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PUERTO RICO NUCLEAR CENTER

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Insect Sterility Program Technical Report 7

David W. Walker, Program Director

April 1973

OPERATED BY UNIVERSITY OF PUERTO RICO UNDER CONTRACT

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PUERTO RICO NUCLEAR CENTER

Insect Sterility Program

Technical Report No. 7: February 1972 to April 1973

(Formerly Potential for Gana-Induced Sterility

in Control of the Sugarcane Sorer,

is (Fab.) in rusrto Rico)

Research supported by the USAEC Report prepared in April 1973 by

Division of Blonadical and David We Walker, Program Director,

Environmental Research under Puerto Rico lwuclear Center,

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---Page Break---

List of Tebles

List of Figures ....

?TABLE OF CONTENTS

References

Appendix - Experimental Design for Cage Test

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20

10

2k

---Page Break---

2

8.

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LIST oF TABLES

F, Population in First cage Test

Frequency of Successful Mating and Survival of

Offspring An Outbred Lines, Fy to Fy

Percent Eze Hateh «

Porcent Adult Snergence

Bnbryonte Hortality and Egg Hatch in IPS Lines ss...

Larval Mortality and Adult Brergency in IPS Lines

from a Male Parent

Larval Mortality and Adult Energence in IPS Lines

from a Fonale Parent «.

Wax Hoth Prolintnary Test: Male Sterility

Wax Motht Test 1 se

Wax Hoth: Tost 2 ssesees

TPS 4n the Stink Bug ses.

---Page Break---

Figure

Le

8.

LIST OF FIGURES

Lines from Irradiated Malo Parent te 18

Dose Effect on Larval and Pupal Survival in IPS

Lines from Irradiated Female Parent «. Seer

Generation Effect on Beg Hatch in IPS Lines from

Irradiated Male Parent « ae »

Generation Bffect on Beg Hatch in IPS Lines Crom

Irradiated Fonale Parent ..+esevees see »

Generation Effect on Larval and Pupal Survival in

IPS Linos from Irradiated Male Parent seseeseeeeeee re)

Generation Bffect on Larval and Pupal Survival in

IPS Lines fron Irradtated Fonale Parent

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---Page Break---

Introduction

among the devolopments in insect control the most important. break-
[frou nas boon the development of the conaost of Integrated Control (IC).
It was first proposed by B. P. Knipling (1966). Ic, Tumlasentallp e

systens approach applied to monccultural crop production practices, ts an
attonpt to integrate natural and artificial control measures to srevert,
pest population outbreaks. It shifts the burden of pest control? fve
single method (insecticides) to « variety of preventive checks and therefore
At emphasizes anticipatory rather than corrective nears:

Specific insecticides are used in IC under certain conditions, with
great care given to the tine of application, the application method cay the
?Mount needed to produce the desired effect. In the crop phase of I¢ pests,

resistant plants are selected, and attention 4s focusi- oi tie tine of?
planting to avoid insect attack. In addition irrigation practices are
Podified to take fullest advantage of irrigation for pest control. ?Clean
harvesting 48 recommended for sons crops to reduce the plant residues efter
harvest that harbor pests.

In the post control of IC entomologists are stuiying trapping
tachniguos, thet ose jivaatle Romnonae, sor eee are choristerilants,
and oviposition lures. Specific parasites, predators, and insect disesses

are cultured and released to control pest populations. Guarantines are
wed to prevent introduction of peste.

Seek populations are also being suppressed by overflooding thon with
Andividuals that havo genetic defects. Smith and von Borstal (1972) wade
an extensive review of insect control by genetic manipulation. "Sone of
forir suggestions apply specitically to lepidoptera programs,? For exeeple,
Amproved methods are needed for producing sterility by mutations wits ne
uniesirable effects on the sperm itself or on sperm transfer nechentons,
We also need moro efficient methods for introducing genotic insults ints
the natural population, e.g.5 # single overflooding Felease 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 lapiioptere

and presented it as a population model (Walker and Petersen, 1069). the basis for the two models was the data fron IPS laboratory observa tions sith fe sugarcane borer fron 1965-9 (Technical Reports 1 through i, and walnor, al.) 1972). The hypothesis and the mechanism is discussed in the ?Spperdix of this report. We think we have solved the maior protlon with fie IFS. technique by having found a satisfactory way of Tully sterilizing Jepidopteran fonsles (with fractionated doses). We discuss this in section D under Accomplishments.

Many additional inprovenents are needed before the sterile release sethod can be used for eradicating lepidopteran pests efficiently. ae peed to improve rearing metiods to be able to produce sillions of motns in

g factory systen, This 1s difficult because the food requirements of depidopters are rot woil known and th larval life sian is lone. we also need to improve methods for producing genetic defects, Ovfey recone use

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for danaging the chromosomes of precursor plans are discussed in the text of this report.

IL. 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 andthe F population was sampled in April. The population in the control eage was nearly five tines the nimber rele.

(See Table 1), One hundred and fifty plants appesrs to be adequate for

providing sufficient oviposition sites for 15 females. Only 8 of the
255 plants had larvae, and the range in infestation rote per plant was

0 to 19, Overflood release - the first ovorflooding release in March
apparently failed booause the males available were of mixed ages. The Fy
population of this cage will be sampled at the end of april.

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

May Decision nade to move the cage to a new location,

Sites and chose one at the Fe
Station, submitted formal request through Ai

to USDA:

une Pormission granted by USDA

July and DAsmantled and moved cage frane

Banst

Septamoor Reconstructed frane at new site

Qotober Received and installed screen

November Planted first eycle com

Deconbor Corn blight destroyed first cycle, due to

heavy rains

sanuary Planted Ibadan variety

February ?First insects released - control test

arch First overflooding release, planted second

eyele corn

pend Sampled F, population of control cage

2. Colony Phase ~ There have been sone problems in developing

the rearing nethod to the production capacity neoded. In September we had

accumulated over 700 pupse in cold storage fer reserve. These wore killed

Woon toe tanperature Setting was sot too low by accident. Present pro-

duction is approximately 20 to 30 pupae per days

Diets the diet thet we are now using contains:

---Page Break---

canned pinto beans 600. gm

brewer's yeast 80. ex

ascorbic acid 8. em

tegosept (nothyL-hydroxy-benzoate) 8x

ar 32. gm

Linseed 011, raw oem

sucrose WO. gm

molassc 100. gm

corn syrup (Karo) 60. gm

wheat gore 30. gm

powdered cellulose Bs.

vitamin solution (Vanderzant) 15. ex

vater 2,000, gm

Ingredients are boiled for five minutes (excluding the ascorbic acid)

before mixing in the blender.

Wo have separated the laboratory into thres work are:

(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 fron dirty food and washed before transferring

to clean food in area 2. Area 3 has a hood with an

exnaust fame

aseptic as

We are contimuing to use the one-ounce jelly cups for rearing larvae instead of the plastic disnes ss we had planned originally because we oan control sold spread with the cups. We exanine tho larvae every day (including weekends and holidays). Larvae are transferred when nold appears on the food. We often transfer larvae every second days

Diseasos have been problem. We have a virus-like disease in the golony. Black atominal prolege and a white waxy appearance t0. the lethargic larvae are the main eymptons. Larvae die in the fourth or fifth stages. Wold kills larvae in all stages, out most of the larvae are killed as second or third instars if they die of mold.

Foeding and tunneling 4s good in thie diet. The adults are larger than those fron previous diets, but snaller than individuals grown on corn oF cone stalks. Oviposition rate, adult longevity, mating frequency and nating 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 nade three relessost

(2) overtlooding the nomal malos with fully sterile males in 8 10:1 ratio,

(2) overflooding? the normal nalos with partially sterile males An a 10;1 ratio,

(3) ne ovarflooding.

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They found the F] population reduoud 62 percent in relation to the control population in tests where fully storile males were released, and 92 percent Feduced in taste where partially sterile males were released. Overflooding wath partially sterile males was more effective than overflosding with Tully? sterile males.

B, IPS: Analysis of Laboratory Data in Relation to Sex Ratios, Dose Effect and Generation sffect (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 « formal individual, 11 offspring wore outbred with normal individuals a Sinele pair matings, Keeping the lines separated in the inmaturo stages. Data include offepring in the Fy to Fg generations. Forty-one Lines were observed. The dose given to the P gonoration parent was 1, 2, ly 64 10, 12, or 1d krads. ?The exporinental work wae done in the laboratory during tas yours 1967-70. These date are shown in Tables 2-25 and Figures 1-28 in Report 6.

1. Sex ratios. ?he previous report showed the Lineage for 1,072 outbred matings that produced fertile egcs and that were descendents Sfon an drradiated parent in tho P generation, In the F) through Fj generations observed 56 percent of tno adults were males. Unsuccessful Ratings were not included in this tabulation. For convenience we have Limited successful matings to only those in which fertile eggs were prom duced, matings where a spermatophore was transferred to the fenale. Matings An widen tere wore no fertile eggs because of apyrene or innobile sperm are not inclided, In mary instances mating occurred, fertile oggs

wore laid and embryonic development proceeded, but no eggs hatched;

are included.

ALL 590 afflicted males of the F) to Hg generations were mated to females that produced fertile eggs, and in M8 of the mating instances sone of the oggs in each mating hatched and sone of the ensuing larv developed to tho adult stage of the following generation (fable 1 and Figure 1). There wore 47lh afflicted fonalas that produced fertile oggs in the Fy to Fp generations: of these G6 instances sone larvae fron exch nating survived to tne adult stage. Comparing reproduction in afflicted fale offspring fron a P generation afflicted male or fenale we find ths

the afflicted male offspring were successful in continuing the line in 2b,6 porcent of the instances and the afflicted fonale offepring in 18.2 percent.

Hales are superior to fonalos in ability to transnit the affliction
in IPS Lines for to reasons: there are more of then, and survivel of
offapring from afflicted males is higher tzan from afflicted fenalos.
Tois'sox difference in reproductive potential in outbred afflicted Lines
Gay be due to differences 4n the sex chronosones of tie sugarcane borer.
Lopideptoran fonales are nemizygous for sex ci:roxosomes.

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2. Dose effect. (a) Table 3 and Figures 1 and 2 show a
comparison of ggg Fateh in the Fy to Fg generations at different doses.
There is a nogative correlation between increase in dose and epg Sater of
Fr and #g enbryos (oxgs produced by the P and Fy generation aleite,
respectively). Seyond the Fp the correlation i¢ hot consistent fee hateh
from afflicted ines descending from afflicted P generation fonales sere
lover than from afflicted P generation miles in the Fy generation,

(b) Table 4 and Figures 3 and 4 snow adult emergence which is a gonparison of larval survival and dose for P generation sales and fonales. Percent survival of Fy Tarvae and pupso is low at 2 krade, higher at 6 kradsy and low in 12 and 14 Krad Lines from both males and fonalo:

(ec), Table 5 snows @ comparison of Lines for of death of tn rolation to dose. ?There 4s a correlation betiea Srcreieet ee earlier death in the Fy generation in lines from both afflicted P gener= ation males and fensles. Al] fertile oggs develop to the orange spot stage, unfertiliced ova only develop to tne bright yellow stage. Eworyonic develomental stages were described proviously (Walker and Guintana, 1966),

3. Gonorstion effect. (a) Table 5 and Figures 5 and 6 also show 4 comparison of stage of death of enbryos beyond the Fy generations Death occurs at. progeasttvaly Inter atases Mi conmeoition Pinte et pearly all doses and generations. hore is « partial recovery in ecg hatch from the offspring produced by the afflicted P generation male Line

from the second to the fourth generations. Generation effect on exe hatch As not consistent, although egg hateh is lower fron descendents of P generation afflicted females.

« (2) Tables 6 and 7 and Figures 7 and 8 show a comparison of relative garvival and stages of death of larvae and pupae in lines from afflicted Pisles and Tomales, rospectivelys again Usctsaconients or afierie fenale lines are nore damaged than the male and fenele offepring of afflicted male Mines. Adult survival in fable 2 and Tables 6 nd? are not comparable since only 2, 6, 12 and il krad doses are tabulated 17 facies

@ ant 2.

Cy IPS in Hemiptera (Restrepo)

Virgin adult fonale stinkbues Miridula (L.), Pentatomidse)
were exposed to 1.55 7.5, or 1540 krads ard then mated with normal males

45 discussed in the previous report. gach generation the eges were cole
lected and the offspring were carried through the fifth generations hone
of the offspring fron tho two higher doses survived beyond the Fy, rymphal
stagess the offspring from fenalos treated at 1.5 krads surviveds Repro-
guetion and survival in the 1.5 krad line and the normal line were equal?
in generations Fz to Fes We interpret this to nave boon a recoveryy ie+y
selection against the sffected genomes. sex ratics of offspring wore equal
in both toe normal and irradiated lines. Survival deta are shown

in table 11.

Pontatonid chronosones are reported to be holokinetic as are

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lepidopterans, Gonoz-Nunez in Venezuela and LaChanco with the USDA in
Nort Dakota Aavo studied the IPS effects in other nenipters. They found

?the afflicted Lines recovered in tho first or second post-irradiation generation, Although they worked with group satings rather than single pair matings, I believe that their data can be correctly interpreted to ?moan taat 2 selection mechanism occurred. The only known difference between the lepidopteran genetic mechanism and tho benipteran 4s in sales. Nezara ard other hemipterans have abnormal spers production from the ?tarlequin lobe of the testes. This may have no bearing on the relationship With the recovery phenonons observed. However, it 1s of academic interest and possibly of significance, It is more likely that the genone duplication mechanism 4s different in gone respect betwoon the tio orders, and this could explain the clear difference between recovery in nemiptoran lines land incomplete recovery in lepidopteran lines, In addition Virkkt (1963) reported asynapsis in tho meiosis of the sigarcane borer males. Agynapsie An mofosis has also boon observed in coceida (homoptera) and Cocidomyidae (dtptora). Vink states (p. 119)¢

"Those examples show that the classte pairing of homologues is not unique method of controlling ?tho Feduction division of tho chromosomes. There are some factore latent in the prophasie coll nich are capable of taking care of a correct, segregation in lack of pairing of homologues. In

our subject, Diatraca saccharalie, such factors
apparently operate in the asynaptic spermatocyte
menue toe anaphase grouping 7 + 27 (or nearly
80) ocours so often.?

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

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

Galleria mellonella (L.) moths wore reared in one-gallon jars on Waterhouse (1959) medium, This contains honey, glycerine, brewer's yoasts water, dry Pablus infant formula, and vitanins. ?Food was autoclaved and after? it had cooled the mature larvae were added. The onerging adults deposited eggs on the modiun, and the next generation of mature larvae and pupae wore collected as they energed 30 days later. Jars were held in the dark with the temperature maintained at 32 + 1°.

?The sex of pupa was determined and each wae maintained in a seperate fone-ounce jelly cup. Upon energing the adults wore irradiated at 0 to 24

hours age, and placed with an individual of tie opposite sex after irradiating. Mating occurred inmediately. Most of the eggs were laid inside the fold of a sali pleoe of wax paper. sege were counted, scored for development ant hatch 10 to 15 days after mating. In order to provent larvae from eating renvining enbryos 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 wore contucted to deternts

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a+ the sterilizing dose to adult males (tio tests);

Bs the sterilizing dose to adult fenaies;

© the sterilising dose as either a single dose or = fractionated dose, 24 hours between the two fractions.

ALL tests were reposted three tines with five or more replicates in ?each, Data reported in tables are averages of all taste.

Kosutte

?Single Exposures The preliminary tests indicated that males could be sterilized at approxinately 22 krads or higher (Table 8) and thet eng production of normal females mated with irradiated males declined sone siderably, particularly if the males nad boon treated at higher doses of radiation. Practically all of the oggs laid were fertile, however the Proportion of nonefertilized aggs increased with dose and age, possibly duo to sporm inactivation, Sterilized males did not recover virility wen ?nated with the second virgin female, nor did the first fomaio mated with the irradiated male produce viable eggs in the absonce of the irradiated male. Most of tne eggs were laid in the first five dys after pairing.

In the second five-day veriod (6-10 days after pairing), og production declined drastically, however this redustion in oviposition was greater

in the treated than {n the control pairs. Similarly, a second female mated with the sone mals failed to produce viable exes, indicating that, ales that had teon irradiated with starilizing doses did not Tessin virility (see Table 8).

Fonales aro sore susceptible to radiation darage than are males (Table 9). Whore both sexes wore irradiated, tha sterilizing effect Ss mare or less equal to that on the females

Tho wating ability, adult longevity and sexual attractiveness of the oth roceiving up to 22.0 krads did not appear to be affected. It was further observed that treated fonale moths started ect laying earlier and erm develoment was longer than the control groups

Fractionated Exposures: In the second series of tests single and fractionated doses were compared. Fonales wore nore radio sensitive to Sractionated doses than were nales (Table 10). Fractionate? doses, pro-Guced signer sterility in both sexes. Mating ability, adult longevity? and sexual attractiveness were not apparently affected ty doses used hovevory eR production and ogg heteh effects were greater in females thay had received fractionated doses as compared toa single dose. i fractionated Gose of 6.6 krads to females sterilized thon, as compared to 12 percent omg haten fron fomsles regeiving 6.6 krads in a single exposures

discussion

The utility of this concept in tee content of lepidopteran control
would appear to Gs great Af the experience with tre wax mete sedise Te
Stier Lepidepterss ?for exanpley Tessle pops oF aS SE

snal¥ conditioning dose of radiation--sufficient to disrupt the repair

---Page Break---

nechanism capability--and later a sufficient done to cause the bulk
of the genetic damage. It is conceivable that this combined dose
Gould be substantially less than the ammunt needed for producing
complete dominant lethality from a single acute dose. This would
Silow us fo use considerobly smaller dosages to achieve the same
Gnount of genetic danape, and thus we could avoid the inherent pro-
Diews encountered at the high doses necessary to sterilize Lepidoptera
The most important of these

tion in mating competitiveness, shorte

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. Tt oe not necessarily mean that the total genetic damage is necessarily preater from fractionated doses, but simply that the effect of repair hechanisna is rendered inoperative in such a manner that the genetic danage becones apparent earlier, in the developing F, embryo stage in this case instead of in the Fp embryonic stage, or in the developing Larval and pupal stages of the F, generation.

?ractionated 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 cool for panipulating lepidopteran sterility through the production of genetic danaze at considershly lover doses.

Host plant resistance (Vakili and Kaiser)

brs. Vakili and Kaiser at the Federal Experinent Station in Mayaguez are field testing hundreds of varieties of beans (Phaseol) and coupeas (Vigna sinensis). ?The objective of this vork is ig 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 Anerican tropics, We have cooperated with then to develop methods for detersining the nature of the attractiveness of Susceptible varictivs, and conversely the factors responsible for re sistance 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 ovn tine. The bean program and a corn and sorghum program with similar objectives directed by Dr. Webster provide an excellent opportunity for us to de~ velop a progeum.

I would Like to begin by studying the differences in profiles of the aromatic compounds from the most Fesistant and the most susceptible varieties of beans and coupeas to the bean veevil, Ch

The compounds producing odors will be solvent extract: hompyenates using mineral oil in blotting paper to absorb the volatiles, Chen extracting this vith a solvent and then analyzing by gas chronato- uraph, This extraction method was used to evaluate the attractiveness

---Page Break---

of volatiles in banana varieties against the banana weevil. PRNC hes the equipsent necessary to begin thie work. Solvents and ather chemicals and columns would be needed, but Little ele is required.

LIL, Relation to other work

The population collapse technique for eradicating lepidopteran grecies needs extensive field testing both in cages and on an ares basis. Since the latter prograns vould be of considerably greater seale I do not think it would be wise to attempt this with the supar-cane borer yet. After 4 mass-rearing method has been developed this can be considered, but until then it would be doomed to failorey

'y the only other IPS cage test (Toba, et al., 1973)
fo the Fz generation. It was based on the hypothenis
that several overfloodings (10:1) would be made ina field progr
using a high dose for producing the seai-sterile males. It isevery

expensive to laboratory rear large nusbers of lepidosterans.

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

1, that the high dose causes lover survival in earlier genera~ tions, therefore requiring higher overflooding ratios to compensate for the smaller nunber of F2 and fy survivors; and

2. that @ saaller dose yields @ higher proportion of F1, Fp and ¥3 genoration individuals with genetic Ioad, enhancing the Azequency of dissemination of this genetic load into she ?atural population, but becoming effective at a relatively later times

the incerrclationship of these two aspects needs to be more defi~ pitively explored and the population collapse concept needs to be teaved further under natural conditions of durvival, ives ia the field,

I feel that ve should stimulate interest in using lover doses so that ve can develop the best method for effectively disseminating artlic~ted genomes into a population.

IV. Future work planned

Completion of the field tests is the first priority of the progras.

Although we had a stow start and difficulties with ehe colony, we shectd he able to complete the cage texts within the end of TY 1998,

Further work with fractionated doses to determire if we can produce Souptote dominant Lethality in fonale sugarcane horere Will be explored, ihe results with the vax soth show considerable promise. Poseiviy thes lepidopteran species could be included in these experiaente,

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1 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

+ Gamma

(ko 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) .

1S in the southern etink bug (Walker and Restrepo) «

Varietal susceptibility of cowpeas co pod borer (Vakili and Walker)

Bean pod and seed damage by the bean pod borer (Walker and vakini)

Differences in susceptibility of bean varieties to pega pers Vakili and Walker).

A ateategy for lepidopteran pest eradication (Walker and Pedersen) «

VE, Program Personnel

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

Hr. Ruben Restrepo, a graduate student from the Universidad Nacional de Bogota, Colombia, vorked officially with the program during June through August on an OAS grant. He has worked for the last vo years on a voluntary basis. We have coupleted the preliminary work with the stink bug, Nezara viridula, ive., diet evaluations (see last report) and IPS. Me. Restrepo will complete the requirements for the master of Seience degree in Siolocy in mid 1973. Wis thesis research is a taxa~ nomie revision of a group of honopterans.

De. Warpal Singh worked fro June through mid Sentenber on a grant from the Oak Ridge Associated Universities. le evaluated fractionated dose effect in the great wax moth.

Alba Rivera-Detres is completing her course work for the mater of science in Biology. Sie will eontinaw hier investigations of henolycnh proteins of sugatcanc borer larvae at the beginning of summer vacation.

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table 2

Frequency of Successful tating and survivgl of offspring

in Outbred Lines, F, to Fg

thaber of adult

offspring produced

Number of matings

Percent of matings

producing adulte in

?successful in cone

in afflicted Mines the following nuance of te

Fw Fy generation outbred ine

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 ean WL average ata

offspring from irradiated P generation female;

Males 235 60 2545

Fonalos 225 bs 20.0

Total 460 205 avorage 22.8

* tis tabulation shovs Fy through Fy individuals fron ines drradiated a8

the male or fenale aault

successively outbred ?ith

Fy through Fe

Ww. 6 agr)t

An the P generation (outbred with « normal), and

normal of the opposite sex in every instance

?Seo Tables 2-25, and Figures 1-26, Teennicel Report

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rable 6

ax Hoth Preliminary Tests Male Stertisty

Exposure Fertile tess 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 Percent age Percent

?eas Produced Hatched Produced Hatched

1st virgin 2nd virgin

irradiaced female (b) irradiated "tema

male (unirradiated) male (onirrediated)

0 se 5 daye 39 95 soo 85

and 5 days 153 %0

10 ast 5 days 456 e 850 0

2nd 5 days o2 2»

20 tat 5 days 433 vo 450 °

ed 5 days us °

30 tat 5 days eas ° ar °

2a 5 days 150 °

4 se 5 days 20 ° 125 °

aed 5 daya 100 °

50 ee 5 days 15 ° 00 °

dod 5 ayn m °

* Hale sterility from a single exposure

© After 5 days irradiated males vere isolated fron the first fenate (2)

and mated with the second fenaie (>),

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Cage Test of Inherited Partial Sterility in the Sugaroane Borer

Experinental Design

Hypothesis being tested:

That overflooding natural population vith subusterile males over

Single generation at the rate of 14 to 1 will eradicate the sopaletion

or will effectively suppress the population for two oF More eeroretinnan

?ihe hypothesis is based upon laboratory experinents that led to

EAS deysloment of a hypothetical model for population control ty Welker

and Federsen (1969, ann. Enton. Soc. Aner. 62i21-6),

Explanation of the nechanten:

The predictability of the success of this model 4s due to two

factors:

A+ A atively high reproductive rate in the P to F, generation

(4.2) An outbred afflicted Lines, and

+ Aarastic reduction in tho chances of « normal to normal nating as F) adults and therefore a drastic reduction in the Pepréductive potential in the F) and subsequent generations.

The introduction of sub-sterile males provides the mechanisa for Antroducing a biological insult into a large projortion of the nated population,

Az shoe in abe Z the overtloding advantage in the P generation 45 1 tolls Gn the nace (PS gunenation fae rece ati genera noma alesse exzoctet te S°5F igo Gl tay, corde eg generation the ratio is expected to be 29 to 745 Ged to 1). fBegreticalyy the coance Fora mrael ate eltin sat sem tomate sein LS Shake egarorathony Din a p'ay ep Wot tome fonae ay ate A gett Soe

This tost is actually measuring:

14 Whether P generation treated males and Fy generation afflicted

males ad tonalos aro equal mating competitors to normal ascies;

2 Wotser devetojmont tine of Fy and Fy affeted anivsduala <e

sypenrorsned with'F ?and fy rdvuis fo" taat hr aefliciad tata

Shiprwoont at Ua hae fde"the's sont aya

2+ Whother tho favorable racio of afflicted to somal individuale

is the F, mating ovourrencos is 0! aulficient advantage to te

sunerior to a hypothetical reisasu or li fully sterile males

to eset sormal wale in tie poulscion: and

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4 Whether the mating between two afflicted iniividuale will

result in viable offspring.

Insectary:

?A room with 100 square feet of floor space is used. It 4s cooled

by an air conditioner and hgated 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 yiven in the text of the accompanying report.

Rearing:

Before energence pupae are placed in open woz plastic cups in a cardtoard 4ee-crean carton, This J-gallon carton is ¥ 1/2tnenes in Gianetor and I0-inches high and 1s ined witn wax paper folded into an fceonitan pleated ring. The creases in the paper provide good sites for Sen laying. Adulte energe from the pupal case in the afternoon and formally nating and egg laying take place shortly after emergence.

Seg clusters are placed in sunlight to spood embryonic develomont. fags laid on the wax paper in the carton can bo clipped off and placed in the plastie eupe, The larvae hatened in the cups are harvested daily and placed in the food as doseribed, They are transferred to clean food a3 hecessary. As they develop Yso pupse are removed from the food and Stored for future tse at 343°C OF are used to continue the colony.

Adults used in tests are sexed at pupse, and are collected daily.

In, this manner they can be irradiated or packaged for release in the cage.

?The subssterilized adults are irradiated on the day toat they are to be released. All of the adults to be released in a given cage are maintained Geperately aa virgins until release. Releases are at dusk to avoid predation by lizards.

Gagess

Bight cages are available, oach 1s 40 foot long and 40 foot wide (approximately 4 percent of an acre), and approxinately 10 feet from floor to ceiling. ?This is a structure 80 feet wide and 160 feet long,

two rows of four cages. The supporting franework is 2ainch dlancter galvanized stool pips bolted toethor, with uprights imbedded in concrete. he uprignts gre ton foot apart. The top is covered with natural

colored Saran? shade fabric with $/16-inen openings. The top hag a

6 poreorit shade factor. Cage sides are covered with green fabrie with 0 openings per equere inch ania 37 percent shade factor.

can be applied by hose, sprinkler or watering cans

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Host plant

Tbidan A or Bis used as the host plant. It is a fastegrowing
Succulent variety, and is well-adapted to the growing condition i& the
cage Tt 18 not highly resistant to rusts nor to aphids, It respons
welt to chemical fertilizer and to moderate irrigation, and it resches
moderate size upon maturity in the cares This is an important factor
since ?the cago provides a shaded growing condition. The corn plants are
eslorotic und tend to develop tall slender stexs,

Gor plants are planted in plastic nursery pails (12-anches diameter,
AO-anshes deep) that aro used for cultivating yourg plants; with 2 109
Flants per pail. Plants are crown in the cages to provect, then agaicst
Anfestation. The cage floor is covered with strips of black mulck
Plastic. The floor covering serves two purposes: to control weds and.
fants and to provide a contrasting surface from which ts collect tre
adults after they have dled.

Soil in the pails is mixed and fortilized in batches. Nomally the plants are watered by hoses

Corn is the preferred host plant for the sugercane borer. corn has ghagher incidence of selection for oviposition ey gravid fonales, better feedings higher survival and faster development tine than cane or other plants (uintana and Walkor, 1965 a, by anda). the soil as Prepared and cory seods planted 25 days before the first day of releas fhe corn is planted in cyeles beginning with the first generation of insects. Fortunately the sugarcane borer tunnels into she stes ona Pupates there; it does not migrate from the plant in which it has funneled. Separate cycles of corn plantings can be made, Approxinately 20'days after tho beginning of the generation tine (release date or tne {nsects) a sample can be taken fron that cycle of com planter or all

the corn plants can be harvested and all tho larvae eat be recovered from the stalks. Theso larvac are counted and ten aaintained on corm etale Vieces An cups held in the cage. {ais de comparable to develosene in rowing stalks. The corn planting syclas sre,

Hirst: 25 days before release

Seconds 6 days after release

Tint 36 days attor release

Fourth: 66 days aftor release

Sufficient planted pails are started to have 200 pails with at least

one plant in each. Plantings are in the cages described using twe cagess

In the cage tests of control vroups in whitch only momma] adults are

Toleased we expect approximately S-(eld incre. ses enol, generation,

Sitrongh this has beer audte varicilu. Pupllation cunee on movleus

control cage tests wares

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?able II

Population Increase in Cages Where hormal Adulte Were Released

pats sme 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 wa 0,68 (2/60)

0 1? 0.28 (17/60)

* Means of those 3 tosts is 4.55 fold Anore

?These samples are too small to have a high reliability. However,
Sf wo assime 9 5-fold increase each generation the population model for
control groups should be as shown in Table III. Since the increase is
Roonetric and the cage sizo limited, it 1s obvious that the nunber of
nost plants that can be grovn in each cage is inadequate for the
population by te 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 4s the test cage with normal ard Stradiated insects.

With edgnt cages available, four test replicstes are being conducted simultaneously.

Control cages

In the control cage 15 pairs of nomal adults are released at dusk into the eage containirs 150 plants. We expect that the population in this cage will increase to the Linit of ts food supply in one generation. Therefore, we hava Limited the population by removing snough larvae in ach generation so that the nimber of adults emerging actually remains constant at appr vinately 15 pairs each generation. Sampling involves removing 15 plants (10s) 20 days aftor the release date, cuttin: the Stens lengthwise in orcer to renove and count the Larvad. In order to Raintain a stable population of 15 mating pairs in each generation we Oxpect to have =) move and replace 60f of the plunts, or 120 of the 150 plants. Tho nuiber of plants actually removed is based on the nmbor of Larvae that vo ovtain in the sample, Kost of tue normal larvae die in the first larva st: je: we estinato that 50 pereent of the Ly stage larvae

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survive to becone adultes

?he following sssumptions are the basis for this population model:

+ ALL of tho females will mate. This assumption 4s based on field collections tron light traps mado ty Rafael Pores in Fortuna, P. Re bie collected nearly 400 adult fonaiee from ght. traps, and found the average mating per fenale was 1.2 tines, and approxinately 97 percent had mated,

Bach mated fomale will lay 300 fertile eggs. There 4s considerable variation in ope production anong 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 15 ne evidence to indicate that the afflicted lines will lay smalier numbers of fertile esgs tian nonmafflicted linese

3+ Fertile fomales will choose plants for ovipositing in a random fasion and there will be 5 to 10 egg clusters fron each female.
we are providing 10 plants for each gravid female.

We expect that 95 percent or more of the fertile eggs from normal, ines will hstch and that the survival from fertile eggs to adult, will be fron 1 to 2 percent resulting in a net population increase of Sefold each goneration.

Therefore, the Limiting factor in the normal population cage 4s the amount of host? plant material available.

Ten plants per female are provided, and 4f 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 Fonales into the care containing 150?corn plants ie at dusk similar to the normal test. Although the normal and overfloodire tests were not Bogun on the sane evening they were started at two or three day intervals With one anoter so that both teste are under the samc Weather conditions. fe wish to avoid the possipility of interaction between males and females sn diferent capex, This is the nain reason for begining the two tests 1B dacferent day's. It 4s possible that female pheromone fron one cage Aght influence nating in another cage and we wish to avoid thise This

'S more important in the overflooded cages.

in both cages rolsase in the late afternoon helps prevant predation

hy Wears, The Lizards sleep during the nights It allows dase diate tits on the nivnt released. Churtshin behavior begins as early ao 200" my and sstiry and org laying begin Jurang the first migut of les

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ow corn plants wore started in a soparsta cage 10 days after the release date so that they are ready for the F, adults and PF, goneration oeges ?Twenty days after release all the leavbs of the dnfedted corn were removed ay cutting thom at the bases New corn plants are placed botwoon the old plants so that there are plenty of oviposition sites for the Py adults.

Planting seouence and sampling schedule are shown in Table IV and Figire Ls

At the tine of removal of loaves from the sorn in each cycle, the plants are sampled. Fifteen plants, 10$ of the sample, are removed. carefully cut longitwdse and examined for larval tunreisy and larvae are counted. After the adults have enorged and laid their eggs on the new

corn plants, the old stalks are renoved ani larval tunnels counted, as previously described. This sequence can be continued as long es the larvae continue.

Bata ton:

Tt ds necessary to estinate the populstion 4n each generation 4n onier to test the hypothosis. Estimating or counting the munber of eggs deid io difficult with the nimber of plants used because of the small? size of the clusters ani the difficulty of seeing then on the losvese However, egg clusters aro counted in a portion of the plants, sstimtes of larval populations are made by cutting the corn stalks as described, This gives an estinate of tho larvae of thind stays and older. Counting larval tunnels 4s the most accurate nethod for assaying population size in this experinent.

Adults can bo observed at nignt (using red Mant) provided the counts aro mde at a tine wien the moths are active, 1. e:y during meting fiierts Population size estinates for such generation are nade as ToLlowt

2s fgg counts aro made on 10 percent or more of the plants.

2, Larval popululion 4s sampled 20 days after adult emergence. This allows sufficient tine for adult emergence and oviposition, ege hatching, and larval development in the tunnels in tne stakes It does not give us an accurate estinate of the mortality that occurred in the embryonic sti,s nor in the first } larval stages

3 Direct counts of adults during mating hours are mute on the night of

Feleace and again forty days after release. In the second instance Fy adults aro oountet. Dead adults aro collected from the floor of the cave caer mornirs: Dead females are dissected to detarcine the number of tines enc! mate?,

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Ser FEE?

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Table IE

Theoretical Movie) of Normal Population Growth

ee

Adults

An Population ?feproduetive adults Produced

Generation ?'M. F Rate 4 Fo Total

wee

e 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

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75-80

80-84

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15-120

20-124

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Activity Schedule

operation

Plant first corn eyelay 250 to 300 plants

Release insects into cages

Count adults during mating pertod, collect deed adults

Bee counts

Plant second eycle of corn plants, count eggs

Gut leaves from first eycle of com plants

Sample plants to estimate larval population

Install 150 second cycle plants in cage

Count adults during mating period, collect dead adults

Beg counts

Renove first cycle plants and make tunnel counts from

each stalk

Plant thint cycle com plants

Cut leaves from second cycle planta, sample plants to

timate Larval population

Install third oyele plants in cage

Count adults during mating period, collect dead adults

Bee counts

Renove second cycle plants and ake tunnel counts from

each etalk

Plant fourth eyele com

Renove leaves from third eyele plants, sanple plants

?to ostinato larval population

Install fourth cycle plants in cage

Count adults during mating period, collect dead adults

Count orgs

Renove third cycle plants and make tunnel counts from

each stalk

Remove leaves from fourth eycle plants, sanplo plants

to estinate larval population, and if larval

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