Physical control of locust *Schistocerca gregaria* : The use of gamma-irradiated males.

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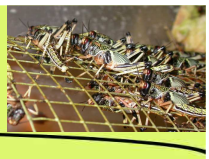


Introduction

Schistocerca gregaria (Forsk.) (Orthoptera, Acrididae) with other locusts and Grasshoppers are major insect pests in Africa, particularly in the Sahelian zone. Major damages were recorded in the past paired with severe socio-economic consequence (Uvarov 1966, Popov *et al.*, 1991, Showler 1995, Pener and Yerushalmi 1998). Among the control options used against grasshopper and locust, Sterile Insect Techniques (SIT) was never tempted against locust althought SIT have been successfully applied against a number of pest species (Klassen 2002). Sterilisation by irradiation is the most practical way to sterilise the insects and then the most used. To reduce the potential fecundity of females in wild population, released irradiated males must still be able to copulate efficiently with several females and win competition with wild males.

In this study, our aim was first to determine the best dose to applied to males of *Schistocerca gregaria* by comparing survival, mating and offspring production of normal and irradiated males. After the dose choice, we observed the competitiveness of irradiated male by comparing with normal male reproductive parameters such as the duration of precopulation period, mating duration and number of sperm stored per females after copulation. To estimate the potential influence of irradiated male on the offspring production of females in the field, the number of females mated in succession has been observed for the both type of males. The sperm competition between the both types of males have also been assessed.

Materiels and Methods

Locusts were exposed to gamma rays generated by a cobalt-60 source (Compact Cell) with a dose of 4 Gy/1.19sec (200 Gy/min).

1°Male survival
Effect of the different irradiation doses on the male survival has been measured. Males were monitored every days and dead individuals were counted. Survival curves were analysed using Kaplan-Meier survival analyses (SAS). The obtained survival curves were compared using log-rank test.

2° Reproduction parameters
For each tested dose, the male was proposed to virgin female. If copulation took place, the females were isolated after mating in a cage containing sand pots placed to allow oviposition. Pots were examined daily. The number of offspring emerging per pots was recorded each day.

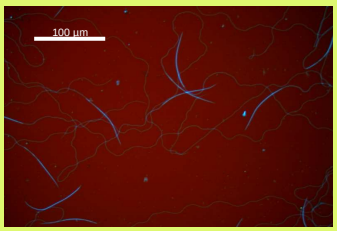
3° Sperm transfer
Virgin normal males (N=16) and irradiated males (N=16) were individually placed with one virgin female to ensure mating. The duration of each courtship and copulation was recorded. After the copulation, a proportion of inseminated females were dissected to count the quantity of sperm transferred per mating. Females were dissected in a saline solution (PBS).The *receptaculum seminis* and the complete spermathecal duct were removed and put in an ependorf containing 1ml of PBS solution.They were slightly ruptured using a potter and homogenized by drawing the solution 10 times trough a syringe. Three 20µl drops of the homogenised solution were deposited on clean microscopic slides. The slides were then dried at ambient temperature prior to fixation in ethanol and stained 10 minutes in a solution of DAPI (4',6-diamidino-2-phenylindole dihydrochloride, 2 µg/ml). The number of sperm present in the storage organ of females was counted using a fluorescence microscope (X-200 magnification).

4°Male mating ability
Virgin normal males (N=10) and irradiated males (N=8) were presented daily with virgin females. The number of sperm present in the storage organ of females was counted.

5°Male competition
In a first experiment, the hatchability of eggs laid by females (N=10) after copulation with normal males following 14 days latter by a copulation with irradiated males has been investigated. A virgin female was firstly mated with an irradiated male. In a second experiment the hatchability of eggs laid by female after copulation with irradiated males following copulation with normal male has been observed

Irradiator


Spermatheca



Sperm of Schistocerca g.

Results

Table1:Male survival after irradiation and reproductive parameters

Doses	150Gy	50Gy	25Gy	10Gy	4Gy	4Gy mature	Control
Survival after irradiation in days	5.7±1.1 a	8.9±3.3 b	11.1±2.8 bc	12.1±1.6 cd	12.1±1.9 c	10.42±2.1 bc	25,24±11.0 d
Number of tested males	0	0	10	16	24	19	18
Number of males that mated	0	0	2	6	20	19	18
Number of females producing ootheque	0	0	1	4	20	19	18
Number of eggs hatching	0	0	0	0	3.2±2.9 a	3.5±2.2 a	45.1±4.1 b

Table 2 : Duration of precopulation and copulation , the quantity of sperm transferred by normal and irradiated males .

	Normal males	Irradiated males	t Test
Precopulation duration (sec)	10.67±4.23	11.65±4.40	t= -0.61, ddl=19, P=0.55
Copulation duration (min)	235.04±50.44	274.42±66.92	t= -2,89, ddl=23, P<0.01
Number of sperm stored	24040.62±6887.36	15359.37±8935.74	t= 3.18, ddl=15, P<0.01

Fig1:Sperm number stored in female spermatheca according to the female rank in a successive mating .

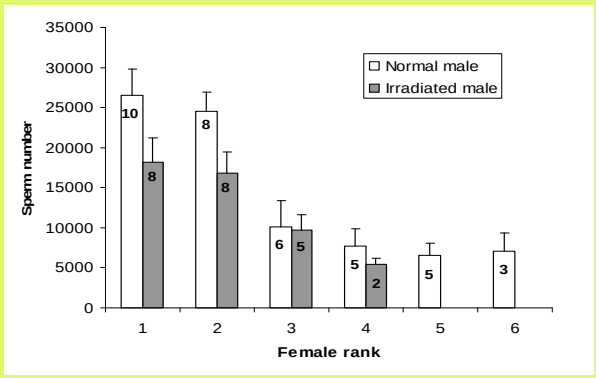
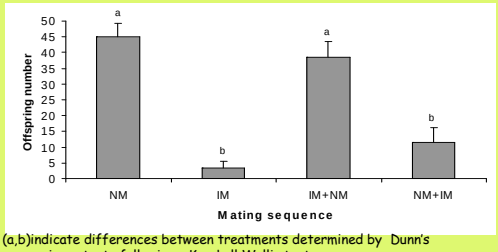


Fig 2: Offspring production of females inseminated by normal male alone (NM), irradiated male alone (IM), irradiated male then normal male (IM+NM) and normal male followed by irradiated male (NM+IM).



Letters (a,b)indicate differences between treatments determined by Dunn's multiple comparisons tests following a Kruskall Wallis test

Discussion

We determine that an irradiation of 4 Gy reduced significantly the number of offspring produced by females mated with irradiated males without reducing the male ability to obtain copulation (Table1). However, irradiated *S. gregaria* showed an increase in mortality when compared to controls probably due to effect on non-specific damage on somatic cells (Proverbs, 1969). Normal male transfer about 24000 sperm while irradiated male have longer copulation duration but transfer less sperm (15000)(Table2). The decrease of sperm number is probably due to the destruction of secondary spermatogonia (Coggins 1973).

Males which are mated every day transfer a great number of sperm during two first mating and then transfer less sperm during the following ones (Fig 1). That probably means that in the field, at least two females could be affected per released male, perhaps more as we do not know the impact of reduce sperm transfer by already mated males on the female loading.

In our observations, *S. gregaria* exhibited great last-male sperm precedence (Fig 2) as observed also by Hunter-Jones (1960). This result suggests that although the impact of irradiated male on a female fecundity could be limited by a subsequent copulation by a regular male, an irradiated male have also the ability to removed the sperm of precedent mating with a regular male.

According to our first results a virgin female which encounters a sterile male have a high probability to produce sterile eggs during her all life. However the degree of gregarization and so the frequency of male encountering can modify this situation.

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