Qatar Univ. Sci. J. (2006), 26:51-58

Gamma Irradiation of 4th Instar Larva of Angoumois Grain Moth and Effects on Parent and Their Generations

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التشعيع بأشعة جاما للطور الرابع اليرقى لفراشة أنجومو الحبوب وتأثير ذلك على الآباء والأجيال

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قسم التطبيقات البيولوجية ، مركز البحوث النووية ، هيئة الطاقة الذرية

صندوق بريد 13759 ، أبى زعبل ، القاهرة ، مصر

تم تشعيع فراشة أنجومو الحبوب في العمر ليرقى الرابع المتأخر بجرعات صفر (الضابطة), 25, 50, 75, 100, 125, 150, جراى من أشعة جاما. الفراشات الناتجة من يرقات شععت بالجرعة 150 جراى أصبحت عقيمة عقما كاملا . أدى تشعيع الذكور كيرقات بالجرعات قبل المعقمة 25, 50 جراى لتوارث العقم في الجيل الأول وانخفاض في أعداد الفراشات وكانت فراشات الجيل الأول أكثر عقما من جيل آبائهم كذلك أن وراثة العقم كان أكثر وضوحا في ذكور الجيل الأول عنها في الإناث .أتضح من النتائج أن خصوبة الفراشات قد عادت في الجيل الثاني والثالث. وقد أدى زيادة النسبة التزاوجية للذكور المشععة وفراشات الجيل الأول من 1 : 1 إلى 5 : 1 (مشععة : غير مشععة) لانخفاض في خصوبة البيض كما أن النسب المئوية لعقم البيض زادت بزيادة الجرعة الإشعاعية. كانت القيمة التنافسية المحسوبة كل النسب التزاوجية المستعملة حول الرقم 1 مما يعنى أن كل من ذكور الآباء في المشععة في العمر اليرقى الرابع المتأخر و فراشات الجيل الأول ذات كفاءة تنافسية عالية مع الفراشات غير ا لمشععة و ذو فاعلية في خفض الكثافة التعدادية لفراشة أنجومو الحبوب .

Key words: *Angoumois grain moth, Gamma irradiation, Inherited sterility, Mating competitiveness.*

ABSTRACT

Late fourth stage larvae of Angomous grain moth, *Sitotroga cerealella* (Olivier) were gamma irradiated with doses 0 (control), 25, 50 75, 100, 125 and 150 Gy. The moths originated from larvae irradiated with 150 Gy became sterile. Irradiation of males as larvae with substerilizing doses of 25 and 50 Gy induced inherited F_1 sterility which reduced the population. F_1 progeny exhibited more sterility than their parent generation. Also F_1 males inherited more sterility than F_1 females. Adult fertility recovered in F_2 and F_3 generations. Increasing the competition ratio in favour of irradiated male parents or F_1 males and females from 1:1 to 5:1 (I:U) gave more reduction in egg viability. The percentage of egg infertility was increased by increasing the dose. The calculated competitiveness values for all ratios and doses used around 1.0 which mean that irradiated males 4th stage larvae or their F_1 moths were full competitive with normal moths and more effective in suppressing population of Angomous grain moth.

Introduction

The Angoumois grain moth, *Sitotroga cerealella* (Olivier), is one of the most frequent and dangerous pests of stored grains. The use of sterile insect technique (SIT) is a new approach that could prove to be useful for the control of a number of major lepidopterous pests throughout the world. Brower and Tilton [1]; Brower [2]; La Chance and Klassen [3].

The release of sterile moths could not suppress the wild population effectively. In order to increase the mating competitiveness of the moths, the substerile insect technique for inherited sterility has been developed. Brower [4]; La Chance [5]; Carpenter *et al.* [6] and Bloem *et al.* [7].

Ashrafi *et al.* [8] and Bartlett and Lewis [9] suggested that, irradiating larvae had the following advantages over adult treatment: a much lower dose of radiation was required to obtain partial sterility, the partially sterile males which developed from treated larvae were highly competitive, and reduced fecundity was transmitted through the F_2 generation, this effect may be a result of more chromosomal translocation when exposures to radiation are made in larval stage than when adult are irradiated. The purpose of the present study is determine the levels of sterility in both males and females of *S. cerealella* irradiated as 4th stage larvae and effects of substerilizing doses on the number of filial generations descended from irradiated male parents and also to determine competitiveness values of partially sterilized males and F_1 generation (males and females).

Material and Methods

Test insects were taken from laboratory cultures of *S. cerealella* that were maintained at 27±1°C and 25±5% R.H., and reared on wheat. 4th stage larvae (24 day-old) were selected on the basis of elapsed time from the infestation date of the culture, 27 to 29 days old cultures were used Shenouda [10].

4th stage larvae were irradiated in the Co-60 gamma cell unit 220 at Atomic Energy Authority at a dose rate of 2.273 Krad/min. The doses were 25, 50, 75, 100, 125 and 150Gy.

To study the effect of gamma irradiation on egg fertility after irradiation of the 4th stage larvae, each kernel containing larva was kept separately in small glass tubes. After emergence, 5 one-day-old males and females were paired with non irradiated opposite sex or with each other in plastic vials (23x83 m) containing black zigzag papers as an ovipositional site. The jar was covered with muslin secured with rubber band. All eggs were collected after death of the females and placed on black paper circles in petri-dishes containing rearing medium for determining percentage of egg hatch after 7 days.

For studying the effect of substerilizing doses on the number of the progeny in the filial generations, larvae were irradiated with 25 and 50 Gy and each kernel containing larva was kept separately in small glass tube. After emergence, ten single P irradiated males (one-day-old) were paired with ten single unirradiated virgin females for each dose. Equal numbers of untreated control pairs were set up. Samples of 100 eggs were placed in 0.75 liter gars containing a standard diet, and incubated as before. The number of emerging F_1 progeny was recorded for each 100 eggs. One-day old F_1 adults were selected for 10 pairs of each of the following combinations: UM x UF (control), F_1M x UF, UM x F_1F and F_1M x F_1F. The total number of F_2 progeny from each 100 eggs was counted. Adults from the F_2 and F_3 were paired in the same way as adults from the F_1 generation and the number of F_3 and F_4 progeny per 100 eggs were determined. Competitiveness of irradiated males was determined by using thesubsterilizing doses 25 and 50 Gy. After emergence the irradiated males as larvae were combined as follows: UM x UF (control), IM : UF, IM : 1 UM : 1 UF and 5IM : 1 UM: 1UF. The same combinations were used for F_1 males and females by replacing F_1 males or females instead of irradiated male parents. The following treatment groups were tested using 10 replicates in each group. The number of hatched egg in each population was recorded. The mathematical method of competitiveness value of Ahmed *et al.* [11] was used.

Results and Discussion

Data in Table 1 show that increasing irradiation dose decreased percentage of egg hatchability. Females were more radiosensitive than males receiving the same doses. The reduction in fertility was more pronounced when both sexes were irradiated and paired together. When males only were irradiated as larvae with 50 and 100 Gy. the percentage of egg hatchability was 48.0% and 18.8%, respectively, and was reduced to 40.0% and 13.2%, respectively, when females only were irradiated, when both sexes were irradiated with the same doses and mated together, 28.8% and 0.0 of the produced eggs hatched as compared with 86.2% in the control. There was a complete sterility for both, males and females, produced from irradiated larvae with 150 Gy and paired with the unirradiated opposite sex.

Genchev and Genchova [12] showed that in males as well as in females the sterility is due chiefly to emergence of dominant lethal mutations in spermatosoa or oocytes which cause egg mortality. These findings were reported by several authors. Cogburn *et al.* [13]; Qureshi *et al.* [14]; Brower [15]; Rosada [16] and Tuncbilek and Akif [17].

Table 1. of gamma irradiation on percentage egg hatchability of *S. cerealella* treated as fourth instar larvae.

Dose	% egg hatchability		
Gy	TM x UF	UM x TF	TM x TF
0	86.8	85.6	86.2
25	66.0	56.4	45.0
50	48.0	40.0	28.8
75	36.5	25.0	11.6
100	18.8	13.2	0.0
125	10.6	4.5	0.0
150	0.0	0.0	0.0

M = Male , F = Female , T = treated
U = untreated , 0 = control

From the data presented in Table 2 it is clear that an increase in radiation dose applied to fourth instar larvae decreased the number of adults in the filial generations. Matings in which

adult males were treated with 25 and 50 Gy produced only 50% and 27%, respectively as many F_1 offspring as did the controls. F_1 males mated with untreated females produced 18.6% and 2.7%, F_2 progeny of controls, F_1 females mated with untreated males produced 28.3% and 5.5%, F_2 progeny of controls. Also, there was greater reduction in number of progeny when both were irradiated, F_1 males and F_1 females were mated together than when F_1 males only and F_1 females only mated with the untreated opposite sex. These results indicate that F_1 progeny exhibited more sterility than their parent generation, also F_1 males inherited more sterility with better reproductive competence than F_1 females. The reduction in number of adult progeny was substantially less in F_3 and F_4 generation. Bahari and Mohamed [18] revealed that 3 main types of chromosomal abberations induced in F_1 male larvae of irradiated *Plutella xylostella*_(L.) by dose of 100, 150, 200 Gy and these results indicate the possibility of using chromosome translocations as the genetic marker. Sutrisno *et al.* [19] showed that in dimondback moth F_1 progeny inherited more sterility than their irradiated parent, while the sterility of the F_2 population was nearly the same as that of the irradiated parents. Also, Zhang *et al.* [20] reported that chromosomal aberrations in spermatocyte of the F_1 generation were directly related to high F_1 sterility, however, the sterility was observed for only one generation and fertility was recovered in the next generation.

Table 2. Effect of substerilizing doses of gamma irradiation on number of adult progeny/100 eggs from various matings of *S. cerealella* males parents irradiated as fourth instar larvae.

Generations	Dose	Treatment					
		TM x UF		UM x TF		TU x TF	
	Gy	Avg.	% of control	Avg.	% of control	Avg.	% of control
F_1	0	43.0 aA	100	--	--	--	--
	25	21.5 b	50	--	--	--	--
	50	11.6 B	27	--	--	--	--
F_2	0	45.2 aA	100	45.2 aA	100	45.2 aA	100
	25	8.4 c	18.6	12.8 b	28.3	2.3 b	5.1
	50	1.2 C	2.7	2.5 B	5.5	0.0 B	0.0
F_3	0	47.0 aA	100	47.0 aA	100	47.0 aA	100
	25	23.2 b	49.4	30.2 c	64.5	18.2 c	38.7
	50	14.3 B	30.4	20.2 C	42.6	0.0 B	0.0
F_4	0	45.8 aA	100	45.8 aA	100	45.8 aA	100
	25	43.4 a	93.9	46.0 a	100.4	40.0 a	87.3
	50	32.0 D	69.9	36.2 A	79.0	0.0 B	0.0

F : filial generation Mean followed by the same small or capital letter in the same column are not significantly different (P> 0.05)

These results are in agreement with results obtained on other lepidopterous insects reported by Ashrafi *et al.* [8]; Brower, [4]; Al-Hakkak *et al.* [21]; Carpenter *et al.* [6] and Bloem *et al.* [7]. The results of mating competitiveness of irradiated males and F_1 progeny are given in Table 3. Increasing the ratio of irradiated males to normal males from 1:1 to 5:1 increased the percentage of egg infertility. Also egg infertility increase proportionality with

the increase in the radiation dose. When males irradiate with 25 and 50 Gy and confined with unirradiated males and unirradiated females at 1:1:1 ratio caused 24.1 and 40.2% egg infertility, respectively, when the ratio of sterile males was increased to 5:1:1 the egg infertility reached 33.2% and 51.6%, respectively. The competitiveness values for irradiated males for the two doses and flooding ratios used were over 1.0 which means that irradiated males were fully sexually competitive with unirradiated males. Carpenter [22] indicate that releasing irradiated *Helicoverpa zea* (Boddie) with 100 Gy (substeriling dose) were highly competitive with wild males in infusing genes into the wild population and reduced it. Similar finding were also reported by Ahmed *et al.* [11]; Brower [2]; Sutrisno *et al.* [19] and Villavas*a et al.* [23].

Also, the results represented in Table 3 it is indicated that, when F_1 males descended from irradiated P males as larvae with 25 Gy were placed with unirradiated males and females at a ratio 1:1:1 or 5:1:1 (F_1M: UM : UF), the percentage of egg infertility increased from 50.5% to 66.6%, respectively. At 50 Gy, the increase of the flooding ratio from 1:1:1 to 5:1:1

TABLE 3. Competitiveness of substerilized male parent (irradiated as larvae) and their F_1 males and females confined with unirradiated moths.

Cross ratio	Dose Gy	% Egg infertility		Competitiveness value
		Observed	Expected	
IPM : UM : UF				
0 : 1 : 1	0	13.2		
1 : 0 : 1		35.0		
1 : 1 : 1	25	24.2	24.1	1.00
5 : 1 : 1		33.2	31.3	1.06
1 : 0 : 1		52.8	33.0	1.22
1 : 1 : 1	50	40.2	46.2	1.12
5 : 1 : 1		51.6		
F_1M : UM: UF				
0 : 1 : 1	0	12.0		
1 : 0 : 1		71.2		
1 : 1 : 1	25	50.0	41.6	1.21
5 : 1 : 1		66.8	61.3	1.09
1 : 0 : 1		85.4		
1 : 1 : 1	50	50.8	48.7	1.04
5 : 1 : 1		74.0	73.2	1.01
F_1F : UF: UM				
0 : 1 : 1	0	12.0		
1 : 0 : 1		61.4		
1 : 1 : 1	25	37.0	36.7	1.01
5 : 1 : 1		55.0	53.2	1.03
1 : 0 : 1		77.5		
1 : 1 : 1	50	48.2	44.7	1.08
5 : 1 : 1		69.8	66.6	1.05

U: Unirradiated, I: Irradiated, M: Male, F: Female, P: Parent, F_1: first generation

(F_1M: UM : UF) increased the egg infertility from 50.8% to 74.0%, respectively. The same trend was also observed with F_1 females, the competitiveness values indicated that F_1 males and females were fully competitive with untreated adults. The calculated C.V. for all ratios

and doses were more than 1.0 value. Shonthoram *et al.* [24] demonstrated that incidince of spermatophore transfer without sperm was significantly higher in case of sterilized males compared to F_1 males and appeared to be a major cause behind the reduced mating competitiveness of sterile males. The results of the present study confirmed the findings of others. Ashrafi *et al.* [8] ; Carpenter *et al.* [6] and Bloem *et al.* [25].

Inherited sterility component is flexible in its application and more effective in reducing the reproductive ability of Angoumois grain moth at low release ratios. In addition to laboratory studies, field studies and within warehouses must be initiated to measure the compatibility of inherited sterility.

REFERENCES

[1] Brower J. H. and Tilton E. W. (1975). Potential For Control of *Cadra* Cautella (Walker) By Release of Fully or Partially Sterile Males, *Int. J. Appl. Radiat. Isotop.* **26:** 720-725.

[2] Brower J. H. (1979). Mating Competitiveness in the Laboratory of Irradiated Males and Females of *Ephestia Cautella*, *Florida Entomologist.* **62:** 41-47.

[3] La Chance L. E. and Klassen, W. (1991). *Applying the Sterile Insect Technique to Control Insect Pests*, IAEA Yearbook, Vienna (Austria). **345:** B 23-33.

[4] Brower J. H. (1979). Substerilizing Irradiation of *Plodia Interpunctella* Males. Effects on Three Filial Generation, *Ann. Entomol. Soc. Am.* **72:** 216-220.

[5] La Chance L. E. (1985). *Genetic Methods For The Control of Lepidoptera Species Status and Potential Rep.*, A.R.S-28. USDA. Washington. D.C.

[6] Carpenter J. E., Nelly H. N. and Mullinix B.G. (1997). Effect of Substerilizing Doses of Radiation on Sperm Precedence in Fall Armyworm, *J. Econ. Entomol.* **90:** 444- 448.

[7] Bloem S., Bloem K. A., Carpenter J. E. and Calkins C. O. (1999). Inherited Sterility in Codling Moth: Effects of Substerilizing Doses of Radiation on Insect Fecundity and Fertility, *Ann. Entomol. Soc. Am.* **92:** 222-229.

[8] Ashrafi S. H., Tilton E. W. and Brower J. H. (1972). Inheritance of Radiation Induced Partial Sterility in the Indian Meal Moth, *J. Econ. Entomol.* **65:** 1265-1268.

[9] Bartlett A. C. and Lewis L. J. (1973). Bink Boll Worm Chromosomal Damage and Reproduction After Gamma Irradiation of Larvae, *J. Econ. Entomol.* **66:** 731-733.

[10] Shenouda M. G. (1966). Morphology and Bological Studies on *Sitotroga Cerealella*, M. Sc. Thesis, Fac. Agric., Alex. Univ., Egypt.

[11] Ahmed M. Y. Y., Brower J. H. and Tilton E.W. (1976). Sexual Competitiveness of Adult Indian Meal Moth Irradiated as Mature Pupae, *J. Econ. Entomol.* **69**: 719-721.

[12] Genchev N. and Genchova E. (1995). Radiation Induced Sterility in the Oriental Fruit Moth. *Bulgarian J. Agric. Sci.* **1**: 227.-233.

[13] Cogburn R. R., Tilton E. W. and Burkholder W. E. (1966). Gross Effects of Gamma Radiation on the Indian Meal Moth and Angoumois Grain Moth. *J. Econ. Entomol.* **59:** 682-685.

[14] Qureshi Z. A., Ahmed N., Hussain T. and Ali S. S.(1995). Effect of Gamma Radiation on One-Day Old Adults of Pink Bollworm and Their F_1 Progeny, *Pak. J. Zool.* **27:** 21-25.

[15] Brower J. H. (1980). Irradiation of Diapausing and Non-Diapausing Larvae of *Plodia Interpunctella*: Effects on Larval and Pupal Mortality and Adult Fertility, *Ann. Entomol. Soc. Am.* **73:** 420-426.

[16] Rosada J. (1988). Effects of Gamma Radiation on Testes and on The Reproductive Ability of The *Anagasta Kuehniella* Zell., *Proc. Int. Sym.* 1987, Vienna. IAEA, 479: 369-380.

[17] Tuncbilek A. S. and Akif K. I. (1996). The Influence of Rearing Medium on The Irradiation Sensitivity of Eggs and Larvae of The Flour Beetle, *Tribolium Confusum*, *J. Stored Prod. Res.* **32:**1-6.

[18] Bahari I. B. and Mohamed M. B. (1993). Radiation Induced Genetic Damage in *Plutella Xylostella, J. Sains Nuklear Malysia.* **7:** 69-76.

[19] Sutrisno S., Hoedaya M. S. Sutardji D. and Rahayu A. (1993). Radiation Induced F_1 Sterility in Diamondback Moth and Tropical Army Worm F., *Proc. Final Res. Co-Ordination Meeting*, Phoenix, Arizona 1991, Vienna IAEA **162:** 23-63.

[20] Zhang H. R., Li Y. Y., Kang W., Wang H. S. and Zhang W. (1993). Studies on Chromosomal Aberrations and Inherited Sterility in Asian Corn Borer, *Proc. Final Res. Co-Ordination Meeting*, Phoenix, Arizona 1991,Vienna IAEA **162:** 117-124.

[21] Al-Hakkak Z. S., Al-Sufi A. A. K. and Murad A. M. B. (1988). Biological and Genetical Effects of Visible Leser Radiation on The Fig Moth, *Eshestia Cautella*, *J. Biol. Sci. Res.* **19:** 95-107.

[22] Carpenter J. E. (1993). Radiation Induced F_1 Sterility in *Helicoverpa Zea* Potential For Area-Wild Population, *Proc. Final Research Co-Ordination Meeting*, Phoenix, Arizona, Vienna (Austria) IAEA. **162:** 37-47.

[23] Villavasa E. J., Mc Govern W. L., Wagner T. L. and Willers J. L. (1998). Components of Competitiveness in Sterile Male Boll Weevils, *J. Econ. Entomol.* **91:** 631-636.

[24] Shonthoram K., Tamhankar A. J. and Harwalkar M. R. (1995). Comparative Mating and Reproductive Performance of Radiation Sterilized and Radiation Induced F sub 1 Sterile Males of *Earias Vittella* Fabricuis, *J. Nucl. Agric. and Biol.* **24:** 232-236.

[25] Bloem S., Bloem K. A., Carpenter J. E. and Calkins C. O. (1999). Inherited Sterility in Codling Moth: Effect of Substerilizing Doses of Radiation on Field Competitiveness, *Environ. Entomol.* **28**: 669-674.