8. STUDY ON THE MATING ABILITY AND COMPETITIVENESS OF THE STERILE MALES OF RICE STEM BORER, *CHILO SUPPRESSALIS* (WALKER).

J. S. HYUN*

Introduction

One of the important requirements for the successful application of the sterile-male technique is to sterilize the insects without serious damage to their mating ability or other biological behaviour. Several species of moths have been studied to test the suitability of the sterile males for release program. There has been a considerable amount of accumulated data on the mating competitiveness of the sterile males indicating that the insects can be sterilized without any appreciable alteration of the mating behaviour if treated with adequate dosages at a suitable age of the life stage.

Mating ability of a moth has been routinely tested by holding normal females with various ratios of sterile to normal males and testing the fertility of the eggs produced. The mating of the tested insects is usually assessed by the presence of spermatophores in the bursa copulatrix of the female (Ouye et al. 1964).

The work reported here is a continuation of the feasibility tests on the use of the sterile male technique in the control of rice stem borer, *Chilo suppressalis* (Walker). The purpose of this work is to investigate the effects of radiation on the mating ability and competitiveness of the sterile males of the insect.

Methods and Materials (General)

A large number of overwintered larvae were collected in the field, and stored in a refrigerator (5°C) with pieces of corrugated carton board into which the larvae had crawled. They were transferred into incubators which maintained at 25 ±2°C temperature and 90% relative humidity. The pupae were removed every days and sexed.

Five to seven day old pupae were irradiated, placing them in a small glass vial. Less than twenty pupae were irradiated at once.

The Co-60 sources were pole types and the dose rates were 4.56×10^4 rad/hr and 3.71×10^4 rad/hr at a distance of 10 cm from the two sources. The temperature was 18 ±2°C at the time of irradiation. The calibration of the dose rates was made by Frick's chemical dosimetric method. The exposure time was less than 30 minutes.

The irradiated pupae were held in large glass cylinders which were kept in an incubator. Each cylinder was provided with a water soaked roll of dental cotton that was remoistened daily. The matings were made on the morning following the adult emergence. Further details of the experimental methods will be explained for each experiment.

Results and Discussion

1. The mating ability of the sterile males

a. The number of matings during life of the male.

Each of emerged male moths from irradiated pupae was confined individually with

* Department of Agricultural Biology, College of Agriculture, Seoul National University, Suwon, Korea.

a female in a small glass bottle (8.5 cm in diameter and 8 cm in height), which was provided with a water-soaked roll of dental cotton, remoistened daily. Ten replicates were used for each treatment. A parchment paper was provided for oviposition on the inside wall of the bottle. A fresh virgin female was provided every day until the irradiated male died; consequently the females were of constant age even though the male became progressively older.

The removed females were preserved separately in 75% alcohol until the examination for spermatophores in the bursa copulatrix. The presence of spermatophores was regarded as evidence of mating. The results obtained are shown in Table 1.

Table 1. Mating performance of males when a fresh female was provided daily throughout the male's life time.

Doses	Mated male (%)	Max. no. of matings for a male	Mated females (%)	Total no. of females provided	Longevity of Male* (days)
0	100.0	2	37.5	32	3.3(1–5)
25	100.0	2	30.0	40	4.0(1–7)
30	100.0	2	30.0	40	4.0(1–9)

* Average longevity of the treated males (range in parenthesis).

There is no difference in percentage of males that mated, nor in the maximum number of matings of the males. The percentages of mated females were 37.5% for the control, and 30.0% for the treated males.

The day of the first mating and the interval between the first and the second mating are shown in Table 2.

Table 2. Average time of first mating and interval between matings when a fresh female was provided daily to a male throughout his life time.

Doses (Krad)	First mating (days)	Interval (days)
0	1.5	1.3
25	2.4	3.0
30	2.2	3.0

The time of the first mating averaged 1.5 days for the control and 2.4 and 2.2 days for the irradiated males, depending on the dosage. The average interval between the first and the second mating was 3.0 days for the treated males, while it was 1.3 days for the control.

This may mean that the irradiation affects the vigour of mating and/or causes some delay in matings. It was observed that 28% out of the 37.5% of the total matings were made in the first two days in the control group, 15% out of the 30% in the treated groups.

b. *Mating ability of the sterile males when 5 or 10 virgin females were provided together in a cage.*

Five or ten normal females were confined together with a single freshly emerged male moth in a screen cage (15 × 15 × 30 cm) in which rice seedlings were provided.

The male moths were removed after five day confinement. Ten replicates were used.

The female moths were preserved in 75% alcohol after their death. Matings were determined by dissection of the bursa copulatrix. The results obtained are shown in Table 3.

Table 3. Number of mated females when 5 or 10 virgin females were provided to one male in a cage for a period of five days.

Doses (Krad)	Mated females (%)	Mating per male			Hatched eggs (%)	Females surviving after 5 days
		Average	Max.	Minn.		
1 : 5						
0	24.0	1.2	2	1	38.14	9
25	34.0	1.7	3	1	8.69	8
30	24.0	1.2	2	0	0	12
1 : 10						
0	25.0	2.5	4	1	32.28	13
25	18.0	1.8	3	1	7.63	13
30	18.0	1.8	4	1	0	12

The total number of mated females was greated when 10 females were provided per male than when 5 females were provided, but the percentage of mated females seemed to be smaller with the greater number of females per male in the case of the irradiated males. The average number of matings per male was greater when the greater number of females was provided, especially for the control group. The average numbers of matings per male were 1.2 and 2.5 respectively for the normal males when 5 and 10 females were provided. No significant increase in total number of matings occurred for the treated males, even though a considerable increase in the maximum number of matings was observed.

2. Mating competitiveness of rice stem borer with different ratios of irradiated and normal males

Five virgin females were confined with various ratios of irradiated to normal males per female (1:0:1, 1:1:1, 2:1:1, 1:2:1, 5:1:1, 1:5:1, 0:1:1). The sizes and the conditions of the mating cages were the same as in experiment **1**-b. Ten replicates were used. The eggs were removed with a piece of the rice leaf on which they had been deposited and placed in a petri dish in which filter paper and a moistened roll of dental cotton were provided to maintain moisture. The results are shown in Table 4.

The treated males were not fully competitive with untreated males, regardless of the irradiation doses. The greatest reduction of egg hatch was obtained with 5:1:1 (30 krad), in which 26.6% of the eggs hatched.

Competition of the irradiated males has been studied for several insects. Walker and Brindley (1963) reported the results of a study on the effect of X-ray irradiaton on *Ostrinia nublalis*(H.), in which the hatchability of the eggs produced by the females confined with males in the ratio of 8:4 (sterile: normal males) was 39.4%, while it was 1.1% when the females were mated with irradiated males only (35 kr of X-ray in adult stage). Flint and Kressin (1968) reported that in their experiments with *Heliothis verscens*(F.) using a 3:1:1 ratio of sterile males: normal males: normal femaels, the hatchability of the eggs produced by the females was 52.4%, while the

Table 4. Mating competitiveness of treated males of rice stem borer.

Ratio*	Mated femals (%)	No. of egg masses/F.	No. of eggs/ egg mass	Hatched (%)	Expected (%)	Adjusted** (%)
25 krad						
0 : 1 : 1	55.70	129.3	35.92	78.62	—	—
1 : 0 : 1	50.60	93.6	25.30	24.96	—	18.07
1 : 1 : 1	57.30	88.7	21.63	59.83	50.7	41.04
2 : 1 : 1	57.70	105.6	27.08	57.68	45.0	47.11
1 : 2 : 1	59.60	108.3	25.79	68.42	60.9	57.31
5 : 1 : 1	59.20	119.2	31.37	40.72	34.4	37.54
1 : 5 : 1	65.90	110.3	26.90	65.76	61.6	56.10
30 krad						
0 : 1 : 1						
1 : 0 : 1	45.50	87.6	18.25	0.3	—	
1 : 1 : 1	53.40	92.3	17.42	42.82	39.0	30.57
2 : 1 : 1	53.70	95.7	19.53	43.62	25.0	32.28
1 : 2 : 1	55.40	93.9	19.78	66.32	50.9	48.16
5 : 1 : 1	54.80	87.8	18.68	26.62	13.7	18.07
1 : 5 : 1	57.90	124.6	23.07	63.28	65.8	60.98

* Ratio of irradiated male: normal male: female.
** Hatched eggs as per cents of total number of eggs produced by females mated with nomal males (see text).

expected egg hatch was 22.3%, or 30.1% lower.

The data of the present study suggest that the irradiated males were not fully competitive with untreated males, even though the irradiated males were not significantly different in apparent mating behaviour from the normal males, when they were separated from the normal males. The reduction in the competitive ability of the treated males may be attributed to the reduction in mating vigour suggested by the delayed mating and longer interval between the first and the second matings (Table 3).

Secondly, the calculated reduction in mating competitiveness of the irradiated males may be an indirectly affected to the reduced number of eggs produced by the females mated with the irradiated males, since the percentages of hatched eggs were calculated from the number of hatched eggs and the total numbers of eggs produced. If the females mated with treated males produce smaller number of eggs than those mated with the normal males, it may result in overestimated hatchability resulting in an under-estimation of the competitive ability of the irradiated males.

Table 5. The number of eggs produced by a female.

Doses (Krad)	Number of eggs per female*	
	Mated	Unmated
0	96.37 (10–235)	57.88 (33–150)
25	62.75 (15–110)	49.33 (8–215)
30	68.83 (38–178)	49.0 (10–90)

* Average number of eggs and range in parenthesis.

Table 5 shows the number of eggs per female obtained from experiment **1**-a.

The number of eggs produced by an unmated female was less than the number produced by a mated female regardless of whether she mated with a treated or an untreated male. The average number of eggs laid by mated females was 75.98, while it was 52.07 eggs for the unmated females. Flint and Kressin (1969) reported that the virgin females of *H. verscens* (F.) produced an average of 49.6 eggs, while the mated females produced 85.2–438.8 eggs depending on the conditions of the sperm transfer: the mated females without sperm in the spermathecae or spermatophore and mated females with sperm in the spermatophore laid 98.6 eggs and 85.2 eggs respectively, while mated females with sperm in their spermathecae laid 438.8 eggs. They concluded that the presence of sperm in the spermathecae, rather than the act of mating, was required for the normal oviposition.

In Table 5, the females mated with treated males produced less eggs than those mated with the normal males. Flint and Kressin (1969) reported that, when the females had crossed with sterile males, the percentages of mated females without sperm increased (26% to 48%), and the number of eggs per female was 178 for the mated females without sperm, while it was 501 eggs for the mated females with sperm. Thus the transfer of the sperm increased the oviposition of the females.

The data shown in Table 5 suggested a similar trend in oviposition, and such a reduction of the oviposition of females mated with irradiated males might have some relation with the reduced competitiveness.

Thus it might be reasonable that in evaluating the mating competitiveness of an insect we should consider both the direct effects of the irradiation: mating vigour, sperm transfer, etc., which are the detrimental effects of the irradiation on mating, and the indirect effects: sperm transfer, sperm competability, etc., which are likely to reduce oviposition by the females.

The figures shown in the last column of the Table 4 are calculated as the ratio of the hatched eggs to the total number of eggs produced by the females mated with normal males. The figures show that the reduction in competitiveness of the irradiated male seems not to be serious from a practical point of view. This kind of defect can be overcome by release of large numbers of treated males.

References

1. Flint, H. M. & Kressin, E. L. (1967): Gamma irradiation of pupae of the tobacco budworm. J. Econ. Ent. 60: 1655–9.
2. Flint, H. M. & Kressin E. L. (1968): Gamma irradiation of the tobacco budworm: Sterilization, competitiveness and observations on reproductive biology. J. Econ. Ent. 61: 477–483.
3. Flint, H. M. & Kressin, E. L. (1969): Transfer of sperm by irradiated *Heliothis verescens* and relationship to fecundity. Can. Ent. 101: 500–7.
4. Ouye, M. T., Graham, H. M., Richmond, C. A. & Martin, D. F. (1964): Mating studies of the pink bollworm. J. Econ. Ent. 57: 222–5.
5. Raun, E. S., Lewis, L. C., Picken Jr., J. C. & Hotchkiss, P. K. (1967): Gamma irradiation of European corn borner larvae. J. Econ. Ent. 60: 1724–1730.
6. Tantawy A. O., Abdel-Malek, A. A. & Wakid, A. M. (1967): Studies on the eradication of *Anopheles pharoensis* by the sterile-male technique using Cobalt-60. V. Mating competitiveness in radiosterilized males. J. Econ. Ent. 60: 696–9.
7. Taylor, O. R. (1967): Relationship of multiple mating to fertility in *Atteva punctella*. Ann. Ent. Soc. Am. 60: 583–590.
8. Walker J. R. & Brindley, T. A. (1963): Effects of X-ray exposure on the European corn borer. J. Econ. Ent. 56: 522–5.

Discussion

M. T. Ouye: In Table 1 of your paper what is the average number of mating for the males?

J. S. Hyun: I don't know.

N. D. Ngoan: What was the temperature at the time of irradiation?

J. S. Hyun: The temperature at the time of irradiation was 25°C.

K. Kiritani: What is the peak period of *Chilo suppressalis* in your country?

J. S. Hyun: Peak periods of moths are middle of June and middle of August.