PRNC 100

PUERTO RICO NUCLEAR CENTER

RADIATION-INDUCED STERILITY FOR POPULATION CONTROL OF THE SUGARCANE BORER (DIATRAEA SACCHARALIS) IN PUERTO RICO

Summary Progress Report

1964 - 1967

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

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I- INTRODUCTION

A. Original Objectives

1. to determine if the sugarcane borer, Diatraea saccharalis (Fab.), can be rendered sterile by subjecting it to gamma irradiation, and

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

B. The Problem in Relation to the Economy

The sugarcane borer Diatraea saccharalis is tropical and subtropical in distribution and is known to infest crops from northern Louisiana to Peru and Brazil. It is of economic importance to the production of sugarcane, corn, milo, rice, lemongrasses, pasture and forage grasses. Principal crops affected in Latin America are sugarcane, lemongrass, citronella, and corn. Serious losses of sugar production have been reported in the United States, Mexico, Guatemala, El Salvador, Nicaragua, Costa Rica, Panama, Columbia, Puerto Rico and the rest of the West Indies. Heavy infestations have been reported in commercial plantings of lemongrass and citronella grass in Guatemala. World annual loss is difficult to estimate. Martorell (1965) reports an annual loss of three million dollars to the Puerto Rican sugar industry and Charpentier, et al (1965) cite six million dollars in United States cane production. The infestation rate is two or three times higher in the continental United States than in Puerto Rico (Charpentier, et al, 1965).

Damage to young plants of less than two months age is more severe than in older cane. Infestation in young plants is called "dead heart" because the terminal bud is killed by the feeding larva. Usually these young plants are killed and when there is widespread damage to young plants the whole field must be replanted. Older plants are more resistant to attack and usually outgrow damage. Yield is greatly reduced by the borer through stunted growth, reduction in sucrose content, sugar inversion, and increased susceptability to plant diseases and other insect pests.

In Puerto Rico cane mortality is high when the young succulent plants are subjected to heavy attack by cane borers and aphids at the same time.

Under natural conditions, the sugarcane borer lays its eggs on the leaf of the sugarcane plant. After hatching and initial feeding on the leaf during the first and second stages, the third stage larva tunnels into the plant stem inside of the leaf axil, where it is protected from virtually all types of conventional insecticidal

applications that are of practical use under commercial growing conditions. Pupation takes place in this tunnel within the stem. During the early summer months when sunlight, temperature, and rainfall are optimum for rapid cane growth the larval period can be completed in twenty-five days. However average larval growth period is 35 days and during the less favorable times of the year the larval period is more than 45 days.

When diapause occurs it takes place as fifth or sixth stage larvae and lasts two or three months. In Puerto Rico larvae begin diapause in December. It is believed that the onset of diapause is controlled by hormonal factors that are controlled by exposure to short-day length in the preceding generation. If this is so, the regulating mechanism must be in very delicate balance because the change in day length in Puerto Rico is less than one hour from summer to winter. Adults and larvae are most abundant during the summer, since population increase is dependent upon the rapid cane growth caused by high rainfall and high temperature. Thus, optimum conditions for larval growth and reproduction occur in Puerto Rico during April, May and June. After August the cane plant is not as suitable for supporting larvae because of its maturation.

The borer's long larval life-span and short adult life-span are the main biological obstacles inherent in using the mass-release method for population control of this species.

In order of their effectiveness natural control agents are: ants (egg predator); Trichogramma minutum (Hymenoptera, Chalcididae); (egg predator); Lixophaga diatraea (Diptera, Larvavoridae); microorganisms such as Cordyceps spp. (attack larvae and pupae); and other pathogenic fungi and bacteria. The most effective of these natural control agents kill the larvae before they have had a chance to damage the plant. Natural control by predators and parasites may reach 70% during dry periods although this varies considerably throughout the year.

The practice of burning standing cane before harvest and of burning cane trash after harvest has helped reduce the level of adult population. Probably re-infestation takes place from alternate hosts each year under these cultural practices.

C. Relation of Other Work to PRNC Program

The USDA and other Agencies have several research and operational programs devoted to eradication of insects by the sterile male release technique. These include the following insects under investigation:

Diptera

Screw-worm fly (operational phase)
Melon fly
Mexican fruit fly
Oriental fruit fly (operational phase)
Mediterranean fruit fly (advanced field testing phase)
Olive fruit fly

Lepidoptera

Gypsy moth
Codling moth
Pink bollworm
Corn earworm
European Cornborer
Oriental fruit moth

This list includes projects in which sterility is radio-induced or chemically-induced.

The Diptera projects are in a more advanced state than the Lepidoptera projects. In general the plant-feeding Diptera are much more easily mass-produced under artificial conditions than Lepidoptera. This is due to their simpler dietary requirements, higher resistance to bacteria and fungi, behavior differences and to other factors. In addition the food requirements of plant-feeding Diptera are better known than those of the Lepidoptera. Yet many of our damaging agricultural pests are Lepidoptera.

The discovery and subsequent evaluation of the importance of sex pheromones has added a new potential in control of Lepidoptera. This method is particularly promising for the suppression of those insects that have a short adult life span and that pass through their immature stages in the soil, in plant stems and fruits, or in other places that are inaccessible by conventional chemical control methods; or those insects for which conventional control measures are ineffectual or only partly effective. This approach is new and so little knowledge has been gained from studies of it that it is difficult to accurately predict the future potential of its usefullness. Natural chemical attractants produced by the insect are usually very specific in their activity. Most of the preliminary biological testing has been done with natural substances produced by the insects themselves. During

this phase of development the results have been spectacular. Solution of the problems associated with isolating the chemically-active substance and subsequently evaluating specific activity has been a very painstaking task in the gypsy moth project and in similar programs. Only a few of the substances have been isolated and chemically identified. Among those that are known are the cabbage looper pheromone isolated by Berger (1966). Hensley is attempting isolation and chemical identification of the sex pheromone produced by female D. saccharalis.

Tests with synthetic pheromone have not been as conclusive as was anticipated. This is due to the fact that the synthetically manufactured sustances contain more than the single laevorotatory isomer that serves as sexual releaser in the insect. Trace impurities neutralize or alter the effectiveness of the specific isomer.

Gamma-induced sterility of the codling moth has been studied by Proverbs (1962), spruce budworm by Berryman (1966), the pine shoot moth by Lee (1966), the European Corn Borer by Walker (1966), corn earworm by Garcia (1966), pink bollworm by Ouye, et al (1964) and the navel orange worm by Husseiny and Madsen (1964). All of these projects are oriented to applied insect control. Gamma exposure doses required to sterilize the adults have been found for most of these insects, but the radiation effect on longevity, mating capacity, and behavior has not yet been fully studied in all of them.

II- ACCOMPLISHMENTS

The reader is referred to Technical Reports 1 (1965), 2 (1966), and 3 (1967) for complete reports of this Project. Conclusions may be summarized as follows:

A. Sterility, Adult Longevity, Mating Behavior and Vigor:

Adult males are sterilized by exposure to 30 Kilorads, and females to 25 Kilorads. No apparent somatic damage occurs when adults of either sex are exposed to 50 Kilorads or less (Walker, 1965). Thirty-five Kilorads was chosen for sterilizing adults to be liberated in cage tests. There is no apparent effect on longevity, oviposition rate, fecundity nor mating ability by this treatment. There is indirect evidence that mating capacity is actually increased by radiation (Walker, 1967). We do not anticipate that there will be problems associated with adverse effects due to irradiation in an eradication program with the sugarcane borer.

B. Delayed Lethal Effect:

Survival and egg hatchability have been measured in the offspring of adults subjected to exposure to sub-sterilizing levels of radiation by Walker (1967). Husseiny and Madsen (1964) report a similar effect in the navel orange worm moth, Paramyelois transitella, and North (1966) cites similar response with the cabbage looper moth, Trichoplusia ni. At exposures as low as 4 Kr. there is a striking reduction in hatchability of the F_2 offspring from irradiated borer parents. This effect occurs regardless of whether the male or female parent (in the P generation) was treated. We believe that the delayed lethal effect is due to the character of the diffuse kinetechore (centromere) in Lepidoptera chromosomes. In the preliminary series of tests in which forty-nine matings were made the net hatch was 31 percent of the eggs produced by the P generation and 26 percent of the eggs produced by the F_1 generation.

At the present time the results of a second series of tests are being evaluated. The following matings were made in the parental (P) generation in this series:

Mating combinations of P generation Adults

Series	Male	Female
A	R (irradiated)	N (normal)
B	N	R
C	R	R
D	N	N

Exposures were made at 1, 2 and 4 Kr. in this second series. The survival rate of F_1 larvae as well as rate of growth are being scored. After ecdysis these F_1 adults have been mated to test the effect on egg hatch in the F_2 generation.

The F_1 mating tests were made in April and May, 1967 and the F_2 rate of egg hatch is now being observed.

In these tests the lethal effect was greater on egg hatchability of the F_2 generation than F_1 generation. In F_2 matings we expect the greatest lethal effect in C x C sibling matings, and the least effect in the A or B with normal outbreeding matings. If we have enough time to do so, we will continue the delayed lethal effect into the fourth generation. Our preliminary results indicate that the lethal effect can be observed in larval survival of the F_2 generation.

C. Cage Testing:

Eight cages were built on the College Farm in April, 1966. These were built to test population change under field conditions.

Each cage is 40 ft. wide, 40 ft. long and 10 ft. high. The supports are made of 1 1/2" galvanized steel pipe and are covered with saran screen. The screen has a 37 percent shade factor. Mesh size is 40 openings per square inch. We release sterile and normal adults into these cages and measure the reproductive rate. Reproductive rate is measured by harvesting the corn plants and extracting the F_1 larvae from them.

Results from 14 tests are shown in Table 1. Mean increase in tests where normal males and females were released was approximately six fold. In comparison competition tests yielded less than unity. By comparison the rate of infestation in corn plants outside the cage were nearly double the highest infestation within the cage (18 °/o of stalks infested outside as compared with 11 °/o inside the cage).

D. Host Range:

Two hundred species of grass have been examined for Diatraea larvae utilizing specimens from the living grass museum at the University in Mayaguez subject to heavy borer attack. Larvae were found in twelve species of these grasses as shown in Table 2.

E. Diet Tests:

Our ultimate goal is to develop an inexpensive food that fulfills the following criteria:

1. high rate of survival to adult
2. rapid development through larval stages
3. production of vigorous adults as measured by length of life span, oviposition rate and mating frequency.

Artificial diet studies have gone through three developmental phases since the project began. In the first phase a number of diets made from sugarcane fiber, cane stem, and cane leaf extracts were tested. Some of these contained supplementary ingredients for increased vitamin and protein content. Also tested were numerous modifications of diets that had been successfully used for rearing other species of lepidoptera. Resulting survival was very low and the few survivors that reached adulthood developed very slowly. The second phase began when Dr. Kenneth Hagen of the University of California recommended carrot powder supplement. Fifty formulations of this basic diet were tested. From the best three formulations further modifications were tested to determine if any specific ingredient could be substituted or eliminated. The resulting PRNC diet is the culmination of this second phase. This diet contains brewer's yeast, carrot powder, corn stalk fiber, ascorbic acid, agar, and mold inhibitor.

Table 1

Population Change in Cage Tests

Tests completed[1]	Type of test	Adults Released				Mean Reproductive Rate	Range in Reproductive rate[2]
		Normal male	Sterile male	Normal female	Sterile female		
5	control	30	0	30	0	5.85	0.47 - 11.33
4	male competition	10	20	30	0	0.95	0. - 3.40
2	female competition	30	0	10	20	0.42	0.37 - 0.47
3	male and female competition	10	20	10	20	0.81	0.73 - 1.70

[1]Completed in May, 1967.

[2]Reproductive rate is the number of F_1 larvae harvested in the test divided by the number of females released.

Table 2

Known Host-range of Diatraea saccharalis in Puerto Rico

	Scientific Name	Spanish Common name	English Common name	Use*
1.	Zea mays	maiz	corn	FC
2.	Saccharum officinarum	caña de azucar	sugarcane	FC
3.	Sorghum vulgare	millo	milo	FC
4.	Cymbopogon citratus	limoncillo	lemon grass	FC
5.	Cymbopogon nardus	yerba de limon	citronella	FC
6.	Eleusine indica	pata de gallina	goosegrass	P
7.	Cuchlaera mexicana	teosinite	teosinite	P
8.	Paspalum secans	yerba dulce	sweetgrass	C
9.	Leptochloa filiformis	yerba de hilo	arrowgrass	C
10.	Axonopus scoparius	----------	---------	C
11.	Tripsicum laxum	----------	guatemala grass	P
12.	Oryza sativa	Arroz	rice	FC

*Grasses used as pasture grass (P), field crop (FC), and wild grass (C) of no commercial value.

Plants are listed in the order of susceptibility to attack by Diatraea larvae. Some of these plants are not common commercial varieties here. No doubt there are other hosts among other native grasses not in the collection.

In the third phase we are seeking to improve survival rate and promote faster development. Formulations to which we have added ingredients known to be required by other lepidoptera are being tested. Tests have been made with alpha-tocopherol, wheat germ oil, corn oil, and mineral supplements. None of these have improved our basic diet.

Further diet tests will be based upon the known nutritional requirements of phytophagous lepidoptera. Vanderzant and other USDA investigators are testing essential requirements by working with holitic diets (wholly defined). When it becomes possible to predict the general nutritional requirements of phytophagous lepidoptera, we will be able to select natural food materials that supply large amounts of the specific requirements.

In general, lepidoptera larvae are considerably more fastidious in their food requirements than diptera larvae. Probably the specific nutritional requirements change during growth, since substances required during early larval stages may not be essential later, and vice versa. The narrow host-range of many species of lepidoptera confirms this.

The long larval growth period is a particularly serious problem in artificially rearing this species. The food decomposes during the twenty to thirty day period required for larval growth. Because the larvae tunnel into the food, extracting them from the food is difficult and time-consuming. It is necessary to extract them when the food decomposes and they must be transferred to fresh food. There are two possible solutions to this problem:

(1) to develop a diet that is nutritionally superior so that larvae complete development before the food decomposes,

(2) to select a fast-growing genetic strain of Diatraea for the laboratory culture.

We have been studying both of these approaches. Relative to the first, larval life span during winter has been shortened from 45 days in nature to 30 days or less in the laboratory as a result of improved diet. Even under best conditions the fastest developing larvae required 17 days. In relation to the second possibility, tests have been made with 197 lines with short larval life span. Apparently larval life span is a function of many genetic factors. Even within the best pure strain there was a great deal of variation in development rate among offspring. Lines were also selected for longer adult life span. Variation among these lines was less than in the larval experiment. It will be difficult to develop a laboratory strain that has a short and uniform growth-rate and is long-lived in the adult stage.

F. Rearing Method and Handling Techniques

The rearing and handling techniques presently utilized to maintain our colony of approximately 10,000 larvae are the result of testing several factors, as these bear on feeding response. Larval feeding behavior suggests that the stimulant to taste food (phagostimulant) is an olfactory response that develops after larvae mature, whereas taste stimulates actual feeding. Immediately after hatching young larvae wander about over the food surface. Later they feed vigorously after first sampling the food. Conditions of food content, light intensity, temperature, humidity and carbon dioxide level have been studied, to provide relatively satisfactory larval feeding response.

Table 3 summarizes the life span of Diatraea saccharalis in the laboratory on PRNC diet. In artificial culture more larvae die in the first stage before feeding than at any other time during their life. During the period of early larval growth (first to third larval stadia) a large surface area with rough texture is needed to promote vigorous feeding. At the third stage when the larvae form tunnels in the food they need a large volume of food. Overcrowding causes accidental cannibalism if there is insufficient food mass for tunnel making.

We have tested the PRNC diet in containers of different size and shape to obtain the optimum relation of surface area and volume. Glass shell-vials (32 ml.) with cotton stoppers have produced the best yield. Rearing in trays would be easier since they can be filled and cleaned easily. Mortality has been high in tray tests because of dessication. Dr. Frank Howell (1965) suggested pouring the food in trays and spraying the surface with paraffin wax to prevent drying. This method is successful for laboratory rearing the codling moth. Survial was low in tests with Diatraea larvae in paraffin-coated food.

Table 3

Synopsis of life-span of D. saccharalis in the laboratory[1]

Stage	Lifespan of Stadium[2] (days)	Length mm.	Activity at this Stage	Main Cause of Death
Egg	4 to 9 (5)	0.6 to 0.8	embryonic development	mold
First Larval	4 to 18 (5)	2 to 4	surface feeding	dessication and starvation
Second Larval	3 to 18 (6)	3 to 7	tunnel forming	bacteria
Third Larval	3 to 18 (7)	6 to 18	tunnel forming	mold and bacteria
Fourth Larval	5 to 21 (11)	15 to 29	tunnel forming, stops feeding before pupating	mold and bacteria starvation
Pupal	5 to 10 (6)	18 to 27	quiescent in tunnel	accidental cannibalism by tunneling larval, dessication, bacteria
Adult Male	1 to 11 (3.5)	15 to 25	rests during day, nuptial flight and mating at night	old age, dessication mold, starvation
Adult Female	1 to 13 (5.2)	18 to 29	same as males oviposits during day and night	old age, dessication, mold, starvation
Total	25 to 107 (45)	2 to 29	----------	----------

[1] from Walker, 1966

[2] Range, the average is shown in parenthesis.

III- PUBLICATIONS

A. The following have been published:

1964. Bionomics of the Sugarcane Borer Diatraea saccharalis (Fab.). III. Oviposition Rate, Jour. Econ. Ent. 57 (4): 515-6.

1965. Bionomics of the Sugarcane Diatraea saccharalis (Fab.) I. A Description of the Mating Behavior, Proc. Entom. Soc. Wash. 67 (2): 80-3

1965. Bionomics of the Sugarcane Borer Diatraea saccharalis (Fab.) II. Longevity of Adults, Proc. XII Intl. Cong. Sugar Technologists: 3pp. (in press)

1966. Improved Xenic diets for the Sugarcane Borer in Puerto Rico, Jour. Econ. Ent. 59 (1): 1-4.

1966. Potential for control of the Sugarcane Borer Through Radio-induced sterility, IAEA/FAO Symposium on Radiation, Radioisotopes and Rearing Methods in the Control of Insect Pests, Oct. 17-21, 1966, Tel Aviv, Israel, in press.

B. The following are in preparation:

1. Gamma-induced sterility in D. saccharalis.

2. Mortality-staging in eggs of D. saccharalis.

C. Lectures have been given in Central America in cooperation with the Atoms for Peace Program. Lectures were given at the National Universities and Graduate Instructional Centers in Guatemala, El Salvador, Nicaragua and Costa Rica. In addition S. Garcia-Rivera has been trained to study the potential for controlling the corn earworm, Heliothis zea in El Salvador and Juan Rodriguez in Nicaragua has been given similar training to evaluate the potential with Laphygma frugiperda, the "cogollero del maiz". Both of these pests cause serious damage to crops in Central America. Training was also given for study of the coffee leaf miner, and the pests affecting lemon grass in Guatemala and El Salvador.

IV- SUMMARY

The ultimate objective of the project has been to determine if the sugarcane borer could be eradicated by the release of sterile adults. Implicit in this objective are a number of related aspects:

1. to determine the stage and point within the stage to be sterilized,

2. to determine if the life-span, fecundity, mating capability and general vigor is affected by sterilizing,

3. to develop a laboratory method for mass-producing this insect capable of producing large numbers of individuals at a low cost,

4. to study the life cycle in the laboratory and under field conditions with particular reference to growth rate, reproductive potential, mating behavior, and other aspects of the bionomics and ecology relevant to a successful overflooding program.

The first objective has been completed. Only the adult stage can be sterilized without producing excessive somatic damage and subsequent high mortality. Larval and pupal stages can be sterilized at much lower exposures. The dose/sterility effect relationship appears to be roughly exponential, but there is considerable variation in individual tests at sub-sterilizing exposures.

Life span and oviposition rate is not affected by sterilizing the adults of either sex. Mating capability of sterile adults is equal or slightly superior to normal adults. This is true whether the individuals have been reared on the host-plant or from artificial diet. We have succeeded in rearing this species in the laboratory in small numbers at high labor cost.

The artificial diet developed is well-adapted to rearing the insect if the insect is continually provided with fresh food. Since 17 to 30 days are passed as larval stages it is not possible to maintain the food free of bacterial and mold growth. If larvae are transferred to fresh food before contamination becomes too heavy survival is improved. The high labor investment of manually removing larvae from contaminated to fresh food is a problem given considerable attention but it is not yet solved.

The second major problem in mass-rearing is to develop a low cost diet. The ingredients for our present diet cost approximately two dollars per thousand larvae. This is about one hundred fold greater than the cost of producing phytophagous diptera and about three hundred times the cost of mass-producing the screw worm. High food-cost and contamination problems are inherent in rearing lepidoptera under artificial conditions.

We have obtained considerable data on growth rate in laboratory rearing but there have not been extensive field studies on this insect in Puerto Rico. We sorely need data on the population of adults in the field, and the variation of adult populations throughout the year. Unfortunately we have not been able to study this because of lack of personnel. Some indirect inferences on the net natural reproductive rate can be made from our field tests in cages, but it would be risky to extrapolate this data to field conditions of cane production. It is expected that the study of Hensley (1966) on the sex pheromone will provide us with greatly improved methods for trapping adult males and that this method will enable us to make accurate estimates of field population. Hensley's group expects to be able to make a chemical analysis of the pheromone in the near future.

The potential for eradicating this species through a combined program of trapping using synthetic pheromone in combination with the release of sterile adults apperars to be promising.

Limitations of this program are: 1) lack of a good rearing method, and 2) incomplete knowledge of the biology of this species under field conditions. Since we were not responsible for these under the 1964 Memorandum of Understanding we did not direct our attention to these problems. Unfortunately the cooperating agencies were unable to solve these problems before terminating their programs.

The PRNC commitment proposed in project objectives under the Memorandum of Understanding between the USDA Entomology Research Division, UPR Agricultural Experiment Station, and PRNC will be completed at the end of Fiscal Year 1967. Further field work and a mass-rearing method will have to be developed before the program will be ready for large scale field testing. A logical sequence of necessary stages is shown in Table 4 of the Appendix.

V- RECOMMENDATIONS

If the work is to be continued with the objective of population suppression or eradication by release of sterile adults I recommend concentrating on these objectives:

1. Field studies, to determine:

    a. fluctuation of the adult population in nature,
    b. host range in Puerto Rico,
    c. rate of population suppression by sterile releases in nature,
    d. effect of multiple releases on subsequent generations.

2. Laboratory studies, with the aims:

    a. to develop an improved diet for mass-rearing,
    b. to develop rearing and handling techniques for mass-rearing,
    c. to study the lethal effect over several successive generations, and,
    d. to select an improved genetic strain for laboratory rearing.

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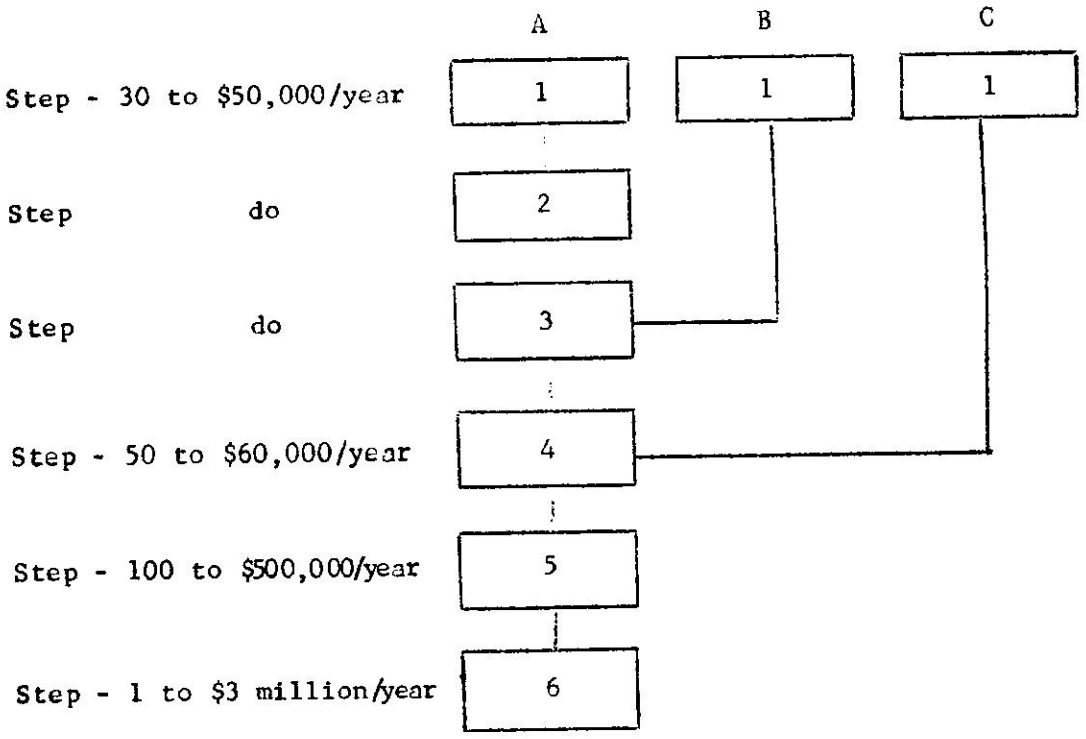
_____ 1966. T. R. No. 2: Radiation-induced Sterility for Population Control of the Sugarcane Borer in Puerto Rico. P. R. Nuclear Center, Mayaguez: 41 pp.

_____ 1967. T. R. No. 3 (in press)

VII- APPENDIX

TABLE 4

Development Stages in a Species Eradication Program*



Series A: for species that have not been raised under artificial conditions and species for which the biology is poorly known.

Series B: for species that can be grown in the laboratory and for which the biology is fairly well-known.

Series C: for species that can be mass-reared in the laboratory and for which the biology is well-known.

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*This was prepared by John Munro and David Walker at the request of the participants of the Symposium on Insect Sterility and Mass-rearing Methods in Tel Aviv, 1967. It will be included as part of the published proceedings of this meeting.

Step 1. Using field-collected material should test the following against all stages of life cycle, and should include differences in response within stage if possible:

a. radiation dose response of:

1) lethality and sterility
2) life-span
3) oviposition rate
4) mating behavior

b. normal performance for the above as a basis for comparison.

Step 2. Laboratory Rearing

The principal objective of this phase of the program is to develop a reliable method of producing the insect in the laboratory under artificial conditions. One should strive to develop a method that produces predictable results, not necessarily a mass-rearing method. Cost of production should not be an important criterion, and it is recommended that natural (host plant) food substances be tested.

a. Diet:

Starting with the natural food material, prepare diets using added food substances to augment nutritional level. Diet tests should be performed on the neonatant larvae, and all immature stages should be tested because specific nutritional requirements are apt to change during the course of the development.

Criteria to be measured should include: length of time required for each stage (i.e. time between molts), rate of size increase (length and diameter), amount of food consumed, pupal weights, longevity of adult life span and of the pupal stage, observation of morphological and behavior aberrations in adults (crumpled wings, active movement, mating behavior, etc.), and survival rate over several generations of laboratory rearing.

b. Develop handling techniques, and test containers in preparation for larger production. With the techniques and diet developed at this stage, one should strive for results that will give the assurance of a predictable survival. The survival rate obtained at this phase may not be as high as needed for mass-production.

c. If possible a laboratory strain of the species should be selected for the qualities desired. These qualities might include

short larval life-span, long adult life-span, high reproductive rate, high rate of survival and vigorous adults having high mating capability.

d. In addition, if a distinct phenotype, i.e. one that can be easily recognized in the field by casual observation, can be selected, it would greatly help in later field work.

Step 3. Bionomics and Ecology in nature

Diurnal behavior, including daily movement, host-range and host-preference, time of day and frequency of mating in nature, natural abundance, seasonal population fluctuation, and other pertinent aspects of the ecology should be emphasized at this stage of the program.

It is of no use to be able to produce sterile adults if these adults are not suitable for their intended use. The sterile adults released should be equal to or superior to adults in nature in respect to longevity and mating capacity. High reproductive rate in the laboratory strain is desirable as it facilitates laboratory rearing.

Step 4. Small Scale Field test

Test the mating ability, behavior and longevity of sterile adults under natural conditions in contained field environment. The test site should be isolated from the greater environment, preferrably it should be a small island or an isolated land mass surrounded by suitable geographic barriers such as mountains.

Size and seasonal change in the natural population should be measured before, during and after this test. Trapping methods, marking techniques and distribution studies can be the main tools for measuring population changes in this phase of the program.

Step 5. Mass-rearing Methods

The diet selected for mass-culture must be cheap, and should be of materials of uniform quality that are readily available. The food selected for mass-rearing should be easy to prepare in large quantity.

Disposal of food after use may be a problem in some programs. Attention should be given to obtaining large numbers of vigorous adults.

Step 6. Eradication Program

Measurement of effectiveness, strigent quarantine measures for the future, suitable release and recapture methods, low-cost, and

related factors are likely to be the most important considerations in this phase of the program.

General Comments

Cost will escalate drastically as the program procedes from phase 4 to 5 and again from phase 5 to 6. Rough estimates of increase are about a five-fold increase from phase 4 to 5, and 100 to 200 fold from phase 5 to 6.

The policy of determining the limiting factors most likely to prevent success should receive the most attention in early phases of the program rather than leaving them till later.