PRNC - 161 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 US ATOMIC ENERGY COMMISSION ---Page Break--- PRNC - 161 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 Sorer, (Draeculacephala minerva) in Puerto Rico) Research supported by the USAEC Report prepared in April 1973 by Division of Biological and Environmental Research under Puerto Rico Nuclear Center, contract No. AT(40-1-1833), Mayaguez, Puerto Rico. OPERATED BY UNIVERSITY OF PUERTO RICO UNDER CONTRACT NO. AT(40-1-1833) FOR US ATOMIC ENERGY COMMISSION ---Page Break--- List of Tables List of Figures .... TABLE OF CONTENTS Introduction Accomplishments A. TPS Cage Tests B. IPS: Analysis of Laboratory Data in Relation to Sex Ratios, Dose Effect and Generation Effect (Nackay) C. IPS An Hemiptera (Restrepo) D. Fractionated Dose Effect with the Greater Wax Moth (Singh) E. Host Plant Resistance (Vakili and Kaiser) F. Relation to Other Work G. Future Work Planned Publications H. Program Personnel References Appendix - Experimental Design for Cage Test 20 10 24 ---Page Break--- LIST OF TABLES F. Population in First Cage Test Frequency of Successful Mating and Survival of Offspring in Outbred Lines, F1 to F2 Percent Egg Hatch Percent Adult Emergence Embryonic Mortality and Egg Hatch in IPS Lines Larval Mortality and Adult Emergence in IPS Lines from a Male Parent Larval Mortality and Adult Emergence in IPS Lines from a Female Parent Wax Moth Preliminary Test: Male Sterility Wax Moth Test 1 Wax Moth: Test 2 TPS in the Stink Bug ---Page Break--- LIST OF FIGURES Dose Effect on Egg Hatch in IPS Lines from Irradiated Male Parent Dose Effect on Egg Hatch in IPS

Lines from Irradiated Female Parent seeeeeeee w Dose Effect on Larval and Pupal Survival in IPS Lines from Irradiated Male Parent the Dose Effect on Larval and Pupal Survival in IPS Lines from Irradiated Female Parent. Generation Effect on Egg Hatch in IPS Lines from Irradiated Male Parent. Generation Effect on Egg Hatch in IPS Lines from Irradiated Female Parent. Generation Effect on Larval and Pupal Survival in IPS Lines from Irradiated Male Parent. Generation Effect on Larval and Pupal Survival in IPS Lines from Irradiated Female Parent. ---Page Break--- Introduction among the developments in insect control the most important. break- through has been the development of the concept of Integrated Control (IC). It was first proposed by B. P. Knipling (1966). IC, a transdisciplinary 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 emphasizes anticipatory rather than corrective measures. Specific insecticides are used in IC under certain conditions, with great care given to the timing of application, the application method, and the amount needed to produce the desired effect. In the crop phase of IC, pests, resistant plants are selected, and attention is focused on the timing 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 post control of IC, entomologists are studying trapping techniques that use juvenile hormones, some are chorister plants, and oviposition lures. Specific parasites, predators, and insect diseases are cultured and released to control pest populations. Quarantines are used to prevent introduction of

peste. Seek populations are also being suppressed by overflooding them with individuals that have genetic defects. Smith and von Borstal (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 Petersen, 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 5, and Walnor, et al., 1972). The hypothesis and the mechanism are 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 lepidopters are not well known and the larval life span is long. We also need to improve methods for producing genetic defects. Overview regarding the use of damage to the chromosomes of precursor plants are discussed in the text of this report. II. Accomplishments As IPS Cage Tests (200 appendix for the experimental design) 1. Cage Phase, control release – The first group of adults was released in

February and the F population was sampled in April. The 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 8 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 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, sites were chosen and one at the FE Station, submitted formal request through AI to USDA; June Permission granted by USDA July Dismantled and moved cage frame August 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 pending Sampled F 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 due to the temperature setting being set too low by accident. Present production is approximately 20 to 30 pupae per day. The diet that we are now using contains: ---Page Break--- canned pinto beans 600 gm brewer's yeast 80 gm ascorbic acid 8 gm Tegosept (methyl-hydroxy-benzoate) 8 gm Linseed oil, raw 32 gm sucrose 100 gm molasses 60 gm corn syrup (Karo) 30 gm wheat germ 30 gm powdered cellulose 5 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: (2) clean area.

for food preparation, maintained 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 aseptic as we are continuing to use the one-ounce jelly cups for rearing larvae instead of the plastic dishes 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 corn stalks. Oviposition rate, adult longevity, mating frequency and mating behavior are 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. ---Page Break--- They found the F1 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 formal individual. Offspring were outbred with normal individuals in single pair matings, keeping the lines separated in the immature stages. Data include offspring in the F1 to Fg generations. Forty-one lines were observed. The dose given to the P generation parent was 1, 2, 4, 10, 12, or 14 krads. The experimental work was done in the laboratory during the years 1967-70. These data are shown in Tables 2-25 and Figures 1-28 in Report 6. 1. Sex ratios. The previous report showed the lineage for 1,072 outbred matings that produced fertile eggs and that were descendants from an irradiated parent in the P generation. In the F1 through Fg 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 590 afflicted males of the F1 to Fg generations were mated to females that produced fertile eggs, and in 58 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 47 afflicted females that produced fertile eggs in the F1 to Fp generations; of these 66 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 26.6 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. ---Page Break--- 2. Dose effect. (a) Table 3 and Figures 1 and 2 show a comparison of egg hatch in the F1 to F2 generations at different doses. There is a negative correlation between increase in dose and egg hatch rate of F1 and F2 embryos (eggs produced by the P and F1 generation females, respectively). Beyond the F1, the correlation is not consistent for

hatch from afflicted lines descending from afflicted P generation females; it was lower than from afflicted P generation males in the F1 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 F1 larvae and pupae is low at 2 krad, 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 the timing of death in relation to dose. There is a correlation between increased dose and earlier death in the F1 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 Guintana, 1966). 3. Generation effect. (a) Table 5 and Figures 5 and 6 also show a comparison of stage of death of embryos beyond the F1 generations. Death occurs at progressively later stages in combination with 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 hatching is lower from descendants of P generation afflicted females. « (2) 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 Pisles and Tomales, respectively; again, the descendants 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 11 krad doses are tabulated. 17 species @ ant 2. Cy IPS in Hemiptera (Restrepo) Virgin adult female stinkbugs Miridula (L.), Pentatomidae were exposed to 1.5, 7.5, or 1540 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 fifth nymphal stage; 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 F2 to F5. We interpret this to have been a recovery due to 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 ---Page Break --- lepidopterans. Gonoz-Nunez in Venezuela and LaChanco with the USDA in North Dakota have studied the IPS effects in other hemipterans. They found that 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 tarlequin lobe of the testes. This may have no bearing on the relationship with the recovery phenomenon 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 nematopteran lines and incomplete recovery in lepidopteran lines. In addition, Virkkt (1963) reported asynapsis in the meiosis of the sugarcane borer males. Asynapsis in meiosis has also been observed in Coceidae (Homoptera) and Cocidomyidae (Diptera). Vink states (p. 119): "Those examples show that the classic pairing of homologues is not the unique method of controlling the reduction division of the chromosomes. There are some factors latent in the prophase cell which are capable of taking care of a correct segregation in the lack of pairing of homologues. In our subject, Diatraea saccharalis, such factors apparently operate in the asynaptic spermatocyte which lead to anaphase grouping 7 + 27 (or nearly 80) 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 Pablus 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 + 1°. The sex of each pupa was determined and each was maintained in a separate four-ounce jelly cup. Upon emerging, the adults were irradiated at 0 to 24 hours of 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 6 ---Page Break--- 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, with 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.

Kosutte Single Exposures

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 nonfertilized 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 males failed to produce viable eggs, indicating that males that had been irradiated with sterilizing doses did not retain 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 females. The mating ability, adult longevity, and sexual attractiveness of the males 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 groups.

Fractionated Exposures: In the second series of tests, single and fractionated doses were compared. Males were more radio-sensitive to fractionated doses than were females (Table 10). Fractionated doses produced similar 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 suggests the sterility of Lepidoptera, for example, testing pops of 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 could 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 include a reduction in mating competitiveness, shorter 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 F1 embryo stage in this case instead of in the F2 embryonic stage, or in the developing larval stage.

pupal stages of the F generation. Fractionated dose technique deserves further attention because of the potential use in lepidopteran control. If the mechanism works for other 89%, it is apparent that we have a powerful tool for manipulating lepidopteran sterility through the production of genetic damage at considerably lower doses. Host plant resistance (Vakili and Kaiser) brs. Vakili and Kaiser at the Federal Experiment Station in Mayaguez are field testing hundreds of varieties of beans (Phaseolus) and cowpeas (Vigna sinensis). The objective of this work is to evaluate 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, Ch. The compounds producing odors will be solvent extracts, homophenates using mineral oil in blotting paper to absorb the volatiles, then extracting this with a solvent and then analyzing by gas chromatography. 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. 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 by the only other IPS cage test (Toba, et al., 1973) for the F2 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 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 F2 and F3 survivors; and 2, that a smaller dose yields a higher proportion of F1, F2 and F3 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 examined 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 altered 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 TY 1998. Further work with fractionated doses to determine if we can produce super-dominant lethality in female sugarcane borers will be explored, the results with the wax moth show considerable promise. Possibly these lepidopteran species could be included in these experiments, ---Page Break --- I feel that the

Host plant resistance project has great potential. The extensive USDA Field programs provide an excellent platform for this research. V. Publications + Gamma (to be induced 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 pests (Vakili and Walker). A strategy for lepidopteran pest eradication (Walker and Pedersen). Program Personnel Dr. Kenneth P. Mackay has worked full-time on the program since September 1972. He has had 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 Bogotá, Colombia, worked officially with the program from 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, including 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 taxonomic revision of a group of homopterans. Dr. Warpal Singh worked from June through mid-September on a grant from the Oak Ridge Associated Universities. He evaluated fractionated dose effect in the greater 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 the sugarcane borer.

larvae at the beginning of summer vacation. w ---Page Break--- VI. References Aimods M.S: He et al. 1972 Inherited sterility in the fig moth, Spodoptera (Gphestia) cautella Walker, 383-9. In Possible uses of ‘tonto Energy, Vol. 12, TABA, Vienna, Anon, 2972. Integrated Pest Management. Council on Environmental Quality, Supt. of Documents, Washington. Car M. le Crores 4 Xorsgnd De 3. North: 1972. Inherited sterility in the F1 progeny of irradiated male pink bollworms. J. Econ. Entomol. 65112715, Graham H. M., Anbertted 652615-50, Al. 1972. Dosages of gamma radiation for fertility in adult pink bollworms, J. Econ. Entomol. Kntpling, E. F. 1966. Some basic principles in insect population suppression. Bull. Entom. Soc. Amer. 1217-25. North D. Tey and G. G. Holt. 1971, Inherited sterility and its use in population suppression of Lepidoptera, pp. 99-11. In Applications of Induced Sterility for Control of Lepidopterous Populations, TASK Symposium, Nov. 1970, Vienna, Austria, Proshold, F. I-, and J. ks Bartell. 1970. Inherited sterility in progeny of irradiated male tobacco budworms: effects on reproduction, developmental time, and sex ratio, J. Econ. Entomol. 63128005, Smith R. S. and R. C. von Borstel, 1972, Genetic control of insect populations. (AAAS) Science 178;1164-76, 1973. Reduction of populations of caged cabbage (Gn press, J. Econ. Entomol. Virkid, Mitlo- 1963, Gametogenesis in the sugarcane borer moth, Diatraea

saccharalis (F.). J. Agric. Univ. of P. Re 47(2):102037, Walker D. Wey and V. Quintanacinize 1968. Mortality staging of dominant lethals induced in the F1 generation of the sugarcane borer, Diatraea saccharalis (F.). Rad. Research 36113843, Walker Dasa and K. B. Pedersen. 1969. Population models for suppression of the sugarcane borer by inherited partial sterility. Ann. Entomol. Soc. Amer. 6212106, Walker D. We 1972. Insect Sterility Program Technical Report 6. P. a. Nuclear Center, Mayaguez, Puerto Rico 73 10, n ---Page Break--- eéep 06 pardave ‘Aaensqed (z suou 1910q auPoz¥Fne aTnpe TwElOU 30 BATE ST

PIHEOTOY a9qany, a9qenn sequence wr ee 30 sagen, 801 9849 as2rd uF wotavindoa Ta Tore ---Page Break--- table 2 Frequency of Successful mating and survival of offspring in Outbred Lines, F1 to Fg number of adult offspring produced Number of matings Percent of matings producing adults in ‘successful in cone in afflicted Lines the following nuance of the Fw Fy generation outbred line Males 598 we ake Females om % we ‘Total yore 234 vonage 21.8 Offspring from irradiated P generation male: Males 369 90 2h 252 a ws total mean WL average data offspring from irradiated P generation female; Males 235 60 2545 Fonalos 225 bs 20.0 Total 460 205 average 22.8 * this tabulation shows Fy through Fy individuals from lines irradiated a8 the male or female adult successively outbred ‘with Fy through Fe Ww. 6 agr)t An the P generation (outbred with ‘normal), and normal of the opposite sex in every instance ‘See Tables 2-25, and Figures 1-26, Technical Report % ---Page Break--- st ator wer atsy 8 wee cree wee ure L tor cor a ter we 96 9 enue ous re oer ser 8°96 s see atte ve ot owe cue 7 a eee ee Ott 696 € see eee re se Tk oe ce z oro rst te ose out ez tly tM STH t wopynxousy mow 9 2 nm m@ 9 2 0 e200 POWWTPRLET sogesouy oTwuog WoReH Jog qoateg € sree PeWwTPRAIT soxeeoWy OTvK ae ---Page Break--- To sot oo ort oo @ a7 se og ate oe L ee 9 en oe ©9 te 9 wy rot er 16 Te os9 s ere est wer ors siz ateg , oo gut atte urot_ zoe ESTEE rtp € eet wee gree we 96 ror eet ety e vote ase gta 87 96 ome re onsg t worse, nm mam 9 2 mo ot 9 2 6 Pew HHRZsT doyssowy oTeuag sousauvig my queaiag 4 orae, 00 bopetemsar soyesouy TH ---Page Break--- 4 : 2 i 1 Tolmer rarar ---Page Break--- ---Page Break--- TTT 1 TT4 ---Page Break--- ---Page Break--- table 6 ax Hoth Preliminary Tests Male Sterility Exposure Fertile tests Fees Percent Kilorads aia Watched Watched ° 800 72 94.0 7.70 766 ost 85.0 11.00 maz 313 80.0 13.75 70 490 70.0 19.25 oor 209 31.0 22.00 750 ° ° 30.9 337 ° ° 40.9 s70 ° ° 50.9 375 ° ° Tale sterility induced by « single exposure. ---Page Break--- Table 9

Wax Moths Tost one? Exposure Sees Percentage Percent ‘eas Produced Hatched Produced Hatched 1st virgin 2nd virgin irradiated female (b) irradiated "tema male (unirradiated) male (unirradiated) 0 se 5 days 39 95 100 85 and 5 days 153 100 10 at 5 days 456 850 0 2nd 5 days 2 20 at 5 days 433 450 0 5 days us 30 at 5 days eas 0 ar 0 2a 5 days 150 0 4 se 5 days 20 125 0 5 days 100 50 ee 5 days 15 0 0 dod 5 ayn m 0 * Male sterility from a single exposure © After 5 days irradiated males were isolated from the first female (2) and mated with the second female (>), ---Page Break--- saved sanoy z F Zz s9s0p Tenby om vy uoAED ssouy2 902y3 poaeoytée2 ‘auvuz9e23 304 sBuy3ea 920m 0 9A;3 Jo aBEsIAV smnjon oq pur oqmmes 04 sommsodxe eTauye PUY peyMUOTIONES Jo eyO0I50 LAFTHINS | 96 ns 089 oo (eteuo3 poseypeszyun!eywa pazeypezztun) T0230) (p) ° ° sus eer o ° 096 ret * zor z19 99 ‘21ea95 porwrpeazy ‘opwu poxerpeaay (9) st 98 us eer oz 6 est ser 6 ate zo ueT 0 eee ‘00 zen oe for esp +9 6 16 oy 09 pyouo3 Yeusou ‘eywe posespezar (4) © ° 109 eer ° o ase ser ° 0 29s rer ° ° one zen 1 o ws +9 ° ° wt +9 21ee Twaxou tereusy poavTpesay (e) paysaey —peyorey paonpoad pexy —payoaey —pagoaey posepoad uaased 832 so 9773293 sansodxa ——aueaz0d e839 ee oTF3I03 302 oTauTs pporeuoyasead — ont asl OH mH ot stare ---Page Break--- sete sere ow x0 8 Ta 991K ¢ x ra Te sopemae ore 96% soreuadyt Woyaonporday uoyieisua Ty ---Page Break--- ---Page Break--- Cage Test of Inherited Partial Sterility in the Sugarcane Borer Experimental Design Hypothesis

being tested: That overflooding natural population with substerile 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 Welker and Pedersen (1969, Ann. Entomol. Soc. Amer. 62:21-6). Explanation of the mechanism: The predictability of the success of this model.

4s due to two factors: An actively high reproductive rate in the P to F generation (4.2) an outbred afflicted lines, and a drastic reduction in the chances of normal to normal mating as F adults and therefore a drastic reduction in the reproductive potential in the F and subsequent generations. The introduction of sub-sterile males provides the mechanism for introducing a biological insult into a large proportion of the mated population, as shown in the overtly loading advantage in the P generation. The ratio in the next (PS generation) is expected to be 29 to 745 compared to 1. Theoretically, the chance for a male to elicit mating is expected to be significant. This test is actually measuring: 1. Whether P generation treated males and F1 generation afflicted males are equal mating competitors to normal species; 2. Whether the development time of F1 and F2 affected individuals is significantly different; 3. Whether the favorable ratio of afflicted to normal individuals in F1 mating occurrences is a sufficient advantage to be superior to a hypothetical release of fully sterile males to replace normal males 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 Storey Bean Diet, is given in the text of the accompanying report. Rearing: Before emergence, pupae are placed in open wide plastic cups in a cardboard tea-crate carton. This 1-gallon carton is 12 inches in diameter and 10 inches high and is lined.

witn wax paper folded into an octagonal pleated ring. The creases in the paper provide good sites for egg laying. Adults emerge from the pupal case in the afternoon and formal 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, pupae are removed from the food and stored for future use at 34°C or are used to continue the colony. Adults used in tests are sexed at pupae and are collected daily. In this manner, they can be irradiated or packaged for release in the cage. The substerilized 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. Gages flight 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 pipes bolted together, with uprights embedded in concrete. The uprights are ten feet apart. The top is covered with natural colored Saran shade fabric with 3/16-inch openings. The top has a 60 percent shade factor. Cage sides are covered with green fabric with 0 openings per square inch and a 37 percent shade factor. Water can be applied by hose, sprinkler, or watering cans. ---Page Break --- Host plant Tibish 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 stalks. Corn plants are planted in plastic nursery pails (12-inches diameter, 40-inches deep) that are used for cultivating young plants; with 2 10 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 pests 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 hoses. 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, 1965 a, by hand). 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 stalks 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 S-(eld increases enol, generation, Strong this has been audited variably. Population curve on mouse control cage tests was ---Page Break--- Table II Population Increase in Cages Where Normal Adults Were Released past some Ty gay 15 pairs 3 Lar (34/30) 30 2H 94.23 (254/60) 30 226 $3.77 (226/60) 30 uO #5467 (340/60) 30 was 0.68 (2/60) 0 1? 0.28 (17/60) * Means of those 3 tests is 4.55 fold More ‘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 (10s) 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 move and replace 60% 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 L2 stage larvae ---Page Break--- survive to become

adults ‘The following assumptions are the basis for this population model: + ALL of the females will mate. This assumption is based on field collections from light traps made by Rafael Pores in

Fortuna, P. 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. 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. 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. 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 several 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 60 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 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 wasps. The lizards sleep during the night. It allows days to date the night released. Courtship behavior begins as early as 200 my and oviposition and egg laying begin during the first night of les. ---Page Break--- Corn plants were started in a separate cage 10 days after the release date so that they are ready for the F1 adults and P1 generation cages. Twenty days after release, all the leaves of the affected corn were removed by cutting them at the bases. New corn plants are placed between the old plants so that there are plenty of oviposition sites for the F1 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 longitudinally, and examined for larval tunneling, and larvae are counted. After the adults have engorged 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. Note: 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, and estimates of larval populations are made by cutting the corn stalks as described. This gives an estimate of the larvae of third instars and older. Counting larval tunnels is the most accurate method for assessing 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 flights. Population size estimates for such generations are made as

To allow 2s counts are made on 10 percent or more of the plants. 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 stakes. It does not give us an accurate estimate of the mortality that occurred in the embryonic stages nor in the first larval stages. Direct counts of adults during mating hours are made on the night of release and again forty days after release. In the second instance, 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 they mated. ---Page Break--- Ser FEE? 32 3 ---Page Break--- ---Page Break--- Table IE Theoretical Model of Normal Population Growth of Adults An Population —reproductive adults Produced Generation —M. F Rate 4 Fo Total 6 os 5 % aso 5 % 5 5 35s 750 % ms ms 3 ers 17s 050 ¥ 1875 1675 5 B75 BIS 18750 R975 35 5 Mors 46875 ga750 SSeS ---Page Break--- E os, 6-10 6210 10 20 35 35-40 oc 45 55 25 75-80 80-84 5 100 us 15-120 20-124 ws wo 20 Table 1¥ Activity Schedule operation Plant first corn cycle 250 to 300 plants Release insects into cages Count adults during mating period, collect dead adults Bee counts Plant second cycle of corn plants, count eggs Cut leaves from first cycle of corn plants Sample plants to estimate larval population Install 150 second cycle plants in cage Count adults during mating period, collect dead adults Bee counts Remove first cycle plants and make tunnel counts from each stalk Plant third cycle corn plants Cut leaves from second cycle plants, sample plants to estimate larval population Install third cycle plants in cage Count adults during mating period, collect dead adults Bee counts Remove second cycle plants and make tunnel counts from each stalk Plant fourth cycle corn Remove leaves from third cycle plants, sample plants to estimate larval population Install fourth cycle plants in cage Count adults during mating period,

collect dead adults Count orgs Remove third cycle plants and make tunnel counts from each stalk Remove leaves from fourth cycle plants, sample plants to estimate larval population, and if larval ---Page Break--- animals »10H seven 9239 put 20509-8400 out ‘of 08 0 or 0 ve sta003 nye ff eaaway snag otreeg pre syyeey sonnet 988g x09 4 wyaL fa sure pret sey ‘u209 a awed 1 ---Page Break--- Notice “This pot was prepared on account of work approved by the United States Government. Neither the United States nor the United States Atomic Energy Commission or any of their employees nor any of their contractors, subcontractors, or their employees, makes any warranty, express or implied, or assumes any liability or responsibility for the accuracy, completeness or usefulness of any information, products or processes, or represents that it would not infringe privately owned rights ---Page Break---