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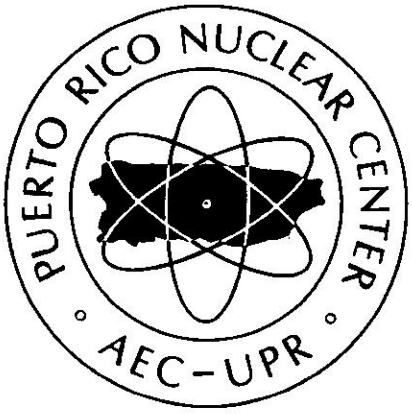
PUERTO RICO NUCLEAR CENTER

Insect Sterility Program Technical Report 7

David W. Walker, Program Director

April 1973



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

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PUERTO RICO NUCLEAR CENTER

Insect Sterility Program

Technical Report No. 7: February 1972 to April 1973

(Formerly Potential for Gamma-Induced Sterility
in Control of the Sugarcane Borer
D. saccharalis (Fab.) in Puerto Rico)

Research supported by the USAEC
Division of Biomedical and
Environmental Research under
contract No. AT(40-1)-1833

Report prepared in April 1973 by
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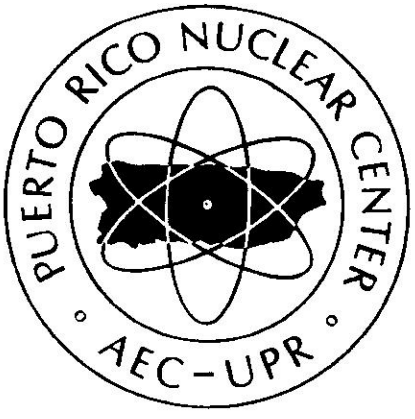

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I. Introduction

Among the developments in insect control the most important breakthrough has been the development of the concept of Integrated Control (IC). It was first proposed by E. F. Knipling (1966). IC, fundamentally a systems approach applied to monocultural crop production practices, is an attempt to integrate natural and artificial control measures to prevent pest population outbreaks. It shifts the burden of pest control from a single method (insecticides) to a variety of preventive checks and therefore it emphasizes anticipatory rather than corrective means.

Specific insecticides are used in IC under certain conditions, with great care given to the time of application, the application method and the amount needed to produce the desired effect. In the crop phase of IC pest-resistant plants are selected, and attention is focused on the time of planting to avoid insect attack. In addition irrigation practices are modified to take fullest advantage of irrigation for pest control. Clean harvesting is recommended for some crops to reduce the plant residues after harvest that harbor pests.

In the pest control phase of IC entomologists are studying trapping techniques that use juvenile hormones, sex pheromones, chemisterilants, and oviposition lures. Specific parasites, predators, and insect diseases are cultured and released to control pest populations. Quarantines are used to prevent introduction of pests.

Pest populations are also being suppressed by overflooding them with individuals that have genetic defects. Smith and von Borstel (1972) made an extensive review of insect control by genetic manipulation. Some of their suggestions apply specifically to lepidoptera programs. For example, improved methods are needed for producing sterility by mutations with no undesirable effects on the sperm itself or on sperm transfer mechanisms. We also need more efficient methods for introducing genetic insults into the natural population, e.g., a single overflooding release with partially sterile individuals as opposed to several overflooding releases with dominant lethal carriers. With this end in mind we developed the hypothesis for population collapse by Inherited Partial Sterility (IPS) in lepidoptera and presented it as a population model (Walker and Pedersen, 1969). The basis for the two models was the data from IPS laboratory observations with the sugarcane borer from 1965-9 (Technical Reports 1 through 4, and Walker, et al., 1971). The hypothesis and the mechanism is discussed in the appendix of this report. We think we have solved the major problem with the IPS technique by having found a satisfactory way of fully sterilizing lepidopteran females (with fractionated doses). We discuss this in section D under Accomplishments.

Many additional improvements are needed before the sterile release method can be used for eradicating lepidopteran pests efficiently. We need to improve rearing methods to be able to produce millions of moths in a factory system. This is difficult because the food requirements of lepidoptera are not well known and the larval life span is long. We also need to improve methods for producing genetic defects, e.g., techniques

for damaging the chromosomes of precursor sex cells. Future program plans are discussed in the text of this report.

II. Accomplishments

A. IPS Cage Tests (see appendix for the experimental design)

1. Cage Phase, control release - The first group of adults was released in February and the F_1 population was sampled in April. The F_1 population in the control cage was nearly five times the number released (see Table 1). One hundred and fifty plants appears to be adequate for providing sufficient oviposition sites for 15 females. Only 88 of the 255 plants had larvae, and the range in infestation rate per plant was 0 to 19. Overflood release - the first overflooding release in March apparently failed because the males available were of mixed ages. The F_1 population of this cage will be sampled at the end of April.

The order of events that took place in preparing for the cage tests is as follows:

May	Decision made to move the cage to a new location, ordered saran screen covering for cage, looked for sites and chose one at the Federal Experiment Station, submitted formal request through AEC to USDA.
June	Permission granted by USDA
July and August	Dismantled and moved cage frame
September	Reconstructed frame at new site
October	Received and installed screen
November	Planted first cycle corn
December	Corn blight destroyed first cycle, due to heavy rains
January	Planted Ibadan variety
February	First insects released - control test
March	First overflooding release, planted second cycle corn
April	Sampled F_1 population of control cage

2. Colony Phase - There have been some problems in developing the rearing method to the production capacity needed. In September we had accumulated over 700 pupae in cold storage for reserve. These were killed when the temperature setting was set too low by accident. Present production is approximately 20 to 30 pupae per day.

Diet: the diet that we are now using contains:

canned pinto beans	800. gm
brewer's yeast	80. gm
ascorbic acid	8. gm
tegosept (methyl-hydroxy-benzoate)	8. gm
agar	32. gm
linseed oil, raw	4. gm
sucrose	40. gm
molasses	100. gm
corn syrup (Karo)	60. gm
wheat germ	30. gm
powdered cellulose	135. gm
vitamin solution (Vanderzant)	15. gm
water	2,000. gm

Ingredients are boiled for five minutes (excluding the ascorbic acid) before mixing in the blender.

We have separated the laboratory into three work areas:

(1) clean area for food preparation, maintained as aseptic as possible,
(2) clean area for transferring larvae, maintained clean but not aseptic,
(3) area for handling cups with contaminated food where larvae are removed from dirty food and washed before transferring to clean food in area 2. Area 3 has a hood with an exhaust fan.

We are continuing to use the one-ounce jelly cups for rearing larvae instead of the plastic dishes as we had planned originally because we can control mold spread with the cups. We examine the larvae every day (including weekends and holidays). Larvae are transferred when mold appears on the food. We often transfer larvae every second day.

Diseases have been a problem. We have a virus-like disease in the colony. Black abdominal prolegs and a white waxy appearance to the lethargic larvae are the main symptoms. Larvae die in the fourth or fifth stages. Mold kills larvae in all stages, but most of the larvae are killed as second or third instars if they die of mold.

Feeding and tunneling is good in this diet. The adults are larger than those from previous diets, but smaller than individuals grown on corn or cane stalks. Oviposition rate, adult longevity, mating frequency and mating behavior is equal or superior to field-reared adults.

Toba et al. (1973) compared IPS and fully sterile individuals in a cage test with the cabbage looper. They made three releases:

(1) overflooding the normal males with fully sterile males in a 10:1 ratio,
(2) overflooding the normal males with partially sterile males in a 10:1 ratio,
(3) no overflooding.

They found the F_1 population reduced 62 percent in relation to the control population in tests where fully sterile males were released, and 92 percent reduced in tests where partially sterile males were released. Overflooding with partially sterile males was more effective than overflooding with fully sterile males.

B. IPS: Analysis of Laboratory Data in Relation to Sex Ratios, Dose Effect and Generation Effect (MacKay)

These data are the reproductive performance of a group of afflicted individuals in outbred lines. Either the male or the female of the P generation was irradiated, the opposite member of the P generation was a normal individual. All offspring were outbred with normal individuals as single pair matings, keeping the lines separated in the immature stages. Data include offspring in the F_1 to F_8 generations. Forty-one lines were observed. The dose given to the P generation parent was 1, 2, 4, 6, 10, 12, or 14 krads. The experimental work was done in the laboratory during the years 1967-70. These date are shown in Tables 2-25 and Figures 1-24 in Report 6.

1. Sex ratios. The previous report showed the lineage for 1,072 outbred matings that produced fertile eggs and that were descendents from an irradiated parent in the P generation. In the F_1 through F_8 generations observed 56 percent of the adults were males. Unsuccessful matings were not included in this tabulation. For convenience we have limited successful matings to only those in which fertile eggs were produced, matings where a spermatophore was transferred to the female. Matings in which there were no fertile eggs because of apyrene or immobile sperm are not included. In many instances mating occurred, fertile eggs were laid and embryonic development proceeded, but no eggs hatched; these are included.

All 598 afflicted males of the F_1 to F_8 generations were mated to females that produced fertile eggs, and in 148 of the mating instances some of the eggs in each mating hatched and some of the ensuing larvae developed to the adult stage of the following generation (Table 1 and Figure 1). There were 474 afflicted females that produced fertile eggs in the F_1 to F_8 generations; of these 86 instances some larvae from each mating survived to the adult stage. Comparing reproduction in afflicted male offspring from a P generation afflicted male or female we find that the afflicted male offspring were successful in continuing the line in 24.8 percent of the instances and the afflicted female offspring in 18.2 percent.

Males are superior to females in ability to transmit the affliction in IPS lines for two reasons: there are more of them, and survival of offspring from afflicted males is higher than from afflicted females. This sex difference in reproductive potential in outbred afflicted lines may be due to differences in the sex chromosomes of the sugarcane borer. Lepidopteran females are hemizygous for sex chromosomes.

2. Dose effect. (a) Table 3 and Figures 1 and 2 show a comparison of egg hatch in the F_1 to F_8 generations at different doses. There is a negative correlation between increase in dose and egg hatch of F_1 and F_2 embryos (eggs produced by the P and F_1 generation adults, respectively). Beyond the F_2 the correlation is not consistent. Egg hatch from afflicted lines descending from afflicted P generation females were lower than from afflicted P generation males in the F_1 generation.

(b) Table 4 and Figures 3 and 4 show adult emergence which is a comparison of larval survival and dose for P generation males and females. Percent survival of F_1 larvae and pupae is low at 2 krads, higher at 6 krads, and low in 12 and 14 krad lines from both males and females.

(c) Table 5 shows a comparison of lines for stage of death of embryos in relation to dose. There is a correlation between increased dose and earlier death in the F_1 generation in lines from both afflicted P generation males and females. All fertile eggs develop to the orange spot stage, unfertilized ova only develop to the bright yellow stage. Embryonic developmental stages were described previously (Walker and Quintana, 1968).

3. Generation effect. (a) Table 5 and Figures 5 and 6 also show a comparison of stage of death of embryos beyond the F_1 generation. Death occurs at progressively later stages in consecutive generations at nearly all doses and generations. There is a partial recovery in egg hatch from the offspring produced by the afflicted P generation male line from the second to the fourth generations. Generation effect on egg hatch is not consistent, although egg hatch is lower from descendents of P generation afflicted females.

(b) Tables 6 and 7 and Figures 7 and 8 show a comparison of relative survival and stages of death of larvae and pupae in lines from afflicted P males and females, respectively. Again the descendents of afflicted female lines are more damaged than the male and female offspring of afflicted male lines. Adult survival in Table 2 and Tables 6 and 7 are not comparable since only 2, 6, 12 and 14 krad doses are tabulated in Tables 6 and 7.

C. IPS in Hemiptera (Restrepo)

Virgin adult female stinkbugs (Nezara viridula (L.), Pentatomidae) were exposed to 1.5, 7.5, or 15.0 krads and then mated with normal males as discussed in the previous report. Each generation the eggs were collected and the offspring were carried through the fifth generation. None of the offspring from the two higher doses survived beyond the F_1 nymphal stages; the offspring from females treated at 1.5 krads survived. Reproduction and survival in the 1.5 krad line and the normal line were equal in generations F_2 to F_5. We interpret this to have been a recovery, i.e., selection against the affected genomes. Sex ratios of offspring were equal in both the normal and irradiated lines. Survival data are shown in Table 11.

Pentatomid chromosomes are reported to be holokinetic as are

lepidopterans. Gomez-Nunez in Venezuela and LaChance with the USDA in North Dakota have studied the IPS effects in other hemiptera. They found the afflicted lines recovered in the first or second post-irradiation generation. Although they worked with group matings rather than single pair matings, I believe that their data can be correctly interpreted to mean that a selection mechanism occurred. The only known difference between the lepidopteran genetic mechanism and the hemipteran is in males. Nezara and other hemipterans have abnormal sperm production from the harlequin lobe of the testes. This may have no bearing on the relationship with the recovery phenomena observed. However, it is of academic interest and possibly of significance. It is more likely that the genome duplication mechanism is different in some respect between the two orders, and this could explain the clear difference between recovery in hemipteran lines and incomplete recovery in lepidopteran lines. In addition Virkki (1963) reported asynapsis in the meiosis of the sugarcane borer males. Asynapsis in meiosis has also been observed in coccida (homoptera) and Cecidomyidae (diptera). Virkki states (p. 119):

> "These examples show that the classic pairing of homologues is not a unique method of controlling the reduction division of the chromosomes. There are some factors latent in the prophasic cell which are capable of taking care of a correct segregation in lack of pairing of homologues. In our subject, Diatraea saccharalis, such factors apparently operate in the asynaptic spermatocytes, because the anaphase grouping 17 + 17 (or nearly so) occurs so often."

Perhaps this, too, could provide a clue to the difference.

D. Fractionated Dose Effect with the Greater Wax Moth (Singh)

Galleria mellonella (L.) moths were reared in one-gallon jars on Waterhouse (1959) medium. This contains honey, glycerine, brewer's yeast, water, dry Pablum infant formula, and vitamins. Food was autoclaved and after it had cooled the mature larvae were added. The emerging adults deposited eggs on the medium, and the next generation of mature larvae and pupae were collected as they emerged 30 days later. Jars were held in the dark with the temperature maintained at 32 $\pm$ 1°C.

The sex of pupa was determined and each was maintained in a separate one-ounce jelly cup. Upon emerging the adults were irradiated at 0 to 24 hours age, and placed with an individual of the opposite sex after irradiating. Mating occurred immediately. Most of the eggs were laid inside the fold of a small piece of wax paper. Eggs were counted, scored for development and hatch 10 to 15 days after mating. In order to prevent larvae from eating remaining embryos a one-half inch piece of scotch tape was stuck to the inside surface of each cup. Larvae congregated under the tape and were trapped.

Three series of tests were conducted to determine:

a. the sterilizing dose to adult males (two tests);
b. the sterilizing dose to adult females;
c. the sterilizing dose as either a single dose or a fractionated dose, 24 hours between the two fractions.

All tests were repeated three times with five or more replicates in each. Data reported in tables are averages of all tests.

Results

Single Exposure: The preliminary tests indicated that males could be sterilized at approximately 22 krads or higher (Table 8) and that egg production of normal females mated with irradiated males declined considerably, particularly if the males had been treated at higher doses of radiation. Practically all of the eggs laid were fertile, however the proportion of non-fertilized eggs increased with dose and age, possibly due to sperm inactivation. Sterilized males did not recover virility when mated with the second virgin female, nor did the first female mated with the irradiated male produce viable eggs in the absence of the irradiated male. Most of the eggs were laid in the first five days after pairing. In the second five-day period (6-10 days after pairing), egg production declined drastically, however this reduction in oviposition was greater in the treated than in the control pairs. Similarly, a second female mated with the same male failed to produce viable eggs, indicating that males that had been irradiated with sterilizing doses did not regain virility (see Table 8).

Females are more susceptible to radiation damage than are males (Table 9). Where both sexes were irradiated, the sterilizing effect is more or less equal to that on the female.

The mating ability, adult longevity and sexual attractiveness of the moth receiving up to 22.0 krads did not appear to be affected. It was further observed that treated female moths started egg laying earlier and egg development was longer than the control group.

Fractionated Exposures: In the second series of tests single and fractionated doses were compared. Females were more radio sensitive to fractionated doses than were males (Table 10). Fractionated doses produced higher sterility in both sexes. Mating ability, adult longevity and sexual attractiveness were not apparently affected by doses used. However, egg production and egg hatch effects were greater in females that had received fractionated doses as compared to a single dose. A fractionated dose of 6.6 krads to females sterilized them, as compared to 12 percent egg hatch from females receiving 6.6 krads in a single exposure.

Discussion

The utility of this concept in the context of lepidopteran control would appear to be great if the experience with the wax moth occurs in other lepidoptera. For example, female pupae or adults could be given a small conditioning dose of radiation--sufficient to disrupt the repair

mechanism capability--and later a sufficient dose to cause the bulk of the genetic damage. It is conceivable that this combined dose would be substantially less than the amount needed for producing complete dominant lethality from a single acute dose. This would allow us to use considerably smaller dosages to achieve the same amount of genetic damage, and thus we could avoid the inherent problems encountered at the high doses necessary to sterilize lepidoptera. The most important of these are sperm immobility, sperm death, reduction in mating competitiveness, shortened adult life-span, reduced oviposition, and reduced vigor.

However, our data indicate that the net effect of fractionated doses may indeed provide greater dominant lethality than a single acute dose. Possibly this can be explained by repair mechanisms. It does not necessarily mean that the total genetic damage is necessarily greater from fractionated doses, but simply that the effect of repair mechanisms is rendered inoperative in such a manner that the genetic damage becomes apparent earlier, in the developing F_1 embryo stage in this case instead of in the F_2 embryonic stage, or in the developing larval and pupal stages of the F_1 generation.

Fractionated dose technique deserves further attention because of the potential use in lepidopteran control. If the mechanism works for other species, then it is apparent that we have a powerful tool for manipulating lepidopteran sterility through the production of genetic damage at considerably lower doses.

E. Host plant resistance (Vakili and Kaiser)

Drs. Vakili and Kaiser at the Federal Experiment Station in Mayaguez are field testing hundreds of varieties of beans (Phaseolus vulgaris) and cowpeas (Vigna sinensis). The objective of this work is measuring potential yields, resistance to plant diseases and to insect attack. Their program is part of an AID sponsored effort in several countries in the Latin American tropics. We have cooperated with them to develop methods for determining the nature of the attractiveness of susceptible varieties, and conversely the factors responsible for resistance in the resistant varieties.

Dr. Walker helped by identifying the pests and assaying the damage in bean and cowpea trials on a voluntary basis and on his own time. The bean program and a corn and sorghum program with similar objectives directed by Dr. Webster provide an excellent opportunity for us to develop a program.

I would like to begin by studying the differences in profiles of the aromatic compounds from the most resistant and the most susceptible varieties of beans and cowpeas to the bean weevil, Chalcodermes ebininus. The compounds producing odors will be solvent extracted from bean pod homogenates using mineral oil in blotting paper to absorb the volatiles, then extracting this with a solvent and then analyzing by gas chromatograph. This extraction method was used to evaluate the attractiveness

of volatiles in banana varieties against the banana weevil. PRNC has the equipment necessary to begin this work. Solvents and other chemicals and columns would be needed, but little else is required.

III. Relation to other work

The population collapse technique for eradicating lepidopteran species needs extensive field testing both in cages and on an area basis. Since the latter programs would be of considerably greater scale I do not think it would be wise to attempt this with the sugarcane borer yet. After a mass-rearing method has been developed this can be considered, but until then it would be doomed to failure.

Unfortunately the only other IPS cage test (Toba, et al., 1973) was only carried to the F_2 generation. It was based on the hypothesis that several overfloodings (10:1) would be made in a field program, using a high dose for producing the semi-sterile males. It is very expensive to laboratory rear large numbers of lepidopterans.

Comparison of the hypothesis of our test plan with Toba's relates to two factors, dose and overflooding ratio:

1. that the high dose causes lower survival in earlier generations, therefore requiring higher overflooding ratios to compensate for the smaller number of F_2 and F_3 survivors; and

2. that a smaller dose yields a higher proportion of F_1, F_2 and F_3 generation individuals with genetic load, enhancing the frequency of dissemination of this genetic load into the natural population, but becoming effective at a relatively later time.

The interrelationship of these two aspects needs to be more definitively explored and the population collapse concept needs to be tested further under natural conditions of survival, i.e., in the field.

I feel that we should stimulate interest in using lower doses so that we can develop the best method for effectively disseminating afflicted genomes into a population.

IV. Future work planned

Completion of the field tests is the first priority of the program. Although we had a slow start and difficulties with the colony, we should be able to complete the cage tests within the end of FY 1974.

Further work with fractionated doses to determine if we can produce complete dominant lethality in female sugarcane borers will be explored. The results with the wax moth show considerable promise. Possibly other lepidopteran species could be included in these experiments.

I feel that the host plant resistance project has a great potential. The extensive USDA field programs provide an excellent platform for this research.

V. Publications

Walker, D. W., Harpal Singh and K. P. MacKay. (----). Gamma induced sterility of the greater wax moth: 18 pp. (to be submitted to J. Econ. Entomol.)

in preparation.

Dose effect on IPS in the sugarcane borer (Walker and MacKay).
Generation effect on IPS in the sugarcane borer (Walker and MacKay).
IPS in the southern stink bug (Walker and Restrepo).
Varietal susceptibility of cowpeas to pod borer (Vakili and Walker).
Bean pod and seed damage by the bean pod borer (Walker and Vakili).
Differences in susceptibility of bean varieties to pega pega Vakili and Walker).
A strategy for lepidopteran pest eradication (Walker and Pedersen).

VI. Program Personnel

Mr. Kenneth P. MacKay has worked full-time on the program since September 1972. He has had a broad experience in metallurgical research at the University of Michigan Engineering Research Institute and has taught science courses and was an administrator at the high school level for several years. He is directly responsible for the laboratory colony phase of the cage tests, but he has also worked with the IPS laboratory in developing the computer analysis.

Mr. Ruben Restrepo, a graduate student from the Universidad Nacional de Bogota, Colombia, worked officially with the program during June through August on an OAS grant. He has worked for the last two years on a voluntary basis. We have completed the preliminary work with the stink bug, Nezara viridula, i.e., diet evaluations (see last report) and IPS. Mr. Restrepo will complete the requirements for the master of science degree in Biology in mid 1973. His thesis research is a taxanomic revision of a group of homopterans.

Dr. Harpal Singh worked from June through mid September on a grant from the Oak Ridge Associated Universities. He evaluated fractionated dose effect in the great wax moth.

Alba Rivera-Detres is completing her course work for the master of science in Biology. She will continue her investigations of hemolymph proteins of sugarcane borer larvae at the beginning of summer vacation.

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Table 1

F_1 Population in First Cage Test[1]

First Cycle:

Total plants	Total larvae[2]	Number of infested plants	Number of non-infested plants
255	255	88	167

Number of stalks that had tunnels:

Number of tunnels per stalk	0	2	3	4	5	6	7	8	9	10	11	12	13	14	19
Number of stalks in each group	167	21	9	2	3	2	3	2	0	1	0	2	1	1	1

[1] Released 15 pairs of normal adult sugarcane borer moths 27 February, sampled 50 days later.

[2] Total larvae count is based on the number of larval tunnels encountered. Most of the larvae had pupated and emerged, laying their eggs on the second cycle of plants. A residue of 11 pupae, 18 mature larvae and 8 L_3 larvae of the F_1 generation were encountered in the first cycle plants. These were placed on the second cycle plants.

F_2 population in second cycle plants will be sampled 50 days after the last sampling.

Table 2

Frequency of Successful Mating and Survival of Offspring in Outbred Lines, F_1 to F_8 [1]

	Number of adult offspring produced in afflicted lines F_1 to F_8	Number of matings producing adults in the following generation		Percent of matings successful in continuance of the outbred line
Males	598	148		24.8
Females	474	86		18.2
Total	1,072	234	average	21.8
Offspring from irradiated P generation male:				
Males	369	90		24.4
Females	252	41		16.5
Total	621	131	average	21.1
Offspring from irradiated P generation female:				
Males	235	60		25.5
Females	225	45		20.0
Total	460	105	average	22.8

[1] This tabulation shows F_1 through F_8 individuals from lines irradiated as the male or female adult in the P generation (outbred with a normal), and successively outbred with a normal of the opposite sex in every instance F_1 through F_7. See Tables 2-25, and Figures 1-24, Technical Report No. 6 (1972).

Table 3

Percent Egg Hatch

	Male Ancestor Irradiated					Female Ancestor Irradiated			
Dose / Generation	0	2	6	12	14	2	6	12	14
1	91.5	71.6	47.7	28.2	17.0	35.0	25.1	15.4	0.0
2	97.3	31.9	31.4	7.5	19.1	39.8	36.4	29.5	
3	96.9	41.0	21.9	39.4	38.3	48.3	32.9	29.9	
4	87.3	28.0		31.2	26.1	31.8	39.5		
5	96.8	49.5		41.2	53.1	57.9	37.9		
6	91.6	36.7		42.1	36.8	40.3	40.1		
7				28.7	39.7	39.3	33.7		
8				45.8	43.7	40.8	30.6		

Table 4

Percent Adult Emergence

	Male Ancestor Irradiated					Female Ancestor Irradiated			
Dose / Generation	0	2	6	12	14	2	6	12	14
1	65.0	9.4	24.0	9.6	4.8	7.5	25.2	8.0	0.0
2	64.3	13.2	10.4	3.6	28.2	23.5	22.9	18.9	
3	67.2	19.6	15.3	20.2	19.7	24.7	17.6	0.0	
4	63.8	27.5		15.4	12.7	15.9	20.9		
5	65.0	9.1		9.7	4.3	10.4	4.7		
6	70.1	6.3		8.6	14.8	3.6	8.3		
7		3.0		2.8	6.0	8.5	4.8		
8		0.0		1.1	0.0	10.5	0.1		

Table 5

Embryonic Mortality and Egg Hatch in IPS Lines[1]

GENERATION / M. Ancestor Series		Krad	1 A	1 B	1 C	1 D	1 E	2 A	2 B	2 C	2 D	2 E	3 A	3 B	3 C	3 D	3 E	4 A	4 B	4 C	4 D	4 E
Irradiated	Control	0		3.6	3.4	1.1	91.5		0.8	1.4	0.3	97.2		1.[illegible]	1.4	0.[illegible]	96.9		6.3	5.8	1.1	87.3
at Indicated	A1	2	0.4	6.2	21.7		71.6	6.4	21.6	[illegible]9.4		31.9	8.[illegible]	22.6	28.0		41.0	0.0	4.6	67.3		28.0
Doses	A2	5	2.8	11.[illegible]	38.[illegible]		[illegible]7.7	3.6	18.4	[illegible].5		31.4	6.[illegible]	23.7	48.3		21.9					
	A3	12	2.2	[illegible]	[illegible]		[illegible]8.2	19.[illegible]	44.0	[illegible]		[illegible]	11.[illegible]	22.1	22.3		39.4	9.5	2.9	50.7		31.2
	A4	1[illegible]	11.7	26.[illegible]	44.[illegible]		17.[illegible]	19.[illegible]	2[illegible]	45.3		19.[illegible]	[illegible]2.2	12.[illegible]	37.5		3[illegible].3	0.6	3.9	69.4		26.1

GENERATION / F. Ancestor Series		Krad	1 A	1 B	1 C	1 D	1 E	2 A	2 B	2 C	2 D	2 E	3 A	3 B	3 C	3 D	3 E	4 A	4 B	4 C	4 D	4 E
Irradiated	Control	[illegible]		3.6	[illegible].4	1.[illegible]	[illegible]1.5		0.8	1.4	0.2	97.3		1.4	1.4	0.1	96.9		6.3	5.8	1.1	87.3
at Indicated	B1	2	1.0	19.5	44.5		35.[illegible]	8.8	[illegible].8	29.6		39.8	9.3	19.6	22.7		48.3	0.2	5.8	62.2		31.8
Doses	B2	6	8.8	29.9	36.1		25.1	6.6	21.5	35.5		36.4	10.6	26.9	29.1		32.9	0.9	3.3	57.6		39.5
	B3	12	9.6	37.8	37.[illegible]		15.4	7.6	32.0	30.9		29.5	17.9	44.9	71.9		29.9					
	B4	14	29.5	60.6	[illegible]9.9		[illegible].0															

Percent Mortality at Indicated Stage of Development

- A Orange spots
- B Hollow center
- C Head capsule
- D Fully developed
- E Percent larvae hatched

[1] Percent mortality at various stages of embryonic development and percent egg hatch by generation for descendents whose male or female ancestor was biologically insulted at the indicated dose of gamma radiation.

Table 6

Larval Mortality and Adult Emergence in IPS Lines from a Male Parent[1]

GENERATION / M. Ancestor Series		Krad	1 H	1 I	1 J	1 K	2 H	2 I	2 J	2 K	3 H	3 I	3 J	3 K	4 H	4 I	4 J	4 K
Irradiated	Control	0																
at Indicated	A1	2	67.4	18.7	0.0	0.0	40.8	12.6	12.4	19.5	18.2	6.9	22.5	12.3	42.0	11.3	3.8	11.4
Doses	A2	6	71.4	0.0	0.0	0.0	35.9	18.4	12.4	21.1	0.0	30.7	4.0	48.7				
	A3	12	87.2	0.0	0.0	0.0	48.9	0.0	0.0	0.0	44.7	3.7	5.1	13.1	47.8	14.6	6.3	11.8
	A4	14	26.9	0.0	0.0	0.0	0.0	0.0	36.7	32.1	29.6	11.8	11.0	25.2	41.5	11.9	8.8	15.0

F. Ancestor Series		Krad	1 L	1 M (A)	1 N (M)	1 O (F)	2 L	2 M (A)	2 N (M)	2 O (F)	3 L	3 M (A)	3 N (M)	3 O (F)	4 L	4 M (A)	4 N (M)	4 O (F)
Irradiated	Control	0		65.0				64.3				67.2				63.8		
at Indicated	A1	2	4.9	9.4	5.2	4.2	22.2	13.2	7.0	6.2	7.3	19.6	10.6	24.9	3.9	27.5	18.1	9.4
Doses	A2	6	4.6	24.0	12.3	11.7	1.9	10.4	5.7	4.7	1.3	15.3	7.6	7.7				
	A3	12	3.2	9.6	5.9	3.7	4.1	3.6	2.0	1.6	1.8	20.2	9.4	10.8	1.8	15.4	11.3	4.1
	A4	14	7.2	4.8	3.4	1.4	2.9	28.2	19.5	8.7	2.7	19.7	11.3	8.4	12.2	12.7	8.8	3.9

Stage of Death

- H L1, L2
- I L3
- J L4
- K L5
- L Pupae

Emergence

- M Normal Adults
- N M
- O F

[1] Percent mortality at various stages of larval development and percent emergence as adults, male and female, descendents whose male ancestor was biologically insulted at the indicated dose of gamma radiation.

Table 7

Larval Mortality and Adult Emergence in IPS Lines from a Female Parent[1]

GENERATION / M. Ancestor Series		Krad	1 H	1 I	1 J	1 K	2 H	2 I	2 J	2 K	3 H	3 I	3 J	3 K	4 H	4 I	4 J	4 K
Irradiated	Control	0																
at Indicated	B1	2	89.0	0.0	0.0	0.0	35.8	7.3	7.8	22.8	24.0	7.5	12.8	28.1	51.1	13.2	6.9	11.0
Doses	B2	6	39.[illegible]	2[illegible].4	5.2	0.0	25.7	15.3	9.4	22.1	32.4	9.6	8.[illegible]	29.4	51.2	6.6	1.9	12.8
	B3	12	87.7	8.0	0.0	0.0	33.1	6.4	2[illegible]	12.6								
	B4	14																

F. Ancestor Series		Krad	1 L	1 M (A)	1 N (M)	1 O (F)	2 L	2 M (A)	2 N	2 O (F)	3 L	3 M (A)	3 N	3 O (F)	4 L	4 M (A)	4 N (M)	4 O (F)
Irradiated	Control	0		65.0				64.3				[illegible]				63.8		
at Indicated	B1	2	3.5	7.5	4.3	3.2	5.1	23.[illegible]	[illegible]3	[illegible]		[illegible]	14.[illegible]	10.1	2.5	15.9	9.7	7.5
Doses	B2	6	4.[illegible]	25.2	12.5	17.7	5.5	22.[illegible]	[illegible]8	[illegible]		37.[illegible]	10.7	6.9	11.4	20.9	11.8	7.6
	B3	12	1.2	8.0	4.9	3.1	2.9	18.[illegible]		[illegible]		[illegible]						
	B4	14		0.0				0.0										

Stage of Death

- H L1, L2
- I L3
- J L4
- K L5
- L Pupae

Emergence

- M Normal Adult
- N M
- O F

[1] Percent mortality at various stages of larval development and percent emergency as adults, male and female, by generation for descendents whose female ancestor was biologically insulted at the indicated dose of gamma radiation.

5					6					7					8				
A	B	C	D	E	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E
		0.9	.8	0.1	96.8			6.5	1.5	0.1	91.6								
0.0	0.8	49.7			49.5	1.1	0.7	61.5			36.7								
0.0	3.6	57.2			41.2	0.0	0.4	57.4			42.1	2.0	0.1	71.2	28.7	0.0	0.5	53.7	45.8
0.1	3.4	43.4			53.1	0.0	2.2	61.0			36.9	0.0	0.2	61.2	39.7	0.0	0.0	56.3	43.7

5					6					7					8				
A	B	C	D	E	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E
		0.9	0.8	0.1	96.8			6.5	1.5	0.1	91.6								
0.0	3.5	34.5			57.9	0.0	0.4	49.2			40.3	0.0	1.2	59.4	39.3	0.2	0.9	58.1	40.8
0.0	2.2	50.0			37.9	0.0	2.2	57.6			40.1	0.0	0.5	65.8	33.7	0.0	0.6	68.8	30.6

5				6				7				8			
H	I	J	K	H	I	J	K	H	I	J	K	H	I	J	K
	35.4	21.7	23.5	37.7	23.7	10.9	19.8	53.8	18.2	10.0	14.5	10.5	17.5	22.5	42.5
23.9	25.9	22.3	15.0	40.1	15.9	9.7	24.7	65.3	13.7	5.7	12.0	34.3	24.0	26.7	13.0
43.9	31.2	4.1	18.2	17.4	26.3	16.3	22.5	41.3	22.2	14.2	20.2	20.8	42.7	28.8	6.6

L	A M	N	F O	L	A M	N	F O	L	A M	N	F O	L	A M	N	F O
	65.0				70.1										
13.3	9.1	4.9	4.2	1.4	6.3	4.7	1.6	2.0	3.0	1.7	1.3	7.0	0	0	0
1.2	9.7	7.2	2.5	13.8	8.6	5.6	3.0	0.6	2.8	1.8	1.0	0.8	1.1	0.5	0.6
1.9	4.3	2.4	1.9	31.9	14.8	9.0	5.8	1.0	6.0	3.2	2.8	1.1	0.0	0.0	0.0

5				6				7				8			
H	I	J	K	H	I	J	K	H	I	J	K	H	I	J	K
6.8	18.4	10.0	27.3	34.2	16.3	11.0	13.0	33.0	22.6	15.8	17.2	35.2	13.3	18.8	21.3
50.9	13.2	9.5	20.9	30.0	27.7	17.9	14.3	56.9	22.6	5.0	10.1	27.9	54.5	10.4	6.6

L	A M	N	F O	L	A M	N	F O	L	A M	N	F O	L	A M	N	F O
	65.0				70.1										
12.3	10.4	6.3	26.1	1.6	3.6	2.4	2.2	4.0	8.5	5.3	3.2	0.9	10.5	5.9	4.6
0.8	4.7	3.1	1.5	1.8	8.3	5.1	3.2	1.0	4.8	3.0	1.8	0.4	0.1	0.1	0.0

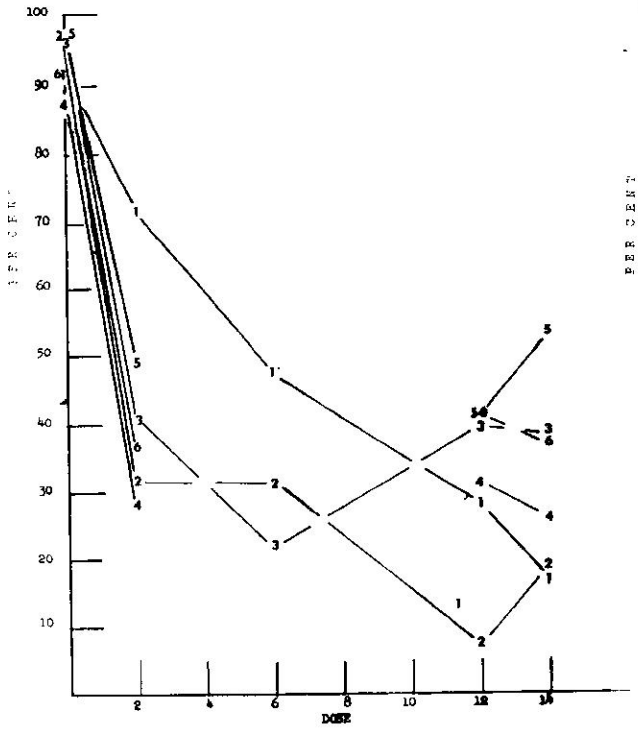


Figure 1. Dose Effect on Egg Hatch in IPS Lines from Irradiated Male Parent

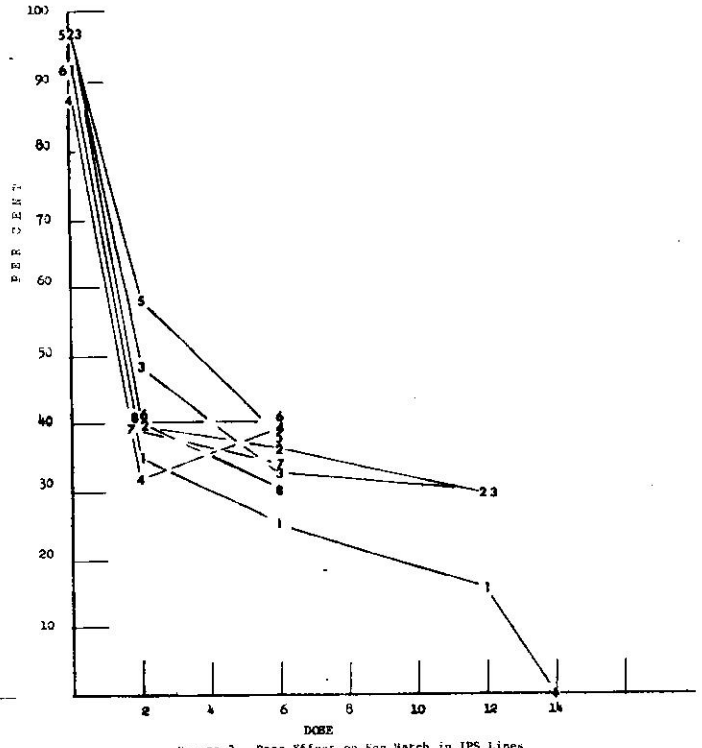


Figure 2. Dose Effect on Egg Hatch in IPS Lines from Irradiated Female Parent

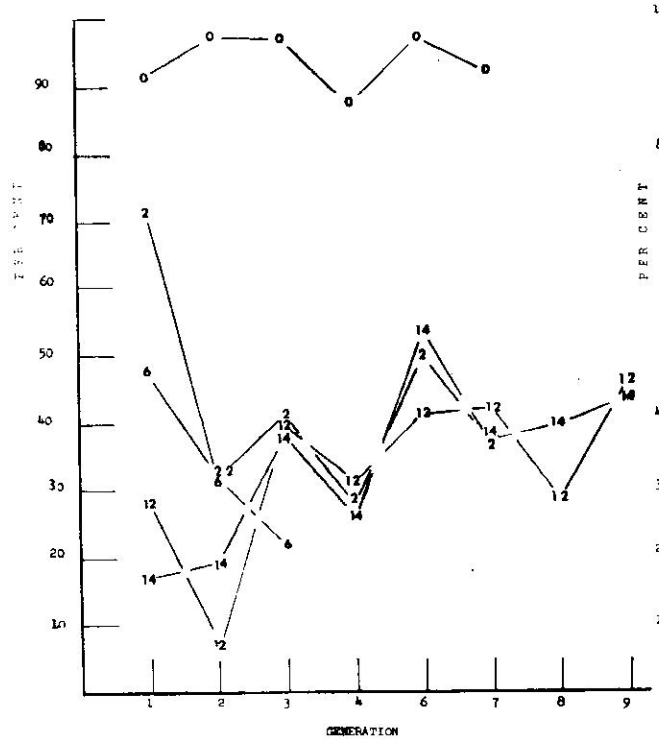


Figure 3. Generation Effect on Egg Hatch in IPS Lines from Irradiated Male Parent

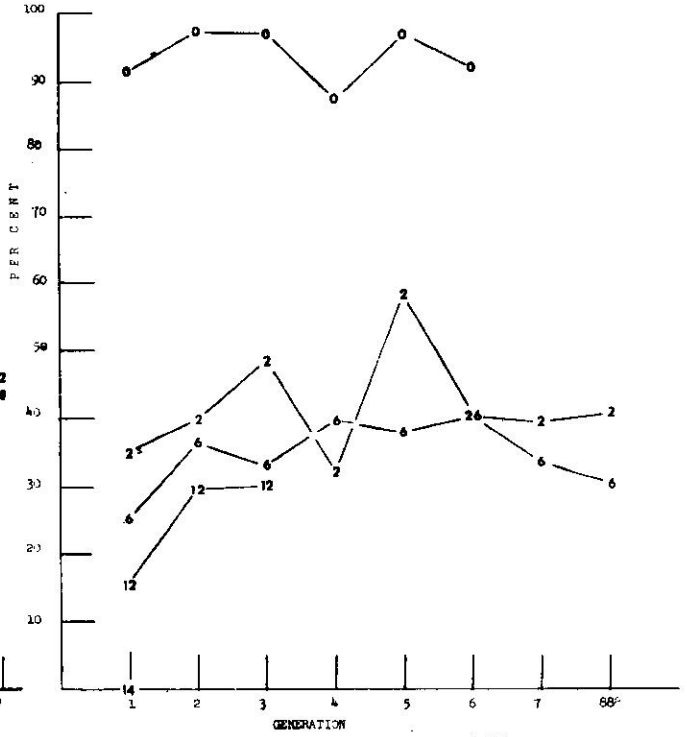
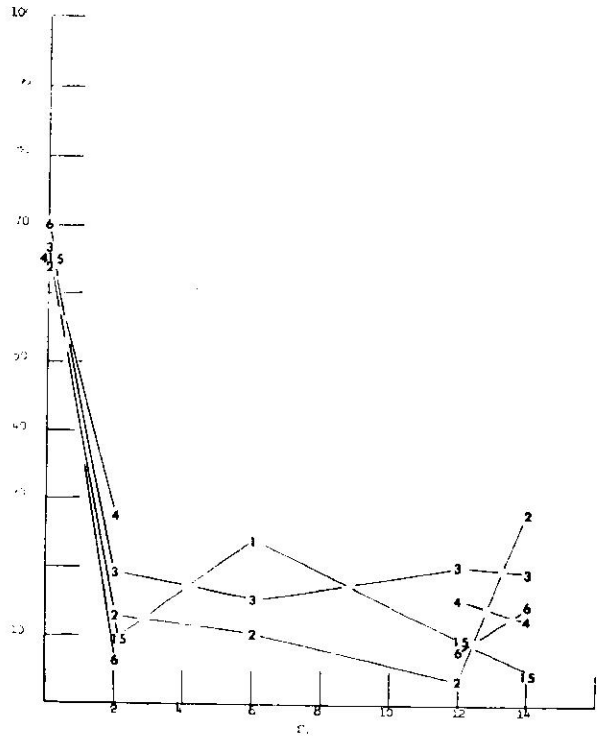


Figure 4. Generation Effect on Egg Hatch in IPS Lines from Irradiated Female Parent



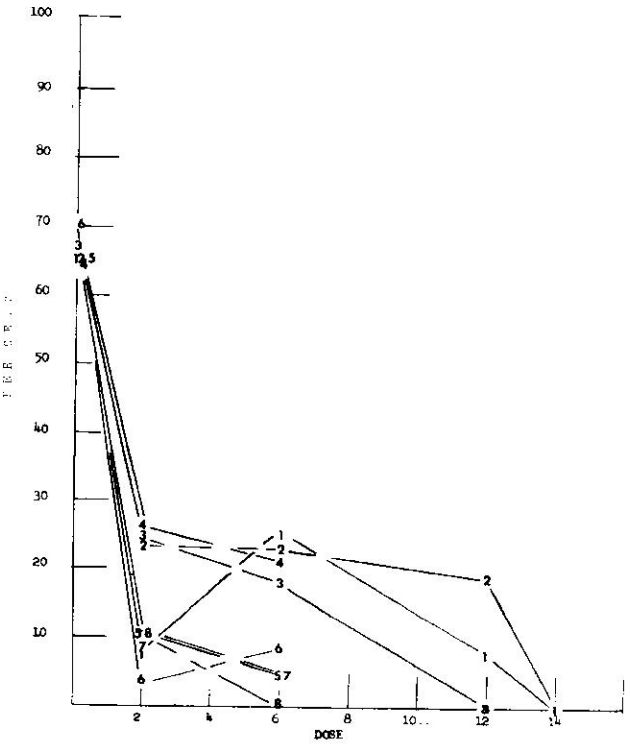
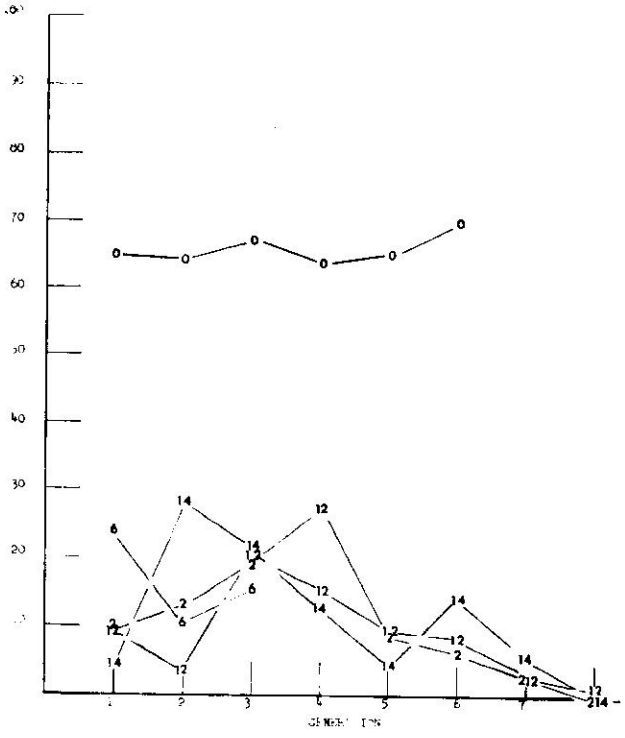


Figure 9. ...dose effect on larval and pupal survival ... IPS ... for ... irradiated female parent



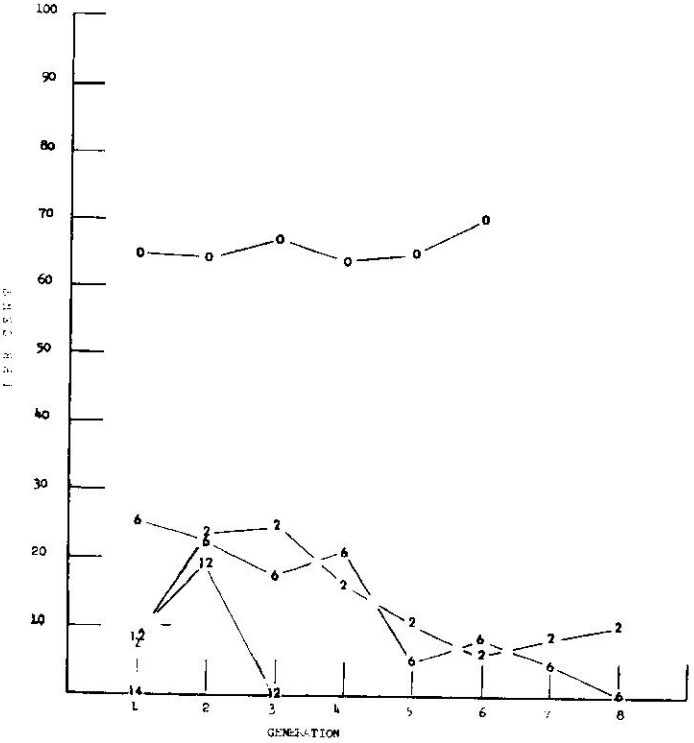


Table 8

Wax Moth Preliminary Test: Male Sterility[1]

Exposure Kilorads	Fertile Eggs Laid	Eggs Hatched	Percent Hatched
0	800	752	94.0
7.70	766	651	85.0
11.00	742	573	80.0
13.75	700	490	70.0
19.25	687	209	31.0
22.00	750	0	0
30.9	537	0	0
40.9	570	0	0
50.9	575	0	0

[1]Male sterility induced by a single exposure.

Table 9

Wax Moth: Test One[1]

Exposure (Krad)		Eggs Produced	Percent Hatched	Eggs Produced	Percent Hatched
		(a)[2] irradiated male	1st virgin female (unirradiated)	(b) irradiated male	2nd virgin female (unirradiated)
0	1st 5 days	579	95	500	85
	2nd 5 days	153	80		
10	1st 5 days	454	82	850	80
	2nd 5 days	92	20		
20	1st 5 days	453	10	450	0
	2nd 5 days	114	0		
30	1st 5 days	623	0	737	0
	2nd 5 days	150	0		
40	1st 5 days	520	0	725	0
	2nd 5 days	100	0		
50	1st 5 days	575	0	500	0
	2nd 5 days	111	0		

[1] Male sterility from a single exposure.

[2] After 5 days irradiated males were isolated from the first female (a) and mated with the second female (b).

Table 10

Wax Moth: Test Two[1]

Fractionated[2]				Single			
total exposure krad	fertile eggs produced	eggs hatched	percent hatched	total exposure krad	fertile eggs produced	eggs hatched	percent hatched
(a) Irradiated female; normal male							
6.6	287	0	0	6.6	567	67	12
13.2	380	0	0	13.2	542	0	0
19.8	358	0	0	19.8	601	0	0
(b) Irradiated male; normal female							
6.6	420	391	93	6.6	453	403	89
13.2	400	239	60	13.2	402	318	79
19.8	453	91	20	19.8	571	86	15
(c) Irradiated male; irradiated female							
				6.6	612	102	16
				13.2	560	0	0
				19.8	575	0	0
(d) Control (unirradiated male; unirradiated female)							
0.0	650	624	96				

[1] Sterility effects of fractionated and single exposures to female and male moths. Average of five or more matings per treatment, replicated three times.

[2] Given in two equal doses 22 ± 2 hours apart.

Table 11

IPS in the Stink Bug[1]

Dose	Female No. of Adults	No. of Fertile Eggs Laid	No. of Fertile Eggs Hatched	% Egg Hatch	No. of Adults Harvested	% Survival
P generation Reproduction						
0	9	659	276	41.8	200	72.4
1.5	9Females	121	20	16.5	10	50.0
7.5	9Females	298	62	20.8	0	0
15.0	9Females	563	8	1.4	0	0
F_1 generation Reproduction						
F_1 0	14Females	496	403	81.3	216	53.6
F_1 1.5	3Females F_1 x 2 Males F_1	163	44	27.1	-	-
Averages F_2, F_3, F_4, F_5						
0		50/F	42.5	85%	21.25	50%
1.5		50/F	42.5	85%	21.25	50%

[1] See text for further information.

APPENDIX

Cage Test of Inherited Partial Sterility in the Sugarcane Borer
Experimental Design

Hypothesis being tested:

That overflooding a natural population with sub-sterile males over a single generation at the rate of 14 to 1 will eradicate the population or will effectively suppress the population for two or more generations.

The hypothesis is based upon laboratory experiments that led to the development of a hypothetical model for population control by Walker and Pedersen (1969, Ann. Entom. Soc. Amer. 62:21-6).

Explanation of the mechanism:

The predictability of the success of this model is due to two factors:

1. A relatively high reproductive rate in the P to F_1 generation (4.1) in outbred afflicted lines, and

2. A drastic reduction in the chances of a normal to normal mating as F_1 adults and therefore a drastic reduction in the reproductive potential in the F_2 and subsequent generations.

The introduction of sub-sterile males provides the mechanism for introducing a biological insult into a large proportion of the natural population.

As shown in Table I the overflooding advantage in the P generation is 14 to 1, in the next (F_1) generation the ratio of sub-sterile to normal adults is expected to be 57 to 10 (5.7 to 1), and in the F_2 generation the ratio is expected to be 29 to 7.5 (3.9 to 1). Theoretically, the chance for a normal male mating with a normal female is 1 in 15 in the P generation, 1 in 6.7 in the F_1 generation, and 1 in 4.9 in the F_2 generation.

This test is actually measuring:

1. Whether P generation treated males and F_1 generation afflicted males and females are equal mating competitors to normal adults;

2. Whether development time of F_1 and F_2 afflicted individuals is synchronized with F_1 and F_2 normals so that F_1 afflicted adults are present at the same time the F_1 normals mate;

3. Whether the favorable ratio of afflicted to normal individuals in the F_1 mating occurrences is of sufficient advantage to be superior to a hypothetical release of 14 fully sterile males to each normal male in the population; and

4. Whether the mating between two afflicted individuals will result in viable offspring.

Insectary:

A room with 100 square feet of floor space is used. It is cooled by an air conditioner and heated by an electric space heater. Temperature can be maintained at 27 ± 2°C. and 50 ± 5 percent relative humidity. Larvae are being grown in the dark in this room in one-ounce jelly cups. The diet, a modification of the Shorey Bean Diet, is given in the text of the accompanying report.

Rearing:

Before emergence pupae are placed in open 4-oz plastic cups in a cardboard ice-cream carton. This 3-gallon carton is 9 1/2-inches in diameter and 10-inches high and is lined with wax paper folded into an accordian pleated ring. The creases in the paper provide good sites for egg laying. Adults emerge from the pupal case in the afternoon and normally mating and egg laying take place shortly after emergence.

Egg clusters are placed in sunlight to speed embryonic development. Eggs laid on the wax paper in the carton can be clipped off and placed in the plastic cups. The larvae hatched in the cups are harvested daily and placed in the food as described. They are transferred to clean food as necessary. As they develop the pupae are removed from the food and stored for future use at 3.3°C. or are used to continue the colony.

Adults used in tests are sexed as pupae, and are collected daily. In this manner they can be irradiated or packaged for release in the cage. The sub-sterilized adults are irradiated on the day that they are to be released. All of the adults to be released in a given cage are maintained separately as virgins until release. Releases are at dusk to avoid predation by lizards.

Cages:

Eight cages are available, each is 40 feet long and 40 feet wide (approximately 4 percent of an acre), and approximately 10 feet from floor to ceiling. This is a structure 80 feet wide and 160 feet long, two rows of four cages. The supporting framework is 2-inch diameter galvanized steel pipe bolted together, with uprights imbedded in concrete. The uprights are ten feet apart. The top is covered with natural colored Saran® shade fabric with 5/16-inch openings. The top has a 6 percent shade factor. Cage sides are covered with green Saran® shade fabric with 40 openings per square inch and a 37 percent shade factor.

Irrigation water is available by 2-inch pipe near the cages, and can be applied by hose, sprinkler or watering can.

Host plant:

Ibidan A or B is used as the host plant. It is a fast-growing succulent variety, and is well-adapted to the growing conditions in the cage. It is not highly resistant to rusts nor to aphids. It responds well to chemical fertilizer and to moderate irrigation, and it reaches moderate size upon maturity in the cage. This is an important factor since the cage provides a shaded growing condition. The corn plants are chlorotic and tend to develop tall slender stems.

Corn plants are planted in plastic nursery pails (12-inches diameter, 10-inches deep) that are used for cultivating young plants, with 2 to 3 plants per pail. Plants are grown in the cages to protect them against infestation. The cage floor is covered with strips of black mulch plastic. The floor covering serves two purposes: to control weeds and ants and to provide a contrasting surface from which to collect the adults after they have died.

Soil in the pails is mixed and fertilized in batches. Normally the plants are watered by hose.

Corn is the preferred host plant for the sugarcane borer. Corn has a higher incidence of selection for oviposition by gravid females, better feeding, higher survival and faster development time than cane or other plants (Quintana and Walker, 1968 a, b, and c). The soil is prepared and corn seeds planted 25 days before the first day of release. The corn is planted in cycles beginning with the first generation of insects. Fortunately the sugarcane borer tunnels into the stem and pupates there; it does not migrate from the plant in which it has tunneled. Separate cycles of corn plantings can be made. Approximately 20 days after the beginning of the generation time (release date of the insects) a sample can be taken from that cycle of corn plants, or all the corn plants can be harvested and all the larvae can be recovered from the stalks. These larvae are counted and then maintained on corn stalk pieces in cups held in the cage. This is comparable to development in growing stalks. The corn planting cycles are:

First:	25 days before release
Second:	6 days after release
Third:	36 days after release
Fourth:	66 days after release

Sufficient planted pails are started to have 200 pails with at least one plant in each. Plantings are in the cages described using two cages.

In the cage tests of control groups in which only normal adults are released we expect approximately 5-fold increases each generation, although this has been quite variable. Population change in previous control cage tests were:

Table II

Population Increase in Cages Where Normal Adults Were Released

Adults	Number of F_1 larvae harvested	Reproductive rate (F_1/P)
15 pairs	34	1.1 (34/30)
30	254	*4.23 (254/60)
30	226	*3.77 (226/60)
30	340	*5.67 (340/60)
30	41	0.68 (41/60)
30	17	0.28 (17/60)

* Means of these 3 tests is 4.55 fold increase.

These samples are too small to have a high reliability. However, if we assume a 5-fold increase each generation the population model for control groups should be as shown in Table III. Since the increase is geometric and the cage size limited, it is obvious that the number of host plants that can be grown in each cage is inadequate for the population by the beginning of the third generation.

Sequence of Cage Activities:

Two cages are needed for each test. One cage is a control cage with only normal insects and the second is the test cage with normal and irradiated insects.

With eight cages available, four test replicates are being conducted simultaneously.

Control Cages:

In the control cage 15 pairs of normal adults are released at dusk into the cage containing 150 plants. We expect that the population in this cage will increase to the limit of its food supply in one generation. Therefore, we have limited the population by removing enough larvae in each generation so that the number of adults emerging actually remains constant at approximately 15 pairs each generation. Sampling involves removing 15 plants (10%) 20 days after the release date, cutting the stems lengthwise in order to remove and count the larvae. In order to maintain a stable population of 15 mating pairs in each generation we expect to have to remove and replace 80% of the plants, or 120 of the 150 plants. The number of plants actually removed is based on the number of larvae that we obtain in the sample. Most of the normal larvae die in the first larval stage; we estimate that 50 percent of the L_3 stage larvae

survive to become adults.

The following assumptions are the basis for this population model:

1. All of the females will mate. This assumption is based on field collections from light traps made by Rafael Perez in Fortuna, P. R. He collected nearly 400 adult females from light traps, and found the average mating per female was 1.2 times, and approximately 97 percent had mated.

2. Each mated female will lay 300 fertile eggs. There is considerable variation in egg production among females, however, the average number in a large sample is consistently 300 to 350. The variation occurs in normal as well as irradiated populations and there is no evidence to indicate that the afflicted lines will lay smaller numbers of fertile eggs than non-afflicted lines.

3. Fertile females will choose plants for ovipositing in a random fashion and there will be 5 to 10 egg clusters from each female. We are providing 10 plants for each gravid female.

4. We expect that 95 percent or more of the fertile eggs from normal lines will hatch and that the survival from fertile eggs to adult will be from 1 to 2 percent resulting in a net population increase of 5-fold each generation.

Therefore, the limiting factor in the normal population cage is the amount of host plant material available.

Ten plants per female are provided, and if the population stress in relation to host plant is kept constant, then 80 percent of the plants will have to be removed in each generation.

Test Cages:

Release of 210 irradiated males, 15 normal males and 15 normal females into the cage containing 150 corn plants is at dusk similar to the normal test. Although the normal and overflooding tests were not begun on the same evening they were started at two or three day intervals with one another so that both tests are under the same weather conditions. We wish to avoid the possibility of interaction between males and females in different cages. This is the main reason for beginning the two tests on different days. It is possible that female pheromone from one cage might influence mating in another cage and we wish to avoid this. This is more important in the overflooded cages.

In both cages release in the late afternoon helps prevent predation by lizards. The lizards sleep during the night. It allows immediate mating on the night released. Courtship behavior begins as early as 7:00 p. m. and mating and egg laying begin during the first night of release.

New corn plants were started in a separate cage 10 days after the release date so that they are ready for the F_1 adults and F_2 generation eggs. Twenty days after release all the leaves of the infested corn were removed by cutting them at the base. New corn plants are placed between the old plants so that there are plenty of oviposition sites for the F_1 adults.

Planting sequence and sampling schedule are shown in Table IV and Figure 1.

At the time of removal of leaves from the corn in each cycle, the plants are sampled. Fifteen plants, 10% of the sample, are removed, carefully cut lengthwise and examined for larval tunnels, and larvae are counted. After the adults have emerged and laid their eggs on the new corn plants, the old stalks are removed and larval tunnels counted, as previously described. This sequence can be continued as long as the larvae continue.

Data Collection:

It is necessary to estimate the population in each generation in order to test the hypothesis. Estimating or counting the number of eggs laid is difficult with the number of plants used because of the small size of the clusters and the difficulty of seeing them on the leaves. However, egg clusters are counted in a portion of the plants. Estimates of larval populations are made by cutting the corn stalks as described. This gives an estimate of the larvae of third stage and older. Counting larval tunnels is the most accurate method for assaying population size in this experiment.

Adults can be observed at night (using red light) provided the counts are made at a time when the moths are active, i. e., during mating flight. Population size estimates for each generation are made as follows:

1. Egg counts are made on 10 percent or more of the plants.

2. Larval population is sampled 20 days after adult emergence. This allows sufficient time for adult emergence and oviposition, egg hatching, and larval development in the tunnels in the stalks. It does not give us an accurate estimate of the mortality that occurred in the embryonic stages nor in the first 3 larval stages.

3. Direct counts of adults during mating hours are made on the night of release and again forty days after release. In the second instance F_1 adults are counted. Dead adults are collected from the floor of the cage each morning. Dead females are dissected to determine the number of times each mated.

Table I

Theoretical Model of a Population Overflooded with Treated Males

Gen.	Adults in Population	Type of Mating	Ratio S: N: Total	Per Cent	Number of Mating	Rate of Increase	Number of Adults Expected in Next Generation		
	$M_N:M_S x F_N:F_S$						M	F	Total
P	15:210 15:0	$M_N x F_N$	1:15	6.7	1	10	5	5	10
		$M_S x F_N$	14:15	93.3	14	4.07	28.5	28.5	57
		$M_N x F_S$	0						0
		$M_S x F_S$	0						0
F_1	5:28.5x5:28.5 (33.5)	$M_N x F_N$	$\frac{5}{33.5} \times \frac{5}{33.5}$	2.2	0.75	10	3.75	3.75	7.5
		$M_S x F_N$	$\frac{28.5}{33.5} \times \frac{5}{33.5}$	12.7	4.26	3.4			
		$M_N x F_S$	$\frac{5}{33.5} \times \frac{28.5}{33.5}$	12.7	4.26	3.4	14.5	14.5	29.0
		$M_S x F_S$	$\frac{28.5}{33.5} \times \frac{28.5}{33.5}$	72.4	9.073	0	0	0	0
F_2	3.75:14.5x3.75:14.5	$M_N x F_N$	$\frac{3.75}{18.25} \times \frac{3.75}{18.25}$	4.2	0.77	10 Est.	3.8	3.8	7.7
		$M_S x F_N$	$\frac{14.5}{18.25} \times \frac{3.75}{18.25}$	16.3	3.0	4.5 Est.			
		$M_N x F_S$	$\frac{3.75}{18.25} \times \frac{14.5}{18.25}$	16.3	3.0	4.5	13.5	13.5	27.0
		$M_S x F_S$	$\frac{14.5}{18.25} \times \frac{14.5}{18.25}$	63.2	11.53	0	0	0	0

Table I (Cont.)

Gen.	Adults in Population	Type of Mating	Ratio S: N: Total	Per Cent	Number of Mating	Rate of Increase	Number of Adults Expected in Next Generation		
	$M_N:M_S \times F_N:F_S$						M	F	Total
F_3	3.8:13.5x3.8:13 (17.3)	$M_N \times F_N$	$\frac{3.8}{17.3} \times \frac{3.8}{17.3}$	4.8	0.83	10	4.15	4.15	8.3
		$M_S \times F_N$	$\frac{13.5}{17.3} \times \frac{3.8}{17.3}$	17.14	3.0	3.7			
		$M_N \times F_S$	$\frac{3.8}{17.3} \times \frac{13.5}{17.3}$	17.14	3.0	3.7	11.1	11.1	22.2
		$M_S \times F_S$	$\frac{13.5}{17.3} \times \frac{13.5}{17.3}$	60.9	10.5	0	0	0	0
F_4	4.2:11.1x42:11.1 (15.3)	$M_N \times F_N$	$\frac{4.2}{15.3} \times \frac{4.2}{15.3}$	7.55	1.15	10	5.7	5.7	11.5
		$M_S \times F_N$	$\frac{11.1}{15.3} \times \frac{4.2}{15.3}$	19.9	3.06	3.8			
		$M_S \times F_S$	$\frac{11.1}{15.3} \times \frac{11.1}{15.3}$	52.65	8.0	0	0	0	0

Table III

Theoretical Model of Normal Population Growth

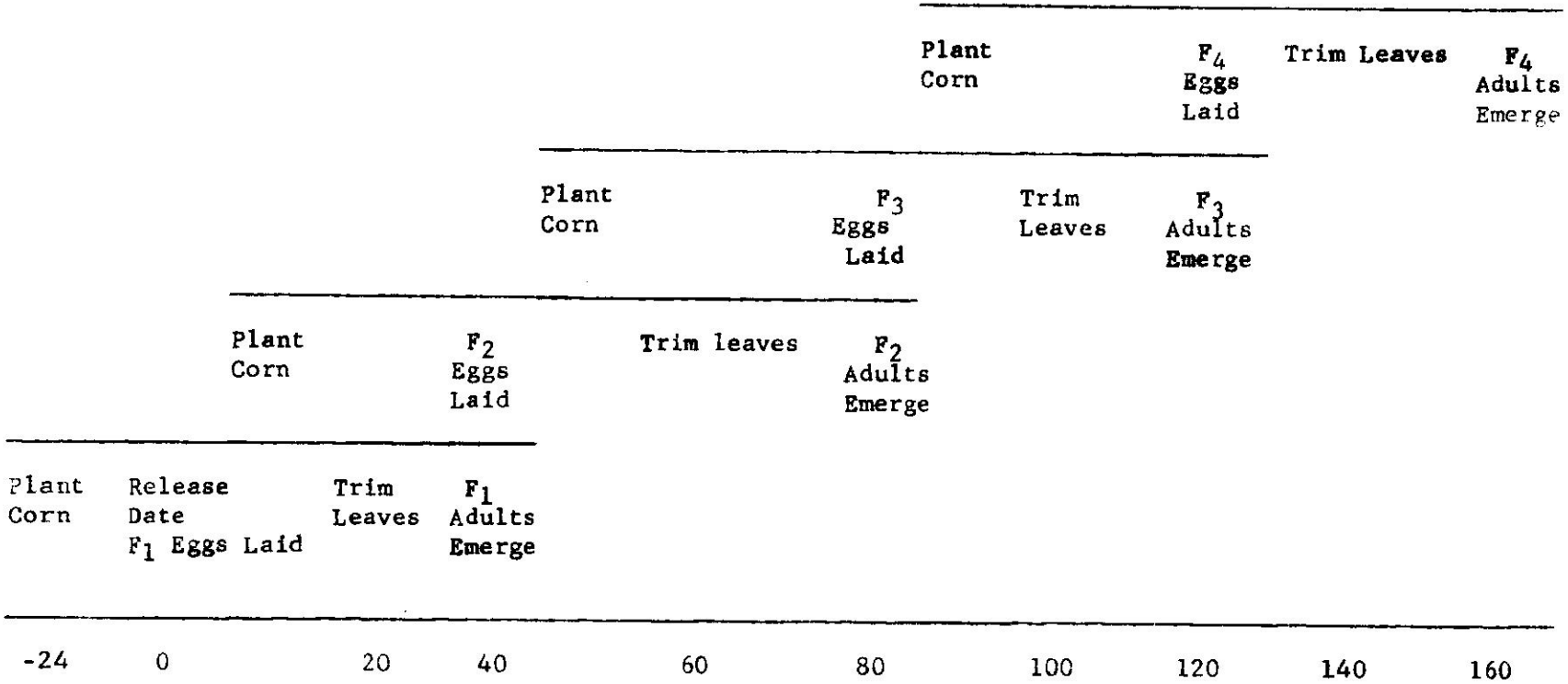
Generation	Adults in Population M	Adults in Population F	Reproductive Rate	Adults Produced M	Adults Produced F	Total
P	15	15	5	75	75	150
F_1	75	75	5	375	375	750
F_2	375	375	5	1875	1875	3750
F_3	1875	1875	5	9375	9375	18750
F_4	9375	9375	5	46875	46875	93750

Table IV

Activity Schedule

Day	Operation
-25	Plant first corn cycle, 250 to 300 plants
0-5	Release insects into cages
6-10	Count adults during mating period, collect dead adults
6-10	Egg counts
10	Plant second cycle of corn plants, count eggs
20	Cut leaves from first cycle of corn plants
	Sample plants to estimate larval population
35	Install 150 second cycle plants in cage
35-40	Count adults during mating period, collect dead adults
40-44	Egg counts
45	Remove first cycle plants and make tunnel counts from each stalk
55	Plant third cycle corn plants
60	Cut leaves from second cycle plants, sample plants to estimate larval population
75	Install third cycle plants in cage
75-80	Count adults during mating period, collect dead adults
80-84	Egg counts
85	Remove second cycle plants and make tunnel counts from each stalk
90	Plant fourth cycle corn
100	Remove leaves from third cycle plants, sample plants to estimate larval population
115	Install fourth cycle plants in cage
115-120	Count adults during mating period, collect dead adults
120-124	Count eggs
125	Remove third cycle plants and make tunnel counts from each stalk
140	Remove leaves from fourth cycle plants, sample plants to estimate larval population, and if larval population is low harvest all plants and make complete tunnel counts

Figure I



WORK SCHEDULE