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CAPABILITY OF *GLOSSINA TACHINOIDES* WESTWOOD (DIPTERA: GLOSSINIDAE) MALES TO MATE AND INSEMINATE FEMALE FLIES IN DIFFERENT MATING RATIOS TO SUSTAIN A LABORATORY TSETSEFLY COLONY FOR STERILE INSECT TECHNIQUE CONTROL PROGRAMME IN GHANA

C. E. ANNOH, H. F. BANOR, D. LUGER AND D. A. ADABIE-GOMEZ

C.E.A. and D.A.A.-G: Biotechnology and Nuclear Agriculture Research Institute, Ghana Atomic Energy Commission, P.O. Box AE 50, Accra, Ghana; H.F.B. and D.L.: International Atomic Energy Agency Laboratories, Entomology Unit, P.O. Box 100, A-1400 Vienna, Austria

Abstract

Loboratory studies were conducted to determine the capability of *Glossina tachinoides* males to transfer sufficient sperms to the females when mated in different ratios. Mature male and female flies were mated in different female: male ratios of 15:15 (1:1), 16:8 (2:1), 15:5 (3:1) and 15:3 (5:1) in separate fly-holding cages, respectively. Percentage insemination of spermathecae ranged from minimum 76 per cent in 5:1 to maximum 100 per cent in 1:1 mating ratios. 'Used' male flies could be reused more than three times in mating to successfully inseminate female flies to sustain the *G. tachinoides* colony.

Résumé

ANNOH, C. E., BANOR, H. F., LUGER, D. & ADABIE-GOMEZ, D. A.: *Capacité des mâles de* Glossina tachinoides *Westwood (Diptera: Glossinidae) à inséminer les mouches femelles dans les proportions d' accouplement différentes pour soutenir une colonie de mouche tsé-tsé de laboratoire pour la technique d'insecte stérile du programme de contrôle au Ghana.* Des études de laboratoire se déroulaient pour déterminer la capacité des mâles de *G. tachinoides* de transférer les spermes suffisants aux femelles lorsqu'ils sont accouplés dans les proportions différentes de 15:15; 16:8; 15:5; et 15:3 (femelle : mâle) respectivement dans les cages de mouche separées. Pourcentage d'insémination de spermathéque varie entre un minimum de 76% en 5:1 et un maximum de 100% en 1:1 de proportion d'accouplement. Les mouches mâles 'utilisées' pourraient être réutilisé plus que trois fois d'accouplement pour accoupler les mouches femelles avec succés pour soutenir la colonie de *G. tachinoides*.

Introduction

The Sterile Insect Technique (SIT) control programme essentially involves laboratory mass rearing of the target insect species, irradiating large numbers of the males and releasing them into the wild to mate and inseminate the female species of the natural population (Knipling, 1955). During field releases of sterile male flies, the tsetse colony should be self-supporting by maintaining a degree of stability in numbers, fecundity and survival of fertilized females. Usually a fewer proportion of male flies are retained to mate with the reproductive females in order to sustain the fly colony. It is, therefore, necessary to conduct studies to determine the acceptable proportions of female to male mating ratios of the species of tsetse colony for such programmes.

The present study was, therefore, conducted on *Glossina tachinoides* Westwood to assess the male flies capability to mate and inseminate female flies in different sex ratios. The re-use of mated male flies to re-mate several females in the tsetse colony was also determined.

Experimental

Matured male flies of *G. tachinoides* (7 - 8 days old) from the tsetse colony in Seibersdort laboratory, Austria, were used in the study. They were mated with 2 - 3 days old females in different mating ratios of 15 females to 15 males (1:1), 15 to 8 (2:1), 15 females to 5 males (3:1) and 15 females to 3 males (5:1), respectively. The mated ratios of flies were kept separate in four fly-holding cages of diameter 12 cm and the sexes were separated after 48 h. The experiment was replicated three times.

The mated male flies were kept in cages for re-use in another mating exercise, while the females were observed for their mating scars, egg or larval abortions, longevity and pupal production, 35 days post-mated. The claspers at the posterior end of the male tsetse are used to grip the end of the female abdomen during mating and, hence, leave mating scars on the mated females. The number of scars represented by (+) determines the number of times that the female had mated. Abortion occurs when an egg or larva fails to reach its full size and is expelled from the uterus of the pregnant female fly. All the surviving females were dissected after 35 days of observation and percentage inseminated spermathecae, an important factor for assessing reproductive performance, determined. All experimented flies were kept under laboratory conditions of 25.0 ± 1.0 °C and 85.0 ± 10% temperature and relative humidity respectively. Flies were fed *in vitro* once a day for 6 days in a week with defibrinated blood, mixed in a proportion of 75% bovine and 25% porcine.

In another laboratory experiment, male flies, which had been used in previous mating exercises were re-used in mating 'virgin' female flies in 1:1 ratio. The mated males were divided into five groups of 15 each and put into five separate fly-holding cages containing 15 virgin females each. The sexes were separated after 48 h and the males dissected within 24 h to measure the male accessory gland by using graticule graduated microscope, and to examine both testes and sperm mobility. The female flies were also dissected and the percentage filled spermathecae recorded.

Statistical analysis, ANOVA, was performed by using Statistical Graphics Corporation Software, USA.

TABLE 1

Percentage survival, inseminated spermathece and reproductive performance of female Glossina tachinoides *mated with males at different mating ratios*

Mating ratios Female: Male	*Surviving females at 35 days (%)*	*Inseminated spermathecae (%)*	*Reproductive Performance (N=15*		
			Mating scar per female	*No. of eggs aborted*	*Av number of pupae per female in 35 days*
15:15 (1:1)	93.3	100.0[a]	+	0	2.0 ± 0.0
15:8 (2:1)	96.7	92.5[ab]	+	1	2.0 ± 0.1
15:5 (3:1)	93.3	80.6[ab]	+	1	2.0 ± 0.0
15.3 (5:1)	96.7	76.0[c]	+	1	2.0 ± 0.0

Mating scars (+) occur as imprint on lower abdomen of mated female flies. The number of +'s determines number of times that the female had mated.

Percentage of insemination with different letters are significantly different at $P < 0.05$

Results

The reproductive performance of female *G. tachinoides* mated in different ratios is shown in Table 1. All female flies dissected had mating scars on the lower abdomen, indicating activity of mating. Viable pupae were produced by all the female flies during the first reproductive cycle which occurred between 18 and 20 days post-mating. Average pupae produced was two for each mating ratios during the observation period of 35 days. The pupae were viable because adult flies emerged from all the pupae at eclosion. There was one abortion each for the mating ratios of 2:1, 3:1, and 5:1 but no abortion at ratio of 1:1.

Percentage inseminated spermathecae of females of mating ratios of 15 females to 15 males and 15 females to 3 males was 100 per cent and 76 per cent, respectively. The diference was statistically significant at $P < 0.05$ (Table 1). However, the difference between ratios of 15 females to 8 males and 15 females to 5 males was statistically not significant ($P > 0.05$). In general, percentage of inseminated spermathecae decreased with lower proportions of male flies.

Table 2 shows measurements of accessory glands of re-used males for re-mating female flies and percentage inseminated spermathecae of mated females. The testes of all dissected male flies showed normal 'bean' shape and coloured reddish brown. The sperms were very active and mobile (shown by double positive symbols, ++). The diameter of the apical body (accessory gland) ranged from minimum size of 50 μm (at narrow section) to maximum of 164 μm (at expanded section). The gland fluid filling the apical body was almost full, 98-100 per cent. All female flies mated with re-used male flies had mating scars and showed spermathecae fully inseminated (100%).

TABLE 2
Measurement of accessory gland of re-used males of G. tachinodes *and inseminated spermathecae of females mated with the males*

Mated groups (10 pairs per group)	*Sperm mobility*	*Diameter of accessory gland size (μm)*		*Accessory gland filled (%)*	*Inseminated spermathecae of female (%)*
		Minimum	*Maximum*		
1	++	50	89	100	100
2	++	84	164	100	100
3	++	98	151	100	100
4	++	75	143	100	100
5	++	57	110	100	100

Sign (++) indicates very active sperm movement

Discussion

Williamson *et al.* (1983) established a self-supporting colony of *G. mortisans* Westwood in Tanga, the United Republic of Tanzania. Between the period of November 1977 and January 1979, the mean number of reproducing females ranged between 50,000 and 53,000. The percentage of male puparia was 94 per cent out of which 68 per cent was used for sterilization and releases. Only 26 per cent (13,160) male flies was retained for colony mating. The estimated female to male ratios of Tanga colony was 4:1. The present study

of *G. tachinoides* mating ratios indicated that percentage inseminated spermathecae for both 2:1 and 3:1 ratios were relatively high, 92.5 per cent and 80.6 per cent, respectively. However, the difference was not significant and, therefore, both ratios were equally competitive of high insemination as observed for the 1:1 ratio. The mating capability of *G. tachiniodes* males in the fly colony in Ghana which originated from the Seibersdolf laboratories) was, therefore, comparable with those of *G. morsitans* at the Tanga laboratory.

Jordan (1972) observed that male *Glossina* species (i.e. *G. morsitans morsitans*) are capable of mating up to six times under laboratory conditions without apparent loss of inseminating ability. This observation could mean that one male fly could be used to inseminate five or more females in extreme cases of very few males in a fly colony.

Re-use of mated male tsetse flies for mating virgin females of stable colonies in SIT programmes has been in practice. Oladunmade *et al.* (1990) radiosterilized and released laboratory 'used' males of *G. palpalis palpalis* during SIT programme in Nigeria between 1985 and 1987. Males available for mating the colony of *G. morsitans morsitans* at Tanga laboratory were used not more than seven times (Williamson *et.al.;* 1983). The present study has also shown that at 5:1 mating ratios, percentage inseminated spermathecae was substantial (76%) and produced viable pupae comparable with other ratios of higher proportions of males. Thus, a colony of *G. tachinoides* with a mating ratio of five females to one male could be managed to maintain a stable and self-sustaining tsetse fly colony during SIT release programmes in Ghana.

The present study revealed that the diameter of accessory gland of *G. tachinoides* males was relatively large and remained almost 100 per cent filled with seminal fluid after mating two times, indicating that re-used males were still sexually active and could be used for further matings. Pollock (1974) also observed that increases in diameter was due to accumulations of accessory gland fluid in sexually active male of *G. morsitans morsitans.* Langley (1977) proposed that, generally, the inseminating ability of male flies of *Glossina* species could improve if they were allowed to rest between mating, presumably for replenishment of male accessory-gland secretions. This strategy could be applied to small numbers of male flies for re-mating virgin females to sustain the tsetse colony during releases of sterile males in SIT programme to be embarked on in Ghana.

Conclusion

G. tachinoides males could mate and inseminate females at females to males ratios of 3:1 normally and 5:1 in extreme situations of very few males. The male flies could be re-used to mate more than three times in the colony without loss of inseminating ability if allowed rest periods between mating.

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References

JORDAN A. M. (1972) The inseminating potential of male *Glossina austeni* Newst. and *G. morsitans morsitans* Westw. (Diptera; *Glossinidae. Bull. ent. Res.* **61**, 669-672.

KNIPLING, E. F. (1966) Some basic principles in insect population suppression. *Bull ent. Soc Am.* **12** (1), 7 -15.

LANGLEY, P. A. (1977) Physiology of tsetse flies (*Glossina* spp.) (Diptera; *Glossinidae*): A Review. *Bull ent. Res.* **67**, 532-574.

OLADUNMADE, M. A., FELDMANN, U., TAKKEN, W., TENABE, S. O., HAMANN, H. J., ONAH, J., DENGWAT, L., VAN DER VLOEDT, A. M. A. & GINGRICH, R. E. (1990) Eradication of *Glossina palpalis palpalis* (Robineau-Desvoidy) (Dipteria; Glossinidae) from agropastoral land in Central Nigeria by means of the Sterile Insect Technique for Tsetse Control and Eradication. *Pro-*

ceedings of the Research Co-ordinating Meeting, Vom, Nigeria. 6-10 June, 1988, pp. 5-23.

POLLOCK, J. N. (1974) Male accessory secretions, their use and replenishment in *Glossina* (Diptera; Glossinadae). *Bull ent. Res.* **64**, 533-539.

WILLIAMSON, D. L., BAUMGARTNER, H. H., MTUYA, P.V., TARIMO, S. A. & DAME, D. A. (1983) Integration of insect sterility and insecticides for control of *Glossina morsitans morsitans* Westwood (Diptera: Glossinidae) in Tanzania. 1. Production of tsetse flies for release. *Bull ent. Res.* **73**, 259-265.

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