Competitiveness of Boll Weevils (Coleoptera: Curculionidae) Sterilized with Low Doses of Fractionated Irradiation[1]

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ABSTRACT Male boll weevils, *Anthonomus grandis* Boheman, sterilized with nine fractionated doses totaling 48 gray (Gy) of irradiation successfully competed with normal untreated males for virgin females. The ratios of Smale:Nmale:Nfemale tested were 1:1:1, 5:1:1, 10:1:1, 20:1:1, and 40:1:1. We determined that Smales, collectively, at a 20:1 or 40:1 ratio of Smale:Nmale were 90.8-95.1% as competitive as Nmales. A 40:1:1 ratio of Smale: Nmale:Nfemale resulted in no matings between Nmales and Nfemales alone. There were an average of 1.8 matings when both Nmale and Smales mated with the Nfemale resulting in 99.9% sterility (based on F_1 adult emergence from eggs collected).

KEY WORDS Insecta, boll weevils, irradiation, competitiveness, *Anthonomus grandis*.

A major cost associated with producing cotton in the United States is the cost of controlling cotton insect pests. Since the cotton boll weevil, *Anthonomus grandis* Boheman, first entered the United States in the late 1800s, it has infested more than one-half of the acres devoted to cotton production, resulting in > $20 billion in economic losses to date. In 1991, arthropod pests reduced crop yields by about 5% ($263 million), despite control measures totaling $437 million; thus, the estimated total loss was at least $700 million (Hardee and Herzog 1992).

An alternative to insecticide control is needed as an element in an area control strategy. Environmentally-sensitive areas and areas with very low populations of weevils would be ideally suited for such strategies as the sterile insect technique. The sterile insect technique involves the rearing, sterilization, and release of treated weevils into fertile weevil populations. The sterile weevils mate with the wild (fertile) weevils, resulting in the production of nonviable eggs.

How effective are sterile boll weevils in controlling untreated boll weevils prior to use in field studies? It was found that boll weevils, *A. grandis* sterilized with 25 fractionated doses of irradiation totaling 62.5 gray (Gy) (Haynes et al. 1977) were only half as responsive in mating and transferring sperm to virgin females as were control males (Haynes and Mitchell 1977). In a more detailed study (Haynes and Wright 1982) showed that a 40:1 ratio of sterile males (Smales) to normal males (Nmales) reduced egg hatch and adult emergence to

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2.0% when mated with normal virgin females; the treatment consisted of an acute dose of 100 Gy plus feeding 100 ppm of diflubenzuron in the diet for 5 days. Although very effective, this method was too labor intensive. While developing an improved method of sterilization, Haynes and Smith (1989) found that boll weevils dipped in a 400 ppm aqueous solution of diflubenzuron for 30 min plus 100 Gy of acute irradiation resulted in only 5.5% F_1 emergence compared with 49.6% emergence in the control with a 40:1 ratio of Smale:Nmale. Continuing research for the last 10-15 years has shown significant improvements in methods to produce large numbers of sterile weevils. The purpose of this study was to determine the mating competitiveness of sterile male weevils (at five ratios) vs untreated males for virgin untreated females for four days following treatment.

Materials and Methods

Boll weevils used in these experiments were from an ebony mutant strain (Bartlett 1967) reared at the Gast Rearing Laboratory, Mississippi State, MS, using the methods of Roberson and Wright (1984). When the immature weevils began to pupate 9-10 days after implantation on the larval development diet, they were moved to the irradiator room at the Harned Research Laboratory, Mississippi State, MS.

The 3107 ± 5% curie Cesium 137 double encapsulated irradiator emitted 0.78 Gy/h during this study. The trays, in which the weevils were reared, were placed in the irradiator and nine doses of 5.3 Gy each were administered over the next 72 h. the irradiator was programmed to automatically irradiate every eight hours. After the total dose of 48 Gy was completed, the newly eclosed adults were removed from the trays and fed adult diet pellets for one day. Also, normal untreated weevils were held and fed. The following day the boll weevils were sexed and counted. Seven matings were conducted with each of the five ratios tested. Normal males and virgin females were set up seven days in advance and fed untreated diet. Virgin weevils at this age will readily mate. Following irradiation the 1-2 day old treated and 7 day old untreated males were mated with 7 day old virgin females. By using 7 day old weevils, which were more sexually active, rather than 3 day old weevils, the test was biased in favor of the Nmales. In eradication experiments in the field the Smales would mostly be competing against feral weevils more than three day old. The seven matings/ratio/day were replicated on days 1-4 after treatment (total of 28 observations). The ratios of 1:1:1, 5:1:1, 10:1:1, 20:1:1, and 40:1:1 of Smale:Nmale:Nfemale were selected for study. In order to identify the weevils, the normal males were painted blue and the females were painted yellow (single dot on elytra); the treated males were left unpainted. The treated males, normal males, and virgin females were placed separately in a different corner of a 35.6 (L) × 26.7 (W) × 10.2 (H) cm plastic container. The sides of the container were coated with petroleum jelly to confine weevils to the floor of the container. The 2 h test began between 9:00 and 10:00 AM. Each female was allowed to mate freely with any male during this period. If a mating started in the last 30 min of the test period, an additional 30 min was allowed for completion of copulation. After mating the male weevils were destroyed. Females were fed and held for only one day, during which time the eggs were collected. The eggs were divided into two groups and held for hatch up to 7 days or implanted in diet for adult emergence 2 weeks later. All females were dissected and their spermathecae were examined to confirm whether mating had occurred. The number of aspermic matings were recorded at each ratio tested.

Fried (1971) developed the following method which we used to determine male mating competitiveness. It is as follows:

$$C = \frac{Ha - Ee}{Ee - Hs} \times \frac{N}{S}$$

C = male competitiveness; Ha = Nmale × Nfemale % egg hatch; Ee = experimentally determined hatch; Hs = Smale and Nfemale % egg hatch; N = the number of normal males used; S = the number of sterile males used.

An analysis of variance (ANOVA) was used to determine whether any significant differences existed in the number or length of matings between Nmales and Smales at the five ratios tested. A further analysis was made to determine significant differences in egg laying, hatchability and adult emergence from females mated to these same males. Means were separated with Duncan's (1955) multiple range test (P = 0.05).

Results and Discussion

The number or percent of matings by Nmales, only, decreased as the number of Smales, only, increased; the differences were most significant when the ratio of Smale:Nmale was increased from 1:1 to 5:1. When the number of matings to females by both Nmale and Smale were considered, we found that increasing ratios of Smale:Nmale from 5:1 to 10:1 and from 10:1 to 20:1 had the most significant effects in reducing these mixed matings. At a 1:1 ratio of Smale:Nmale, there were about 1.5 times more matings by Nmales, indicating Smales were approximately 38.0% competitive. When a 40:1 ratio of Smale:Nmale was tested, the Smales were 100.0% competitive, as a group, in preventing the Nmale from mating with the female (Table 1).

The number of Smale, only, matings to females doubled when ratios of Smale:Nmale:Nfemale were increased from 1:1:1 to 5:1:1 and from 5:1:1 to 10:1:1. These matings increased by only 12.0 to 16.0% at higher ratios. Usually, aspermia decreased as the ratio of Smales increased (Table 1). We suspected that fewer aspermic matings would occur at the lowest ratios tested because there was less harassment occurring in the small containers enabling them to successfully mate and transfer sperm. We discovered that one out of every seven matings was aspermic at a ratio of 1 Smale: 1 Nmale; aspermia increased to one out of every 3-4 matings at ratios of > 20:1 of Smale:Nmale. This increase in aspermia at the higher ratios is understandable since some mating couples were harassed by as many as six other males attempting to cling to them.

As the number of matings by Smales, only, increased, the number of Nmale, only, matings decreased by ca one-half until, at a 40:1 ratio of Smale:Nmale, all matings by Nmales, only, were prevented. About 19.0% of the Nmale, only, matings were aspermic at the lower ratios, but no aspermic matings were observed at higher ratios.

There were about five matings per test by both Smale and Nmale which dropped to about two when the ratio of Smale:Nmale increased to 20:1 or 40:1. The number of mixed aspermic matings varied from about 1 out of 3 at the highest ratios tested to 1 out of 6 matings at the lower ratios of Smale:Nmale (Table 1).

Table 1. Competitive mating tests (1h) between males treated with fractionated irradiation and normal male boll weevils for virgin females.

Ratio of *,† Smale: Nmale:Nfemale	Total no. matings by Nmale	Total no. matings by Nmale	No. and % of matings to Nfemale by: ‡,§ Nmale only No.	Nmale only %	Smale only No.	Smale only %	Nmale and Smale No.	Nmale and Smale %
1:1:1	13.6	11.6	8.7 (1.7)	44.1 a	6.0 (1.7)	30.4 c	5.0 (0.7)	25.4 a
5:1:1	9.3	20.0	3.7 (0.7)	16.5 b	13.0 (4.3)	58.0 b	5.7 (1.0)	25.4 a
10:1:1	6.6	32.0	2.0 (0.0)	6.2 c	25.7 (6.0)	79.3 ab	4.7 (1.0)	14.5 b
20:1:1	3.3	32.6	1.0 (0.0)	3.0 cd	29.3 (4.0)	89.9 a	2.3 (0.7)	7.0 c
40:1:1	1.3	35.8	0.0 (5.1)	0.0 d	33.5 (0.5)	94.4 a	1.8	5.1 c

* Nmale = 7-day-old normal male;; Smale = sterile male irradiated with 9 treatments totaling 48 Gy; Nfemale = 7=day-old normal virgin females.
† Numbers in parenthesis indicate the number of aspermic matings.
‡ Four replications of 7 individually paired matings/ratio/day tested for days 1-4 after treatment.
§ Means followed by the same letter in the same column are not significantly different (P = 0.05) using Duncan's multiple range test.

When the total number of matings were considered, we found that a ratio of 1:1 of Smale:Nmale resulted in slightly more Nmale matings to the female than Smale matings; a 10:1 ratio of Smale:Nmale resulted in 5 × more Smale matings; and a 40:1 ratio of Smale:Nmale resulted in about 18 × more Smale than Nmale matings to females.

Normal males mated with females for 38.2 min at the lowest ratio of Smale:Nmale tested to 26.5 min at the highest ratio, as compared with Smales which mated for 36.4 to 28.4 min. The differences were not significant (P = 0.05). These data were omitted from Table 1 for brevity.

Hatch and emergence of eggs from females that mated with both Smale and Nmale, at a 1:5 ratio, was not significantly different from eggs collected in matings at a 1:1 ratio of Smale:Nmale. Also, there was no significant differences in progeny development of matings at 5:1 ratios of Smale:Nmale compared with 10:1 ratios. When matings at a 20:1 or 40:1 ratio of Smale:Nmale was evaluated, we found there was significantly less F_1 adult emergence compared with a F_1 emergence at a 10:1 ratio (Table 2).

Table 2. Resultant progeny development from competitive matings between normal females mated with both sterile males and normal males.*,†

Ratio of ‡ Smale:Nmale:Nfemale	No. eggs	% hatch	% F_1 adult emergency
1:1:1	37.0 ± 21.2	39.3 a ± 5.7	24.5 a ± 1.7
5:1:1	14.7 ± 11.0	24.6 ab ± 6.3	14.3 ab ± 4.1
10:1:1	41.0 ± 15.2	12.0 b ± 11.1	7.8 b ± 7.5
20:1:1	20.7 ± 4.0	4.3 b ± 5.1	0.4 c ± 0.7
40:1:1	50.9 ± 7.2	3.0 b ± 1.7	0.1 c ± 0.3

* Nmale = normal (5-8 day old) male weevils; Smale = sterile male irradiated with 9 treatment totaling 48 Gy; Nfemale = normal virgin females (mated when 5-8 days old).

† Means followed by the same letter in the same column are not significantly different (P = 0.05) using Duncan's multiple range test.

‡ Four replications of 7 individually paired matings/ratio/day tested for days 1-4 after treatment.

When females mated only with Smales, egg hatch varied from 1.6% to 8.0% and adult emergence varied from 1.0 to 4.9 at the ratios tested, but the differences were not significant at the 5% level. Likewise, when females mated only with Nmale's, we found that egg hatch varied from 85.6% to 90.0% and adult emergence varied from 40.0% to 65.5% at the ratios tested, but the differences were not significant at the 5% level. These data were omitted from Table 2 for brevity. The fact that no significant differences were found in these matings by Nmale, only, or Smale, only, substantiates the accuracy of these tests.

In an earlier study Haynes and Wright (1982), using Fried's formula, evaluated weevils sterilized with diflubenzurion plus irradiation and determined that 40 Smales, as a group, were 73.0% as competitive as one normal weevil (Fried 1971) in the laboratory. In the present study, 40 male weevils sterilized with nine fractionated doses totaling 48 Gy were 85.1% as competitive, as a group, as one normal weevil in the laboratory using Fried's formula.

Villavaso et al. (1979), in two field studies (0.2 ha plots), reported that weevils dipped in a 0.2% acetone solution of diflubenzuron + 100 Gy acute irradiation treatment were 16.9 - 36.0% competitive on a 1:1 basis (Smale:Nmale) using Fried's formula.

We conclude that treatments using a fractionated irradiation regime rather than acute irradiation produced healthier, more viable weevils. One reason that multiple fractionated treatments were more successful was because cells became 2.5 - 3.0 × more sensitive due to hypoxia when subsequent doses were administered (Withers 1975). Based on the total number of matings in the competitive mating tests, we found that 20 or 40 Smales treated with a nine fractionated treatment totaling 4.8 Gy were 90.8-95.1% as competitive, as a group, when compared with a single Nmale and conducted under laboratory conditions.

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