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Insect Population Control by the Sterile-Male Technique

A.W. Lindquist, Scientific Editor

COMPREHENSIVE REPORT OF A PANEL
HELD IN VIENNA,
16-19 OCTOBER 1962

INTERNATIONAL ATOMIC ENERGY AGENCY - VIENNA, 1963

INSECT POPULATION CONTROL
BY THE STERILE-MALE TECHNIQUE

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IAEA, VIENNA, 1963
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FOREWORD

The successful use of the sterile-male technique to eradicate the screw worm fly from the Southeastern part of the United States demonstrated that an entirely new biological method using radiation-sterilized insects could not only control but even eradicate harmful insect pests. This is an excellent example of the peaceful application of atomic energy for the benefit of mankind, the primary aim of the International Atomic Energy Agency.

The Agency has received numerous inquiries regarding the possible use of this technique against other important insect pests, such as the Mediterranean fruit fly, the olive fly and the tsetse fly. In view of this interest and the tremendous potential of the method, it was felt that the Agency should actively pursue and develop the inherent possibilities of this breakthrough. Therefore, a panel of experts was convened from 16-19 October 1962 in Vienna at the Headquarters of the International Atomic Energy Agency not only to discuss the various aspects and applications of the sterile-male technique and to assess both its usefulness and its current short-comings, but also to suggest future lines of action.

At the conclusion the panel members felt that the panel proceedings should be summarized in a comprehensive technical report. Dr. A.W. Lindquist, one of the panel members, was asked to edit the information presented. All of the panel members should be considered as contributors to this report, even though individual contributions are not listed by name in it.

Since the report contains a wealth of information as well as many references, it was decided to give it as wide a distribution as possible and to publish it as one of the Agency's Technical Reports Series.

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I. INTRODUCTION

The following report represents some of the important contributions of the members of the Panel on Insect Population Control by the Sterile-Male Technique. The Panel was sponsored and convened by the International Atomic Energy Agency in Vienna, Austria, 16-19 October 1962. The objective of the Panel was to review available information on the subject and advise the Agency as to how it might encourage and support research in this area of work. The discussions were informative and stimulating. It is anticipated that there will be greater research efforts on many species of insects to determine if the sterile-male technique can be used for control or complete eradication. At the conclusion of the discussions the Panel presented its recommendations, which are found at the end of this report.

The panel members were as follows:

J.O. Bull	United Kingdom Atomic Energy Authority, Wantage Research Laboratory, Wantage, Berks., England
L.D. Christenson	United States Department of Agriculture, Beltsville, Maryland
F. Evens	Biographic Institute and Laboratory for Ecology, University of Ghent, Ghent, Belgium
M. Feron	Centre de Recherches Agronomiques du Sud-Est, Station de Zoologie Agricole, Cantarel, Montfavet (Vaucluse), France
Mostafa Hafez	Ministry of Agriculture, Dokki, Cairo, United Arab Republic
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H.O. Königshöfer	Food and Agriculture Organization of the United Nations, Viale delle Terme di Caracalla, Rome, Italy
A.W. Lindquist	formerly: United States Department of Agriculture; retired, Bridgeport, Kansas
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M.D. Proverbs	Canada Department of Agriculture, Summerland, British Columbia
Miss H. Thomou	Atomic Energy Commission, Merlin 5, Athens, Greece
F. Wilson	Australian Scientific Liaison Office, Africa House, Kingsway, London, W.C.2, England
J.W. Wright	World Health Organization, Palais des Nations, Geneva, Switzerland

The Scientific Secretaries of the Panel were C.H. Schmidt and M. Fried, both of the IAEA.

II. THE SCREW-WORM SAGA

The idea of using sterile male insects in a natural population was considered by E.F. Knipling as early as 1938 for possible control of the screw-worm fly, Cochliomyia hominivorax (Coq.), a destructive livestock pest in the United States. Mating is one of the strongest instincts in animal life, and the use of sterile males to seek out native females and destroy reproduction presents new opportunities. By no other single means, including dispersion of insecticides, is it possible with such efficiency to reach and destroy all of the insects in a normal native population.

The sterile-male technique consists essentially in the rearing and release of male insects, made sexually sterile by exposure to gamma radiation, in numbers greater than exist in nature. Continued releases increase the ratio of steriles to normals so that extinction of the species results.

KNIPLING (1959) [1] has calculated the theoretical population decline of insect and other animal species subjected to a treatment which causes sterility compared with one that produces only direct kill, as with insecticides. His calculations are given in Table I.

He makes the following assumptions in the hypothetical model: (i) The female is monogamous in mating habit, and each sterile male in the population is fully competitive with normal fertile males in mating with normal females. (ii) The sterilizing agent or procedure will induce sexual sterility in 90% of the males and females of each generation, and the killing agent will kill 90% of both sexes in each generation. (iii) The biotic rate of increase in an untreated population is fivefold in each generation, and survivors in the two treated populations increase at the same rate.

a. Requirements for the sterile-male technique

The requirements for the success of the technique seem to be rather severe; they have been mentioned in various publications but they are listed here for the sake of completeness.

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TABLE I

THEORETICAL POPULATION TRENDS OF AN INSECT SPECIES SUBJECTED TO NO TREATMENT AND TO CHEMICAL OR OTHER TREATMENT THAT AFFECTS 90% OF THE POPULATION (i) BY KILLING AND (ii) BY SEXUAL STERILIZATION

Generation	No treatment	Treatment that kills	Induced sterility
Parent	1 000 000	1 000 000	1 000 000
F_1	5 000 000	500 000	50 000
F_2	25 000 000	250 000	2 500
F_3	125 000 000	125 000	125

(1) An economical method of rearing large numbers of insects must be available, or at least the problem must be reasonably amenable to development.

(2) The insect must be of a type that can be readily dispersed by aircraft and other means, and the males must have the ability to search effectively for the opposite sex and mate in competition with native males.

(3) The sterilizing procedure must not adversely affect mating behaviour or injure the males appreciably.

(4) The species to be controlled must have a comparatively low population or be subject to reduction by insecticide or other means. Advantage may be taken of seasonal fluctuations, since most insects are less numerous at some seasons of the year than at others.

(5) The area to be treated must be reasonably protected against reinfestation, preferably isolated by water, mountains or other barriers.

(6) The males to be released must not be harmful to man, animals or plants. Mechanical and low-cost means for separation of sexes before release must be available in cases where the female is harmful. For example, the release of female mosquitoes in large numbers would create an intolerable condition for man and animals within the area. This problem could be exceedingly difficult although progress has been made in the mechanical separation of mosquito pupae.

(7) A thorough knowledge of the habits and ecology of the insect is essential. This will include the number of annual generations, the length of various stages under different conditions, the rate of emergence in natural habitats, the distribution of emerged broods, when and where mating occurs, the populations per unit area and other factors. We know very little regarding the incidence of an insect species per unit area. Most assessments of population density are on the basis of rates of incidence rather than on total numbers. A great deal of careful research is needed, and it may be found that total numbers, especially at a low level of annual incidence, are not nearly as great as supposed. For example, the screw-worm fly was

originally considered to number tens of thousands per square mile. Research at Uvalde, Texas, in the 1930's indicated, however, that this species exists at a rate of not over 500 to 1000 per square mile (LINDQUIST, 1955 [2]).

b. Screw-worm eradication on an area basis

The use of the sterile-male technique in elimination of the screw-worm, C. hominivorax, from the Southeastern area in the United States in 1959 is an outstanding achievement. The successful eradication project was the result of a research programme that included a few small-scale field trials conducted over a period of several years. Research on ecology and behaviour of this pest suggested certain sterilization experiments which were reported by BUSHLAND and HOPKINS in 1951 and 1953 [3, 4] . They found the optimum time for sterilizing this insect to be five to six days after pupation at 80°F. A dose of 2500 r caused sterility of males and 5000 r, of females without interfering appreciably with their normal behaviour. When 100 sterile males were introduced into cages containing 10 normal males and 10 females, approximately 75% of the resulting egg masses did not hatch. Eradication in the Southeast was thought to have a good chance of success, because the screw-worm overwintered in Florida, a peninsular area, and the only source of reinfestation was from the Southwest.

The eradication programme was begun in August 1958 when the United States Department of Agriculture joined with the State of Florida to provide funds for an eradication project. The U.S.D.A. Animal Disease Eradication Division was given the administrative responsibility for the project, and the Florida Livestock Board carried out the State's obligations.

Because of the complexity of the programme, several entomologists from the Entomology Research Division were asked to assist in its technical direction.

An enormous rearing facility was completed at Sebring, Florida. Production was aimed at rearing 50 million screw-worm flies (half of them males) for release each week. For prevention of reinfestation from the Southwest a quarantine line was set up for inspection and treatment of infested animals along the Mississippi River.

The sterile flies were released systematically in specially designed cardboard containers by aircraft over the entire State of Florida and parts of Georgia and Alabama. After a period of 17 months no screw-worm infestations or fertile adult flies could be found in these states. Approximately 3.5×10^9 reared sterile flies were released. This has resulted in an annual saving to the livestock industry of more than $20 million. The savings paid for the cost of the programme in several months.

c. Eradication attempt in Texas and the Southwest

The great success of the Southeastern screw-worm eradication project stimulated much interest among livestock growers and others in Texas and the Southwestern States. The livestock interests felt a similar programme might be successful in the Southwest. Numerous conferences between Federal and State research and regulatory personnel and livestock industry leaders were held. Research leaders were well aware of the climatological and other differences between the Southeast and the Southwest. Of greater importance, however, was the fact that Florida was a peninsula while Texas was not and could be easily reinfested from adjoining Mexico. The problem was then to determine if some natural barriers in Mexico could be utilized to prevent the fly from migrating into Texas. Apparently no suitable barrier existed.

Serious consideration was given to a continuously treated barrier zone about 100 miles wide along the Rio Grande River separating Texas from Mexico. If successful, the barrier line would be extended to the southern part of New Mexico, Arizona and California. Once the species was eradicated in Texas, sterile flies would be released on a year-around basis in this zone. The calculated cost would not be prohibitive. There is some evidence that such a zone would be effective in preventing fertile flies from migrating into uninfested territory. There is a possibility also that Mexico might want to join in an eradication programme which would eliminate the fly to the south, perhaps to the Isthmus of Panama.

The demand from Texas livestock interests to begin a screw-worm eradication programme in that State gradually grew to the initiation of a co-operative endeavour early in 1962. The U.S.D.A. joined with the Southwest Animal Health Research Foundation and other Texas groups to set up an eradication project. A screw-worm rearing plant had to be constructed and staff employed. This took several months. In the meantime, however, a small temporary rearing plant was set up at Kerrville, Texas, to rear and release sterile flies during March and April when the incidence of the pest was low. This plant did not have the capacity to produce more than about 20 million flies per week, which was not enough to cover adequately the overwintering area in Texas. However, there was evidence that the release programme was partially effective. The large new plant became operational in July (Fig. 1), and it is expected that an all-out effort to eradicate the screw-worm in Texas will be under way during late 1962 and 1963. The barrier zone of released flies will be tested at that time and during the spring to determine if migration from Mexico can be prevented. Figs. 2, 3 and 4 show some of the operations of mass rearing and handling of millions of screw-worms in the Mission, Texas, plant.

III. FACTORS INFLUENCING THE INDUCTION OF STERILITY IN INSECTS

One of the major requirements in the successful use of the sterile-male release technique is that the insect should be sterile but normal in every other respect. The use of radiation to sterilize an insect can produce adverse effects, the magnitude of which will be influenced by a number of factors.

a. Developmental stage

The optimum stage for irradiation is when there is the greatest difference in sensitivity between the somatic and gametic tissues. This is

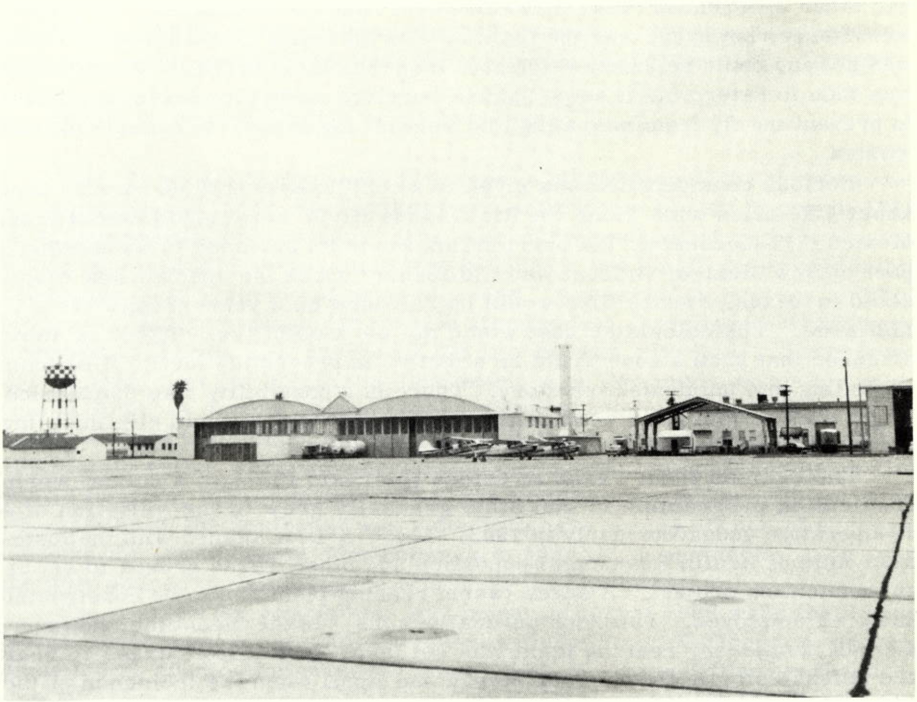

Fig. 1

The screw-worm rearing facility at Mission, Texas
Aircraft buildings at an air force base were converted to house the insect production plant.
(U. S. D. A. photograph)

found in the pupal or adult stage when the imaginal tissues have differentiated and cell division is most active in the gonads.

b. Species

There are wide variations in the susceptibility of species to both the lethal and the sterilizing effects of radiation. In a number of adult beetles infesting stored products, doses which produce 99% sterility also produce considerable mortality within four weeks of treatment; complete sterility also is accompanied by 100% mortality in about three weeks (BULL, 1962 [5]). Mature pupae and adults of Lepidopteran pests, e.g. Anagasta kühniella Zell, Carpocapsa pomonella L., Cadra cautella, are highly resistant to the lethal effects of radiation, doses of up to 50 000 rad having no effect upon longevity. A dose of 50 000 rad produces a high degree of sterility. Dipteran species such as C. hominivorax, Lucilia sericata (Meig.) and certain of the fruit flies have susceptibilities rather lower than do either beetles or moths. Irradiation inhibits egg development and prevents oviposition but also reduces longevity and mating activity. There is, however, a considerable difference between a dose which produces 100% sterility and one which gives

Fig. 2

Screw-worm egg masses scraped from egg tray preparatory to being placed in large vats containing a mixture of meat and blood for rearing of the larvae (U.S.D.A. photograph)

100% mortality; this contrasts sharply with the effects on beetles, where the difference is small.

c. Sex

Susceptibility of the sexes varies from species to species, males being more resistant than females in Oryzaephilus surinamensis (L.), Anagasta kühniella Zell., Carpocapsa pomonella L. and L. sericata, but females being more resistant in Sitophilus granarius (L.), C. Hominivorax and Tribolium castaneum (Herbst).

d. Dose rate

It is well known in radiobiology that chronic irradiation is less effective than is acute irradiation. An increase in dose rate from 4000 rad/h to 250 000 rad/h reduced the LD 50 of S. granarius from 6000 rad to 5150 rad. Reduction of the LD 50's for Tribolium confusum (Duv.) and O. surinamensis was about 1000 rad when the dose rate was increased from 2000 to 4000 rad/h. The number of eggs laid and percentage hatch were markedly higher at a

Fig. 3

System for separating larvae and pupae from sawdust and conveying them to storage areas
(U. S. D. A. photograph)

low dose rate than at a high dose rate when Anastrepha ludens (Loew) were given a dose of 2000 rad.

e. Temperature

Survival of adults is markedly influenced by temperature before, during and after irradiation. Experiments on S. granarius showed that insects maintained at 30°C before irradiation were more sensitive than were those at 15°C; this situation was reversed when insects were irradiated at these two temperatures. Survival after irradiation was considerably higher at 15°C than at 30°C. No effect on progeny production could be found with any temperature combination.

f. Dose fractionation

Experiments with the grain weevil S. granarius showed that, if a radiation dose is given in a number of fractions separated by various time intervals, insects could recover from the effects of the dose, the amount of recovery being a function of the dose, the time lapse between doses, the number

Fig. 4

Packaging screw-worm pupae by means of an automatic loading machine, which loads an exact number of specimens into each carton
Cartons are stored until ready for dispersal by aircraft. (U. S. D. A. photograph)

of fractions and the temperature at which insects were kept in the intervals between doses. The rate of recovery was greatest in the first few hours after irradiation. Weevils irradiated as eggs, larvae and pupae showed recovery in reproductive capacity when doses were fractionated; the reproductive capacity of adults subjected to similar treatment did not recover, and redevelopment of fertility did not occur.

g. Culture environment

Experiments, again with S. granarius, showed that conditions of rearing can affect radiation susceptibility. The dose for 50% kill of adults reared in jars fell from 6750 rad to 5000 rad when the initial inoculation density was increased from 10 adults per 800 g wheat to 1000 adults per 800 g. This increase in susceptibility was largely associated with high temperatures induced by the metabolic activity of the immature stages.

h. Gas tension

Irradiation under anoxic conditions can increase survival and progeny production in the grain weevil. In the limited experiments carried out, no differential could be demonstrated between increased survival and increased progeny production.

The deleterious effects of radiation can be mitigated by judicious choice from the factors outlined above. Irradiation studies should be restricted to mature pupae and adults and the susceptibility of both sexes examined in detail by cross-mating experiments. An insect with a short life cycle and a "batch" type of oviposition is unlikely to have the extended range of fertility found in the beetles and may thus be a more favourable insect for use in the exploitation of the sterile-male technique. Dose rate should be standardized and doses administered under controlled conditions of aeration; fractionation of a dose into two or three parts with a minimum of one hour between fractions might increase survival and leave sterility unchanged. Rearing of insects at low temperatures might improve survival, but this would need to be balanced against the economics of producing massive numbers at high temperatures. Irradiation at a high temperature might allow the maximum recovery of somatic tissues but leave sterility unchanged. Maintenance of insects at a high temperature in the intervals between dose fractions could also aid the recovery process.

The best balance between these factors needs considerable research, but improvements in longevity and maintenance of normal behaviour are worth while, since the result is an increase in the effective overflooding ratio.

IV. SOME ASPECTS OF NUTRITION CONCERNED WITH THE MASS CULTURE OF INSECTS

One of the prime requisites in the use of the sterile-male technique for pest control is the availability of a continuous supply of a large number of insects that are relatively standard. If a male sterilization programme is not possible by manipulation of field populations, the species to be controlled must be mass-cultured in the laboratory or insectary. Some insect species can be mass-cultured on their natural hosts in the laboratory, but in most cases it will be necessary to rely on a prepared or artificial medium (HAGEN, 1962 [6]).

Natural insect host material that is inexpensive and available throughout the year, such as dried foods, tubers, some fruits, meat or fish, may be satisfactory food for mass culturing certain insects. However, most insects that are foliage, fruit or root feeders as well as many sucking insects are difficult to culture continuously on their natural hosts, for it is often difficult to find, produce, store or present such material in its natural form the year round.

a. Nutritional requirements

The nutritional problems that will arise in the development of a medium for mass culture will not mainly be provision of the nutritional requirements (chemical factors essential to the adequacy of the ingested diet) since these requirements are quite similar for all insects; but it may be necessary to consider the ratio of one nutrient to another and the extent of hydrolysis of the major nutrients presented, for these conditions may vary interspecifically. The chief problems, however, will be encountered as one departs further from the natural hosts, since "odd" chemicals which are not nutritional requirements in the strict sense must be present in the diet of some insects in order to obtain a feeding response. Such chemicals are necessary to satisfy the chemical feeding requirements. The physical feeding requirements, such as the texture of the medium, are important and if not satisfied may disrupt feeding and development.

The addition of some natural host material or an extract from the host in a prepared medium may satisfy the chemical feeding requirements. There are, however, insects that can be cultured without any of their host material included in a medium, and often the adult stage of holometabolous insects will feed if some sugar is present in their food.

b. Physical form of the medium

Perhaps the most difficult problem in developing a medium is providing the food in a physical form which will permit the insect to eat and live under optimum conditions. Besides permitting ingestion, the medium must not interfere with respiration and locomotion. Not only is the coarseness of medium important, which can be regulated by grinding or homogenization to obtain good particle size of the fibre, but the amount of water and oil to be used will differ between species. The insects that will feed and develop upon relatively dry solid foods are the simplest types to culture. The larvae of species which will tolerate a soupy type medium are also rather easy to culture. The addition of agar to rather fluid diets may permit borers to develop. The most difficult type of insect to culture on an artificial diet is the foliage feeder, for to date no satisfactory artificial substrate that simulates the physical nature of a leaf has been developed.

However, Plutella maculipennis (Curtis), an important pest of cruciferous crops, has been fed on agar gels containing powdered dehydrated plants alone and in combination with glucosides, mustard oil and myrosin (THORSTEINSON, 1953 [7]). There may be no special difficulty in rearing leaf-feeding insects on such artificial foods.

c. Contamination of the medium

Preventing spoilage of a medium by harmful micro-organisms will be necessary if non-aseptic techniques are used, and it is more practical in mass culture to work under non-aseptic conditions. If aseptic techniques are employed, this will involve autoclaving of the media; and after the eggs are sterilized chemically and carefully placed on the substrate, such a system remains closed until pupation. Under non-aseptic conditions an inclusion of a mould inhibitor and adjustment of the pH of the medium toward the acid side will often control harmful contaminants. Mould inhibitors such as sodium benzoate, "Butoben" (n-butyl parahydroxybenzoate), "Nipagin" (methyl p-hydroxybenzoate), sodium propionate and sorbic acid can be tested alone or in combination in a medium. It must be determined which of the inhibitors the species to be cultured will tolerate.

The pH of the medium when acidic will often control the bacteria. Hydrochloric acid or citric acid can be used. Some insects will tolerate pH 3.5, but usually a pH 4 or 4.5 is satisfactory if the species being cultured develops within a week or two. Antibiotics can be used for bacterial suppression, but this method is more costly and may interfere with symbiotes if they are involved. Since many adult insect foods contain rather high concentrations of sugar, no special treatment of such foods is necessary.

d. Food for adult insects

Mass culture is only possible if a good continuous supply of fertile eggs can be obtained. Many insects in the adult stage do not require a complex diet for ovigenesis. Others require only water or water plus a carbohydrate which lengthens longevity and hence permits more eggs to be deposited. There are, however, some insects in which the adult receives little metabolite transfer from the larval stage. In such species a complex diet has to be ingested for egg production.

If such species will ingest carbohydrate solutions or eat solid foods, an enzymatic protein hydrolyzate of yeast could be added to the carbohydrate or exposed separately. Such a hydrolyzate is often effective since it contains not only protein in a digested state but also has B vitamins and salts which may be required. A carbohydrate should either accompany or be mixed with the hydrolyzate, and if a sterol-like cholesterol is added to this mixture, it then contains nearly all the nutrients required by insects for growth and development.

e. Obtaining eggs

The method of obtaining eggs will, of course, vary with each species; but if artificial substrates are tested, it may be advisable to include some of the natural host for emission of perhaps attractant odours. It is most important that some control of the number of eggs placed in or on the medium be observed, either by natural oviposition directly on the substrate for a definite period of time at one temperature or measurement of the eggs volumetrically or by weight and the placing of definite numbers on a certain volume of media.

Although not necessarily a nutritional problem, mating must of course occur in the laboratory. Therefore, since daily diurnal light conditions may induce a mating response, the insectary should have some exposure to normal lighting. Also, if cages are crowded and mating involves odour gradients, it may become necessary to flush the air periodically to permit directional gradients to exist.

If there is no medium known for a species that is under consideration for laboratory culture, it may pay to attempt homogenization of the natural host material and supplement this material with about 5 to 15% brewers' yeast, adding a mould inhibitor as well as adjusting the pH to around 4.0. This type of medium will support species that will tolerate rather a soupy medium. If a borer is involved, a 2% boiling agar solution can be added to the homogenate while it is being blended.

It is always advisable to place on the medium eggs close to hatching or newly hatched larvae.

V. SPECIES BEING CONSIDERED OR BEING USED IN PROGRAMMES OF STERILE-MALE RELEASE

a. The Australian sheep blowfly

Sheep strike is a problem caused by flies which lay their eggs in the wool or on the skin of live sheep; the young larvae can develop and cause extensive wounds and may cause death in untreated animals. Strike is associated largely with Merino sheep, which constitute 75% of Australian flocks. The flies involved in strike can be divided into primary flies - those which can initiate an attack on sheep, secondary flies - those which cannot initiate an attack but closely follow infestation by primary flies, and tertiary flies - those which follow and supplement attack by secondary flies. All species are native to Australia with the exception of Lucilia cuprina (Wied.) and L. sericata. Of all species, L. cuprina was present in 95% of 732 strikes observed and occurred alone in 57% of strikes. Both FULLER [8] and WATERHOUSE [9] concluded that measures should be directed against primary blowflies and L. cuprina in particular, which, since its establishment 60 yr ago, has become widespread and is particularly abundant in the subtropical and semi-arid regions of southern Queensland and northern New South Wales.

(i) Comparison of the sheep blowfly and the screw-worm

The two species, L. cuprina and C. hominivorax, are remarkably similar in their life histories: both feed gregariously as larvae and produce alkaline wound reactions (BULL [5]). Adults have maximum activity at similar temperatures, and the highest infestations are found following wet weather. Natural populations of blowflies are maintained by carrion, but screw-worm is sustained by wounds on wild animals.

Mating habits, flight range and longevity of the two species are similar, but the sterilization dose for L. cuprina is unknown. Females of L. sericata are sterilized by 3000 r and males by 4000 r, the relative susceptibilities of the sexes being the reverse of those of the screw-worm. The sterilization dose for L. cuprina would probably be between 4 000 and 10 000 r.

(ii) Suitability of the sheep blowfly for sterile-male release

(1) Mass rearing of L. cuprina has been carried out for a number of years and could be expanded to any desired extent. The techniques developed for screw-worm could be applied with very little modification.

(2) Dispersal could be accomplished similarly to that used for screw-worm dispersal.

(3) Sterility would probably be best achieved by irradiation of mature pupae since adults of other species irradiated at this stage retain near-normal longevity and mating behaviour.

(4) Females normally mate once, but this is unimportant so long as sterile sperm competes effectively with fertile sperm.

(5) Density of adults varies widely with season and latitude. The low densities found in the spring correspond approximately to the initial densities of screw-worm found on Curaçao.

(6) Non-destructive adults mean that released sterile flies would not cause additional damage; also L. cuprina only forms a small fraction of the total blowfly population.

Sheep blowfly fulfils the primary requirements for control by sterile-male release. The major factor limiting its use is the vast area to be controlled and the concomitant cost of the the operation. Even if the method is applied efficiently, other species of blowfly may become increasingly important; many of the control measures already applied are essential to good sheep husbandry.

Additional information would be required on a number of points:

(1) Flight range and distribution of adults under "release" conditions;
(2) Behaviour of laboratory strains in the field;
(3) The effect of protein and season on adult nutrition;
(4) The relative importance of sheep and carrion in the maintenance of the wild population;
(5) Effect of radiation on pupae

(a) Susceptibility of sexes,
(b) Mobility and flight range,
(c) Age of pupae,
(d) Mating behaviour both in small cages and under dynamic conditions,
(e) Effect of different swamping ratios,
(f) Dose required to inhibit oviposition,
(g) Interactions of temperature and oxygen on susceptibility;

(6) Interaction of sterile and fertile females at oviposition sites.

(iii) Use of the technique for the control of sheep blowfly in Australia?

(1) Since two-thirds of Australia is infested, i.e. 2 million square miles, the cost would be about £ 200 million (based on Florida costs).
(2) Total costs of blowfly control in Australia by cultural practices and insecticides average £ 8 million per annum; but, since cultural practices are desirable, actual cost of blowfly control is estimated at £ 4 million.
(3) Eradication of L. cuprina would reduce crutch strike to 15-25%, but incidence of body strike would remain unchanged.
(4) Cordons sanitaires of at least 25 miles would be required since no natural barriers impede blowfly dispersal.
(5) The effect of density-dependent factors on population of flies would complicate the use of this technique.

(6) Insecticide resistance has not yet reached the stage where all insecticides are impotent in control of sheep blowfly.

These facts indicate that sheep blowfly is unlikely to be used in an eradication programme by sterile-male release in the near future.

b. The New Guinea screw-worm

The possibility of eradication of the New Guinea screw-worm, Chrysomyia bezziana Villen. has been considered. This African and Asiatic species is an important pest in New Guinea and New Britain, and there is fear that it could establish itself permanently in Australia (WILSON [10]). Its eradication in New Guinea and New Britain would therefore be an important quarantine step for Australia. It seems this insect could probably be mass-cultured, and further basic ecological information is being sought on matters relevant to its control or to an eradication campaign: its distribution in the islands north of Australia, its mass culture, mating habits, flight range, population densities, hosts, etc. The importance of C. bezziana in retarding the development of the cattle industry in New Guinea cannot be over-emphasized, and its invasion of northern Australia would be disastrous for the cattle industry there under present conditions. However, the eradication of screw-worm from New Guinea and New Britain by the sterile-male technique seems virtually impossible under present circumstances because of the size of the infested areas, the problems of fly production and distribution and other difficulties. Local eradication might perhaps be practicable.

c. The tropical ox warble

Dermatobia hominis (Linn.), "tórsalo" or the "tropical warble fly", is an insect which causes damage in many Latin American countries, mainly in cattle. It has not been recorded in any part of the world outside Latin America. The parasitic larvae of Dermatobia are the causal agent of a cutaneous myiasis which clinically resembles largely the condition caused by the Hypoderma species.

(i) Economic importance

The total cattle population is about 12 million in Central America and about 160 million in South America.

Dermatobia is generally considered a major economic problem. Ticks and Dermatobia are the most important external parasites of cattle in Latin America. The value of the skins of Dermatobia-infested animals is considerably reduced, the hides of heavily infested animals being valueless. In some parts of Latin America the incidence of damaged skins is so high that the development of a leather industry cannot even be envisaged. In heavily infested herds the indirect losses resulting from lowered production and lowered resistance against other diseases are certainly even more important than the direct losses.

As in the case of most animal diseases, data are too scarce to permit an assessment of the total economic damage. A few attempts have, however, been made. Estimates in Costa Rica show that one-third of the cattle population is infested and 30-35% of the skins are damaged [11]. Losses in Central America and Panama are approximately estimated at more than US $4 million annually [12, 13], while estimates in heavily infested areas show Dermatobia is responsible for 30% losses in production, i.e. losses of US $50-70 per animal [14].

(ii) Distribution and incidence

Dermatobia hominis occurs in most Central and South American countries. Chile is the only South American country from which Dermatobia has not been reported. In each of the infested countries the distribution is limited to certain areas. The intensity of infestation differs in the various areas, and there are also remarkable seasonal fluctuations. In Central America average larval counts in cattle vary from figures like 32 [13] or 64 [15] up to 846 [16] per animal. In the heavily infested areas of South America infestations of more than 1000 larvae per animal are not uncommon during the season of highest incidence. Other areas of Latin America are free and remain free even if heavily infested animals are brought into the area: the warbles of the imported animals heal spontaneously after some time, and the parasite does not spread to other animals.

Areas at high altitudes are usually free from infestation above 5000 feet [17] or above 4000 feet [11]. In some countries also the low coastal areas, up to 1000 feet, are free; in other countries Dermatobia occurs at sea level as well. In continental Central America some work has been done to determine the exact geographic distribution [18]. In many parts of South America, however, detailed information is lacking. The causes which prevent the spread of Dermatobia in the free areas are not fully explained at the present time. Laboratory investigations [19] suggest that the temperature and relative humidity requirements of the pupae are the factors which determine its distribution.

(iii) Biology and life cycle

Imago

In captivity the life span of the adult fly ranges from 1 to 19 d. Pairing begins the day after emergence; oviposition, three days later.

Oviposition and vectors

Several workers have published information on the biology and life cycle [17, 19, 20, 21]. A single female produces about 200 eggs. A most peculiar biological feature of Dermatobia hominis is the transmission of its eggs to the mammalian host by intermediary insect vectors; as far as is known, this is unique among the parasitic insects. The female Dermatobia catches a mosquito or small fly and releases it after attaching 20 to 60 eggs to its abdomen. Oviposition has also been observed on ticks and, exceptionally, on the host animal. The vector insects are either mammalian blood suckers -

mostly mosquitoes - or non-parasitic small flies, which usually approach warm-blooded animals; 36 species of insects have been observed as egg vectors in seven Latin American countries. The nature of the stimulus which induces oviposition in the female Dermatobia is entirely unknown.

Larval stage

The young larvae emerge from the egg when the vector alights upon a host. Near the site of deposition, using a hair follicle or the puncture made by the vector-mosquito, the larva penetrates into the skin of the host and lodges in the subcutaneous tissue. In contrast to Hypodermia species, Dermatobia larvae do not migrate. The reaction of the host is a pus-filled warble around each larva. The duration of the larval stage in dogs is 33 d but will vary according to host and other conditions.

Pupal stage

The mature larvae leave the host, drop to the ground and pupate. The duration of the pupal stage seems to depend on environmental factors, mainly temperature, and may last from 20 to 60 d. Humidity is essential.

Host

The bovine species appear to be the main hosts of Dermatobia at the present time, but many species of mammals, including man, can act as hosts.

It is interesting to note that no reference to the importance of Dermatobia in cattle is found in the early literature. In the beginning of this century, Dermatobia was still considered mainly a human health problem. In São Paulo, Brazil, 44% of 819 persons examined were found to be infested with Dermatobia in 1927 [15]. In fact, the bovine species, which was introduced to Latin America not more than about 400 yr ago, can hardly be considered the original and natural host reservoir of Dermatobia. It is certainly possible that the prevalence in cattle represents a relatively new development, which perhaps has not yet even reached its peak. Such development may result from changes in the predilections of Dermatobia itself or to a relative increase of those species of vector insects which approach cattle.

The epizootical importance of host species other than cattle may differ in the various infected areas, but the information available at present is too scarce to be conclusive.

(iv) Control

During the last twenty years much progress has been made in the development of new control methods, all based on the use of chemical insecticides [2]. At present it seems that a considerable decrease of incidence can be obtained by regular treatment of cattle, either by external treatment, e.g. sprays, or by oral or parenteral systemic treatment. Most authors agree that the treatment should be given at intervals of 15 d over a period of 5 or 6 months. Recently it was suggested that treatment at monthly intervals over a period of 5 months could also have highly beneficial effects.

(v) Promising aspects of sterile-male release

The sterile-male technique offers promising prospects: It could be effective even in areas where the Dermatobia population is maintained by reservoirs in free-living host animals or where part of the parasitized domestic livestock is not accessible to individual treatment.

At the present stage it seems not impossible that a sterile-male technique for the control of Dermatobia might be developed. There is certainly only one species involved. During certain seasons the incidence is low; with available chemical methods it would be possible even in heavily infested areas to decrease the population density sufficiently to come within the reach of the sterile-male technique. The release of large numbers of sterile males would have no adverse effect, because the adult Dermatobia as such does no harm.

Another favourable circumstance is the fact that Dermatobia occurs in limited areas and that it occurs on one continent only. The economic damage done by Dermatobia is certainly high enough to justify the expense required for developing and applying the sterile-male technique.

(vi) Research problems

Whenever the use of the sterile-male technique is envisaged, certain specific research problems have to be resolved. In the case of Dermatobia the situation is different in that not only do the usual specific research problems have to be dealt with but also a considerable amount of basic research has to be done.

From the purely scientific point of view, the biology of Dermatobia is fascinating; certainly Dermatobia is one of the most interesting insects. There will be no difficulty in enlisting the services of the most highly qualified entomologists for research on it once the necessary funds have been made available.

In view of the possible use of the sterile-male technique, many problems of ecology which till now have seemed to be of academic interest only have to be investigated. It will also be necessary to investigate the distribution and the role of the various hosts in the various areas: some of the "free areas" may be free only in the sense that the prevalence of Dermatobia in cattle is too low to constitute an economic problem. For purposes of using the sterile-male technique, however, it will be essential to know which areas are entirely free in the strict biological sense. It will also be important to investigate the nature of the limiting factors, the normal flight range, eventual migrations, environmental physiology, etc. Even the life cycle and factors influencing the duration of the various stages have thus far not been adequately investigated.

The specific research problems are the same as in other insects: methods of mass-culturing on an artificial medium, methods of sterilizing without affecting longevity and ability in competitive mating, methods of releasing the sterile flies under optimal conditions, methods of marking and trapping in order to follow up the progress of eradication, etc. A specific problem found in Dermatobia is that oviposition in genetically normal individuals has to be induced under laboratory conditions which, of course, eliminate the use of vector insects. It is not known whether Dermatobia mates only once or repeatedly. Repeated mating would not necessarily preclude the use of the sterile-male technique but would affect certain details.

At the present stage none of the existing problems appears to be unresolvable; there is no indication that the nutritional requirements of Dermatobia might be specially complicated ones and none that sterilization would meet with exceptional difficulties; it has already been demonstrated accidentally that sterilization by chemical means is possible [22], in principle.

d. The tsetse fly

Trypanosomiasis is an illness in man and animals caused by a blood flagellate, trypanosome, and transmitted in Africa through the bite of tsetse flies, in South America through the excretions on the site of a bite by a triatoma, Rhodnius. There are 25 species of Glossina in Africa infesting approximately 25 million square miles of area, but only a few species are efficient vectors of trypanosomiasis.

(i) West African trypanosomiasis in man

This is the typical sleeping sickness - a chronic disease caused by Trypanosoma gambiense and transmitted in nature through the tsetse fly, Glossina palpalis R.-D., of the riverine forest type of situation.

The virus reservoir seems to be man himself although wild and domestic swine can keep these trypanosomes in their blood for a long time without any harm or symptoms.

(ii) East African trypanosomiasis in man

This is an acute disease caused by Trypanosoma rhodesiense and transmitted in nature through the Savannah tsetse flies, especially Glossina morsitans Westw. and pallidipes Aust. Wild deer can act as virus reservoir. It is very difficult to differentiate T. rhodesiense from T. gambiense on morphological grounds.

(iii) Animal trypanosomiasis in Africa

Very many different animals are infected with trypanosomes. Only the most important are worth mentioning here:

T. brucei	especially dogs and horses
T. congolense)	especially cattle and sheep
T. vivax and T. uniforme)	
T. simiae	especially swine

These trypanosomes can be transmitted through the bite of different Glossinae of the palpalis, morsitans or fusca groups. Besides the Glossinae, other bloodsucking insects such as Tabanidae seem to be able to transmit the infection mechanically.

The tsetse flies present interesting biological features which suggest that the sterile-male technique might be feasible for eradication. However, a great deal of research is needed especially to determine the conditions under which radiation sterilization can be achieved without undue injury to the insect and whether sterilized flies are competitive with normal flies. POTTS (1958) [23] published preliminary information on radiation effects on tsetse flies.

Tsetse flies, both males and females, are exclusively bloodsucking species. They are fast, noiseless, flying insects that can, however, be captured in fairly large numbers by means of simple mechanical traps. The females copulate the first few days after emergence. The spermatozoa are stored in special sperm receptacles so that the ovulating eggs are fertilized throughout the life of the fly after only one successful copulation. Only one larva is extruded at a time. The first larva is deposited approximately 20 d after copulation, and an overall larval production averages about 12 to 20 in the life of the fly. The larvae migrate into sandy soil along streams and under decaying trees. Flies emerge from the pupae in about 25 to 40 d. Non-fertilized females will readily copulate with males at any time during life and will eventually become fertilized. However, females copulating for the first time at the age of 8 to 10 d rarely survive because of the extensive wounds inflicted on the hardened anal plates during copulation.

The possibility of sterilizing trapped flies by exposure to gamma radiation or chemicals and releasing the treated specimens in the area could after a period of time bring about a remarkable reduction of the insects. However, in time, the trapping operations would reduce the number of flies, and the final eradication would have to be made by the release of reared sterile specimens or by some other means such as, possibly, insecticides.

e. The Mediterranean fruit fly

The Mediterranean fruit fly, Ceratitis capitata (Wiedman), is one of the most damaging insects affecting fruit in the world. It is wide-spread and is especially important as a pest of tropical fruits in the Mediterranean area. Efforts to control the pest include the use of insecticides and parasites, neither of which is entirely satisfactory. Insecticides have created problems of residues in foods and in some cases preclude the usefulness of the beneficial parasites and predators. The possibility of using the sterile-male technique is promising since this technique offers the possibility of complete eradication, which is preferable to partial control. Much more experimental work needs to be done before the method is practicable.

(i) Hawaii

Studies on the use of the sterile-male method were initiated at the United States Department of Agriculture's Hawaiian fruit laboratory several years ago. STEINER and CHRISTENSON (1956) [24] discussed the possibilities of the method for tropical fruit fly eradication. It was found that the Mediterranean fruit fly was made sterile when exposed as pupae, 3 or 4 d before emergence, to 10 000 r of gamma radiation from a cobalt-60 source. In cages the reproduction of normal flies was strongly inhibited and fertility of eggs reduced even though these flies mated frequently. Releases of many millions of sterile flies in a pilot test in a semi-isolated area in Hawaii

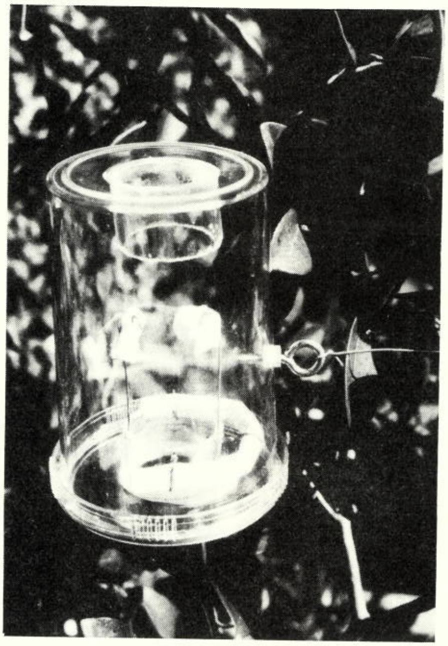

Fig. 5

The Steiner dry trap made of plastic used to estimate populations in sterile-fly release tests
The chemical lure is applied to the cotton wicks. (Photograph, L.F. Steiner, U. S. D. A., Honolulu, Hawaii)

appeared to reduce the wild fly population and prevented normal infestations in a non-replicated experiment.

The Mediterranean fruit fly can be reared economically in vast numbers on a special carrot medium. Biological and sterility studies suggest that the species can be eradicated by this technique in certain types of isolated areas. The species has the advantage of being attracted to powerful artificial lures used in special traps, which makes field studies on population densities more precise (Fig. 5). This information permits the release of sterile insects to be adjusted to the density of the natural population, thereby avoiding waste of sterile insects.

(ii) United Arab Republic

M. HAFEZ (1962) [25] reported on the eradication attempt of the Mediterranean fruit fly in a locality in the United Arab Republic. This project is still in its early stages. Investigations are being made as to the most appropriate areas for releases of sterile males. Oases located in the Western Desert, quite isolated from each other and from the main agricultural areas in the Nile valley, are excellent candidate areas.

The rearing facility has been beset with difficulties in producing large numbers of flies for release. Trouble arose in the production of eggs and in larval rearing. Another problem was contamination of the larval medium by Drosophila. Then there are problems of when and where to release flies so as to study the possibilities of eradication of the species. The problems are gradually being solved, and much useful information is expected to accumulate.

(iii) France

An excellent study of the Mediterranean fruit fly in France has been made by FERON (1962) [26]. In France the overwintering hibernation of Ceratitis has been noted in only two extreme localities near Menton and near Cerbère. In other regions of France and in Europe overwintering hibernations seemed impossible or, at most, very difficult; contradictory observations are poorly explained. Overwintering hibernation of the pupal stage has been made the object of many studies; the conditions for the overwintering of the adults are very inadequately known.

(iv) Israel

In Israel Ceratitis has four generations in the hill areas, with a period of rest from November to May. There are up to eight generations per year in the coastal plains and in the Jordan Valley, where favourable hosts, such as citrus, apples, pears, avocados and guavas, exist all year round because of the sub-tropical climate.

(v) Tunisia

In Tunisia the ecology of Ceratitis is beginning to be particularly well known, thanks to the work of the research staff of the Institut National de la Recherche Agronomique and notably that of F. Soria. The ecology of the cultivated and wild hosts of Ceratitis and knowledge of their relative importance show that there exists a period of very low population density which extends from December to April. During this period the most dangerous host plant is the bitter orange; this plant could be controlled.

Tunisia seems to present very favourable conditions for an eradication trial of Ceratitis by the sterile-male technique, which would, of course, be made in combination with other methods. The borders should not be too difficult to watch: the sea in the north and east, the Sahara in the south and the mountains in the west. This extensive trial could be realized progressively from isolated plantations in oases and thence to the entire territory.

A complementary ecological study would precede these operations, at which time the industrial rearing of Ceratitis would be put into operation. The sterilization technique could be worked out thanks to the previous work done in the United States of America and with the help of French research agencies.

f. The Mexican fruit fly

CHRISTENSON (1962) [27] reported some recent research by the U.S.D.A. on tropical fruit flies. After treatment of 12-d-old pupae of the Mexican fruit fly, Anastrepha ludens (Loew), with 5000 r of radiation from cobalt-60, emerging adults were sterile. In a former study, treatment of puparia was more effective at 90 r/min than at 10 r/min at a marginal sterilization dosage of 2000 r. In recent tests after sterilization of puparia at rates of 2695 r/min and 42.8 r/min there was no apparent effect of the higher dosage rate on the emergence or longevity of adults. Both sexes were sterilized when a 5000 r dosage was fractionated, 2500 r being followed by the same amount after a 24-h interval. When 12-d-old puparia were administered dosages up to 7000 r in an N_2 atmosphere, there was little sterilization effect; when puparia were irradiated in an O_2 atmosphere at 7000 r, some fertile eggs were obtained from emergent adults.

The release of sterile Mexican fruit flies was continued in 1961 at the same two locations in Mexico where sterile flies had been released in 1960. The species can be reared rather easily in the laboratory (Figs. 6 and 7). At Santa Rosa, where 838 000 sterile flies were released in a semi-isolated hacienda with about one acre of citrus (mostly grapefruit) and one square mile of other hosts, only 5.5 larvae per pound were recovered from 2 tons of fruit despite an infestation potentially double that of the preceding year. In the previous season, after release of 1 172 000 sterile flies, infestations averaged 6.9 per pound. At San Carlos similar results were obtained in 1960 and 1961. Releases made at San Carlos in February 1962 and continued until June 6 gave a mean 8 : 1 ratio of non-gravid to gravid flies, but the native fly population was so high that there was little suppression of infestation.

g. The oriental and melon flies

Field tests of the sterile-male release method with the oriental fruit fly, D. dorsalis (Hendel), and the melon fly, D. curcurbitae (Coq.), were started in late 1960 on Rota, a small island located 40 miles northeast of Guam. This was a co-operative U.S.D.A. and U.S. Navy project. Millions of flies were reared and released on Rota (Figs. 8 and 9) until 1961, when work with the melon fly was discontinued because of inability of the researchers to give attention to both species simultaneously. In the continuing studies with the oriental fruit fly a wealth of information with much basic significance has been accumulated. Late in November 1961 the release rate was increased to almost 10 million pupae per week, with ground emergences supplementing aerial drops. Overflooding was attained in all areas, with a ratio as high as 5 sterile flies to 1 wild fly in some areas and an average of at least 4 to 1 on the southwest half of the island where preferred hosts are most abundant. However, the target overflooding ratio of 10 to 1 was never reached, and the experiment was terminated when wild fly populations began to increase.

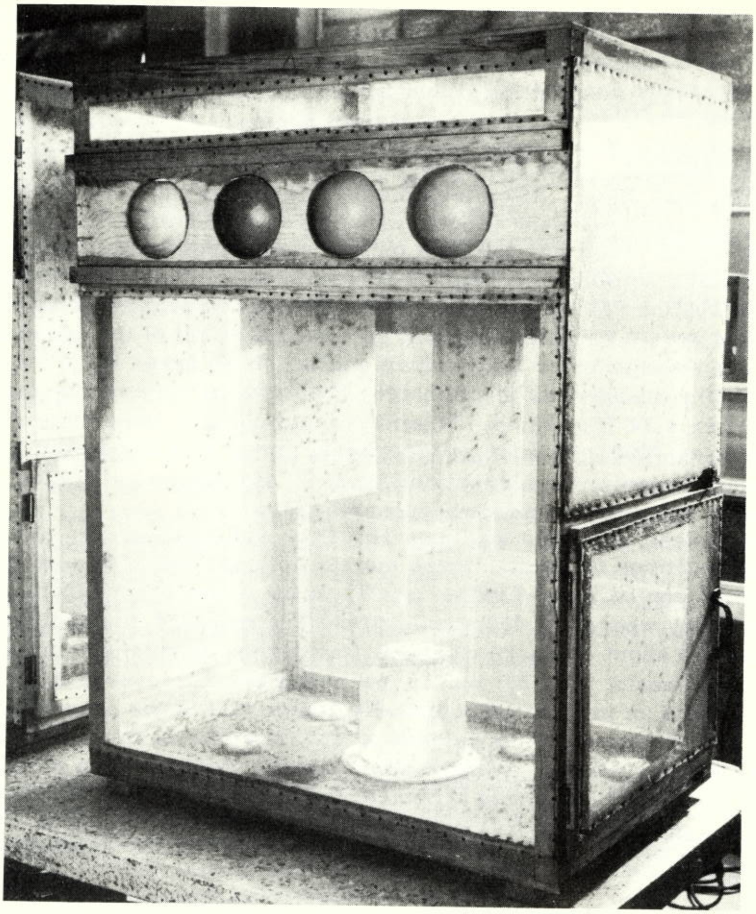

Fig. 6

Breeding stocks of adult Mexican fruit fly flies maintained to produce eggs
The eggs are deposited in special wax shelves inserted at the top of the cage.
(Photograph, Rockefeller Foundation, Mexico City)

Losses of flies during processing and distribution and mortality in the field before the flies attained ability to mate, in all accounting for as much as two-thirds of the total production, plus physical limits on experimental-fly production accounted for failure in this experiment. The test with the oriental fruit fly indicated that with some species it may be necessary to reduce wild fly populations by other means before applying sterile flies. The importance of the calculation of overflooding ratios on the basis of the number of sterilized flies surviving processing and exposure to field conditions until attainment of sexual maturity was also revealed.

Fruit flies from ground releases distributed themselves satisfactorily throughout most areas, and their survival and longevity may have been better

Fig. 7

Rearing of Mexican fruit flies

Mexican fruit flies needed for sterile-fly release experiments are reared in trays containing dehydrated ground carrot medium fortified with special nutrients. Mexican fruit fly eggs on organdy pads are being added to carrot larval rearing medium at the U. S. D. A. fruit fly laboratory in Mexico City. Similar techniques are used in rearing Medflies in Hawaii. (Photograph, Rockefeller Foundation, Mexico City)

than that of flies released from aircraft. Some of the flies from airplanes dropped too close to the shoreline of the small island and drifted out to sea, particularly when boxes broke open at plane height. Chutes designed to deliver boxes into the airstream with less shock were tested, but in the best performance from 18 to 20% were ripped open by the air blast. Throwing the boxes out by hand at a higher level through the open door of airplane eliminated most breakup, and this method is now being used.

Although this field trial did not achieve elimination of the oriental fruit fly, a great deal of information was obtained, especially as to pitfalls in such a programme.

h. The Queensland fruit fly

Dacus tryoni (Frogg) is an important pest of cultivated fruits in Eastern Australia (MONROE [28]). Even where it is now found only sporadically,

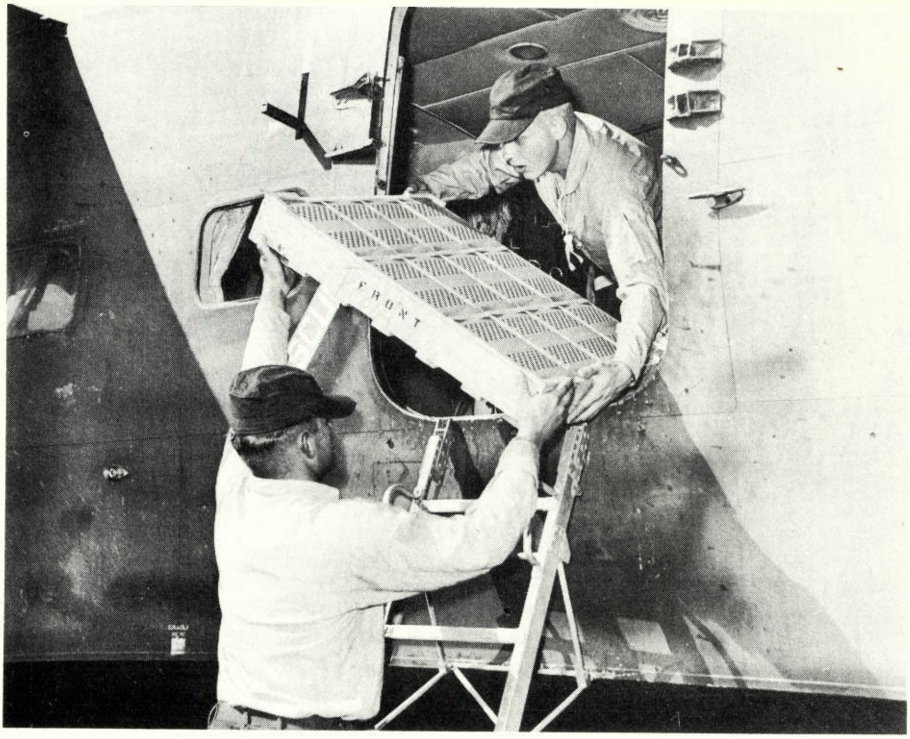

Fig. 8

Loading trays of sterile flies into United States Navy flying boat for distribution over Rota in the Western Pacific (Official photograph, United States Navy)

it may later become established and at present threatens the overseas markets of these areas through quarantine restrictions. First described about 80 yr ago, it has moved from native to cultivated hosts and extended its geographic distribution in a southerly and westerly direction. Populations are self-sustaining along the coastal fringe from Cape York in the north to around Eden in southern New South Wales. Farther west and south, populations are broken up into small pockets whose numbers fluctuate markedly, and in some of these pockets local extinction may occur in some seasons. Larvae transported into these marginal populations in infested fruit are continuously providing candidates for selection of strains adapted to these less hospitable environments. Such geographic strains have been described by BATEMAN [29]. The geographic range of D. tryoni has increased in the past, and this trend continues.

There may be some hope of using sterile males against D. tryoni on the fringes of its distribution, because populations are small, limited to semi-isolated areas and subject to great fluctuation. Furthermore, in the laboratory at least, female flies mate infrequently during life, with long intervals between matings. The population-dynamics and physiology of

Fig. 9

Boxes for dispersal of fruit flies
Fruit fly adults are allowed to emerge from pupae in special boxes containing special food.
The boxes containing adults are then dropped from an airplane. (Official photograph, United States Navy)

D. tryoni should favour sterile males, because the winter population seems to consist only of adults, with most females uninseminated, and the populations reach minimum numbers in spring when mating recommences.

Gamma irradiation sterilizes both sexes, and so do the chemosterilants aphoxide and apholate. The minimum sterilizing dose of irradiation is about 4000 rad given to the 8-d-old pupae at 25°C, but 5000 rad is given to flies released in the field. Only flies sterilized by irradiation have so far been used in eradication trials. In the laboratory cages a swamping ratio of nine sterile to one fertile male almost completely prevented normal females from laying fertile eggs. In field cages irradiated flies were shorter-lived than were wild ones, but sterile males were as efficient as the wild ones were in mating. Irradiated females are unlikely to increase greatly the amount of stinging of fruit, because in the laboratory they probe fruit much less than do unirradiated females.

Methods of mass rearing which differ little from those used in Hawaii have been developed, and a capacity of 10^6 pupae per week has been achieved. It is not proposed to increase the production beyond 10^6 per week during current field trials, because this would require much mechanical equipment and space.

Sterile flies are being released in three semi-isolated towns in north-eastern New South Wales, as pupae in summer and as adults in winter. Populations of D. tryoni are being observed in these treated towns and in four control towns. Sterile males are detected in samples by their shrunken testes, and some are also "bleached" by gamma radiation applied on the sixth day of pupal life.

The relative numbers of adult flies, the ratio of sterile to fertile males (two independent methods), the proportion of females inseminated with normal sperm and the percentage infestation of fruit are being estimated. The absolute numbers of flies in the towns are not known; releases will have to be adjusted according to the degree of flooding achieved.

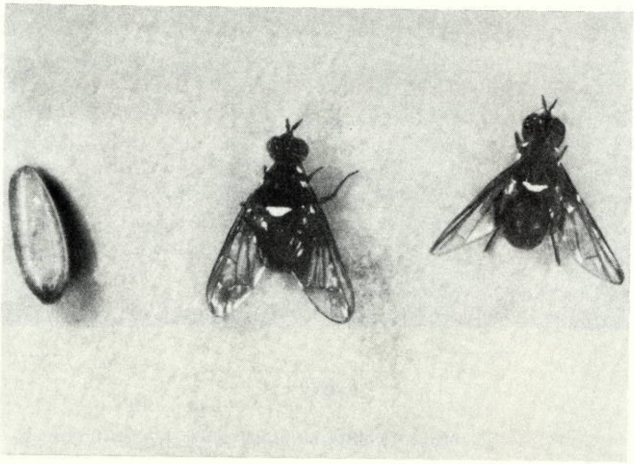

Fig. 10

A puparium; female and male, of Dacus oleae

i. The olive fly

The damages caused by the olive fly, Dacus oleae (Gmelin) (Fig. 10), in the Mediterranean basin are considerable. For example, losses in Greece sometimes exceed $30 million per year.

The most interesting characteristic of this species is its variation in population density from year to year and country to country, ranging from extremely low to enormously high levels. The sterile-male technique could be employed during low-level years.

(i) Italy

Some preliminary work suggests that the sterile-male method may have good possibilities against this insect. MELIS and BACCETTI (1960) [30] reported that pupae of the olive fly exposed to 12 000 r are rendered sterile.

They exposed sterile flies on caged olive trees at a ratio of 4 : 1 and obtained complete control of the species. Fruit of the tree was saved. This pilot test is most encouraging and requires more research for determination of whether practical field trials are indicated.

(ii) Greece

The first requirement was the development of a method of rearing the insect. The IAEA foresaw this need and supported K.S. Hagen in his research in Athens. Rearing of this fly proved to be more difficult than anticipated [6].

However, an artificial diet that permits the culture of D. oleae without olives has now been developed. Two generations of apparently normal flies have been produced on a medium consisting of agar, brewers' yeast, soy hydrolyzate, olive oil, Tween 80, sodium benzoate and a dehydrated carrot powder. A mould inhibitor (sodium benzoate) is added, and the pH is adjusted in the medium to 4.1 - 4.4 for control of the growth of undesirable micro-organisms.

The speed of larval and pupal development is normal, and reproduction occurs in at least two generations. However, the number of puparia recovered from the number of fertile eggs placed on the best medium to date varies between 30 and 60%. Further research is required to increase the percentage of recovery. The expense of the medium is now within a practical range, but further research may lead to a cheaper material to substitute for the soy hydrolyzate.

Obtaining oviposition in the laboratory presented problems. Plastic moulds or thick wax forms with ready-made punctures used successfully with other Trypetids do not work with the olive fly. It will not oviposit in an existing hole but makes a new one each time it deposits a single egg. Therefore an easily penetrated material must be used. Thin paraffin (melting point 52-53°C) domes about the size of a hen's egg were developed and found satisfactory (Fig. 11). These domes with moist cotton underneath were sealed to a piece of glass by gentle heating of the glass on a hot plate.

The number of eggs produced by the olive fly is less than that by other Trypetids. Even though the olive fly appears to live longer, its total fecundity in the laboratory averages 183, ranging from 99 to 276 (Fig. 12), considerably less than the oriental fruit fly, for example.

Two possible limitations may exist at the moment that will prevent shifting satisfactorily into a mass-culture programme. To date only two generations have been produced on the prepared medium, but soon it will be known whether a third generation will develop. In the use of larger units of the medium for mass culture, some difficulties may also arise which were not encountered with the smaller test units; thus, if either one of these limitations should occur, further research besides attempts to increase puparial recovery would be required.

During the course of development of the larval medium, several interesting points concerning the nutrition of D. oleae were discovered. First, the larvae require a much higher protein concentration than do the other fruit flies now being cultured. Secondly, the use of a copper chloride solution on the eggs influences the longevity and reproduction of the adults pro-

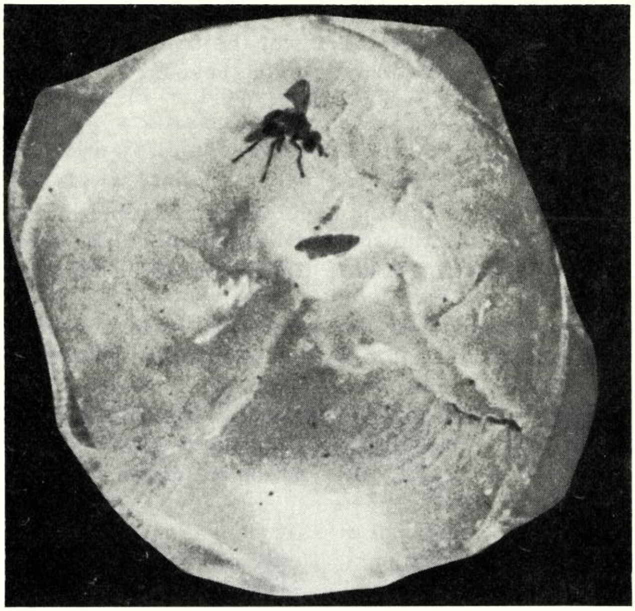

Fig. 11

Upper surface of paraffin dome showing Dacus oleae ovipositing
Note the small dark ovipositional punctures in the foreground.

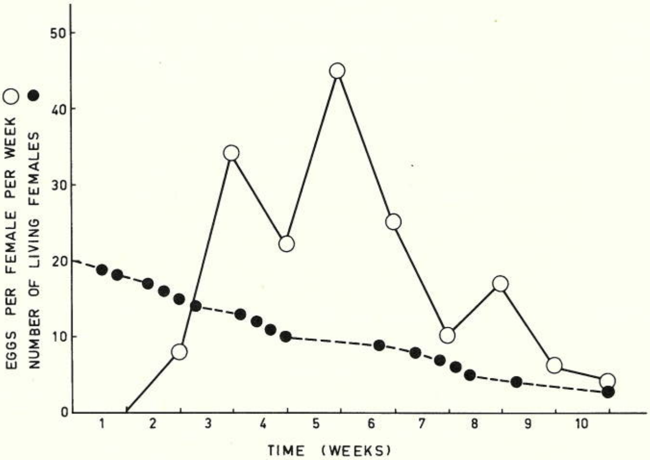

Fig. 12

The average fecundity per female per week and longevity of 20 Dacus oleae females exposed to a diet
of an enzymatic protein hydrolyzate of yeast plus sucrose plus water

duced. The copper may be destroying symbiontes necessary for metabolic activities in the adult. Particularly the excretory function seems to be influenced. Thirdly, there seems to be a reproductive diapause in the adults which emerge during the winter months. Further research concerning these observations will be undertaken.

(iii) Israel

Excellent research on developing an artificial diet and the eco-physiology of the olive fly has been carried out in Israel (MOORE, 1962 [31]). Among the interesting findings were the following:

1. Adult longevity was greatest when flies were fed a diet of sucrose, brewers' yeast, enzymatic yeast hydrolyzate and water. The temperature and longevity curve of females fed this diet exhibited three cardinal points:

(a) a minimum close to 11°C;
(b) an optimum near 22°C;
(c) a maximum at 35°C.

Under the same conditions, males were found to have optimum and maximum temperatures close to those of the females.

2. The temperature and longevity curve of sucrose-plus-water-fed adults fluctuated widely and did not display these cardinal points.

3. Adults emerging in winter were able to close the epidemiological cycle by spanning the period when no olives are available for oviposition when fed on a diet containing a carbohydrate and nitrogen compounds such as the one used in the experiments. As opposed to this, adults which had been fed a carbohydrate plus water, and under conditions not conducive to the development of micro-organisms on that food, were unable to overwinter and resume infestation.

4. In the freshly emerged adult, a substantial proportion of the contents of the larval "fatcells" present in the body is protein. Fourteen different amino acids were identified in the acid hydrolyzate of the protein, and most of them were identical with those of the olive-flesh protein.

5. In summer the fat cells disintegrate within 4 d of the adult's emergence, and their contents are freed in the hemolymph. These cells were always found to have disappeared before ovarian development had been initiated. A similar situation exists in the case of the Mediterranean fruit fly.

6. A carry-over of nutrients from the larva to the adult takes place by means of these temporary cells, but the amounts of metabolites needed for egg production do not always reach the required level.

7. Several free amino acids were identified in the honeydews of various insects present in and around the olive groves. The honeydew produced by the citrus mealybug on pumpkin as host was found to be toxic to the flies unless diluted by a fine spray of water designed to imitate dew fall. Under natural conditions the adult food permitting substantial and continuous oviposition is honeydew containing, among other substances, free amino acids.

With mass-rearing techniques almost perfected and much useful information obtained, field trials for evaluation of the effect of sterile males of the olive fly in natural populations will be possible.

j. The codling moth

The codling moth, Carpocapsa pomonella (L.), is found in almost all apple-growing areas in the world, and frequently it is the most destructive pest in the orchard (Fig. 13). Furthermore, this insect can be a serious pest of pears, peaches, apricots, prunes and English walnuts. In most

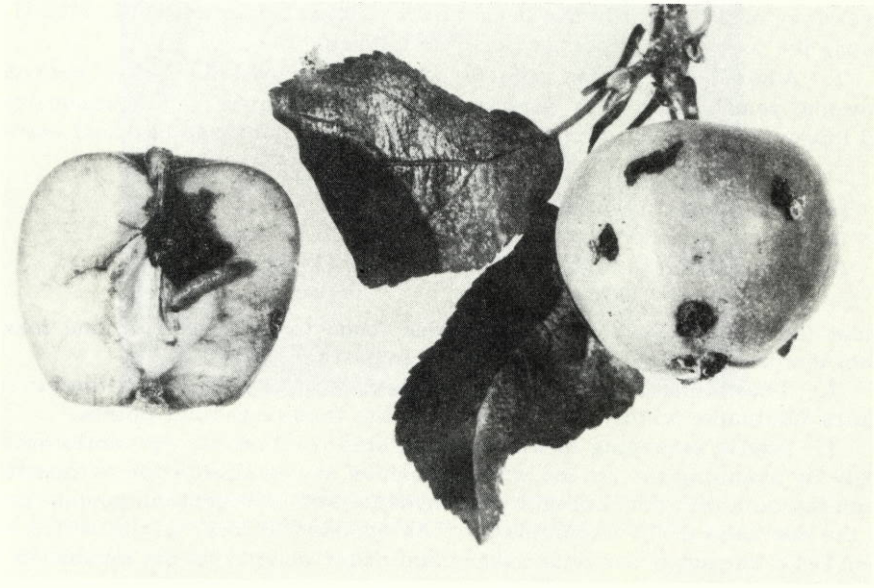

Fig. 13

Apples damaged by the codling moth

Right: four blackish marks on the apple are frass-filled holes where fully developed larvae have left the apple in order to pupate. Left: apple cut longitudinally to show fully developed larva and associated black frass. Four or five chemical sprays are applied annually to control the codling moth in British Columbia.

areas the only satisfactory method of control is by chemical sprays. Unfortunately, the constant application of chemicals has created many problems in the orchard. The use of DDT and Sevin against the codling moth has caused a phenomenal build-up of certain species of phytophagous mites. Also, these persistent organic insecticides are very destructive to many insects that are parasitic or predacious on aphids, scale insects and other potentially dangerous orchard pests. Insecticide resistance is another formidable problem; the insect first became resistant to lead arsenate, has more recently developed resistance to DDT and probably will become resistant to Sevin and Guthion. Also, the use of persistent chemicals may result in soil poisoning. Another important consideration is that of meeting residue tolerances at harvest.

It is certainly evident that some method of controlling the codling moth, other than by the use of chemicals, would be of great value. Control, or possibly localized eradication, by the release of sexually sterile male moths could be the answer. Work on the feasibility of using the sterile-male release technique was commenced in British Columbia in 1956.

Since the female codling moth usually mates more than once, some method of treatment was required which would induce dominant lethality in the sperm without affecting its activity, for such sperm would have to compete with sperm from normal males.

Reproduction in the codling moth is suppressed when the insect is reared at high temperatures (PROVERBS, 1962 [32]). Consequently, there was the possibility that sterility - used in the broad sense - could be induced by subjecting the insect to abnormally high temperatures. Mature larvae, pupae at four stages of development and adults moths were exposed to various high temperatures for different lengths of time. Some of the heat treatments induced complete or almost complete sterility in both sexes of the moth, but these treatments also caused prohibitively high larval or pupal mortalities or they reduced the frequency of mating.

Gamma irradiation was then investigated as a means of inducing sterility. The female was killed by a lower dosage of radiation than was the male, and the female was much easier to sterilize than was the male. Generally speaking, radiosensitivity decreased as development progressed from the egg to the adult stage. Also, young eggs were more radiosensitive than were old eggs, and young pupae were more radiosensitive than were old pupae.

In experiments for the determination of which stage of the insect should be irradiated for induction of sterility, it was found that the egg stage was not satisfactory; dosages that were high enough to cause sterility also caused prohibitively high mortality during post-embryonic development (PROVERBS and NEWTON, 1962 [33]). When mature male larvae were irradiated, dosages that were sufficiently high to cause complete, or almost complete, sterility adversely affected the mating behaviour of the moths that developed from the larvae.

When male pupae were irradiated early in the pupal stage, the dosage of radiation required to cause a reasonably high degree of sterility resulted in considerable pupal mortality (Fig. 14). However, when mature pupae (pupae from which the adults would emerge within about 18 h) were irradiated, the results were much more satisfactory. When mature male pupae were exposed to dosages up to 40 000 rad, adult emergence was not affected. The irradiated males mated satisfactorily with normal females, but only 2% of the eggs hatched. When the dosage was increased to 50 000 rad, the egg hatch was further reduced (0.5%). The longevity of these 50 000-rad-treated males was not affected, but the treatment killed a small percentage of the pupae; and, what is more important, the irradiated males only mated one-half as frequently as did controls. When the dosage was further increased to 65 000 rad, the egg hatch was zero. However, this high dosage of radiation killed 66% of the pupae, and the moths that did emerge only mated one-third as often as did normal control males. As far as irradiation of adult moths was concerned, it was found that at any one dosage of radiation the degree of sterility produced in the male was approximately the same as if the insect had been irradiated during late pupal development.

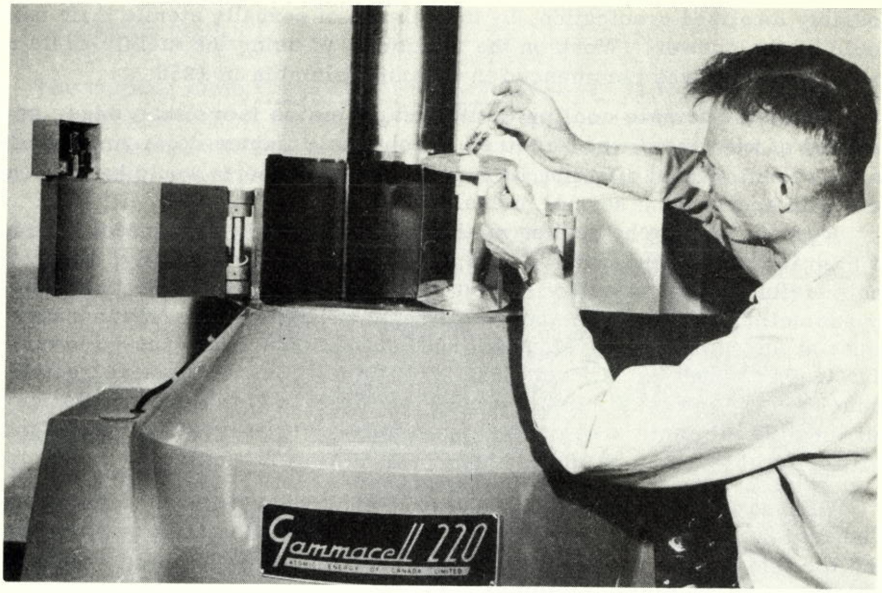

Fig. 14

Irradiation of pupae

Pupae, contained in a glass vial, are placed in an aluminium holder for irradiation. The holder centres the pupae at the approximate mid-point of the sample chamber, where the radiation dose rate is most uniform. This particular gamma irradiator, known as a Gammacell 220, is charged with Co^{60}, delivering a dose rate of approximately 100 000 rad/h.

Sperm from irradiated (40 000 rad) male moths were about two-thirds as competitive as those from normal males. Despite this, the reproductive potential of the moths was reduced about 75% when 50 irradiated male moths (exposed as mature pupae to 30 000 or 40 000 rad) were caged in the laboratory with 5 normal male and 5 normal female moths. The reduction in reproductive potential was less marked when both 50 irradiated (30 000 rad) males and 50 irradiated (30 000 rad) females were added to the normal insects. The reduction was even less marked when 50 irradiated (30 000 rad) females were added to the normal moths.

When irradiated male moths (pupae exposed to 30 000 rad, a dosage inducing dominant lethality in about 90% of the sperm) were caged with an equal number of normal female moths, the sex ratio of the adult offspring was approximately 9 males to 1 female. The female offspring were completely sterile; and the males were mostly so for, when they were caged with normal female moths, less than 2% of the eggs laid were viable. However, when irradiated male moths were caged with normal male and female moths, in the proportion of 10 : 1 : 1, the sex ratio of the adult offspring was 1 : 1 and both sexes were mostly fertile.

Although not as easy to rear in the laboratory, the codling moth can be reared by the thousands (Figs. 15 und 16). In orchard experiments (cages over dwarf apple trees) in which both irradiated males and females (exposed

Fig. 15

Rearing codling moth larvae

The insects are cultured on immature apples, exposed continuously to artificial light and a temperature of 80°F to prevent the mature larvae from entering diapause. The rearing trays are fitted with an electrical barrier to prevent the larvae's escaping.

as pupae to 40 000 rad) were caged with normal male and female moths, in the proportion of (a) 10 : 10 : 1 : 1, and (b) 20 : 20 : 1 : 1, the number of adults in the F_1 generation in (a) remained about the same as the number of normal adults present in the parent generation, whereas the number of F_1 adults in (b) was reduced to one-sixth of the number of normal adults present in the parent generation. However, when irradiated males alone were caged with normal male and female moths, in the proportion of (c) 10 : 1 : 1, and (d) 20 : 1 : 1, the number of adults in the F_1 generation was reduced in (c) to about one-third and in (d) to about one-tenth of the number of normal adults in the parent generation.

These results indicate that the release of sterile males may be a promising method for the control or localized eradication of the codling moth. The method is being tested in the field.

k. Crop insects

Research into the possible application of the radiation sterilization method of population suppression is now under way on a number of insects that attack a variety of plant crops. For the most part, only basic laboratory studies have been conducted, and, thus far, field trials are lacking. These exploratory investigations have shown that ionizing irradiation will

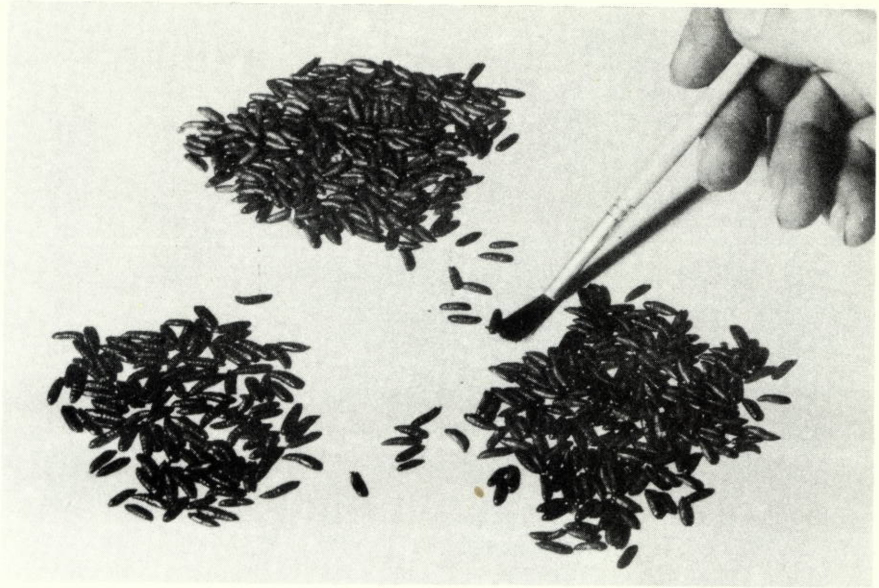

Fig. 16

Sorting pupae of the codling moth before irradiation
Pupae are irradiated when they assume a characteristic grey colour, i.e., about 6 h before adult emergence. Manual sorting probably could be replaced by electronic colour sorting.

induce sterility in all species, but considerable variation in amounts needed is evident. The research also suggests that radiation damage may limit application of the method for some insects. A common obstacle that must be overcome is the lack of practical mass-rearing methods. Some insects also appear to be so abundant that the technique may not be appropriate unless the infested area is first processed with other control measures to bring wild populations within workable limits. The activity patterns and behaviour of some insects may be such that it may be difficult for the sterile individuals to distribute themselves effectively throughout natural environments. Despite these difficulties, in situations where conditions are favourable few other approaches to the control of pests are so potentially rewarding.

The radiation sterilization method should also be thought of as a possible means of delaying development of infestation until crops are harvested.

The following summary of basic radiation research conducted at the United States Department of Agriculture laboratories may be of interest [27]:

(i) Sugar-cane borer

In basic studies on the sugar-cane borer, Diatraea saccharalis (Fab.) (Houma, Louisiana), dosages of 10 000 to 60 000 r severely affected moth emergence and fecundity of irradiated females. When pupae less than 3-d-old were irradiated at dosages ranging from 10 000 to 30 000 r, moth emergence was severely depressed. No viable eggs were obtained when pupae of either sex were irradiated at dosages above 15 000 r. When 3- to 6-d-old pupae were treated, moth emergence was not affected severely; however, some viable eggs were always obtained from the cross of irradiated males with non-irradiated females regardless of the dosage rate at which the males had been treated. When pupae older than 6 d were irradiated at dosages ranging from 20 000 to 60 000 r, emergence was not affected, and normal numbers of viable eggs were obtained. These data indicate that any level of irradiation which results in complete male sterility may also reduce moth emergence severely.

(ii) European corn borer

Studies with the European corn borer, Ostrinia nubilalis(Hābn.) (Ames, Iowa), permit the following conclusions concerning possible utilization of sterile males as a possible method for controlling the European corn borer: (1) One-day-old male moths can be "sterilized" by exposure to 32 000 r. (2) "Sterile males" can compete equally with untreated males for virgin females under laboratory conditions, suggesting that similar results might be obtained under field conditions. (3) If the irradiated males outnumber the normal males in nature by 2 to 1, the number of viable eggs may be reduced by 39%. (4) Because of erratic results obtained with irradiated pupae, the use of irradiated adults will be preferable in future trials. (5) The cost of rearing and treating large numbers of moths with X-rays may be economically unsound. A cobalt-60 source is desirable for any large-scale test. (6) In general, the use of irradiated-male releases for controlling the European corn borer appears to be promising enough to warrant further research in small isolated fields.

(iii) Pink bollworm

Radiation studies on the pink bollworm, Pectinophora gossypiella (Saunders) (Brownsville, Texas), show that sterilization can be achieved by irradiation of the fourth-instar larvae, pupae and adults. The safety margin between the sterilizing and lethal doses in larvae seemingly is rather narrow. Irradiation of pupae now seems to be the most promising method because of ease of handling and other factors. No morphological change has been observed in irradiated moths, but there have been behavioural changes. When pupae were exposed to doses above 110 kr, there was little movement of moths, and the latter clustered near the sugar-water solution used for food. Moths from pupae treated with higher doses were darker than normal and some had wing deformities. When larvae were treated at doses greater than 15 kr, there was a marked effect on colour and behaviour. When larvae were treated at doses of more than 10 kr, deformities and colour changes were observed in the adults.

(iv) Boll weevil

Preliminary studies of the boll weevil, Anthonomus grandis Boheman, (Starkville, Miss., and College Station, Texas) permit the following conclusions: (1) 2 500 r did not affect the longevity or egg-laying capacity of reproducing boll weevils but greatly reduced egg hatch. (2) 5000 r and higher doses greatly reduced longevity, egg-laying capacity and egg hatch of reproducing weevils. (3) Exposure of young, virgin males to 5000 r resulted in very low egg hatch for 20 d after mating with unexposed females, and this was followed by nearly normal egg hatch for the balance of the 30-d test period. (4) Exposure of young, virgin males to 10 000 r resulted in transient sterility and rapid mortality. (5) Exposure of young, virgin females to 10 000 r or 15 000 r virtually eliminated egg laying and resulted in rapid mortality. (6) Thirty-eight sterilized males did not affect egg laying or hatch when they were caged with 10 pairs of unirradiated weevils. (7) There appeared to be little, if any, effect of adult boll weevil age on susceptibility to the lethal effects of gamma rays. (8) Emergence of adults from prepupae, young pupae and old pupae was respectively prevented or greatly reduced or unaffected, following exposure of pupae to 10 000 r. All emerging adults died before the 14th day after emergence. (9) Exposure of boll weevil eggs to 150 and 600 r did not affect hatch or subsequent growth and development; a dosage of 2 300 r caused a drastic drop in hatch and prevented subsequent development.

(v) Fall army worm

Five- to nine-day-old pupae of the fall army worm, Laphygma frugiperda (J. E. Smith) (Tifton, Georgia), tolerated dosages from 5 000 to 25 000 r, with mortality equivalent to that in the controls. The mortality of three- to four-day-old pupae was directly proportional to increasing levels of radiation from 10 000 to 25 000 r (46% at 10 000 r; 91% at 25 000 r). A few moths emerged with a wing deformity after exposure to 10 000 r.

Generally, more egg masses and more eggs per mass were produced from crosses of treated males with untreated females than were produced from the reciprocal crosses. Conversely, fewer eggs hatched after crosses of treated females with untreated males than after the reciprocal crosses. The percentage of egg hatch was usually inversely proportional to the total irradiation dosages.

(vi) Drosophila

Research by the U.S.D.A. has shown that Drosophila, which are pests of tomatoes and other fruits, can be sterilized at practical dosages of gamma radiation without pronounced effect on behaviour. Certain chemosterilants also produce sterility. Field studies conducted in the summer of 1962 in the vicinity of Beltsville, Maryland, revealed that overflooding wild populations of Drosophila in tomato fields may regulate population growth and prevent development of objectionable numbers.

Preliminary laboratory tests were made on the effect of gamma radiation on the fertility of Drosophila melanogaster Meigen. Untreated females mated with males exposed to gamma radiation at 5000 r in the larval stage, 10 000 to 20 000 r in the pupal stage or 2000 r in the adult stage deposited the normal number of eggs, but none of them hatched. At the same dosages females irradiated in the pupal or adult stage and mated with untreated males produced few or no eggs, and females irradiated in the larval stage produced fertile eggs but in smaller numbers. The longevity of males or females irradiated in the pupal or adult stage was not affected while the longevity of those irradiated in the larval stage was reduced.

In multiple mating tests untreated female flies mated with irradiated males produced sterile eggs until a subsequent mating with untreated males, after which they produced viable eggs that developed into adults. Untreated female flies mated with normal males produced viable eggs and continued to do so after a subsequent mating with irradiated males. This effect of multiple mating needs further study before any conclusions can be drawn. In limited tests sterile and normal males in the ratio of 5 to 1 gave 55 to 60% reductions of the progeny of normal females with which they mated. The 2000-r dosage of gamma radiation was effective on adult males 1, 5 and 10 d after emergence. One treated male which mated with five different untreated females in an 8-h period caused each to produce sterile eggs. This male repeated the performance after 5 d and again after 10 d. At Beltsville progress in the development of techniques for sterilizing Drosophila by chemicals has paralleled that with gamma radiation. Effective dosages of apholate have been developed and exploratory tests initiated on the control of Drosophila by sustained releases of sterile males in small isolated fields of tomatoes. The results indicate that population growth may be inhibited by a ratio of 16 sterile flies to one normal wild fly.

1. The Mediterranean flour moth

Investigations have been made at Wantage into the biology, ecology and radiosensitivity of Anagasta kühniella Zell, the Mediterranean flour moth, to determine the feasibility of using the sterile-male technique to control infestations (BULL, 1962 [5]). This technique is not to be confused with the radiation disinfestation of grain, which is a promising method of destroying insects in infested grain.

(i) Biology and ecology

Development rate of the moth was examined on numerous milling fractions both in the laboratory and in the mill; fastest growth occurred on fractions with high bran content and slowest growth on good flours (Fig. 17). The rate of emergence was correlated with the total yield. Development in the mill was most rapid in the summer (average temperature, 25°C) and most retarded in the winter (16°C). The degree of infestation in various machines, ducts and elevators was attributed to the type of flour fraction, machine process, screen size and flow sequence.

Reinfestation could occur by means of infested sacks returned to the mill; 10% of returned sacks were infested – smaller firms returned a higher proportion of infested sacks than did large firms.

Free-flying adults were present on the milling floors a few weeks after annual fumigation although treatment had been completely effective against large test cultures.

Populations increased in January, with a subsequent two-fold increase per month until fumigation in July. Changes in the abundance of other

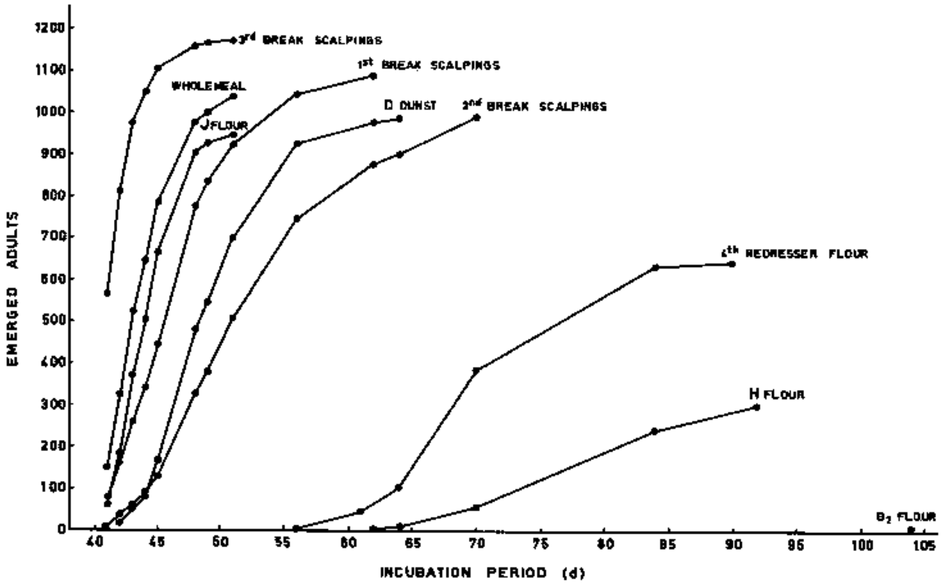

Fig. 17

Accumulated emergence of adult A. kühniella from cultures on 8 milling fractions and wholemeal in relation to incubation period

species were also examined. Movements of adult moths were examined by the release/recapture technique; dispersal of males on the milling floors was slow, but some movement occurred between floors. Adults released in machines were able to escape into the body of the mill. Males comprised 70-85% of the catches in light traps.

(ii) Mating behaviour of males

Males offered a virgin female on successive days were able to mate about seven times but only 4.4 matings were fertile. Multiple mating by the males reduced their longevity significantly, and a proportion of females - up to 50% - mated more than once.

(iii) Large-scale rearing

Laboratory culture methods were developed into a method by which 100 000 pupae per day could be reared in two small constant-temperature rooms by one man. Distribution of the corrugated cardboard blocks containing the pupae would also require the services of one man. Flour ground from English wheat proved to be a rearing medium superior to Manitoba wheat, development from egg to adult being two days faster on the former medium than on the latter. No more than 5000 eggs per kilogram of flour should be used in order to avoid excessive culture temperature.

(iv) Radiosensitivity

Studies in other species and work on the large-scale rearing of the moth indicated that pupae represented the best stage for ease of handling and distribution and the production of sterility by radiation. Experiments were, however, carried out on all stages of development. The criteria of all radiation experiments were adult emergence, longevity and fertility.

(v) Emergence

Radiation reduced total adult emergence and caused considerable delay in cultures up to the age of 32 d (larval/pupal moult) (Fig. 18). At 34 d there was a sharp transition in the emergence pattern: delay was no longer apparent, and emergence was unaffected by radiation throughout the pupal stage. The greatest sensitivity was shown by young eggs and by larvae beginning to moult into pupae.

More males than females emerged when eggs and larvae were irradiated, but in the pupal stage the sex ratio was approximately unity. Wing deformities, largely confined to adults irradiated in the immature stages, also occurred; these deformities were partly responsible for the low incidence of mating at high doses.

(vi) Longevity

Male longevity was reduced by radiation, but the extent depended upon the physiological state and dose. The most marked reduction was found when mature larvae and young pupae were irradiated, but with older pupae the effect of radiation was much reduced. Similar effects were observed with females, but the position was complicated by the interaction of mating and longevity. Normal virgin females lived nearly twice as long as did mated females, but irradiated unfertilized females did not live as long as did the fertilized females.

(vii) Fertility

Fertility was assessed as the ability to produce adult progeny. Irradiated males and females were intermated and also mated with virgin stock. The highest fertility was found when irradiated males were mated with normal females, indicating that males were more resistant than females. The fertility of males was markedly reduced by radiation but persisted at low levels up to 45 000 rad. The fertility of females did not extend beyond 25 000 rad.

More spermatophores were found in irradiated females which had mated with irradiated males than when irradiated adults had mated with normal adults. The most marked increases were found with old females, which indicates that multiple mating had taken place and also that irradiated males did not satisfy the females as effectively as did normal males.

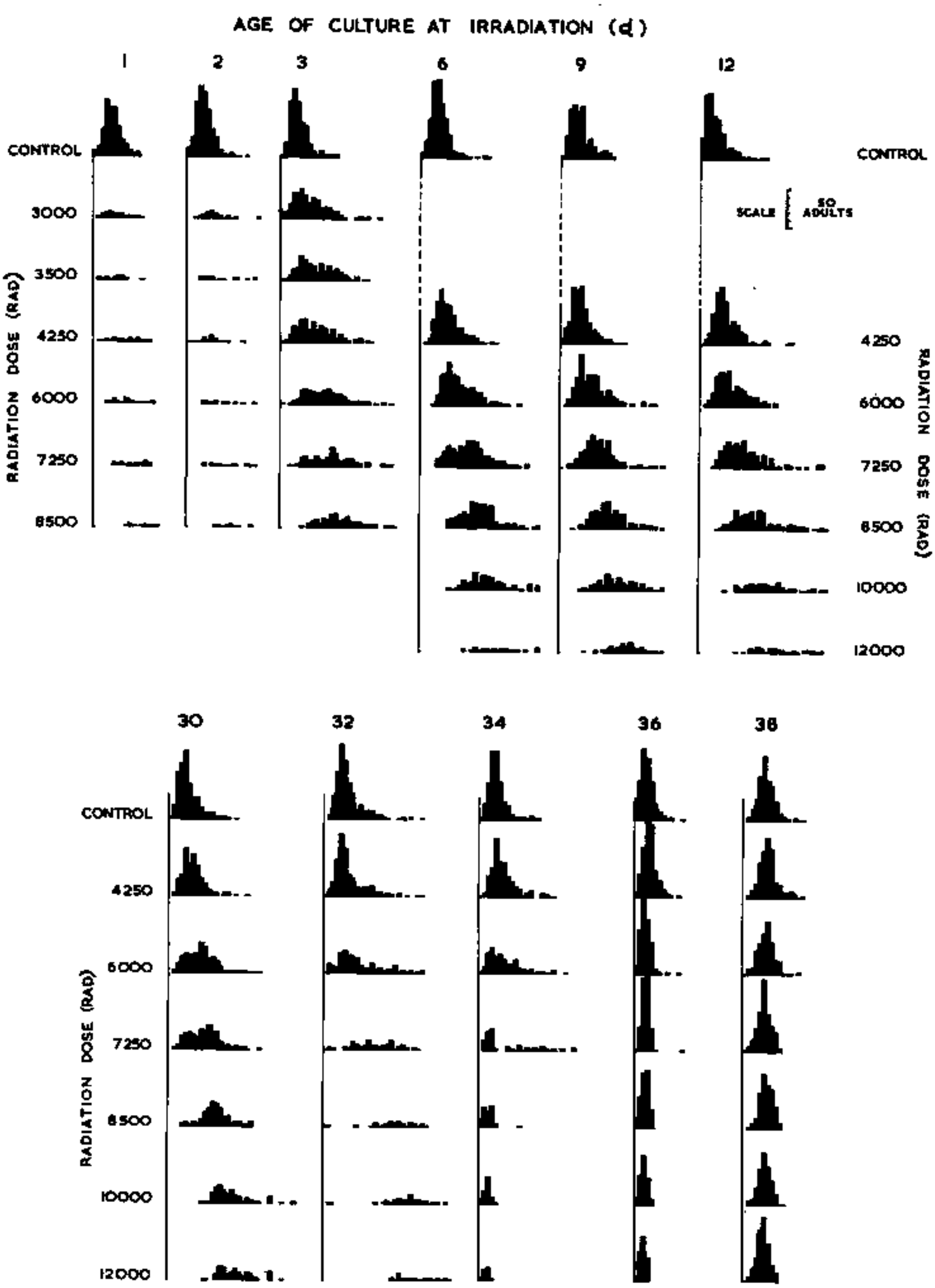

Fig. 18

Histograms of daily adult emergence of moths from cultures irradiated at different times in the life cycle *(viii) A. kühniella and sterile-male release*

Experimental work has shown that large numbers could be reared for release and that the pupae could be sterilized adequately. Ecological circumstances, however, militate against the use of the technique; the distribution of the wild population is exceedingly variable owing to a number of factors, i.e. milling fraction, position in mill flow and temperature.

Certain portions of ducts and elevators are relatively inaccessible: since efficient operation of the technique demands a reasonably uniform ratio of sterile to fertile adults, "hot spots" of infestation could prevent complete eradication. The long life-cycle of stages growing in certain milling fractions and in certain machines would necessitate release being carried out over a considerable period, possibly two or three years.

Reinfestation may also occur via returned sacks and from other portions of the premises which have not been completely treated.

Adult mobility is relatively slow in the body of the mill and is probably even more restricted in the machines and duct systems. The poor dispersal of released adults would, therefore, have to be compensated by an increase in the number of release points. Finally, since the sterile-male technique is specific, other pests would not be eliminated and would require treatment every two or three years. It is concluded, therefore, that the technique is unsuited for the economic control of the Mediterranean flour moth.

m. Other grain pests

The pests of grain and stored foods are confined principally to the Lepidoptera and Coleoptera. To a large extent the arguments which apply to the mill moth apply to other grain pests:

(1) Infestations are often of two or more species which may be competing to some degree. Eradication of one species would still leave others which might then increase more rapidly than usual.

(2) Commodities are frequently stored in large bulk, which would make efficient distribution of sterile insects very difficult, particularly where "hot spots" may occur.

(3) The majority of the adult Coleoptera do extensive damage, which would be amplified by released sterile adults.

(4) Many species of beetles are difficult to sterilize while retaining normal longevity. Lepidoptera can be sterilized and still retain normal longevity; doses in the region of 50 000 rad are necessary.

(5) Contamination of food materials by large numbers of insect fragments may render them unfit for human consumption.

At present, therefore, it seems unlikely that the sterile-male release technique can be applied effectively for the control of stored products pests.

n. Recent research on the screw-worm

During and following the Southeastern programme it became clear that more basic research was needed to make eradication less costly and more effective. Because the screw-worm and other insects are injured and are less vigorous following a sterilizing dose of radiation, it was decided to intensify studies on radiation effects on the reproductive system. Further research was also initiated on the ecology of this insect and on the development of an effective attractant.

At the Kerrville, Texas, laboratory of the Agriculture Research Service of the U.S.D.A., following an increase in funds, Bushland, LaChance, Crystal, Baumhover and others intensified investigational work on the screw-worm. LaChance studied radiation effects on the screw-worm. He found that there are two ways of causing sterility by radiation. In one the treatment inhibits the formation or development of mature ova or sperm while the other does not prevent the production of these cells but rather induces lethal changes in the hereditary material of the gametes, thus rendering them incapable of sustaining embryonic growth. Sterility is achieved in both cases, but the processes involved are different. LaCHANCE and LEVERICH (1962) [34] showed that newly emerged females can be sterilized with about 2500 r. However, when females are 24 h of age, 8000 r does not affect ovarian growth and development. They also showed that in the screw-worm the nucleus of the reproductive cells is far more sensitive to ionizing radiation than is the cytoplasm. Demonstrated also was the fact that adult females 1, 2 and 3 d of age are quite resistant to radiation when exposed to cobalt-60 and tested for dominant lethals. Large numbers of eggs are deposited and most of them hatch. However, females 4 and 5 d old are very sensitive, and complete sterility is caused by low doses. Thus, sterility can be achieved by induction of dominant lethal changes. With any insect under study doses of radiation in relation to the stage and age must be carefully worked out under constant environmental conditions.

In radiation of the male screw-worm it was found that sensitivity varies according to different stages of development similar to that of the female. When this sex is irradiated as 4-5-d-old pupae, a dose of 2500 r will induce dominant lethal changes in the spermatids. Adult males 24 h of age are resistant, and doses of 5000 to 6000 r have not produced complete dominant lethality.

The Kerrville workers found that packing and storing screw-worm pupae before radiation treatment may cause changes in biological effects. Anaerobic conditions tend to reduce the effectiveness of radiation. They found that 3500 r given to pupae in air reduces egg production and hatchability. However, the same exposure in pure nitrogen or carbon dioxide has little effect on egg production and hatchability. Treatment with the same dose in an atmosphere of 50% air and 50% CO_2 is more damaging than is treatment in air alone. Some gases therefore enhance the biological effect of radiation while others reduce the effect. These findings may have considerable importance in the sterilization of some species that are injured by the treatment.

Temperature which affects the growth rate of insects is important in radiation work. Also, splitting the radiation dose over a period of time affects damage to tissues. Some workers have found there is no advantage to splitting the dose so as to reduce somatic tissue damage and yet obtain sterility.

o. Chemosterilant research

There has been much interesting research in the Entomology Research Division of the U.S.D.A. on the use of chemicals to sterilize insects during the past two to three years (LINDQUIST, 1961 and 1962 [35, 36]). A few of the ethylenimine compounds known as alkylating agents and commonly referred to as radiomimetic chemicals have shown exceptional promise in causing sexual sterility of the housefly, stable fly, screw-worm and several species of mosquito. Several excellent papers have been published by LaBRECQUE (1961) [37], HARRIS (1962) [38], MORGAN and LaBRECQUE (1962) [39], WEIDHAAS (1962) [40], WEIDHAAS and SCHMIDT [41] and others. The practical small-scale use of a chemosterilant for control of houseflies has been reported by LaBRECQUE, SMITH and MEIFERT (1962) [42]. An isolated refuse dump in the Florida Keys was treated for nine consecutive weeks with a dry granular cornmeal bait containing 0.5% of aphoxide. The chemosterilant bait was applied in a manner very similar to that recommended for insecticide baits in such situations. Housefly populations were reduced from 47 per grid count to 0 within four weeks. The number of egg masses from female flies collected at the dump was reduced from 100% to 10% within four weeks, and the hatch of eggs from these flies was reduced more than 90%. A similar, but untreated, refuse area maintained a high population of flies throughout the test period. This preliminary experiment shows great promise for the control of houseflies but needs confirmation under a variety of conditions, including tests in non-isolated areas.

VI. CONCLUSIONS

Although the screw-worm fly mates only once, the principle of sterility in a population should work with polygamous insects, provided that mating is with males producing competitive sperm bearing dominant lethals. The deleterious genetic material should thus be introduced into the population. Experiments with polygamous tropical fruit flies have shown that continued introduction of sterile males in cages of normal males and females decimates the populations of flies. Sperm from sterilized males were competitive with sperm from normal flies, and because of a preponderance of steriles the population in cages was annihilated.

It seems obvious that an insect species with a low natural population would be more desirable, because it would be easier to outnumber the native population by a rearing programme. Apparently the tsetse flies, <u>Glossina</u>, have a low natural incidence of about 200 to 2000 per square mile of area. They are, of course, exceedingly difficult to rear, but this problem might be overcome. Furthermore, it is possible that rather large numbers of native tsetse flies could be trapped, exposed to radiation and released to supplement a rearing and release programme. Another possibility is the development of powerful lures that would attract both sexes of tsetse to a central point where they could be made sterile by radiation. Chemical sterilants exposed with a bait might also be highly effective.

Sterilizing the native population of an insect either by radiation or chemicals, rather than rearing and releasing them, presents exciting possibilities of control. If this could be accomplished, eradication of a species would be faster and the cost would probably be less. Sterilizing the males of a natural population would solve any problem that might be created by introduction of large numbers of a species that are blood suckers, carriers of diseases or annoying to man, animals or crops. Furthermore, if 90% of a native population could be sterilized, reproduction would immediately cease in the females and the males would be available to compete with non-sterile males in mating with non-sterile females. Theoretically there should soon develop a preponderance of sterilized males in the area treated. In a few generations reproduction would be enormously reduced, and the insect population would be depleted to the vanishing point.

It should be recognized that the sterile-male method of insect control is not a "ready-to-go" technique for general insect elimination. An immense amount of research is necessary with any insect before it can be ascertained if the method has practical application. The method probably will have use on only a comparatively few species and only in certain types of situation, such as in isolated areas. Besides a thorough knowledge of behaviour and ecology, much radiation research on the insect is required.

VII. PANEL RECOMMENDATIONS

I. Overall statement

In view of the excellent results obtained in the use of the sterile-male technique for the eradication of the screw-worm in the Southeastern United States and the promise of this technique shown against tropical and subtropical fruit flies, the Panel recommends that the Agency should take active steps to support existing programmes, initiate new programmes and disseminate technical information on the application of this technique to the control or eradication of suitable insect pests, such as the Mediterranean fruit fly, the oriental fruit fly, the olive fly and the Queensland fruit fly, in particular, and also the tsetse fly, the leopard moth, the tropical ox warble, cotton boll weevils, etc.

II. Introduction

Insects are among man's foremost competitors for food, fibres, forest commodities and other useful products of plant growth. They also transmit numerous serious diseases of man, animals and plants. This results in world losses that total many hundreds of millions of dollars annually. Failure of insecticides to provide practical control measures for certain insect pests and growing concern over problems associated with insecticide usage emphasize the urgency of research to find effective remedies not requiring the application of toxic materials.

The sterile-male release procedure has eradicated the screw-worm fly, a serious pest of cattle, in the Southeastern United States and has demonstrated promise against tropical fruit flies. Further evaluation against fruit flies and other selected crop pests is highly desirable.

The sterile-male release technique has possible usefulness as

(a) an insect eradication procedure, whether for established or incipient infestations;
(b) a method for delaying development of damaging infestations until crops are harvested; and
(c) a method to prevent re-establishment of infestations by using sterile caretaker populations.

This method may be used alone or in combination with other control practices. It is obviously not a panacea for all insect problems. An extra bonus lies in the ecological and biological aspects of pest insects that must evolve from such investigations.

III. Recommendations

A. Field operations and research

1. Mediterranean fruit fly

The Mediterranean fruit fly is of great economic importance in the Mediterranean basin. It is also a pest in many of the subtropical areas such as Central America, Australia and certain islands. Evaluation of the sterile-male method is under way in Hawaii, Central America and the United Arab Republic. A programme is in the planning stage in Tunisia. The Panel encourages support of this type of programme.

2. Queensland fruit fly

An action programme on the Queensland fruit fly is now under way in Australia. The Panel feels that this type of programme should also be supported.

3. Olive fly

Present progress in olive fly investigations, particularly in Greece, has been so satisfactory that such programmes should be encouraged and supported wherever practicable. Particular areas of support could be related to evaluation techniques, population estimates, field trials, labelling techniques, and release and recapture.

4. Tsetse fly

As the current methods aiming at the eradication of human and animal trypanosomiasis are still not satisfactory, and as the sterile-male technique is a new and promising approach towards the control and eradication of the vector, the tsetse fly, the Panel recommends that immediate steps be taken to initiate and promote the research needed.

With this in mind, the Panel further suggests that a consultant be engaged to develop and present a programme to the Director General. The problems involved being of an exceptional magnitude and importance for science as well as for humanity, the Panel hopes that the World Health Organization and the Food and Agriculture Organization and other organizations will give their help and support.

5. Tropical ox warble

Dermatobia hominis, the "tropical ox warble" or "tórsalo", is a parasite of considerable economic importance in Latin America. The present methods of control are unsatisfactory. The sterile-male technique offers promising prospects. The Panel recommends that the Agency encourage and support such research in co-operation with other interested organizations.

6. Other insect pests

The Panel considers that other insects might be controlled by this method. The collection and dissemination of information on such insects is desirable. Eradication of the Australian sheep blowfly is most unpromising at the present, mainly because of economic considerations. The use of the sterile-male technique cannot be applied economically or efficiently for the control of stored grain insects. However, note was taken of a previous panel report suggesting that irradiation disinfestation of grain was both feasible and practicable.

7. Labelling techniques

Effective means of labelling insects with radioisotopes are extremely important. Work to develop more suitable and standardized methods should be supported and encouraged.

B. Exchange of information

1. Collection and dissemination

The Panel recommends that:

(a) A scientific report on the work of the Panel be prepared for the internal use of the Agency and for distribution to members of the Panel;

(b) The Agency arrange for a consultant to prepare a fuller account of the Panel's findings, primarily for the information of Governments and similar bodies. In this might be included a description of the principle upon which the sterile-male technique is based together with an objective evaluation of the limitations and potentialities of the procedure. The Agency might consider publishing the report; and

(c) The Agency undertake the publication, at regular intervals, of a scientific information circular containing abstracts from the published literature and summaries of articles in press when these are available. Scientific workers might be encouraged to provide summaries when material is submitted for publication and to provide progress reports at frequent intervals. A current bibliography should also be included. The first issue of the circular might contain reference to this panel and a copy of the account referred to in (b) above.

2. Co-operation

In the opinion of the Panel, the IAEA should work co-operatively with WHO and FAO and with any other interest bodies in the development of research on the sterile-male release procedure.

3. Training

The Panel recommends that the IAEA support requests for training of scientists from countries where research on the radiation sterilization technique is either in progress or contemplated. Long-term training as well as shorter study periods in laboratories where active research or field programmes are under way are highly desirable. This is important because the field is new and highly technical. Effective studies require an adequate knowledge of radiation and its effect on insects. The IAEA should also support requests for exchange visits by scientists engaged in research on this subject when such visits could be expected to facilitate progress or are considered desirable to reconcile differences between critical experimental data.

4. Future panel meetings

In view of the rapid expansion, fluidity of research results and great interest in this field, the Panel, which represents the first international gathering of its kind, recommends that the IAEA consider in 1964 another meeting of research scientists engaged in studies on this method to review progress and exchange new information.

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