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Effects of Gamma Radiation on the Southern Corn Rootworm

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EFFECTS OF GAMMA RADIATION ON THE SOUTHERN CORN ROOTWORM

BY

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ON THE SOUTHERN CORN ROOTWORM

This thesis is approved as a creditable and independent investigation by a candidate for the degree, Master of Science, and is acceptable for meeting the thesis requirements for this degree. Acceptance of this thesis does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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INTRODUCTION

Insects continue to be one of the major problems of man. It is, however, becoming more and more difficult to find substances that are effective in controlling insects and that are environmentally safe. It has become apparent that to control or eliminate harmful insects, alternate methods of control must be developed to replace or reduce the use of insecticides.

One possible alternate method of control is to use insects against themselves in a process called the sterile male technique. This technique generally uses gamma radiation to induce sterility in males. When they are released into the native population and mate with females, no offspring are produced.

The purpose of this work was to test the effects of gamma radiation on the southern corn rootworm (*Diabrotica undecimpunctata howardi*) to determine if this insect is a possible candidate for the sterile male technique. The first objective was to determine if the southern corn rootworm could be sterilized with sublethal doses of gamma radiation. The second objective was to compare the longevity and sexual competitiveness of the sterilized males with untreated males.

LITERATURE REVIEW

The Southern Corn Rootworm

The southern corn rootworm (SCR), *Diabrotica undecimpunctata howardi*, is also commonly called the spotted cucumber beetle and belongs to the class Insecta order Coleoptera. The SCR is widely distributed, occurring in most parts of the United States east of the Rocky Mountains, in southern Canada, and in Mexico (2, 32).

Even though it is a widely distributed insect, the SCR does most of its economic damage in the Gulf Coast states. For some specific examples, seventy-five percent of the corn crop in Marengo County, Alabama was destroyed by SCR larvae in the spring of 1977 (10). In several corn fields in Houston County, Alabama, twenty percent of the corn crop was damaged by larvae (10).

The SCR damages other crops in addition to corn. In 1974 SCR larvae destroyed 100 acres of grain sorghum on a dairy farm in Perry County, Alabama (8), and during 1976 in Houston County, Alabama SCR larvae infested thirty percent of the peanut plants (9).

The adult SCR is a pest of the South Carolina vegetable industry causing damage to cucumbers, watermelons, squash, and cantalope. Combined damage estimates from both larvae and adults in South Carolina alone is $200,000 a year (15).

At the present time in South Carolina 250,000 acres of corn are treated with Furadan® (carbofuran). It is estimated that twenty percent of its usage is attributed to the control of rootworms (15). Earlier, several other southern states recommended the use of aldrin,

heptachlor or chlordane for treatment in fields where rootworms were expected to be a major problem (24, 32).

One effective method of control for the SCR that does not involve pesticides has been late planting of corn on land that is frequently cultivated before planting to keep vegetation down (32). This technique is used where possible, mainly because it is extremely difficult to eliminate SCR larvae from the soil once the crop is germinated.

Nature can be an effective aid in the control of the SCR under certain conditions. It has been reported that as many as twenty-four species of birds feed upon the adult SCR. Temperature is another natural control, because the adult SCR cannot withstand extremely low temperatures. Hot, dry weather is detrimental to all developmental stages (2). It is, therefore, common for SCR damage to increase in years having adequate moisture and heavy vegetation and to decrease in years that are dry with little vegetation.

The SCR survives the winter in the south. Adults become inactive on days when the temperature drops below 21° C and females oviposit very few eggs during the winter months. As the temperature increases in the spring, the adult SCR will migrate northward. The females, which copulate only once, deposit eggs in the soil around the base of plants or randomly throughout the soil. An early study by Arant (2) showed one female laying a total of 1198 eggs. Guss and Krysan (18) found in the laboratory that the SCR female deposits an average of about 20 eggs/day. The time of incubation for egg development is directly related to the temperature. Eggs held constantly at 25° C will usually hatch in six to ten days.

The larvae, upon hatching, immediately bore into the roots of surrounding plants. The larval period consists of three instars, and the larvae increase in size until the formation of the prepupa, at which time feeding stops. The time to complete larval development, about 13 days in the laboratory (4), depends on temperature and the amount of feeding that occurs. Prepupae form a cell in the soil and turn into pupae from which the adults emerge after five to seven days (4). The SCR generally has three generations a year in the south (2); however, only a partial second generation occurs in the north (32).

Control of Insects by the Sterile Male Technique

Some insect pests can be effectively controlled by a method called the sterile male technique. In brief, this process involves the release of overwhelming numbers of sterile males into a native population thereby disrupting reproduction. There are three basic requirements for this type of insect control to be successful: 1) the technology and materials to rear the insect on a large scale must be available and economically feasible, 2) there must be an effective procedure to sterilize the male with one hundred percent certainty and yet not interfere with its longevity or ability to compete sexually with the native males, 3) there must be a method of releasing the sterile males so that they will be undamaged and well mixed with the native population (25, 26, 27, 30, 33, 44). It is preferable that the female mates only once; however, this is not essential (26). It is also preferable that the area to be infested with the sterile males is isolated so that the problem of reinfestation does not occur (44).

A study of the area to be infested is required so that an estimated number of sterile males to be released can be decided upon before the rearing procedure is started (29, 44). After the releases are made, the temporary increase in insect population must create no serious damage to crops, animals or man (26, 44). Delucchi (11) stated that before work is started, studies should be made to see what the possible impact could be on the entire ecosystem.

The first successful attempt to control an insect by the sterile male technique involved the screw-worm fly. In the initial test, the screw-worm male pupae were sterilized with a dose of 2500 roentgens (3, 26, 30, 31), and the adult sterile males were released at a rate of 100 per week per square mile for three months on Sanibel Island (26). After three months the native population had virtually disappeared; however, reinfestation occurred from the United States two miles away. Another test was made on the island of Curacao. During this test 400 sterile males were released per square mile per week, which for the entire island totaled 170,000 screw-worm flies per week. After four generations one hundred percent of the egg masses found were sterile (26).

A much larger test was conducted in Florida where 50 million sterile screw-worm flies were released per week. Both sexes of pupae were irradiated with 8000 roentgens from a cobalt-60 source furnished by the Oak Ridge National Laboratory (26). The flies were distributed by airplanes. A year after initiation of the program and six months after all areas (which included Florida and parts of Georgia and Alabama) were receiving sterile fly releases, the insect appeared to be eradicated. The entire southeast was free of the screw-worm fly

because Florida was free of the insect, and Florida was the overwintering site of the insect. The area between Texas and Florida is unfavorable for overwintering and thus serves as a natural barrier against reinfestation.

The sterile male technique proved to be very successful against the screw-worm fly. However, several alterations may make this technique more feasible with other insects. On the Mariana Islands the oriental fruit fly was eradicated with a combination of sterile insect releases and male annihilation. The male pupae were sterilized by irradiation with 10 Krad of gamma radiation. Final eradication was not achieved, however, until male annihilation caused by fiberboard squares, impregnated with methyl eugenol and naled, were distributed on the island every two weeks for four and a half months (41). Male flies were attracted to the fiberboard squares by the methyl eugenol and were killed by the naled (1,2-Dibromo-2,2-dichloroethyl dimethyl phosphate).

An early laboratory study showed irradiated melon fly males were as competitive as normal flies (1). The island of Rota, M.I. had a release program consisting of 257 million melon flies sterilized with a dose of 9.5 Krad from a cobalt-60 source. This release was preceded by a low cost application of concentrated protein hydrolysate, malathion bait sprays. This was a successful eradication attempt (40).

The Mediterranean fruit fly has been partially controlled by the sterile male technique. The Mediterranean fruit fly pupae were irradiated with a cobalt-60 source with a dose of 10 Krad (6). Tunisia was the test site; however, only semi-isolated areas were used. An insecticide barrier was used to help safeguard against reinfestation

into the test areas. The results improved toward the end of the program when an aerial distribution method was introduced. The estimated population was down by 82.3 percent over the previous year when no sterile releases were made (6). The problem of reinfestation and inadequate sterile fly distribution made it impossible to completely suppress the Mediterranean fruit fly population in this experiment.

There has been continued research on the Mediterranean fruit fly aimed at improving longevity and sexual competitiveness of the irradiated insects (34, 21). Studies have shown that although the male pupae are sterilized with a dose of 10 Krad in air, sterilization did not occur in nitrogen, carbon dioxide, helium, or partial vacuum until a dose of 16 Krad is used (34). Another study showed that when males were irradiated in nitrogen two days before eclosion (emergence as adults), the dose had to be increased by approximately 2.5 Krad over that required in air to achieve the same level of sterility (21). Even at these higher doses the males treated in the artificial atmospheres, especially nitrogen and helium, were significantly more competitive, and in some tests males irradiated in nitrogen and helium were up to three times more competitive than the males irradiated in air (34). The longevity remained unchanged (22). Irradiation of pupae in a nitrogen atmosphere rather than in air increased longevity (42).

Other examples of the beneficial effects of artificial atmospheres are also known. Clark (7) reported that the parasitic wasp *Habrobracon* was three times more resistant to irradiation in nitrogen than in air. Male mosquitoes irradiated with 10 Krad in the presence of nitrogen were as competitive as normal males, while males irradiated in air were

less competitive than untreated males (19).

Some insects are unsuitable for the sterile male technique because it requires almost lethal doses of radiation before sterilization is achieved. For example, adult males of the longheaded flour beetle, sterilized with 20 Krad for males and 10 Krad for females, have their longevity greatly reduced. Survival was less than three weeks after the exposure to 20 Krad (5).

Sterilization of the adult fall armyworms was achieved with a dose of 35 Krad for males and 15 Krad for females. The sterilization process caused no alteration in the adult lifespan, no change in the ability of the female to attract the male, and no change in the ability of the adults to copulate (38), but this work also showed that the sterilized male was only 0.46 times as competitive as normal males (38).

Many studies have gone as far as field tests. When low numbers of sterile horn flies were released in Texas no effect was obtained (28). Later when much larger numbers of sterile horn flies were released, a decrease in population was achieved and approximately 90 percent control was obtained (12, 28).

Corn earworm males were irradiated with 32 Krad. In 1968, 1,289 sterile males were released daily on St. Croix, U.S. Virgin Islands. This attempt was unsuccessful because a significant ratio of sterile males to native males was not achieved; however, in 1969, frequent "locking" of the released males and native females during copulation contributed to the partial suppression of the insect because moths that lock are reproductively dead (37).

The codling moth was sterilized with either a cobalt-60 source or

a cesium-137 source (46). Males require 40 Krad of gamma irradiation for sterilization (36, 45, 47). Work has shown that the irradiated males were less competitive than normal males (23). Nevertheless, damage to orchards in Washington due to the codling moth has been decreased due to the release of sterile insects (36).

Control of the tobacco budworms has been best achieved with a combination of gamma irradiation and chemosterilants. Females were first subjected to a substerile dose of reserpine and then gamma irradiation of a 10 Krad and 15 Krad dosage (17). Later work has shown treatment with chemosterilants or gamma radiation alone was not as effective as the combination of the two (16). In these studies, the tobacco budworm was first subjected to a substerile dose of either sulfanilamide, dicumarol, beta-sitosterol, 2-imidazolidenethione, or imidazole in the larval stage and later subjected to 7.5 Krad or 15 Krad of gamma irradiation during the pupal stage. None of the various combinations affected larval growth and development, adult eclosion, or adult longevity (16).

When SCR adults were subjected to apholate, the viability of the eggs decreased. The apholate reduced the size of the ovaries of the females and inhibited the transfer of the sperm in the males (20). Use of the sterilizing agent, thiotepa, on mosquitoes resulted in suppression and elimination of a population on Seahorse Key, an island off the coast of Florida, after ten weeks (35).

The basic requirements for the effective use of chemosterilants are similar to those of the sterile male technique involving gamma irradiation. Chemosterilants can be used directly in the field

involving only the native population (43); however, their effect on nontarget species must be of no consequence in the environment under consideration.

MATERIALS AND METHODS

The SCR used in experimentation were maintained and reared according to the standard procedures used at the Northern Grain Insects Research Laboratory (4). Two modifications were made in the techniques for maintaining the laboratory colony: 1) the SCR adults were given a moisture source of 0.075 percent sorbic acid in water placed in one ounce plastic cups fitted with a paper lid through which was placed a cotton wick, and 2) gauze which had previously been used as the oviposition medium was replaced with a pink Handi-wipe® (Colgate-Palmolive).

In preparation for irradiation, 0-24 hour old SCR adults were collected and sexed according to established criteria (4). Females were placed in a 1 ft^3 cage until they were needed for mating. The males were prepared for irradiation as described below.

When the cobalt-60 source located on the South Dakota State University campus was used, the males were placed in a half-inch plastic (Tygon®) tubing which contained small holes for air exchange. After the males were in the tubing, the ends were interlocked to form a circle 10 inches in diameter. The tubing was then placed on a platform so that any point in the interior of the tubing would be equidistant (5 inches) from the cobalt-60 source when raised into position. A stop watch was used to monitor the length of irradiation exposure. The cobalt-60 source produced a dose rate of 0.011 Krad per minute at a distance of 5 inches.

When the cesium-137 source located at the Northern Grain Insects

Research Laboratory was used, the males were placed in a small cage situated on top of a brass base. This device was then placed on a small turntable inside the radiation unit to ensure an equal radiation exposure. Later when the males were irradiated in special atmospheres they were placed in three-dram vials. These vials were fitted with a special top consisting of a two-hole rubber stopper through which small diameter glass tubing was placed. The glass tubing was connected to short lengths of rubber tubing to which clamps were attached. This arrangement allowed complete flushing of the vials with either nitrogen or helium, and subsequent sealing of the vial to ensure the integrity of the artificial atmosphere. Enough nitrogen or helium was added to the vial to anesthetize the insect and to remove oxygen so that the insect would remain anesthetized during the irradiation process. The insects were revived immediately following the required exposure time. The cesium-137 source was equipped with an automatic timer that could be set to within 0.1 minutes. The cesium-137 source produced a dose rate of 1.76-2.38 Krad per minute.

When a test was made involving the same dosage with more than one type of atmosphere, the insects were irradiated simultaneously to guarantee all treatments received the same amount of radiation. This was possible because eight three-dram vials could be placed on the brass base at one time.

When using either cobalt-60 or cesium-137, Fricke dosimetry was used to check the dose received in all experiments. The Fricke dosimetry solution was placed in three-dram vials, and the dose received was determined using standard procedures (13). Fricke dosimetry was

used immediately prior to the irradiation of insects to adjust the exposure time for the proper dose and during the irradiation of the insects to verify the actual dose received.

Following irradiation the males were placed in a 1 ft^3 cage prior to mating. For studies to determine the sterilization dose, three males irradiated at the same rate and three females were placed in a small plastic cage. Five of these cages were set up identically for each radiation dose used. Food consisting of a dry diet (4) and water were changed twice weekly. Dead insects were removed regularly and sexed to keep an accurate record of insect longevity. Twelve days after the females and males were mated, a pink Handi-wipe® oviposition medium was placed in the bottom petri dish of the cage.

Eggs were collected weekly from each cage. Twenty-five eggs per replicate were kept each week and screened for hatchability according to standard techniques (4). The egg hatch was checked daily, and empty chorions and larvae were removed and recorded. Egg collection procedures were done once a week for four to six weeks depending on the study. This technique was used to determine the sterilization dose in both air and helium.

In preparation for a competitive study, 0-24 hour SCR adults were collected and sexed as before. The females were placed in a cage for future mating. One-sixth of the males were placed in another cage to be used as non-irradiated controls, and the remaining five-sixths were irradiated in a helium atmosphere with 5.14 Krad from the cesium-137 source and placed in another cage. Since it was not possible to obtain sufficient numbers of 0-24 hour adults in a single day, this procedure

was continued daily until the necessary number of males were obtained.

Sexual competitiveness of sterilized males was determined essentially by the method of Fried (14). In this method, insects from an original pool are separated into three groups: 1) untreated males and females in a ratio of 1:1 (untreated control); 2) sterile males and females in a ratio of 1:1 (irradiated control); and 3) a mixture of sterile males, untreated males, and females in a ratio of 5:1:1 (competitive experiment).

Two separate competitive studies were performed for a period of six weeks. In the first study, the design included 400 irradiated males, 80 untreated males and 80 females. The appropriate controls contained 80 pairs each (see groups 1 and 2 above). In the second study the design included 250 irradiated males, 50 untreated males, and 50 females while the control contained 50 pairs each (see groups 1 and 2 above).

For these two competitive studies, 50 eggs were collected twice a week from each cage and checked for hatch. This procedure was conducted for the entire six week period. Dead insects were sexed periodically and recorded during the six-week period for the two control cages.

Analysis of variance and the t-test were used to interpret data from the sterilization studies (39). The competitiveness value and expected egg hatch were computed as described by Fried (14) as follows:

$$\text{Competitiveness value} = \frac{Ha - Eobs}{Eobs - Hs} \div \frac{S}{N} \quad \text{and}$$

$$\text{Expected egg hatch} = \frac{N(Ha) + S(Hs)}{N + S} \qquad \text{where}$$

Ha = percent egg hatch of N males x N females, Hs = percent egg hatch of S males x N females, Eobs = percent egg hatch at a given ratio of irradiated males: non-irradiated males, N = number of non-irradiated males and S = number of irradiated males.

RESULTS

Sterilization Dose

Initial studies to determine the amount of radiation necessary for sterilization of SCR males were done with a cobalt-60 source in an air atmosphere. Table 1 shows the percent hatch at the various radiation levels. Analysis of variance showed highly significant differences among the dosages.

A similar study was done using a cesium-137 source. Table 2 shows the percent egg hatch at the various dosages. Analysis of variance again showed highly significant differences among the dosages.

Both sources successfully sterilized the males at about the same radiation level; however, since the exposure time was considerably less with the cesium-137 source, this source was used for the remainder of the studies. Both sources produced ninety-five percent sterility with a dosage of 3.6 Krad in an air atmosphere.

Preliminary studies involving the irradiation of males in nitrogen or helium indicated helium to be the atmosphere of choice because of better survival of the irradiated males in the helium atmosphere. Table 3 compares the percent egg hatch and the death rate for three key dates using helium or air during irradiation. Analysis of variance on egg hatch data for both helium and air showed highly significant differences among doses. According to the t-test there was a highly significant difference between helium and air.

Analysis of variance showed highly significant differences among doses for the death rate on all three dates for those insects

Table 1. Average percent hatch of eggs from female SCR mated with males irradiated 0-24 hr. after emergence with various doses of gamma irradiation from a Cobalt-60 source (ave. of 5 replications).

Dose (KRAD)	% Egg Hatch[a]
0.00	47.6
0.99	34.4
2.19	6.2
3.67	4.8
4.19	2.6
4.68	0.6

[a]Analysis of variance showed highly significant differences among doses.

Table 2. Average percent hatch of eggs from female SCR mated with males irradiated 0-24 hr. after emergence with various doses of gamma irradiation from a Cesium-137 source (ave. of 5 replications).

Dose (KRAD)	% Egg Hatch[a]
0.00	75.6
1.18	30.4
2.22	13.0
2.40	17.6
3.27	8.8
3.64	0.4
4.50	0.8

[a]Analysis of variance showed highly significant differences among doses

Table 3. Comparison of average percent egg hatch and male death for insects irradiated in helium and air.*

	AIR				HELIUM			
Dose (Krad)	Percent Hatch[b]	No. Dead on Day 7[b]	No. Dead Day 14[b]	No. Dead Day 45[b]	Percent Hatch[b]	No. Dead Day 7[a]	No. Dead Day 14[a]	No. Dead Day 45[a]
0.0	64.0	1	8	9	58.1	2	9	10
1.0	34.0	2	5	6	59.1	0	1	4
2.0	15.5	1	6	10	41.6	1	8	9
2.5	12.8	5	9	10	33.7	0	2	6
3.1	10.1	2	6	7	32.3	4	7	10
3.7	3.1	5	12	15	13.9	2	5	7
4.0	2.9	4	13	14	14.1	2	2	3
5.0	0.5	11	15	15	3.5	4	6	6

[a]Analysis of variance showed no significant difference between doses.
[b]Analysis of variance showed highly significant differences between doses.
*Egg hatch - ave. of 5 replications, no. dead - total no. for 5 replications.

irradiated. However, there was no significant difference in death rate on any of the three dates when helium was used during irradiation. According to the t-test there were no significant differences in the death rates between air and helium for any of the dates.

Ninety-five percent sterility was achieved with a dose of 3.7 Krad in air, but not in helium until a dose of 5 Krad was reached. To achieve ninety-nine percent sterility 5 Krad was required for the treatment in air, a dose that severely affected longevity.

Because ninety-nine percent sterility was not achieved with the helium atmosphere in the first series of treatments, a second test was performed. In this test ninety-nine percent sterility was achieved in the helium atmosphere at a dose of 6 Krad. Table 4 shows the percent egg hatch and the death rate for this study. Analysis of variance showed highly significant differences among doses for egg hatch. Even at these higher doses analysis of variance of the death rate data showed no differences among doses when irradiation was performed in the helium atmosphere.

Competitive Studies

The first competitive study involved males irradiated in helium with a dose of 5.14 Krad. Table 5 shows the percent egg hatch both observed and expected and the competitive value as calculated by the method of Fried (14). These values are shown for the individual six-week test period and as an average for the six weeks. The average competitive value was 0.81.

Results of the second competitive study, which was performed under

Table 4. Average percent egg hatch and death rate for males irradiated in helium.*

Dose (Krad)	Percent Hatch[b]	No. Dead Day 7[a]	No. Dead Day 14[a]	No. Dead Day 45[a]
0.0	71.0	2	4	5
3.6	15.0	6	7	7
5.0	3.0	6	6	7
6.0	1.0	4	6	8
6.8	0.0	5	10	12

[a]Analysis of variance showed no significant difference between doses.
[b]Analysis of variance showed highly significant differences between doses.
*Egg hatch - ave. of 5 replications, no. dead - total no. for 5 replications.

Table 5. First study of sexual competitiveness of SCR males irradiated at 5.14 Krads in helium 0-24 hr after emergence.

	Situations			1st Week			2nd Week			3rd Week			4th Week			5th Week			6th Week			Ave.		
				% Egg Hatch		Comp.	% Egg Hatch		Comp.	% Egg Hatch		Comp.	% Egg Hatch		Comp.	% Egg Hatch		Comp.	% Egg Hatch		Comp.	% Egg Hatch		Comp.
	I♂[a]	N♂[b]	N♀	Obs.	Ex.	Value	Obs.	Ex.	Value	Obs.	Ex.	Value	Obs.	Ex.	Value	Obs.	Ex.	Value	Obs.	Ex.	Value	Obs.	Ex.	Value
Non-Irradiated Control		1	1	95			97			92			95			78			63			86.3		
Competitive Test	5	1	1	19	20.5	1.14	31	21.2	0.53	25	18.7	0.64	27	20	.62	18	15.5	.80	3.0	11.3	6.0	20.5	17.9	.81
Irradiated Control	1		1	6			6			4			5			3			1			4.167		

[a] I = irradiated
[b] N = untreated

the same conditions, are shown in Table 6. The average competitive value for this study was 1.8.

Table 6. Second study of sexual competitiveness of SCR males irradiated at 5.14 Krads in helium 0-24 hr after emergence.

	Situations			1st Week			2nd Week			3rd Week			4th Week			5th Week			6th Week			Ave.		
				% Egg Hatch		Comp.	% Egg Hatch		Comp.	% Egg Hatch		Comp.	% Egg Hatch		Comp.	% Egg Hatch		Comp.	% Egg Hatch		Comp.	% Egg Hatch		Comp.
	Id[a]	Nd[b]	N♀	Obs.	Ex.	Value	Obs.	Ex.	Value	Obs.	Ex.	Value	Obs.	Ex.	Value	Obs.	Ex.	Value	Obs.	Ex.	Value	Obs.	Ex.	Value
Non-Irradiated Control		1	1	85			90			90			95			94			92			91		
Competitive Test	5	1	1	14	17.5	1.42	17	20.8	1.46	19	22.5	1.42	17	23.3	1.95	24	24	1.00	8	24.5	-5.6	16.5	22.1	1.8
Irradiated Control	1		1	40			7			9			9			10			11			8.3		

[a]I = irradiated
[b]N = untreated

DISCUSSION

For the sterile male technique to be successful in the field, many preliminary tests must be conducted in the laboratory. First, the sterilization dose must be determined. After the insect has been successfully sterilized, studies must be made to determine if there were any detrimental effects concerning longevity and sexual competitiveness. This study was involved with these aspects of the sterile male technique for the SCR.

The SCR males irradiated in air with 5 Krad were ninety-nine percent sterile. However, when SCR males were irradiated in helium a dose of 6 Krad was required to achieve the same level of sterility. Even though no significant differences were found in the death rate comparing irradiation of males in helium or air with the t-test, all males were dead after irradiation in air at 14 days following a dose of 5 Krad while twenty percent of the males survived 6.8 Krad in helium for at least 45 days when the experiment was terminated. Although the SCR male can be successfully sterilized in both helium and air, the results obtained in this study suggest that the SCR males irradiated in helium retain vigor and viability for a longer period of time than those irradiated in air. Ohinata (34) and also Hooper (21) found that higher irradiation doses were required in helium and nitrogen compared to the doses required in air for sterilizing the Mediterranean fruit fly. Clark (7) reported that the parasitic wasp *Habrobracon* was also more resistant to irradiation in nitrogen as compared to air.

Sexual competitiveness is essential for a successful sterile male

technique. This study found that on the average the sexual competitiveness value after the sterilization process in helium was 1.305. This competitive value appears to be high because it would seem unlikely that the irradiation process would actually improve sexual competitiveness. It is probably more realistic to say that there was no real difference found between the sexual competitiveness of the irradiated male and the untreated male. Hallinan (19) found that when mosquitoes were irradiated in nitrogen they were as competitive as normal males, while when mosquitoes were irradiated in air they were not as competitive as normal males. The Mediterranean fruit fly was also more sexually competitive when irradiated in helium or nitrogen than when irradiated air (34). These studies suggest that the irradiation process in nitrogen or helium helps maintain the sexual competitiveness of the insect probably by reducing somatic damage as a result of irradiation in an oxygen-rich environment.

Even though this study showed that the SCR male could be successfully sterilized, final judgement as to its suitability as a candidate for the sterile male technique would have to be made after a field test. Several successful field tests have been conducted by releasing both sterile males and females. The screw-worm fly release program was successful in Florida when both sexes were sterilized with 8000 roentgens and released (26). An attempt to suppress the Mediterranean fruit fly on the island of Tunisia also involved the irradiation of both sexes after a dose of 10 Krad (6). It would seem most beneficial for the sterilization dose to be determined for the SCR female, if research were to continue with the SCR as a possible candidate for the

sterile male technique. It would be more practical to sterilize both sexes, because one would eliminate the time and handling required for the sexing of the insects before the irradiation process.

Many problems could hinder further development of this technique for the SCR. Because the SCR is widely distributed (2, 32), it would be very difficult to find an isolated test area. Attacking the entire SCR population on a regional basis is at present a technological impossibility. If an actual attempt were made, a technique would have to be developed to rear the SCR in large numbers easily and economically. A technique would also have to be devised for an efficient and effective distribution and release program.

SUMMARY

Laboratory reared SCR males were sterilized with gamma irradiation from either a cobalt-60 or a cesium-137 source. A higher irradiation dose was required for sterilization in helium compared to air. Irradiation doses in air were significantly different from irradiation doses in helium. When SCR males were irradiated in helium, they retained their sexual competitiveness.

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