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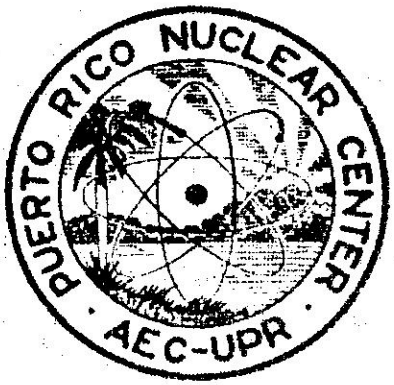
PUERTO RICO NUCLEAR CENTER

PROGRESS REPORT

SUGARCANE BORER PROJECT



OPERATED BY UNIVERSITY OF PUERTO RICO UNDER CONTRACT NO. AT (40-1)-1833 FOR U. S. ATOMIC ENERGY COMMISSION

Induced Sterility for Population Control of the Sugarcane Borer (*Diatraea saccharalis*) in Puerto Rico

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Technical Report No. 1

Work performed at Puerto Rico Nuclear Center
under U. S. Atomic Energy Commission
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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

I. Introduction

The principal objectives of this project are:

1. To determine if Diatraea saccharalis can be rendered sterile by subjecting it to gamma irradiation,

2. To study the bionomics of this species with reference to phases of the life cycle that are applicable to the prosecution of a mass-release program of sterile adults in Puerto Rico.

The sugarcane borer, Diatraea saccharalis (Fab.) (Crambidae, Lepidoptera) causes direct destruction of plant tissues, often completely destroys young plants, as well as causes sugar inversion in cane stalks. In addition this pest allows for secondary invasion of fungi and bacteria which in turn reduces sucrose yield. This pest is cosmopolitan in distribution, and is of major importance in most major sugar producing areas of the world.

This project is part of a cooperative effort with the Commonwealth Experiment Station and the U.S. Department of Agriculture. Cooperation does not include exchange of funds.

Work was begun on a modest basis in the Spring of 1963. Preliminary work included irradiation of the pupal stage and larval stages. Artificial rearing methods were attempted at this time, and were unsuccessful. Wongsiri and Randolph (1962), Pan and Long (1961), and Katyar (1963) were able to rear successfully the continental strain of this species on a modest laboratory basis. The diets suggested by these workers have not been suitable for rearing the Puerto Rican strain of this species. Survival with the diets recommended by these workers has never exceeded 20.$^{o}/_{o}$ with the Puerto Rican strain.

Lacking a good diet, our earlier work was greatly retarded due to our inability to produce sufficient numbers for testing purposes. During the earlier phases of the project much of our time was spent in field collecting larvae and pupae from sugarcane and corn. Although we were able to collect considerable information of value concerning field conditions during this time, we were not able to concentrate fully on our primary objectives with the intensity desired. Very recently we have developed a satisfactory diet and we no longer have this problem.

We have attempted to give consideration to all of the factors of importance to a mass-release program of sterile adults. This has entailed considerable adjustment in our working arrangements such that each objective was evaluated on the basis of its immediate need, as well as our capacity to obtain an immediate solution. Our policy has been to investigate thoroughly all factors that would prevent the use of the mass-release method at the earliest possible date in the project history. In terms of the economy of time, and the capacity to predict the ultimate success of this method, we have explored some aspects of the biology of this species, while at the same time concentrating on our principle objective. Although this approach has been cumbersome at times it has given us a clear-cut basis for evaluating the potential success at each individual stage in the project's development. Stated more directly, our approach has been to try to explore all of the reasons why this method could not succeed.

A summary of the important progress made during the last year includes the following:

1. development of a satisfactory diet for rearing this species in large numbers in the laboratory,

2. determination of the sterilizing dosage for adult males, and females,

3. determination of the stage to be irradiated,

4. development of methods of evaluating radiation effects, and the kind of effect produced,

5. determination of mating behavior, time of day when this species mates, and the method by which the male is able to discover the female under field conditions,

6. the determination of longevity of the adults of the species,

7. the determination of oviposition rate of this species.

The following portion of this report gives a brief summary of our work during the previous fiscal year.

II. Accomplishments

A. Effects of gamma irradiation

Under normal conditions there are five distinct larval stages each of 4 to 6 days duration. Under conditions of stress there may be as many as 7 to 9 larval stages, of longer duration.

Larvae: Subjecting larval stages 1 through 5 at dosages of 6 to 12 krads caused mortality to 50% or higher, without producing sterility.

Pupae: The pupal stage is six days in duration. Pupae one to three days age are killed by dosages of 8 kr and higher, pupae three to five days duration are killed by 12 kr and higher, and pupae 5 days and older are killed by radiation in excess of 14 kr. The few irradiated pupae that survived these treatments did not mate. Therefore we have concluded that it is not practicable to irradiate larval or pupal stages. The data obtained in this laboratory are substantiated by the data of Hensley (1962).

Adults: It is possible to induce sterility in this species by irradiating adults, this is discussed in the section on egg hatchability.

1. Oviposition rate

There is no reduction in egg production at exposures below 60 kr in adult males and females. Tables 1, 2 and 3 present data for dosage in kilorads versus the number of eggs produced per female, and the number of egg clusters produced per female.

Normal virgin females grown on corn lay 350 eggs, in 15-20 clusters after mating.

Although the sample is small it is apparent that the number of clusters produced is independent of dosage.

2. Egg hatchability

a. mating of irradiated males with normal females.

Table 4 and figure 1 provide a comparison of dosage and egg hatch. It will be noted that the sterilizing dose for males is approximately 25 kr.

TABLE 1

Diatraea saccharalis Dosage and Egg Production

♂ Irradiated X ♀ Normal

Dose KR	No. of ♀	Average No. eggs/♀	Average No. eggs/cluster
0	111	350.0	17.5
8	8	207.2	10.76
10	11	114.6	9.13
18	8	284.4	14.40
20	17	253.7	15.31
22	12	371.4	17.96
24	6	125.0	4.42
25	22	374.6	25.77
30	25	248.9	13.66
40	84	280.3	13.5
50	28	162.2	11.2
60	15	245.4	44.6
70	7	146.8	19.8

TABLE 2

Diatraea saccharalis Dosage and Egg Production

♂ Normal X ♀ Irradiated

Dose KR	No. of ♀	Average No. eggs/♀	Average No. eggs/cluster
0	111	350.0	17.5
8	8	152.5	16.44
10	12	100.5	8.87
18	8	297.5	17.61
20	19	182.8	12.97
22	11	210.0	11.33
24	6	110.0	25.81
25	16	257.7	18.33
30	42	158.8	16.69
40	84	174.0	10.87
50	28	221.8	14.64
60	19	139.4	9.72
70	8	44.4	13.32

TABLE 3

Diatraea saccharalis Dosage and Egg Production

♂ Irradiated X ♀ Irradiated

Dose KR	No. of ♀	Average No. eggs/♀	Average No. eggs/cluster
0	111	350.0	17.5
8	13	152.58	8.34
10	13	140.42	11.58
18	8	246.25	29.51
20	21	254.18	18.90
22	11	268.30	15.71
24	6	86.00	17.43
25	19	162.70	21.65
30	21	213.90	13.77
40	24	357.72	12.94
50	32	169.27	16.35
60	12	107.00	14.25
70	4	63.00	11.30

TABLE 4

Hatchability of Eggs of Diatraea saccharalis Following Co^{60} Gamma Exposure to Adult Males

Exposure	Hatchability		
(Kr)	Eggs Laid	Eggs Hatched	% Hatched
0	39,034	38,927	99.7
0.5	750	750	100.0
1	642	642	100.0
6	700	700	100.0
8	1,400	1,175	83.9
10	250	250	100.0
14	325	160	49.2
15	3,000	300	10.0
16	1,340	370	27.6
16.8	5,041	1,426	28.3
18	1,175	247	13.9
20	420	60	14.3
22	1,250	32	2.56
25	1,100	29	2.64
30	1,850	14	0.757
40	11,565	2	0.000173

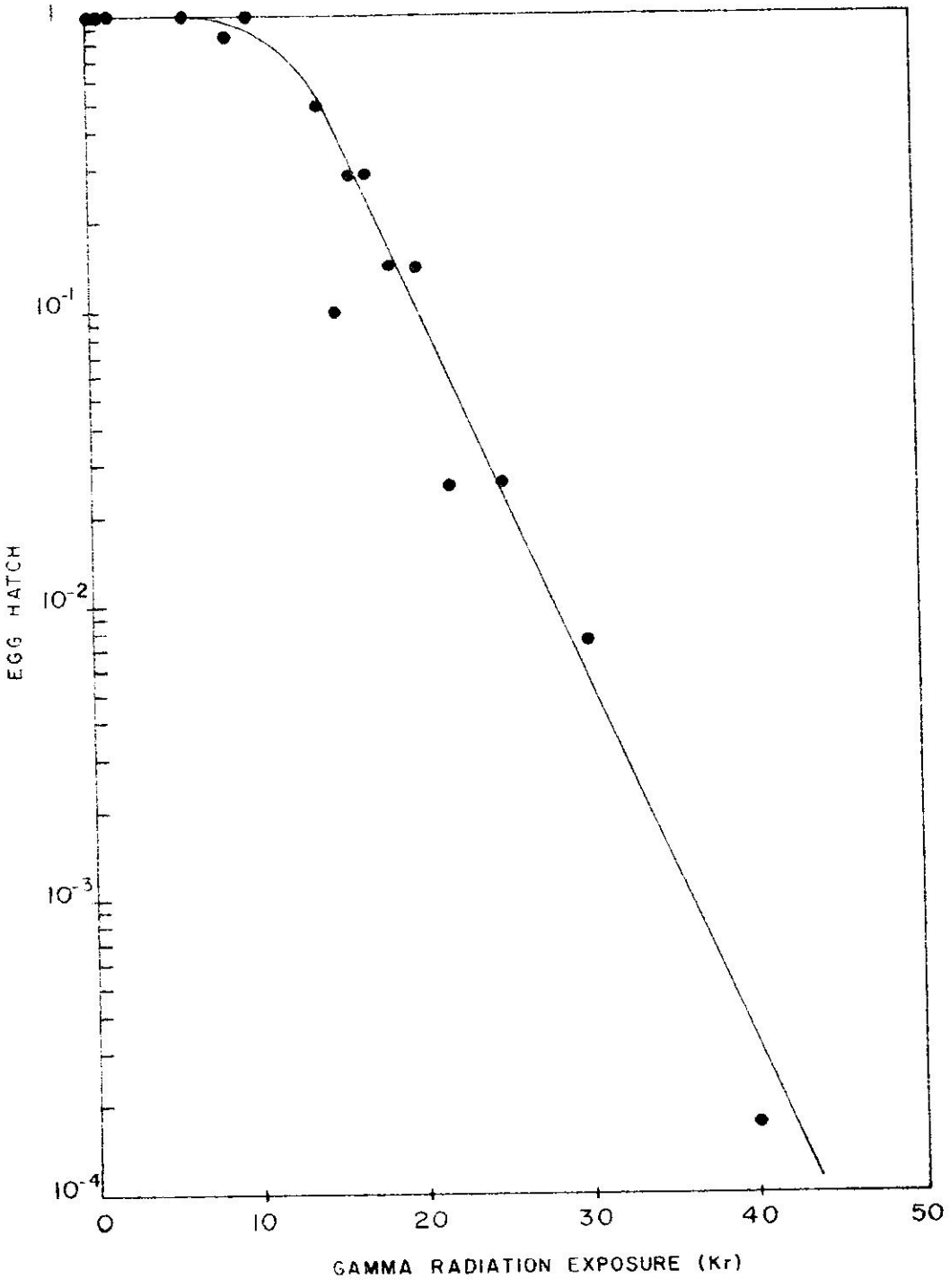

Figure 1. Adult male irradiated with female normal.

TABLE 5

Hatchability of Eggs of Diatraea saccharalis Following Co^{60} Gamma Exposure to Virgin Adult Females

Exposure	Hatchability		
(Kr)	Eggs Laid	Eggs Hatched	% Hatched
0	39,034	38,927	99.7
8	1,218	410	29.4
10	1,129	5	0.444
12	448	2	0.406
14	450	13	2.910
15	2,930	57	1.945
16.8	10,735	379	3.534
18	2,380	0	0
20	3,686	0	0
22	894	0	0
24	660	0	0
25	3,540	16	0.454
30	6,150	1	.0163
40	10,000	0	0

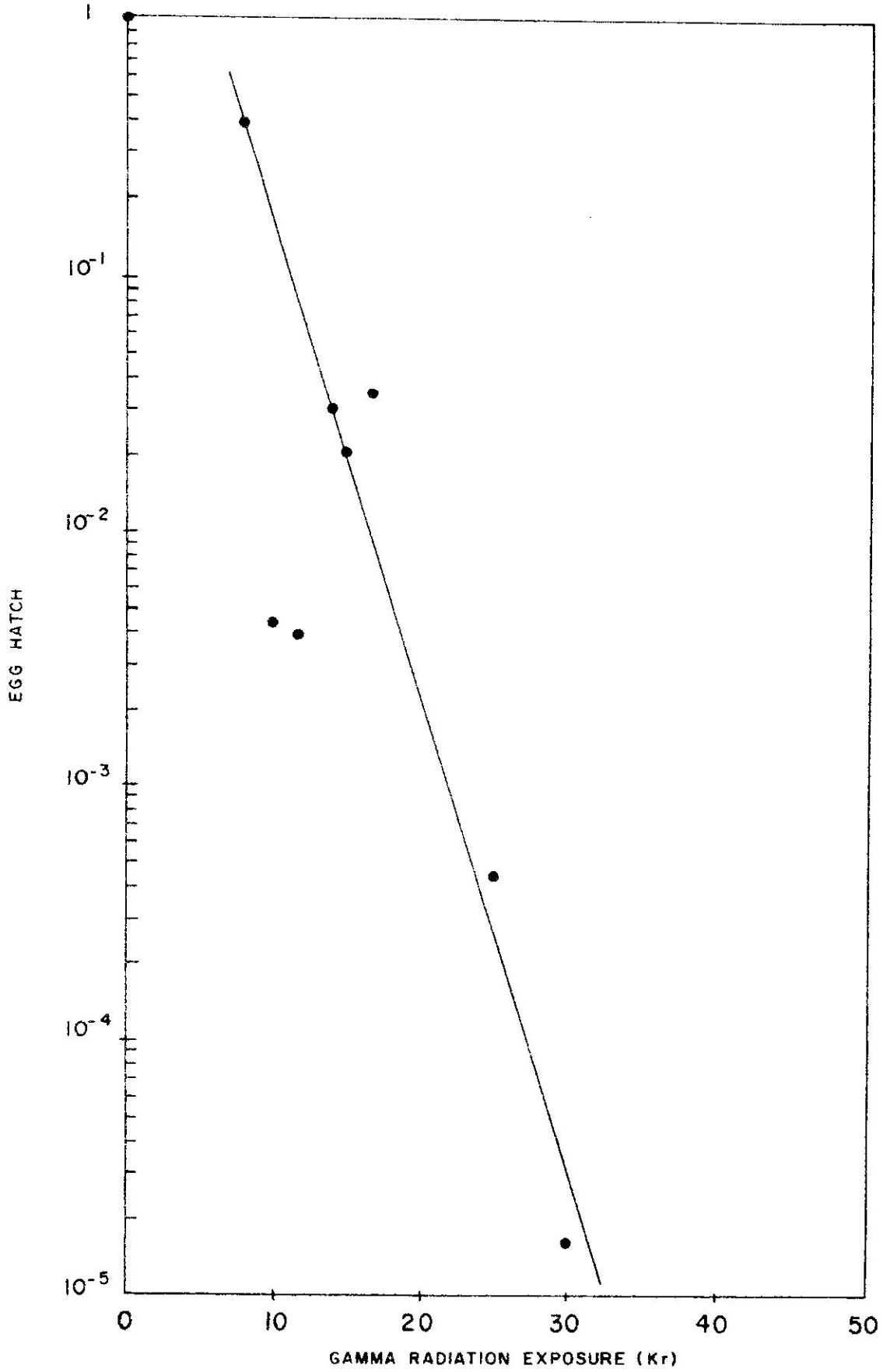

Figure 2. Male normal with adult female irradiated.

TABLE 6

Hatchability of Eggs of Diatraea saccharalis Following Co^{60} Gamma Exposure to Both Adults (Before Mating)

Exposure	Hatchability		
(Kr)	Eggs Laid	Eggs Hatched	% Hatched
0	39,034	38,927	99.7
8	2,480	358	14.4
10	1,659	6	3.62
12	840	0	0
14	771	0	0
15	1,535	3	0.130
18	2,180	0	0
20	5,460	0	0
22	2,970	0	0
24	700	0	0
25	3,209	1	0.031
30	4,250	0	0
40	7,670	0	0

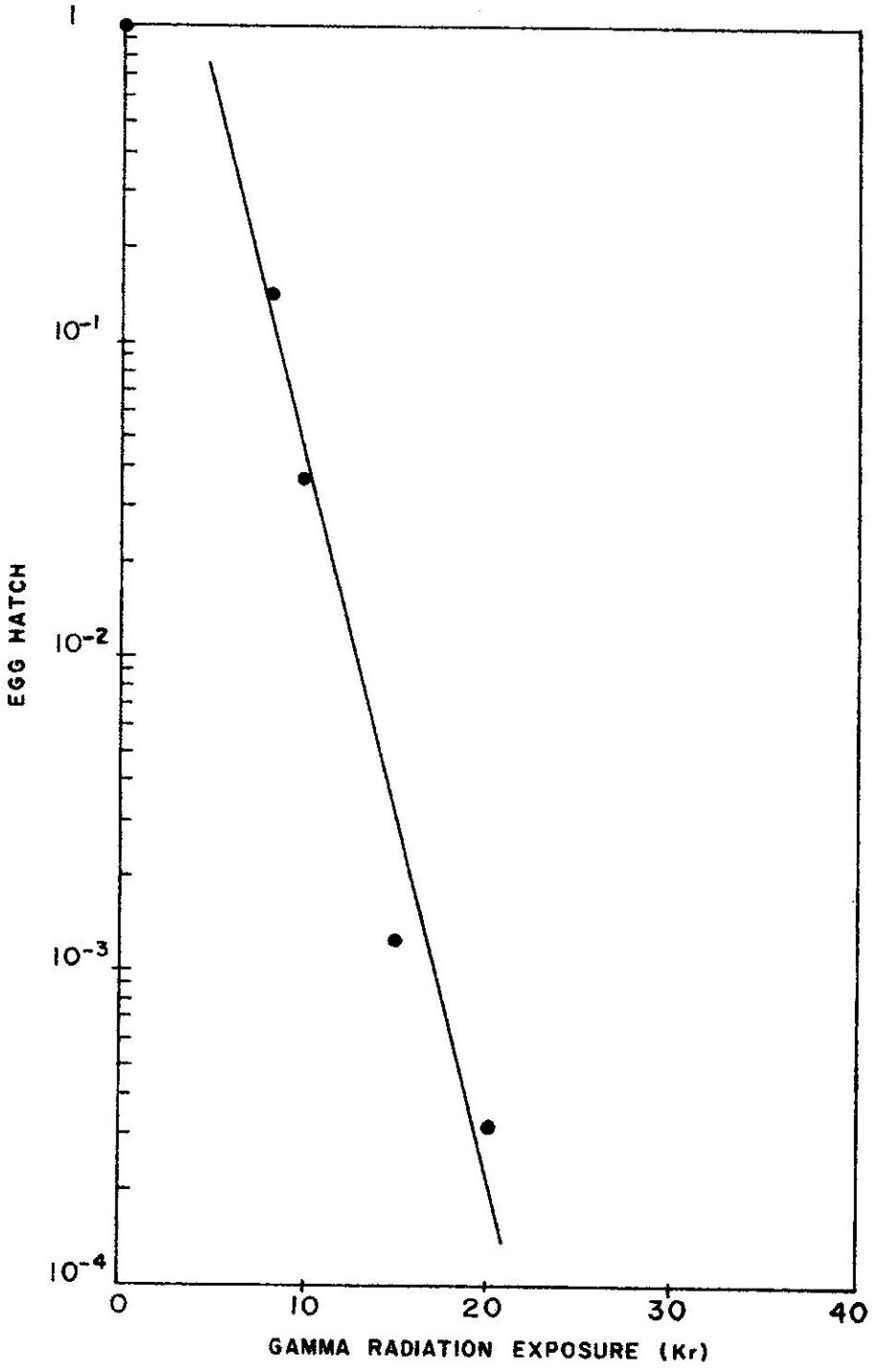

Figure 3. Adult male irradiated with adult female irradiated.

b. Egg hatchability produced by irradiated females.

Table 5 and figure 2 compares egg hatch from irradiated females. The dosage for induction of sterility in female adults is 22 kr.

There are two dosage ranges for irradiated females, depending on the age of the female when irradiated. If young females of less than 12 hours of age are irradiated, most of the ova will be in the female oviduct in the prophase division of meiosis. Prophase ova are more resistant to radiation than metaphase ova. As a result there are actually two curves for females, one for the females containing metaphase oocytes and the second prophase oocytes.

If irradiated virgin females are to be released a sufficient dosage must be used to sterilize the prophase oocytes.

c. Results obtained from irradiation of both parents before mating is shown in table 6 and figure 3. Two curves were obtained in this case also.

In summary we have concluded that even though the sterility-effect in the female is more radiosensitive, the biological effectiveness is less subject to error in treating males. This may be due to the fact that all of the sperm are in the same stage of meiotic division at the time of irradiation. There is some evidence for this because of the method of transfer of sperm in this species.

In general the Lepidoptera do not transfer individual sperm to the female in mating, but rather transfer a package of sperm in the form of a spermatophore. In this species the spermatophore is actually manufactured at the time of mating. This is not an unusual occurrence in the order lepidoptera. This will be further discussed under section C-3.

3. Adult longevity is not affected by irradiation at dosages of 70 kr and below. Table 7 and figure 4 shows the survival of normal adults, and table 8 presents the similar survival of irradiated adults.

It may be concluded that longevity is independent of the radiation dose received in the range of 70 kr and below.

4. Behavioral changes

Virgin males irradiated at 100 kr were capable of mating with normal females. Virgin males irradiated at 2 to 4 kr mated sooner than non-irradiated males when placed with females. We do not have conclusive data on the ability of sterile males to compete with normal males for mating with females.

Diurnal activity of irradiated adults: both males and females do not appear to be affected by irradiation at dosages sufficient to sterilize them.

5. Second generation effects

There was a reduction in mating capacity, longevity, and weight of adults produced from mating of irradiated individuals in the previous generation (irradiated at a sub-sterilizing dosage). We have also observed aberrant morphological types in the F_1 generation. The most common aberration was a double set of palpi.

From the limited data available it is apparent that radiation stress is effective in subsequent generations, but the full extent of this effect is not known.

TABLE 7

Survival of Adult Diatraea saccharalis

Number of Surviving Adults by Day		Day of Death	°/o Survival to this day	
Males	Females		Males	Females
353	696	0-1	100.0	100.0
343	681	1-2	97.0	97.8
322	660	2-3	91.0	94.8
217	582	3-4	61.4	83.6
110	465	4-5	31.1	66.8
48	125	5-6	13.5	17.9
8	57	6-7	2.2	8.3
2	16	7-8	0.5	2.2
0	3	8-9	0	0.4
0	2	9-10	0	0.2
0	1	10-11	0	0.1
0	0	11-12	0	0

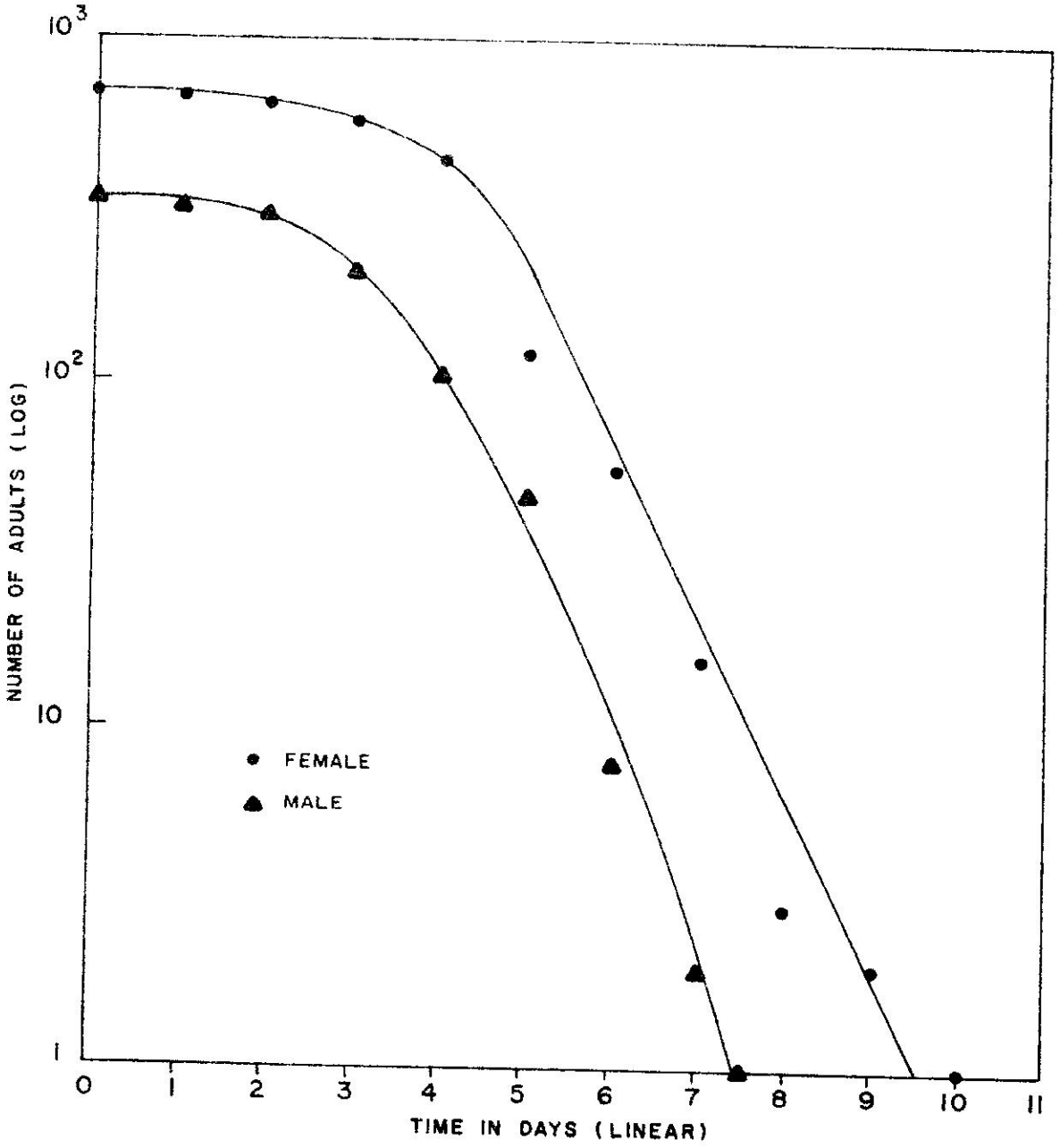

Figure 4. Life span adult Diatraea saccharalis. (Fab.)

TABLE 8

Longevity of Irradiated Adults

Dose KR	No. of Inds. ♂	No. of Inds. ♀	Life Span ♂	Life Span ♀
8	27	29	3.7+	3.7+
10	9	15	3.3+	3.9+
18	16	16	3.7+	3.9+
20	23	33	6.1+	4.2+
22	24	19	4.3+	5.1+
24	12	12	3.2+	3.3+
25	13	12	4.7+	5.0+
30	30	30	6.1+	5.1+
40	70	60	5.5+	4.2+
50	65	58	5.3+	5.5+
60	14	8	6.0+	6.0+
70	11	10	5.5+	5.3+
0	353	696	3.5+	5.2+

6. Embryology

A complete description has been made of normal egg development and was used for comparison with the development of eggs produced by irradiated parents. The dosage selected for comparison was 16.8 kr (irradiated male parent) due to the fact that 5 per cent of the eggs produced at this dosage developed beyond the preliminary "orange spot" stage.

This method is being used to interpret the type of damage produced in the irradiated male parent. If the egg develops to a bright yellow color and does not progress further, we know that the damage produced was sperm death. If the egg develops to the orange spot stage and develops a head capsule which subsequently deteriorates, we know that a dominant lethality has occurred. A full description of the types of sperm damage may be found in Von Borstel (1963). Table 8 gives a comparison of egg development using normal eggs, unfertilized eggs, and eggs produced by females mated with irradiated males as the basis for comparison.

7. Egg counts

In our preliminary experiments it was necessary to make estimations of egg hatch due to lack of personnel. In our present study we are making actual counts of egg hatch and total egg production, as well as the number of egg clusters produced.

8. Testing pure genetic strains

The data presented in the previous tables is from individuals collected from the field as larvae or pupae and individuals reared in the laboratory on artificial diet. The dosage-effect is presently being investigated using a pure genetic strain reared on artificial diet in the laboratory. By using a pure genetic strain we will be able to obtain the dosage-effect relationship precisely.

Unfortunately, this pure genetic strain does not have any distinctive morphological characteristics. Development of this pure strain is discussed in some detail under section B.

9. Determination of copulation by dissection of females after mating.

As was previously mentioned copulation in lepidoptera envolves the transfer of a packet of spermatozoa in the form of a spermatophore. The spermatophore is placed into the vulva of the female and transferred by the female to a sac-like structure, the bursa copulatrix. One portion of the spermatophore dissolves, however the tube-like structure remains. Therefore it is possible to determine the number of times that a female has mated by dissecting the female and counting the number of spermatophore tubes present in her bursa copulatrix. Thus, we can tell whether a female has mated, or whether she is still a virgin. We have found that females mate as many as three times, and the average number of times that she will mate is between 1 and 2 times. This provides a useful method for determining mating capacity of irradiated males.

B. Artificial Rearing

1. Diets

A good dietary medium must satisfy the following criteria:

a. result in high survival rate,

b. produce vigorous adults,

c. cost less than 1/5 cent per adult produced,

d. be easily prepared from readily available material,

e. provide uniform development without a prolonged adult emergence period,

f. have good keeping quality.

The diet must inhibit mold, bacteria, and virus development. It must maintain a stable pH, and suitable consistency throughout the period of larval growth.

Seventy-five diets were tested during the previous year. Approximately 150 diets have been tested since the beginning of this project. These diets are artificial, but they are not synthetic diets. The ingredients used in the diets proposed are essentially preparations of the normally-preferred host-plant material with the addition of supplementary nutrient material.

TABLE 9

Dietary Formulae

Ingredients Used:	Quantity Used in Each Diet 1	2	3
Corn fiber (blended)	20 gm.	20. gm.	20. gm.
Carrot powder	10. gm.	10. gm.	10. gm.
Agar	2. gm.	2. gm.	2. gm.
Brewer's Yeast	15. gm.	15. gm.	10. gm.
Enzymatically hydrolized casein	1. gm.	1. gm.	2. gm.
Sodium benzoate	0.3 gm.	0.3 gm.	0.3 gm.
Methyl-p-hydroxybenzoate	.05 gm.	.05 gm.	.05 gm.
Ascorbic acid	.32 gm.	.32 gm.	.5 gm.
Distilled water	---	125. ml.	125. ml.
Corn filtrate	125. ml.	---	---
pH	5.8	5.8	5.8
Hydrochloric acid (1.N)	---	10. ml.	10. ml.

TABLE 10

Comparison of Development on Different Diets

	Diet Number				
	1.	2	3	4*	5**
Percent Survival from egg to adult	95.	90.	90.	<30.	<40.
Average length of larval life span, days	25.	22.	23.	>90.	50.
Range, larval life span	18-32	18-35	18-33	90->210	40-65
Average length of adult life span, days, male	8	9	8	>4	3.5
female	10	10	10	>5	5.2
Average number of eggs produced per female	600.	500.	550.	360.	350.
Range, egg production	300-800.	300.-850	300.-850		
Number of molts	5	5	5	7	5 or 6
(time between molts (days)	3-5	3-5	3-5	>20	5-6
Tunneling	Excellent	Excellent	Excellent	poor	good
Feeding	Excellent	Excellent	Excellent	fair	Excellent
Texture	good	good	good	liquifies	poor
Mold and bacterial inhibition	good	good	good	fair	very poor
Color changes in medium during larval growth	slight	slight	slight	fair	------
PH stability	good	good	good	poor	poor
Odor changes	none	none	putrifies	slight	putrifies

*Diet by Miskimen (1964), MM-B.

**Rearing on cut sections of corn stalk.

TABLE 11

Evaluation of Diets on an Arbitrary Scale

	Highest Possible	1	2	3	4*	5**
Survival, egg to adult	5	4.9	4.7	4.7	1.5	1.8
Speed of larval growth	2	1.8	2.0	2.0	0.1	0.5
Length of adult life span	2	2.0	2.0	1.9	1.0	1.0
Egg Production	1	1.0	0.9	1.0	0.6	0.6
Total Score	10	9.7	9.6	9.6	3.2	3.9

*MMB diet by Miskimen
**Cut pieces of fresh corn stalk

Table 9 presents the dietary formulae for the three most promising diets developed in this laboratory, and table 10 presents a comparison of development with various diets. Table 11 presents an evaluation of diets on an arbitrary scale.

It is possible to rear as many as 10 larvae in a vial, whereas the diets previously recommended (Miskimen, 1964, Martorell, 1964) were limited by the fact that only one larva could be raised in each vial. This provides a considerable economy in time, in yield, and in convenience. A high degree of efficiency is possible with these improved diets, and it is believed that they will provide a suitable method for rearing sufficient quantities of adults for a small field test with the facilities available in this laboratory. We do not have adequate space, personnel, nor equipment to rear sufficient quantities for population overflooding of a large land mass.

2. Handling methods

Larvae are transferred to 8 dram vials (25mm. in diameter, and 95mm. in length) after hatching. These larvae readily enter the food, and pupate before the diet becomes moldy. The pupae are recovered from these vials, after which the sex of each individual pupa is determined. Males are isolated from the females, by placing each sex in a separate container. Complete data is kept of the genetic line, and the speed of development of each group of larvae. This ensures the prevention of brother-sister matings. Upon ecdysis the virgin males are irradiated, and then are transferred to a small sputum cup of six ounces capacity. A virgin female is then placed in the same cup. Each mating pair is given a separate code number and a record is kept of egg hatchability, longevity of each adult, and other observations.

After the female dies her abdomen is dissected to determine the number of spermatophores in her bursa copulatrix.

The cycle is completed when the new eggs hatch and the larvae emerging are placed in new food medium.

3. Renewing colony from field collected material

It has been found that hybrid lines, produced by brother-sister matings do not have the vitality of a pure line. Therefore it is necessary to maintain a field colony of homogeneous genetic content for periodic renewal of the colony.

From this we hope to improve the present pure genetic strain, on a pure-line basis rather than on a hybrid-line basis. We are selecting adults that are more vigorous, have longer adult life span, have shorter larval life span, and have high egg production. We would also like to have a pure line that is morphologically distinguishable from field collected material. As yet we have not encountered easily recognizable genetic "sports" in nature. There is considerable color variation in males and females in nature, but this color variation appears to be influenced more by food than by any other single factor. Darker colored males appear to be more sexually vigorous than lighter colored males. Conversely lighter color females appear to be more sexually vigorous than darker colored females. Color intensity, or the lack thereof, is related to the degree of sexuality in this species. There is even some color variation within individuals of the same sex that develop from the same egg cluster.

C. Needed biological information for a mass-release program.

1. Normal mating behavior

During the previous year we have not substantially increased our knowledge of normal mating behavior, however we have been able to confirm our earlier observations. Briefly they may be summarized as follows: The female initiates courtship. The virgin female flies or climbs to a prominent leaf on a cane plant and extends the tip of her abdomen above her thorax. Then she extrudes a pair of bulb-like structures from the openings near her vulva, and emits a chemical substance. Later she actively beats her wings at a frequency of 5,000 beats per minute. Usually her presence is discovered by a male in less than 30 minutes.

The male flies in a random fashion among the higher cane leaves. When he detects a female in early courtship stance he alights posterior to her and nuptial behavior proceeds.

Average time of 20 observed matings was in excess of 65 minutes from the onset of copulation. A detail description of the mating process has been accepted for publication in the Proceedings of the Entomological Society of Washington. Extrinsic factors important in influencing mating are the following: mating takes place in the dark, and occurs in nature

as early as 8:00 P.M. to as late as 3:30 A.M. in Puerto Rico. Reduction of light intensity appears to be the most important extrinsic factor. Increase in relative humidity is correlated with the onset of copulatory behavior. The relative importance of humidity increase is not known.

D. Field observations

1. Seasonal population fluctuations in nature

Although complete information is not available it is apparent that the adult population varies considerably throughout the year. High adult population levels can be found during the months of March through July in Puerto Rico, with a gradual reduction from early September to middle November. From middle November until early January population levels continue to decline, reaching the lowest level in early February. From middle February the adult population increases. Larval populations follow roughly the same course with a lag of approximately 30 days in the field. Approximately 30 % of the larvae found during December through January are large, light-colored fifth stadium larvae. This indicates that there is diapause in the fifth statium in Puerto Rico. The factors causing diapause in this species have not been fully evaluated. Decreasing temperature, and decreasing day-length are probably the most important factors in the initiation of diapause. Normally sugarcane is harvested in January through February in Puerto Rico. As a consequence, population level of larvae and adults in the field is at a minimum after harvest, however population again increases at the time of new growth.

2. Field collecting methods

We do not have sufficient personnel to make evaluations of the infestation rate in sugarcane in Puerto Rico. However information on the general status of larval population has been obtained by cutting field cane and removing the larvae from these stalks. Adult population sampling has been made by the use of a large cage equipped with an ultra-violet light. This light is suitable for making rough adult population estimates in the field. This light is not suitable for attracting all adult Diatraea from a field.

Previous experience has shown that the infestation rate is approximately 10 times greater in field corn as compared

with sugarcane. Population fluctuation throughout the year follows the same cycle in corn and cane.

III. Future Needs

A. Field Test

Much of our previous information on behavior and mating capacity is based upon laboratory observations. It will be necessary to conduct a field test to confirm our laboratory observations. It is suggested that Mona Island be the site for such a field test. A preliminary survey of Mona Island has shown that the sugarcane borer is established there. Mona Island is 32 miles west of Puerto Rico and approximately 40 miles southeast of the Dominican Republic. The primary deterrent is the lack of irrigation water. Transportation can be arranged on the weekly Police boat. In addition some of the personnel from the Fish and Wild Life Service (Commonwealth) stationed there have training in agronomy and they are willing to cooperate with us in a field test. A cooperative project can be arranged if the proper clearance for a test can be negotiated. A field test is essential for the verification of the laboratory tests.

B. Competitive Behavior of Irradiated Males

A laboratory test will be initiated during the forthcoming year to evaluate the effectiveness of irradiated males for competing with normal males. Preliminary studies have been made using dyes such as Rhodamine B, fluorescein, and other markers. Tests were made by marking virgin females to determine if the color was transferred to a male during copulation. Conversely marked males were used to test if dyes were transferred to the female. We have not been able to develop a satisfactory combination of dye and solvent. We propose to use irradiated males tagged with radioactive phosphorus for laboratory tests of competitive mating behavior with normal males.

The need for this information is critical when we consider that the female will mate as many as three times in nature. Multiple mating and its ramifications are discussed by Von Borstel (1963). The success of a mass-release program of sterile males is dependent upon the superior mating capacity of irradiated males in the field. Our laboratory-produced males now are longer-lived than normal field males. However we have the disadvantage of the factor of multiple mating.

C. Improved Mass-rearing Method and Handling Techniques

Considerable attention has been given to more suitable containers for rearing this species in the laboratory. We have found that the surface area to volume relationship is of great importance in rearing larvae. Although the larva readily enters the food medium, they prefer to tunnel near the surface at the wall of the container. Containers of many different types of plastic have been tested. The larvae were capable of tunneling through all types tested. Acrylic plastics have been found to be toxic to this species, but polyethylene, polypropylene and nalgene plastics are not toxic. Glass vials have been found to be the most satisfactory rearing containers for the larval stage. Container size is important and 20 to 30 grams of medium is the optimum quantity of food for approximately 10 to 15 larvae, depending on the type of diet used. Free air exchange has been found to be necessary for optimum larval development. Many types of containers were tested (refrigerator containers, glass dishes, petri plates, many types of plastic containers, etc.) with varying degrees of success. Taking into consideration contamination by the larvae themselves, the optimum amount of food is approximately 2 to 3 grams of food per larva. When ready to pupate the larva first forms an exit hole of sufficient size to emerge as an adult. Often this exit hole will be made to the light even though the glass wall of container prevents exit.

Vial-rearing will be difficult for a mass-rearing method. Considerable attention to this problem is needed to perfect mass-rearing techniques before beginning a large-scale release program.

D. Cooperation with Interested Groups

The Puerto Rico Sugarcane Growers Association has shown considerable interest in this project, and it is believed that their cooperation may be obtained in carrying on a field test. Preliminary discussion should be initiated in the near future to determine the best means for developing a cooperative working relationship with the sugar producers of Puerto Rico.

E. Program Financing for Field Test

It is hoped that there will be sufficient funds in the present budget to carry on a one-half acre field test in Mona Island. This may not be possible due to insufficient funds, however an early decision should be made of the extent

of support to which AEC may be committed for a large field test in the future. In terms of the long-term future, the means of program financing a island-wide program should be considered at the present stage of the project.

F. Diurnal Cycle

We do not have complete information on the daily activities of the adults in the field, except for those observations made during the time of mating at night. It is essential to evaluate the important factors in nature that are controlling population levels of the adults of this species. Predation by birds, spiders, and other natural agents of control (under field conditions) must be evaluated to determine the expected field mortality.

We are not equipped and staffed to carry on a study of this magnitude.

IV. Publication and Scientific Papers

A. A paper was presented at the Entomological Society of America Annual meeting in Philadelphia, December 1964. The abstract is as follows: "Irradiation of larvae of pupae at 8 kr and higher produced excessive mortality. Irradiation of virgin, newly-emerged adult males and/or females at 20 to 40 kr produced 2% egg hatch or less, irradiation at higher dosages produced mixed results. Behavior and methods are discussed".

B. A paper was presented on the oviposition rate of Diatraea saccharalis at the Twelfth International Congress of Sugarcane Technologists in San Juan, April, 1965. This has been accepted for publication in the Proceedings.

C. A description of the mating behavior has been accepted for publication in the Proceedings of the Entomological Society of Washington.

D. A paper on the longevity of adult Diatraea saccharalis was published in the Journal of Economic Entomology in July 1964.

E. In addition the following manuscripts are in preparation for submission to an editor;

1 - Improved Xenic Diets for Diatraea saccharalis

2 - Embryology in Diatraea saccharalis

3 - Gamma-induced sterility in Diatraea saccharalis adults

4 - A Description of the Reproductive System of Diatraea saccharalis

V. Present Work in Progress

1. Development of a pure genetic line

2. Investigation of improved mass-rearing methods

3. Study of effects of gamma irradiation on a pure genetic line of adult males.

VI. Relation of this Project To the Economy of Puerto Rico and the Scientific Community.

1. The economy of Puerto Rico: There is approximately two and one-half million dollars annual loss by this species in Puerto Rico. If this project is successful considerable interest can be stimulated on the part of sugar producers in this area.

2. The relationship of this program and the advisory capacity of the PRNC: the Atoms in Action Exposition has developed two programs on insect sterility in El Salvador, and we will probably cooperate with Guatemalan scientists in the beginning of a third project.

During the course of our consulting with the scientists of El Salvador we were able to suggest diets for rearing Heliothis zea and Leucoptera coffeela. Both insects are serious pests to crop production in Central America. Development of an adequate background through the Diatraea project has enabled us to be of help in initiating investigations with pest species in Central America.

VII. References cited

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