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Advances in Insect Population Control by the Sterile-Male Technique

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ADVANCES IN INSECT POPULATION CONTROL BY THE STERILE-MALE TECHNIQUE

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ADVANCES IN INSECT POPULATION CONTROL BY THE STERILE-MALE TECHNIQUE

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SCIENTIFIC EDITORS

REPORT OF A PANEL
HELD IN VIENNA
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FOREWORD

Prior to 1962 only the screw worm in the Southeastern United States had been successfully eradicated by the sterile-male technique. Since then, this technique has been successfully used in the control or eradication of at least eight species of insect in either experimental or field trials. This method of insect population control is unique in that it utilizes an inherent mating instinct to the detriment of a species without harm to any other species in the environment, with perhaps the exception of obligatory parasites of the species under attack. In addition, the utilization of the radiation from radioisotopes to sterilize insects and to label them so that they can be detected after release is one example of the peaceful use of atomic energy.

The International Atomic Energy Agency convened the first Panel of experts on this subject in 1962, and in view of the increasing number of inquiries regarding the application of the sterile-male technique against many insects of agricultural importance, the Agency convened a second Panel at its Headquarters in Vienna from 20 to 24 July 1964. The Panel of experts reviewed the progress that has been made in research on the application of this technique and suggested recommendations for future action. These findings are published in this Technical Report.

Much of the information in this Report was submitted by persons not participating in the Panel and has not been published elsewhere, therefore the Agency would like to gratefully acknowledge the contributions that are not otherwise documented.

CONTENTS

I. INTRODUCTION

This Report reviews the recent progress made in inducing sexual sterility in insects by means of irradiation, chemosterilants or mutations. Although emphasis is placed on fruit flies and tsetse flies, the discussions deal also with other insects of economic and medical significance. The summary expresses the Panel members' hope that research will be accelerated on the species already under study and broadened to include other fields where the concept can be applied.

The Report is compiled by the scientific editors Dr. G.C. La Brecque and Dr. J.C. Keller from the papers read at the Panel.

II. EARLY DEVELOPMENTS IN THE STERILE-MALE TECHNIQUE (11)*

The concept of utilizing sexually-sterilized members of an insect species to act as agents in the elimination of the species has been vividly demonstrated by the eradication of many insect populations in various parts of the world. The history of the sterile-male technique is frequently recorded from this point. Unknown to many is that this concept was nurtured during a period of more than 20 years of diligent research, often with negative results, before its significance and value was finally established by the success of the screw worm eradication in Curacao. It is well worth recalling some of the early work in order that many research workers in this restricted field will not be discouraged and abandon efforts because of the frequent reversals. Success is often dependent upon the knowledge of a multiplicity of factors specific to the insect species.

Early interest in the sterile-male technique began about 1937-38 when Knipling was investigating the screw worm, one of the most destructive livestock pests in the southern parts of the United States. He was impressed by certain features of the life history, biology and population dynamics of this insect, realizing that for an obligatory parasite the total number of the insect in a given area was relatively low. Moreover, as it is a tropical species, the winter months greatly restricted its distribution and reduced the number of insects in the population. By about 1937, Melvin and Bushland had developed a method of rearing this insect on artificial media, which was composed principally of ground meat and blood. Thus it was reasoned that the combination of low population density and the ability to rear the insect in considerable numbers (even though mass production methods had not been developed), might make it possible to rear and release enough insects carrying some genetic deficiency to overflood the natural population and in this manner achieve population control. At about the same time it was also noted that progress had been made in producing sterility in Drosophila by the use of X-rays. The possibilities of a sterile-male technique were discussed, but unfortunately conditions were not favourable for the pursuance of the

* Throughout this Report the numbers in brackets at the end of some section headings and subheadings refer to the Panel member who presented the information (see numbered List of Participants).

project until about 1946 when Knipling made a renewed effort to obtain information on this possible approach. He discussed the possibilities with a number of scientists, especially geneticists, but found them somewhat skeptical regarding the feasibility of this approach as they did not seem to fully understand the theoretical potential that was involved. Finally Knipling wrote to Dr. Mueller, who is perhaps the outstanding mutation geneticist in the world, outlining the over-all theoretical plan. Mueller felt that, based on his experience with Drosophila, sterility could, technically, be achieved. He expressed some doubts about the ability of these sterile flies to mix and compete in nature, but his queries were of a type that indicated his lack of understanding of the insect and not the principle. He actually suggested the dosage range and the age of the flies that one might use to get effective results. With this encouragement, Bushland in 1948-49 undertook exploratory investigations and arranged for the use of X-ray equipment. He demonstrated within relatively few months that it was possible to completely sterilize both sexes of the screw worm flies and determine the dosage that would be necessary to sterilize these flies, and found in effect that about 3000 r of X-ray would sterilize males although it required about 4000 r to sterilize the females. It was determined that the best time to expose the insect was just prior to emergence from the pupal stage, and that there were no apparent serious adverse effects on the length of life and competitiveness of the males. By placing various ratios of sterile and fertile males in cages with a given number of fertile females, it was demonstrated that these sterile males would compete quite effectively. For example, if the ratio of 50% sterile males and 50% fertile males competed with a given number of females, approximately 50% of the eggs produced by the females did not hatch. If the ratio was 9 steriles : 1 fertile, the approximate ratio of sterile to fertile eggs was 9 : 1. It was not exactly 9 : 1, but averaged 83% sterile egg masses as compared to a theoretical 90%. This 7% reduction in terms of percentage may not seem to be substantial but it does indicate that sterile males are not completely competitive. Actually, this would mean almost a 50% reduction in competitive ability which was still well within the range regarded as being feasible. The most difficult task remained — to demonstrate whether or not the sterile males would compete with normal males in natural conditions of the insect population. If the research workers had not been persistent they would certainly have dropped this project before they had demonstrated its feasibility. In the first attempt on a small island, half a mile off the coast of Texas, they released some sterile flies. The results demonstrated absolutely nothing. The habitat was unsuitable, and neither sterile nor wild fertile flies could be captured 24 h after release. The next point was to undertake a reasonable experiment in a suitable habitat. The first attempt was to try a release within an area of about 1000 square miles. It took practically all the resources of the division at that time to secure the funds to try to rear enough screw worms to release within 1000 square miles. This test was conducted in Florida. One side of the release area was along the Atlantic Ocean coast, to minimize migration. Unfortunately, when the tests were started, there was a three-week period of wet, rather cool climate. Again, the release demonstrated nothing of any significance. Then the next effort was on the island of Sanibel,

which is off the west coast of Florida. It had a natural infestation of screw worms, was separated from the mainland by about two miles of water, and had an area of about 16 square miles. The work was undertaken during the winter months when the natural population was at a low level. Screw worm flies which had been reared on animals (to be sure that they were at maximum vigour) were first tagged with radioisotopes and released unsterilized on the island in order to derive some idea of the natural population density. The females tagged with P^{32} would produce egg masses which could be detected because of the presence of the radioisotopes. About 100 males and females each were released per square mile. Then, by collecting egg masses on wounds of animals, it was demonstrated that this level of release would result in a higher ratio of released insects as compared with the natural population. This was the first evidence of natural population density of screw worms. According to estimates, the natural population density of screw worms on this island was of the order of about ten per square mile. This had actually confirmed early estimates made four or five years previously. Rearing and sterilizing of screw worms was initiated and 100 males per square mile per week were released. Within two weeks after the release of this number, the egg masses produced by native wild flies showed about 80% of the egg masses to be sterile. This was extremely encouraging, and after about three months of release the natural population on the island virtually reached zero. Occasionally an egg mass would be collected on wounds of animals and in most cases these were fertile. It was assumed that the two-mile water barrier was not adequate, and that a few insects were flying from the mainland onto the island. Although suspected, this could not be absolutely ascertained, but it was demonstrated that the flies would leave the island and go to the mainland because considerable numbers of radiotagged flies released on Sanibel were captured on the mainland. Nevertheless, the principle had been established, although elimination was not yet proven. A few months later the veterinary officer on the island of Curacao asked for suggestions on how to deal with the screw-worm problem on the island. Through communications, the type of control programme carried out on Sanibel was outlined, and a co-operative agreement arranged. After the preliminary work, it was found that the natural population was quite high, but within a short period of time the native population was annihilated, demonstrating for the first time the absolute validity of the sterile-male principle in field trials.

III. SPECIES BEING CONSIDERED OR USED IN PROGRAMMES OF STERILE-MALE RELEASES

The Trypetids occur as the most destructive fruit and vegetable pests present in most tropical, subtropical, and some temperate areas of the world. Basic food sources in many countries are jeopardized by the presence or threat of introduction of at least one of these species. Initial research with these insects indicates that the sterile-male technique can be utilized for their control or eradication.

A. OLIVE FLY (Dacus oleae)

Rearing

GREECE*

Larval medium. Research on the culture of the olive fly is proceeding satisfactorily. A systematic study for larval rearing was begun with "Hagen's formula" in which different techniques of preparation were tested. The most satisfactory method of preparation proved to be the following: the Butoben (n-butyl parahydroxybenzoate) is added to the olive oil and heated lightly. After cooling the Tween 80 is added. The mixture can be made in large batches and stored until used. It must be well agitated before using. The brewer's yeast and soy hydrolysate are mixed in the proper proportions and can be stored in large quantities also. The agar is boiled in the required amount of water, cooled to 90 ± 3°C and agitated while the benzoate of soda, choline chloride, and olive oil-Butoben are added. When a satisfactory emulsion is obtained, the brewer's yeast-soy hydrolysate is added while agitation is continued. After mixing the ingredients well, sufficient HCl (either 2N or concentrated) is added to obtain a pH of 4.1 to 4.5. The mixture is then immediately placed in an homogenizer at a velocity of 70 units for 1 min after which the dehydrated carrot is added and the mixing continued for 20 to 25 seconds at a velocity of 20 to 25 units.

After 160 different combinations, the maximum and minimum requirements were determined which produced a medium 95 to 100% less susceptible to fungus infection than the Hagen formula without affecting its nutritional qualities. The formula used as a control and the ranges in the amounts of the ingredients required are shown in Table I.

In rearing tests with the control medium, 680 eggs set on 31 preparations yielded 119 pupae for an average recovery of 17.5%. Average pupal weight at 0 to 24 h ranged from 5.8 to 8.9 mg with an average of 6.9 mg. Recoveries from formulations without olive oil, Tween 80 and Butoben in six tests averaged 14% with mean pupal weights of 6.7 mg. In all tests with rearing medium, it was observed that consistency is very important as recovery from those too wet or too dry was very poor.

Emergence of adults from pupae reared on medium containing agar as well as those without agar, and those without olive oil, Tween 80 and Butoben was 85 to 95%. Adult behaviour with respect to longevity, oviposition and fertility was similar to that obtained by Hagen.

Adult care and handling. The maintenance of a temperature of 23°C and a relative humidity of 60% in the rearing room appears to be the most satisfactory as better oviposition was noted under these conditions in preliminary tests. Since food for the olive fly adults is liquid, it was supplied once or twice daily. It can be kept in acceptable condition for two or three days when spread on an organdy panel backed by a moist cotton pad and separated from the organdy by about 5 mm, thus providing moisture without saturation.

Egging and handling. Eggs are removed from the oviposition shells with a 0.03% solution of sodium benzoate either by immersing the shells or by

* Contribution by F. Lopez and read in absentia.

TABLE I

FORMULATION USED AS CONTROL AND THE RANGES IN AMOUNTS OF INGREDIENTS WHICH PRODUCED A MEDIUM 95 TO 100% LESS SUSCEPTIBLE TO FUNGAL INFECTION THAN THE MEDIUM USED FORMERLY

Ingredients	Control (%)	Ranges determined (%)
Water	68.500	Remaining difference
Agar	0.500	0.250 - 0.500
Sodium benzoate	0.175	0.125 - 0.175
Choline chloride	0.050	0.050
Olive oil	2.000	0.000 - 2.500
Butoben	0.025	0.000 - 0.025
Tween 80	0.750	0.000 - 1.000
Brewer's yeast	7.500	4.000 - 7.500 } 10.5*
Soy hydrolysate	3.000	3.000 - 8.500 }
HCl (2N)	4.000 (pH 4.4)	3.000 - 5.000 (pH 4.1-4.5)
Dehydrated carrot	13.500	12.000 - 15.000

* Percentage of combined ingredients.

washing them out with a fine spray of the solution. The eggs are then concentrated in a small container, drawn into a pipette, discharged into a small funnel with a calibrated stem and allowed to drop onto a 2.5 cm organdy disc in the bottom of a Petri dish. The eggs are then dispersed with the above sodium benzoate solution. The Petri dishes containing the eggs are covered and placed within a container lined with either blotting or filter paper soaked with the sodium benzoate solution. The container is then sealed and held at 25 ± 2°C for approximately 48 h. An average hatch of 70% and ranging from 65 to 90% was obtained.

Placing of eggs on medium. After the incubation period, the discs with the first larval instars and hatching eggs are placed on the medium by either placing the organdy discs directly upon the surface of the medium or by inverting the discs and touching them lightly at several places on the surface of the medium to obtain better distribution of larvae and eggs. When the former method is used, it is best to remove the disc after three days to avoid severe bacterial contamination. The contamination is probably caused by alteration of the pH and dilution of mould inhibitors where the disc is in contact with the medium.

With these methods of placement of larvae and eggs, recovery was 65% lower than with the method regularly used in which larvae and eggs are placed individually on the medium. The low recoveries were due in part to some cannibalism as well as the use of eggs obtained over a 24-h

period with the resultant difference in hatching time. Although recoveries were lower with the organdy method of placement, handling time was reduced by 80%. The lower rate of recovery may be compensated for by increasing the number of eggs per medium as well as by collection of eggs over a 4- to 5-h period.

It is necessary to score the surface of the rearing medium in all the methods of egg placement to enable larvae to penetrate. Trays of medium must be covered to protect them against contamination and to prevent desiccation.

Handling of pupae. A more satisfactory method for separation of pupae from the medium was employed. The trays of medium were inverted upon first appearance of the larvae on the surface and the larvae pupated on the inverted top of the tray.

Adult behaviour. The results of the photoperiodicity experiments showed that time of copulation can be varied by exposure to artificial lighting and indicate that oviposition may be influenced by it, enabling standardization of fly production during different seasons of the year. Further studies are needed on the period of exposure to artificial lighting, intensity, distribution, and colour.

ISRAEL (14)

Rearing medium. Some years ago research was carried out in Israel on the eco-physiology of the olive fly. The purpose was to determine the nutritional requirements of the adults for fecundity and fertility as well as optimum environmental conditions. In brief, the main relevant findings were for good egg production. The female needs a protein source (preferably hydrolysed), a carbohydrate source, B vitamins and water. On the other hand, it was found that the male requires only a carbohydrate source and water. Although evidence for a nutrient carry-over from the larva to the adult was revealed, the adults were found to be mainly dependent on extrinsic sources of nutrients for fecundity and therefore the greater their longevity, the larger the number of eggs deposited. In this connection it was found that on a given food, the females lived longest and therefore were able to deposit the largest number of eggs when kept at about 22°C, which is approximately the prevailing autumnal temperature when the olives are most heavily damaged. In addition, an artificial oviposition substrate was developed to replace olives so that the basic requirements for the development of an artificial medium for rearing the larvae were at hand. Such a medium was then developed and several generations of flies obtained under aseptic conditions.

Consequently, interference with the symbiotic microorganisms harboured by all stages of the olive fly was avoided. The presence and the carry-over of bacteria from generation to generation had been shown originally by Petri as early as 1910. Furthermore, over 50 years ago Petri made an extremely important observation: he called attention to the fact that when the normal symbiotes were absent from the harbouring organs, these symbiotes were replaced by other microorganisms to the detriment of their host. One of the fundamental tenets of success with the sterile-male technique is that mass produced and irradiated males should be able to compete with their

wild counterparts. It follows then that any detrimental influence ought to be avoided, particularly since irradiation itself may be, and often is, debilitating. Therefore, the second phase of the work which is the problem of rearing the olive fly for mass production, was attacked from two aspects, namely: i) the development of a simpler and cheaper artificial medium than the one now available, and ii) the exclusion from such a medium of bacterial and fungal contaminants without interfering with the two symbiotes present in the olive fly.

Among the many proteins tested in the medium, only with peanut butter was consistently satisfactory larval development obtained on non-contaminated medium. Peanut butter is readily available, comparatively inexpensive, and offers the further advantage of contributing to the medium quite an array of important nutrients: 1) high quality protein in the form of globulins and therefore water soluble in the presence of minerals; 2) carbohydrates and lipids; 3) sterols; 4) minerals; 5) emulsifying phospholipids; and 6) some B vitamins.

The medium used at present, therefore, contains five ingredients: peanut butter, brewer's yeast, sucrose, agar, and distilled water.

In order to effect bacteriostasis and fungistasis within limitations, three compounds were added: bile salts, 8-hydroxyquinoline sulphate (oxine) and methyl-p-hydroxybenzoate. The symbiotic bacteria normally harboured by Dacus oleae are both Gram-negative rods, hence the inclusion of compounds controlling Gram-positive organisms was permissible. This is effected with bile salts which, in addition to being strong emulsifiers, are commonly used in bacteriological practice for the selective growth of Gram-negative bacteria. Further control of Gram-positive bacteria was obtained with 8-hydroxyquinoline sulphate, which is strongly bactericidal for these bacteria when potentiated by ferrum and calcium ions. Because the balance with 8-hydroxyquinoline and the activating ions is so critical the use of oxine for routine work was not contemplated. A search for something to replace it is being continued. Methyl hydroxybenzoate is used as a fungistat.

By inoculating this medium with neonate larvae, the yield in terms of adults per larva was quite high: 50 to 80% and three successive generations were reared until the population was eliminated by an epizootic and a contamination with Tyroglyphus.

It seems that not very much will have to be done to improve the medium from a nutritional standpoint. However, there are still two stumbling blocks on the road to a true system of mass production — the screening of better bacteriostats and bactericides, and the elimination of the need for using neonate larvae instead of eggs.

Laboratory studies

GREECE (20)

Radiation. Eight hundred 7- to 8-day-old pupae were divided into five equal lots and irradiated at dosages ranging from 2 to 12 krad in a Co^{60} source. Upon emergence, the adults were fed Hagen's liquid diet. Irradiated males were placed in cages containing various numbers of virgin females and the percent hatch and the number of eggs laid by these females

was recorded. Radiation at 2 and 4 krad dosages reduced the hatch but male fertility was regained within 20 days. Significant reduction in hatchability (98.3 to 100%) was noted at the 8 to 12 krad dosages provided the insects had mated before the males were 24 days old. Effective mating behaviour was initially delayed at the higher dosages. Dosages of 4, 8, and 12 krad all caused permanent sterility in females and had little effect on the longevity of either sex.

From the very start it was realized that expressing the age of pupae in terms of days from pupation, at a given constant temperature and air humidity, was not satisfactory for the determination of the irradiation date. In a constant temperature room the temperature is seldom constant. Fluctuations within the temperature range may vary sufficiently to delay or speed up the development of pupae of one batch as compared to those of another. Furthermore, it is often desirable to determine the proper time for the irradiation of pupae of unknown age which are field collected or taken from within the olive fruit. Morphological criteria of pupal age would, therefore, be desirable. Eye colour offers a practical way of determining pupal age in some species of fruit flies.

Pupae were reared on olive fruit and maintained in Petri dishes with moist cotton at 25 ± 1°C and evident eye colour changes were noted. The colour of the eyes changed from light brown to orange on the 6th day, were brown on the 7th, dark red-brown to purplish on the 8th, and dark brown-blue or purple on the 9th day. Adult emergence started on the 10th day. Iridescence on the eyes started to appear to a very limited extent on the 7th day and was generalized on all individuals on the 8th day. The antennae on the 7th day were of the same light colour as the frons. On the 8th day they were brown and distinctly darker than the frons. On the 7th day, the majority of the cephalic hairs had the same light colour as the head, whereas on the 8th day the hairs were dark in contrast to the rest of the head except for the eyes and the antennae which were still light yellowish. The colour changes, therefore, which were observed from the 7th to the 8th day of life at 25°C may prove of practical value in determining a certain stage of development and therefore the proper time for irradiation of an olive fruit fly pupa.

ITALY (3)

The action of radiation on the male germ cells. In Dacus oleae and in Drosophila melanogaster the testis is bounded by a double sheath – an outer epithelial layer, rich in inclusions and organules, and an inner striated muscular coat. The germ cells are divided into groups and bundles by the nurse cells and the cyst cells, and are characteristically rich in organules (mitochondria, lysosomes, ergastoplasm, etc.) which also exert a phagocytic function on the residual germ elements [1].

Spermatids are organized in bundles made up of a fixed number of elements: 64 in Drosophila, and 256 in Dacus. These are connected by cytoplasmic processes and bridges which confer to each group a syncytial organization. In the almost mature spermatid the syncytium becomes particularly

evident owing to a complete loss of the inner cell boundaries, which are restored during the conversion into spermatozoon. In the two species, the spermatids differ by some peculiarities of the mitochondrial bodies originated from Nebenkern. In Dacus they are two in number, equal, and invaded by a long osmiophilic fibre, whereas in Drosophila one of them is larger, strongly osmiophilic, enwrapping the flagellar axial filament, and the other is reduced to a very small tubule, running laterally to the first. Other differences are seen in the arrangement of the elements at the area that marks the junction of the head and tail.

The spermatozoon is similar in both species, except for the arrangement of the mitochondrial bodies (already noticeable in the spermatid) and for the insertion of the flagellar axial filament which is more cranial at the beginning of the Nebenkern bodies in Drosophila, and more caudal in Dacus. Interesting information was obtained on the ultra structure of the flagellar axial filament, equal in both species. A typical pattern was revealed, consisting of nine thick outer osmiophilic fibres, nine primary double fibrils at the periphery, nine secondary fibrils, nine Afzelius radial fibres, two primary central fibrils, and a central sheath. The simple embedding with osmium revealed, however, that the outer fibres appear to be made up of subunits. The double fixation, i.e. osmium preceded by glutaraldehyde, showed in addition that the secondary fibrils are made up of two rather minute subfibrils, joined by a transverse bridge.

In the sperm tail of the examined Diptera the axial filament complex was found to be accompanied for a very long distance by the two mitochondrial bodies. When these terminate, a transformation is produced also in the inner ultrastructure, owing to disappearance of the secondary fibrils. The above data, in accordance with the current literature, suggest that it would be advisable to take, as the reference point for the subdivision of the spermatozoon into segments, the axial filament ultrastructure. The length of the mitochondrial bodies would indicate that they are the "connecting area" traversing almost the full length of the tail. It is therefore suggested to subdivide the whole sperm into a "head" and a "tail", applying this terminology to the entire length of the axial filament after nucleus disappearance. The tail is composed of an anterior "principal piece" containing secondary fibrils, and an "end piece" devoid of secondary fibrils, independently of the mitochondrial length. The "neck" may be indicated as the area of the "head-tail" junction. The term "connecting piece" or "middle piece" (not an intermediate area between the head and the tail) is a restricted area as in mammalians, where the mitochondrial bodies show helical configuration [1].

The timing of spermatogenesis of Dacus oleae Gmel. has been studied at different stages of development from last larva to adult, by determining the type of germ cells present at the examined stages.

The results are summarized in Table II.

For the study of the action of radiations, Dacus were irradiated in the last instar larval and pupal stages by a cobalt-60 teletherapy unit with a focal distance of 80 cm at a dose rate of 30 r/min. The specimens were placed side by side in a depression cut into the top of a block of paraffin-wax to a depth equal to the width of the larvae or pupae. The depression was covered with a layer of plexiglass 5 mm thick, to obtain electron equilibrium.

TABLE II

THE TIMING OF SPERMATOGENESIS IN VARIOUS DEVELOPMENTAL STAGES OF DACUS OLEAE

Stage of development	Spermatogonia	Spermatocytes 1st	Spermatocytes 2nd	Spermatids	Spermatozoa
Last larva	+++	+	-	-	-
Pupa 3 days old	+	+++	+	+	-
Pupa 5 days old	+	+	+	+++	+++
Pupa 7 days old	+	+	+	+++	+++
Pupa 9 days old	+	+	+	+++	+++
Adults	+	+	+	++	+++

Four lots of Dacus were irradiated; namely, last instar larva, 1-, 7- and 10-day-old pupae. The doses of irradiation were 1 kr for the larvae, and 5.8 and, in a few instances, 30 kr for the pupae.

Only adults have been examined cytologically, one day after the eclosion.

The material was fixed in Dubosq-Brazil, embedded in paraffin-wax, serially sectioned and stained with Feulgen light green reagent. In many cases the testes were tridimensionally reconstructed.

The sheaths of the testes were unaffected by radiation.

It was determined by several observations that in the specimens irradiated in the oldest stages of development (pupae 7 to 10 days old) the adults have fewer cells in prespermatid stages than do the controls. In the testes of the animals irradiated in the young stages (last instar larvae and 1-day-old pupae) mature sperms are always found, which were not present at the time of irradiation. This demonstrates that in Dacus phases of the spermatogenesis are not completely inhibited by ionizing radiations.

By considering the stage of the gonads at the time of irradiation it seems that late spermatogonia, and particularly the first spermatocytes, were the cells most sensitive to irradiation, and therefore degenerated so that they do not contribute to the sperm pool of the adult testes. Among the other stages, the young spermatogonia seem to be rather insensitive, and from these are derived the spermatocytes and spermatids present in the adults. The evolution of the spermatids is not blocked, but spermatids are sometimes damaged in ultrastructure.

From electron microscope observations of gonads of male Dacus it can be demonstrated that the bundles of sperm from insects irradiated in the 7- to 9-day pupal stage, with doses up to 30 kr, are unmodified in the number of elements in each bundle that is constantly typical of the species, i.e. 256.

It can be concluded that the irradiation does not bring about degeneration of single cells in the bundles. This can be justified because of the syncytial organization of the germ cells in each bundle. Degeneration, if present, concerns all the cells of the bundle, and the reduction of volume of testes is probably caused by the degeneration of entire bundles.

Many degenerating or degenerated germ cells cannot be found in the flies irradiated in pupal or larval stages. But it is certain that degenerating bundles are reabsorbed by the cyst cells and nurse cells.

A very important point of the research is the ultrastructure of the mature sperms in adults from irradiated pupae. Motility and fecundity of the sperm are associated with the ultrastructure. Research on this problem is still in progress.

Doses of 20 to 30 kr are very close to the pupal lethal dose. In preliminary studies concerning the resistance of a very sensitive tissue, the mesenteron epithelium of pupae does not tolerate more than 32,500 r, and in these conditions the lethality is very high [2].

In the few flies obtained from the pupae irradiated at more than 20 kr, and in the more numerous flies emerging from pupae irradiated at 8 to 12 kr, the mature sperms seem to have normal structure upon examination of the head, the flagella filament of the tail, and in the mitochondrial bodies.

Other studies are needed to test the resistance to irradiation of the energy supply of the sperms.

AUSTRALIA (15)

Planning of rearing facilities. Because the success of a sterile-male programme is often dependent upon an economical and rapid method of rearing great numbers of vigorous insects, serious consideration should be given in planning insect rearing facilities that can be rapidly enlarged or slowed down as the situation demands. By considering the life and fecundity tables of a species, one may choose conditions which allow production of a maximum number of adult insects from a stock of breeding adults. The associated costs are thus minimized. These tables were originally used in ecology to estimate the rates of increase of various species under different environmental conditions [3, 4].

Under given conditions, the harvested population of adults (N_H) per week from an insect factory is given by:

$$N_H = \frac{N_0 Z}{t_d} \left[\left(K \sum_0^{t_d} \lambda_x m_x \right) - 2 \right]$$

where Z = Total number of cages available.
N_0 = Number of newly emerged females/cage.
λ_x = Proportion of females surviving at age "x".
m_x = Available number of eggs produced per female at age "x".
K = Proportion of eggs surviving to adult emergence.

It is assumed that a cohort of $N_0 Z / t_d$ newly emerged adult females are

added to the population each week and that the survivors of this cohort are removed from the breeding population t_d weeks later.

As an illustration, Fig. 1 shows the expected harvest plotted against the age at destruction for two strains of Queensland fruit fly (Dacus tryoni)

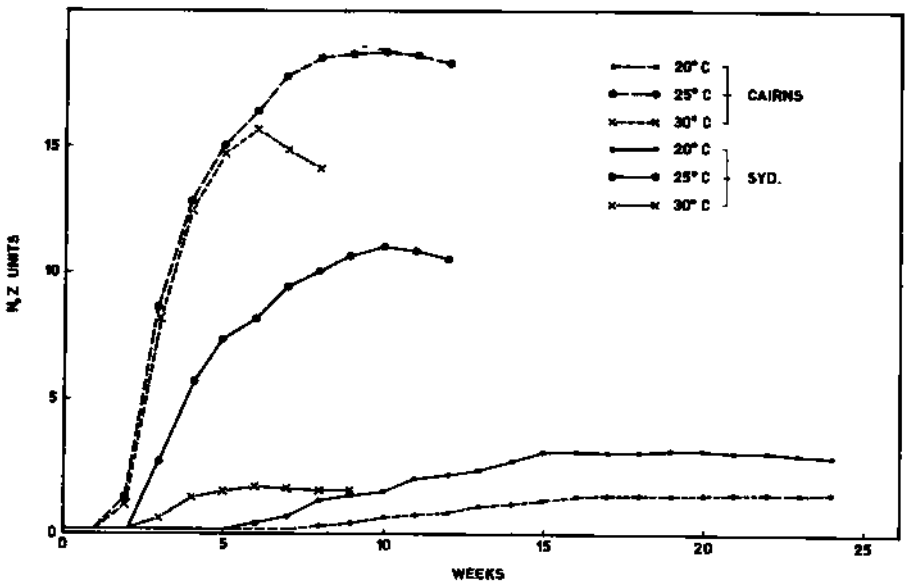

Fig. 1

Calculated productivities of two strains of Dacus tryoni at three temperatures plotted against the age at which weekly cohort is discarded.

at different temperatures. These graphs were calculated from the data of BATEMAN [5]. These two strains have very different maximal productivities as shown in Table III.

For comparison, similar graphs are shown for Dacus oleae as calculated from HAGEN's [6] published data (Fig. 2).

In practice, cultures are much more crowded than in these experiments which could lead to changes in λ_x and m_x. Artificially cutting off the survival curve at the optimal age (t_d) may also select a different pattern of survival and fecundity. The equation suggests various ways in which productivity may be increased. Increased crowding raises N_0 and gives a proportional increase in production if λ_x and m_x remain unchanged. Also one can select for a shortened preoviposition period and increased λ_x, m_x and K.

In adult rearing, capital and overhead costs are usually much more important than costs of food and handling, which explains the emphasis in earlier paragraphs on the most efficient use of space and cages. In larval rearing, the costs of food and handling become very important. Here economics may be improved through increased crowding, through changes in concentration of dietary components and through cheaper kinds of food. At the same time one should try to maintain or improve the quality of the harvested insects. For example, the average production of Dacus tryoni for a single

TABLE III

MAXIMAL PRODUCTION OF CAIRNS (QLD.) AND SYDNEY (N.S.W.) STRAINS OF DACUS TRYONI AT 25°C

Strain	Input of new females per week	Age when destroyed	Harvested adults per week
Cairns	5×10^4	10 weeks	9.26×10^5
Sydney	5×10^4	10 weeks	5.42×10^6

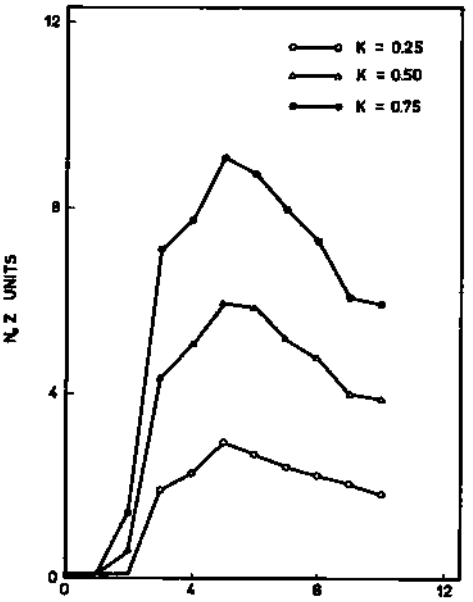

Fig. 2

Productivity of Dacus oleae at 25°C for different levels of egg to adult survival (K) plotted against age at which each cohort is discarded.

tray (2.5 l) of the standard carrot-yeast medium [7] was improved from 3040 pupae using 0.75 ml of eggs and a yeast concentration of 26 g/l to an average of 14 000 pupae from 2 ml of eggs at a yeast concentration of 102 g/l. At the same time the average weight of pupae (a measure of quality) rose from 9.3 mg to 10.8 mg.

With almost every species much work remains to be done on the economics of mass rearing and on the selection of animals adapted both to mass rearing and to their task in the field.

on a single wooden frame are optimal. All the eggs produced by the unit are collected in a single receptacle. The position of the cages on the frame is staggered so that the top cage extends forward on the frame and each succeeding cage is slightly inset so that all the eggs have a free line of fall to the receptacle. Each unit is used for a period of two weeks at which time the survivors are discarded. It is convenient and economical to work on a surplus egg system allowing at least two eggs for each pupa finally required. In a continuous production schedule of one million pupae per week, the output of six producing units (thirty cages) of ovipositing flies is required. On the average, each unit produces approximately 50 000 eggs per day. Collection and volumetric estimation of the number of eggs of all the producing units require about ten minutes per day for the 300 000 eggs.

Larval medium. The chief improvement in rearing is, however, the development of a larval medium in which inexpensive and readily available wheat bran replaces the carrot powder allowing the production of one million pupae at a cost in larval food of less than $5.

Larval medium is prepared weekly. The medium is held in a cold room at 5°C until required. The expected return per 2326 g portion is 30 000 or more pupae from approximately 50 000 eggs. The medium formula is given below.

Constituent	Quantity(g)
(1) Water	1200
(2) Nipagin (the control of contaminating microorganisms is affected) (Merck)	3.0
(3) Nipasol (Merck)	3.0
(4) Sucrose	300.0
(5) Brewer's yeast, dry	200.0
(6) HCl, 1N 23%	20.0
(7) Wheat bran, small grains	600.0
Total	2326.0

The best results are obtained by heating the water to boiling point, then adding the preservatives Nipagin (methyl p-hydroxybenzoate) and Nipasol (propyl p-hydroxy benzoate) and heating until these ingredients are completely dissolved. This solution is transferred to a large capacity mixer and the sugar and dry brewer's yeast added. Next, approximately 20 ml of 1N HCl is added and finally the wheat bran. After mixing, the pH of the medium should range between 4.3 and 4.5, but sometimes further adjustment with HCl may be necessary. According to the particle size of the bran, an adjustment in the amount of water may prove advisable. The diet mixture, loose but not flowing, is then transferred to plastic refrigerator boxes (30 × 25 × 7.5 cm) to a depth of about 2.5 cm. The boxes are covered immediately with a glass plate.

B. MEDITERRANEAN FRUIT FLIES AND RELATED SPECIES

Rearing

ISRAEL (16)

Oviposition. A system of mass rearing of the Mediterranean fruit fly without leakage even under the most primitive insectary conditions has been developed. One laboratory technician can produce one million pupae per week with a weekly work load of 6 to 8 h when laboratory conditions are held at a temperature of 25°C and a relative humidity of 60%.

The oviposition cage is a rectangular plastic box (35 × 19 × 20 cm), closed by a removable nylon-screened frame. One face of the box is cut out and a fine-mesh silk cloth is heat sealed into the opening. The cage is placed with the silk screen facing a light source and the female flies oviposit through it, most of the eggs falling to a receptacle containing water (see Fig. 3). Each cage is set up with about 3000 pupae, a water source and about

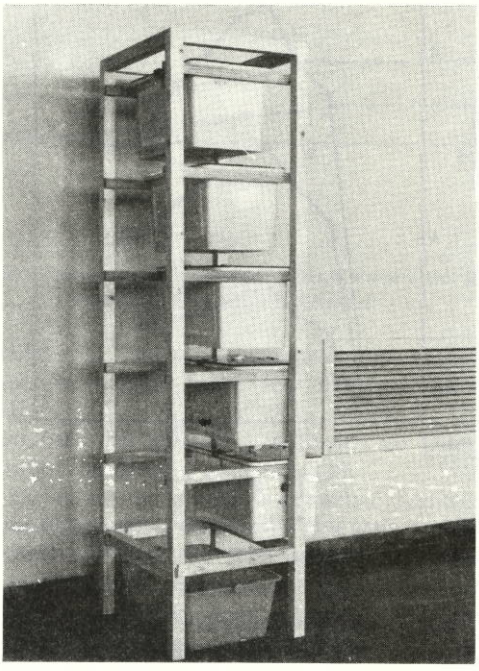

Fig. 3

Oviposition cages staggered to permit eggs to fall into receptacle on the floor.

90 g of food consisting of 3 parts sucrose to 1 part of Fleshman's Type M yeast mixed together. After loading, the cage is closed by clamping the nylon-screened frame (see Fig. 4) in place. The cage remains closed through the entire oviposition period. For convenience, units of five cages

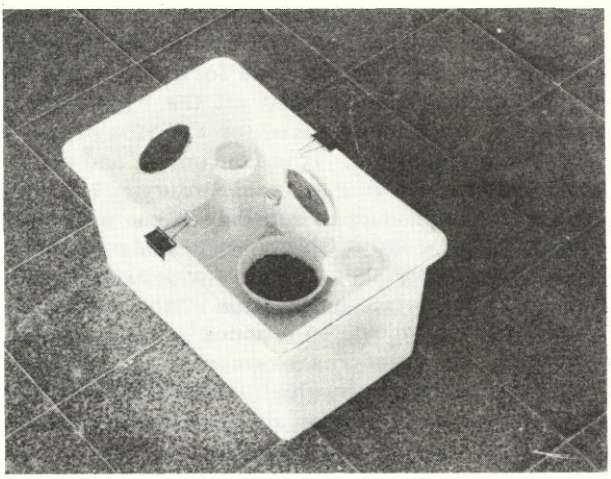

Fig. 4

Oviposition cage with food, water and pupae. The lid is clamped in place.

Egg collection. Eggs are collected daily and their numbers estimated volumetrically: 20 000 = 1 ml. Approximately 250 000 eggs are segregated and placed in a common table salt shaker with a small amount of water to act as a carrier. The appropriate number of larval food portions are placed side by side on a work bench and the eggs are shaken evenly over the entire surface area of the portions resulting in a distribution of approximately 50 000 eggs per portion. The boxes are again covered with glass plates to ensure maintenance of maximum humidity and then placed on open-bottom holding racks where they remain until one day before pupation. Control of the humidity and temperature of the medium by adjustment of the glass plate is essential. For the first three days the plate covers the box entirely resulting in a high humidity bath for the eggs and newly hatched larvae. From the fourth to the seventh day, it is necessary to progressively open the box so that only a few if any larvae are visible on the surface of the medium. If a box inadvertently remains closed, the temperature rises and the medium becomes unsuitable. Proper adjustment of this simple temperature-humidity control device is the key to successful production. On the seventh day the glass plates are removed and a thin crust is allowed to form on the surface of the medium. On the eighth day the crusts are removed and the moist larvae-filled media are transferred to plastic strainers on an emergence stand. The stand, of sheet metal or wood, is simply a series of chutes set at an angle, each chute capable of holding a number of strainers loosely covered to insure against drying and unwanted pupation in the medium. The emerging larvae fall from the plastic strainers to the chute and make their way to a collection box set at the bottom (Fig. 5). The portions are held in the chutes for a maximum of four days after which they are discarded. Maximum larval emergence takes place on the tenth day. The pupal collection boxes containing fine sand to discourage larval movement and permit quicker pupation are changed twice daily. Following complete pupation of

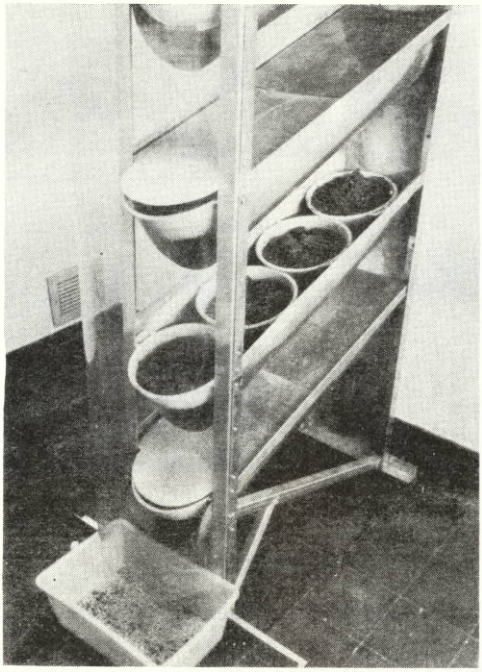

Fig. 5

Larval collection arrangement.

one day's collection, the sand is sifted off and the pupae are measured – 50 000 pupae per litre. The pupae are then transferred to screen-bottomed holding boxes (Fig. 6).

Laboratories initiating or conducting rearing research should be particularly aware that humidity plays a significant role in successful larval rearing. Also mould formation can be eliminated by replacing sodium benzoate with Nipagin or Nipasol; moreover, additional benefits can be derived. It was noted that upon change of these materials, Drosophila larvae could not compete successfully in the two litres of medium containing approximately 30 000 Ceratitis larvae. Insecticide contamination of the wheat bran can also be a serious problem, but if one purchases a bran that is slightly infested with grain beetles, this hazard is eliminated.

The production time for one million pupae per week is given in Table A.

TUNISIA (18)

Rearing. Adults are usually provided with solid food consisting of sucrose and powdered yeast hydrolysate which are placed separately in the cage before the introduction of the pupae which are about to hatch. A mixture of these two nutrients is used since tests have shown that higher laying yields may be obtained in this way while avoiding deliquescence of the hydrolysate (50 g of sucrose and 25 g of hydrolysate to each cage containing 5000

TABLE A

PRODUCTION TIME FOR ONE MILLION PUPAE PER WEEK

Operation	Time (min)	Frequency of tasks			Weekly Total
		Daily	Semi-weekly	Weekly	
Egg					
Collection and counting	15	X			105
Distribution on diet portions	2	X			14
Larvae					
Preparation of larval diet	60			X	60
Adjustment of glass plates	2	X			14
Mixing of portions on emergence rack	5	X			35
Transfer of diet portions to strainers	10	X			70
Pupae					
Collection and counting	3	X			21
Adults					
Preparation of adult diet	11			X	11
Preparation of five cages	15		X		30
Miscellaneous					
Cleaning and transferring of cages, discarding used portions, etc.	20	X			140
				Total	500

The total production time for 1 million fly pupae is approximately eight hours per week.

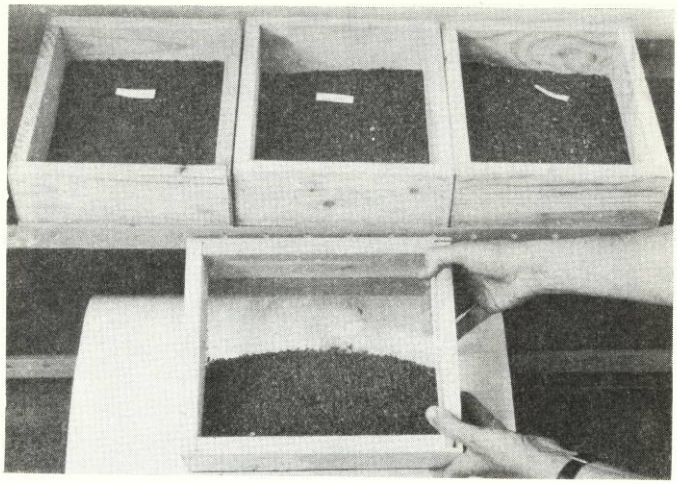

Fig. 6

Pupal holding boxes.

Ceratitis). A piece of glass-fibre screening is placed on the mixture to prevent the flies from sticking to the food.

Water is supplied by means of a damp cloth-sponge strip which rests on the glass-fibre mesh forming the roof of the cage and is immersed in the water tank fixed between the oviposition domes at the front of the cage. To avoid pollution of the water, a solution of two parts per thousand of benzoic acid is recommended.

To achieve a certain degree of automation, cages were modified having the entire front portion removable. This consists of a sheet of transparent plastic in which are inserted and fixed the oviposition domes in the form of pliable red plastic cups pierced when cold with a very large number of fine punctures. The oviposition dome, which is accessible from the outside, is closed by a plastic plug fitted with a button. A high level of humidity is maintained by means of a strip of sponge-cloth attached to the interior surface of the cup.

Each morning the eggs are collected by means of a jet of water directed into the oviposition domes from a small hand sprayer from which the anti-splash nozzle has been removed. The eggs are collected in a tray together with the water and are then separated by means of a fine nylon filter. The sponges for maintaining the humidity of the oviposition domes are rinsed separately. The total quantity of eggs collected may be measured volumetrically in a graduated tube, the bottom of which consists of a nylon plug. The amount of eggs necessary to impregnate the host material is measured by means of a spoon of triangular section which can easily be filled with the drained eggs.

The spoon, with a volume of 0.5 ml, holds 14 500 eggs (average of 20 counts) when filled level. With a spoonful of this volume 1 kg of larval medium can be impregnated; the eggs are spread over the medium by allowing benzoic acid solution to drip on the spoon, causing a gradual overflow.

Larval medium. The larval medium is obtained by mixing the following ingredients:

Powdered carrot	94.0 g
Powdered brewer's yeast	55.5 g
Granulated sucrose	80.0 g
HCl solution (165 parts per 1000)	20.0 ml
Benzoic acid solution (2 parts per 1000)	638.5 ml
Fresh pure hard wheat bran	112.0 g
Total:	~1000.0 g

The medium is placed in polythene trays with an internal diameter of 30 cm (over-all diameter 35 cm), each tray holding 1 kg of medium. The lid consists of another tray from which the bottom has been removed and to the sides of which has been welded a fine metallic screen forming a barrier to Drosophila. The loaded trays are stored at 25°C and 85% relative humidity for seven days which is the time needed for the earliest larvae to develop. The medium, which has so far not required stirring or any additional ingredients, is transferred to the pupal holding room where it remains until the 12th to 13th day. In the holding room the medium must be stirred every day; this helps the larvae to emerge, but dries out the medium somewhat so that it may have to be moistened with a two parts per thousand benzoic acid solution.

One kilogram of the medium produces on an average about 10 000 pupae, a yield of 69%. One hundred pupae consistently weigh 0.9 g.

Under these conditions, and bearing in mind the percentage of pupae which must be set aside for breeding purposes, a daily output of one million pupae for sterilization requires about 100 kg of medium at a cost of approximately US $50.

Laboratory studies

COSTA RICA (9)

Radiation. In tests on irradiation dosages and mating behaviour in the Mediterranean fruit fly, a dosage of 10 kr or higher is necessary to destroy all egg-forming tissue present in the ovaries of the female pupa regardless of its age as well as to produce almost 100% dominant lethals in all the sperms of males irradiated either at the 7-, 8- or 9-day-old pupal stage. With dosages less than 10 kr, fewer dominant lethals are produced as the pupal development progresses. From the practical point of view, this wide range of optimum stage of pupal irradiation provides ample time for the mass irradiation needed for field releases.

Since 10 kr and 12.5 kr seem to be the practical dosages for inducing at least one dominant lethal in every sperm, the effect of these doses on the longevity of the adult was studied. The adult longevity was not affected ad-

versely by these levels of radiation. On the contrary, the 10 kr treatment enhanced the longevity of the males slightly, and the females to a greater extent.

As the 7-day-old pupal stage seems to be the first practical stage for mass irradiating, it was desirable to know the upper limit of radiation dosage that has no effect, either on adult emergence from the pupae, or on the longevity of the emerged flies. Dosages beyond 70 kr are 100% lethal to the pupae. The treatment with 20 kr does not influence adult emergence or subsequent survival. Radiation dosages greater than 20 kr reduce the longevity of the emerged adult; the higher the dosage, the greater the reduction of the adult's life.

In mating competitiveness tests the results indicate that the sterile males reduce the fertility of normal females at all the ratios tested, but the irradiated males do not seem to compete equally in mating with normal males. If the mating competition were equal between normal and sterile males, in the population with a ratio of 1 irradiated male : 1 normal male, the egg fertility should be 50% in the normal females, instead of 74% as observed in the experiment. With a flooding ratio of 39 irradiated males : 1 normal male, the female fertility was reduced to 89%. This indicates that field population should be overflooded with irradiated flies at the rate of 39 or higher.

Radiation reduces the insemination vigour of the males. During the 4th week of adult life, a normal male mates on an average 14.64 times compared to 9.76, 7.58, 4.02, and 1.52 times for the males irradiated in the pupal stage 0, 24, 48, and 72 h before emergence, respectively. The normal males are about twice as vigorous in mating as the males irradiated 0 or 24 h before emergence. The closer the irradiation time to adult emergence, the higher the insemination efficiency of the treated males. The males irradiated 0 h before emergence inseminate at a higher rate and for longer periods than those irradiated 24, 48, or 72 h before emergence.

FRANCE (8)

Radiation. Tests were initiated in September 1963 to determine the optimum irradiation dosage and insect age necessary to produce a sterile population of Ceratitis capitata that would be competitive with normal flies. The flies were reared in a carrot medium, at temperatures of 26°C and 80% relative humidity with a light-dark period of 12 h. The flies were irradiated in a cobalt source of 2000 curies and the dosages used ranged from 5 to 17 kr.

Complete sterility was obtained in all females that were irradiated as 4- to 8-day-old pupae at dosages of 5 kr or above, but it was found that 5 to 15 kr produced 100% mortality in 2- to 4-day-old pupae. Five and 10 kr on 5-day-old pupae resulted in poor eclosion, but the same dosage did not affect eclosion in pupae from 6 to 8 days old. At 15 kr, eclosion was poor in pupae irradiated when six days old and normal in pupae irradiated on the 7th and 8th day.

Males were more severely affected by irradiation than females. Dosages of 15 kr caused the males to be very weak and produced high mortality within a few days. Male fertility (as assessed by crosses with virgin females)

reached 30% after a 5 kr dosage but was lower after dosages of 7 or 10 kr, in cases of pupae irradiated on the 6th, 7th or 8th day of age. As irradiated males mature, there is an obvious loss of sexual vigour.

In mating-competitiveness experiments it was found that irradiated males do not compete as readily as normal males. A 16 : 1 ratio (16 sterile males to 1 normal male) was necessary to produce an 80% reduction in fertility and 32 and 64 : 1 ratios were needed to produce more than 90% sterility.

These are the results of a first series of experiments. From these preliminary results it appears that the optimum age and dose is 8-day-old pupae irradiated with 8 to 10 kr. To be effective the sterile : wild male ratio should be in the order of 30 : 1.

Field studies

AUSTRALIA (15)

"Flushing" of Dacus tryoni. "Flushing" or population displacement by means of sterile animals was put forward as a hypothetical model [8] at the first International Atomic Energy Agency (IAEA) Panel on sterile males in 1962. The method depends on overloading resources with sterile animals thus causing a loss of both sterile and fertile individuals. By continued releases the wild population would become more and more diluted with sterile animals. In principle, after sufficient successive releases the wild population would be completely replaced by a sterile one.

Empirical evidence now shows that this type of displacement can be induced in the Queensland fruit fly. Near Sydney during the winter of 1963, three out of four small wild populations (each of which ranged in numbers from about 100 to 200 individuals) were reduced to 15 to 30 by a single release of about 500 sterile flies (see T_1, T_3 and T_4 in Fig. 7). One release (T_2) failed, probably because the flies had inadvertently been irradiated too early during morphogenesis. They were "bleached" of yellow pigment and sluggish. In the successful trials "flushing" followed soon after release and was almost certainly not due to exhaustion of food. The most likely explanation is that increased social contact and fighting caused the flies to move away. Three control populations showed no such sudden fall in population in the middle of the experimental period, although one showed a continuing downward trend from the beginning.

Another experiment with single massive releases of flies in the centre of the town of Warren in autumn 1963 seemed to push the natural infestation to the periphery of the town in the following spring, but this observation should be repeated.

By releasing heavily in the centres of aggregation of a pest, "flushing" might drive the population out into the less favourable periphery. If carried out at the onset of an unfavourable season, this could increase the mortality of the wild population. Similar central releases might also force a pest away from a threatened crop.

Experimental control of Dacus tryoni. After favourable results in laboratory and field cages, sterile males were released in two towns in New South Wales (N.S.W.): in Manilla during 1962-63 and in Warren during 1963-64. Probably because our factory failed to produce its intended 0.5 to

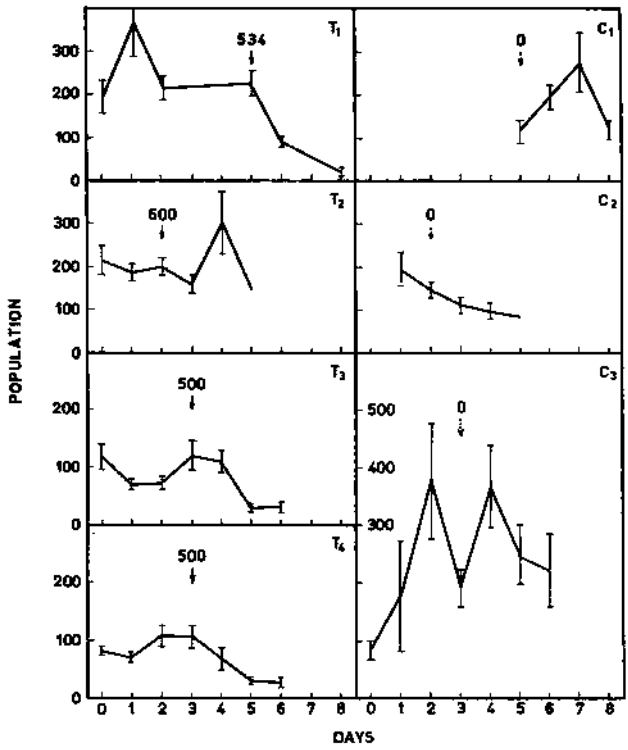

Fig. 7

Estimates of numbers in four populations T_1, T_2, T_3, and T_4 into which sterile laboratory reared adults were released at various times and in the numbers shown by arrows. C_1, C_2, and C_3 are comparable populations into which no flies were released.

1.0 million pupae per week, control was not achieved in Manilla. However, the following observations were made in this town:

(a) Infestation of the loquat crop in spring 1963 was lower than in the control towns.

(b) Released sterile females caused negligible damage by stinging fruit with their ovipositors.

(c) From the eggs laid into loquats in spring 1963, only 49% hatched compared with 95 and 94% in two comparable control towns.

(d) Adults released in Manilla in winter 1963 did not seem to survive well in comparison with the wild population. Releases of Dacus tryoni in the southern part of the range should probably not be made in winter. But selection of a strain resistant to cold could reverse this conclusion.

From late spring 1963, the population in Manilla was obviously out of control so releases were concentrated only in the small town of Warren (area 1.5 to 2 square miles). An average of 1.17×10^5 pupae per week were released here from April 1963 to March 1964. Control was achieved

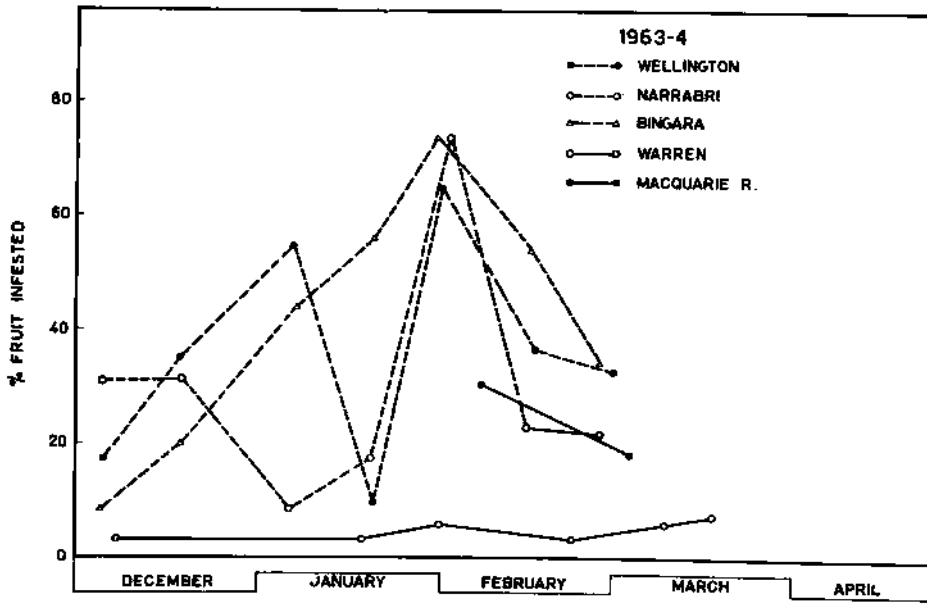

Fig. 8

Fruit infestation (Dacus tryoni) at Warren in summer 1963-64 compared with 3 control towns and an area along the Macquarie River adjacent to the treated area.

as shown in Fig. 8. Three untreated towns showed much higher rates of fruit infestation than in Warren. Eradication is difficult to demonstrate in such towns in central and western N.S.W. because they are in contact with outside populations which seem to move into them along the water courses. However, these results give every reason to believe that isolated populations could be eradicated and southern outbreaks suppressed by means of sterile males.

COSTA RICA (13)

Field releases. Field tests were initiated at Puntarenas, Costa Rica (a 2.5 km^2 peninsula) to determine the effect of releasing sterile Mediterranean fruit flies on a very dense population of wild flies. The area selected is fairly well isolated but some migration into the area from adjacent breeding areas was likely. The principal fruit fly host was the tropical almond, a very abundant and preferred host of this insect. Releases were begun in July 1963 and continued until May 1964, when the last observations were made for this Report. Releases in 1963 ranged from 0.5 to 2.2 million per month. In 1964, the releases ranged from 5.5 to 6.5 million per month; all releases were from emergence cages placed in the field. One application of protein hydroloysate and Lebaycid (O, O - dimethyl O-[4-(methylthio)-m -tolyl] phosphorothioate) was made in both February and March to reduce the numbers of fruit flies to reasonable amounts.

The results of these releases reduced the per cent of infested fruit from 38% in August 1963 to 22% in May 1964, but the number of larvae per fruit was lowered from 3.13 in August 1963 to 0.6 in May 1964. These results suggest that while there was only a moderate reduction in the per cent of infested fruit, there was at least a five-fold reduction in the number of larvae per fruit. Further research is in progress.

ISRAEL (16)

Release studies. Initial experiments were carried out in 1963 to study the techniques of mass field release of irradiated Mediterranean fruit flies. Over three million irradiated flies were tagged with either Calco blue or P^{32} or both and released as pupae and adults. Preferential male trapping at the release site was carried out with trimedlure (trans-6-methyl-3-cyclohexene-1-carboxylic acid hydrochloride-tert-butyl ester) in Steiner-type dry traps. The number of released flies recaptured was extremely low. It was noted, however, that in three field trials the number of wild flies captured was greater than the number of irradiated flies. Moreover, the number of irradiated flies recaptured was inversely proportional to the dosage – the higher the dosage, the lower the number trapped.

In 1964, after having improved rearing techniques, a more vigorous fly was released. With these flies, results were encouraging and over 10% of the released insects were recaptured. The high female fly recovery in the trimedlure trap was attributed to the absence of natural food.

TUNISIA (18)

Population dynamics and ecology. Population dynamics were studied mainly by applying the Lincoln index. Each month 2000 Ceratitis males, labelled with P^{32}, were released and recaptured within a non-irrigated apricot orchard one hectare in area. As from 1964, a second orchard with the same area and situated more than 1 km from the first is also being used for population estimates. This orchard is irrigated and contains citrus fruit trees. We shall thus be in a better position to determine what happens in winter when the trees of the first orchard are almost all without leaves and no longer contain any fruit flies.

Dispersion observations. The presence of labelled flies also makes it possible to observe the tendency of the flies to stray from the orchards.

A first set of data was provided by three groups of ten medlure (sec-butyl trans-4(or 5)-chloro-2-methylcyclohexene-1-carboxylate) traps – identical to those used for the population estimates – set up at about 50 m from the release area and maintained for 24 h. Moreover, between 300 and 500 m around the same area, a cordon of 150 medlure traps was maintained for the three days following release in order to detect dispersion over greater distances. Such dispersion is very slight at Ras el Djebel, since the region is interspersed with wind-breaks. No labelled flies were caught either in summer or winter; the most ever caught being four.

Capture curves. Eleven control stations (each consisting of ten fruit trees of the same species) dispersed widely throughout the orchards at Ras el Djebel and containing an equal number of fly traps baited with protein hydrolysate, provide data from which the capture curve is plotted. Since 1 January 1964 dry medlure traps, which are definitely more attractive to the insects, have replaced the fly traps.

Degree of infestation. Sample batches of ripe fruit weighing 5 to 10 kg were gathered each week and placed in holding rooms for the purpose of determining the degree of infestation.

In 1963, infestation counts of the crop as a whole, involving large quantities of fruit (more than 40 000 units), were carried out in order to check the results obtained in the holding rooms. However, the difficulty of detecting by direct observation in the orchard late punctures and the beginnings of larval development caused us to abandon this method. Consequently, larger samples of ripe fruit were gathered in 1964 and placed in holding rooms, this being the only reliable way of ascertaining the importance of various species of host fruit in the rapid multiplication of Ceratitis.

"Fauna" shock treatment was applied at monthly intervals in one orchard and subsequently in two, situated at equal distances from the release area, with the object of confirming the population estimates. Four bitter orange trees, four apricot trees and four fig trees — representing winter, spring and summer crops — were used. However, the rather strong winds which characterize the Ras el Djebel region often made it somewhat difficult to fix in place beneath the trees the sheets of paper for catching the poisoned insects; in addition a proportion of the insects were blown away. For these reasons, although it did provide some information, shock treatment was not repeated in 1964.

From January to June 1963 the number of insects trapped was low or zero. Nevertheless, the bitter oranges were infested, although very late. A few hours of sunshine are, of course, sufficient for Ceratitis to oviposit and, when the fruit is subsequently brought into the laboratory, the temperature allows the larvae to develop rapidly. In the case of the bitter oranges, almost 15 larvae per fruit were observed during May. This confirms, for the fourth consecutive year, the responsibility of this host fruit in the early multiplication of Ceratitis. The bitter orange trees provide a basis for the rapid multiplication of the fly during the warm season.

In May, June, and July relatively few pupae were found in the medlars, or even in the apricots and summer figs. This was doubtless due to the late start of the fine season, since the two latter species are generally subject to severe attack. Some pears were, however, infested.

In July the rapid multiplication of Ceratitis began suddenly (1910 males per hectare) and continued into August (4674 males per hectare). The summer figs, and later the peaches (rare at Ras el Djebel) and late figs showed considerable degrees of infestation, whereas the prickly pears were much less infested. The largest numbers of flies were caught between 4 and 28 August in the fly traps on the bitter orange trees and medlars (cultivated by intercropping).

In September the population in the release area fell sharply (2034 males per hectare). Allowing for underestimates due to the light rain falling at the time of release, this decline may be ascribed to the fact that this orchard

contains (with a few exceptions) only deciduous trees. These were no longer bearing fruit and, in particular, the apricot trees in the non-irrigated orchard were beginning to lose their leaves.

At the same time the traps in the dispersion study cordon, which were generally kept in position for three days, benefited from the hours of sunshine following the rain. Moreover, the cordon of traps skirting several citrus fruit orchards picked up flies which had come to find new host fruit.

During September the highest degree of infestation was observed in late peaches, late figs and prickly pears, as well as a few apples. From 11 to 25 September the figures for flies caught by the fly traps showed a high concentration of Ceratitis on the prickly pears and figs.

In October there was a fall in the capture curve and in the population estimates. The degree of infestation of the fruit also remained fairly low.

In November there was an increase in population (1745 males per hectare) while the infestation of citrus fruit also became more serious, particularly that of tangerines and clementines. The bitter oranges were again seen to be very important host fruit.

From December 1963 to January 1964 the capture curve fell away almost completely. It was at this time that the dry medlure traps replaced the fly traps baited with protein hydrolysates. Laying continued on the bitter orange and other citrus fruit trees and these infestations persisted at a moderate level until the end of March. During the winter months the release-recapture operations showed that the populations were remaining extremely low, falling to 116 males per hectare in December and 40 males per hectare in March.

Beginning in April the Ceratitis populations resumed the same customary cycle of infestation as is observed during the normal year. The first spate of rapid multiplication in the year again took place on the bitter orange trees, with an average of seven larvae per fruit collected. The population estimates rose to 126 males per hectare.

In May, with the bitter orange trees exhausted, a few oranges still remaining on the tree were subject to attack, although of much less severity. At the same time the first apricots with maggots were noted, apricots being subject to severe attack during the following month. The estimated population was 99 males per hectare.

In June, the population suddenly underwent a tenfold increase (959 males per hectare); during one week the capture curve rose from 13 to 1042 flies. This sudden increase in population was a consequence of the very high degree of infestation observed in summer figs and late apricots. The peaches and apples were also attacked.

Subsequently pears, late figs and other warm-season fruit enabled the following generations of Ceratitis to reach their annual maximum.

This study has shown the extraordinary ability of Ceratitis to multiply even during a year with a hard winter (1963). During the same year a warm autumn again produced severe attacks on citrus fruit.

This extremely rapid increase shows clearly the need to make the first releases of sterile males at the end of winter or the beginning of spring, during the very first warm days, even if these warm days are followed by a period of bad weather. In a normal year this period starts about 15 March.

At present, however, the release of sterile males serves only to neutralize the Ceratitis emerging from the bitter oranges.

It can be seen from the above statements that, in the special case of Tunisia, these operations must be preceded by the elimination of the bitter orange tree. This species of tree bears a fruit which is the favourite host fruit of Ceratitis over a period of six months.

USA (19)

Field releases. The first field test of the sterile-fly method on a tephritid species utilized a 12.5 square mile area in Hawaii, where 18 million Mediterranean fruit flies were released during a 13-month period in 1959-60. This produced a mean 90% reduction in infestations in English walnuts and various deciduous and wild fruits for the final six months; but immigrating wild flies from several miles away prevented greater suppression. The presence of a low infestation of oriental fruit flies that maintained its normal level throughout the experiment indicated that the decrease in Mediterranean fruit flies from normal levels was not caused by any peculiar climatic conditions. Following termination of releases of sterile Mediterranean fruit flies, reproduction within the area brought the infestation up to its usual level within two generations.

Rota is an island located in the Mariana Islands about 37 miles northeast of Guam. It covers an area of 33 square miles and has a plateau of 500 meters' elevation at the southwest end and another of about 150 meters' elevation at the northeast end. Winds during the dry season are predominantly ENE trades that tend to push fly populations to the southwest end of the island where conditions are most favourable for fly reproduction throughout the year. During the rainy season winds are more variable, the host situation improves on the lower plateau, and the flies spread back over the whole island. Annual temperatures range from 23 to 34°C with precipitation from 80 to 100 inches.

Fly production and logistics. The Western Pacific work was supported by the fruit fly rearing facility at our Honolulu Laboratory. Generally 5 to 11 million pupae of the species being used were produced weekly at the laboratory. Pupal development was synchronized by using two holding temperatures (about 20 and 27°C) so that pupae from many lots would reach the required stage of development for irradiation and shipment one day before emergence each week. The shipments were made on regularly scheduled commercial airline jets, and the 3800-mile flight to Guam was usually made between midnight and daylight, or within a period of eight hours.

The minimum dosage was 9.5 kr . Dosages less than this permitted some loss of sterility in old flies in each of the three species in Hawaii. Irradiated pupae were packed in six-tray, well-ventilated shipping cartons. For ground releases these cartons were utilized as emergence cages when placed inside specially designed screened cages.

Fly distribution. About 3 000 000 pupae of each week's shipment were placed in approximately 800 drop boxes in an air conditioned room held at 27°C. The boxes were then flown to Rota, opened in the plane, and dropped

in lines half a mile apart on the second day by which time emergence was complete.

During the first experiment, which utilized the oriental fruit fly, a strain with white instead of yellow thoracic markings was segregated and released exclusively for 12 months. Well distributed plastic traps baited with methyl eugenol were used to measure fly abundance and marked sterile: wild fly ratios.

Twenty to twenty-six ground release cages were utilized for releasing up to seven million additional flies each week in areas where wild populations were heaviest. A total of 531 million oriental fruit fly pupae that produced 403 million healthy flies were shipped and released between September 1960 and 1 August 1962. In May 1962, sterile oriental fruit flies outnumbered wild flies by 58 : 1 in the upwind half of the island, but never reached more than 10 : 1 for a one-week period in the southwest portion and never averaged more than 4.6 : 1 for a full month over the island as a whole.

By use of extensive release/recovery data collected over several years, it is possible to construct a formula that will, when based on catches in well-distributed non-competitive traps, give approximate release rates required for 10 : 1 overflooding. These estimates indicate that during that part of the year when the population birthrate reaches its lowest level, generally in April, a release rate of at least 16 million sterile flies per week would have been necessary to obtain 10 : 1 overflooding. When populations on Rota reach their high point during the year, the requirements during the months of September and October in three different years would have ranged from 500 to 800 million sterile flies per week.

In the first experiment with the oriental fruit fly on Rota the fruit infestations were not depressed. Maximum monthly infestations for the three years 1960-62 averaged 110 larvae per pound for breadfruit in October, 73 per pound for mango in June, and 50 to 51 for papaya in July and August.

Rota island. Field releases. On Rota, melon fly hosts were concentrated on less than 40 farms, and melon fly traps averaged nearly 15 times as many insects near host plantings as a short distance away. This was found true of sterile males as well as normal flies. It is significant that sterile flies of both species begin moving into host areas before the males become sexually mature. This indicates that irradiation does not affect the natural behaviour of the male. Where a host is widely distributed, the application of any measures needed to suppress heavy infestations in preparation for a sterile-fly release programme would have to be widespread and suppressive measures could be applied at much less cost. Because of this, it was possible to reduce the mature melon fly population by nearly 75% with 4 to 6 weekly bait sprays of protein hydrolysate/malathion applied in August and September to about 1% of the land area containing melon fly hosts. The total cost was only $300 for the 33 square mile island. Spot spraying could never accomplish complete eradication because it would not kill all the flies that either leave the farms to find food or travel between host areas, but it may prove worthwhile in reducing the number of sterile flies to be released later.

Bait sprays and the natural seasonal decline in late September reduced the melon fly trap catches to 0.65 fly per trap-day at the time of the first sterile fly release.

From 305 million melon fly pupae shipped to Guam for this second experiment between late September 1962 and 4 July 1963, 240 million flies were released (i.e. nearly 7 million per week – two-thirds from ground release cages and one-third from the air). The first three releases were marked with a blue dye by coating the pupae so that upon emergence the fly would retract the dye into the ptilinium and its presence could later be detected by crushing the head with a flat-tipped bolt dipped in acetone. A ratio of 13 marked sterile to 1 wild fly was obtained by the time the unmarked sterile flies from the fourth release began appearing in the traps. Since the dye-marking was not effective against 100% of the pupae, and since fly catches increased to 54 per trap-day at a time of season when the normal catches were one or less, the ratio of more than 30 to 1 was believed to be attained within a month after the start of the experiment.

Typhoon Karen, which devastated Guam on 11 November, struck Rota with 80 mph winds. Tall vegetation on the south side of the island was damaged but most melon fly hosts escaped injury. Fly populations were temporarily reduced but the reduction was greater among sterile flies than among wild flies because egg, larval, and pupal stages of the latter in fruit and soil escaped injury. No ground releases could be made for two weeks and no aerial releases for eight weeks. Following the typhoon, pupae totalling nearly 18 million for the first three ground releases were also dyed. Fly catches then increased from 7 to 53 per trap-day at which time the ratio of marked flies to unmarked flies was 33 : 1, but the unmarked included many sterile flies so the actual ratio was probably 50 : 1. Since total per-trap-day catches reached 92 by the first week of January, the ratio at that time was at least 100 sterile to 1 wild and could have been as much as 500 : 1. Many marked released flies survived eight weeks or more, including the typhoon period.

To establish infestation levels, cucumbers and tomatoes were sampled at random, whereas cucurbits were limited to stung fruit collected in the immature stage. Normal infestations had been heaviest during the months of January to September, when the monthly mean values averaged as high as 15 larvae per pound of cucumbers, 34 for water-melon, 49 for cantaloupe, and 11 for tomatoes. In November and December 1960 to 61, cucumber infestations averaged 12.8 larvae per pound. In late 1962 the infestations began to decline soon after the first release. The last melon fly larvae to be found on the island for more than a year was taken in fruit collected on 26 December 1962, less than four months after the eradication experiment started.

Cage tests had already indicated that sterile females of all three species will puncture the fruit when confined together with it. Under field conditions, many sterile melon flies were observed extending their ovipositors when crawling on young fruit, but they were only occasionally seen making fresh punctures. It was evident that the mechanism that brought the melon fly female to the host was not affected by irradiation or failure of the females to develop eggs. The disappearance of sting injuries coincident with disappearance of the sterile flies from the island now indicates that most of the oviposition punctures observed after February were caused by the released sterile females, but the incidence of stings by these flies was ap-

parently less than 2% of that which would have been caused by an equivalent number of wild flies.

In the mid-summer of 1963 following eradication, more than 200 000 pounds of worm-free cucurbits, cantaloupes, and water-melons were shipped to Guam where at that time the melon fly was infesting 50 to 60% of the hosts grown there.

Late in 1963 and early in 1964 several wild melon flies appeared on Rota after strong southerly winds from Guam apparently helped to transport them. Establishment on Rota was prevented by a series of sterile melon fly releases amounting to about 6 million flies at the rate of 200 000 to 400 000 per week. This procedure required a long enough series of releases to have sterile flies at overflooding levels present when F_1 progeny produced by already fertilized immigrant females emerged.

The results of the melon fly experiment demonstrated, as anticipated, that the single mating habit in an insect species is not an essential requirement for successful adaptation of the method. Multiple mating does require extension of sterile releases until all wild females die. It gives the wild male some advantage over the sterile male, since 2nd matings by wild males (presumably because of their greater sperm content and aggressiveness) are more frequent and effective than those by sterile males. This can be compensated for by greater overflooding.

Guam. Oriental fruit fly eradication. During the summer of 1963 trapping on Guam for both species was intensified in order to measure population densities and distribution. One hundred pairs of methyl eugenol and "cue-lure" traps were well distributed throughout the 210-square-mile island. Although oriental fruit flies had been taken on the island regularly since Typhoon Karen one year earlier, high winds from Typhoon Olive, coming five and a half months later, again destroyed the tree fruits on Guam. Consequently the oriental fruit fly was unable to survive in most places on the island and the surviving population gradually withdrew to steep, narrow gulches or valleys that opened out across the north end of Guam. The valleys facing this direction were protected from the highest velocity winds of Typhoon Olive and still contained oriental fruit fly hosts.

A release of 1.2 million sterile oriental fruit flies of the white-marked strain was made at five points across the north end of the island close to the mouths of the valleys. Flies from these releases spread downwind as far as twelve miles in about the same pattern as had been reported for wild flies captured during the few weeks before the release was made. Most of the marked oriental fruit flies disappeared within a month and at the end of that period no wild flies remained. These results justified an attempt at eradication. However, two wild flies were captured in the middle of the island several miles apart and fifteen miles from the release site. Because of the apparent suppressive effect of the white strain, additional releases were made across the north end of the island and also 1/8 mile upwind of each of the two areas where the wild flies were last known to exist in November. No oriental fruit flies were caught on the island from November to late March when two additional flies were taken near the south end of the long island. It is believed that infested mangoes, imported before the island was placed under quarantine against introduction of oriental fruit fly hosts, supplied flies represented by these last two catches. Each of these areas

was overflooded by releases of 100 to 200 thousand sterile flies per week for several weeks and trapping was intensified. No wild oriental fruit fly has been captured in the last four months, thus the only flies caught on the island since November 1963 were the two taken in March 1964. It is believed that eradication has been achieved by releasing about 17 million flies.

This programme proved that both the white-marked laboratory strain and the wild flies were compatible under natural conditions — a fact that had not been proven because of the failure of the previous sterile oriental fruit fly experiment on Rota. Under laboratory conditions the sterile white-marked strain has been more aggressive and has been entirely compatible with white-yellow flies.

The results on Guam demonstrated the unusual opportunity for eradication that is available in the sterile release method when a species is under severe stress because of unfavourable environmental conditions.

Saipan-Tinian. Oriental fruit fly releases. In February 1964 a fourth programme designed to complete eradication of the oriental fruit fly from the Miarana Islands was started in co-operation with the Trust Territory of the Pacific Islands. This has resulted to date in the production and shipment from Hawaii of more than 90 million sterile oriental fruit flies at the rates of 3 to 5 million per week. Distribution has been by air over Tinian and Aguijan and mainly from ground release cages on the more accessible island of Saipan. The total area under treatment involves 90 square miles. Some factors, as yet undetermined, during a period of six weeks in April and May, accounted for a great reduction in longevity of our sterile flies and reduced our sterile:wild fly ratio of more than 200 : 1 to less than 30 : 1. But during the last generation the situation has improved and the ratio appears to approximate 300 : 1.

C. TSETSE FLIES

Laboratory studies

BELGIUM (7)

Rearing. Before attempting any mass rearing or initiating any eradication programme, a careful study of the particular insect is mandatory. Although much information of this nature is available on the tsetse fly (Glossina palpalis), it is preferable that individual ecological factors be analysed individually or concurrently in laboratory evaluations. With these factors in mind, a cage was designed for a dynamic approach to the problems of tsetse fly ecology whereby desired temperatures, humidities, air movement and illumination within close limits could be maintained as long as necessary or be changed cyclically as necessary.

The basic unit consists of 1 to 5 cages in tandem supplied with apparatus to maintain the cages at temperature gradients of 20 to 30°C with a relative humidity between 70 and 95% and to displace the total air at specific intervals. Although the unit in its present state still necessitates slight modifications, a sound ground has been established whereby very critical studies of an ana-

lytical nature on certain environmental factors in the tsetse fly can be performed.

CENTRAL AFRICA (6)

Status of tsetse fly research. The tsetse fly is distributed over much of Africa, and has been estimated to infest about three million square miles. It is absent from highland with altitudes above 6000 feet and from grassland due to the absence of trees. It feeds exclusively on vertebrate blood and during feeding may ingest blood parasites of the cosmopolitan genus Trypanosoma. During a subsequent meal these parasites, having undergone a cycle of development in the fly, may infect other vertebrates. These parasites have little or no pathogenic effect on indigenous African vertebrates, but they produce the often fatal sleeping sickness in man and nagana in domestic animals. Research and attempts to develop control methods have covered a span of fifty years and large areas have been cleared in central and eastern Africa. The most common methods are: clearing the bush, thereby destroying the fly's habitat; shooting game animals to reduce the food supply, and spraying insecticides either from the ground or from the air. These methods were, however, aided considerably by the Rinderpest disease in the early part of the century which destroyed many of the fly's hosts. In the past few years it has become increasingly apparent that the fly density has increased and that the tsetse is slowly recolonizing its former areas. This has resulted in an increasing number of repeated cases of nagana among the cattle herds along the fly front. If their advance is unchecked, it could mean a considerable loss of grazing land and a decline in the good economy of central and eastern Africa.

Extensive investigations since 1953 have shown that the present control methods are slow, expensive and not fully effective. They can slow the advance of the fly front but they cannot eradicate the tsetse fly except in small isolated areas. The spectacular successes achieved against the screw worm fly in the United States of America aroused interest and discussion on the possibilities of sterile-male release. POTTS [9] made a preliminary investigation on the possibility of sterilizing the tsetse fly with gamma-rays from a cobalt-60 source. He concluded that the fly could be sterilized with both 6000 and 12 000 r and that the irradiated flies were probably capable of competing with normal flies, though their longevity was reduced.

The initial objection against sterile-male release was the very low reproduction rate. The tsetse fly is larviparous and produces only one-third instar larvae every ten days. This larva is deposited on the ground it enters before pupation and remains there for about a month before the adult fly emerges. According to GLASGOW [10] a female fly may produce up to ten larvae during her life span. A mass-rearing technique was a necessary research problem especially as the fly is difficult to rear in laboratory colonies.

Research projects designed to investigate the problem of obtaining enough pupae or flies for sterilization are under way in Southern Rhodesia. Another project is concerned with climate and its limitations on tsetse flies and their breeding. So far this team has collected over 200 000 pupae from

around two permanent field camps. The majority of these pupae are used to supply the requirements of the chemo- and radiosterilization programmes. In addition, detailed records are being kept of the classes of sites, such as river beds, warthog holes, fallen logs, etc. from which the pupae are obtained. Seasonal shifts in pupae sites both within classes of sites and within vegetation patterns are recorded on a statistical sampling basis from the main ecological associations. The temperatures within these pupae sites are also being investigated with a view to applying the data derived from laboratory and meteorological studies.

Another line of research is radiosterilization. Investigations were initiated at the end of May 1964, and the first preliminary results can be considered only as trends at the present time. The irradiation was done with a low rate of emission source of cobalt-60 against field-collected pupae of undetermined age. Results indicated that irradiation between 3 and 12 kr reduced emergence. The untreated flies gave an 82% emergence while 6000 r reduced it to 63% and 12 000 r reduced emergence to 56%. The period of emergence from the untreated pupae was 30 days, but only 21 days for the irradiated pupae.

Irradiation appeared to increase mortality among pupae 9 to 16 days old, and mortality reached 100% among the pupae less than 9 days old. Examination of these pupae indicated that imago development was completed and that death occurred just before or during emergence. Male flies were more susceptible than female flies to irradiation.

Mortality during the first day after emergence was generally low, about 2%, but flies emerging from pupae less than 14 days old when irradiated were less viable, and mortality increased through the 6000, 9000 and 12 000 r treatments to a maximum of 18%.

Fly survival was followed during a 28-day period after emergence. Mortalities among untreated and treated pupae were similar for the first three weeks, and it was only during the fourth week that mortality in the irradiated flies increased over the controls.

Combinations of virgin male and female flies were caged for periods of 3 or 28 days in order to determine the success of mating and insemination. At least 83% of the female flies were inseminated and it was not possible to demonstrate that either the length of the mating period or the treatment caused any variation.

CENTRAL AFRICA (3)

Mass rearing and chemosterilization. Studies have recently been initiated on mass rearing and chemosterilization of Glossina morsitans (Westw.). Major emphasis is being given to rearing the tsetse fly in its natural environment. The philosophy of outdoor rearing stems from demonstrated difficulties in producing vigorous tsetse flies under confined laboratory conditions. A site has been selected amidst an existing natural tsetse population in the Zambezi River valley and research facilities for the rearing studies are now near completion. Cages enclosing areas of up to several acres of favourable natural tsetse habitat will be utilized in an attempt to obtain the most suitable conditions for reproduction.

Laboratory evaluations of chemosterilants require flies in numbers which can currently be obtained only by collecting pupae in the field. Emerging adults are sexed daily to insure the virginity of the experimental stock. To evaluate the reproductive capacity, 25 pairs of males (6 to 7 days old) and females (2 to 3 days old) were confined in cages. The caged flies were fed daily by placing a guinea pig under the wire enclosure portion of the cage. Throughout the third and fourth week, the larvae produced crawled through the wide-mesh cloth bottom of the cage and then pupated in a sand tray which remained under the cage. On the 28th day, the tray was examined and the collected pupae were then kept for six weeks to determine adult emergence. Under these conditions the insemination rate normally exceeds 95%, female survival averages 76%, and pupal production averages 24 per cage.

The chemosterilants tested gave promising results. Males were exposed when two days old to residues of 10 mg per ft^2 of tepa (tris (1-aziridinyl) phosphine oxide), metepa (tris (2-methyl-1-aziridinyl) phosphine oxide) or apholate (2,2,4,4,6,6,-hexakis (1-aziridinyl)-2,2,4,4,6,6-hexahydro-1,3,5,2,4,6-triazatriphosphorine) for either 2 or 4h. The tepa treatments resulted in the complete absence of adult progeny, while metepa allowed an adult production of 0.5 adults per cage. Apholate reduced pupal production but the results were inconsistent. The control groups produced 23 adults per cage.

TABLE IV

GLOSSINA MORSITANS (WESTW.) MALE SURVIVAL (%) AT INDICATED DAYS FOLLOWING EXPOSURE TO 10 mg/s ft^2 of TEPA

Days after exposure	13	20	27	34
VIRGIN				
Tepa	94	86	68	57
Control	95	84	77	65
MATED				
Tepa	82	56	50	43
Control	86	80	68	58

The effect of the tepa treatment on male survival is shown in Table IV. Both virgin and mated males which were exposed to tepa exhibited a moderate decrease in survival rate.

In another series of tests pupae were dipped for 60 s in 5% aqueous tepa solutions. The males emerging during the first week following treatment were completely sterile, whereas those emerging during subsequent weeks were not.

Preliminary tests on multiple mating by the females indicate that the effectiveness of an initial sterile mating is not reduced by subsequent mating. These encouraging results are being followed by further investigations in the laboratory and field.

D. SCREW WORM (Cochliomyia hominivorax(Coq.))

Laboratory studies

USA (17)

Even though the screw worm Cochliomyia hominivorax (Coq.) has been successfully eradicated from Curacao, the Southeastern United States and now has almost reached that goal in the Southwestern United States, concurrent work on the effect of gamma-radiation on this insect is still going on. The research has been of a developmental nature of immediate benefit to the eradication programme, and in the area of basic radiation research. BAUMHOVER [11] studied the effect of aeration during gamma-irradiation of screw worm pupae. This work was initiated because some of the irradiated females produced eggs subsequent to exposure to a presumed sterilizing dosage of gamma-rays. His studies indicated that this unexpected and variable response was due to the unavailability of oxygen during irradiation. A dosage of 11 000 r was required to sterilize females in CO_2 atmosphere as compared to 5500 to 6250 r in oxygen or air under forced ventilation.

In an eradication programme it is very important to have not only as many insects as possible per man-hour of rearing time, but also to have high quality insects which are as vigorous and competitive as possible. One of the problems encountered during the Southeastern screw worm eradication programme was the pupal mortality caused by desiccation. Under the system used at that time the larvae about to pupate were collected in dry sand. The amount of dry sand available had to be kept to a minimum because the trays had to be lifted by hand, thus imposing a weight limitation. The problem was solved by substituting sawdust for the sand as the pupating medium. Now 99% survival is routine, as compared to about 80% with sand.

BAUMHOVER [12] studied the problem of desiccation and found that the critical period occurred during the larval and prepupal stages (0 to 48 h after larvae leave the rearing medium) and that the losses sustained in the Southeastern programme were primarily due to delay in providing protection. He reported that screw worms exposed as full-grown larvae to 32°C and 40% relative humidity for 48 h incurred a 53.5% weight loss and a 19.6% survival as compared to 24% and 94%, respectively, for controls protected with sand cover and room conditions of 85% relative humidity. Screw worms protected for 24 h after pupation suffered minor losses, even when transferred to conditions of low humidity.

The standard tests for male sexual vigour are laborious and time consuming as they involve either constant observation of mating activity, keeping track of egg mass hatching data, or the checking of female spermatheca for sperm. This led BAUMHOVER [13] to develop the "SAG" or sexual ag-

gression test. This is a technique for measuring sexual activity of male screw worms based on the mortality of females exposed to a high male ratio of 3 : 1. Female mortality after 15 days rose sharply when the ratio of males was increased; for example, it was 35% at a 1 : 7 ratio and 83% at the 3:1 ratio as compared to 26% for females caged alone. The increase in mortality was attributed to harassment of the females by vigorous males as the female screw worm is monogamous. Baumhover also mentioned that the criteria of longevity, which has long been the basis for evaluation of vigour in screw worms, may have little or no correlation and can be misleading if the interplay of the sexes is not considered. Several different mutant strains of screw worms have been evaluated by means of the SAG test. For example, males of the black body mutant strain, and several wing venation mutant strains are not as competitive as the males of the Florida strain when tested with the Florida females. Studies on the basic radiobiology are being pursued vigorously.

LaCHANCE [14] while at the Mission, Texas, Laboratory, studied the effect of different gas mixtures, CO_2, N_2, or O_2 on the radiosensitivity of the screw worm. Irradiation of 5-day-old pupae in N_2 or CO_2 proved to have a distinct radioprotective effect, whereas O_2 had very little effect over that of ambient air. However, when pupae were irradiated in an equal mixture of CO_2 and air, the damage to the female reproductive system was greater than when irradiated in air alone. All three components of female fertility were reduced: the number of females depositing eggs, the number of eggs per fecund female, and the hatchability of the eggs. For enhanced radiation damage to occur, the pupae were pretreated in the CO_2-air gas mixture for about 45 min prior to irradiation. Complete sterility was achieved in a CO_2-air mixture at a dosage of 4500 r whereas 5500 to 6000 r were required in air alone.

In another investigation, LaCHANCE and BRUNS [15] reported on the cytopathology of normal and irradiated screw worm ovaries when the radiation (2000 or 4000 r) was applied during various developmental stages. The cytology of the reproductive system, from 5 day-old pupae to the sexually mature female, 4 to 5 days old, is described in detail. They found the most radiosensitive stage to be the period during which the egg chambers contained nurse cells undergoing endomitotic replication of chromosomal material.

Field studies

USA (17)

Ecological studies are being pursued on this insect to obtain a better knowledge of the local distribution of sterilized flies released in different habitats.

HIGHTOWER [16] used sterile flies tagged with fluorescent dyes to determine the nocturnal resting places. Flies were found along streams and livestock pens, 90% of them on leafless twigs 4 to 5 feet above the ground in low-canopy trees. So for the first time actual proof of where the flies spend the night has been obtained.

In another series of experiments, HIGHTOWER and ALLEY [17] released marked flies in southwest and central Texas to determine their local distribution relative to streams and stock ponds. In multiple releases they used flies marked with either acetone-soluble or ultraviolet fluorescent dyes. In the first experiment, tagged flies that had been released in a brushy, semiarid cattle land with no running streams tended to congregate around the stock ponds. In the second experiment, the tagged flies released near two parallel streams of water continued to disperse beyond one stream one mile from the release point to the other stream two miles away. The result indicated that flies continued to disperse even after they reached favourable habitats. Fly activity was restricted by hot dry weather but intensified after rains.

Using dye-tagged sterilized screw worms, HIGHTOWER et al. [18] showed that these flies can migrate over much greater distances than suspected heretofore. Under favourable conditions, adults live only a little more than two weeks, but it was believed that they could fly only about 35 miles per week. The largest number of tagged flies recaptured were caught within 50 miles of the release point; however, two flies were caught at 140 miles and one each at 165, 170 and 180 miles. These results have an important bearing on the eradication programme with regard to the width of the barrier zone. In addition, isolated screw worm infestations may arise at greater distances from the barrier zone because of the greater flight range capabilities of screw worms.

The screw worm eradication programme in Texas has made good progress since its co-operative inception in February 1962. A joint statement issued by three organizations on 1 February 1964 indicated that both objectives have been achieved: namely, a) to eradicate the screw worm from the Southwest and b) to prove the feasibility of a barrier to prevent reinfestation of the fly-free areas from migrant flies from Mexico.

From 20 December 1963 to 12 March 1964, only two confirmed cases of screw worm infestation occurred in Texas. For all practical purposes, the screw worm can be considered eradicated. The 73 cases in Texas reported from 12 March to the end of May 1964, were probably caused by flies coming over from Mexico or by their first generation descendants. No infestations persisted in any Texas county. A comparison of the screw worm and non-screw worm* cases reported in Texas on a monthly basis for a 3-year period is shown on page 39.

It is very interesting to note that up to the end of May 1964, during a 5-month period, no screw worm cases were reported in New Mexico, Oklahoma, Arkansas, and Louisiana. In addition to the 73 cases reported in Texas, only one case was found in Arizona.

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* Larvae of other species of flies, some closely related to the screw worm (such as the secondary screw worm (Cochliomyia macellaria (Fab.)) also may be found in wounds of livestock. Larval samples collected and sent in by cattlemen were identified at the Mission, Texas, laboratory. The results of these identifications are presented here; each separate wound containing larvae is considered a case.

	Screw worm cases			Non-screw worm cases		
	1964	1963	1962	1964	1963	1962
January	1	157	8	114	70	0
February	0	10	113	106	58	92
March	7	50	331	519	416	105
April	29	357	2 633	1217	1465	507
May	36	450	6 308	856	1151	522
June	15	439	8 300	436	556	254
July	8	304	10 267	204	362	168

At the beginning of the programme, it was hoped that a 100-mile barrier zone would be adequate; however, further research on screw worm migration showed that the fly can occasionally travel as far as 180 miles during its lifetime. This finding indicated that the old barrier zone would not provide adequate protection under all conditions. The width has been increased to a maximum of 200 miles in areas where terrain features are favourable for such extended migrations.

The expanded barrier zone is almost completely effective in keeping areas now free of screw worms from becoming reinfested by screw worms migrating from Mexico. Reinfestations are prevented by the continued release of irradiated flies within this expanded barrier zone and special fly releases in localities where migrating flies cause occasional infestations. Arrangements are now being made with Mexico to move the barrier zone progressively further south in Mexico, thus extending the fly-free zone.

A vast undertaking such as the Southwest screw worm eradication programme can provide some very interesting statistics, namely:

(a) In the fly-rearing plant in Mission, Texas, built at a cost of about $650 000 in five months, as many as 150 million flies per week have been produced.

(b) In the Southwest, the annual loss attributed to screw worms was estimated to be about $100 million.

From February 1962 to July 1964, the approximate cost breakdown of the programme is as follows:

United States Department of Agriculture	$6 300 000
Southwest Animal Health Research Foundation	$3 200 000
State of Texas	$2 700 000
Total	$12 200 000.

In 1962, 1.8×10^9 sterile flies were dispersed, whereas in 1963 almost 6×10^9 were released. The magnitude of such numbers is difficult to grasp. If, for example, the 6 billion flies released in 1963 were placed end to end,

they would form a line more than 36 900 miles long that would girdle the earth over 1.5 times.

It has been calculated that it costs $855 to produce and distribute 1 million sterile screw worms. This cost includes the amount for the food necessary to rear the flies – more than half a ton of meat and 26 gallons of blood.

E. OTHER SPECIES

Laboratory studies

USA (17)

Horn fly, Haematobia irritans (L.). The effects of gamma-irradiation on the horn fly were investigated by LEWIS and EDDY [19]. Both sexes of horn fly were sterilized at a dosage of 5000 r by irradiating pupae in a cobalt-60 source. At this dosage the longevity of adults was not affected. At higher dosages, 10 000 and 25 000 r, adult flies were weakened and had a much shorter lifespan. To determine the competitiveness of irradiated males, irradiated pupae and untreated pupae at a 10 : 1 ratio were caged together. Females laid 66% fewer eggs than the controls. Thus, the irradiated male flies did not fully compete with the untreated males as the reduction in female fertility was less than the theoretical value. However, the data might not represent an accurate analysis of the effects of radiation on the fertility of females since they were derived from only one-tenth of the females; the irradiated females did not lay eggs. Even though the test design could have been faulty, this was a very interesting preliminary study, much complicated by the necessity for developing methods of obtaining eggs, mass rearing, and sexing newly emerged adults.

Drosophila melanogaster Meigen is a pest of economic importance. Because it breeds in fermenting fruits and vegetables, it is especially troublesome to the tomato canning industry. Since conventional field control methods were not satisfactory, the possibility of using the sterile-male technique under certain conditions was investigated. HENNEBERRY [20] studied the effects of gamma-radiation on the fertility and longevity of this fly. Normal females, mated with males exposed to 4000 r in the larval stage or 16 000 r in the pupal or adult stage, produced normal numbers of eggs which did not hatch. Longevity was not significantly affected by irradiation treatment up to 16 000 r. Normal females mated with irradiated males (16 000 r) produced sterile eggs but when mated a second time with untreated males produced viable eggs. Sterile males confined with normal males and females at various ratios (1 : 1 : 1 to 20 : 1 : 1)* caused a reduction in the number of progeny.

In another series of experiments, HENNEBERRY and McGOVERN [21] studied the effects of gamma-radiation on mating behaviour and competitiveness of Drosphila males. Three- to four-day-old males treated at 16 000 r

* This refers to the ratio of treated males to untreated males to untreated virgin females used in competitive mating tests.

and exposed immediately to virgin females did not mate as many times or as readily as males treated at 8000 r. However, if the mating trials were delayed for 24 h, the males treated at 16 000 r mated as readily as the group treated at 8000 r and the controls. Results of multiple mating tests, in which one male was confined with ten virgin females, indicated that both irradiated and non-irradiated males mated on the average about seven times.

The effects of radiation on the fertility of Drosophila were also investigated by HENNEBERRY and McGOVERN [22]. Normal females mated with males irradiated as adults with 16 000 r at one, five, or ten days after emergence produced about the same number of eggs as the controls and very few or no adult progeny emerged. However, females irradiated when ten days old produced more eggs than females treated when one to five days old. Virgin females mated to irradiated males that were held six days after being irradiated at 16 000 r produced the same number of eggs as those mated to males immediately after irradiation. Thus, sperm viability was not restored in the male during the six days.

In further tests, males exposed to 8000 r or 16 000 r were allowed to mate five successive times with virgin females either on the same day or five and ten days after treatment. Fewer eggs were deposited by the fourth and fifth female than the first and second in each series; an indication of sperm depletion.

Codling moth, Carpocapsa pomonella (L.). In anticipation that the sterile-male technique would be applied to the codling moth, some laboratory and field cage studies were made by Hathaway at Yakima, Washington. Eggs, larvae, pupae, and adults were irradiated in a cobalt-60 source at various dosages. Too many side effects, such as decreased larval vitality, high pupal mortality and deformed adults, occurred when eggs and larvae were irradiated at 2500 and 5000 r. When irradiations were carried out during the pupal stage at a dose of 20 000 r, female moths produced no viable eggs. Male moths were less sensitive to radiation since a 25% hatch was obtained in matings with normal females. Mature pupae within 24 h of emergence or adult moths 0 to 24 h old (anaesthetized with CO_2 for convenience in handling) were irradiated at 40 000 r and a 98% sterility was obtained with no undesirable side effects. These results confirmed the excellent work done by PROVERBS and NEWTON [23] in British Columbia.

Field cage tests using 20 : 1 : 1 and 20 : 20 : 1 : 1 ratios * of treated moths (40 000 r) versus normal moths indicated a 10% greater reduction of the F_1 generation when the irradiated females were not present. This same trend was first noted by PROVERBS and NEWTON [24].

Pink bollworm, Pectinophora gossypiella (Saunders). The effect of gamma-radiation from a cobalt-60 source on pupae and adults of the pink bollworm – a polygamous insect – was investigated by OUYE et al. [25]. Fewer side effects, such as deformed wings, occurred when the treatments were made during the late stages of pupal development than when they were made during the earlier stages. Pink bollworms, irradiated in the pupal stage when seven days old, required a dosage of 40 000 r to sterilize the females, whereas a much higher dosage (55 000 r) was required for the males.

* This indicates the ratio of caged moths used in the test as follows: 20 treated males to 20 treated females to 1 normal male to 1 normal virgin female.

The longevity of treated males (about 12 days) was approximately half that of males in the controls. However, the investigators pointed out that this difference in longevity between treated and untreated males may not be a great disadvantage because of the mating habits of the moth. The important factor would be the competitiveness of the irradiated males. Ouye indicated that males from seven-day-old pupae treated with 34 000 r were only partially competitive when released in ratios of 19 : 1 : 1 into large cages containing cotton plants. The mean reduction in population was only 52% instead of the expected 96%.

European corn borer, Ostrinia nubilalis (Hubner). The effect of X-rays on European corn borer, exposed either in the pupal or adult stages, was investigated by WALKER and BRINDLY [26]. Adult males, less than 24 h old, were sterilized by doses of 32 000 r. When these males were mated with untreated females, less than 1% of the eggs hatched. Longevity was about the same for both irradiated and non-irradiated corn borers. The investigators reported that the irradiated males competed equally with normal males for females.

Yellow fever mosquito, Aedis aegypti (2). In an investigation to compare the mating ability and reproduction potential of the yellow fever mosquito WEIDHAAS and SCHMIDT [27] reported that the males treated as pupae either with a chemosterilant or with gamma-radiation at 8000 or 10 000 r, and tested at a 4 : 1 : 1 ratio were not fully competitive. The reason for the lack of competitiveness of these males was not apparent, since they appeared vigorous, lived as long, and mated as readily as normal males when they were not in competition.

USSR (2)

Irradiation effect on six species of agricultural pests. Considerable attention is being paid to research into the effects of radiation on insects. The aim of this research is to develop methods of combating insect pests harmful to stored produce and to agricultural livestock and plants.

The effects of ionizing radiation are demonstrated most clearly in the case of stored product pests, since the effectiveness of radiation can be ascertained more easily when agricultural produce is stored in a confined space.

Tests with the granary weevil (Calandra granaria L.) have indicated that the beetles' life-span decreases and the mortality rate increases with the increasing exposure to radiation of the one-day imaginal stage. In the direct path of the beam they die when a dosage of 325 000 r is applied, and it was established that the dosage required for the sexual sterilization of this weevil lies in the region of 8000 to 9000 r. These data are given in Fig. 9.

It must be concluded that a dosage of 10 000 r guarantees complete sexual sterilization of the granary weevil (Calandra granaria L.). This dosage may be used as the basis of calculation for gamma-disinfestors. Disinfestation of grain with larger dosages is not advisable since it involves higher radiation levels and leads to a decline in the germinative and nutritional properties of the grain.

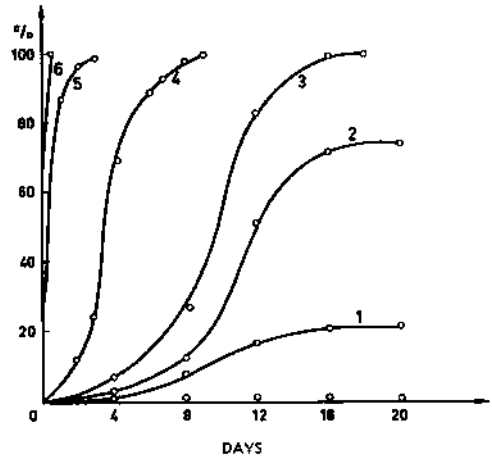

Fig. 9

Mortality of granary weevils after gamma-irradiation.

Dose 1	500 r	Dose rate 230 r/min
Dose 2	3 500 r	
Dose 3	5 000 r	
Dose 4	75 000 r	Dose rate 547 r/min
Dose 5	225 000 r	
Dose 6	325 000 r	

Results with species of agricultural importance have indicated that when overwintering caterpillars of the mallow moth (Pectinophora malvella Hb.) are irradiated in the spring with dosages of 10 000 r and above of X-rays, they suffer 100% mortality and never emerge from the cocoon, but when pupae are irradiated at 5000 r the moths which emerge are so debilitated and deformed that they die within one or two days. Summer-generation mallow moth caterpillars are comparatively resistant to X-rays. Dosages of 5000 r and more either sharply reduce the fertility of the moths or render them completely sterile. If the dosages are increased above 10 000 r, the percentage of deaths rises, [28, 29].

In cutworm moth (Laphygma exigua Hb.) X-ray dosages of 3000 and 5000 r applied to the egg stage produced 85 to 95% sterility in adults. After a dosage of 7000 r, all the caterpillars hatched died on approximately the 19th day, whereas after a dosage of 9000 to 11 000 r, death occurred on the 12 to 14th day, [30].

Gamma-irradiation on the cutworm moth sterilized 36.8% of the males after irradiation of the pupae at 3000 r, 46% after a dosage of 5000 r, and 100% after a dosage of 9000 to 11 000 r. The female became sterile when the dosage reached 5000 r.

Young beetles (Acanthoscelides obtectus Say) were subjected to various doses of gamma-rays in order to study radiation effects. About one month after irradiation observations were made on the hatching of the new generation produced by the irradiated beetles. A periodic count was taken of the

larvae hatched. Even with a dosage of 1000 r, there was a substantial decrease in the hatching of beetles from eggs laid by the females. At a dosage of 6000 r no hatching was observed. This dosage may be considered the sterilizing dosage for Acanthoscelides obtectus Say.

Investigations on the radiation sterilization of the cotton bollworm (Chloridea obsoleta F.) showed that when the males are subjected to a dosage of 4000 r, the fertility of the females after mating with the irradiated males decreases by 49% in comparison with the control sample. When the males are irradiated at 8000 r, the fertility of the females is reduced to zero. This gives grounds for the conclusion that 8000 r is the sterilizing dosage.

When Colorada beetle (Leptinotarsa decemlineata Say) pupae were irradiated with an X-ray dosage of 5000 r and allowed to emerge and mate with untreated females, it was found that the females laid eggs of which only 4% were viable. Irradiation with larger dosages (8000 to 10 000 r) produced complete sterilization of the males.

In mating-competitiveness experiments it was found that, as the percentage of sterilized males placed in the insectary increases, a decrease in the population of the new generation occurs. For example, when the proportion of sterilized males is 20% the population decreases to 34% by comparison with the control, whereas at 60% of sterilized males the population decreases to 9% by comparison with the control.

Great importance is attached to the sterilization method of combating agricultural crop pests. In this connection, a number of scientific institutes are conducting far-reaching research into the use of the sterilizing effects of radiation in combating field pests harmful to agricultural plants.

Research is being conducted along the following lines:

(a) Study of theoretical questions connected with the effects of radiation on the generative organs of insects;

(b) Determination of optimum dosages for the sexual sterilization of insects of various species in relation to their stage of development;

(c) Selection of those species, important as pests, possessing biological characteristics which permit the use of the radiation sterilization method in exterminating the population at sites of mass propagation.

Theoretical investigations on the mechanism of ionizing radiation effects have shown that, as a consequence of irradiation, profound changes take place in the chromosome nucleoproteides and their constituent, deoxyribonucleic acid (DNA) molecules.

Detailed investigations on DNA have shown not only that a breakdown of the polymeric structure of DNA occurs under the action of radiation, but also that various changes take place in the chemical structure of the molecule itself.

In addition to the extensive internal change in biochemical structure, disturbance and reorganization within the chromosome apparatus (breaks, translocation, etc.) are also observed.

The action on the cells at the moment of division causes a disturbance in the distribution of the chromosomes among the daughter cells, which is of fundamental importance for the formation of germ cells since it produces sterility of the gametes, i.e. infertility (DUBININ [31]). The data quoted are of great theoretical importance for understanding the mechanism by which the sterilizing effects of radiation are produced.

In this way ionizing radiation, with its powerful effects on the germ cells, gives rise to profound changes in the germinative system, so causing sexual sterility.

It has been proven by a number of workers that translocational reorganization of the chromosome apparatus during mitosis can be produced by ionizing radiation. This raises enormous possibilities for the use of radiation in achieving sex change.

Development work connected with the use of the sterilization method in combating the more menacing agricultural plant pests of major economic importance renders essential the cooperation of scientists from many countries and of various specializations: biologists, physicists, engineers, etc.

Therefore, the initiative of the International Atomic Energy Agency, directed at coordinating research into the use of radiation in combating agricultural plant insect pests, is to be welcomed. It provides an assurance that the enormous losses caused by pests to agriculture will be reduced substantially in coming years, releasing additional food resources to feed millions of people.

INDIA (23)

Culex fatigans. KRISHNAMURTHY et al. [32] have described the results of a small-scale trial in India in which male Culex fatigans sterilized by irradiation were released to assess their effect on wild populations. Laboratory experiments carried out by Ramakrishnan had shown that C. fatigans conformed to the prerequisites established by Knipling in 1955 for insect control through the release of sterile males. The same workers had shown that a proportion of 2 : 1 : 1 of sterile to normal males and unmated normal females gave a 38 : 40 population reduction under laboratory conditions. The field study performed by Krishnamurthy has particular value as it indicates the type of problem that will probably be encountered when the technique is introduced into developing countries. Chief among these are the rearing and handling of large numbers of mosquitoes in the laboratory, the separation, sexing, irradiation and transportation of pupae and the field releases of the males. Evaluation was also hampered in the experiment by the lack of a practical method of estimating natural populations. There was also considerable opposition from the population in the treated villages regarding the release into their environment of large numbers of mosquitoes.

Notwithstanding these difficulties, however, Krishnamurthy was able to show that although only 24 000 irradiated males were released, there was a viability reduction of 6% for egg rafts collected from the experimental village as compared with the control sample.

Field studies

USA (17)

Yellow fever mosquito, Aedes aegypti (L.). MORLAN et al. [33] released sterilized Aedes aegypti (L.) males in several areas near Pensacola,

Florida. Results were not conclusive since mosquito populations decreased in some test areas but also decreased in some check areas. The authors concluded that before the sterile-male technique can be adapted for mosquito control, additional investigations of mosquito biology are required, especially with regard to male dispersal under field conditions. The necessity for additional investigations applies to many of the other insects that are possible candidates for the sterile-male technique.

Even though this field test by Morlan et al. was not an unqualified success, some very interesting techniques were developed by FAY et al. [34] for rearing and irradiating A. aegypti. Mosquitoes were reared in large trays each containing about 8000 larvae in 6 to 12 l of water. Pupae were separated from the larvae by an ingenious technique adapted from Bar-Zeev and Galun whereby magnetic iron oxide was added to the larval medium. Larvae ingested the oxide and then were separated by means of an electromagnet from the pupae and prepupae which do not feed. Batches of 60 000 pupae were irradiated by placing them around a cobalt-60 point source. The basic irradiation procedure described by McCray was modified for two reasons: firstly, because of the large variations in dosage (from 9400 to 18 750 r) which were obtained, and secondly because of the decrease in emergence and in longevity noted at dosages above 12 000 r. By changing the geometry of the pupae holders in relation to the point source, the limits of the dosage range were narrowed down to between 8800 to 9500 r and pupal survival was increased to 94%.

FAY et al. [34] also ran some tests on the mating competitiveness of sterile and normal males 2 to 7 days of age at 21 : 1 : 1 and 5 : 1 : 1 ratios. The sterilized males were less competitive as they became older compared to normal males.

USA (17)

Anopheles quadrimaculatus. The Lake Okeechobee experiment performed by WEIDHAAS, SCHMIDT and SEABROOK [35] was also inconclusive because of lack of competitiveness of the sterilized males. Field trials conducted by DAME, WOODARD, FORD and WEIDHAAS [36] with A. quadrimaculatus showed that the behaviour of colonized males was significantly different from that of wild males — to the point that they were incapable of incorporating themselves sufficiently into wild populations to bring about the required degree of sterility.

IV. STATUS OF CHEMOSTERILIZATION

USA (12)

History of chemosterilization. The success of the sterile-male-release programmes stimulated research for a substitute method to replace radiation to obtain a more flexible and economical method of achieving sterility.

With radiation, the source is usually permanently fixed; sterility can be induced only in laboratory-reared or field-captured insects which must be brought to the radiation source. These insects are later released into a wild population. Research in the past few years has disclosed that insects can also be sterilized by chemicals; such chemicals can be easily transported to the site of insect infestation. Furthermore, they can be placed in the habitat of the insect as a bait or as a residue, thereby sterilizing natural populations in situ. This approach in many instances precludes the necessity of rearing and sterilizing astronomical numbers of a species that after release might injure a crop or be a disease vector.

By 1961, research workers had detected some chemicals possessing sterilant activity. With these they initiated preliminary laboratory experiments on several different species of insects to determine the sex and stages sterilized, the effect on longevity, cytological development, mating competitiveness, and the optimum concentrations. A continuing screening programme was also maintained to find newer and better chemosterilants by testing chemicals having a greater variety of structure, as well as finding those having less toxic effect on mammalian tissues.

Most chemosterilants fall into the category of alkylating agents. The mode of action of these chemicals is considered by many to be a reaction with deoxyribonucleic acid. The gross biological end effects of alkylating agents on nuclear material often resemble those produced by ionizing radiation, causing them to be labelled "radiomimetic". Promising chemicals of this group that sterilized both sexes of house flies and have been utilized successfully in laboratory and field experiments are tepa, metepa, and apholate [23, 26].

Another group of chemosterilants meriting consideration consists of the antimetabolites, although few compounds of this nature have been investigated. Research workers theorize that these chemicals act by replacing a metabolic product necessary to an enzymatic reaction in the cell, thereby causing a condition that appears to be associated with the specific lack of the essential metabolite. In this group methotrexate (amethopterin; N-{p-[(2, 4-diamino-6-pteridylmethyl) methylamino] benzoyl} glutamic acid) and 5-fluorouracil were among the first sterilants found. Unfortunately, these two compounds produced sterility only in the female house flies, but later the related compound, 5-fluoroörotic acid, produced sterility in both sexes. Because the biological effects produced are not as pronounced as those from the alkylating agents, interest in this group has been renewed.

Another group of compounds recently receiving favourable notice consists of the dimethylamine derivatives. The structure of these chemicals closely resembles some of the more promising alkylating agents, but it is doubtful whether they possess the radiomimetic activity of alkylating agents because they do not contain the aziridinyl ring structure. They appear to have low acute mammalian toxicity; but until chronic toxicity and mode of action data can be obtained, field experiments should only be performed under close supervision of authorized personnel. The same consideration applies with alkylating agents and antimetabolites. Promising candidates from this group are hemel (hexamethylmelamine) and hempa (hexamethylphosphoramide).

Because of the effectiveness and availability of a few specific alkylating agents, research has been conducted largely with this group. Metepa, apholate, and tepa in the diet of adult house flies produced sterility at concentrations of 0.022, 0.025 and 0.0027%, respectively, at the SC_{50} level and substantially shortened the lifespan. More than 90% of the males survived the first ten days, which is probably sufficient time to allow mating with most females that emerge at the same time as the males. Moreover, males sterilized with apholate in the diet were as successful as normal males in competition for females. The percentage of sterile eggs laid by females in cages containing normal and chemosterilized males was as high as, or higher than, would be expected from the ratio of sterile males present.

In studies with these chemicals on the ovarian development in house flies, metepa, tepa, and apholate in the diet inhibited ovarian development in some flies within 24 to 48 h. In other flies ovarian development was not inhibited and the oöcytes in the first egg chambers, though smaller than normal in flies treated with tepa, were able to develop normally. In most females the inhibited oöcytes in the first egg chambers of the treated ovaries began degenerating within 96 h. The degeneration in the inhibited oöcytes and the noninhibited eggs became progressively more severe. The cells of the second egg chambers were either normal or degenerative according to the condition of the cells in the first egg chambers at the time of treatment.

In evaluating the effect of apholate on testicular development, little effect was noted on the gross morphology; but marked aberrations were noted in the chromosomes. They were moderately to severely fragmented, poorly delineated, and stained atypically. The poor delineation appeared to be caused by chromosome clumping. The fragmented and atypically stained chromosomes observed after treatment with apholate were similar to those observed after exposure to 2850 r of gamma radiation.

The chemosterilant concept is receiving more and more widespread acceptance as a promising approach to control. Many research workers in the United States, Mexico, Europe, Africa, Asia and Australia have initiated laboratory evaluations with apholate, metepa, and tepa. The text table below is representative of some of the species sterilized or undergoing screening with chemosterilants.

House fly	Musca domestica L.
Mosquitoes	Anopheles quadrimaculatus Say
	Aedes aegypti (Linnaeus)
	Culex pipiens quinquefasciatus Say
	Anopheles gambiae var. Melas (Theo)
	Anopheles gambiae var. Gambiae Giles
German cockroach	Blattella germanica (L.)
Fruit fly	Drosophila melanogaster Meigen
Mediterranean fruit fly	Ceratitis capitata Wiedemann
Oriental fruit fly	Dacus dorsalis Hendel
Queensland fruit fly	Dacus tryoni Frogg
Olive fruit fly	Dacus oleae (Gmelin)
Screw worm fly	Cochliomyia hominivorax (Coquerel)
Stable fly	Stomoxys calcitrans (Linnaeus)

Citrus red mite	Panonychus citri (McGregor)
Mexican bean beetle	Epilachna varivestis (Mulsant)
Mexican fruit fly	Anastrepha ludens (Loew)
Boll weevil	Anthonomus grandis (Boheman)
Tsetse fly	Glossina morsitans Westwood
	Glossina pallidipes Austen
Chinch bug	Blissus leucopterus (Say)
Eye gnat	Hippelates pusio (Loew)
Face fly	Musca autumnalis De Geer
Horn flies	Haematobia irritans (Linnaeus)
Blow fly	Phaenicia cuprina (Wiedemann)
Fall armyworm	Spodoptera frugiperda (J.E. Smith)

Field studies

USA (12)

House flies, Musca domestica (L.). Many investigations have progressed to the field-study phase. In the United States experiments have been conducted against house flies with tepa, metepa, and apholate. On a refuse dump in the Florida keys nine weekly applications of a cornmeal bait containing 0.5% tepa reduced house fly populations from a count of 47 per grill* to 0 within 4 weeks. Fly populations rose slightly in the following weeks because of increased traffic at the dump. In another refuse dump study, a cornmeal bait containing 0.75% apholate applied once a week for seven consecutive weeks, and then five times per week, reduced grill counts from 68 to less than 1 within ten weeks and remained at a low level thereafter. Cornmeal baits containing 0.5% metepa were also used in a poultry house containing caged layers. The cornmeal bait was applied to the droppings under the cages at weekly intervals. Fly populations were reduced, but not markedly. The persistence of a noticeable population was attributed to the surrounding breeding sites which provided numerous loci for reinfestation. A later experiment in a caged poultry farm conducted with liquid baits composed of sugar and water and containing 1.0% metepa was highly effective and reduced population indices from 49 to 0 within nine weeks. Except for one day when three flies were counted, flies were absent from this site for the remainder of the experiment. This poultry farm was well isolated and at least six miles from the nearest community. At two other poultry installations (caged layers) apholate and trichlorfon, both at 1.0%, provided only moderate control. Unfortunately, in the apholate treatment area, neighbouring breeding sites were a constant source of reinfestation. The results from these small controlled field experiments, although not conclusive in themselves, indicated that in an isolated area chemosterilant measures could possibly be used to eliminate an insect population. Such an experiment is in progress on Grand Turk Island, B.W.I., where 1.0% metepa in a sugar-water bait is being sprayed twice a week in the pits of

* A grill consists of wooden strips 3/4" wide and 18" long nailed 3/4" apart on a frame 18" wide.

outdoor privies — the major area of fly breeding on the island. Fly populations are checked by grid counts in the kitchens, the site of adult fly congregation. Fly abundance decreased from 34 flies per grid immediately before treatment to less than 2 in the month of June when fly populations are normally at their peak. Moreover, the grid counts of less than two have been at this level for several months.

ITALY (22)

House flies, Musca domestica (L.). In a personal communication to Mr. J. Wright of the World Health Organization (WHO), Sacca reports the preliminary results of the treatment of a garbage dump in Italy with tepa started in April this year. During the first week, 8 l of a solution containing 2 g tepa, with 15% sugar and 10% malt extract, was applied to fresh garbage and the area surrounding the dump. The dump was fenced and the six farms in the area treated with insecticides to prevent the importation of flies. During the second week, the amount of tepa was increased to 2.4 g, and in the third and fourth weeks the amount added was 5 g. During June 1964 a solution containing 5 g tepa (0.06%) was applied twice weekly. The fly population was assessed every 10 to 12 days. It increased steadily during the first month (14 000; 45 000; 153 000) but has since decreased (99 000; 65 000; 16 000). The fertility of flies of both sexes, determined twice weekly, has been shown to be nearly zero a few hours after the application of the spray but proportionately higher in the following days.

USA (23)

Mosquitoes. Lack of ecological knowledge has been at the root of our failure as far as the mosquito is concerned. Extensive investigation will be required on this subject before any experiment can hope to be successful. Two methods are open for the application of the technique. The first involves the sterilization of larvae or pupae in sewage lagoons and similar collections of water, and the second the sterilization of large numbers of pupae in the laboratory and the subsequent release of males in the environment. Both have obvious disadvantages, and here again the development of attractants appears to be vital. If the insect could be brought to a chemical bait, only a short exposure of the adult would be necessary to bring about sterility. This exposure would affect not only eggs awaiting oviposition but also those that might be produced at some time in the future.

MEXICO (12)

Mexican fruit fly, Anastrepha ludens (Loew). In another study in Mexico [37], tepa-treated bait stations effectively sterilized a low population of adult Mexican fruit flies in a small semi-isolated mango grove during the dry season but were less effective in the rainy season, when an influx of flies increased population densities.

USA (12)

Boll weevils, Anthonomus grandis (Boheman). Another approach under study is the utilization of the chemical as an inexpensive and highly mobile source of sterility production in laboratory-reared insects. Preliminary studies have indicated that the boll weevil can be sterilized with apholate. Results of experiments conducted in widely separated areas of the Cotton Belt (Virginia, Tennessee and Lousiana) indicated that, at present, extensive natural populations of boll weevils cannot be controlled with existing chemosterilant measures. But, as was indicated in one test, eradication can be caused by a high ratio of sterile males competing with a low population of normal males and females. If boll weevil populations could be reduced to low numbers, whatever the method, it appears feasible that a population could be eradicated by the introduction of chemosterilized males [38].

Other research. There is another fascinating aspect of chemosterilant treatments. Since mosquitoes are readily sterilized by resting on tepa-treated surfaces, it was reasoned that enough chemosterilant should be translocated to affect not only the reproductive organs but also the parasites within the insect [39]. In a study connected with tepa residues against Aedes aegypti infected with Plasmodium gallinaceum, it was found that malaria development in the mosquito and the transmission rate was sharply reduced in mosquitoes exposed to the chemosterilant.

Unfortunately, it appears that at least with some species of insects, resistance to the sterilants can develop although it is of a low order [40]. The yellow fever mosquito, A. aegypti, selected by exposing the larval stage of each of 11 generations to concentrations of apholate, developed a 4- to 5-fold degree of resistance; but this same phenomenon has not been noted in house flies selected in the adult stage for over 40 generations with this same compound.

MEXICO (19)

Mexican fruit fly suppression at Tijuana. The sterile-fly-release programme may be useful for the prevention of new introduction into uninfested areas. This procedure has been used several times during the past year on Rota to overflood melon flies coming from Guam. Entomologists have been releasing male Mexican fruit flies along the Mexican border near Tijuana instead of applying bait sprays. Although an effort is being made by the co-operating agencies to prevent infested mangoes from reaching border areas, some do reach markets on the Mexican side where flies can emerge and fly across into southern California. The flies used are sterilized with chemosterilants and fed for several days. All the females are removed before sexual maturity and all the males are marked with lacquers for subsequent identification. The traps used to measure populations on the Mexican and United States sides of the border contain the liquid protein hydrolysate lure that is more attractive generally to females than males. The appearance of a female in the traps would suggest that it was a wild fly. Any males caught in the traps would, if sterile, be identified by the lacquer.

Although the production, separation of the sexes, marking of the flies, and the transport from Mexico City by air to the Tijuana area requires considerable labour and time, this may prove less objectionable and more effective than the bait sprays previously used. Where genetically marked strains can be developed for identification purposes, there would be less need for segregating sexes and for artifically marking released flies, so that the procedure used against the Mexican fruit fly may be adaptable to other species.

Gamma-irradiation has one distinct advantage in that the dose rate required to insure 100% effectiveness of treatment can be controlled and adjusted. Because of more variation in intake of chemosterilants or in the amount contacted during or after emergence, assurance of complete sterility in the female is less certain, and the release of only the male sex may be necessary where chemosterilants are used in lieu of gamma-irradiation.

WHO (23)

Toxicology. The toxicity of some of the presently available chemosterilants greatly limits their use and will in all probability restrict them to application in well-controlled baits. This was reviewed by a meeting of toxicologists convened by the WHO in April of this year. It indicated that tepa, metepa, and tretamine are all known to injure the bone marrow of mammals when given in the appropriate doses, and that smaller doses could produce damage to the spermatogenic cells in the testes. This statement was based to a large extent upon the work performed by GAINES and KIMBROUGH [41], which indicated that the oral toxicity of metepa is about the same as that for DDT, but – unlike DDT – it is almost as poisonous by the dermal route as when given by a stomach tube.

The oral toxicity of apholate is between those of metepa and tepa but its dermal toxicity is less than either of the two compounds. However, the organ primarily affected by repeated small doses of metepa is the testes. Metepa at the daily oral dose of 5 mg per kg (about 4% of acute oral LD-50 level daily) produced severe reduction in fertility of male rats within 22 days, sterility within 70 days and testicular atrophy within 77 days. Half that dosage produced a smaller reduction in fertility and only partial testicular atrophy in some rats in 197 days. Daily dosages of 1.25 mg per kg or less produced no detectable effect on fertility and no histological change in the testes in 197 days. The survival of new-born was not affected by any dosage given to their fathers.

At the same time the group stated that there was both chemical and experimental evidence that the action of these compounds on both bone marrow and testes could be reversible under some circumstances, but the exact conditions of exposure that would permit full reversibility of these lesions was at present quite unknown.

Although a practical chemosterilant has not yet been found, progress has been very promising with the experimental materials now available.

V. GENETIC MANIPULATIONS

WHO (23)

To ensure that the sterile-male technique is exploited to its maximum, the WHO is not only encouraging research and investigation directed towards finding solutions to the problems referred to, but is also actively supporting investigations into other possibilities for the manipulation of genetic mechanisms already present in natural populations, and has under consideration a number of field trials involving one or more of these. They may be described briefly as follows.

Incompatibility. Two forms of incompatibility are considered to have feasibility: the first, which has the greatest promise, is related only to mosquitoes and is based upon the principle that with certain complexes cytoplasmic agents cause incompatibility between populations. A Scientific Group on Genetics of Vectors and Insecticide Resistance which met in 1964 summed this up as follows:

"Crosses between certain populations give no offspring at all. In other cases females of one population may cross with males of another and fertile offspring are produced, but in reciprocal crosses all embryos die. The sterility is due to a cytoplasmic factor transmitted through the egg and before karyogamy."

Control in the field would be brought about by the mass rearing of males of one crossing type, separation of the sexes in the pupal stage and the release of these males into an area populated by an incompatible crossing type. The value of this procedure is that it achieves the objectives of the sterile-male technique without the difficulties inherent in the use of irradiation.

Two trials in which incompatible strains will be used to reduce field populations are being considered. One will be related to Culex fatigans control and will be performed by the Research Unit on the ecology, biology and control of C. fatigans established by the WHO in Rangoon. Although this Unit has been in existence for only 18 months, it has already been able to collect ecological information that will be of value in any programme involving the release of large numbers of males. In addition, Burma lends itself well to this type of study. During the dry season rice-growing villages become biologically isolated as far as mosquitoes are concerned, with little chance of intermixing occurring. One has in fact an 'island' situation. Populations of C. fatigans from Rangoon have been established in a number of laboratories in different parts of the world and their genetic characteristics are being studied; the establishment of an incompatible strain is being undertaken by Dr. H. Laven of Mainz, and procedures for the large-scale rearing of suitable material are under way.

Cytoplasmic incompatibility is also known to exist in the Aedes scutellaris complex; this fact, plus its island distribution, appears to make it particularly suitable for control through genetic manipulations. In addition, the work done by Fay, McCray and Kilpatrick on the mass rearing of Aedes will do much to solve the problem of mass rearing. However, less is known of the genetic characteristics of the group than about those of C. fatigans and a considerable amount of research will be required before the first trial can be started. This will include the collection of laboratory colonies from

different island groups, the carrying out of hybridization experiments to isolate suitable crossing types, cage experiments to determine the proportion of compatible and incompatible males in a population necessary to reduce or stop production of fertile eggs, and the adaptation of known mass rearing methods to the scutellaris material.

Hybrid sterility. The rapid advances made in recent years on the speciation of Anopheles gambiae have shown that control of this mosquito might be achieved through hybrid sterility. A number of crossing types have already been isolated; crossing in either direction results in fertile females but sterile males. The latter have normal sexual activity and in some cases this may even be enhanced by heterosis. In cage experiments sterile males have been able to compete favourably with normal males, resulting in a reduction in the number of fertile eggs proportional to the number of sterile males introduced. The elements of a field trial have been outlined, but the stumbling block will again be the development of mass rearing techniques. This will be particularly difficult for A. gambiae.

Deleterious genes. The introduction of deleterious genes in natural populations is also being given attention. These would include lethal factors, conditional lethals and genes reducing viability. The mutation load of field populations could be increased by continued introduction of these genes, which would result in the elimination of a proportion of the population with each generation. Sex-linked or sex-influenced lethals would be of particular value.

Candidate genes are already available in well-studied species such as Aedes aegypti, and although the amount of work to be done is extensive, the promise is high. Insecticidal control of this species is becoming increasingly difficult in Latin America, where resistance has brought a number of national eradication programmes to a halt. The increase in the incidence of haemorrhagic fever and other dengue-like conditions in the South East Africa and Western Pacific regions has brought the importance of controlling this mosquito into sharp focus. It may be that genetic control will be the only avenue open to us.

Gene propagation. The Scientific Group on Genetics of Vectors and Insecticide Resistance also recommended that special attention should be given to perfecting procedures by which certain genetic mechanisms, such as meiotic drive, can be used to propagate genes in populations. These procedures, which have shown themselves to be effective in Drosophila, have not yet been fully explored for other species. It is probable that the greatest promise lies in the distortion of segregation ratios brought about by meiotic drive, by which the characteristics produced by the chromosomes of a few individuals can sweep through a whole population affecting the fitness of that population. Thus, a meiotic-drive chromosome containing a gene for female sterility could eradicate a population. A meiotic-drive chromosome is available in A. aegypti and it is hoped that this will be developed to the maximum in the future.

VI. ADJUNCTS TO THE STERILITY APPROACH

UAR (1)

Feeding habits of male mosquitoes. Little has been published on the feeding habits of male mosquitoes, and the subject has not previously been investigated in respect to the important oasis malaria vector, Anopheles sergenti. The present study was designed to investigate the suitability of the various plants of an ecologically isolated habitat as food for male A. sergenti Theo., and the natural feeding habits of these insects. The project was undertaken at Siwa Oasis, from 19 May to 28 June 1963.

Male A. sergenti needed for experimental purposes were reared in a field laboratory from larvae brought in from the field. Drainage canals from the springs and seepages proved to be the most favoured larval habitats. The different plants present in and around the larval habitats of A. sergenti were collected and brought to the laboratory for experimental purposes. In the laboratory, the cut end of a stem from each plant (carrying leaves, and flowers where present) was wrapped in a piece of cotton and immersed in 30 ml of 20 μc per ml of radiophosphorus ($Na\ H_2P^{32}O_4$) in distilled water inside a 50-ml wide-mouth bottle. The opening of the bottle was then covered with cheese cloth to prevent the mosquitoes from coming into direct contact with the radioactive solution. Every plant exposed to radiophosphorus was set aside for 24 h before being placed inside an experimental cage having the top and sides of metal screening, and a sliding glass front. Fifteen A. sergenti males were introduced into the experimental cage through a 5-cm opening in the centre of the glass front, which was then closed with a cork. The males were left for 48 hours with the radioactive plant. After that exposure time, all the mosquitoes inside the cage were taken out, killed (if still alive) by chloroform vapour, and washed in three changes of distilled water. After being dried between two pieces of filter paper, each was transferred to the centre of a copper planchet and was covered with a piece of cellotape. The mosquitoes on the planchets were then assayed for their radioactivity.

The results of these experiments showed that out of forty different species of plants (both wild and cultivated) belonging to 24 families and representing almost every species encountered in the oasis, only three proved suitable as food for A. sergenti males. These plants, in descending order of their importance in this respect, were: Salicornia fruticosa (L.) L., Alhagi maurorum Medic. and Juncus arabicus (Asch. et Buch.) Adams. These three species grow wild in different parts of Siwa, in and around the breeding sites of A. sergenti. Males of this anopheline were subsequently collected from them in the field in much greater numbers than from other plants. They comprise a relatively small proportion of the local flora, and should further studies prove the males of this anopheline unable to adjust to alternative food sources, it is submitted that their selective control could be of practical vector control significance. For example, in lowering the normal ratio of A. sergenti males to females immediately prior to the mass release of males sterilized by means of chemosterilants or radioisotopes.

VII. MAJOR PITFALLS IN RELEASE PROGRAMMES

(a) Lack of preliminary population studies to determine the number of insects in the experimental area may well lead to an underestimation of the necessary overflooding rate.

(b) Too limited facilities (rearing, distribution, evaluation) to permit adequate and rapid expansion when and if the need arises can seriously jeopardize a programme.

(c) All mechanical details used in rearing and distribution should be keyed to the habits and biology of the insect in nature. For example, screw worm flies were ineffective after being released by air in the early morning when the temperature was too cold for insect flight.

(d) Undue mortality of insects due to poor release techniques, i.e. flies that are dropped and allowed to emerge on hot soil or flies that are released during poor weather conditions.

(e) Variation of ecological conditions in the test area must the considered. Insects must be dropped in an environment where they can survive. If the test area is not uniform a grid release cannot be relied on as too many flies will be wasted. A straight strategic release cannot be substituted as pockets of infestation can be overlooked. Thus a combination grid and a strategic release are needed for variable environments.

(f) In making island tests, avoid releasing insects in areas where they will drift out to sea. For some of the fruit flies, drifting will be in the order of 3/4 mile for each 1000 ft of elevation and 10 mph velocity.

(g) For economy, insects should be packaged as densely as possible. It is essential that they are released within 24 h after emergence to obviate starvation; food and water should be added. Boxed fruit flies have their longevity reduced by nearly 50% and wing multilation increased to 10 or 20%, depending on the species.

(h) Insects packaged for release generate much heat from normal oxidative metabolism, therefore release boxes should be well aerated and loosely packed with spacers between the cartons to keep the losses from overheating to a minimum.

(i) Fly pupae should be handled with care. Fruit fly pupae do not tolerate concussion.

(j) Anoxia during irradiation reduces the dosage effect as much as 50%. Avoid overheating. Load canisters as close to the time of irradiation as possible. Keep dose rate high to reduce time of confinement.

(k) The age of the pupae to be irradiated should be as uniform as possible. The exact age will depend on the species used. With fruit fly pupae, lots that vary as much as two days in date of emergence must be irradiated 1 or 2 days before first emergence if excessive radiation damage to the younger pupae is to be avoided.

(l) There is a likelihood that the mass reared strain may be adapted to laboratory conditions; before a large scale release, make sure that the reared strain is competitive with the native field strain. Small scale observations are necessary to ensure that insects have not lost migration and other flight behaviour patterns as well as the ability to seek their mates.

(m) In the selection of genetical marker strains it was noted that colonies produced via inbreeding may have diminished sexual aggressiveness.

(n) If females are also released they should be permanently sterile; this is especially important in long-lived species.

(o) Underestimation of immigration hazard from outside the treated (presumed isolated) area.

In addition to the above-mentioned pitfalls encountered in release programmes, some of the essential requirements are:

(a) A knowledge of fly population explosions or density variations that occur seasonally, or in certain parts of the area.

(b) Data on wind direction and velocity and information of their effects on dispersal in the area treated.

(c) A knowledge of predator species and probable activity in release areas. This could affect the method of dispersal selected.

(d) A genetic or other convenient marker for the released flies. The alternative is extension of releases long beyond the expected end point, for security reasons.

(e) Quarantines or holding procedures to prevent reinfestation during and after the programme.

(f) Quality control checks at all stages of operations from rearing to longevity.

VIII. PROPOSED APPROACH TO THE STUDY OF RADIATION STERILIZATION AND CHEMICAL STERILIZATION.

The requirements for successfully initiating and conducting a control or eradication programme by the utilization of the sterile-male technique are demanding. The success is governed by a multiplicity of factors which, individually or collectively, could result in failure if improperly assessed. The principal question is whether the insect or its environment is suitable for the sterility approach or whether adequate control can be derived by less specific measures available, today. If the sterility approach is feasible, preliminary considerations should determine whether the release of sterile insects into the population or direct sterilization of the natural population would be the most advantageous. Sterilization can be derived by irradiation, chemosterilization, or genetic manipulation. The investigations and recommendations listed below should be seriously considered when releasing insects into a natural population.

The laboratory should be centrally located with the sterilizing source within its confines. Facilities should be suited for rapid expansion and large enough to permit both research and rearing.

Initial studies should be carried on in the laboratory to investigate the optimum dosage, stage sterilized, optimum time of sterilization, effect on mating competitiveness and longevity, and the physiology of the insect, insects' reproduction and other vital processes prior to and followed irradiation. Later experiments on genetic selection for desirable characters to give maximum effect in the field, as well as methods of sexing, may be

desirable. The use of tagging with either isotopes or genetic markers should be evolved to assist in field studies.

Field studies should be conducted at the same time as laboratory studies to derive information on insect densities, population cycles, migration, dispersal, and behaviour as well as to obtain information on the optimum time to initiate a sterile-release programme and to determine techniques whereby the rates of saturation of sterile insects will affect the natural population.

At this point, prior to the consideration of a large-scale programme, it is appropriate to state that the sterile-male approach in most instances is not suited to the control or eradication of populations of great densities. This can be achieved by using other techniques such as insecticides or similar measures. On the other hand, low population densities render the above-mentioned procedures costly but ideally suited for the sterile approach. Therefore, an integrated programme should be conducted whereby the population densities could be initially reduced by means other than the sterile approach and then succeeded by a release of sterile insects. In addition, a predator or parasite release would perhaps assist in maintaining populations at manageable densities during the release programme.

Mass rearing procedures should be developed. Some insects can be mass cultured on their natural host in the laboratory but, in most cases, it is necessary to rely on a prepared or artifical medium. This involves studies on nutritional requirements in order to adopt an economical and readily available source of ingredients to rear the astronomical number of insects at an economically feasible cost. The facilities should be designed to minimize handling of the insects and the rearing procedure mechanized to the greatest extent to reduce personnel costs. Quality control samplings should be established at various stages to ensure that quality and number remain at an optimum level.

The method and source of sterility induction should be mechanized to the ultimate in order to minimize hazards. Moreover, their capabilities should far exceed their normal demands, should the necessity arise for increasing numbers of sterile insects.

This is considered by many to be the most critical phase of a release programme. The success of an entire programme depends on the dispersal of a vigorous, sterile insect, at the proper time and place, and in adequate numbers. The problems of confining, transporting and releasing should all be critically analyzed.

Serious consideration should be given to the results of the elimination of an insect population, particularly in the prevention of reinfestation from outside sources. Releases should be continued for a definite period of time to establish a guardian population to prevent establishment of immigrating insects. Rearing facilities should be kept on a standby basis should the need of reactivation be necessary to prevent sporadic outbreaks of the pest.

Many of the investigations and recommendations listed above are equally applicable to the induction of sterility into a natural population. The concepts of sterility induction are the same but, in this instance, the source is mobile and brought to the field to sterilize the insects in their environment and preclude mass rearing. The inherent hazards of this approach in the field are significantly greater than those encountered under laboratory conditions.

The source of sterility should be made available to a large number of the natural population. With the use of chemosterilants, the compound of least potential hazard should be utilized. This does not necessarily indicate the chemical of lowest toxicity, but residual effectiveness and suitability to the environment merits consideration and would permit the use of a chemosterilant of higher toxicity and sterilant effectiveness.

Care must be taken to ensure that all personnel are trained in the proper use of these toxic compounds prior to initiating laboratory or field experiments.

The acute toxicity of some of the presently available chemosterilants greatly limits their usefulness especially because of the long-term effect of these chemicals. The majority are mutagens and reputed carcinogens, and until more data is assembled on their chronic toxicity they should be used with care.

The chemosterilant approach is an exceptional tool for investigating sterility induction in a natural population. With the chemicals available today, sterility induction can be investigated with the judicious application of the material. Chemosterilants have significantly reduced insect populations when included in a bait and sprayed in selected sites of insect congregation; also they have sterilized mosquitoes when applied as residuals in resting sites. By using a bait or a physical lure, such as light traps that attract an insect to rest or feed, the insect can be drawn to a selective environment where the chemosterilant can extend its effect on the natural population without harm to other species in the environment. Attractants offer the greatest potential to the practicality of the chemosterilant method. Studies should be initiated and maintained for evaluating various compounds, natural or synthesized, that may act as good sex attractants. If a good attractant can be found and incorporated with a chemosterilant, an inexpensive technique which excludes rearing and dissemination of insects would be available for species extermination.

PARTIAL LIST OF INSECTS WITH WHICH SEXUAL-STERILITY RESEARCH AS DESCRIBED IN THE PREVIOUS SECTION HAS BEEN INITIATED

Number	Species	Common name
1	Musca spp.	House fly
2	Glossina spp.	Tsetse fly
3	Anopheline spp.	Malaria mosquito
4	Aedes aegypti (Linnaeus)	Yellow fever mosquito
5	Culex fatigans (Wiedemann)	House mosquito
6	Aedes scutellaris (Walk)	---
7	Simulium spp.	Black flies, etc.
8	Anthonomus grandis (Bohemann)	Boll weevil

Number	Species	Common name
9	Pectinophora gossypiella (Saunders)	Pink bollworm
10	Heliothis spp.	---
11	Protoparce sexta (Johannson)	Tobacco hornworm
12	Dacus oleae (Gmelin)	Olive fly
13	Ostrinia nubilalis (Häbn)	European corn borer
14	Dermatobia hominis (Linnaeus, Jr.)	Human bot fly
15	Anastrepha ludens (Loew)	Mexican fruit fly
16	Diatraea saccharalis (Fab.)	Sugar cane borer
17	Zeuzera pyrina (L.)	Leopard moth
18	Dacus tryoni (Frogg)	Queensland fruit fly
19	Ceratitis capitata (Wiedemann)	Mediterranean fruit fly
20	Dacus dorsalis (Hendel)	Oriental fruit fly
21	Dacus cucurbitae (Coq.)	Melon fly
22	Stomoxys calcitrans (Linnaeus)	Stable fly
23	Musca autumnalis (Degue)	Face fly
24	Pectinophora malvella Hb.	Mallow moth
25	Prodenia litura F.	Cotton leaf worm
26	Carpocapsa pomonella (Linnaeus)	Codling moth
27	Porthetria dispar (Linnaeus)	Gypsy moth
28	Papillia japonica (Newman)	Japanese beetle
29	Rhagoletis cingulata (Loew)	Cherry fruit fly
30	Nezara viridula (Linnaeus)	Southern green stink bug
31	Ceratitis rosae	Natal fruit fly
32	Laphygma exigua Hb.	Cutworm moth
33	Liptinotarsa decemlineata (Say)	Colorado potato beetle
34	Acanthoscelides obtectus Say	Bean weevil
35	Bruchus pisorum (Linnaeus)	Pea weevil
36	Eurygaster integiceps	Sun-pest
37	Rhynchites cupreus var. auratus	Weevil
38	Calandra granaria L.	Granary weevil
39	Lucilia sericata	Blowfly

IX. THE INTEGRATION OF THE STERILE-INSECT-RELEASE METHOD WITH OTHER MEANS OF CONTROL (11)

The sterile-insect-release method will be useful alone for specific insect pests or vectors under three circumstances, namely:

(a) For the elimination of incipient infestations in new areas of spread before the population has reached high levels and before the infestation involves large areas.

(b) For the elimination or control of populations that have suffered severe natural catastrophes; such as severe adverse weather, which reduce the natural population to abnormally low levels. The elimination of the oriental fruit fly from Guam by the Hawaii Fruit Fly Laboratory following two severe typhoons, which reduced the natural population to a very low level, is an example of a situation where this method may be practical alone, but under ordinary circumstances would have required prior reduction of the natural population by other means of control.

(c) For the elimination or control of insect populations that normally reach very low levels due to severe seasonal weather, limited host material, marginal areas of distribution, or for other reasons.

However, due to the large numbers of insects present in established populations of most insects, even during the low periods in the seasonal cycles of abundance, the prior reduction of the natural population by chemical, cultural, biological or other means will usually be essential before the method can be used to advantage.

The sterile-insect-release method offers an effective supplementary system to other methods for the control of insect populations or, conversely, it can be stated with equal justification that conventional methods of insect population control provide an effective supplementary method to the sterile-insect-release system.

Current methods of insect population control such as the application of chemicals and use of cultural measures are highly efficient when the natural population density is high, but highly inefficient in terms of numbers of insects killed when the natural population density is low. In marked contrast, the sterile-insect-release system is highly efficient when the natural population density is low and highly inefficient when the population density is high.

Thus, we have two systems that complement each other in the overall control or elimination of insect populations. The validity of this conclusion is strikingly shown in Table V by noting the theoretical trends of comparable but hypothetical insect populations subjected to four conditions of treatment, namely:

(a) No control (showing the theoretical normal trend).

(b) Chemical or cultural treatments that are 90% effective in every generation.

(c) An initial overflooding rate of 9 to 1 with fully competitive males.

(d) An integrated programme of chemical control for one generation followed by sustained release of sterile insects that equal the original natural population.

TABLE V

RELATIVE TRENDS OF HYPOTHETICAL INSECT POPULATIONS SUBJECTED TO: (1) NO CONTROL; (2) CONTROL BY INSECTICIDES ALONE; (3) CONTROL BY THE USE OF STERILE INSECTS ALONE; AND (4) CONTROL BY THE INTEGRATION OF CHEMICAL CONTROL AND STERILE INSECT RELEASES

Generation	(1) Uncontrolled population [a]	(2) Insecticide treatments (90% kill each generation)	(3) Sterile insect releases (9 000 000 each generation)	(4) Integrated programme of insecticides and sterile-insect releases
Parent	1 000 000	1 000 000	1 000 000	1 000 000
F_1	5 000 000	500 000	500 000	45 450
F_2	25 000 000	250 000	131 625	9 880
F_3	125 000 000	125 000	9 540	485
F_4	Same	62 500	50	0
F_5	Same	31 250	0	
F_6	Same	15 625		
F_7	Same	7 812		
F_8	Same	3 906		
Total requirements for theoretical elimination of populations		Treatments for 18 generations	45 000 000 sterile insects	Insecticide treatments for 1 generation plus 4 000 000 sterile insects

[a] It is assumed that the uncontrolled population increases at a 5-fold rate until the maximum density for the environment is reached, which is assumed to be 125 000 000.

It should be noted that, theoretically, insecticide treatment would be required for 18 generations before the population reaches theoretical zero. It would be necessary to release 45 000 000 sterile insects during the course of five generations to reach theoretical zero by the use of sterile insects alone. However, when these two systems are combined only one generation would, theoretically, require insecticide treatment together with the release of only 4 000 000 sterile insects to achieve theoretical zero.

The integrated programme is far more effective in population control than either system alone or the sum effect of either system alone.

No other generally employed system of insect population control has the feature of increasing effectiveness as the natural population declines. However, it should be noted that the mass and sustained liberation of parasites or predators for a series of generations would also, theoretically, possess the feature of an increasing ratio of parasites or predators to the host insect as the natural population declines. The efficiency of certain parasites or predators under low host density circumstances is not too well known. It would seem reasonable to assume that sterile males would be more efficient in seeking out low density survivors of the natural population than would parasites or predators.

The integration of mass liberations of efficient parasites or predators on a sustained basis plus the use of sterile insects should make a highly complementary and desirable approach to complete population control. This is a totally unexplored area of research in the field of biological control. Tables VI and VII are theoretical models of integrated programmes involving the use of chemicals or cultural measures and sterile-insect releases for the control of populations of the boll weevil and the tobacco horn worm, two of the more important of the agricultural pests in the United States.

Table VIII shows the results of theoretical calculations of the effect on a low density tsetse fly population subjected to sterile-male releases. Tables VI and VII show the estimated effect of an integrated programme of insecticides and sterile-male releases on a high density tsetse fly population.

The models projected are hypothetical, but they are based (i) on the assumption that reasonably competitive sterile males can be developed, and (ii) on estimated population densities that must be dealt with after employing control measures that are known to be effective in drastically reducing natural population densities.

On the basis of estimates of the number of sterile males that would be required and an approximation of the cost for mass producing the insects, it should be possible to estimate the general magnitude of the total cost for developing this sterile-male procedure for a given pest. With this information, together with the cost involved in the prior reduction of the natural population, an appraisal can be made of the general magnitude of a full scale operational programme. The estimated cost of such a programme can then be related to the grower's costs for control by conventional methods together with the losses that result in spite of treatment. If the rough estimates of cost for the integral programme are favorable from an economic standpoint, intensive investigations on the sterile-insect-release method would seem to be fully justified.

In the case of the boll weevil, if the projections in Table VI are valid, it is possible to make a reasonable estimate of the cost of such a programme.

TABLE VI

HYPOTHETICAL MODEL SHOWING TRENDS OF BOLL WEEVIL POPULATIONS ON 1000 ACRES OF COTTON WHEN SUBJECTED TO: (1) NO TREATMENTS; (2) FULL SEASON TREATMENTS WITH INSECTICIDES INVOLVING 18 APPLICATIONS; (3) AN INTEGRATED PROGRAMME OF INSECTICIDES INVOLVING 7 APPLICATIONS AND THE RELEASE OF STERILE MALE BOLL WEEVILS AVERAGING 300 000, 200 000, AND 100 000 RESPECTIVELY FOR THE F_1, F_2, AND F_3 GENERATIONS. THE MODEL ASSUMES A 5-FOLD INCREASE PER GENERATION OF ALL SURVIVING BOLL WEEVILS THAT REPRODUCE AND A 20-DAY DEVELOPMENTAL PERIOD FOR EACH GENERATION

	Parent generation	F_1 generation	F_2 generation	F_3 generation
	Number of boll weevils	Number of boll weevils reproducing	Number of boll weevils reproducing	Number of boll weevils reproducing
1. Uncontrolled population	200 000	1 000 000	5 000 000	25 000 000
2. Population controlled by insecticides only	10 000	5 000	2 500	1 250
3. Population controlled by insecticides plus sterile males	10 000	2 000 [b]	120	0

[b] The model assumes 6 insecticide applications against the parent (overwintered) population and 1 additional insecticide application of insecticides on the last day of the F_1 emergence period to destroy accumulated fertile males and females. Sterile males as indicated are then added again to compete with the lowered emergence rates in the F_2 and F_3 generations.

TABLE VII

ESTIMATED TREND OF A TOBACCO HORN WORM POPULATION EAST OF THE MISSISSIPPI RIVER (USA) SUBJECTED TO STERILE MOTH RELEASES IN A 2-YEAR PROGRAMME. AN INCREASE RATE OF 5-FOLD FOR EACH BROOD IS ASSUMED FOR A NORMAL POPULATION. STERILE INSECTS ARE ASSUMED TO BE FULLY COMPETITIVE. AFTER A RIGID CULTURAL PROGRAMME IN THE FALL, THE INITIAL SPRING POPULATION IS ASSUMED TO BE 8 MOTHS PER ACRE ON 1 250 000 ACRES OF TOBACCO AND TOMATOES, MAKING A TOTAL OF 10 000 000 MOTHS IN THE TOTAL POPULATION AT THE START

Brood	Natural moth population	Sterile moth population	Ratio sterile to fertile moths	Number of moths reproducing
First year				
1	10 000 000	100 000 000	10 : 1	909 090
2	4 545 450	100 000 000	22 : 1	197 454
Second year				
1	246 818 [c]	50 000 000	202 : 1	1 215
2	6 075	50 000 000	8230 : 1	0

[c] A mortality of 75% due to hazards during hibernation was assumed. Theoretically, in the absence of winter hazards, a moth population of 987 270 would have resulted from the 197 454 reproducing moths in the second brood.

TABLE VIII

SHOWING THE THEORETICAL TREND OF A LOW-DENSITY TSETSE-FLY POPULATION AVERAGING 200 FLIES PER SQUARE MILE, WHEN SUBJECTED TO STERILE MALE RELEASES AT THE RATES AND PERIODS INDICATED

Period of release	Natural population density at beginning of each period	Number of sterile males released each period	Estimated ratio of sterile to fertile males	Assumed natural population density at the end of each period [d]
0-3 months	200 (100 males + 100 females)	900 (300 per month)	3 : 1	75
4-6 months	74 (37 males + 37 females)	450 (150 per month)	4 : 1	22
7-9 months	22 (11 males + 11 females)	225 (75 per month)	7 : 1	4
10-12 months	4 (2 males + 2 females)	112 (37 per month)	16.5 : 1	<1
		1687 Total		

[d] In calculating the theoretical effect of the sterile-male releases, it is assumed that the released males are fully competitive with normal wild males and exert full effect in reducing the reproductive potential of the total population. The assumption is also made that in the absence of control efforts the natural population from a low level would increase by 50% each 3-month period (each generation). Thus, in the absence of sterile-male releases, the population trend would be as follows: 1st period, 200 flies per square mile; 2nd period, 300 flies per square mile; 3rd period, 450 flies per square mile; 4th period, 675 flies per square mile.

Each treatment with an insecticide costs about $2.00 per acre. A complete season's insecticide regime would involve about 18 treatments costing a total of $36.00 per acre. However, according to our theoretical projections, complete elimination of the population would not result even with this effort and cost. If we assume that the integrated programme of sterile insects and insecticides would involve seven insecticide treatments and that the release of 1000 sterile male boll weevils (almost twice the number of insects that theoretically would cause elimination as projected in Table V) would eliminate the population, we would have an economical and practical procedure for boll weevil eradication. If the production and release of 1000 sterile male boll weevils cost $5 per acre and the seven insecticide treatments $14 per acre, the total cost of the integrated programme would be $19 per acre. Many growers spend this amount or more annually to control the boll weevil. If we further assume that in the United States there are 10 million acres of cotton in the boll weevil infestation areas, the total cost of complete eradication would amount to $190 million. Release of the sterile insects would cost less than half the cost of insecticide treatments. From a theoretical standpoint, the sterile insects would contribute most to the eradication effort. A programme of this nature would involve substantial additional costs for facilities, equipment, and overall operations. However, if the potential possibilities, as projected, could be realized, we would have a system of boll weevil eradication costing less than the losses this insect causes annually in the United States. These losses are estimated to be about $350 million.

With regard to the tobacco horn worm a programme as projected in Table VI appears to be entirely feasible and economically advantageous in relation to the importance of the problem. It is estimated that annual costs of control by current methods amount to $10 000 000 annually. In addition, however, annual losses are estimated to amount to $35 million.

Based on research by the Tobacco Insects Laboratory at Oxford, North Carolina, it is estimated that tobacco horn worm moths can be mass produced at a cost of $5.00 per 1000 moths. If we assume that the natural population density, after the institution of a rigid cultural programme, can be reduced to the level indicated and that a ratio of 10 sterile moths to 1 fertile moth will start a further downward trend in the natural population, then the total number of moths required would be 300 000 000. The cost for rearing the moths would therefore amount to $1 500 000.

A programme of this kind would involve an area of perhaps 500 000 square miles. We are not accustomed to think in terms of controlling an insect population in such a large area. However, it is the view of some observers that the size alone of an area should not be a serious concern. The screw worm programme in the Southwest involves fly releases in an area of 200 000 square miles or more.

If all other costs for the programme were equal to the costs for rearing the insect, the total cost for achieving complete population control for the tobacco horn worm by the method projected would be approximately $3 million. If complete domination of the existing horn worm population can be achieved the maintenance of complete domination over migrants from other areas at a cost of several hundred thousand dollars each year should be pos-

sible. Since the current cost for tobacco horn worm control amounts to about $10 million per year when based largely on the use of insecticides, the use of the sterility technique might make possible complete control of the problem at less cost and a maintenance control programme at a mere fraction of current costs. In addition, the estimated annual loss of $35 million resulting from reduced yields and quality of tobacco would be avoided.

Thus, there appears to be a good justification for a concerted research effort to develop the sterile-insect-release method for dealing with this pest.

With regard to tsetse flies, if the projection in Table VIII is realistic in principle and if it costs 5¢ to produce and release each sterile male tsetse fly, this part of the cost for eradication would be only about $85 per square mile. Since current costs for eradicating tsetse flies by using residual insecticides on vegetation in the habitat amount to about $500 per square mile, the use of sterile male tsetse flies alone for eliminating low level populations would appear to offer great promise even though rearing costs will be high.

Table VIII is a hypothetical model that projects the use of sterile males alone. An integrated programme involving the minimum use of an insecticide to destroy the existing fertile adult population followed by the release of sterile males would seem to be the most logical approach to tsetse fly control. The integration of sterile-male releases with insecticide mist treatments should be feasible and practical in almost any tsetse fly situation. Estimates have been made along lines discussed for the boll weevil and have concluded that if the density of a natural population were high, involving populations of about 1000 flies per square mile, the use of one mist spray application and properly integrated releases of sterile tsetse flies should lead to a much more economic eradication than the use of insecticides alone or sterile insects alone. According to tsetse fly authorities, about 6 to 8 insecticide mist sprays, applied from an airplane, are required to eliminate a moderate to high population of tsetse flies. If the projections in Tables IX and X and in the graph (Fig. 10) reasonably reflect the potential for the sterile-male technique for controlling populations of the insect, about 1500 sterile tsetse males per square mile would be required to eliminate a population following one mist spray treatment. If each insecticide application costs $100 per square mile and if male tsetse flies could be produced and released at a cost of 5¢ each, the combined cost of the integrated programme would be in the order of $175 per square mile instead of the $600 to $800 required when insecticide mist sprays alone are used. Thus, from a theoretical standpoint, the potential exists for employing the sterility technique to great advantage in tsetse fly eradication. The elimination of high natural populations with sterile tsetse flies alone probably would not be feasible even if rearing costs could be developed below current estimates. If the sterility technique were used alone, the release of several thousand sterile males per square mile over a period of six months or a year might be objectionable from a hazard standpoint. Thus, except for low density areas, an integrated sterile-male-insecticide programme should be the most practical and feasible way to use the sterility technique for these economically important insects.

TABLE IX

EFFECT OF STERILE-MALE RELEASES ON THE REPRODUCTIVE POTENTIAL OF A HYPOTHETICAL TSETSE FLY POPULATION DURING THE FIRST 30 DAYS AFTER THE APPLICATION OF ONE INSECTICIDE MIST TREATMENT THAT IS ASSUMED TO DESTROY 99% OF THE EXISTING ADULT POPULATION. THE EMERGENCE RATE OF NEW FLIES FROM PUPAE ALREADY IN THE ENVIRONMENT IS ASSUMED TO BE 16.7 FLIES PER DAY FOR THE FIRST 30 DAYS

	Days after initiation of programme						
	1	5	10	15	20	25	30
Estimated accumulated natural population, both males and females	27 [e]	94	176	256	340	426	505
Assumed ratio of sterile to fertile males	300 : 13	300 : 47	300 : 88	300 : 128	300 : 170	300 : 213	300 : 252
Accumulation of females mated to fertile males	0.3 5/5.3	3.6 5/8.6	11.4 4/15.4	22 4/26	36 4/40	51 3/54	70 2/72
Overall decline in reproduction	99%	98.3%	97%	95%	92%	89%	85.5%
30 day average = 92.3%							

[e] Includes the newly emerged flies plus 10 flies of the original population surviving the insecticide treatment (5 fertile mated females and 5 fertile males). Half of the original surviving adults are assumed to disappear by the 30th day, whereas the newly emerged flies are assumed to accumulate at the rate of 16.7 flies per day until the 30th day.

TABLE X

EFFECT OF STERILE MALES ON THE REPRODUCTIVE POTENTIAL OF THE HYPOTHETICAL TSETSE FLY POPULATION DURING THE 31st TO THE 150th DAY AFTER AN INSECTICIDE APPLICATION

	Days after initiation of the programme							
	31	45	60	75	90	105	120	150
Estimated population of natural flies (both sexes) [f]	505	380	260	228	190	133	47	34
Estimated daily emergence rate of new flies	0.2	0.2	0.8	2.4	1.8	1.4	0.8	0.2
Assumed ratio, sterile to fertile males [g]	300 : 250	225 : 190	400 : 180	400 : 114	400 : 95	300 : 67	100 : 25	100 : 17
Approximate further overall decline in reproduction during 30th to 150th day = 80%								

[f] These estimates include survivors of flies emerging during the first 30 days, plus flies emerging during the 31st to 150th day. Natural mortality is assumed to be 50% per month during the first 2 months and complete mortality by the 90th day after emergence.

[g] Includes estimated survivors of sterile males released during the first 30 days plus enough sterile males added to maintain numbers indicated.

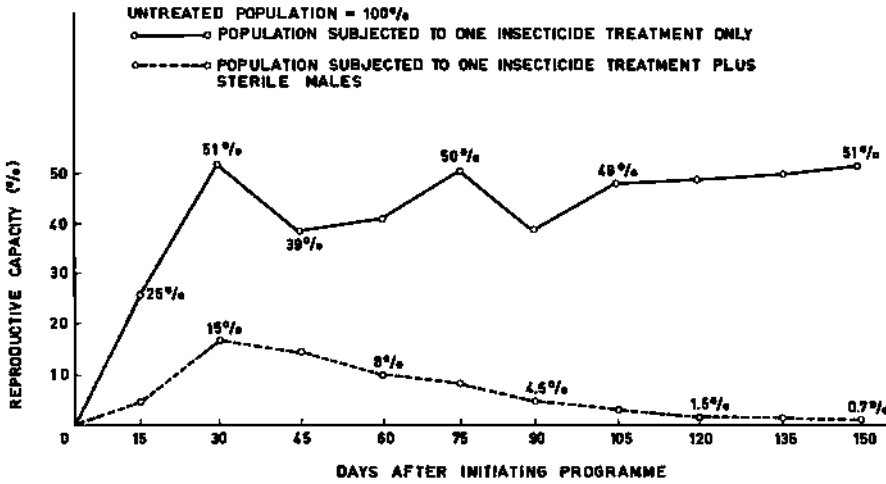

Fig. 10

Estimated trend in the reproductive capacity of a tsetse fly population originally consisting of a 1000 adults per square mile after the application of 1 insecticide mist treatment followed by the release of sexually sterile males as projected in Tables VIII and IX in comparison with the trend of a population treated once with insecticides alone.

X. THE THEORY OF DOMINANT LETHALITY (21)

A theoretical model, though not a unique one, provides a reasonable explanation for most of the parameters associated with dominant lethality induced in sperm. The theory is important for the sterile-male method of insect control because it may explain the resistance to radiation and the apparent multitarget kinetics of the sperm of Lepidoptera.

There is considerable circumstantial evidence that induced dominant lethality is associated with chromosome breakage. One of the best pieces of evidence for this is that a diploid sperm is exactly twice as sensitive to radiation as a haploid sperm (MORTIMER and von BORSTEL [42]), and this effect is equivalent to the capacity of radiation to induce chromosome breaks in a ploidy series (CONGER and JOHNSTON [43]). These are the only two radiation effects in ploidy series where sensitivity increases with higher ploidy, since higher ploidies usually confer a kind of protection against radiation in growing plants or in insect embryos (cf. von BORSTEL [44]).

Let us assume that dominant lethality from X-irradiation of the wasp Habrobracon or the fly Drosophila is induced by formation of chromosome bridges — an assumption based on a considerable body of data (cf. von Borstel, [45] . The initial exponential component of a dose-hatchability curve following irradiation of sperm could come from terminal deletions of the chromosome arms which form chromosome bridges when a new chromosome is synthesized, and this would follow the expression

$$S = e^{-kD}, \qquad (1)$$

where S is the surviving fraction, D is the dose of radiation, and k is the sensitivity of the chromosome. The multihit component of the dose-hatchability curve could possibly come from formation of dicentric translocations at a limited number of binomially distributed sites in a sperm where the chromosomes lie closely enough together for a translocation to be formed when both are broken. This would follow the expression

$$S = [1 - (1 - e^{-kD})^2]^m , \qquad (2)$$

where m is the number of sites. The 2 in the exponent refers to the two chromosomes that must be broken within the site.

Together, equations (1) and (2) are

$$S = e^{-k_1 D}[1 - (1 - e^{-k_2 D})^2]^m , \qquad (3)$$

which can give an explicit, though, as mentioned before, not necessarily a unique, description of the dose-hatchability curve following irradiation of the sperm of Habrobracon or Drosophila.

The model derived from Eq. (2) has been developed earlier (von BORSTEL [45], BENDER and WOLFF [46] and WOLFF [47] to describe dose-action data on the induction of dicentric translocations, and it was used to analyse data on detachments, through translocations, of attached X chromosomes (WOLFF [48] and PARKER [49]). Since the frequency of total induced reciprocal translocations can be measured independently, and since these should be equal in number to the lethal dicentric translocations formed and measurable by Eq. (3), it is expected that a test of consistency of the above model will be made soon.

The problem of radiation-weakening of males. There are at least five possible ways in which the radiation effect on the weakening of male insects might be lowered, namely: (i) lowering the dosage to the amount just above where 100% killing of the gonial cells can be accomplished, (ii) fractionating the dosages, (iii) irradiating the males with radiation of higher LET, (iv) irradiating as late as possible in the life cycle, and (v) irradiating only the portion of the body containing the gonads. Items (ii) and (v) were also suggested at this conference by Knipling.

For the success of the sterile-male method it is not necessary that 99.9, 99, nor 90% of the sperm contain induced dominant lethals. Even lower lethal limits would give positive results provided that the females (which usually have to be released as well) have no viable eggs and that neither males nor females have repopulation of the gonial cells. This may be possible in certain insects and should certainly be investigated when other methods fail. Also, although hatchability is a convenient and accurate criterion of damage, reduced adult survival is all that really matters. With flies and wasps the dosage can probably be reduced about 20% below that used to obtain 99% dominant lethality, providing again that the gonial cell populations are eliminated. This reduction of dosage for the same final effect on viability for adults and eggs may vary from insect to insect and should also be investigated.

Dose-fractionation allows time for certain types of radiation damage to be repaired between the doses. Interestingly enough, the dose-fractionation allows recovery of most types of damage except the genetic and

dominant lethal damage induced in sperm. It might be possible to increase the vigour of males without reducing their lethal capacity by using fractionated or chronic radiation.

Dominant lethality is approximately doubled by 1 MeV neutrons over that of equivalent dosages (kilorep) of gamma-or X-radiation, whereas recessive lethality only increases by about 50% (EDINGTON and RANDOLPH [50]). This should be investigated for the effect on weakening of males. It is possible that a nuclear reactor or some type of heavy ion generator may be the radiation source of choice for certain insects.

Weakening of the males is lessened if the insects can be irradiated as adults rather than as pupae, or as late pupae rather than as early pupae. A number of investigations on different insects indicate that, as a rule, the earlier in its life cycle the insect is irradiated, the greater its sensitivity to the radiation.

The advantages and disadvantages of partial-body irradiation procedures are obvious, and probably would be practicable only on larger insects.

The problem of choosing nonhazardous chemosterilants. The great potential for the use of chemosterilants for insect control has barely begun to be explored since it was first suggested by Knipling in 1959. The chemicals are in many ways simpler to use than the radiation and the possibility exists that they can be used in the field. Of course the hazards are also great: long-lived chemosterilants could possibly be as hazardous as heavy fallout from nuclear detonations, and trace amounts might be carried by the same insects that are being treated in the field. Nevertheless, different chemosterilants have different actions. Certain chemosterilants undoubtedly induce differing ratios of dominant lethal to recessive lethal mutations. For example, the alkylating agent ethyl methanesulfonate appears to induce a low ratio of dominant lethal to recessive lethal mutations, and the ratio is increased when nitrogen mustard is used (LÖBBECKE and von BORSTEL [51]). Apholate appears to have a higher dominant lethal to recessive lethal ratio than either APO (aphoxide) or MAPO (methyl aphoxide) in Neurospora (KANEY and ATWOOD [52]) and thus it may be the least hazardous. These relations should be sought among the better chemosterilants and then these should be tested for long-term somatic effects in mice, i.e. for their ability to induce tumors or to reduce the age of senescence. The somatic studies programme directed by A.C. Upton at the Biology Division, Oak Ridge National Laboratory, could perhaps be referred to as a model programme for studying these types of effects. It is possible that an excellent chemosterilant could be found for use in the field which would have no debilitating effects on humans, on vertebrates in general, or on plants.

XI. PANEL RECOMMENDATIONS

In view of the ever-increasing accumulation of evidence that, when judiciously applied, the sterile-male approach is the most promising form of genetic control for the suppression or eradication of insect species, the Panel recommends that the Agency take active steps to initiate and support

existing programmes, particularly against the olive fly, the Mediterranean and related fruit flies, tsetse flies, and other insects of economic and medical importance in which the approach appears feasible. Moreover, as this and related methods are still relatively new, serious consideration should be given to the study of the principle, the standardization of technique, the dissemination by information and other factors that the Panel considers vital. The Panel's recommendations are listed below.

1. Panels of this nature are invaluable and should convene every two years. Consideration should be given to the suggestion that some of the meetings be held at a location where a large-scale eradication programme is in progress, if this is economically feasible.

2. Participation and collaboration at an international level should be carried on with various laboratories and agencies to assess long- and short-term effects of chemosterilants and radiation on mammals.

3. The programme to exchange professional personnel for short periods and trainee personnel for longer periods should be expanded and accelerated.

4. A list of institutions, individuals, insect species, and fields of interest should be drawn up and placed in the bulletin and updated when considered necessary.

5. Specific standards should be devised for dosimetry and irradiation equipment.

6. Standard samples of both isotopically labelled and unlabelled chemosterilants should be made available upon request to derive dosage base lines.

7. Comparative economic assessments and surveys should be conducted to determine whether the sexual sterility approach is more economically feasible than present methods involving insecticides and biological control.

8. Research on irradiation and chemosterilants should be carried on at sub-sterile levels to determine the effectiveness of this approach in insect control, particularly in disease vector species.

9. The potentially hazardous nature and unknown toxicity of chemosterilants now available make it imperative that materials of this type be employed in a selective manner if the potential of this sterility procedure can be realized for insect population control without creating possible hazards to man and his environment. The development of highly effective insect attractants offers one approach to such selective action. Intensified research on various manners to attract insects is therefore mandatory.

10. In the interest of eventually achieving insect control wherever possible without the need of applying potentially hazardous chemicals in the environment, it is recommended that research on promising candidate species of economic pests be undertaken to explore the feasibility of integration of mass and sustained release of sterile insects with the mass and sustained release of parasites, predators, or insect disease organisms controlling or eliminating high density insect populations.

11. Investigations on resistance and cross-resistance, although less susceptible to arise from chemosterilant measures, should be undertaken.

12. Programmes involving the release of insects that may be disease vectors or otherwise harmful should be preceded by adequate public information.

13. Research on the olive fly, the Mediterranean fruit fly, and the tsetse fly should be accelerated. Moreover, sterile-male technique re-

search should be initiated or amplified against other species, particularly against disease vectors not now under intensive study.

14. The findings of the Panel should be published as an Agency Technical Report.

REFERENCES

[1] BACCETTI, B. and BAIRATI, A., Redia 49 (1964) 1.
[2] BACCETTI, B., De DOMINICUS, R. and CAPPELLINI, M., Redia 46 (1961) 205.
[3] BIRCH, L.C., J. Anim. Ecol. 17 (1948) 15-26.
[4] ANDREWARTHA, H.G. and BIRCH, L.C., Distribution and Abundance of Animals, Univ. of Chicago Press (1954).
[5] BATEMAN, M.A., Doctoral thesis, University of Sydney, Australia (1958).
[6] HAGEN, K.S., SANTAS, L. and TSECOURAS, A., Radiation and Radioisotopes Applied to Insects of Agricultural Importance, IAEA, Vienna (1963) 333.
[7] CHRISTENSON, L.D., MAEDA, S. and HOLLOWAY, J.R., J. econ. Ent. 49 (1955) 135-8.
[8] MONRO, J., Science 140 (1963) 496-7.
[9] POTTS, W.H., Ann. trop. Med. Parasit. 52 (1958) 484-99.
[10] GLASGOW, J.P., "The distribution and abundance of tsetse", Vol. 20 in the International series of monographs on pure and applied biology.
[11] BAUMHOVER, A.H., J.econ. Ent., 56 5 (1963) 628-31.
[12] BAUMHOVER, A.H., J. econ. Ent. 56 4 (1963) 473-5.
[13] BAUMHOVER, A.H., (1964) private communication.
[14] LaCHANCE, L.E., Int. J. Radiat. Biol., 7 4 (1963) 321-31.
[15] LaCHANCE, L.E. and BRUNS, S.B., Biol. Bull. 124 1 (1963) 65-83.
[16] HIGHTOWER, B.G., J. econ. Ent. 56 4 (1963) 498-500.
[17] HIGHTOWER, B.G. and ALLEY, D., J. econ. Ent. 56 6 (1963) 798-802.
[18] HIGHTOWER, B.G., ADAMS, A.L. and ALLEY, D.A., (1964) private communication.
[19] LEWIS, L.F. and EDDY, G.W., J. econ. Ent. 57 2 (1964) 275-7.
[20] HENNEBERRY, T.J., J. econ. Ent. 56 3 (1963) 279-81.
[21] HENNEBERRY, T.J. and McGOVERN, W.L., J. econ. Ent. 56 6 (1963) 739-41.
[22] HENNEBERRY, T.J. and McGOVERN, W.L., J. econ. Ent. 56 6 (1963) 819-22.
[23] PROVERBS, M.D. and NEWTON, J.R., Can. J. Zool. 40 (1962) 401-20.
[24] PROVERBS, M.D. and NEWTON, J.R., J. econ. Ent. 55 6 (1962) 934-6.
[25] OUYE, M.T., GARCIA, R.S. and MARTIN, D.F., J. econ. Ent. (1964) in press.
[26] WALKER, J.R. and BRINDLEY, T.R., J. econ. Ent. 56 4 (1963) 522-5.
[27] WEIDHAAS, D.E. and SCHMIDT, C.H., Mosquito News 21 1 (1963) 32-4.
[28] VASILYAN, U.V., Proc. Fourth Conf. All-Union ent. Soc. (1959).
[29] VASILYAN, U.V., Ent. Rev. 34 3 (1960).
[30] RASULOV, F.K., Cotton Growing No. 7 (1963).
[31] DUBININ, N.G., Gosatomizdat (1963).
[32] KRISHNAMURTHY, B.S., RAY, S.N. and JOSHI, G.C., WHO/EBL/6; WHO/Vector Control 14, (1963).
[33] MORLAN, H.B., McCRAY, E.M. and KILPATRICK, J.W., Mosquito News 22 3 (1962) 295-300.
[34] FAY, R.W., McCRAY, E.M. and KILPATRICK, J.W., Mosquito News 23 (1963) 210-14.
[35] WEIDHAAS, D.E., SCHMIDT, C.H. and SEABROOK, E.L., Mosquito News 22 3 (1962) 283-91.
[36] DAME, D.A., WOODARD, D.B., FORD, H.R. and WEIDHAAS, D.E., Mosquito News 24 1 (1964) 6-14.
[37] SHAW, J.G. and SANCHEZ-RIVIELLO, M., J. econ. Ent. in press.
[38] DAVICH, T.B., KELLER, J.C., MITCHELL, E.B., HILL, R.P., LINDQUIST, D.A. and McKIBBEN, G. J. econ. Ent. in press.
[39] ALTMAN, R.A., Amer. J. Hyg. 77 3 (1963) 221-7.
[40] HAZARD, E.I., LOFGREN, C.S., WOODARD, D.B., FORD, H.R. and GLANCEY, B.M., private communication.
[41] GAINES, T.B. and KIMBROUGH, R.D., in press.

[42] MORTIMER, R. K. and von BORSTEL, R. C., Genetics 48 (1962) 1545-9.
[43] CONGER, A. D. and JOHNSTON, A. H., Nature, London 178 (1956) 271.
[44] von BORSTEL, R. C., Biological Basis of Radiation Therapy, Lippincott, Phila. (1964) in press.
[45] von BORSTEL, R. C., Atti Assoc. genet. ital. 5 (1960) 35-50.
[46] BENDER, M. A. and WOLFF, S., Amer. Nat. 95 (1961) 39-52.
[47] WOLFF, S., J. cell. comp. Physiol. 58 Suppl. 1 (1961) 151-62.
[48] WOLFF, S., J. Theoret. Biol. 3 (1962) 304-14.
[49] PARKER, D. R., Repair from Genetic Radiation Damage and Differential Radiosensitivity in Germ Cells, Pergammon Press Ltd., London (1963).
[50] EDINGTON, C. W. and RANDOLPH, M. L., Genetics 43 (1958) 715-27.
[51] LÜBBECKE, E. A. and von BORSTEL, R. C., Genetics 47 (1962) 853-64.
[52] KANEY, A. R. and ATWOOD, K. C., Nature, London 201 (1964) 1006-8.

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