimiento entomológico en aquellas areas donde las densidades de moscas son bajas y especialmente para evitar la exposición potencial una vez que se han completado las operaciones de lucha. *Palabras claves* : *Glossina austeni* - Hembra - Esterilización - Irradiación gamma - Lucha contra los insectos.

REFERENCES

1. CURTIS (C.F.). Radiation sterilization and the effect of multiple mating of females in *Glossina austeni*. *J. Insect. Physiol.*, 1968, **14** : 1365-1380.

2. DUCOFF (H.S.). Causes of death in irradiated adult insects. *Biol. Rev.*, 1972, **47** : 211-240.

3. JOHNS (D.L.). Investigations of *Glossina austeni* in Zanzibar. Annual Report 1949. East African Trypanosomiasis Research Organization, 1951.

4. JORDAN (A.M.). The mating behaviour of females of *Glossina palpalis* (R.-D) in captivity. *Bull. ent. Res.*, 1958, **49** : 35-43.

5. LaCHANCE (L.E.), BRUNS (S.B.). Oogenesis and radiosensitivity in *Cochliomyia hominivorax* (Diptera ; Calliphoridae). *Biol. Bull.*, 1963, **124 :** 65-83.

6. RIORDAN (K.). Development of *Glossina palpalis* R.-D. inside the puparium. *Entomologist*, 1970, **103** : 171-181.

7. SAUNDERS (D.S.). The ovulation cycle in *Glossina morsitans* Westwood (Diptera : Muscidae) and a possible method of age determination for female tsetse flies by examination of their ovaries. *Trans. R. Ent. Soc. London.*, 1960 : 221-238.

8. VAN DER VLOEDT (A.M.V.), BARNOR (H.). Effects of ionizing radiation on tsetse biology. Their relevance to entomological monitoring during integrated control programmes using the Sterile Insect Technique. *Insect. Sci. Applic.*, 1984, **5** (5) : 431-437.

9. VAN DER VLOEDT (A.M.V.), TAHER (M.), CZOCK (K.H.), MALEKGHASSEMI (B.), HASELBERGER (N.). Laboratory studies on the sexual sterilization of the tsetse fly *Glossina palpalis palpalis* (Robineau-Desvoidy) by ionizing radiation. III. Effect of fast neutron irradiation on male and female pupae of different age. IAEA, Technical report presented at the RCM meeting, Bobo Dioulasso, Burkina Faso, 1976.

10. VAN DER VLOEDT (A.M.V.), TAHER (M.), TENABE (S.O.). Effect of gamma radiation on the tsetse fly *Glossina palpalis palpalis* (Rob.-Desv.) (Diptera, Glossinidae) with observations on the reproductive biology. *Int. J. Appl. Radiat. Isotopes,* 1978, **29** : 713-716.