

## EFFECT OF A SUBSTERILIZING DOSE OF RADIATION ON THE MATING COMPETITIVENESS OF MALE AND ON THE MATING PROPENSITY OF FEMALE *HELICOVERPA ARMIGERA* (LEPIDOPTERA: NOCTUIDAE)

VIRGINIA R. OCAMPO

Department of Entomology, University of the Philippines Los Baños, College, Laguna 4031, Philippines

### ABSTRACT

In this article we report the results of experiments conducted with *Helicoverpa armigera* (Hübner) to determine the effects of a substerilizing dose of gamma radiation (100 Gy) on the mating competitiveness of treated males and the effect on the mating propensity of females with which they mate. Mating competitiveness of treated and untreated male moths was measured at two different release ratios inside field-cages in a cabbage field. A 1♂:1♀ and a 4♂:1♀ ratio were used while keeping a constant density of moths per cage. The mean number of matings recorded was not significantly different at either ratio, suggesting that treated males of this species are equally as competitive as their untreated counterparts. In the mating propensity studies, virgin female *H. armigera* were first mated to treated or untreated males and then re-exposed to untreated males 24 hours later. No statistical differences were found in the number of females that re-mated from either group. Thirty point eight percent of the females first mated with treated males and 29.17% of the females first mated to untreated males re-mated in this study. When both types of females were re-exposed to untreated males in the same field-cage, a higher percentage (38.3%) of females that had initially mated with a treated male re-mated than those initially mated with a untreated male (31.7%), although the differences were not significant.

Key Words: F<sub>1</sub> sterility, partial sterility, remating, corn earworm

### RESUMEN

En este artículo se reportan los resultados de experimentos con la especie *Helicoverpa armigera*. El objetivo de estos experimentos era determinar el efecto de una dosis sub-esterilizante de radiación gamma (100 Gy) en la competitividad de copula de machos irradiados y el efecto indirecto sobre la propensidad de re-copula de las hembras con las cuales copularon estos machos. La competitividad de machos fértiles e irradiados se evaluó a dos diferentes tasas de liberación dentro de jaulas de campo colocadas en una plantación de repollo. Las tasas de liberación utilizadas fueron 1 macho:1 hembra y 4 machos:1 hembra mientras se mantuvo el número de insectos constante dentro de las jaulas. El número promedio de copulas que se recobraron en cada jaula no fue significativamente distinto, lo que sugiere que los machos irradiados pueden competir efectivamente con los machos fértiles. En estudios sobre propensidad de re-copula, hembras vírgenes fueron apareadas con machos fértiles o irradiados y luego fueron re-expuestas a machos fértiles después de 24 horas. No se encontró una diferencia estadística en el porcentaje de re-copulas entre los grupos de hembras. Treinta punto ocho por ciento de las hembras que primero se aparearon con machos irradiados re-copularon después de 24 horas mientras que 29.17% de las hembras primero apareadas con machos fértiles re-copularon después de 24 horas. Cuando se expusieron estas hembras (inicialmente apareadas con distintos tipos de machos) simultáneamente a machos fértiles después de 24 horas, un porcentaje más alto (38.3%) de hembras inicialmente apareadas con machos irradiados se re-aparearon que las hembras apareadas con machos fértiles (31.7%), pero las diferencias no fueron estadísticamente significativas.

The corn earworm, *Helicoverpa armigera* (Hubner), is one of the most destructive pests of agricultural crops in the Philippines. It is a highly polyphagous insect, feeding on 84 different plant species (Gabriel 1997). One environmentally acceptable control strategy currently being explored for the control of this insect is the release of irradiated partially sterile males capable of transferring sterility to the next generation. The advantages of using partial, inherited or F<sub>1</sub> sterility over classical sterile insect release methods is that partial sterility produces a more competitive

insect that will actively mate with wild females and, as such, will effectively introduce heritable sterility into the native population (Mastro & Schwalbe 1988). Inherited sterility has been investigated in a number of lepidopteran pests such as *Helicoverpa zea* (Carpenter & Gross 1993), *Spodoptera frugiperda* (Carpenter et al. 1997), *Manduca sexta* (Seth & Reynolds 1993) and *Ephestia kuehniella* (Marec et al. 1999).

*Helicoverpa armigera*, a closely related species of *H. zea* (Laster & Hardee 1995), is a potential candidate for population suppression by means of

the  $F_1$  sterility technique. Ocampo et al. (1996) were able to induce partial sterility in this species by irradiating pharate adult males with 100 and 150 Gy. When the treated males were mated to untreated females, female fertility was greatly affected. Percentage egg hatch in females mated to 100 Gy or 150 Gy treated males was only 40-60% while that of females mated to untreated males was 80-100% (V. Ocampo, unpublished data). In a related study, Bella et al. (1997) reported that when 100 Gy was used to treat *H. armigera* males, the dose caused chromosomal translocations in 86% of the spermatocytes examined while 150 Gy caused chromosomal translocations in 98% of the spermatocytes in the testes of treated males. Similarly, 100 Gy has been shown to be most efficacious in the codling moth, *Cydia pomonella* (Anisimov 1993) and the fall armyworm, *Spodoptera frugiperda* (Carpenter et al. 1997). In these species, the dose induced a high level of inherited sterility in the  $F_1$  generation without reducing competitiveness of parental males treated.

Mating competitiveness is essential to the effectiveness of a sterile insect release program. However, the refractory period of wild females must not be adversely affected by mating with the released males (Snow 1988). An irradiated male must be able to transfer a full complement of sperm and accessory gland fluid to the female, and this complement must reach the spermatheca to elicit the female refractory period. Giebulowicz et al. (1990) showed that presence of sperm in the spermatheca is necessary to induce the switch from virgin to mated behavior in the gypsy moth, *Lymantria dispar*. During the refractory period, pheromone production by the female remains low, oviposition behavior is triggered and the female will refrain from remating. If sperm and accessory gland fluid do not reach the spermatheca, pheromone production will increase and the female will resume calling for mates.

In this article we report the results of experiments conducted with *H. armigera* to determine the effects of 100 Gy of gamma radiation on the mating competitiveness of treated males and the effect on the mating propensity of the females with which they mate.

## MATERIALS AND METHODS

*Helicoverpa armigera* used in these experiments came from a laboratory colony maintained at the Department of Entomology, University of the Philippines Los Baños. Larvae were reared under ambient laboratory conditions ( $27 \pm 2^\circ\text{C}$ ) in 50 ml plastic cups and fed a soybean-corn based diet as described in Ocampo et al. (2000). Pupae were collected and sexed and stored at ambient conditions until needed. Late pupae containing pharate males were irradiated at a dose of 100 Gy

using a Cobalt<sup>60</sup> Gammacell 220 irradiator delivering a dose of 4 Gy/min. Treated and untreated pupae were held separately until adult emergence. Newly emerged (<24 h-old) virgin male and female moths were used in all mating experiments.

### Mating Competitiveness of Males

Male competitiveness was assessed in field-cages ( $n = 4$ ;  $6\text{m} \times 5\text{m} \times 2.15\text{m}$ ) placed over cabbage plants within 1-ha cabbage field. Three types of males were used in the experiments. Males treated with 100 Gy ( $T\delta$ ), as indicated above;  $F_1$  progeny males ( $TF_1\delta$ ), obtained from crossing treated (100 Gy)  $P_1$  males to untreated virgin females, and untreated males ( $N\delta$ ). In order to distinguish the male "types" the wing tips of treated and  $F_1$  males were differentially colored with a felt-tip marker and untreated males were left unmarked. Moths were allowed to acclimate to field conditions for 3 hours (from 1700 hours Philippine Standard Time (PST) until they were released at 2000 hours PST). Males and females were released at opposite ends of the cages. In each cage mating pairs were collected once every hour for five hours, from 2100 to 0200 hours PST, coinciding with the peak of mating activity as reported by Morallo-Rejesus and Alcalá-Carilo (1981). These workers also report that copulation lasted an average of 30 minutes.

Mating competitiveness was measured at two different release ratios. A  $1\delta:1\text{f}$  ratio, maintaining equal numbers of male "types" while keeping a density of 60 moths per cage. Field-cages in this study received 30  $N\delta$  each, and the following male treatments: 30  $T\delta$ ; 30  $N\delta$ ; 15  $T\delta$  + 15  $N\delta$ ; 10  $T\delta$  + 10  $TF_1\delta$  + 10  $N\delta$ . The next set of experiments was conducted at a  $4\delta:1\text{f}$  ratio, while keeping a constant density of 50 moths per cage. Field-cages in this study received 10  $N\text{f}$  each, and the following male treatments: 40  $T\delta$ ; 40  $N\delta$ ; 30  $T\delta$  + 10  $N\delta$ . All combinations were replicated once per night for four nights. Male types with the highest number of recorded matings were considered the most competitive. Data were subjected to analysis of variance and differences between means were tested for significance using Waller-Duncan's K-ratio t-test (Ott 1993).

### Mating Propensity of Females

Mating propensity was assessed inside field-cages (see above). Insects were released into separate cages in the following combinations: 100 untreated *H. armigera* females ( $N\text{f}$ ) + 100 treated males ( $T\delta$ -100 Gy), and 100  $N\text{f}$  + 100 untreated ( $N\delta$ ) males. Pairs in copula were collected once per hour as described above. Females that mated with treated ( $N\text{f}$ - $T\delta$ ) and untreated ( $N\text{f}$  -  $N\delta$ ) males were differentially colored using a felt-tip marker as above. On the following

evening, the mated females (from both groups) were given the opportunity to remate with untreated males ( $N\delta$ ) by releasing them into field-cages in the following combinations (at  $1\text{♀}:1\delta$  ratio):  $30\text{ N♀-}N\delta + 30\text{ N}\delta$ ,  $30\text{ N♀-}T\delta + 30\text{ N}\delta$ ,  $15\text{ N♀-}N\delta + 15\text{ N♀-}T\delta + 30\text{ N}\delta$ . Pairs in copula were collected as above. All treatments were replicated four times. Females were then dissected to determine the number of spermatophores in the bursa copulatrix. A twice-mated female should have two spermatophores to confirm two successful matings/copulations.

## RESULTS

### Mating Competitiveness of Males

The results of the mating competitiveness tests are summarized in Table 1. At the  $1\text{♀}:1\delta$  ratio, the mean number of mating pairs collected in cages containing treated ( $T\delta$ ) and untreated ( $N\delta$ ) males was not significantly different ( $F = 1.07$ , d.f. = 6,  $\alpha = 0.05$ ). In addition, when both male types were together in the same cage ( $30\text{ N♀}:15\text{ T}\delta:15\text{ N}\delta$ ), both  $T\delta$  and  $N\delta$  males appeared to be equally competitive. The mean number of matings for  $N\delta$  was 4.25 and this number was 3.76 for  $T\delta$  ( $F = 0.17$ , d.f. = 6,  $\alpha = 0.05$ ). Mating competitiveness of  $F_1$  male offspring ( $TF_1\delta$ ) was also evaluated. Results show that the mean number of matings for  $TF_1\delta$  was 4.76, which did not differ statistically from the number of matings with other male types ( $F = 0.21$ , d.f. = 9,  $\alpha = 0.05$ ).

When experiments were conducted at a  $4\delta:1\text{♀}$  ratio, there was no significant difference in the mean number of mating pairs collected in cages containing  $T\delta$  (7.00) or  $N\delta$  (6.00) males ( $F = 0.46$ , d.f. = 6,  $\alpha = 0.05$ ). When three times more treated males than untreated males were released into the field-cage ( $10\text{ N♀}:10\text{ N}\delta:30\text{ T}\delta$ ) the number of observed matings for the treated males significantly exceeded the expected number ( $F = 216$ , d.f. = 6,  $\alpha = 0.05$ ).

### Mating Propensity of Females

The results of the mating propensity studies, where virgin female *H. armigera* were first mated to treated or untreated males and then re-exposed to untreated males 24 hours later are shown in Table 2. Thirty point eight percent of the females first mated with treated males ( $N\text{♀-}T\delta$ ) and 29.17% of the females first mated to untreated ( $N\text{♀-}N\delta$ ) males re-mated in this study. The difference was not statistically significant ( $F = 0.04$ , d.f. = 6,  $\alpha = 0.05$ ). When both types of females were re-exposed to untreated males in the same field-cage ( $15\text{ N♀-}T\delta + 15\text{ N♀-}N\delta + 30\text{ N}\delta$ ), a higher percentage (38.3%) of females that had initially mated with a treated male re-mated than those initially mated with an untreated male (31.7%), although the differences were not significant ( $F = 0.25$ , d.f. = 6,  $\alpha = 0.05$ ). Carpenter et al. (1987) report similar trends for *Helicoverpa zea*, a closely related species to *H. armigera*.

## DISCUSSION

An important concern in sterile insect release programs is that treated males destined for release retain the ability to perceive/orient to pheromone signals from females and, as such, are able to compete with the wild males in locating and mating with calling females in the field. Data presented herein suggest that partially sterilized male *H. armigera* and their male progeny were as competitive as untreated males in seeking and securing mates in a field-cage situation. Males treated with 100 Gy,  $F_1$  males (from 100 Gy treated fathers) and untreated males appeared to be equally as competitive in mating with virgin females when placed together in field-cages. It appears that a dose of 100 Gy of gamma radiation does not cause sufficient physiological damage to alter male mating behavior, but induces sufficient genetic damage in the spermatocytes to reduce sperm viability, as reported by Bella et al. (1997).

TABLE 1. MATING COMPETITIVENESS OF TREATED (100 GY- $T\delta$ ), UNTREATED ( $N\delta$ ) AND  $F_1$  OFFSPRING ( $TF_1\delta$ ) OF *HELI-COVERPA ARMIGERA* MALES FOR VIRGIN FEMALES INSIDE FIELD-CAGES (6M  $\times$  5M  $\times$  2.15M) IN A CABBAGE FIELD AT UPLB, LAGUNA, PHILIPPINES, 1996.

Ratio	Number and type of moths	Mean number of mating pairs ( $\pm$ SD) <sup>1</sup>		
		N $\times$ N	N $\times$ T	N $\times$ $TF_1$
1 $\delta$ :1 $\text{♀}$	30 N $\text{♀}$ :30 N $\delta$ or 30 N $\text{♀}$ :30 T $\delta$	10.00 $\pm$ 2.00a	12.75 $\pm$ 4.92 a	- <sup>2</sup>
	30 N $\text{♀}$ :15 T $\delta$ + 15 N $\delta$	4.25 $\pm$ 2.22a	3.76 $\pm$ 0.96 a	- <sup>2</sup>
	30 N $\text{♀}$ :10 T $\delta$ + 10 $TF_1\delta$ + 10 N $\delta$	6.25 $\pm$ 4.50a	5.25 $\pm$ 3.30 a	4.76 $\pm$ 1.26 a
4 $\delta$ :1 $\text{♀}$	10 N $\text{♀}$ :40 N $\delta$ or 10 N $\text{♀}$ :40 T $\delta$	6.00 $\pm$ 2.31a	7.00 $\pm$ 1.82 a	- <sup>2</sup>
	10 N $\text{♀}$ :30 T $\delta$ + 10 N $\delta$	0.50 $\pm$ 0.58a	6.50 $\pm$ 0.58 b	- <sup>2</sup>

<sup>1</sup>Means  $\pm$  SD on the same row followed by the same letter are not significantly different (K ratio = 500).

<sup>2</sup>Not applicable.

TABLE 2. MATING PROPENSITY OF ONCE-MATED FEMALE *HELICOVERPA ARMIGERA* MATED TO EITHER UNTREATED (N♀-N♂) OR TREATED (N♀-T♂) MALES AND EXPOSED TO UNTREATED MALES AFTER 24 HOURS INSIDE FIELD-CAGES (6M × 5M × 2.15M) IN A CABBAGE FIELD AT UPLB, LAGUNA, PHILIPPINES, 1996.

Moths released into field-cages	Mean number of re-matings (±SD) 24 h after the first mating (percent) <sup>1</sup>	
	N♀-N♂ × N♂	N♀-N♂ × N♂
30 N♀-N♂:30 N♂ or 30 N♀-T♂:30 N♂	8.75 ± 3.86 (29.17) a	9.25 ± 2.99 (30.83) a
15 N♀-T♂:15 N♀-N♂:30 N♂	4.75 ± 2.87 (31.67) a	5.75 ± 2.75 (38.33) a

<sup>1</sup>Means ± SD (percentage) on the same row followed by the same letter are not significantly different (K ratio = 500).

This dose also induced a high level of inherited sterility in the F<sub>1</sub> male progeny of males treated with 100 Gy without reducing their capacity to seek and secure mates. The competitiveness of other irradiated Lepidopterans has been reported by Carpenter et al. (1989, 1997) and Anisimov (1993). In these species, 100 Gy has also been shown to be most efficacious. Carpenter et al. (1989) further showed that the irradiated *H. zea* males were competitive with normal laboratory reared insects in their ability to survive under field conditions.

*Helicoverpa armigera* has a tendency to mate more than once. In our experiments, about 30% of mated females re-mated regardless of the type of male with which they had first mated (Table 2). These findings agree with an earlier study (Ocampo et al. 2000) that found 31-36% of *H. armigera* females mated twice. Our data also suggests that females mated to treated (100 Gy) males were no more attractive to untreated males on the night following the first mating than were females mated to untreated males. One explanation for this finding might be that there is no discernible difference in the quality of the sperm complement and accessory fluid transferred by untreated and treated males during mating. Thus, the type of male with which the female mates first does not affect her mating propensity and refractory period.

#### ACKNOWLEDGMENTS

This research was supported by Research Contract No. 8156/RB under the FAO/IAEA Coordinated Research Program entitled "Evaluation of Population Suppression by Irradiated Lepidoptera and Their Progeny" of the International Atomic Energy Agency, Vienna, Austria. The author is very grateful to the two anonymous reviewers for valuable suggestions on the manuscript. The author would also like to thank Ms. Josephine B. de Leon, Ms. Gaudencia M. Añober and Ms. Rosita M. Crisologo for their technical support during the conduct of field-cage experiments.

#### REFERENCES CITED

- ANISIMOV, A. I. 1993. Study of mechanisms and possibilities of using F<sub>1</sub> sterility for genetic control of codling moth, pp. 135-155. *In* Proc. Final Research Coordination Meeting: Radiation Induced F<sub>1</sub> Sterility in Lepidoptera for Area-Wide Control, Phoenix, Arizona, 1991. International Atomic Energy Agency, Vienna, STI/PUB/929.
- BELLA, A. P., G. L. JERUSALEM, A. A. BARRION, V. R. OCAMPO, AND J. B. DE LEON. 1997. Gamma-ray induction of translocations in the corn earworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae). *Philippine Ent.* 11: 65-69.
- CARPENTER, J. E., A. N. SPARKS, AND H. L. CROMROY. 1987. Corn earworm (Lepidoptera: Noctuidae): Influence of irradiation and mating history on the mating propensity of females. *J. Econ. Entomol.* 80: 1233-1237.
- CARPENTER, J. E., A. N. SPARKS, S. D. PAIR, AND H. L. CROMROY. 1989. *Heliothis zea* (Lepidoptera: Noctuidae): Effects of radiation and inherited sterility on mating competitiveness. *J. Econ. Entomol.* 82: 109-113.
- CARPENTER, J. E., AND H. R. GROSS. 1993. Suppression of feral *Helicoverpa zea* (Lepidoptera: Noctuidae) populations following the infusion of inherited sterility from release substerile males. *Environ. Entomol.* 22: 1084-1091.
- CARPENTER, J. E., HIDRAYANI, AND B. MULLINIX. 1997. Effect of sterilizing doses of radiation on sperm precedence in fall armyworm (Lepidoptera: Noctuidae). *J. Econ. Entomol.* 90: 444-448.
- GABRIEL, B. P. 1997. Insects and Mites Injurious to Philippine Crop Plants. National Crop Protection Center. UPLB, College, Laguna. 171 pp.
- GIEBULTOWICZ, J. M., A. K. RAINA, AND E. C. UEBEL. 1990. Mated-like behavior in senescent virgin females of gypsy moth, *Lymantria dispar*. *J. Insect Physiol.* 36: 495-498.
- LASTER, M. L., AND D. D. HARDEE. 1995. Inter-mating compatibility between North American *Helicoverpa zea* and *Helicoverpa armigera* (Lepidoptera: Noctuidae) from Russia. *J. Econ. Entomol.* 88: 77-80.
- MAREC, F., I. KOLLAROVA, AND J. PAVELKA. 1999. Radiation-induced inherited sterility combined with a genetic sexing system in *Ephesia kuehniella* (Lepidoptera: Pyralidae). *Ann. Entomol. Soc. America* 92: 250-259.
- MASTRO, V. C., AND C. P. SCHWALBE. 1988. Status and potential of F<sub>1</sub> sterility for control of noxious Lepidoptera, pp. 15-40. *In* Proc. Symp.: Modern Insect Control: Nuclear Techniques and Biotechnology.

- IAEA/FAO, Vienna, Austria, 16-20 November 1987. International Atomic Energy Agency, Vienna.
- MORALLO-REJESUS, B., AND E. ALCALA-CARILO. 1981. The attacus juvenile hormone studies. II. Sterilization of *Helicoverpa armigera armigera* (Hübner) with attacus juvenile hormone and dimilin. Philippine Entomol. 4: 389-403.
- OCAMPO, V. R., J. B. DE LEON, AND M. T. TABUR. 1996. Mass-rearing and effects of substerilizing doses of radiation on the corn earworm, *Helicoverpa armigera* (Hübner) and its progeny, p. 51. In Working Material, Second FAO/IAEA Research Coordination Meeting: Evaluation of Population Suppression by Irradiated Lepidoptera and Their Progeny, Vienna, Austria, 2-6 September 1996. IAEA-D4-RC-561. 57 pp.
- OCAMPO, V. R., M. T. TABUR, R. D. FULGENCIO, A. C. LIM, AND F. D. OROZCO. 2000. Diet development and mass rearing of the corn earworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) in the Philippines. Philippine Agric. Sci. 83: 282-291.
- OTT, R. L. 1993. Introduction to the analysis of variance and multiple comparisons, pp. 769-841. In An Introduction to Statistical Methods and Data Analysis 4th edition. Duxbury Press, Belmont, CA. 1051 pp.
- SETH, R. K., AND S. E. REYNOLDS. 1993. Induction of inherited sterility in the tobacco hornworm *Manduca sexta* (Lepidoptera: Sphingidae) by substerilizing doses of ionizing radiation. Bull. Entomol. Res. 83: 227-235.
- SNOW, J. W. 1988. Radiation, insects and eradication in North America. An overview from screwworm to bollworm, pp. 8-10. In Proc. Symp.: Modern Insect Control: Nuclear Techniques and Biotechnology, Vienna, Austria, 16-20 November 1987. International Atomic Energy Agency, Vienna.