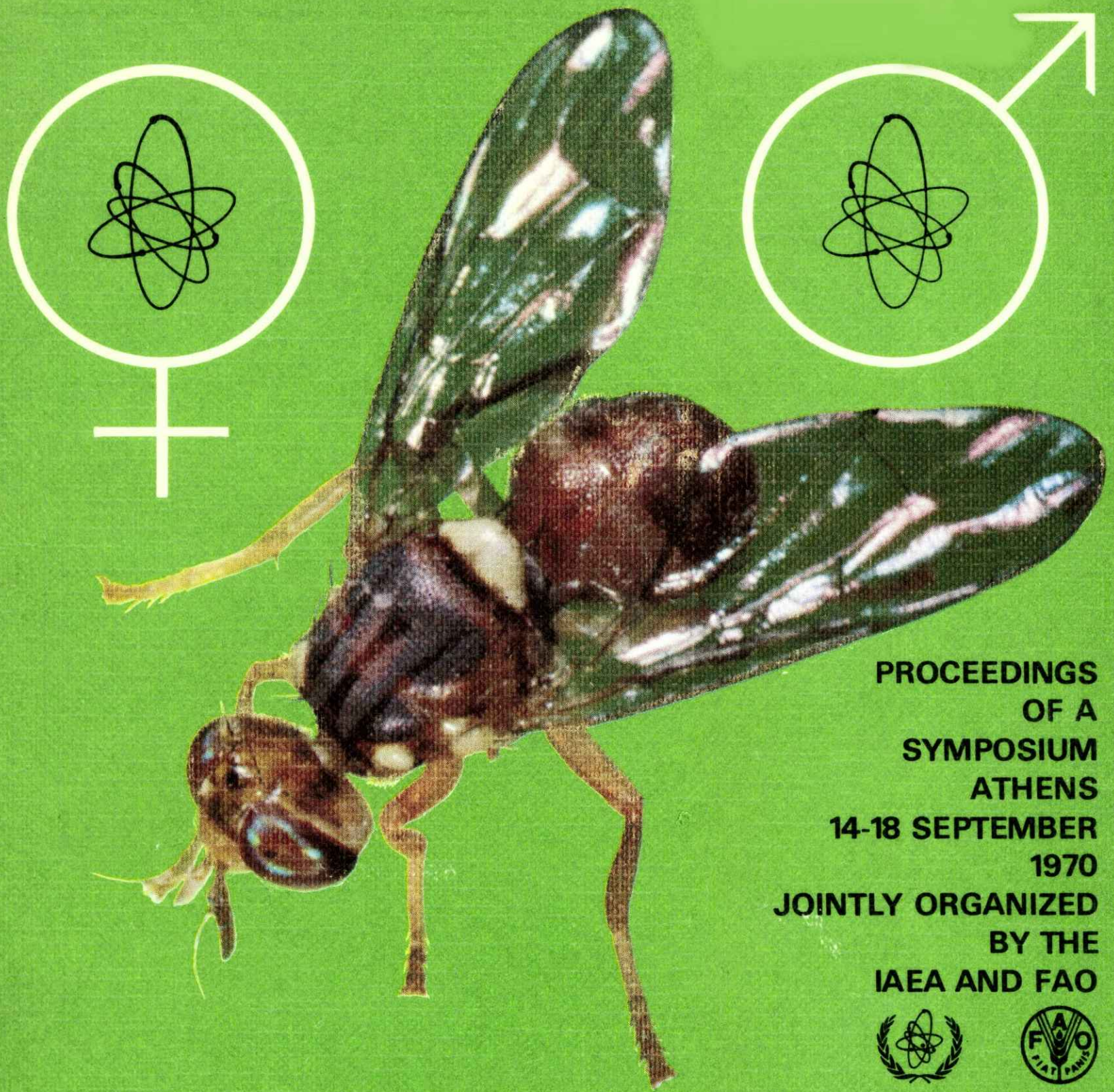


PROCEEDINGS
OF A
SYMPOSIUM
ATHENS
14-18 SEPTEMBER
1970
JOINTLY ORGANIZED
BY THE
IAEA AND FAO

Sterility Principle for Insect Control or Eradication

INTERNATIONAL ATOMIC ENERGY AGENCY, VIENNA, 1971

STERILITY PRINCIPLE FOR INSECT CONTROL OR ERADICATION

PROCEEDINGS SERIES

STERILITY PRINCIPLE FOR INSECT CONTROL OR ERADICATION

PROCEEDINGS OF A SYMPOSIUM
ON THE STERILITY PRINCIPLE FOR
INSECT CONTROL OR ERADICATION
JOINTLY ORGANIZED BY THE IAEA AND FAO
AND HELD IN ATHENS, 14-18 SEPTEMBER 1970

INTERNATIONAL ATOMIC ENERGY AGENCY
VIENNA, 1971

STERILITY PRINCIPLE FOR
INSECT CONTROL OR ERADICATION
IAEA, VIENNA, 1971
STI/PUB/265

Printed by the IAEA in Austria
February 1971

FOREWORD

The symposium in Athens on 14 - 18 September 1970 was the fourth held since 1961 by the FAO and IAEA on the subject of radiation and radioisotope application in entomology; however, it was the first symposium devoted entirely to the sterility principle and related entomological studies for insect control or eradication. A total of 89 participants from 39 countries and six international organizations attended, and the 49 papers presented, together with the discussions, are published in the present volume.

The meeting was held against a background of mounting interest in the use of non-pesticide methods of insect control. Increasing food production calls for better and more effective protection of the food in the field and in storage against insects and pests which are estimated to devour about one-third of the world's potential food production.

There is general agreement that continuing reliance on pesticides as a sole method of insect control has numerous disadvantages, which have been well publicized, yet effective alternative methods are not immediately available. The alternative methods of pest control discussed at this symposium have distinct advantages, since they leave no residues, are species-specific, and if successfully and continually applied can lead to eradication. The sterile-insect release method has already been successfully applied for the control and eradication of several insect species and with further development could be used against numerous other insects. Theoretically the technique is applicable to all sexually reproducing species.

In the past ten years hundreds of insect species have been studied with the eventual aim of applying the sterility technique. Significant advances have been made in insect mass-rearing technology, and in the use of ionizing radiation and chemicals to induce sterility. An impressive number of field trials have demonstrated that the sterility principle is indeed promising for the control of many insect species, yet much additional work remains to be done before the technique can be used on a large-scale routine basis.

One of the most persistent barriers to the application of the technique in area-wide insect suppression programs is the difficulty in obtaining funds for the conduct of large-scale field demonstrations for those species where the required parameters have been developed. In addition to high initial cost, these programs require a smooth and efficient organization of experienced scientists and technicians; consequently the laboratory research phase is more easily within the reach of the individual scientist. However, as long as insects remain our primary competitors for food and fibre and continue to transmit disease to man and livestock while we at the same time strive to avoid the continued and increased use of pesticides there can be no pause in the development of all alternative pest-control methods.

The organizers are very grateful to the Government of Greece for acting as host for the symposium. The close co-operation of the staff of the Greek Atomic Energy Commission, External Relations Office, was indispensable in making this symposium a success.

EDITORIAL NOTE

The papers and discussions incorporated in the proceedings published by the International Atomic Energy Agency are edited by the Agency's editorial staff to the extent considered necessary for the reader's assistance. The views expressed and the general style adopted remain, however, the responsibility of the named authors or participants.

For the sake of speed of publication the present Proceedings have been printed by composition typing and photo-offset lithography. Within the limitations imposed by this method, every effort has been made to maintain a high editorial standard; in particular, the units and symbols employed are to the fullest practicable extent those standardized or recommended by the competent international scientific bodies.

The affiliations of authors are those given at the time of nomination.

The use in these Proceedings of particular designations of countries or territories does not imply any judgement by the Agency as to the legal status of such countries or territories, of their authorities and institutions or of the delimitation of their boundaries.

The mention of specific companies or of their products or brand-names does not imply any endorsement or recommendation on the part of the International Atomic Energy Agency.

CONTENTS

STERILITY PRINCIPLE FOR CONTROL OF INSECTS AFFECTING MAN AND ANIMALS (Session III)

CHEMOSTERILIZATION AND REPRODUCTIVE PHYSIOLOGY (Session IV)

BASIC BIOLOGY AND ARTIFICIAL REARING OF INSECTS
(Session V)

STERILITY PRINCIPLE AND INSECTS ATTACKING FOOD AND FIBRE CROPS (Session VI)

GENETIC MECHANISMS OF INSECT CONTROL (Session VII)

INSECT RADIOBIOLOGY (Session VIII)

STUDIES IN RADIATION STERILIZATION OF INSECTS

(Session I)

Chairman

M.E. TZANAKAKIS (Greece)

Survey paper

STERILITY PRINCIPLE FOR INSECT CONTROL
Historical development and recent innovations

R. C. BUSHLAND
Entomology Research Division,
Agricultural Research Service,
United States Department of Agriculture,
Fargo, N. Dak., United States of America

Abstract

STERILITY PRINCIPLE FOR INSECT CONTROL: HISTORICAL DEVELOPMENT AND RECENT INNOVATIONS.

The sterile-male technique of insect control, in the form so well known for its success in the eradication of the screw-worm, Cochliomyia hominivorax (Coquerel), utilizes radiation-induced dominant lethal mutations. Laboratory-reared insects are sterilized by exposure to ionizing radiation and released in overwhelming numbers to compete for mates in a native population. Many species of Diptera tolerate a dose in the range of 5 to 10 krad and can be effectively sterilized in this manner.

A similar dose administered to the boll weevil, Anthonomus grandis Boheman, causes so much radiation damage to the midgut that it is necessary to use a chemosterilant with selective action on the gonads to avoid somatic injury.

Sterilization of Lepidoptera requires about 50 krad of gamma radiation to induce dominant lethal mutations in all the sperm. Somatic damage from this large dose reduces vigour and mating competitiveness. Aberrations in spermatophore transfer commonly result so the sperm of irradiated males are not competitive.

A dose of about 15 krad does not completely sterilize moths, but their chromosomes undergo so many modifications, such as reciprocal translocations, that their F_1 progeny, though active at mating, are almost all sterile. The irradiated adults, since they are treated with only about one-third the sterilizing dose, do not suffer the severe somatic damage associated with complete sterilization and are vigorous and competitive. Further, these males are able to transfer their spermatophores properly so that their sperm are competitive.

The sterile-male technique, as practised to date, requires that insects be reared in the laboratory or be collected in the field, be treated, and then be released to compete for mates in nature. Except for those instances where irradiation causes somatic damage that could be avoided by a chemosterilant with selective action, there is no advantage in using chemical mutagens for insect sterilization.

Many female insects mate only once because those females develop a mating avoidance response as a result of a chemical received in the semen. A chemosterilant for males of such species need not be a mutagen but could be a substance with physiological rather than genetic effects since competitive males – but not competitive sperm – are required. If such a chemosterilant were used to sterilize insects in nature, the expense of rearing, treating, and distributing sterile insects would be avoided, and the economic application of the sterile-male technique would be greatly advanced.

INTRODUCTION

I was invited to give this paper because I had the good fortune to be involved in the first insect sterilization experiments done with the practical goal of developing a technique for pest eradication. In the ensuing 20 years of research in this field, I have had the privilege of associating with and learning from young men especially trained in insect genetics, cytology, and radiation biology. I will not bore you with a long list of insects subjected to sterilization experiments. This information was reviewed by LaChance

et al. [1] and is further documented in recent publications of the International Atomic Energy Agency. In my talk today, I will attempt to tell you how this still new field of entomology got started and what I consider to be promising developments in control of insect reproduction.

SCREW-WORM ERADICATION

Our story begins in 1933 when the screw-worm, Cochliomyia hominivorax (Coquerel), appeared in Georgia after accidental introduction through shipment of infested cattle from Texas. The species is an obligatory parasite of warm-blooded animals, and it is found only in the Western Hemisphere, living year around only in tropical and sub-tropical areas. In the United States, the insect survived the winter in the warmest parts of those states bordering on Mexico. It spread several hundred miles northward each summer but did not get far east of the Mississippi River.

The 1933 infestation in the South-east spread from Georgia into Florida, a sub-tropical peninsula where it was able to survive the winter cold. In the summertime, screw-worms attacked livestock over much of the South-east, but their range did not go far enough to merge with screw-worms migrating north from the Texas overwintering area. Hence, there was a new and isolated population widespread in summer, but in the wintertime occupying only about 50 000 square miles of the Florida peninsula.

Workers of the United States Department of Agriculture studied the biology of the insect in this new habitat and co-operated with livestock growers and State agricultural officials in attempting to eradicate screw-worms by treating infested wounds with a larvicide and by protecting livestock with a fly repellent. Since the insect is an obligatory parasite in its larval stage and breeding is chiefly in livestock, it was hoped that an intensive program of wound treatment would so reduce the insect population that eradication would result. This control program, conducted from 1935 to 1937, reduced but did not eliminate the screw-worm population; it was able to maintain itself in neglected livestock and wild animals.

E. F. Knipling was one of the investigators studying the biology of the screw-worms in the South-east in 1935 - 1937. In 1937, he transferred to the laboratory in Menard, Texas, where Melvin and Bushland [2] had established a thriving colony of screw-worms by growing the larvae in a medium composed of ground meat, blood, water and preservative. The adults were kept in cages where they could be readily observed, and Knipling noted that there was a great deal of mating activity in the cages during the 3rd to 5th day of adult life but that subsequently the flies seldom mated though males continued to pursue females. In conversation with his associates at the Menard laboratory, he speculated that the females mated only once. He proposed that if females were monogamous it might be economically feasible to eradicate the Florida screw-worm population if some way could be found to sterilize the males since they could be reared so cheaply in the laboratory. He thought it would be practical to overwhelm the native population by releasing sterile flies in an integrated program involving chemical control and good animal husbandry practices to restrict the breeding of native flies. At that time, Knipling and I knew that Muller [3] had developed a technique of increasing the mutation rate in

Drosophila by irradiating males with X-rays. However, we were not aware of the sterilizing effects of radiation. Our work assignment at Menard had to do with laboratory and field testing to improve larvicides for use against screw-worms. We were soon assigned to other problems, and we did not get back to screw-worm research until 1946.

By 1946, Knipling had been promoted to leadership of the Division of Insects Affecting Man and Animals, Bureau of Entomology and Plant Quarantine, and livestock insect research had been consolidated at a new laboratory at Kerrville, Texas, where I was working. Thousands of organic compounds had been screened as military insecticides during World War II and were now to be screened as toxicants and repellents against insects and arachnids affecting livestock and poultry. Knipling therefore suggested a concurrent study of the mating behaviour of screw-worm flies and the techniques of sterilization that could be done by checking the fertility of the flies that survived exposure to various chemicals. However, those screening tests did not uncover any sterilants.

It was not until 1950 when A.W. Lindquist, then investigations leader at the Entomology Research laboratory at Corvallis, Oregon, called our attention to the paper by Muller [4] that we learned of the sterilizing effects of radiation-induced dominant lethal mutations. Knipling wrote to Muller, outlined the Florida eradication problem, and inquired whether it would be possible to induce complete sterility in a fly and still have an insect that could survive and compete for mates in nature. Muller was not certain but felt that the experiments were worth trying. So we went ahead.

On preliminary experiments with X-rays [5] were successful. Then we tried gamma radiation and were again successful [6]. Testing indicated that it was most efficient to irradiate pupae 2 days before adult emergence; that a dose of 2500 rad sterilized males, and a dose of 5000 rad sterilized females; and that pupae apparently tolerated doses of as much as 20 000 rad. Then, since it was not feasible to separate the sexes, we used the 5000-rad dose in our first field tests. However, females sterilized with 5000 rad sometimes laid a few sterile eggs, and these eggs confused our results because we could not tell whether they had been oviposited by released females or by native females that had mated with sterile males. Therefore, in 1954, we used a dose of 7500 rad because females treated as pupae with that dose cannot produce eggs. Ever since, the standard dose has been 7500 rad ±10% administered to pupae 2 days before eclosion.

Beginning in 1952, we made field tests on Sanibel Island off the west coast of Florida. First we released fertile flies labelled with ^{32}P and used a portable survey meter to detect the radioactivity in both the released flies and their eggs [7]. Some released flies were reared on the ground meat medium, and others were reared on cattle injected with ^{32}P to establish that flies reared on animals or on artificial diet responded similarly. The test results also showed that we could greatly outnumber the native population if we released 100 sterile males/mile^2 per week – plus an equal number of sterile females since it was not practical to separate the sexes. At this rate of release, we controlled the screw-worms on Sanibel Island, but we could not eradicate them because the untreated mainland was within the flight range of fertile flies.

A field test on the island of Curacao [8] was the first in which we actually achieved eradication of the screw-worm. The early releases of 100 sterile males/mile^2 per week did not succeed, but a rate of 400 sterile males/mile^2 per week did.

After the success on the island of Curacao, Knipling [9] wrote his first paper describing theories he had begun developing 18 years earlier. At the same time, Lindquist [10] published his estimates of the dimensions of the screw-worm populations.

Curacao has an area of only 170 mile^2, so the experiment had involved the release of only 136 000 flies/week. This scale was too small to allow us to use the results there to project plans for eradication throughout Florida. Therefore, a final pilot test was necessary in a larger area. We selected a 2000-mile^2 section bordering the Atlantic Coast near Cape Kennedy where screw-worms were even more abundant than on Curacao. Then we released 500 males/mile^2 per week for 10 weeks. Our pupose was not eradication of the flies because the area was not isolated; however, we found that egg masses collected on trap wounds on animals located near the centre of the treated area had the same degree of sterility as egg masses collected on Curacao at the time when the population there began to decline. Therefore, we considered that the results could serve as a base for realistic planning for eradication of the screw-worm from Florida. Moreover, the experience in mass rearing and large-scale distribution of sterile flies had shown that the techniques were feasible.

The screw-worm eradication program in the United States has been conducted by the Animal Health Division of the Agricultural Research Service, U.S. Department of Agriculture, in cooperation with agricultural agencies of the States concerned and with the research support and advice of the Entomology Research Division.

The release of sterile flies for eradication in Florida began in January 1958 with flies reared at the facilities constructed for the pilot test. From January until July, while a new 60-million-fly-capacity mass-rearing facility was being constructed at Sebring, Florida, the pilot plant was used to produce increasing numbers of sterile flies ranging from 1 to 14 million flies per week. Those early releases were made to take advantage of unusually cold weather which reduced the overwintering area of screw-worms to about the lower half of the peninsula. Because the pilot plant could not produce enough flies to treat the entire overwintering area, the production that was available was used to make a sort of barrier zone about 200 miles wide in northern Florida and southern Georgia to keep the flies from extending their range with the onset of warm weather. (It was the success of this barrier in restricting spring migration that subsequently led us to conclude that a South-western program would be feasible). Then, in July, the Sebring facility began to operate, and it was possible to treat the whole Florida peninsula as a unit. The procedure was as follows. Flies were released at the rate of 400 males and 400 females/mile^2 per week 6 days a week in flight lanes 12 miles apart. Each day the flight lanes were moved so that by the end of a week the area had been covered by 2-mile swaths and by the end of 2 weeks by 1-mile swaths. The release zone was divided into treatment areas with each area assigned to a pilot. Pilots flew 6 days a week with their rest days staggered so the daily production of sterilized insects was distributed as freshly emerged flies. In addition, wherever screw-worms occurred, there was 'hot spotting', additional releases of sterile flies. The last recorded screw-worm infestation was found in June 1959; releases were terminated in November.

The Florida eradication program cost 10.6 million dollars, but the savings were estimated to exceed 20 million dollars annually. As a result,

South-western livestock producers sought a similar program: there, screw-worms were estimated to cost them about 100 million dollars a year.

Our original concept of screw-worm eradication had encompassed only the isolated population in Florida which could be treated as a unit. However, the success of the barrier zone and of the hot spotting during the first few months of the Florida program encouraged us to believe that a South-western program had a good chance of success. The overwintering area in Texas in average years was about 50 000 mile^2, approximately the same as the area treated successfully in Florida; overwintering areas in the other South-western states were much smaller. Moreover, from the experience in Florida, we were able to estimate that a South-western effort would cost about 5 million dollars a year and that it would require 2 or 3 years to achieve eradication and test a barrier zone. Livestock producers organized the South-west Animal Health Research Foundation and collected over 3 million dollars in donations from ranchers and farmers to provide half the estimated cost of the program until funds to match the federal half could be appropriated by State legislatures.

Within 2 years of the beginning of the South-west program in 1962, screw-worms were eradicated from Texas and New Mexico; in another 2 years, the whole south-western United States was free of this pest. Migrant flies still invade the border states from Mexico and cause minor infestations which sometimes persist a few generations before they are wiped out, but there has been no continually breeding population in Texas since 1964 or anywhere in the United States since 1966.

With inflation, the cost of maintaining the 1500-mile-long barrier between the United States and Mexico has increased to 6 million dollars a year. The Mexican government has co-operated so wholeheartedly that in some areas the releases of sterile flies are made as much as 350 miles into Mexico. It is hoped that arrangements can soon be made to extend the program to the Isthmus of Tehuantepec in Mexico where the continent is only 150 miles wide or perhaps eventually to the least expensive location for a barrier zone, the Isthmus of Panama. Wherever the zone is located, recurring infestations will be caused by migrating flies so the program will be unending. However, there is no doubt about its complete and unqualified success.

STERILIZATION OF THE BOLL WEEVIL

One insect that has proved difficult to sterilize is the boll weevil, Anthonomus grandis Boheman. Irradiation damages its midgut so severely that sterile males are not competitive and soon die of starvation [11]. American investigators are therefore seeking a chemosterilant that will selectively attack the gonads and spare the midgut.

Klassen and Earle [12] were successful with busulfan when it was mixed with the synthetic diet at a concentration of 0.1% and fed for 6 days, except that the females laid a small number of viable eggs. However, busulfan cannot be used at a higher concentration without causing too much damage to the gut; therefore, weevils sterilized in this way will probably have to be separated by sex so that only males are released. Such separation will itself be another problem because there is no pronounced sexual dimorphism, and some females are mistaken for males.

being done today. However, his paper did not influence research in our organization until recently when it was called to our attention by Curtis [19].

Several workers have reported progress in translocation research. Curtis [20] has made remarkable advances with the tsetse fly, Glossina austeni Newstead, in view of the difficulty of rearing these insects and the lack of genetic markers for their chromosomes. Laven [21] used translocations to eradicate a cage population of Culex pipiens pipiens L. McDonald and Rai [22] described a translocation involving three chromosomes in Aedes aegypti (L.) that was derived from a double translocation heterozygote. Whitten [23] proposed that Lucilia cuprina (Wiedemann) can be controlled in Australia by displacing the present wild-type population through release of homozygous translocation stocks that bear genes for insecticide susceptibility or a conditional lethal mutation for cold susceptibility.

By using translocations, Wagoner [24] reconciled differences between the Italian and Japanese systems of numbering chromosomes and established a standard terminology for the house fly karyotype.

He has genetic marker stocks to identify the chromosomes and had no difficulty producing 193 translocation heterozygotes by irradiating males with 2000-2500 R of X-rays [25]. When these translocations involved two chromosomes, sterility of the heterozygotes averaged 55%; when they involved three chromosomes, it averaged 66%; and when they involved four chromosomes, it averaged 79%. However, the development of vigorous, fertile, translocation homozygotes has been difficult.

Last year, Wagoner, lacking a translocation homozygote, released male heterozygotes at a semi-isolated location in Florida in co-operation with G. C. LaBrecque of the Division's Gainesville, Florida, laboratory. (Mating of these released males with the native females was then indicated by the recovery of recessive genetic markers and the translocation in the progeny of native females.) Wagoner (unpublished) now has two homozygous translocation stocks in the laboratory which, through back-crossing to wild types, he is attempting to develop into strains sufficiently vigorous for release as homozygotes.

I should emphasize that even with the chromosomes appropriately marked, the development of a homozygous translocation stock is difficult. One reason is probably the induction of detrimental and recessive lethal mutations in males irradiated to induce reciprocal translocations. The treatment to induce translocations may cause dominant lethal mutations in more than 90% of the sperm. Muller [4] published a figure that dramatically illustrates the extent of radiation damage in Drosophila. He showed that for every dominant lethal mutation, four or more recessive detrimental mutations occur that do not kill the progeny before they can reproduce but do reduce vigour, longevity, and general fitness for the environment. In addition, when breeding stocks are maintained in cages, spontaneous mutations that could be harmful in nature accumulate but are not expressed as lethals or detrimentals in the shelter of the laboratory. Therefore, it is important to back-cross a laboratory strain to a wild-type strain and to test progeny for vigour, competitiveness, and ability to survive in nature before the stock is used in a field test of genetic control.

Just how difficult the task of developing a homozygous stock will be was indicated by experiences in obtaining a genetic marker. Ten years ago I thought it would be easy to get a good genetic marker to identify released sterile screw-worm flies, and, in fact, LaChance et al. [26] had no

difficulty producing visible mutations that lived well in laboratory cages. However, only 1 of 15 visible mutations could be used to mark a strain vigorous enough to pass a preliminary field screening test. For this screening, the same laboratory conditions were used to rear the regular wild-type stock and a genetically marked strain. Then the two kinds of flies were released simultaneously at a given location, and the catches in fly traps placed at appropriate distances were observed. For example, the traps were emptied at intervals that would indicate how quickly each strain moved from the release point to the traps. Also, trapping was continued long enough for the lifespan in nature to be determined. It took 3 years of this work before a genetically marked strain was obtained that was not obviously inferior to the wild-type, whether the genetic markers were spontaneous or had been induced by radiation or chemicals.

The problems with translocation stocks will probably be similar, but this line of research has such merit that it justifies setting out on the difficult course.

Another approach to house-fly control is the use of chemosterilants. The first house-fly chemosterilants were antimetabolites that affected only females [27]. When it was found that apholate would sterilize both sexes [28], research was greatly stimulated, but so far all the effective sterilants are mutagens, that is, they sterilize flies by producing dominant lethal mutations. I consider that mutagens are an especially hazardous group of chemicals that may be useful in some special situations when conditions are carefully controlled such as those cited earlier for the boll weevil, and even with the boll weevil we will have to do metabolism studies to establish that the released insects will not be hazardous. I cannot foresee the widespread use of a chemical mutagen for house-fly control. However, a mutagen is not needed to sterilize house flies. All that is needed is a competitive male. Even competitive sperm are not necessary: house flies are mostly monogamous [29, 30]. Riemann et al. [31] showed that even castrate male house flies could induce a mating avoidance response in the females if their copulatory ducts were left intact when the testes were surgically removed. Adams and Nelson [32] found that a water-soluble extract of these ducts could cause mating avoidance if it was injected into virgin female flies.

Subsequently, R. H. Leopold and A. C. Terranova of this laboratory (unpublished data) showed that the copulatory ducts of house flies secrete a mixture of proteins and that more than one substance in the mixture is probably involved in the mating avoidance. Treating males with tritiated arginine caused the seminal fluid, but not the sperm, to become radioactive. Then when they dissected female flies during mating and at intervals thereafter, the autoradiograms showed that the radioactive material penetrated the copulatory pouches of the vagina about 40 min after copulation began but while mating was still continuing. In another 20 min, the material reached the brain of the female and elicited a rejection reaction. The female then dislodged the male. From 95 to 97% of female house flies mate only once. Moreover, Riemann et al. [31] showed that even those females that did remate usually oviposited repeatedly before they would accept a second male. Therefore, the mating inhibition may actually be much more than 95% effective since most females in nature would not survive long enough to prepare themselves for a second mating.

In doubt whether precise identification of the proteins involved in the mating avoidance response will suggest the structure of a chemical that can

be synthesized for house-fly control. However, if a natural product produces this reaction in female house flies, many synthetic substances might elicit the same response, and a frigidity factor might be used to control the breeding of house flies. On farms, flies seek shelter every night and frequently rest inside buildings during the day. If such resing sites were sprayed with a substance that caused mating avoidance, the virgin females would be exposed to it during the 2 days before they were old enough to mate. The effect on a fly population would be the same as that of any toxicant that killed female flies, but the anti-mating substance might well be one that would have no effect on organisms that do not have this reaction. This work may therefore lead to the development of a new way of controlling monogamous insect pests, especially since Nelson et al. [33] found that virgin female house flies also responded to extracts of males of other monogamous species, Phormia regina (Meigen) and screw-worms. A chemical that could cause mating avoidance in house flies therefore might be useful against such monogamous Diptera as the stable fly, Stomoxys calcitrans (L.); the horn fly, Haematobia irritans (L.); and the face fly, Musca autumnalis DeGeer.

Also, many mosquitoes are monogamous. Craig and Fuchs [34] described a substance they called matrone which is produced by the accessory glands of male A. aegypti. Matrone does not prevent repeated matings on the part of the female yellow fever mosquito, but it does cause a blocking of the vagina so the female uses sperm from the first male only.

I think that much more important than developing a chemical to cause a mating avoidance response in virgin females is the development of a chemical that will inactivate insect sperm but not be a mutagen. Such a chemical variously applied in baits and sprays might be a highly practical chemosterilant for many monogamous pest insects. Knipling [35] published models illustrating that it would be much more efficient to sterilize 90% of a field population than to kill the same proportion with an insecticide.

However, investigators will need to consider the possibility that insects could develop resistance to a mating avoidance chemical or a sperm inactivator. Whitten and Taylor [36] reported that L. cuprina were 95 - 99% monogamous but that in 10 generations of selection for multiple mating, the monogamous response was decreased to 70%.

STERILIZATION OF OTHER DIPTERA

In extending the sterile-male technique, scientists have found Diptera fairly easy to sterilize with radiation. I will not cite the many references. When they are treated as adults, both males and females can be sterilized with 10 000 rad or less of gamma radiation; when they are treated as pupae, the insects suffer somatic damage if treatment is administered too early during pupal development, but radiation is tolerated if it is applied after the adult organs have been largely formed. I therefore think that with this order the major problems are associated with developing inexpensive mass-rearing techniques or with establishing effective release procedures.

F_1 STERILITY

It is generally accepted that the main cause of dominant lethal mutations in most insects is the breaking of chromosomes by radiation or chemicals. Easily sterilized insects have a single centromere in each chromosome,

and the loss of broken pieces at the time of cell division causes a genetic imbalance, which is the most common lethal mutation induced by radiation.

Lepidoptera and Hemiptera have holokinetic chromosome. Therefore, when they are broken, pieces are not lost at the time of cell division because the multiple spindle fibres, with their many points of attachment in each chromosome, pull the fragments along to the poles. Thus, each daughter cell has all the hereditary material needed for survival. Investigators believe that this is the reason that sterilization of moths requires about 10 times the dose of radiation needed to sterilize flies.

When male moths are treated with the massive doses of radiation required to cause sterility, somatic injury occurs which reduces mating effectiveness and results in sperm that are not competitive. North and Holt [37] recently reviewed this situation and pointed out that fully sterile males frequently fail to place their spermatophores in the proper position for the sperm to reach the spermathecae. Also, sterile males often fail to incorporate sperm in their spermatophores.

When North and Holt [38] worked with the cabbage looper, Trichoplusia ni (Hübner), they administered less than sterilizing doses of radiation and tested the fertility of the F_1 progeny. They found that males treated with about a 50% sterilizing dose had progeny that were more than 90% sterile. They explained this effect as the result of the many broken chromosomes in the sperm that had rejoined with fragments of other chromosomes to form multiple translocations. The F_1 progeny thus became translocation heterozygotes with the extent of sterility dependent on the number of induced translocations. The male parents treated with the semi-sterilizing dose naturally suffered less somatic damage than fully sterilized males and hence were more competitive. Also, their deposition of spermatophores was more nearly normal as was the incorporation of sperm. The F_1 progeny were found to mate competitively in laboratory cages [39] and also in a field cage test made at Riverside, California, in 1969, by H. H. Toba, A. N. Kishaba, and D. T. North.

North and Holt [37] reviewed the literature on F_1 sterility in Lepidoptera and reported that inheritance of sterility was known for 10 species. Thus, in Lepidoptera, the progeny of irradiated male moths are usually more sterile than their parents.

Since Hemiptera also have holokinetic chromosomes, LaChance et al. [40] irradiated males of the milkweed bug, Oncopeltus fasciatus (Dallas), and found that chromosomal fragments, translocations, and sterility effects were inherited through three subsequent generations in this species.

In their laboratory tests with the cabbage looper, North and Holt [38] found that inherited sterility was most pronounced in the F_1 generation, much less evident in the F_2 generation, and had largely disappeared by the F_3 generation. The fact that some fertile progeny are produced in the F_1 generation and that almost normal fertility is recovered by the F_3 generation indicates that this technique has more value for suppressing lepidopterous populations than it does for actual eradication. Knipling [41] showed that a single release at a 9:1 ratio of 80% sterile males would have a greater effect at the end of two generations than a single release of 100% sterile males at the same ratio. Knipling [42] recently extended his calculations and professed his hope for the application of F_1 sterility to control the cabbage looper in the eastern United States and the corn

earworm, Heliothis zea (Boddie), in California. He pointed out that F_1 sterility could be used in eradication programs against the codling moth, Laspeyresia pomella (L.), and the pink bollworm, Pectinophora gossypiella (Saunders), for its great suppressive effect at the beginning of a program; final eradication would be accomplished by release of totally sterile males.

CONCLUSIONS

As you can see, in the 20 years since we found that screw-worms could be sterilized with radiation, there have been many developments in the use of sterility to control insect populations.

The first practical technique – attaining sterility by inducing dominant lethal mutations in 100% of the sperm – has proved promising in laboratory and small-scale field tests against many insects. It needs further development by application either to geographically isolated insect populations or to very large areas where migration and intermingling of fertile insects from adjacent untreated areas will not interfere. The sterile released insects must be vigorous and competitive. They must be released at a frequency that will maintain adequate numbers to compete for mates in the native population. They must be released into the environment at such intervals of distance that they are rather uniformly dispersed and will outnumber native males in all areas of the environment. It is especially important that realistic estimates of native insect populations be made so that enough sterile insects will be released.

I think there is a real future in relating research in insect reproductive physiology to population control. The difficulties associated with mutagens as male sterilants would not be factors to hamper use of a non-mutagenic sperm inactivator for species with monogamous females.

The use of conditional lethal mutations is a promising form of genetic control. Such a mutation, inability to diapause, already exists in the boll weevil and in many other species. Inability to survive extremes of heat or cold is being sought in other insects.

Sterility in insects heterozygous for reciprocal translocations is another new and promising area. This work may involve rearing fertile laboratory strains homozygous for translocations and releasing a strain to interbreed with wild-type insects, or it may involve making crosses in the laboratory so that sterile heterozygotes can be released. Basic work along these lines is underway with house flies, mosquitoes, and tsetse flies.

A simpler adaptation of the translocation heterozygote principle involves F_1 sterility in the progeny of partly sterilized Lepidoptera. I am enthusiastic about the prospects of early practical use of this approach to genetic control.

REFERENCES

[1] LaCHANCE, L.E., SCHMIDT, C.H., BUSHLAND, R.C., "Radiation-Induced Sterilization," Ch. 4, Pest Control (KILGORE, W.W., DOUTT, R.L., Eds), Academic Press, New York (1967).
[2] MELVIN, R., BUSHLAND, R.C., J. econ. Ent. 33 (1940) 850.
[3] MULLER, H.J., Science, N.Y. 66 (1927) 84.
[4] MULLER, H.J., Am. Scient. 38 (1950) 33, 126.

[5] BUSHLAND, R.C., HOPKINS, D.E., J. econ. Ent. 44 (1951) 725.
[6] BUSHLAND, R.C., HOPKINS, D.E., J. econ. Ent. 46 (1953) 648.
[7] RADELEFF, R.D., BUSHLAND, R.C., HOPKINS, D.E., J. econ. Ent. 45 (1952) 509.
[8] BAUMHOVER, A.H., GRAHAM, A.J., BITTER, B.A., HOPKINS, D.E., NEW, W.D., DUDLEY, F.H., BUSHLAND, R.C., J. econ. Ent. 48 (1955) 462.
[9] KNIPLING, E.F., J. econ. Ent. 48 (1955) 459.
[10] LINDQUIST, A.W., J. econ. Ent. 48 (1955) 467.
[11] RIEMANN, J.G., FLINT, H.M., Ann. ent. Soc. Am. 60 (1967) 298.
[12] KLASSEN, W., EARLE, N.W., J. econ. Ent. 63 (1970) 1195.
[13] McCOY, J.R., LLOYD, E.P., BARTLETT, A.C., J. econ. Ent. 61 (1968) 163.
[14] KLASSEN, W., KNIPLING, E.F., McGUIRE, J.U., Jr., Ann. ent. Soc. Am. 63 (1970) 238.
[15] RIVOSECCHI, L., Riv. Parsitologia 23 (1962) 71.
[16] WAGONER, D.E., Nature, Lond. 223 (1969) 187.
[17] McDONALD, L.C., Can. J. Genet. Cytol. (accepted for publication).
[18] SEREBROVSKII, A.S., Zool. Zh. 19 (1940) 618.
[19] CURTIS, C.F., Bull. ent. Res. 57 (1968) 509.
[20] CURTIS, C.F., Genet. Res., Camb. 13 (1969) 289.
[21] LAVEN, H., Nature, Lond. 221 (1969) 958.
[22] McDONALD, P.T., RAI, K.S., Science, N.Y. 168 (1970) 1229.
[23] WHITTEN, M., "Genetics of pest species in their management," (RABB, R.L., GUTHRIE, F.E., Eds.), Concepts in Pest Management, N.C. State Univ. Press, (1970, in press).
[24] WAGONER, D.E., Genetics 57 (1967) 729.
[25] WAGONER, D.E., NICKEL, C.A., JOHNSON, O.A., J. Hered. 60 (1969) 301.
[26] LaCHANCE, L.E., DAWKINS, C., HOPKINS, D.E., J. econ. Ent. 59 (1966) 1493.
[27] MITLIN, N., BARODY, A.M., J. econ. Ent. 51 (1958) 384.
[28] LaBRECQUE, G.C., J. econ. Ent. 54 (1961) 684.
[29] ZINGRONE, L.D., BRUCE, W.D., DECKER, G.C., J. econ. Ent. 52 (1959) 236.
[30] SACCA, G., BENETTI, M.P., Rc. Ist. sup. Sanita 23 (1960) 423.
[31] RIEMANN, J.G., MOEN, D.J., THORSON, B.J., J. Insect Physiol. 13 (1967) 407.
[32] ADAMS, T.S., NELSON, D.R., Ann. ent. Soc. Am. 61 (1968) 112.
[33] NELSON, D.R., ADAMS, T.S., POMONIS, J.G., J. econ. Ent. 62 (1969) 634.
[34] CRAIG, G.B., FUCHS, M., "Sterilization of female mosquitoes with a hormone derived from male mosquitoes," U.S. Patent Office 3450816.
[35] KNIPLING, E.F., U.S. Dept. Agric. ARS 33-98 (1964) 54 pp.
[36] WHITTEN, M.J., TAYLOR, W.C., J. econ. Ent. 63 (1970) 269.
[37] NORTH, D.T., HOLT G.G., Expert Panel on Application of Induced Sterility for Control of Lepidopterous Populations, (Proc. Panel Vienna, 1970) (in press).
[38] NORTH, D.T., HOLT, G.G., J. econ. Ent. 61 (1968) 928.
[39] NORTH, D.T., HOLT, G.G., Can. Ent. 101 (1969) 513.
[40] LaCHANCE, L.E., DEGRUGILLIER, M., LEVERICH, A.P., Chromosoma 29 (1970) 20.
[41] KNIPLING, E.F., Sterile-Male Technique for Eradication of Control of Harmful Insects (Proc. Panel Vienna, 1968) IAEA, Vienna (1969) 19.
[42] KNIPLING, E.F., Bioscience 20 (1970) 465.

EFFECT OF COBALT-60 IRRADIATION OF MALE PUPAE OF THE GYPSY MOTH, Lymantria dispar L., ON BIOLOGICAL FUNCTIONS OF MALE MOTHS

M. MAKSIMOVIĆ
Institute for Plant Protection,
Belgrade, Yugoslavia

Abstract

EFFECT OF COBALT-60 IRRADIATION OF MALE PUPAE OF THE GYPSY MOTH, Lymantria dispar L., ON BIOLOGICAL FUNCTIONS OF MALE MOTHS.

The effect of irradiating male pupae of the gypsy moth with ^{60}Co was investigated at doses of 30 000 and 40 000 rad. Certain unfavourable results were noted with male moths. Mortality among irradiated pupae was found to increase proportionally with radiation dose as compared with unirradiated pupae. The age of pupae when irradiated is an important factor. The lowest mortality is obtained by irradiation with 30 000 rad, when carried out within the last 5 days before emergence. The ability of irradiated males to mate is related to their development as measured by their body length in dry condition. The longer the body, the more the males copulated. Those with body length in dry condition not greater than 7 mm did not copulate at all. The stated radiation doses have a considerable effect on the life span of male moths, as well as on the duration of copulation. Both are shortened as compared with unirradiated males. Sterility induced in males not only makes the eggs obtained on copulation sterile, but also, very probably, affects the female biological functions in mating. The oviposition period and duration of the female life were found to be shortened. Hatchability of eggs is reduced to 43.2% with male pupal irradiation of 30 000 rad. In 56.8% eggs, although the caterpillar larva was formed, it did not hatch. The unfavourable effects of overdoses point to the need for investigating lower radiation doses for gypsy moth pupae.

1. INTRODUCTION

The effect of sterilizing the male of the gypsy moth with cobalt-60 has been investigated by, amongst others, Godwin et al. [1] and Vasiljević [2]. These studies have shown that it is questionable what are the most reliable radiation doses, i.e. doses that will sterilize without harming the biological functions of the male moths.

In the present work we give some results on the effect of high radiation doses of 30 000 and 40 000 rad on the biological functions of male moths and the indirect effect on females with which they copulated.

Investigations were carried out within the framework of the project on the radiological sterilization of the gypsy moth, undertaken by the Institute for Plant Protection, Belgrade, with the assistance of a Research Contract from the International Atomic Energy Agency and support from the Research Fund of the Socialist Republic of Serbia.

2. METHODS

The gypsy moth caterpillars were reared to the third instar in the greenhouse and then put in cages to be reared in the insectarium till they become pupae.

TABLE I. EFFECT OF IRRADIATION, ADMINISTERED AT VARIOUS AGES, ON MORTALITY OF MALE PUPAE OF GYPSY MOTH

Age at which pupae were irradiated (days)	Pupae irradiated with						Unirradiated		
	30 000 rad			40 000 rad					
	Total no. of pupae irradiated	Dead		Total no. of pupae irradiated	Dead		Total no. of pupae	Dead	
		Number	%		Number	%		Number	%
2-5	13	12	92.3	14	9	64.2			
6-10	11	2	18.1	10	1	10.0	11	2	18.1
11-15	6	0	0.0	5	1	20.0			

TABLE II. EFFECT OF BODY LENGTH OF MALE MOTHS ON ABILITY TO MATE

Number of male moths	Body length (mm)	Number of matings	Average number of matings per male moth
1	7	0	0.0
3	10	1	0.33
5	11 - 12	4	0.80
9	13 - 17	12	1.33

Male pupae of various ages were exposed to ^{60}Co irradiation at doses of 30 000 and 40 000 rad. The effect on mortality was investigated.

Males obtained from irradiated pupae aged 8 – 13 days were crossed with females. The number and duration of matings and the life span were then observed individually. The course of oviposition, the life span and egg hatching were also observed for females.

3. RESULTS OF INVESTIGATION

3.1. Effect of irradiation on pupal mortality of male

The mortality of male pupae after irradiation is shown in Table I. The general characteristic is the decline of mortality with pupal age, i.e. the highest mortality occurs when pupae are irradiated at 2 - 5 days. At 30 000 rad, mortality increases to 92.3% in this period. When pupae were irradiated at 11 - 15 days, or when moths were already formed in the pupae, mortality was nil. With pupae irradiated at 6 - 10 days the 30 000-rad dose produced a mortality, 18.1%, equal to that of the control. It can therefore be taken that, even at this early stage, 30 000 rad appears to have no effect on mortality.

With the 40 000-rad dose, it can be seen that there was a 20% mortality in pupae irradiated at 11 - 15 days. The reason for the lower mortality in pupae irradiated at 2 - 5 days, as compared with the 30 000-rad dose for the same age group, is not clear.

3.2. Effect of the body size of moths on ability to mate

A certain consistency was noted when we compared the number of matings for sterile males irradiated at 30 000 rad with their body length measured in dry condition at death. Table II shows that there was no mating by the male with the shortest body length of 7 mm; such moths were impotent. There were 66.7% impotent moths even when the body was 10 mm in length. More than one mating per moth was observed among moths with body length over 13 mm.

TABLE III. EFFECT OF IRRADIATION DOSE ON DURATION OF COPULATION

Irradiation dose (rad)	Number of males	Temp. (°C)	Duration of copulation		
			Minimum	Maximum	average
30 000	11	21 - 26	1h 35min	2h 43 min	2.07 h
40 000	9		0h 45min	1h 53min	1.11 h
Unirradiated	14		1h 25min	2h 10min	1.36 h

TABLE IV. EFFECT OF IRRADIATION DOSE ON LIFE SPAN OF MALE MOTHS

Irradiation dose (rad)	Number of males	Temp. (°C)	Life span of male moths (days)		
			Minimum	Maximum	Average
30 000	11	21 - 26	1.04	2.75	1.83
40 000	9		1.30	2.50	2.12
Unirradiated	14		1.00	4.00	2.97

3.3. Effect of irradiation on duration of copulation and life span

Irradiation of male pupae with doses of 30 000 and 40 000 rad affected the duration of copulation. From Table III it can be seen that the average duration of copulation in males irradiated with 30 000 rad was twice that of males irradiated with 40 000 rad. At 40 000 rad the minimum and maximum duration of copulation are much lower than for unirradiated moths.

The harmful effect of irradiation on life span is more marked, as Table IV clearly shows. The maximum durations are shorter for 40 000 than for 30 000 rad, and both are shorter than for the unirradiated males. The average figures also show the same trend, except that the 40 000 and 30 000 figures are reversed. This larger average duration at 40 000 rad may not be reliable and may result from variation due to the small number of moths used.

3.4. Effect of mating of irradiated males on oviposition and life span of female moths

Table V shows that the average period of oviposition for females crossed with males irradiated with 30 000 rad, was considerably lower than for females crossed with unirradiated males. Nevertheless this figure was twice as long as that for females crossed with males irradiated with 40 000 rad. The maximum and minimum figures are also noticeably lower than for females crossed with unirradiated males.

These trends are paralleled by the results obtained for the life span of female moths, as shown in Table VI. After copulation with a male irradiated at 30 000 rad the life of a female is on average 1.89 days shorter than that of a female copulating with an unirradiated male. The life of a female after copulating with a 40 000-rad-irradiated male is 2.31 days shorter.

3.5. Effect of sterile males on egg hatching

Eggs obtained from mating of sterile males with normal females showed wide differences in comparison with the control moths. Table VII shows that there was no egg hatching after mating, as compared with 82.6% hatched in the control. However, among eggs resulting from crossing with sterile males there were 56.8% containing larvae that died unhatched, whereas there were 17.2% such eggs in the control. There were 43.2% completely sterile eggs, and only 0.2% in the control.

4. DISCUSSION AND CONCLUSIONS

Cobalt-60 irradiation with the high doses of 30 000 and 40 000 rad had a harmful effect on the biological functions of male moths. A marked effect was noted in the mortality, which increased to 92.3% (Table I) when pupae were irradiated at 2 - 5 days. However, in spite of there being no mortality when pupae were irradiated at the end of the pupal period, i.e. at 11 to 15 days, the effect of irradiation was very noticeable when

TABLE V. EFFECT OF IRRADIATED MALE MOTHS AFTER MATING ON DURATION OF OVIPOSITION

Irradiation dose of male moths (rad)	Number of female moths	Temp. (°C)	Duration of oviposition (days)		
			Minimum	Maximum	Average
30 000	5	21 - 26	1.58	4.81	2.96
40 000	6		1.00	2.66	1.65
Unirradiated	4		3.56	5.72	4.40

TABLE VI. EFFECT OF MATING OF IRRADIATED MALES ON LIFE SPAN OF FEMALES

Irradiation dose of male moths (rad)	Number of female moths	Temp. (°C)	Life span of female moths (days)		
			Minimum	Maximum	Average
30 000	5	21 - 26	1.16	8.62	4.50
40 000	6		1.70	5.00	4.08
Unirradiated	4		4.75	7.75	6.39

TABLE VII. PERCENTAGE OF HATCHED EGGS AFTER MATING WITH STERILE MALES IRRADIATED WITH 30 000 RAD

Egg-mass from copulation with:	Number of egg-masses	Total number of eggs	Percentage of eggs		
			Non-hatched		Hatched
			Embryonated	Non-embryonated	
Male moth irradiated with 30 000 rad	3	562	56.8	43.2	0.0
Unirradiated male moth	3	819	17.2	0.2	82.6

applied at an earlier stage. It was also shown that the size of the male has a bearing on the ability to mate.

Irradiation decreased the duration of copulation (Table III) and shortened the male life span (Table IV). From the data on moths with the maximum life span, i.e. the strongest moths, it is shown that their life is shortened by 1.25 and 1.5 days by doses of 30 000 and 40 000 rad, respectively.

The sterility of the males is transferred not only to the eggs obtained on mating, but affects also the female biological functions in mating. This is shown by the shorter period of oviposition observed (Table V) and the shorter female life span (Table VI).

Sterility caused by the radiation dose of 30 000 rad is complete, since no caterpillar hatched (Table VII). No larva was formed in 43.2% eggs, larvae being formed in the remaining 56.8%. Vasiljević [2], under the same conditions, observed that there was hatching in 11.4% of the eggs.

The results show that high radiation doses for male pupae produced a range of unfavourable effects in males, causing the decline of their biological functions. There is therefore a need to investigate lower radiation doses with the gypsy moth. Godwin et al. [1] have already worked on lower doses, and have found that, when a dose of 20 000 rad was applied to male pupae aged 6 - 8 days, only 0.9% of the eggs hatched. When pupae were irradiated more than 8 days old, no eggs hatched. These authors showed that a dose of 10 000 rad also caused a very low percent of hatching (1.5 - 1.6%), and even lower doses, down to 2500 rad, had a considerable inhibiting effect. Vasiljević [2] found that not more than 5.5% eggs hatched with a dose of 20 000 rad.

Increasing attention has been given to partially sterilizing doses. Henneberry [3], in work on the cabbage looper, Trichoplusia ni (Hübner), showed that excessive exposures or overdoses of radiation do diminish mating and sperm transfer, and can cause other adverse effects. He found that males exposed to partially sterilizing doses of radiation were more competitive and that their progeny also were sterile. Similar results were given by North and Holt [4] on the same species. For Laspeyresia pomonella L., Fossati et al. [5] reported on the noxious effect on the male of overdoses of radiation and the beneficial effect of lower doses.

For the gypsy moth a trial field release was carried out with pupae irradiated with 30 000 rad. Maksimović [6] pointed to unfavourable factors that influenced the results of this trial. So the need to investigate lower radiation doses on male pupae of the gypsy moth is clear.

REFERENCES

[1] GODWIN, P.A., RULE, H.D., WATERS, W.E., Some effects of gamma irradiation on the gypsy moth, Porthetria dispar, J. econ. Ent. 57 (1964) 986-990.

[2] VASILJEVIĆ, Lj., Sterilizacija gubara radioaktivnim zracima (1967) (in manuscript).

[3] HENNEBERRY, T.J., "Research on sterility methods for control of the cabbage looper, Trichoplusia ni (Hübner)", Panel on Application of Induced Sterility for Control of Lepidopterous Populations, 1-5 June, FAO/IAEA, Vienna (1970).

[4] NORTH, D.T., HOLT, G.G., "Inherited sterility and its use in population suppression of Lepidoptera", Panel on the Application of Induced Sterility for Control of Lepidopterous Populations, 1-5 June, FAO/IAEA, Vienna (1970).

[5] FOSSATI, A., STAHL, J., GRANGES, J., "The effect of gamma irradiation dose on the reproductive performance of the P and F_1 generations in the codling moth, Laspeyresia pomonella L.", Panel on the Application of Induced Sterility for Control of Lepidopterous Populations, 1-5 June, FAO/IAEA, Vienna (1970).

[6] MAKSIMOVIĆ, M., "A trial with application of sterile male technique for the gypsy moth, Lymantria dispar L., in the open", Panel on the Application of Induced Sterility for Control of Lepidopterous Populations, 1-5 June, FAO/IAEA, Vienna (1970).

DISCUSSION

E. FYTIZAS: From the results obtained it is apparent that there is a critical period between the fifth and the sixth day of the pupal stage, as the mortality is definitely high in the case of pupae aged from 2 to 5 days and definitely low in that of pupae aged from 6 to 10 days. Is this difference in sensitivity related in any way to the changes that the insect is undergoing at that time? I should like to have some information on the condition of the nervous system, the muscular system and the digestive tract.

M. MAKSIMOVIĆ: So far I have no information on these factors.

M. FRIED: I wonder whether it is advisable to draw conclusions about the effect of radiation levels on various biological factors when the number of individuals in the tests was so small. For example, I should be very surprised if mating with irradiated males had any real effect on the adult life span of the female, particularly as the time of irradiation of the individual pupa before emergence has such a marked influence on later biological factors.

M. MAKSIMOVIĆ: These are merely the results of preliminary observations, which I think are worth reporting. In further investigations which I am conducting more female individuals are involved, and I hope to establish whether in fact mating with irradiated males does have any effect on the life span of the female.

M.E. TZANAKAKIS (Chairman): Mr. Maksimović, you mentioned that you irradiated some gypsy moth pupae aged 11 to 15 days. How long is that before eclosion?

M. MAKSIMOVIĆ: At this temperature the pupal stage is completed at a pupal age of 14 or 15 days. The irradiated pupae contained fully developed moths. At lower temperatures the pupal stage can be prolonged to 16 to 18 days.

M.E. TZANAKAKIS: Was the variation in temperature between 21°C and 26°C the daily fluctuation?

M. MAKSIMOVIĆ: Yes, it was.

INFLUENCE DE L'IRRADIATION DES GRAINS DE BLE SUR LE DEVELOPPEMENT ET LA REPRODUCTION DE Sitophilus granarius L. *

E. BAGHERI-ZENOUZ
Faculté d' agriculture
de l' Université de Téhéran,
Téhéran, Iran

Abstract — Résumé

EFFECT OF THE IRRADIATION OF WHEAT GRAINS ON THE DEVELOPMENT AND REPRODUCTION OF Sitophilus granarius L.

The author has studied the effect of ^{60}Co gamma irradiation of wheat grains on the conditions of development and reproduction of Sitophilus granarius which normally lives off this product.

The wheat grains were irradiated with doses of 25, 100, 500 krad and 1 Mrad. In a series of experiments, the fertility and longevity of insects reared on irradiated and on normal wheat were compared. The author also attempted to compare the same data for second generation insects originating with these irradiated media.

In experiments on 5 batches of 10 pairs, with 5 replications, the following facts were established with regard to fertility: the average fertility is significantly higher for media irradiated at 100 and 500 krad and 1 Mrad; the difference between the control medium and the medium irradiated at 25 krad is no longer significant. This difference is also found in the case of adults from the second generation reared on the various media.

Longevity was found to have increased more significantly in the males than in the females in rearings on irradiated media. This effect is observed at irradiation doses of 25 krad but is greatest for the medium irradiated at 500 krad. The differences in average longevity between the control batch and batches reared on irradiated media, calculated without distinction of sex, remained insignificant. It would seem, therefore, that the irradiation of wheat grains at doses of between 25 krad and 1 Mrad can modify this foodstuff so as to create factors that promote longevity and fertility in adults of Sitophilus granarius.

INFLUENCE DE L' IRRADIATION DES GRAINS DE BLE SUR LE DEVELOPPEMENT ET LA REPRODUCTION DE Sitophilus granarius L.

L' auteur a étudié l' influence de l' irradiation de grains de blé par le rayonnement gamma du ^{60}Co sur les conditions de développement et de reproduction de Sitophilus granarius, qui vit normalement aux dépens de cette denrée. Les grains de blé ont été irradiés à des doses de 25, 100, 500 krads et 1 Mrad.

Dans une série d' expériences la fécondité et la longévité d' insectes élevés sur du blé irradié et sur du blé normal ont été comparées. L' auteur a également tenté de comparer ces mêmes données pour des insectes de deuxième génération issus de ces milieux irradiés.

En ce qui concerne la fécondité, des expériences portant sur 5 lots de 10 couples et répétées 5 fois permettent d' établir que la fécondité moyenne est significativement plus élevée sur les milieux irradiés à 100, 500 krads et 1 Mrad; la différence n' est plus significative entre le milieu témoin et le milieu irradié à 25 krads. Cette différence se retrouve pour des adultes issus de la deuxième génération élevés sur ces divers milieux.

Pour la longévité, on remarque que, dans les élevages sur milieux irradiés, celle des mâles est accrue plus significativement que celle des femelles. Cet effet se manifeste pour des doses d' irradiation de 25 krads, mais il est maximal pour le milieu irradié à 500 krads. Les différences de longévité moyenne entre le lot témoin et les lots élevés sur milieux irradiés, calculées sans distinction de sexe, restent significatives. Il apparaît donc que l' irradiation des grains de blé à des doses variant de 25 krads à 1 Mrad peut modifier cet aliment de façon à créer des facteurs favorables à la longévité et à la fécondité des adultes de Sitophilus granarius.

* Ce travail a été effectué au Laboratoire de zoologie de l' INA (Paris).

TABLEAU I. FECONDITE MOYENNE DES FEMELLES DE Sitophilus granarius ELEVEES SUR DU BLE NORMAL (TEMOIN) ET DU BLE IRRADIE DE 25 krads A 1 Mrad

Durée de l'expérience (j)	$1A_T$			$1A_{25}$			$1A_{100}$			$1A_{500}$			$1A_M$		
	Nombre de femelles pondeuses	Nombre total de descendants	Moyenne	Nombre de femelles pondeuses	Nombre total de descendants	Moyenne	Nombre de femelles pondeuses	Nombre total de descendants	Moyenne	Nombre de femelles pondeuses	Nombre total de descendants	Moyenne	Nombre de femelles pondeuses	Nombre total de descendants	Moyenne
0 - 15	50	599	11,98	50	635	12,70	50	718	14,36	50	647	12,94	50	556	11,12
15 - 30	50	995	19,90	49	1119	22,83	50	1318	26,36	50	1193	23,86	49	1297	26,46
30 - 45	50	991	19,82	49	1016	20,73	50	1394	27,88	49	1211	24,71	48	1174	24,45
45 - 60	50	871	17,42	49	843	17,20	50	956	19,12	48	988	20,58	48	868	18,08
60 - 75	49	524	10,69	46	436	9,45	47	554	11,78	49	557	11,18	47	578	12,29
75 - 90	45	184	4,08	42	142	3,38	44	212	4,81	44	135	3,06	46	240	5,19
Moyenne par femelle au bout de 60 j		59,12			73,46			87,72			82,09			80,11	

INTRODUCTION

L'objet de ce travail était de déterminer si l'irradiation de grains de blé par le rayonnement gamma du ^{60}Co pouvait modifier les conditions de développement et de reproduction de Sitophilus granarius L., qui vit normalement aux dépens de cette denrée et dont les larves se développent à l'intérieur des grains.

Nous avons comparé successivement la fécondité et la longévité d'insectes élevés sur du blé irradié et sur du blé normal (témoin).

Nous avons également tenté de comparer ces mêmes données sur des insectes de deuxième génération issus de ce milieu irradié.

1. INFLUENCE DE L'IRRADIATION DU MILIEU SUR LA FECONDITE

1.1. Expériences

Nous avons constitué un important élevage de Sitophilus granarius L. en vue de comparer la fécondité des insectes élevés sur milieu témoin et sur milieu irradié.

Le milieu d'élevage était du blé de la variété Capelle-Vilmorin, qui avait été irradié par le rayonnement gamma du ^{60}Co à des doses de 25, 100, 500 krads et 1 Mrad.

Pour l'étude de la fécondité nous avons isolé 50 couples de charançons qui venaient d'éclore sur un milieu non irradié; nous nous sommes efforcés de récolter les couples dès leur sortie du grain, pour avoir des géniteurs de même âge, d'une part, et éviter qu'ils ne s'alimentent sur ce milieu non irradié, d'autre part.

Les 50 couples ont été répartis en cinq lots de 10 couples dans des boîtes en matière plastique contenant chacune 50 grammes de blé. L'un des cinq tubes contenait du blé sain, les autres du blé irradié à 25, 100, 500 krads et 1 Mrad.

Les boîtes ont été placées dans une étuve afin que les mêmes conditions de température et d'humidité règnent dans tous les lots. Tous les 15 jours les couples ont été transférés dans d'autres tubes contenant le même milieu. Au moment de ces transferts nous avons noté le nombre d'individus morts, mâles ou femelles, dans chacun des lots. Pour éviter de confondre par la suite les différents lots, ceux de la première génération ont été notés 1A ($1A_T$, $1A_{25}$, $1A_{500}$, $1A_M$) et ceux de la deuxième génération 2A ($2A_T$, $2A_{25}$ ....).

Pour évaluer la fécondité nous avons dénombré tous les imagos qui sortaient utlérieurement des divers lots de grains. Le premier comptage des imagos a toujours été fait 33 à 35 jours après la mise en place des géniteurs, ce qui correspond à peu près à la date des premières sorties de jeunes imagos.

Dans toutes nos expériences (0 à 15 j, 15 à 30 j, etc.) les comptages d'éclosions ont été effectués tous les trois jours et se sont poursuivis pendant 40 j; passé ce délai on est certain qu'il n'y aura plus d'éclosion.

La même expérience a été répétée 5 fois afin de fournir des données plus valables, c'est-à-dire que chaque test porte sur 5 lots de 10 couples dont on détermine la fécondité de 15 jours en 15 jours.

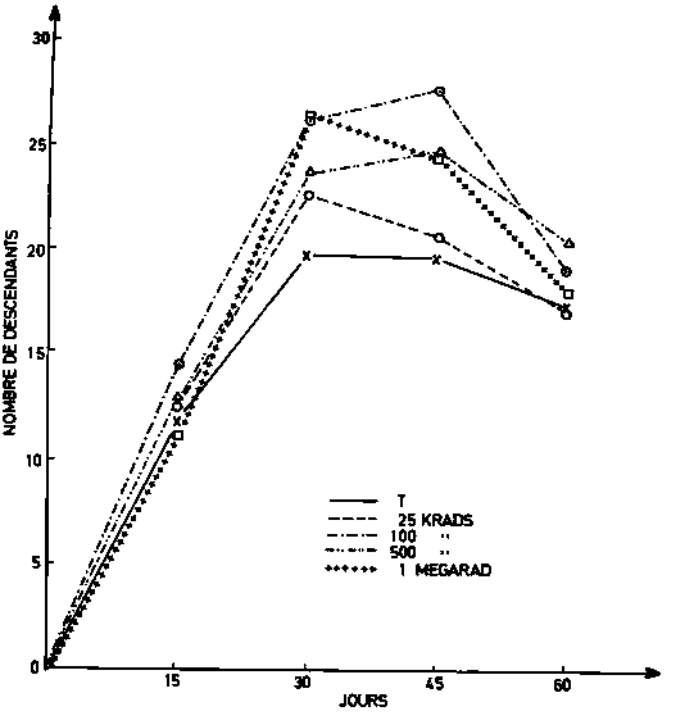


FIG. 1. Evolution de la fécondité moyenne, par femelle et par quinzaine.

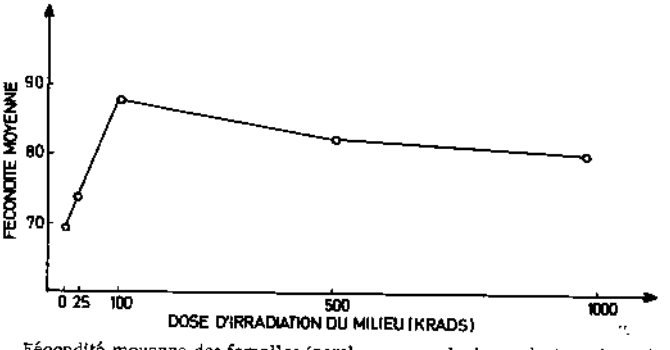


FIG. 2. Fécondité moyenne des femelles (nombre moyen de descendants au bout de 60 j).

Ces expériences ont été prolongées en outre jusqu'à la mort de tous les géniteurs afin de connaître l'influence éventuelle du milieu sur la longévité des adultes.

1.2. Résultats

Nous donnons au tableau I les résultats obtenus pour chaque série de 50 géniteurs soit, dans chaque cas, 5 répétitions effectuées avec 10 couples.

Ce tableau permet de comparer la fécondité moyenne des femelles pour l'ensemble des 50 femelles et sur chacun des milieux (témoin et

milieux irradiés), pour chaque quinzaine. Ces données permettent en outre d'établir la fécondité moyenne d'une femelle pour une période de 60 jours.

On peut remarquer que dès le premier contrôle, c'est-à-dire celui qui correspond à la période de 0 à 15 j, le nombre de descendants est déjà plus élevé dans les lots sur milieux irradiés que dans le lots témoin. Seul le lot $1A_M$ (sur milieu irradié à 1 Mrad) montre une légère diminution du nombre des descendants.

Au cours de la période de 15 à 30 j on a noté une femelle morte dans le lot $1A_{25}$ (milieu irradié à 25 krads) et une autre dans le lot $1A_M$; malgré cela le niveau de la population de descendants dans les milieux irradiés est toujours plus élevé que dans le lot témoin.

Au cours de la troisième période (30-45 j) il y a eu une femelle morte dans les lots irradiés à 25 krads et 500 krads et deux femelles mortes dans le lot irradié à 1 Mrad, alors que dans le lot témoin et le lot irradié à 100 krads il n'y a pas eu de mortalité.

Les figures 1 et 2 soulignent cette incidence de la nature du milieu, irradié ou non, sur la fécondité moyenne.

Nous avons limité nos calculs à une période de 60 jours, car au-delà de 60 jours on note qu'il se manifeste dans tous les lots une mortalité plus ou moins sensible, qui rend la comparaison de la fécondité moyenne plus difficile. L'analyse statistique révèle que la différence observée entre le lot $1A_T$ (témoin) et le lot $1A_{25}$ n'est pas significative, mais qu'elle l'est entre ce même lot $1A_T$ et les lots $1A_{100}$, $1A_{500}$ et $1A_M$.

Les résultats de nos expériences relatives à des adultes de deuxième génération sont difficiles à comparer avec les précédents. En effet nous avons dû utiliser pour ces expériences un nouveau stock de blé (lot témoin et lots irradiés aux mêmes doses que précédemment) dont les grains étaient plus petits.

Pour cette raison sans doute, et pour d'autres encore inexpliquées, les lots expérimentaux $2A_{25}$, $2A_{100}$, $2A_{500}$, $2A_M$ et le lot $2A_T$ ont donné en 60 jours beaucoup moins de descendants que les lots de la série 1A. Malgré cette différence, on retrouve dans la série 2A une plus grande fécondité moyenne globale, évaluée par femelle et pour 60 jours, en particulier dans le lot $2A_{500}$ et $2A_M$ (tableau II). Seul le lot $2A_{25}$ a donné moins de descendants que le témoin, la différence n'étant toutefois pas significative.

Ces expériences ne portaient d'ailleurs que sur 20 couples (deux répétitions de 10 couples) au lieu de 50 couples pour les lots de la série 1A.

2. INFLUENCE DE L'IRRADIATION DU MILIEU SUR LA LONGEVITE

Nous avons étudié la longévité de S. granarius sur les milieux irradiés et les milieux sains. Les expériences décrites ci-dessus nous ont permis de noter, à l'occasion des transferts de géniteurs sur de nouveaux milieux, la mortalité des mâles et des femelles.

Nous avons poursuivi ces contrôles pendant 210 j. Passé ce délai très peu d'individus étaient encore vivants.

Comme dans les expériences sur la fécondité nos résultats se rapportent pour chaque milieu à 5 lots de 10 couples.

TABLEAU II. FECONDITE DE LA DEUXIEME GENERATION

Durée de l'expérience (j)	$2A_T$			$2A_{25}$			$2A_{100}$			$2A_{500}$			$2A_M$		
	Nombre de femelles pondeuses	Nombre total de descendants	Moyenne	Nombre de femelles pondeuses	Nombre total de descendants	Moyenne	Nombre de femelles pondeuses	Nombre total de descendants	Moyenne	Nombre de femelles pondeuses	Nombre total de descendants	Moyenne	Nombre de femelles pondeuses	Nombre total de descendants	Moyenne
0 - 15	20	164	8,20	20	144	7,20	20	247	12,30	20	215	10,75	20	279	13,90
15 - 30	20	184	9,20	20	182	9,10	20	219	10,90	20	198	9,90	20	315	15,70
30 - 45	20	199	9,95	20	165	8,25	20	292	14,60	20	206	10,30	20	255	12,70
45 - 60	20	185	9,20	20	155	7,75	20	279	13,90	18	214	11,88	20	271	13,50
Moyenne par femelle au bout de 60 j			36,55			32,90			51,70			42,83			55,80

TABLEAU III. COMPARAISON DE LA LONGEVITE (en jours) DES ADULTES, MALES ET FEMELLES, DE Sitophilus granarius ELEVES SUR DU BLE NORMAL ET DU BLE IRRADIE DE 25 krads à 1 Mrad

	$1A_T$			$1A_{25}$			$1A_{100}$			$1A_{500}$			$1A_M$		
Sexe	min.	moy.	max.	min.	moy.	max.	min.	moy.	max.	min.	moy.	max.	min.	moy.	max.
Mâle	22,5	116,4	172,5	22,5	139,25	181,5	22,5	135,3	172,5	52,5	144	202,5	22,5	139,8	172,5
Femelle	67,5	121,95	210	22,5	123	202,5	67,5	129,6	172,5	37,5	130,8	202,5	22,5	132,3	187,5

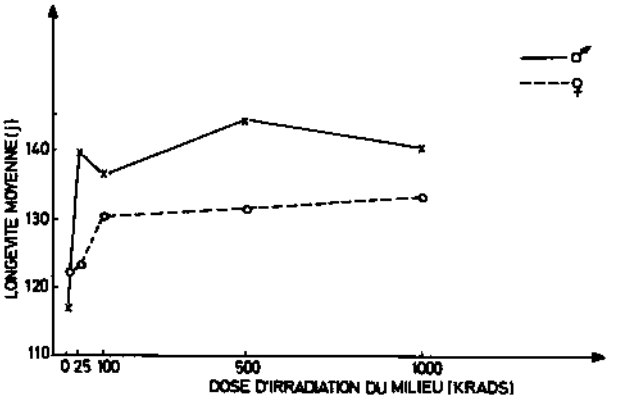


FIG. 3. Longévité moyenne des mâles et des femelles.

Résultats

Nous donnons au tableau III les résultats de ces observations et indiquons pour chaque milieu les longévités minimale et maximale observées pour chaque sexe, ainsi que la longévité moyenne calculée par pondération.

La figure 3 fait ressortir un accroissement plus important de la longévité des mâles par rapport aux femelles, dans tous les lots irradiés, alors que dans le lot témoin les femelles ont une longévité supérieure à celle des mâles. A la différence de ce que nous avons observé à propos de la fécondité, les milieux irradiés à une faible dose (25 krads) sont déjà favorables à un accroissement de la longévité; celle-ci est toutefois maximale sur les milieux irradiés à 500 krads.

Enfin si nous soumettons à l'analyse de variation l'ensemble des résultats observés, en ne tenant pas compte du sexe des individus, nous trouvons que les différences de longévité observées entre le lot témoin et les lots irradiés sont significatives à mieux que 1‰.

On peut ainsi imaginer que l'irradiation des grains de blé, à des doses variant de 25 krads à 1 Mrad, peut modifier cet aliment de façon à créer des facteurs favorables à la longévité et à la fécondité des adultes de Sitophilus granarius L.

Cet effet favorable peut se manifester parfois dès 25 krads, mais il est en général maximal pour une dose d'irradiation de 100 krads.

REMERCIEMENTS

Je me fais un très agréable devoir d'exprimer ma respectueuse gratitude à Monsieur le professeur P. Pesson, Directeur du Laboratoire de zoologie de l'INA, qui a bien voulu m'accueillir dans son laboratoire et a mis à ma disposition toutes les ressources matérielles désirables.

BIBLIOGRAPHIE

BLETCHLY, J.D., RONALD, C., FISHER, R.C., Lethal and sterilizing effects of gamma radiation on insects infesting cereal commodities, Nature, Lond., 179 (1957) 670-72.

BOLLY, L., PIERARD, A., PROOST, M., de, Essais d' application de l' irradiation gamma à la stérilisation des légumes, Bull. Inst. agron. Stns Rech. Gembloux (1967/68) 821-35.

CORNWELL, B.P., «Susceptibility of the grain and rice weevils, Sitophilus granarius (L.) and S. zeamais Mots. to gamma radiation», The Entomology of Radiation Disinfestation of Grain, Oxford, Pergamon Press (1966) 1-19.

JEFFERIES, D.J., «Effects of continuous and fractionated doses of gamma radiation on the survival and fertility of S. granarius», The Entomology of Radiation Disinfestation of Grain, Oxford, Pergamon Press (1966) 41-56.

LAVIOLETTE, L., NARDON, P., Action des rayons gamma du cobalt-60 sur la mortalité et la fécondité des adultes d' un charançon du riz, Bull. biol. Fr. Belg. XCII 1 (1963) 305-34.

MANIL, P., Radiostérilisation et radiopasteurisation des aliments, Bull. Inst. agron. Stns. Rech. Gembloux (1960) 808-21.

PENDLEBURY, J.B., «The influence of temperature upon the radiation susceptibility of S. granarius L.», Entomology of Radiation Disinfestation of Grain, Oxford, Pergamon Press (1966) 27-39.

PESSON, P., L' énergie nucléaire est-elle utilisable pour la protection des stocks de céréales contre les insectes?, Inf. Doc. agric. 2 (1965) 58-69.

PESSON, P., VERNIER, J.M., La protection des denrées contre les insectes ravageurs par l' emploi des radiations ionisantes en vue d' obtenir la stérilité des insectes adultes, Annls. Nutr. Aliment. 17 6 (1963) B 487 - B 497.

PROOST, M., de, Les facilités pour des irradiations gamma au C.E.N. à Mol et leur possibilité d' emploi dans l' agriculture et l' alimentation, Bull. Inst. agron. Stns Rech. Gembloux (1960) 834-44.

DISCUSSION

G.H.S. HOOPER: The moisture content of grain influences the development of Sitophilus granarius as well as that of other stored grain insects. Was any attempt made to standardize the moisture content of the grain during the various treatments?

E. BAGHERI-ZENOUZ: Yes, we tried to maintain exactly the same moisture content and temperature throughout all the experiments.

G.R. SETHI: There have recently been some reports that rearing on irradiated food resulted in some genetic changes in the insects/organisms thus bred. Could you please throw some light on this aspect?

E. BAGHERI-ZENOUZ: I have no detailed information on this aspect. I have compared histological sections of the genitalia of females bred in irradiated and unirradiated media and have not found any differences between them. Nor did a comparison of the general appearance of the genitalia of adult females bred in irradiated and normal media reveal any apparent differences.

W.F. BALDWIN: What would your explanation be for the interesting effect of irradiated media on the fecundity and survival of Sitophilus granarius?

E. BAGHERI-ZENOUZ: In order to find out the reasons it would be necessary to study the effects of irradiation on the protein chains of the vitamins, enzymes, etc. in irradiated media. It is possible that a biochemical change occurs in the wheat grains which promotes the increase in fecundity and longevity.

EFFECT OF GAMMA RADIATION ON THE REPRODUCTIVE ORGANS OF Dacus zonatus (SAUNDERS)

HESHAMUL HUQUE
Department of Plant Protection,
Ministry of Agriculture and Works,
Karachi, Pakistan

Abstract

EFFECT OF GAMMA RADIATION ON THE REPRODUCTIVE ORGANS OF Dacus zonatus (SAUNDERS).

The effects of gamma radiation on various life stages of fruit fly Dacus zonatus (Saunders) has been studied in detail to explore the possibilities of its control in the Karachi area by the sterile-male release technique. One aspect of this work, namely the gross changes which take place in the gonads of irradiated male and female flies, is described.

The insect material used in these experiments was collected from the local orchards. Pupae from the infested guava fruit (Psidium guajava), 20 - 30 in number, were irradiated 5 - 7 days after pupation and the study was made in adults at various time intervals after emergence. Each test was replicated three times. Haematoxylin and eosin were used as stains and Carnoy's fixative for fixation.

The main findings are that the size of both male and female reproductive organs of irradiated insects was reduced. At 5 kR the formation of spermatozoa was completed on the 16th day as against the 12th day with the normal insect. At 8 kR spermatozoids and spermatozoa were not observed even after the 15th day of emergence. At 16 kR necrosis started in the apical region of the testes which could be seen up to mid-region when the dose was increased to 18 kR. The testicular sheath also shrank.

INTRODUCTION

Fruit flies (Diptera, Tephritidae) are serious pests of fruits and vegetables in the Karachi area. The fact that the small cultivated area around Karachi on the Arabian Sea coast is separated from the main agricultural hinterland by a desert belt, permits a good chance of eradicating these flies from the area by the sterile-male release technique. After the initial studies of Steiner and Christenson (1956) [1] and Steiner et al. (1962) [2], doses of gamma radiation were determined which would induce sterility in the males and females of three important species of fruit flies prevalent in this area, Dacus zonatus (Saunders), D. cucurbitae Coquillett and D. ciliatus (Loew). Work has since been done by Huque and H. Ahmed (1966) [3], Huque and Malik (1967) [4] and Huque and C.R. Ahmed (1969) [5], and further detailed studies have been undertaken against D. zonatus, by far the most injurious of the three species mentioned. A preliminary report on the effect of ionizing radiation on eggs and larvae of this species in situ has been published by Huque and C. R. Ahmed (1967) [6]. The present paper describes in detail the major changes brought about by ionizing radiation in the reproductive organs of D. zonatus at different dose levels.

MATERIAL AND METHODS

The insect material used in these studies was obtained from guava (Psidium guajava) fruits infested with larvae of D. zonatus. The infested fruit, collected from the Malir gardens near Karachi, were placed in

TABLE I. STATE OF SPERMATOGENESIS IN IRRADIATED MALES OF Dacus zonatus ABOUT TWO WEEKS AFTER EMERGENCE FROM PUPAE

Dose of gamma radiation applied to pupae (kR)	Pairs of testes examined	Testicles appearing normal when compared to control (%)	Testicles appearing partially normal (%)	Remarks
5	20	85	15	(1) Spermatocytes in abundance. (2) Spermatozoa few in number.
8	20	0	4	(1) Testicles reduced in size. (2) Primary cells very few in number and scattered.
10	20	0	1	
16	20	0	0	
18	20	0	0	

FIG. 1. Necrosis in testis irradiated at 16 kR.

glass jars containing sand at the bottom. The full-grown larvae which pupated in the sand were collected for laboratory trials.

To irradiate the 5 - 7-day-old pupae, 20 - 30 of them were kept in a small glass vial and placed in the centre of a Gammacell 200 irradiation chamber. The source used for these experiments was cobalt-60 housed in

TABLE II. THE MEAN LENGTH AND WIDTH OF OVARIES OF IRRADIATED FEMALES OF Dacus zonatus 16 DAYS AFTER EMERGENCE FROM PUPAE

Dose of gamma radiation applied to pupae (kR)	Pairs of ovaries measured	Length (mm) Range	Length (mm) Mean	Width (mm) Range	Width (mm) Mean
3	20	4.8-5.3	4.9	3.1-3.5	3.4
5	20	3.8-4.0	3.99	2.5-2.6	2.25
7	15	3.5-3.7	3.68	1.6-1.8	1.75
9	20	2.0-2.1	2.05	1.5-1.75	1.6
Untreated	20	7.3-7.5	7.4	4.0-4.5	4.3

a fool-proof cell manufactured by Atomic Energy of Canada Ltd. A range of doses varying from 3 to 18 kR was applied. Male and female flies emerging from irradiated pupae were dissected at different intervals up to 24 days after emergence. Ovaries and testes were removed and stained with haematoxylin and eosin and fixed in Carnoy's fixative.

RESULTS

Effect of radiation on testes

At a dose of 5 kR there was almost no effect except that the formation of sperm was slightly delayed. It took about 16 days of adult life for a spermatozoon to form in irradiated males as compared to 12 days in unirradiated males. Of the 20 testes studied under the microscope, 85% showed normal spermatogenesis while in 15% some stages were missing (Table I). With an 8-kR dose, however, the size of the testes was reduced and the normal process of spermatogenesis was also affected (Table I). But a few scattered cells, resembling spermatocytes, were visible under high magnification. The spermatocytes disappeared when the dose was increased to 10 kR. Further observation revealed that at 16 kR (Fig. 1) necrosis started in the apical region of the testes which could be seen up to mid-region with increase in dose to 18 kR, when the testicular sheath also shrunk.

Effect of radiation on ovaries

From Table II it can be seen that gamma radiation has been responsible for reducing the size of ovaries when exposed at 3 - 9 kR. At 3 kR the ovaries contained a somewhat compact mass of oogonia at distal ends and the oocytes did not develop for about 12 days. But in dissected ovaries taken from the adults on the 16th post-emergence day, it was seen that oocytes were sparsely distributed in the middle portion (Fig. 2),

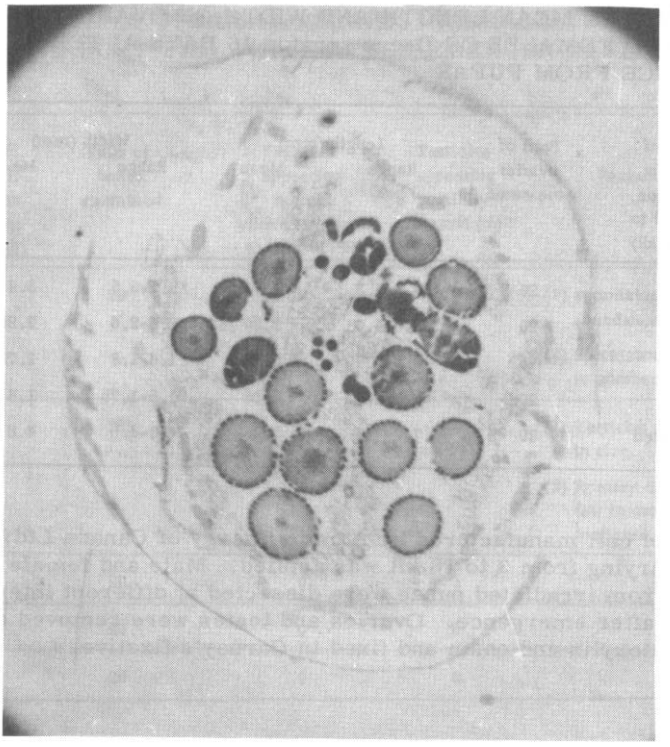


FIG. 2. Cross-section of ovary irradiated at 3 kR.

while in unirradiated females, both on the 12th and 16th post-emergence days, the ovaries were quite normal, being loaded with matured oocytes. Oocytes in ovaries irradiated at 3 kR, however, started maturing from the 20th day, but the eggs contained little yolk. Of the various doses applied, the effect at 5 kR was more pronounced as compared to that at 3 kR, and no development was observed after the 8th day of the emergence of females from irradiated pupae. At 7 and 9 kR the size of the ovaries was markedly reduced (see Fig. 3(a) and (b) for normal and 9 kR-irradiated ovaries). In addition, at 9 kR follicular degeneration was also observed and no differentiation could be made between oogonia, oocytes and eggs.

DISCUSSION

From the above observations it appears that the development of the reproductive organs of both males and females of D. zonatus is adversely affected by gamma radiation. The extent of damage is dose-dependent, i.e. the higher the dose applied, the more extensive will be the damage. As compared to females, males of this species seem to be slightly more resistant. With a 5 kR dose, the reproductive organs of the males remained almost normal, while the reproductive organs of the females were adversely affected even at 3 kR.

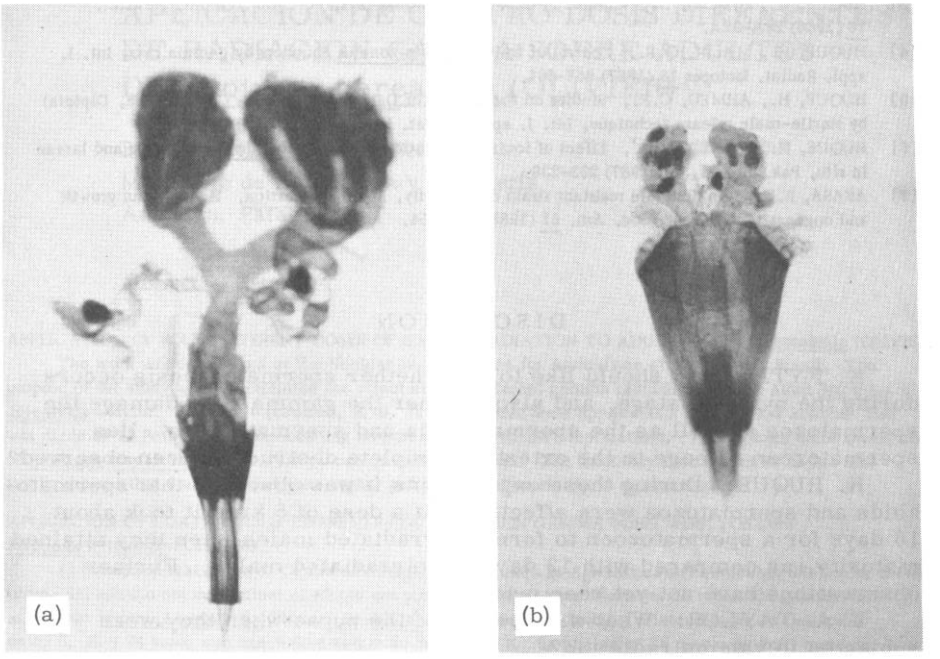


FIG.3. Corss-section of (a) normal ovaries, (b) ovaries irradiated at 9 kR.

Huque and Malik (1967) [4] reported that males of D. zonatus emerging from pupae irradiated at 7 - 9 kR showed no adverse effects except that they could not fertilize the normal females. Females which emerged from these pupae, however, reacted differently in the sense that the pre-oviposition period was noticeably prolonged and in many cases egg formations were completely retarded. Thus the results reported in this paper point to the explanation that this difference is due to the differential susceptibility of the sexes to gamma radiation.

The report of Abasa (1968) [7] on the reduction in size of ovaries tallies with the observations reported here.

ACKNOWLEDGEMENT

A part of the work reported here is from the author's Ph.D. thesis (University of Karachi, 1969). Technical assistance by Mr. Mohammad Sardar Alam is gratefully acknowledged.

REFERENCES

[1] STEINER, L.F., CHRISTENSON, L.D., Potential usefulness of the sterile fly release method in fruit fly eradication, Proc. Hawaii Acad. Sci. (1956) 19.

[2] STEINER, L.F., MITCHELL, W.C., BAUMHOVER, A.H., Progress of fruit fly control by irradiation sterilization in Hawaii and Marianas islands, Int. J. appl. Radiat. Isotopes 13 (1962) 427-34.

[3] HUQUE, H., AHMED, H., Control of fruit fly by gamma rays, Fd. Irrad. Q. Int. Newslet. 16 (1966) 28A-32A.
[4] HUQUE, H., MALIK, Q.R., Control of fruit fly Dacus zonatus Saunders by gamma rays, Int. J. appl. Radiat. Isotopes 18 (1967) 658-661.
[5] HUQUE, H., AHMED, C.R., Studies on the control of Dacus ciliatus Loew (Tephritidae, Diptera) by sterile-male release technique, Int. J. appl. Radiat. Isotopes, 20 (1969) 791-95.
[6] HUQUE, H., AHMED, C.R., Effect of ionizing radiation on Dacus zonatus fruit fly egg and larvae in situ, Pak. J. Sci., 19 (1967) 233-238.
[7] ABASA, R.O., An apholate resistant strain of house fly, Musca domestica. II. Ovarian growth and oogenesis, Ann. ent. Soc. Am. 61 (1968) 1351-54.

DISCUSSION

E. FYTIZAS: I should like to ask whether spermatogenesis occurs during the nymphal stage, and also whether the gamma rays damage the spermatozoa as well as the spermatogonia and spermatocytes. Has spermatozoan damage to the extent of complete destruction been observed?

H. HUQUE: During these experiments it was observed that spermatozoids and spermatozoa were affected. At a dose of 5 krad it took about 16 days for a spermatozoon to form in irradiated males when they attained maturity, as compared with 12 days in unirradiated males. Further observations have not yet been made.

E. A. TAYLOR: What was the age of the pupae when they were subjected to gamma radiation?

H. HUQUE: They were 5-7 days old when irradiated. The ovaries or testes were removed within 24 hours after emergence.

J. THEUNISSEN: Can you summarize what standards you used in order to determine whether a testis or ovary was normal, nearly normal or more heavily damaged?

H. HUQUE: The standard method was used. Ovaries were removed and kept on glass slides stained with haematoxylin and eosin and fixed in Carnoy's fixative. Sizes were measured with an optical micrometer. Under the high magnification of a phase microscope it was a simple matter to detect the damage done by ionizing radiation.

APLICACION DE CUATRO DOSIS DIFERENTES DE RADIACION GAMMA SOBRE ADULTOS DE Sitotroga cerealella (OLIVIER)

B. S. ARANDA CENTURION
Ministerio de Agricultura y Ganadería,
Asunción, Paraguay

Abstract — Resumen

APPLICATION OF FOUR DIFFERENT DOSES OF GAMMA RADIATION TO ADULT Sitotroga cerealella (OLIVIER).

The work was carried out at the Nuclear Energy Centre for Agriculture at Piracicaba, Brazil. The purpose of the study was to determine the effect of four different gamma radiation doses on adult Sitotroga cerealella Olivier. Doses of 0 (control), 3, 6, 12 and 27 krad were applied at a rate of 30.2 krad/h. Use was made of 25 Petri dishes, representing five treatments with five replications. Ten unsexed adult specimens of Sitotroga cerealella Olivier were placed on each Petri dish. The mortality counts took 13 days. The results showed that 6 and 12 krad gave higher percentages of mortality in shorter time.

APLICACION DE CUATRO DOSIS DIFERENTES DE RADIACION GAMMA SOBRE ADULTOS DE Sitotroga cerealella (OLIVIER).

El trabajo se realizó en el Centro de Energía Nuclear para la Agricultura en Piracicaba, São Paulo, Brasil. El objeto del estudio era determinar el efecto que producen cuatro dosis diferentes de radiación gamma sobre adultos de Sitotroga cerealella Olivier. Se aplicaron dosis de 0 krad (testigo) e intensidades de radiación de 3, 6, 12 y 24 krad, con una «dose-rate» de 30,2 krad/h. Se utilizaron 25 placas de Petri correspondientes a cinco tratamientos con cinco repeticiones. Se colocaron 10 ejemplares no sexados de adultos de Sitotroga cerealella Olivier en cada placa de Petri. Los contajes de mortalidad llevaron 13 días. El resultado mostró que 6 y 12 krad produjeron mayor porcentaje de mortalidad en menor tiempo.

1. INTRODUCCION

El uso de radiaciones ionizantes gamma para el control de plagas, especialmente las que atacan granos almacenados, ha despertado bastante interés en entomólogos especialistas de varios países en estos últimos años. Debido a la descendencia de especies o razas de insectos cada vez con mayor resistencia a productos insecticidas clorados y fosforados, se hace necesario intensificar la investigación y la aplicación de otros métodos de control como el uso de radiaciones ionizantes. De acuerdo a la bibliografía consultada, en la América del Sur se realizaron pocos trabajos en materia de uso de radiaciones en entomología. Gallo [1] efectuó trabajos de esterilización de pupas de machos de Ceratitis capitata (Wiedemann) y Diatraea saccharalis F. Wiendl [2] aplicó radiación gamma en dosis de 2, 5, 10, y 20 krad sobre adultos de Sitotroga cerealella (Olivier) y Simon [3] trabajó con la cría masal y esterilización con rayos gamma de Dysdercus peruvianus G. En el Paraguay, puede constituir actualmente un aspecto interesante la iniciación de trabajos relacionados con el uso de radiaciones para el control de plagas que atacan granos almacenados, entre las cuales Sitotroga cerealella (Olivier) ocasiona anualmente graves perjuicios durante el período de almacenamiento de la producción nacional de maíz.

El presente trabajo fué realizado en el mes de septiembre de 1969, en el Centro de Energía Nuclear para la Agricultura anexo a la ESALQ,

EFECTO DE RADIACION GAMMA SOBRE ADULTOS DE Sitotroga cerealella (Olivier)

Dosis (krad)	Mortalidad en 3 días	Mortalidad en 6 días	Mortalidad en 9 días	Mortalidad en 12 días
0	1	10	32	48
3	9	13	37	48
6	9	18	40	50
12	16	21	41	50
24	9	12	44	49

en Piracicaba, Estado de São Paulo, Brasil. El objetivo del estudio fué determinar los efectos que producen cuatro dosis diferentes de radiación gamma en la longevidad de adultos de Sitotroga cerealella (Olivier).

2. MATERIAL Y METODOS

La fuente de radiación ionizante gamma fué un irradiador de ^{60}Co marca «Gammabeam 150, Atomic Energy of Canada Ltd. Otawa, Canada - Model GB-^{150}B», con una actividad de 931 Ci en septiembre de 1969.

El material a tratar consistía de ejemplares adultos no sexados de Sitotroga cerealella Olivier proveniente de maíz infestado.

Se utilizaron 25 placas de Petri, provistas de humedad adecuada y correspondientes a cinco tratamientos con cinco repeticiones inclusive testigo. Se colocaron dentro de cada placa de Petri diez adultos no sexados del material a tratar.

Las dosis usadas fueron de 0 krad (testigo); las intensidades de radiación de 3, 6, 12 y 24 krad, con una «dose-rate» de 30, 2 krad/h. El tratamiento fué realizado el día 8 de septiembre de 1969.

Los contajes de mortalidad comenzaron al día siguiente después de 24 h de la aplicación. Las observaciones continuaron con intervalos de 24 h, hasta el 13° día para obtener el dato de mortalidad total.

3. RESULTADOS

El tratamiento testigo con 0 krad evidentemente demostró menor porcentaje de mortalidad en los días de observación en relación a los demás. Las dosis de 6 y 12 krad parecen ser las más indicadas por haberse observado mayor porcentaje de mortalidad en menor tiempo (véase tabla).

REFERENCIAS

[1] GALLO, D., Radioisotopos no controle das pragas, O Solo 52 1 (1960) 30.

[2] WIENDL, F.M., Influencia da radiação-gamma na longevidade de Sitotroga cerealella Oliv., Resumos da I Reunião Anual da Soc. Brasil. Entomol (1968) 22.

[3] SIMON, J.E., «Cría masal de Dysdercus peruvianus G. y su esterilización mediante rayos gamma», Isotopes and Radiation in Entomology (Proc. Symp. Vienna, 1967), IAEA, Vienna (1968) 287.

STERILITY PRINCIPLE FOR CONTROL OF FRUIT FLIES

(Session II)

Chairman

E.A. TAYLOR (USA)

Survey paper

MEDITERRANEAN FRUIT FLY SUPPRESSION USING THE STERILITY PRINCIPLE
Prospects and programs

E.A. TAYLOR
Entomology Research Division,
United States Department of Agriculture,
Beltsville, Md.,
United States of America

Abstract

MEDITERRANEAN FRUIT FLY SUPPRESSION USING THE STERILITY PRINCIPLE: PROSPECTS AND PROGRAMS.
When the sterility principle was used against relatively low populations of two species of tropical fruit flies and integrated with other techniques of population suppression, eradication resulted. Research on the Mediterranean fruit fly, Ceratitis capitata (Wiedemann) has increased several-fold during the past 5-10 years, and this extra effort has now resolved some of the problems encountered in the effective use of this insect in integrated suppression programs. This is a brief résumé of the progress and a discussion of some of the problems that still must be resolved.

INTRODUCTION

The value of the sterility principle for the eradication of tropical fruit flies was clearly demonstrated in two separate pilot tests with the melon fly, Dacus cucurbitae (Coquillett), and the oriental fruit fly, Dacus dorsalis Hendel, in the Mariana Islands [1, 2]. When the principle was used against relatively low populations and integrated with other techniques of population suppression, eradication resulted. The principle has also been successful with the Mexican fruit fly, Anastrepha ludens (Loew); thus releases of sterile insects are now used in a regulatory program undertaken by the U.S. Department of Agriculture and the California Department of Agriculture to prevent the spread of this pest from Mexico into California [3, 4].

Eradication technology may be more advanced for the melon fly and oriental fruit fly than the Mediterranean fruit fly, Ceratitis capitata (Wiedemann), because of the successful pilot test against isolated island populations. However, these species and the Mexican fruit fly also appear to be more tolerant of sterilizing doses of irradiation than the Mediterranean fruit fly and are generally more competitive when they are released after treatment. However, research on the Mediterranean fruit fly has increased several-fold during the past 5-10 years, and this extra effort has now resolved some of the problems encountered in the effective use of this insect in integrated suppression programs. My objective is to provide a brief résumé of the progress and to discuss some of the problems that still must be resolved.

1. RELEASING A COMPETITIVE STERILE FLY

1.1. Rearing

The efficiency of mass rearing of the Mediterranean fruit fly has increased greatly as a result of the research project, "Eradication of the Mediterranean Fruit Fly in Central America". In 1965, United Nations Development Programme, International Atomic Energy Agency (UNDP/IAEA) worked with the Organismo Internacional Regional de Sanidad Agropecuaria (OIRSA) in the establishment of mass-rearing facilities at San Jose, Costa Rica, and by July 1967 they were capable of maintaining a normal production of 40 million flies per week. Concurrently more effective techniques for egg collection, larval rearing, pupal recovery, adult holding, and feeding and handling of tremendous numbers of insects were developed [5-8]. As a result, fly-rearing costs were reduced from $50 per million to about $15 per million. In addition, certain changes made in the rearing techniques and methods of subsequent handling of pupae and adults seemed to markedly improve overall fly quality; this has increased the recapture of released flies about three-fold [9, 10].

The technology is now adequate to produce hundreds of millions of Mediterranean fruit flies per week at a very low cost. Additional efficiency in mass-rearing research and methods of handling might further reduce the cost, but improvements in the quality and competitiveness of flies would now seem to offer the best opportunity to increase the efficiency and effectiveness of sterile Mediterranean fruit flies in eradication and suppression programs.

1.2. Handling pupae

The detrimental effect of the overheating of pupae during treatment and movement from the rearing facilities to release sites has been recognized by several investigators. For example, the results of recent tests by N. Tanaka of the Hawaiian Fruit Flies Investigations Laboratory, indicate that 15 litres of pupae can be held and shipped in a single small package without overheating if the pupae are packaged in polyethylene bags under 5-inch NAP vacuum. Apparently the limited amount of oxygen available for normal metabolism results in slower development and a reduction in the amount of heat produced. Pupae packaged in this manner have been held at room temperature for 3-4 days and have been shipped by air without any adverse effects on adult emergence, behaviour, or longevity.

If subsequent tests corroborate these preliminary findings, this method of handling pupae could greatly improve the prospects for the use of the sterility principle against the Mediterranean fruit fly. One large production centre could produce Mediterranean fruit flies and could ship them for use in action programs in any part of the world.

1.3. Sterilizing adults

In all large-scale programs involving the release of sterile insects to eradicate fly populations, the insects have been irradiated in the pupal state [1, 2, 11, 12]. The success of these eradication programs against

tropical fruit flies has caused many investigators to defer investigation of the effects of radiation on stages of flies other than pupae. However, the initial large-scale tests involving the release of Mexican fruit flies made use of flies that were sterilized with tepa as they emerged. Laboratory tests indicated that these flies were more aggressive than flies sterilized by gamma irradiation in the pupal stage 1-2 days before adult eclosion [3]. This difference in fly behaviour was therefore attributed to the method of sterilization rather than to the stage of the insect at the time of exposure. However, several investigators have since reported that sterilization by irradiation in the pupal stage reduces the mating competitiveness of male Mediterranean fruit flies. That is, mating ability of males irradiated as pupae with 10-krad gamma irradiation 2 days before adult emergence is about 50-65% that of untreated males, though the mating ability of treated females is not appreciably affected [13-16]. There is some question whether relative mating competitiveness in a laboratory cage condition represents relative competitiveness in the field. However, the results of recent tests in Hawaii indicate that male Mediterranean fruit flies irradiated as 2-day-old adults with 10 krad of gamma irradiation have no reduction in mating effectiveness compared with a 50% reduction when the same treatment is applied to pupae 2 days before adult eclosion [17]. This superiority of Mediterranean fruit flies irradiated as 2-day-old adults was then established in tests in outdoor cages and in small cages and verified by a study of sperm transfer. Apparently, the increased mating competitiveness of males irradiated as adults over that of males irradiated as pupae may be their larger supply of sperm at the time of irradiation [18].

The possibility therefore exists that the relative competitiveness of flies sterilized by either method will be even more favourable for those sterilized as adults when released in the field. This possibility should be further explored.

1.4. Distributing sterile flies

The success or failure of the suppression experiment carried out by the project in Central America depended in large measure on adequate dispersal of sterile flies by air. So a major portion of the research effort was concerned with this phase of the program. As a result, certain of the problems involved in aerial release were resolved with the paper bag method [11]. This system of distribution improved the quality of released flies, but it was still a very costly method of distribution. Furthermore, flies held in such containers are subject to the hazards of overheating while they are being transported to airfields, during loading, or within the plane.

Therefore, if full advantage were to be taken of irradiation in the adult stage, new techniques need to be developed for handling and distributing adults. However, during the past 3 years, the Plant Protection Division, Agricultural Research Service, U.S. Department of Agriculture, has made significant progress in developing a method for aerial distribution of free release of sterile insects [19]. This method, which involves chilling adult moths for treatment and handling, has greatly reduced the cost of distribution and could be readily adapted to the Mediterranean fruit fly if the fly can tolerate temperatures of about 5°C for a few hours. Preliminary tests in Hawaii have shown that adult Mediterranean fruit

flies can be chilled and held at 5° C for as much as 24 hours without any apparent adverse effects. At this temperature, flies become inactive and are easily irradiated and released by air or ground equipment.

2. INTEGRATION OF SUPPRESSION TECHNIQUES

2.1. Bait sprays

Both the successful and the abortive attempts to eradicate tropical fruit flies by releasing sterile insects have indicated that a combination of two or more techniques will in all likelihood be necessary if sterile insects are to be used successfully and effectively in eradication or suppression programs. For example, before the overflooding of the melon fly population on Rota in 1962, all the farm sites received two or more applications of protein hydrolysate-malathion bait sprays [1]. Also, when sterile oriental fruit flies were used to eradicate the population of oriental fruit flies on Guam, full advantage was taken of the suppression that had resulted from two hurricanes, and supplemental suppression was used to eradicate reinfestations. Thus, as soon as the incipient reinfestation was delineated, two applications of protein hydrolysate-malathion bait sprays were made to all fruiting hosts, and methyl eugenol-naled bait stations were put in operation. Thereafter, 0.5 million sterile flies were released each week for 26 weeks. In contrast, when attempts were made to eradicate this species with the sterility technique in Saipan and Tinian without the use of supplemental methods, eradication was not achieved, even though an apparently favourable ratio of sterile to native flies was maintained for several months [2].

Again, during the initial phases of a successful test of population suppression with sterile Mediterranean fruit flies (Carazo, Nicaragua; September 1968 to May 1969; Rhode et al., in press), the 45-km^2 test area was isolated from surrounding infestations, as follows. A 2-km border was bait-sprayed 6 times at 2-week intervals with 60 ml of malathion plus 540 ml of protein hydrolysate bait per acre applied from the air. One-third of the total border was covered in each application by delivering the spray over every third swath. Thus the whole border area was treated three times.

2.2. Male annihilation

Male annihilation proved effective as an eradication technique against the oriental fruit fly on Rota, Saipan and Tinian in the Mariana Islands [2]. Also, an incipient infestation of oriental fruit flies that occurred in Southern California during the fall of 1969 was eliminated by spraying the tree trunks in a 10-mile^2 area with 3 - 5 ml of methyl eugenol plus naled [20].

The male annihilation with cue-lure is now being pilot-tested on Guam against the melon fly by the Hawaiian laboratory under the direction of D. L. Chambers and R. T. Cunningham in co-operation with the Department of Agriculture, Government of Guam, as a method of supplementing a sterility program that is already in progress. Thus, on 11 July, 1970, about 200 000 1.25-cm cane fibre cubes, each containing 1 ml cue-lure

plus 1 ml of naled, were distributed over more than half of the 210-mile^2 island. In the next 3 - 4 weeks, the captures of native flies in the 200 evaluation traps were reduced to about 80% of the pre-treatment captures. Therefore, a second application was made on 22 August. Meanwhile, the weekly releases of 5 - 10 million sterile melon flies have been continued, and it is planned to continue both techniques for about 6 months. In addition, areas in which wild mormordica occur along highways will receive frequent ground applications of protein hydrolysate-malathion bait sprays, and a mixture of cue-lure plus naled is being applied to tree trunks with ground equipment through the populated areas of Guam at the rate of 1 - 2 ml per spot. Theoretically, this combination of methods should eradicate the population of melon flies on Guam: the applications of cue-lure plus naled will kill both the released sterile and native male flies, and the bait sprays applied to the wild hosts that are known to produce melon flies will reduce the residual fertile female population and kill many released sterile females. However, the bait sprays are applied to very limited areas and are effective for only a few days. So the sustained releases of sterile insects should provide the necessary additional suppression and the released sterile females should increase the number of females available to attract emerging native males that might escape the cubes or bait sprays.

The male annihilation technique was also tested against the Mediterranean fruit fly by Steiner and Cunningham on Terceira in the Portuguese Azores in 1967. They used a formulation containing trimedlure, 5% naled, and a thickening agent, and applied about 800 lb (1760 kg) of this mixture aerially to most hosts of the Mediterranean fruit fly that occurred in dooryard fruit plantings within the villages. Each application (rate of 2.25 kg/km^2) reduced the captures of flies in traps for a few days. However, such a high rate of trimedlure could only be used in a very limited area in an actual pilot test. Furthermore, aerial application of this lure mixture damages the paint of automobiles in a manner similar to that of bait sprays and would be at least as objectionable since the spots from the thickened mixture are much larger than those from bait sprays.

Subsequently, a 19:1 formulation of trimedlure plus naled applied to 5 cm^2 × 1.25-cm cane fibreboard suspended 2 - 3 ft above the ground at the rate of one per acre reduced catches of male Mediterranean fruit fly in Hawaii. Tray tests are therefore being conducted by Cunningham in Hawaii to determine the most efficient rate of trimedlure plus toxicant applied to fibreboard or other materials. The preliminary results of these tests showed that 10 days after the treatment began, 1 ml of lure plus 1 ml of toxicant on 1.25 cm fibre cubes attracted and killed more Mediterranean fruit flies than a 5 cm^2 × 1.25-cm piece of fibreboard saturated with 22 ml of lure plus 1 ml of naled. These tests suggest that Mediterranean fruit fly males may not be in contact with the trimedlure on the saturated fibreboard long enough to be killed by the toxicant.

Similar tests in which cue-lure was used against the melon fly also showed that less than 1 ml of lure plus 1 ml of toxicant applied to 1.25 cm fibre cubes was as effective as 5 cm^2 × 1.25 cm pieces of fibreboard saturated with 24 ml of cue-lure and 1 ml of naled. Then if further testing indicates that 1 ml of trimedlure plus 1 ml of naled on 1.25 cm cube

fibreboard attracts and kills Mediterranean fruit flies as efficiently as the lower rate of cue-lure attracts melon flies, a combination of male annihilation and release of sterile insects may be the most promising method of suppressing or eradicating populations of Mediterranean fruit flies.

3. LARGE-SCALE DEMONSTRATION

3.1. Central America

The final phase in the development of the sterility principle against the Mediterranean fruit fly must be a large-scale demonstration that this insect can actually be eradicated over a sizeable area if the total population is subjected to sterile-fly releases integrated with a thorough bait-spray program in all areas of high population density; male annihilation method with trimedlure may also be advantageous. Such a program has been proposed for Nicaragua, Central America, as a follow-up to the work so far accomplished by the UNDP research project mentioned previously, because the infested area in that State (about 1000 mile^2) is geographically isolated. Moreover, a minimum of quarantine procedures would be necessary to prevent reinfestation from Costa Rica, and no infestations are known to exist north of Nicaragua.

The objectives of the proposed program in Nicaragua would be to develop and demonstrate procedures for eradicating the Mediterranean fruit fly by an integrated program of sterile-insect release plus bait spray plus male annihilation. Such a program would provide the data necessary to establish realistic costs for the eradication of the Mediterranean fruit fly from the whole of Central America and would reduce the chances of further spread of this insect northward into Mexico and the United States.

3.2. Hawaii

One of the Islands in the State of Hawaii would also provide a very suitable location for an investigation of the practicality of the use of the sterility principle against the Mediterranean fruit fly. However, the current major objective of our Hawaii laboratory is the development of highly selective methods for the simultaneous eradication of all three species of tropical fruit flies, the oriental and Mediterranean fruit flies and the melon fly. Thus the immediate plans involve pilot tests against all three species to determine whether eradication can be achieved. Since the male annihilation technique has reduced the cost of eradication of the oriental fruit fly by about 90%, we are confident that it is now possible to eradicate this fruit fly from Hawaii. However, if the Mediterranean fruit fly and the melon fly are not also eradicated, the cost of maintaining quarantines would not be reduced. Moreover, there is a possibility that the population of Mediterranean fruit flies would increase because of the absence of the competition. Therefore a large-scale pilot test should include a bait-spray program in selective host areas, male annihilation with all the highly specific lures, and release of sterile insects of all three species.

Such use of sterile insect releases as a supplementary measure for the eradication of all three species appears very promising. Many basic and applied phases of this research have been completed in the laboratory and in small field tests. Now certain aspects of the combination of the releases and the male annihilation technique need further development in large-scale pilot tests in which these methods can be perfected for use in Hawaii. Such tests should be made against fruit-fly populations on one of the smaller islands, for example Lanai. The support for and interest in such testing is growing among officials in Hawaii, members of the U.S. Congress, and State and local authorities in California for the additional financial resources necessary to undertake the type of research needed to perfect eradication measures for all three species of tropical fruit flies in Hawaii.

REFERENCES

[1] STEINER, L.F., HARRIS, E.J., MITCHELL, W.C., FUJIMOTO, M.S., CHRISTENSON, L.D., Melon fly eradication by overflooding with sterile flies, J. econ. Ent. 58 (1965) 519.

[2] STEINER, L.F., HART, W.G., HARRIS, E.J., CUNNINGHAM, R.T., OHINATA, K., KAMAKAHI, D.C., Eradication of the oriental fruit fly from the Mariana Islands by the methods of male annihilation and sterile insect release, J. econ. Ent. 63 (1970) 131.

[3] SHAW, J.G., PATTON, W.P., SANCHEZ-RIVIELLO, M., SPISHAKOFF, L.F., REED, B.C., Mexican fruit fly control, The Calif. Citrograph 51 (1966) No.5.

[4] SHAW, J.G., SANCHEZ-RIVIELLO, M., SPISHAKOFF, L.M., TRUJILLO, G.P., LOPEZ D., F., Dispersal and migration of tepa-sterilized fruit flies, J. econ. Ent. 60 (1967) 992.

[5] MITCHELL, S.N., TANAKA, N., STEINER, L.F., Methods of mass culturing melon flies and oriental and Mediterranean fruit flies, USDA, ARS 33-104 (1965).

[6] NADEL, D.J., in: Advances in insect population control by the sterile-male technique, Tech. Reports Series No.44, IAEA, Vienna (1965) 14.

[7] PELEG, B.A., RHODE, R.H., New methods in mass rearing of the Mediterranean fruit fly in Costa Rica, J. econ. Ent. 60 (1967) 1460.

[8] PELEG, B.A., RHODE, R.H., New larval medium and improved pupal recovery method for the Mediterranean fruit fly in Costa Rica, J. econ. Ent. (in Press).

[9] NADEL, D.J., MONRO, J., PELEG, B.A., FIGDOR, H.C.F., A method of releasing sterile Mediterranean fruit fly adults from aircraft, J. econ. Ent. 60 (1967) 899.

[10] RHODE, R.H., CALDERON, W., Aerial release techniques for the Mediterranean fruit fly, J. econ. Ent. (in Press).

[11] RHODE, R.H., SIMON, J., PERDOMO, A., GUTIERREZ, J., DOWLING, Jr., C.F., LINDQUIST, D.A., Application of the sterile insect release technique in Mediterranean fruit fly suppression, J. econ. Ent. (in Press).

[12] de MURTAS, I., CIRIO, U., GUERRIERI, C., ENKERLIN, S.D., "An experiment to control the Mediterranean fruit fly on the Island of Procida by the sterile-insect technique", in: Sterile-Male Technique for Control of Fruit Flies, (Proc. Panel Vienna, 1969), Technical Reports Series, IAEA, Vienna (in Press).

[13] FERON, M., in: Advances in Insect Population Control by Sterile-Male Technique (Report Panel Vienna, 1964), Technical Reports Series No.44, IAEA, Vienna (1965) p.21.

[14] KATIYAR, K.P., in: Advances in Insect Population Control by Sterile-Male Technique (Report Panel Vienna, 1964), Technical Reports Series No.44, IAEA, Vienna (1965) p.20.

[15] KATIYAR, K.P., VALERIO, J., Efecto causado por la introduccion de machos esterilizados por radiacion gamma, en una población normal de moscas del Mediterráneo. (Ceratitis capitata) Turrialba 14 (4) (1964) 211.

[16] HOLBROOK, F.R., FUJIMOTO, M.S., Mating competitiveness of unirradiated and radio-sterilized Mediterranean fruit flies, J. econ. Ent. (in Press).

[17] OHINATA, K., CHAMBERS, D.L., FUJIMOTO, M., KASHIWAI, S., MIYABARA, R., Sterilization of the Mediterranean fruit fly by irradiation: comparative mating effectiveness after treatment of pupae and adults, J. econ. Ent. (in Press).

[18] ANWAR, M., CHAMBERS, D.L., OHINATA, K., KOBAYASHI, R.M., Radiation-sterilization of the Mediterranean fruit fly: comparison of spermatogenesis in flies treated as pupae or adults, J. Insect Physiol. (in Press).
[19] HIGGINS, A.H., A machine for free aerial release of sterile pink bollworm moths, USDA, ARS 81-40 (1970).
[20] USDA, ARS, Plant Protection Division 1969 Annual Report.

DISCUSSION

M.E. TZANAKAKIS: Do you plan to use radiation or chemicals to sterilize the three fruit-fly species in the eradication attempt?

E.A. TAYLOR: Sterilization will be carried out by means of gamma irradiation.

M.E. TZANAKAKIS: Are the adult flies chilled during irradiation? If not, in what other way do you keep them anaesthetized?

E.A. TAYLOR: They are chilled.

M. FRIED: You mentioned in your paper the possibility of using pupae shipped in polyethylene bags under vacuum. Can this method be used after irradiation and how does it affect emergence and the nature of the adult that has emerged?

E.A. TAYLOR: The pupae were treated before packaging and kept at room temperature for three to four days without any adverse effects on adult emergence, behaviour or longevity.

L.E. LaCHANCE: You mentioned that irradiation of two-day-old adults produced a "better" sterile fly than irradiation of "old" pupae. How was this measured?

E.A. TAYLOR: The superiority of Mediterranean fruit flies irradiated as two-day-old adults was established by means of tests in large outdoor cages (in which mating was observed) and small cages (with various ratios of treated to untreated flies), and by means of a study of sperm transfer.

Survey paper

LA TECNICA DE MACHOS ESTERILES EN EL CONTROL DE LA MOSCA DEL MEDITERRANEO Programas realizados en España

L. MELLADO
Instituto Nacional de Investigaciones Agronómicas,
Madrid, Spain

Abstract — Resumen

THE STERILE-MALE TECHNIQUE IN THE CONTROL OF THE MEDITERRANEAN FRUIT FLY: PROGRAMS CARRIED OUT IN SPAIN.

The Instituto Nacional de Investigaciones Agronómicas started in 1965 a program of application of the sterile-male technique for the control of Ceratitis capitata (Wiedemann). Artificial mass rearing was started in Madrid in 1965; at present, the rearing laboratory produces an average of 1 million pupae per day.

From 1966 to 1968, field releases of sterile insects (irradiated at a dose of 9 krad) were carried out in the island of Tenerife, over an area of 450 ha. In the first two years no positive results were obtained (partly because of the small number of insects released). In 1968, the total release amounted to 25 million insects; significant differences between the release area (15% infestation) and the control area (70% infestation) were shown.

In 1969, field experiments were carried out in a regular plantation (citrus, apricot and peach) in the province of Murcia. 32 million sterile flies were released over an area of 25 ha during the period March-August. Although the release area was not well isolated, the infestation in the area stayed below 1% (except for the last week when there was very little fruit left on the trees), whereas the infestation in the nearby control areas was higher than 60% during the same period.

In 1970, experiments are being carried out on irradiation doses (7 krad and 9 krad) and on the problem of punctures caused by sterile females on certain fruit varieties.

In conclusion, it has been shown that the sterile-male technique is a fully effective method, when applied to small areas. Experiments on larger areas are being planned for 1971.

LA TECNICA DE MACHOS ESTERILES EN EL CONTROL DE LA MOSCA DEL MEDITERRANEO: PROGRAMAS REALIZADOS EN ESPAÑA.

El Instituto Nacional de Investigaciones Agronómicas viene desarrollando, desde 1965, un programa de aplicación del método de «machos estériles» contra Ceratitis capitata (Wiedemann). La cría artificial se inició en el laboratorio de Madrid en 1965, a pequeña escala; actualmente esta instalación tiene una capacidad de producción media de 1 millón de pupas/día.

De 1966 a 1968 se realizaron ensayos de suelta de insectos estériles (irradiados con una dosis de 9 krad) en la isla de Tenerife, sobre una superficie de 450 ha. Los primeros dos años no se obtuvieron resultados positivos debido, en parte, al escaso número de insectos soltados. En 1968 con una suelta total de 25 millones de insectos, se apreciaron diferencias significativas entre la zona de sueltas (15% de ataque) y la zona testigo (70% de ataque).

En 1969 los ensayos de campo se realizaron en una plantación regular de cítricos, albaricoque y melocotón, de la provincia de Murcia. Se soltó un total de 32 millones de insectos sobre un área de 25 ha durante el período marzo-agosto. A pesar de no estar la zona bien aislada se logró mantener el ataque de Ceratitis inferior al 1% (excepto en la última semana de julio, cuando ya quedaba muy poca fruta). Durante el mismo período, las zonas testigo adyacentes registraron ataques superiores al 60% en todas ellas.

En 1970 se está estudiando, en el campo, el efecto de distintas dosis de irradiación (7 krad y 9 krad) y el fenómeno de las picaduras producidas por las hembras estériles en algunas variedades de fruta.

Como conclusión, los resultados obtenidos muestran que el método es plenamente eficaz en su aplicación a extensiones de terreno pequeñas. Para 1971 se proyecta realizar un ensayo sobre extensiones mayores.

Desde 1965 el Instituto Nacional de Investigaciones Agronómicas viene desarrollando un programa de aplicación del método de «machos estériles» para combatir la mosca de la fruta, Ceratitis capitata (Wiedemann). En la presente comunicación se exponen, brevemente, los trabajos realizados y resultados obtenidos.

TRABAJOS REALIZADOS EN 1965-66

En 1965 se inició la cría artificial masiva de Ceratitis capitata en el laboratorio de Madrid. Se realizaron estudios básicos sobre métodos de cría, dosis de irradiación y marcado de insectos con radioisótopos. En 1966 el laboratorio producía un promedio de 53 000 pupas diarias.

Para los ensayos de campo se eligieron dos valles adyacentes y relativamente aislados en el sur de la isla de Tenerife, uno como zona experimental y otro como zona testigo. En estos valles existían huertos de albaricoqueros, melocotoneros, cítricos, higueras y vides. No existían plantaciones regulares propiamente dichas. En toda la zona, el ataque de Ceratitis era muy fuerte. No se realizaba ningún tratamiento sistemático con pesticidas. Durante todo el tiempo que duraron nuestras experiencias se suprimió totalmente todo tipo de tratamientos.

La suelta de insectos estériles se inició en mayo de 1966, sobre un área de unas 450 ha, en el valle elegido como zona experimental. Las pupas, irradiadas en Madrid, en una fuente de ^{137}Cs, a una dosis de 9 krad, se enviaban por vía aérea a Tenerife. Se ensayaron distintos métodos de empaquetado y suelta. Finalmente, se adoptó el método de envío de las pupas irradiadas en bolsas de papel, rasgándose estas bolsas en el campo al emerger los adultos. No se efectuaron sueltas aéreas. De mayo a diciembre de 1966 se soltaron más de siete millones de insectos. No se pudo comprobar ningún resultado positivo; el ataque de Ceratitis continuaba siendo muy intenso.

TRABAJOS REALIZADOS EN 1967

Se instaló en Madrid un nuevo laboratorio de cría artificial. Se efectuaron una serie de experimentos sobre dietas alimenticias y su influencia en la oviposición. La producción de pupas ascendió a un promedio de 72 000 diarias. Se continuó la suelta de insectos irradiados en la misma zona y con los mismos métodos del año anterior. No se efectuó un muestreo sistemático de la fruta atacada pero algunas tomas de muestras aisladas parecían indicar un descenso del ataque de Ceratitis en la zona experimental. El total de insectos soltados fué de 4 millones.

TRABAJOS REALIZADOS EN 1968

Con la introducción de nuevos métodos de cría se logró elevar la producción a un promedio de 200 000 pupas diarias. Continuaron realizándose las sueltas de insectos irradiados en la misma zona; estas sueltas alcanzaron, en 1968, un total de 25 millones de insectos estériles. Se

PORCENTAJE DE FRUTA ATACADA

Zona experimental	Abril	Mayo	Junio	Julio 1-10	Julio 10-20	Julio 20-25	Julio 25-31
Cítricos	10	-	-	-	-	-	-
Albaricoque	-	0	0	-	-	-	-
Melocotón	-	-	0	0,1	0,2	1,0	10

Zona testigo	Abril	Mayo	Junio	Julio 1-10	Julio 10-20	Julio 20-25	Julio 25-31
Albaricoque	-	0	0	50	90	-	-
Albaricoque	-	0	0	40	90	-	-
Melocotón (tratado con insecticida)	-	-	-	0	0	0	0
Melocotón	-	-	-	40	90	100	-
Melocotón	-	-	-	10	25	100	-
Melocotón	-	-	-	-	-	-	60

realizó un muestreo sistemático del grado de ataque en el melocotón. Los resultados fueron, en resumen:

1) Zona experimental: promedio de fruta atacada = 15% (variando entre el 6% y el 23%);

2) Zona testigo: promedio de fruta atacada = 70% (variando entre el 39% y el 100%).

TRABAJOS REALIZADOS EN 1969

La producción de pupas alcanzó un promedio diario de 400 000. El programa de campo de la isla de Tenerife se abandonó temporalmente, debido a una serie de razones, sobre todo de tipo logístico. Se inició un nuevo programa en la zona de Alhama de Murcia, en el Sudeste de España. En este programa colaboró la División Mixta FAO/OIEA, proporcionando asesoramiento técnico y suministrando pupas durante la primera fase del mismo.

La nueva zona experimental abarcaba unas 25 ha, con plantaciones regulares de cítricos, albaricoque y melocotón. Como zonas testigo se eligió una plantación regular de melocotón y ocho puntos de control en áreas próximas. La plaga de Ceratitis es endémica en toda la región.

El objetivo del programa consistía en controlar el ataque de Ceratitis en el albaricoque y melocotón, mediante la suelta de insectos estériles, sin utilizar ningún otro tratamiento.

Todas las pupas se irradiaron en Madrid, se transportaron por carretera a Murcia y se soltaron en estado adulto. Las sueltas se realizaron desde marzo a agosto, totalizando 32 millones de pupas (26 millones procedentes del laboratorio de Madrid y 6 millones del laboratorio de Seibersdorf, suministradas por el OIEA).

Se realizaron ensayos sobre longevidad y dispersión de los insectos irradiados. Los datos obtenidos muestran que, en condiciones normales, la «vida media» en el campo de la población de insectos irradiados puede estimarse en 6 días (entendiendo por «vida media», el tiempo que la población tarda en reducirse a la mitad). La dispersión de los insectos irradiados es relativamente escasa, inferior a 250 ml del punto de suelta (aunque haya algunos insectos que excepcionalmente recorran distancias de varios kilómetros).

La zona experimental estaba relativamente aislada. Sin embargo, se demostró la posibilidad de penetración de insectos fértiles desde zonas próximas.

El examen de la fruta de la zona experimental y de zonas testigo dió los resultados que se pueden observar en el cuadro.

Estos resultados demuestran que es posible lograr un control eficaz de Ceratitis capitata, mediante el exclusivo empleo del método de «machos estériles», en zonas de extensión reducida, incluso aunque no estén perfectamente aisladas.

TRABAJOS REALIZADOS EN 1970

En los ensayos de campo realizados en años anteriores se puso de manifiesto que existen dos problemas que es preciso resolver para poder aplicar el método de «machos estériles» a gran escala y con máxima eficacia:

a) Mejora de la calidad del insecto criado artificialmente e irradiado.

b) Picaduras «estériles», es decir, picaduras que produce en la fruta la hembra esterilizada; estas picaduras no dan lugar a larvas, pero deterioran el aspecto externo de la fruta y pueden constituir un serio inconveniente. En años anteriores ya se observó que las picaduras «estériles» no constituyen problema en ninguna especie de cítricos, pero sí pueden constituirlo en algunas variedades de albaricoque o melocotón.

Con objeto de estudiar algunos factores de estos dos aspectos, se montaron en una explotación frutícola de la provincia de Murcia un total de veinte jaulas de armadura metálica y malla de plástico, cubriendo cada una de ellas un árbol frutal (melocotón). Dentro de estas jaulas se efectuaron dos tipos de ensayos:

a) Suelta de insectos irradiados a dosis de 9 krad y 7 krad e insectos fértiles en proporción de 10:1 y 50:1. Estos ensayos tienen por objeto comprobar en el campo la eficacia de una y otra dosis de irradiación. Los experimentos se están realizando actualmente y aún no hay datos concluyentes.

b) Suelta de insectos estériles (5000 por semana y jaula en un ensayo y 50 000 por semana y jaula, en otro), sobre distintas variedades de melocotones. En todos los casos, se observan numerosas picaduras «estériles». Sin embargo, en ciertas variedades (Collins, Dixired, Cardinal), las picaduras no son prácticamente visibles, por lo que no afectan al valor comercial del fruto.

En otras (Jerónimo, paraguaya), son muy visibles y constituyen un serio inconveniente. En todos los casos, el número de picaduras está en relación directa con el número de insectos soltados.

Actualmente, la capacidad de producción del laboratorio es superior a 1 millón de pupas/día. Con esta capacidad de producción y los datos que se están obteniendo este año, se proyecta realizar en 1971 un experimento sobre superficies más extensas.

DISCUSSION

I. A. KANSU: The puncturing problem appears to be a very important one and would also be encountered in my own country, Turkey. I believe that, in order to overcome it, release must be carried out earlier – if possible in May or June instead of July. What is your opinion?

L. MELLADO: Early releases, when the fruit is not yet receptive, would solve the problem, as you suggest. However, it is not always possible to apply this solution.

L. E. LaCHANCE: With regard to the problem of 'sterile stings' by released females, I believe it remains to be determined how serious this problem would be if the females were not caged over the trees.

L. MELLADO: The problem is certainly more serious when the females are caged over the trees. The objective of the experiment was to create the worst possible conditions in order to determine the maximum possible damage.

M. FRIED: You mentioned that different varieties of peach were affected differently. Is there any generalization that you can make, such as earlier varieties being affected differently from later ones, and so on?

L. MELLADO: Unfortunately, we found that such a generalization is not possible, at all events not on the basis of data we have at present. We found that both early and late varieties may or may not be affected by 'sterile punctures'.

M. J. WHITTEN: In the light of the possibility of serious damage caused by punctures of the sterile female, have you given consideration to the possible use of genetic techniques for the rapid and automated sexing in the pupal stage of the Mediterranean fruit fly?

Dealing with a related problem, working with the Australian sheep blowfly Lucilia cuprina, we isolated a pupal colour mutant controlled by a gene on chromosome 2. We coupled this gene to the male-determining chromosome by means of a translocation. The absence of crossing-over in males of higher diptera make this attachment very stable. Our workshop then built a machine which sorted the pupae on the basis of colour – males had black pupae and females brown – providing a rapid and accurate means of separating the sexes at this early stage.

Any mutant which expresses itself in the pupal stage can be sex limited in this manner in the higher diptera. However, it should be realized that these males will have a reduced fertility of 50% because of the translocation heterozygote. This may be an obstacle to mass rearing in some instances.

With the sheep blowfly our object was not simply to eliminate a troublesome sterile female (in fact she is not troublesome) but to allow us to find ways of giving her a positive role in the sterile insect program. This work

has been described in my article "Genetics of pests in their management" in "Concepts in Pest Management", edited by F. Guthrie and R. Rabb, published by North Carolina State University Press, Raleigh, N. C. (1970).

L. MELLADO: Your suggestion is very valuable. An easy method for sexing the fruit fly in the pupal stage would certainly solve the problem of 'sterile stings'.

R. PAL: May I please ask one general question? How was the release ratio determined in these control and eradication programs in the absence of any knowledge of the size of the natural target population of fruit flies?

L. MELLADO: We did not determine the release ratio. However, the positive results of the experiment in Alhama show that a knowledge of the size of the normal target population is not essential if enough sterile insects are released. I agree, however, that this knowledge is highly desirable for large-scale experiments.

R. PAL: Mr. Taylor, may I put the same question to you?

E. A. TAYLOR (Chairman): In the case of the melon fly, we experimented with a 100:1 ratio of overflooding, and the situation improved week by week. In some other programs that we have carried out infested spots developed, the ratio of sterile to wild flies declined and the program had to be abandoned. A ratio of 50:1 to 100:1 over the entire area is a good starting point.

D. WALKER: What would be the relative advantage, Mr. Mellado, of releasing only one sex, particularly with respect to the avoidance of intermating between sterile males and sterile females?

L. MELLADO: I think there would be no significant differences in the results if only males were released.

G. H. S. HOOPER: In laboratory experiments with cage populations of approximately 400 flies, we have found that the addition of sterilized females to untreated males and females to give a ratio of 10:1:1 has little effect on the resulting egg hatch. When both sterilized males and females are added to untreated males and females to give a ratio of 10:10:1:1 the egg hatch is equivalent to that obtained by adding sterile males only. Thus our experiments indicate that the addition of sterile females of Ceratitis capitata neither enhances nor reduces the control provided by sterile males alone.

J. W. WRIGHT: As a general comment, it seems desirable that greater attention be given to ecological studies involving the establishment of accurate estimates of population numbers. If this is not done, and releases are made on the basis of only rough figures, we shall never know, or never be able to prove, the reasons for success or failure. Absolute numbers were accurately determined in those programs that have been successful in the past and sould be the foundation on which future studies are built.

E. A. TAYLOR(Chairman): I agree that more and better ecological studies are needed.

POSSIBILITES DE LUTTE INTEGREE CONTRE Dacus oleae (GMELIN) AU MOYEN DE METHODES AUTOCIDES ET CHIMIQUES

P. S. ORPHANIDIS, P.E. KALMOUKOS
Institut phytopathologique Benaki,
Kiphissia,
Athènes, Grèce

Abstract — Résumé

POSSIBILITIES OF INTEGRATED CONTROL OF Dacus oleae (GMELIN) BY AUTOCIDAL AND CHEMICAL METHODS.

As is well known, the prerequisite for any integrated control of the olive Dacus by autocidal and chemical methods is that satisfactory solutions be found first to a number of questions relating to these techniques. In this study, the authors seek to summarize the main results of research carried out in the last twelve years (1957-1969) on different questions directly or indirectly connected with the integrated control of the olive-tree Dacus by autocidal and chemical methods. More particularly, the research in question has dealt with the action of chemical sterilants, ionizing radiation, insecticides, attractants and repellants; with questions of toxic residues and other secondary effects; and lastly, with some questions relating to the biology and ecology of the olive fly which are directly connected with the problem of controlling this species by combined methods.

POSSIBILITES DE LUTTE INTEGREE CONTRE Dacus oleae (GMELIN) AU MOYEN DE METHODES AUTOCIDES ET CHIMIQUES.

Toute lutte intégrée contre le Dacus de l'olive au moyen de méthodes autocides et chimiques présuppose, comme on sait, la solution préalable d'une série de problèmes relatifs à ces méthodes. Dans ce travail, les auteurs passent en revue les principaux résultats des recherches effectuées au cours des douze dernières années (1957-1969) sur différentes questions concernant directement ou indirectement la lutte intégrée contre le Dacus de l'olive au moyen de méthodes autocides et chimiques. Ces recherches ont trait plus particulièrement à l'action des substances chimiostérilisantes, des rayonnements ionisants, des insecticides et des substances attractives ou répulsives, aux problèmes posés par les résidus toxiques et autres effets secondaires, enfin à certains aspects de la biologie et de l'écologie de la mouche de l'olive se rattachant directement au problème de la lutte contre cette espèce au moyen de méthodes combinées.

INTRODUCTION

La stérilisation d'une certaine espèce d'insectes en laboratoire, à l'aide de radiations ou de produits chimiostérilisants, conduit parfois à conclure prématurément que nous sommes en possession d'une nouvelle méthode de lutte efficace, fondée sur la stérilisation de l'insecte. En réalité, une constatation ainsi établie en laboratoire, en dépit de l'intérêt théorique manifeste qu'elle présente, ne constitue qu'un premier pas dans la voie de la lutte contre cet insecte au moyen de méthodes autocides.

Pour étudier l'efficacité et les possibilités d'application pratique d'une méthode de lutte autocide s'appuyant soit sur la stérilisation d'insectes d'élevage au moyen de radiations ou de facteurs chimiostérilisants, soit sur la chimiostérilisation de populations naturelles, il est indispensable de résoudre préalablement différents problèmes en liaison directe ou indirecte avec la question.

La solution de ces problèmes est absolument indispensable lorsqu'il y a lieu d'appliquer les méthodes autocides, non pas isolément, mais, comme c'est l'usage, en combinaison avec d'autres méthodes, dans le cadre d'une lutte intégrée contre le Dacus de l'olive.

Le présent travail a pour but de présenter en un tableau d'ensemble les principaux résultats de diverses recherches accomplies pendant la dernière période de douze ans (1957-1969) sur l'action exercée sur le Dacus de l'olive par certains produits chimiostérilisants, par les radiations, les insecticides, les substances attractives ou répulsives, ainsi que sur certaines questions biologiques et écologiques relatives à cette espèce, questions étroitement rattachées, directement ou indirectement, à toute application future d'une lutte intégrée au moyen de méthodes chimiques et autocides.

Nous aimons à croire qu'un tel exposé, sommaire mais synthétique, nous permettra de mieux apprécier les résultats obtenus jusqu'à présent et de mieux discerner les possibilités que présente pour l'avenir une lutte intégrée contre le Dacus au moyen de ces méthodes.

1. RECHERCHES SUR DES AGENTS DE CHIMIOTROPISME POSITIF DU Dacus

Le repérage de substances fortement attractives pour le Dacus de l'olive présente un grand intérêt dans toute application de méthodes autocides. En effet, l'emploi de substances de cette nature sous forme d'appâts peut occasionner une diminution considérable de la densité de la population naturelle et, conséquemment, la réduction au minimum possible du nombre d'individus stériles nécessaires pour être lâchés sur la surface d'une oliveraie donnée. Cette réduction du nombre d'individus lâchés, qui se répercute directement sur les dimensions de l'élevage et, par suite, sur le coût de la campagne dacicide, revêt une importance spéciale si l'on considère que la proportion entre individus stériles et normaux est ordinairement évaluée [34], en ce qui concerne le Dacus de l'olive, à plus de 4:1.

Mais, alors même que la stérilisation serait faite, non pas sur des insectes d'élevage mais sur la population naturelle même, l'application de substances attractives sous forme d'appâts en vue de réduire cette population naturelle ne serait pas de moindre importance.

Les principales recherches effectuées dans l'espace des douze dernières années pour découvrir des substances attractives pour le Dacus ont été les suivantes:

A la suite d'expériences effectuées en 1957 [21, 22] il a été possible de constater la puissante action attractive exercée sur le Dacus par différentes substances produites par hydrolyse de protéines (staley 2, staley 7, caséine, levure de bière). A titre d'indication on notera que le rapport d'attractivité entre l'hydrolysat de protéines staley 7 et la mélasse – seule substance attractive largement employée à l'époque dans la méthode de Cillis Berlese – a été dans ces expériences de 47 à 1 adultes de Dacus.

Des recherches ultérieures, au cours desquelles on a employé des solutions aqueuses de 27 acides aminés différents et de leurs sels, ont montré que la forte action attractive des hydrolysats de protéines pourrait être attribuée à la présence de différents acides aminés [42]. Les nombreuses

recherches parallèlement entreprises pour repérer d'autres substances attractives du Dacus parmi différents groupes de substances chimiques (huiles essentielles, alcools, résines, esters, aldéhydes, huiles végétales) ont abouti à des résultats négatifs [44]. Tout aussi négatifs ont été, au point de vue du chimiotropisme positif, les résultats obtenus avec certains produits de synthèse [25, 56] (trimedlure, siglure, medlure, cue-lure, eugénole, méthyleugénole, isoeugénole, méthylisoeugénole, huile d'angélique, huile Citronella) dont la puissante action attractive sur d'autres espèces de diptères était déjà depuis longtemps connue [4, 8, 11, 65, 66].

Ainsi constatée, la puissante attractivité exercée par les hydrolysats de protéines sur les adultes de Dacus a conduit à l'emploi à une échelle de plus en plus grande des principales d'entre ces protéines (staley 7, protéine Rhodia, cératène, atropaz, zitan 85, tarmon 127, 64/3442, PLIH, 66/4180, etc.), en combinaison avec des insecticides organophosphorés dans une nouvelle méthode de lutte préventive modifée. La protection satisfaisante de l'oléiculture assurée par l'application d'appâts de protéines [28, 58, 64], par pulvérisations à faible volume (10-40 litres/ha), d'abord à partir du sol puis à partir de l'air [59, 60], a encouragé pendant ces dernières années l'extension de cette méthode, qui réduit avec une extrême rapidité la densité de la population de Dacus adultes, sur des superficies correspondant à environ cinquante millions d'oliviers par an en Grèce.

Les études effectuées et les perspectives de succès qui se dessinent dans l'application de la nouvelle technique de pulvérisation à très faible volume (1 litre/ha) montrent [43, 49] l'importance que ces résultats peuvent présenter, au point de vue de la diminution de la population naturelle du Dacus, dans l'application d'un programme de lutte intégrée contre cet insecte au moyen de méthodes chimiques et autocides.

2. RECHERCHES SUR DES AGENTS DE CHIMIOTROPISME NEGATIF DU Dacus

Il a été constaté pendant ces dernières années que les huiles essentielles et certaines autres substances aromatiques constituent pour le Dacus de l'olive des agents de chimiotropisme négatif qui contrarient vigoureusement l'action des substances attractives [44, 52].

Ces résultats présentent évidemment un très grand intérêt en ce qui concerne l'application d'un programme de lutte contre le Dacus au moyen de méthodes chimiques et autocides. En effet, certains des agents de chimiotropisme négatif signalés jusqu'à présent se trouvent dans les appâts de Dacus (substances actives d'insecticides, solvants organiques) et agissent dans un sens opposé à l'action des hydrolysats de protéines déjà largement employés dans ces appâts [45, 51].

3. RECHERCHES SUR LA CHIMIOSTERILISATION DU Dacus EN LABORATOIRE

A la suite des travaux effectués dans les années 1962-1963, nous avons pu observer une action stérilisante particulièrement forte exercée sur des adultes de Ceratitis capitata (Wiedemann) – une espèce de la famille des Trypetidæ apparentée au Dacus – par une nourriture contenant trois

différents agents d'alkylation (dérivés d'aziridine) connus sous les noms d'apholate, metepa et tepa [33, 35, 37]. Les résultats obtenus sur Ceratitis ont été postérieurement confirmés par des travaux analogues de Keiser et al. [13] et de Scherney et Haisch [63].

Durant cette même période nos expériences révélèrent une forte action stérilisante de l'agent d'alkylation metepa sur des adultes de Dacus oleæ, tandis que Pelekassis et Mourikis [57] et, plus récemment, d'autres chercheurs [7, 70] signalaient l'action analogue de l'agent tepa. Enfin nos récents travaux dans les conditions de laboratoire ont permis de constater la forte action exercée sur des adultes des deux sexes de la même espèce par un autre agent d'alkylation, l'apholate [47].

Dans les conditions des expériences précitées la stérilisation – toujours plus intense chez les mâles que chez les femelles – était élevée, atteignant même un rapport de 4:1 (80%:20%) entre mâles stérilisés et mâles normaux [34], ce qui concorde avec la proportion indiquée par des chercheurs italiens dans des expériences de stérilisation par les radiations [18].

En ce qui concerne la stérilisation de pupes de Dacus par des agents d'alkylation, elle a été plutôt faible, malgré les fortes concentrations employées [37].

Un des principaux avantages de la chimiostérilisation sur la stérilisation par les radiations consiste, comme on le sait, dans le fait que la stérilisation chimique peut être appliquée, d'après Knipling [15], non seulement à des insectes d'élevage mais aussi à des populations naturelles. Il est évident que cette possibilité présente un intérêt tout spécial pour certaines espèces monophages comme le Dacus de l'olive, dont l'élevage en masse sur substrat artificiel présente encore bien des difficultés.

Malheureusement, toute chimiostérilisation à grande échelle de populations naturelles de Dacus à l'aide des trois puissants agents d'alkylation repérés jusqu'ici doit être présentement exclue pour des raisons purement toxicologiques [10] – à l'exception d'expériences à l'aide de pièges d'autochimiostérilisation – à moins que des méthodes plus sûres et plus simples d'application de ces substances en plein air ne soient mises au point entre-temps.

L'impossibilité d'appliquer à grande échelle à des populations naturelles les agents d'alkylation précités nous a amenés à étudier l'éventualité d'une chimiostérilisation au moyen d'autres substances considérées comme toxicologiquement inoffensives. Parmi les substances de cet ordre, celle qui a été étudiée pour la première fois par Chang et coll. [6] sur Musca domestica, l'hempa (hexaméthylphosphoramide), a montré dans nos expériences une action très forte sur des adultes mâles de Dacus. Parmi les autres substances chimiques que nous avons étudiées ont paru intéressants quelques composés organiques de l'étain, la plupart connus soit par les travaux d'Ascher [1, 2] comme des agents antiappétants, soit par les travaux de Kenaga comme des agents inhibiteurs d'oviposition [14]. On notera que, parmi ces substances, les fongicides connus sous les noms de Du-Ter[1] et Decafentin[2], administrés per os, ont fortement entravé le

[1] Triphénylhydroxyde d'étain de la Maison Philips-Duphar, Pays-Bas.

[2] (Décyltriphénylphosphonium)-bromochlortriphénylstannate de la Maison CELA/Landwirtschaftliche Chemikalien GmbH, Ingelheim, Rép. féd. d'Allemagne. Le Decafentin n'a été étudié que sur Ceratitis capitata (Wiedemann) (les résultats obtenus par le premier des deux auteurs du présent travail n'ont pas été publiés).

phénomène d'oviposition chez les femelles aussi bien de Dacus que de Ceratitis capitata (Wiedemann) [47].

Ajoutons pour terminer que, d'après les recherches expérimentales exposées ci-dessus, la question de la dose efficace à chaque fois ne constitue pas une notion absolue, comme le dit Keiser [13], mais qu'elle est fonction, entre autres, du mode d'administration de la substance. Il semble, par exemple, que le mélange d'agents d'alkylation avec des protéines hydrolysées ait pour effet d'augmenter aussi bien la limite de tolérance que la dose efficace minimale.

4. RECHERCHES SUR LA CHIMIOSTERILISATION DU Dacus EN PLEIN AIR

Pour étudier les possibilités de chimiostérilisation de populations naturelles de Dacus en plein air au moyen d'agents d'alkylation (apholate), nous avons procédé en 1964 à une expérience spéciale sur une oliveraie isolée de l'île de Lesbos, comprenant environ 2000 oliviers [38, 41]. Nous avons employé pour cette expérience des pièges spéciaux d'autochimiostérilisation; les résultats étaient constamment contrôlés par l'observation des pourcentages d'éclosion, tant dans l'aire expérimentale que chez le témoin. Le fait que les pourcentages d'éclosion se sont maintenus constamment, pendant deux mois et demi, à des niveaux nettement inférieurs aux pourcentages correspondants des témoins (45, 3 ± 14, 5 contre 89, 7 ± 5, 3), malgré l'exiguïté de l'oliveraie expérimentale et l'insuffisance de son isolement, dénote clairement croyons-nous l'importance toute spéciale que pourrait présenter à l'avenir la chimiostérilisation de la population naturelle du Dacus de l'olive pour la lutte contre cet insecte.

5. RECHERCHES SUR LA STERILISATION DU Dacus PAR LES RADIATIONS

La méthode de stérilisation par les radiations constitue, comme on le sait, avec la méthode de chimiostérilisation l'une des deux méthodes fondamentales dont nous disposons pour la stérilisation des insectes et, partant, du Dacus de l'olive.

Les travaux effectués pour la première fois en Italie en 1960 par Melis et Baccetti [18] ont permis de constater qu'il est possible de stériliser des pupes de Dacus 3 à 7 jours avant leur éclosion à l'aide des rayonnements gamma (8000 - 12 000 R) émis par une source de cobalt (^{60}Co). Ces travaux ont montré que, avec un rapport de 4:1 entre mâles stérilisés et mâles normaux, le résultat était satisfaisant.

D'autres recherches effectuées plus tard en Grèce [67, 70] ont de nouveau mis en évidence la forte influence des rayonnements gamma, tant sur les pupes que sur les adultes et les larves de Dacus. Tzanakakis a récemment étudié l'influence de l'âge sur la résistance aux radiations et, de plus, les répercussions de ces dernières sur la compétitivité des mâles [71].

6. RECHERCHES SUR DES INSECTICIDES CONTRE LE Dacus DE L'OLIVE

Le repérage de puissants insecticides contre le Dacus de l'olive présente, comme c'est aussi le cas pour les substances attractives adéquates, un grand intérêt pour l'application de méthodes autocides dans le cadre d'une méthode de lutte intégrée contre cet insecte. Cet intérêt réside dans la diminution de la population naturelle du Dacus obtenue à l'aide de ces insecticides et, par conséquent, dans l'augmentation du rapport entre individus stériles et normaux.

Les recherches effectuées pendant les douze dernières années ont porté non seulement sur l'action exercée sur les adultes et les larves du Dacus par divers insecticides organophosphorés et carbamiques [3, 12, 17, 23, 45, 53], mais aussi sur d'autres questions relatives aux effets secondaires des insecticides, comme leur action toxique sur l'olivier [27], la présence de résidus toxiques dans l'huile et les olives [24, 48] ou leur effet sur les espèces utiles vivant dans les oliveraies [29, 30, 31, 54]. Ces recherches, qui ont permis d'étudier parmi les principales propriétés physiques et biologiques des insecticides celles qui sont les plus déterminantes dans leurs effets secondaires (coefficient de répartition, rémanence, spectre d'action), ont déjà conduit au choix des insecticides appropriés, séparément pour chacune des deux méthodes chimiques actuellement en usage dans la lutte contre le Dacus, soit la méthode préventive et la méthode curative.

La diminution des quantités de substance active appliquées en Grèce par unité de superficie du sol et par pulvérisation (100 à 150 g/ha au lieu de 600 à 900 g/ha dans d'autres pays) a eu pour effet, non seulement de réduire le coût de la lutte chimique contre le Dacus et ses répercussions sur l'équilibre biologique [40], mais aussi d'atténuer l'importance du problème, autrefois extrêmement aigu, des résidus dans l'huile et dans l'olive et des problèmes de toxicité. Il est évident que les résultats des recherches en question sont particulièrement utiles en cas d'application d'un programme de lutte intégrée contre le Dacus au moyen de méthodes chimiques et autocides.

7. RECHERCHES SUR LA BIOLOGIE ET L'ECOLOGIE DU Dacus

a) Recherches sur la densité de la population et la dispersion des adultes

Pour l'application efficace de toute méthode autocide il est particulièrement important que, au moment où les individus stériles sont lâchés ou pendant l'application des chimiostérilisants en plein air, la population naturelle se trouve à des niveaux aussi bas que possible.

En conséquence, outre la diminution de la population naturelle, obtenue comme on vient de le voir par application de méthodes chimiques (substances attractives, insecticides), il y a lieu de déterminer les courbes de densité relative de la population et de fixer en conséquence les dates auxquelles il convient le mieux de lâcher les individus stériles. On voit l'intérêt majeur que présente le choix et l'application de méthodes adéquates pour l'évaluation de la densité relative de la population. C'est pourquoi il y a lieu de considérer à cet égard comme une borne l'année 1953, au cours de laquelle il a été établi par Kalopissis et al. que le mode classique

d'évaluation au moyen de pièges de la densité de la population de Dacus aboutit à des résultats trompeurs, étant donné que le nombre de captures dépend de l'humidité relative du milieu [12], et plus spécialement de la différence entre l'humidité relative existant à chaque fois à l'intérieur des pièges et à l'extérieur [26].

Cette précieuse observation a conduit par la suite à évaluer la densité de la population au moyen d'une autre méthode, consistant à appliquer des pulvérisations de couverture (sondages) à l'aide d'un insecticide déterminé et à compter par la suite le nombre d'adultes tombés en 48 h sur des collecteurs en toile de 36 m^2, étendus sous les arbres.

Par cette méthode il a été démontré qu'en Grèce la population maximale est d'habitude observée en avril-mai; une brusque diminution intervient ensuite, pendant les mois secs et chauds de juillet-août, puis une remontée rapide exponentielle jusqu'à fin novembre/mi-décembre, et finalement une baisse constante jusqu'en février [23, 32].

En ce qui concerne la distance de dispersion des adultes, qui présente aussi un intérêt notable pour l'application de méthodes autocides dans la lutte contre le Dacus, les recherches de Pélékassis [55] et les nôtres [32] ont montré qu'elle peut atteindre 2 à 4 kilomètres au moins.

Cependant, en ce qui concerne aussi bien la distance maximale de dispersion des adultes que l'évaluation de la densité absolue de la population (qui est particulièrement importante pour le calcul du nombre d'individus stériles à lâcher) des lacunes importantes subsistent encore, qui exigent de nouvelles recherches.

b) Elevage de Dacus sur substrat artificiel

La possibilité d'un élevage continu de Dacus de l'olive présente de l'intérêt non seulement pour la production d'insectes à stériliser, mais encore parce qu'elle constitue l'unique moyen de procéder à une investigation approfondie de la biologie de l'espèce et du mode d'action des insecticides, des répulsifs, des attractifs et des chimiostérilisants.

Les premières tentatives en vue de développer une méthode d'élevage ont été d'une part celle de Sakantanis en 1953 sur substrat naturel [61], et d'autre part celle de Moore [19] sur substrat artificiel. Depuis lors, notamment pendant la dernière décennie, vu l'importance de la question pour une méthode autocide, les efforts se sont multipliés [5, 9, 16, 20, 36, 39, 46, 50, 62, 68, 69, 71], de sorte que l'élevage de l'espèce sur substrat artificiel est déjà praticable sans discontinuité pendant une longue série de générations[3].

Néanmoins, malgré les progrès incontestables ainsi réalisés en matière d'élevage de Dacus, la question ne peut être encore considérée comme suffisamment élucidée, d'une part parce que le problème de l'antisepsie n'a pas encore reçu de solution entièrement satisfaisante, d'autre part à cause de l'instabilité des rendements, de la main-d'œuvre requise et en général du coût de l'opération.

---

[3] Nous avons réussi jusqu'ici (mai 1970) dans notre laboratoire l'élevage de 21 générations de Dacus sur substrat artificiel. Un nombre encore plus élevé a été obtenu par Tzanakakis et les autres chercheurs du Centre Democritos.

Pour tous ces motifs, il est essentiel, en vue de l'application de méthodes autocides dans la lutte contre le Dacus, de trouver des agents chimiostérilisants toxiquement inoffensifs pour l'homme et les animaux à sang chaud, susceptibles d'être appliqués à la population naturelle du Dacus sans aucun recours à l'élevage artificiel.

CONCLUSIONS

Des progrès remarquables ont été réalisés au cours des douze dernières années (1957-1969) dans différents secteurs de recherche se rattachant directement ou indirectement à une lutte intégrée contre le Dacus oleæ (Gmelin) au moyen de méthodes chimiques et autocides.

Néanmoins, à l'heure actuelle l'application d'une méthode de lutte intégrée se heurte principalement à deux obstacles: en ce qui concerne la stérilisation d'insectes d'élevage au moyen de radiations ou de chimiostérilisants, il n'existe pas encore de méthode simple, peu coûteuse et facilement applicable d'élevage sur substrat artificiel; et, pour ce qui est de la stérilisation de populations naturelles au moyen de chimiostérilisants, il n'existe pas de substances chimiques toxiquement inoffensives pour l'homme pouvant être appliquées facilement à ciel ouvert.

Les données exposées dans ce travail indiquent cependant que la solution de ces deux aspects du problème ne se fera pas trop attendre.

REFERENCES

[1] ASCHER, K.R.S., A review of chemosterilants and oviposition inhibitors in insects, Wld. Rev. Pest Control, 3 1 (1964) 7-26.

[2] ASCHER, K.R.S., MOSCOWITZ, J., Fentin (triphenyltins) has an antifeedant effect for house fly larvæ, Int. Pest Control 10 (1968) 10-13.

[3] AYOUTANTIS, A., PELEKASSIS, C., ARGYRIOU, L., MOURIKIS, P., TSAKAS, L., Rapport sur les travaux expérimentaux de lutte contre le Dacus à Roviès (Eubée), pendant l'année 1953, Annls Inst. phytopath. Benaki 8 2 (1954) 7-75.

[4] BEROZA, M., ALEXANDER, B.H., STEINER, L.F., MITCHELL, W.C., MIYASHITA, D.H., New synthetic lures for the male Melon Fly, Science, N.Y. 131 (1960) 1044-45.

[5] CAVALLORO, R., Orientamenti sull'elevamento permanente di Dacus oleæ Gmelin (Diptera, Trypetidæ) in laboratorio, Redia L (1967) 337-44.

[6] CHANG, S.C., TERRY, P.H., BORKOVEC, A.B., Insect chemosterilants with low toxicity for mammals, Science, N.Y. 144 3614 (1964) 57-58.

[7] FYTIZAS, E., Action du Tepa sur les adultes de Dacus oleæ Gmel. (Diptera, Tephritidæ), en fonction des quantités du chimiostérilisant utilisé, Annls Inst. phytopath. Benaki (Nouvelle Série) 8 (1967) 32-46.

[8] GERTLER, S.I., STEINER, L.F., MITCHELL, W.C., BARTHELL, W.F., Esters of 6-methyl-3 cycloexene 1-carboxylic acid as attractants for the Mediterranean Fruit fly, Agric. Food Chem. 6 8 (1958) 592.

[9] HAGEN, K.S., SANTAS, L., TSECOURAS, A., « A technique of culturing the olive fly, Dacus oleæ Gmel., on synthetic media under xenic conditions», Radiation and Radioisotopes Applied to Insects of Agricultural Importance (C.r. Coll. Athènes, 1963) AIEA, Vienne (1963) 333.

[10] HAYES, W.J., « Toxicological aspects of chemosterilants», Principles of Insect Chemosterilisation (La BRECQUE, G.C., SMITH, C.N., Eds), North Holland Publishing Company, Amsterdam (1968).

[11] HOWLETT, F.M., The effect of oil citronella on two species of Dacus, Trans. ent. Soc. Lond. (1912) 412-18.

[12] KALOPISSIS, I., KARAMANOS, G., ORPHANIDIS, P., VRETTACOS, L., MOROS, H., PAPOUTSIS, E., L'expérience antidacique de Kirra-Itéa en 1953, Athènes (1954) (en grec).

[13] KEISER, J., STEINER, L.F., KAMASAKI, H., Effect of chemosterilants against the Oriental fruit fly, Melon fly and Mediterranean fruit fly, J. econ. Ent. 58 (1965) 682-85.

[14] KENAGA, E.E., Triphenyltin compounds as insect reproduction inhibitors, J. econ. Ent. 58 (1965) 4-8.
[15] KNIPLING, E.F., Potentialities and progress in the development of chemosterilants for insect control, J. econ. Ent. 55 (1962) 782-86.
[16] LOPEZ, E.D., Development of Olive Fruit Fly Mass Production Methods, Report to the Government of Greece (1964) 1-7.
[17] MARTELLI, G.M., Prime prove con gli esteri fosforici contro la mosca delle olive, Agr. Pugliese III (1950) 7-9.
[18] MELIS, A., BACCETTI, B., Metodi di lotta vecchie e nuovi sperimentali contro i principali fitofagi dell'olivo in Toscana nel 1960, Redia, Seconda Serie XLV (1960) 3-27.
[19] MOORE, I., A method for artificially culturing the olive fly (Dacus oleæ Gmel.) under aseptic conditions, Ktavim, Beit-Dagan 9 3-4 (1959) 295-96.
[20] MOORE, I., in: Advances in Insect Population Control (Report Panel Vienna, July 1964), IAEA, Vienna (1965).
[21] ORPHANIDIS, P.S., Recherches expérimentales sur la lutte contre le Dacus de l'olive (effectuées durant l'année 1957), Rapport soumis à la 3e réunion de la FAO sur la lutte contre la mouche de l'olive, Florence, 25-30 novembre 1957 (polycopiés).
[22] ORPHANIDIS, P.S., DANIELIDOU, R.K., ALEXOPOULOU, P.S., TSAKMAKIS, A.A., KARAYANNIS, G.B., Recherches expérimentales sur l'attractivité exercée par certaines substances protéinées sur le Dacus adulte de l'olive, Annls Inst. phytopath. Benaki (Nouvelle Série) 1 (1958) 171-98.
[23] ORPHANIDIS, P.S., KARAYANNIS, G.B., ALEXOPOULOU, P.S., TSAKMAKIS, A.A., DANIELIDOU, R.K., Expériences concernant l'efficacité de certains insecticides phosphorés sur le Dacus de l'olive effectuées en 1957, Annls Inst. phytopath. Benaki (Nouvelle Série) 1 (1958) 229-89.
[24] ORPHANIDIS, P.S., PHYTIZAS, E.A., VASAKAS, D.Th., Résidus de Rogor, Dimécron, Ekatin, Ekatin M, Dipterex, Diazinon et Parathion dans l'huile et dans l'olive, Annls Inst. phytopath. Benaki (Nouvelle Série) 2 (1959) 102-17.
[25] ORPHANIDIS, P.S., SOULTONOPOULOS, C.D., DANIELIDOU-PHYTIZA, R.E., Attractivité exercée sur le Dacus oleæ Gmel. par diverses substances organiques non protéinées, Annls. Inst. phytopath. Benaki (Nouvelle Série) 4 (1962) 29-38.
[26] ORPHANIDIS, P.S., DANIELIDOU-PHYTIZA, R.E., SOULTANOPOULOS, C.D., Contribution à l'étude de l'influence de l'eau sur l'intensité du phénomène de l'attraction d'adultes du Dacus oleæ Gmel. par diverses substances attractives, Annls Inst. phytopath. Benaki (Nouvelle Série) 4 (1962) 39-51.
[27] ORPHANIDIS, P.S., SOULTANOPOULOS, C.D., Action toxique exercée sur l'olive par divers pesticides et substances attractives (Recherches sur quelques facteurs influençant ce phénomène), Annls Inst. phytopath. Benaki (Nouvelle Série) 4 (1962) 89-102.
[28] ORPHANIDIS, P.S., SOULTANOPOULOS, C.D., Olive fruit fly control with proteinaceous bait-sprays (Comparative results to those obtained with arsenated molasse baits), Annls Inst. phytopath. Benaki (Nouvelle Série) 4 (1962) 103-11.
[29] ORPHANIDIS, P.S., SOULTANOPOULOS, C.D., Observations préliminaires sur la différenciation de l'action exercée par certains insecticides polyvalents en fonction de la quantité de substance active appliquée et du temps écoulé depuis la pulvérisation, Annls Inst. phytopath. Benaki (Nouvelle Série) 4 (1962) 125-37.
[30] ORPHANIDIS, P.S., SOULTANOPOULOS, C.D., Action rémanente de certains insecticides organophosphorés sur divers insectes des oliveraies, Annls Inst. phytopath. Benaki (Nouvelle Série) 4 (1962) 138-47.
[31] ORPHANIDIS, P.S., SOULTANOPOULOS, C.D., Observations préliminaires sur les courbes de densité de la population de certains insectes vivant dans les oliveraies en 1961 (Corrélation de ces courbes avec l'époque des traitements aux insecticides organophosphorés), Annls Inst. phytopath. Benaki (Nouvelle Série) 4 (1962) 148-54.
[32] ORPHANIDIS, P.S., SOULTANOPOULOS, C.D., KARANDINOS, M.G., Preliminary experiments with radioactive phosphorus (P^{32}) on the dispersion of olive fruit fly (Dacus oleæ Gmel.), Annls Inst. phytopath. Benaki (Nouvelle Série) 4 (1962) 155-82.
[33] ORPHANIDIS, P.S., Recherches en laboratoire sur la stérilisation d'adultes de Ceratitis capitata Wied. au moyen des substances stérilisantes, I. Expériences avec l'Apholate, Annls Inst. phytopath. Benaki (Nouvelle Série) 5 (1963) 260-87.
[34] ORPHANIDIS, P.S., PATSAKOS, P.G., Recherches en laboratoire sur la stérilisation d'adultes de Dacus oleæ Gmel. au moyen de Metaphoxide et d'Aphoxide (Comparaison avec les résultats relatifs obtenus sur le Ceratitis capitata Wied.), Annls Inst. phytopath. Benaki (Nouvelle Série) 5 (1963) 305-22.
[35] ORPHANIDIS, P.S., SOULTANOPOULOS, C.D., KARANDINOS, M.G., Recherches en laboratoire sur la stérilisation d'adultes de Ceratitis capitata Wied. au moyen de substances stérilisantes, II. Expériences au moyen de Metaphoxide, d'Aphoxide et de chlorure de cuivre, Annls Inst. phytopath. Benaki (Nouvelle Série) 5 (1963) 323-31.

[36] ORPHANIDIS, P.S., SOULTANOPOULOS, C.D., PATSAKOS, P.G., Contribution à l'étude d'une méthode d'élevage du Dacus oleæ Gmel. sur substrat artificiel. Rapport à la 5e réunion de la FAO sur la lutte contre la mouche de l'olive, Lisbonne, 20-27 mai 1963.

[37] ORPHANIDIS, P.S., « Stérilisation en laboratoire de la Ceratitis capitata Wied. et du Dacus oleæ Gmel. au moyen d'aziridines, acaricides, fongicides et de sels minéraux», C.r. 2e Congrès Int. des antiparasitaires, Naples, 15-17 mars 1965.

[38] ORPHANIDIS, P.S., PATSAKOS, P.G., KALMOYKOS, P.E., Rapport préliminaire sur une expérience de stérilisation d'adultes du Dacus oleæ Gmel. en plein champ, Meded. LandbHoogesch. OpzoekStns Gent XXX 3 (1965) 1836-45.

[39] ORPHANIDIS, P.S., Rapport sommaire sur les recherches phytopharmaceutiques relatives à la lutte contre le Dacus et les autres ennemis de l'olivier effectuées en Grèce pendant 1963-1965, Rapport présenté à la 6e réunion de la FAO sur la lutte contre la mouche de l'olive, Madrid, 17-24 mai 1965 (polycopiés).

[40] ORPHANIDIS, P.S., « Aperçu synoptique sur les possibilités, les perspectives et les effets secondaires des différentes méthodes appliquées ou proposées pour la lutte contre la mouche des olives (Dacus oleæ Gmel.)», C.r. 1ères Journées de phytiatrie et de phytopharmacie circum-méditerranéennes, Marseille, 13-15 septembre 1965.

[41] ORPHANIDIS, P.S., PATSAKOS, P.G., KALMOUKOS, P.E., Expérience préliminaire en plein champ sur la chimiostérilisation d'adultes du Dacus oleæ Gmel, Annls Inst. phytopath. Benaki (Nouvelle Série) 7 (1966) 177-90.

[42] ORPHANIDIS, P.S., KALMOUKOS, P E., Les acides aminés et leurs sels en tant que facteurs de chimiotropisme positif des adultes du Dacus oleæ (Gmel.), Meded. Rijksfac. LandbWetensch. Gent XXXIII 3 (1968) 937-43.

[43] ORPHANIDIS, P.S., PELEKASSIS, C.E.D., Preliminary experiments for the control of the olive fruit fly (Dacus oleæ (Gmel.)), with ultra-low-volume technical Malathion in coverage or bait-sprays aerial applications, conducted in Greece during 1965-1966, Annls Inst. phytopath, Benaki (Nouvelle Série) 8 (1968) 102-22.

[44] ORPHANIDIS, P.S., KALMOUKOS, P.E., PATSAKOS, P.G., Chimiotropisme positif et négatif envers les acides aminés, huiles essentielles et autres substances chimiques, des adultes de la mouche de l'olive (Expériences de plein-air, 1966-1967), Annls Inst. phytopath. Benaki (Nouvelle Série) 8 (1968) 199-210.

[45] ORPHANIDIS, P.S., KALMOUKOS, P.E., BETZIOS, B.Ch., Les insecticides carbamiques par comparaison aux insecticides organophosphorés dans les appâts d'hydrolysats de protéines pour la lutte contre la mouche de l'olive (Dacus oleæ (Gmel.)), Z. angew. Ent. 63 (1969) 389-405.

[46] ORPHANIDIS, P.S., PETSIKOU, N., PATSAKOS, P.G., Eléments sommaires concernant un élevage expérimental du Dacus de l'olive sur substrat artificiel, Rapport soumis à la 8e réunion de la FAO sur la lutte contre les parasites de l'olivier, Athènes 8-12 mai 1969 (polycopiés).

[47] ORPHANIDIS, P.S., PATSAKOS, P.G., « Nouvelles expériences sur la stérilisation de deux espèces de Trypetidæ (Dacus oleæ (Gmel.) et Ceratitis capitata Wied.), au moyen de substances chimiques, avec ou sans propriétés d'alkylation», C.r. 3e Congrès int. des antiparasitaires, Milan, 6-8 octobre 1969.

[48] ORPHANIDIS, P.S., Résidus de certains nouveaux insecticides organophosphorés et carbamiques dans l'huile et dans l'olive, Annls Inst. phytopath. Benaki (Nouvelle Série) 9 (1969) 18-19.

[49] ORPHANIDIS, P.S., MOURIKIS, P.A., SOULIOTIS, M.C., Note on the control of Dacus oleæ (Gmel.) with low and ultra-low volume bait sprays, Paper presented to the Joint EPPO/IAAC/CIFAR Conf. on Low and Ultra-Low Volume Applications, Belgrade, Yugoslavia, 28-30 April 1970.

[50] ORPHANIDIS, P.S., PETSIKOU, N.A., PATSAKOS, P.G., Elevage du Dacus oleæ (Gmel.) sur substrat artificiel, Annls Inst. phytopath. Benaki (Nouvelle Série) 9 (1970) 147-69.

[51] ORPHANIDIS, P.S., KALMOUKOS, P.E., Recherche expérimentale sur les facteurs d'efficacité des appâts de la mouche de l'olive, Annls Inst. phytopath. Benaki (Nouvelle Série) 9 (1970) 170-78.

[52] ORPHANIDIS, P.S., KALMOUKOS, P.E., Chimiotropisme négatif d'adultes de Dacus oleæ (Gmel.) envers des huiles essentielles, Annls Inst. phytopath. Benaki (Nouvelle Série) (sous presse).

[53] PELLEGRINI, G., Esperimenti di lotta antidacica svolti nel 1952 con una insetticida a base di p-nitrofenil-dietiltiofosfato, Edit. Montecatini, Milano (1954).

[54] PELEKASSIS, C.E.D., Preliminary note on the influence of Dacus control schedule on natural enemies in the olive-growing area at Rovies, Greece, Paper presented at the 4th FAO Meeting on the Olive-Pests, Tel-Aviv, 5-12 December 1960 (Preprint).

[55] PELEKASSIS, C.E.D., MOURIKIS, P.A., BANTZIOS, D.N., Preliminary studies on the field movement of the olive fruit fly (Dacus oleæ Gmel.) by labelling a natural population with radioactive phosphorus (P^{32}), Annls Inst. phytopath. Benaki (Nouvelle Série) 4 (1962) 170-79.

[56] PELEKASSIS, C.E.D., Field trials with lures and attractants for the olive fruit fly (Dacus oleæ (Gmel.)) conducted in Greece during 1960-1962, Paper presented at the 5th FAO Meeting on the Olive Pests Control, Lisbon, 20-27 May 1963.

[57] PELEKASSIS, C.E.D., MOURIKIS, P.A., Preliminary note on the chemical sterilisation of the olive fruit fly, Paper presented at the 5th FAO Meeting on the Control of the Olive Pests, Lisbon, 20-27 May 1963.
[58] PELEKASSIS, C.E.D., in collaboration with MOURIKIS, P.A., ARGYRIOU, L.C., STAVRAKI-PAVLOPOULOU, H.G., Bait-sprays experiments for the control of the olive fruit fly (Dacus oleæ (Gmel.)) conducted in Greece during 1961-62, Paper presented at the 5th FAO Meeting on the Olive Pests Control, Lisbon, 20-27 May 1963.
[59] PELEKASSIS, C.E.D., ORPHANIDIS, P.S., On the effectiveness of poisoned bait-sprays applied by airplane for the control of the olive fruit fly (Dacus oleæ Gmel.) conducted in Greece during 1964, Paper presented at the 6th FAO Meeting on the Control of the Olive Pests, Madrid, 17-25 May 1965.
[60] PELEKASSIS, C.E.D., ORPHANIDIS, P.S., Preliminary experiments on the effectiveness of poisoned bait-sprays applied by airplane for the control of the olive fruit fly (Dacus oleæ Gmel.) conducted in Greece during 1964, Paper presented at the 6th FAO Meeting on the Control of the Olive Pests, Madrid, 17-25 May 1965.
[61] SAKANTANIS, K.B., Méthode d'élevage en laboratoire de la mouche des olives (Dacus oleæ Gmel.), Revue Path. vég. Ent. agric. Fr. XXXII 4 (1953) 247-57.
[62] SANTAS, L.A., Sur un nouveau milieu nutritif artificiel pour l'élevage des larves du Dacus oleæ Gmel. (Diptera: Trypetidæ), Bull. Bank Agric. 188 8-15 (1965) 1754-61 (en grec).
[63] SCHERNEY, F., HAISCH, A., Zur Zucht und Sterilisation von Schadinsekten, insbesondere der Mittelmeerfruchtfliege Ceratitis capitata Wied., Anz. Schädlingsk. PflSchutz XLI (1968) 7-15.
[64] SOULIOTIS, M.K., « Application d'un appât à hydrolysat de protéine et à Malathion pour la lutte contre la mouche de l'olive durant l'année 1960», C. r. de la décennie 1954-1963 de la Station Phytopathologique de Volos (1966).
[65] STEINER, L.F., Methyl eugenol as an attractant for Oriental Fruit Fly, J. econ. Ent. 45 (1952) 241-47.
[66] STEINER, L.F., MIYASHITA, D., CHRISTENSON, L.D., Angelica oils as Mediterranean Fruit Fly lures, J. econ. Ent. 50 (1952) 505.
[67] THOMOU, H., « Sterilization of Dacus oleæ by gamma radiation», Radiation and Radioisotopes Applied to Insects of Agricultural Importance (C.r. Coll. Athènes, 22-26 avril 1963), AIEA, Vienne (1963) 413.
[68] TZANAKAKIS, M.E., EKONOMOPOULOS, A.P., TSITSIPIS, I.A., Rearing larvæ of the olive fruit fly on artificial food (A progress report, covering the period from March to July 1965), « Democritus» Nuclear Research Center of the Greek Atomic Energy Commission, Athens (1965) (mimeo).
[69] TZANAKAKIS, M.E., EKONOMOPOULOS, A.P., TSITSIPIS, I.A., Improved artificial food media for larvæ of Dacus oleæ Gmel. (Diptera: Tephritidæ) (« Democritus» Nuclear Research Center of the Greek Atomic Energy Commission, Athens), Z. angew. Ent. 58 4-5 (1966) 373-83.
[70] TZANAKAKIS, M.E., Control of the olive fruit fly Dacus oleæ (Gmel.) with radiation or chemical sterilisation procedures, Final technical Report, « Democritus» Nuclear Reserach Center, Aghia Paraskevi, Greece (1967).
[71] TZANAKAKIS, M.E., Experiments with the olive fruit fly at the « Democritus» Nuclear Research Center, Greece, 1967-1968 (A summary of progress), Paper distributed at the 8th FAO Meeting on the Control of Olive Pests and Diseases, Athens, 8-12 May 1969 (Preprint).

DISCUSSION

M.E. TZANAKAKIS: Referring to the title of your paper, I would ask you in what way you think that chemicals (by which I mean insecticides, not chemosterilants) and autocidal control could be integrated or combined against Dacus oleae, for example by using insecticides before releasing sterile flies?

P.S. ORPHANIDIS: An autocidal campaign against Dacus oleae can theoretically be implemented either by sterilization in the laboratory (using irradiation or chemosterilants), followed by release of the sterile individuals, or by sterilization of the wild population.

In the first case chemicals (insecticides or attractants) could be used prior to the release of the sterile flies. This use of chemicals, for the purpose of reducing the density of the wild population, would lead to a considerable reduction in the scale of the rearing necessary and consequently in the cost of the autocidal campaign.

In the second case, i.e. sterilization of the wild populations by means of chemosterilants, it would theoretically be possible to apply insecticides or attractants not only prior to the use of chemosterilants, but also simultaneously with the latter, as Lindquist suggests.

Obviously this presupposes the existence of chemosterilants which are completely safe for man and for warm-blooded animals, and such substances have not so far been developed in practice.

K. KRIMBAS: I would like to suggest another way of combining insecticide control with a sterile-male release programme. First, a strain resistant to a certain insecticide should be created by selection; then a release of sterile males of this particular strain can be combined with sprays of the insecticide in order at the same time to reduce the size of the natural population.

K.S. RAI: Mr. Orphanidis, could you say a little more about the chemical sterilants you have in mind that have no toxicity and that could be used in natural habitats? I should also like to ask whether you draw a line between toxic chemicals and those that are non-toxic, but which are nevertheless mutagenic, hempa for example.

P.S. ORPHANIDIS: As I have just pointed out – and as is stated in the conclusions of my paper – there are for the time being no chemosterilants which are safe and harmless to man and warm-blooded animals and which can be applied in the natural environment for the sterilization of the wild population.

Nevertheless, research carried out in the last few years, mainly in the United States, gives grounds for more optimism in this respect. According to Chang, for example, there are some organic compounds, such as Hempa and Hemel, which have a molecular structure more or less similar to that of tepa and tretamine and which do not possess alkylation properties.

I may also refer to certain organic compounds of tin which have been widely used for a long time as fungicides and which act on insects either, according to Ascher, as antifeedants or, according to the results of work by Kenaga and ourselves, as oviposition inhibiting agents.

We believe that a more systematic and detailed screening of various pesticides from the point of view of their sterilizing properties could lead to a more rapid solution to this problem, which prevents the use of chemosterilants in the field.

C. BORGHI: An 'integrated campaign' against insects could include means other than insecticides and sterilization, possibly other physical means, such as ultra-sonic methods. These are known to have a coagulating effect on organic compounds and could perhaps contribute to control, especially in open areas.

P.S. ORPHANIDIS: I agree with Mr. Borghi that there are other methods, such as biological and physical methods, which could be combined with the autocidal approach.

In my paper, which relates exclusively to Dacus oleae, I only mention the possibilities of an integrated campaign involving the combination of a number of methods (sterilization by irradiation and by chemosterilants, insecticides, agents involving positive or negative chemotropism) on which experimental data are available.

GENETIC CONTROL OF THE EUROPEAN CHERRY FRUIT FLY, Rhagoletis cerasi L.

Progress report on rearing and sterilization

A. HAISCH
Institute for Soil Cultivation, Plant Cultivation and Plant Protection of the Federal State of Bavaria,
Munich, Federal Republic of Germany
and
E.F. BOLLER
Swiss Federal Research Station for Arboriculture, Viticulture and Horticulture,
Wädenswil, Switzerland

Abstract

GENETIC CONTROL OF THE EUROPEAN CHERRY FRUIT FLY, Rhagoletis cerasi L.: PROGRESS REPORT ON REARING AND STERILIZATION.

Rhagoletis cerasi L. is a pest insect of economic importance. Efforts to control it by using the sterile-insect release method are being made. An account is given of egg collecting experiments and of the success achieved by using small black wax domes. Rearing tests with larvae have shown that production under laboratory conditions is possible. 22.8 ± 4.8% pupae from 100 larvae could be obtained by feeding with the best diet. This is a promising basis for further experiments. In irradiation experiments the fly has shown a similar sensitivity to that of the Mediterranean fruit fly. The competitiveness of males irradiated with 5 kR is significantly reduced. The egg production of females irradiated with 2 kR or more is low and can be neglected.

The European cherry fruit fly, Rhagoletis cerasi L., occurs in the moderate climatic zones of Europe and of some parts of Asia. According to unconfirmed reports this pest is also known in some places on the North African coast.

ECONOMIC IMPORTANCE

The species attacks especially sweet cherries (Prunus avium), not so much sour cherries (Prunus cerasus). It is therefore a real danger to cherry production. Since Europe produces about 77% of the annual world crop of cherries (1962 - 1966) the economic importance of this pest in Europe is beyond any doubt [1, 2]. Almost the entire cherry-producing area belongs to the biotope of the cherry fruit fly.

The pest insect can be controlled by chemicals. Because of the widely varying time of emergence of the flies the chemicals are the more effective the longer lasting they are. On the other hand the time of application of the insecticides and the time of harvesting are close together, so that the danger of toxic residues on the fruit for the consumers is an important factor.

For this reason some national regulations forbid the use of the most effective chemicals. In other countries effective chemicals are not being sold because they spoil the taste of the fruit.

The problem associated with the use of chemicals lead to demands for a better method of controlling the European cherry fruit fly, and the genetic method is promising.

BIOLOGICAL ASPECTS

The cherry fruit fly is an oligophagous species which attacks among other plants the honeysuckle (Lonicera sp.) [3, 4]. Under laboratory conditions the females also lay their eggs in other kinds of fruits that allow a development to the larval stage even if the rate of mortality is high. Under natural conditions these fruits are not infested, owing to the differences of time between the oviposition period of the fly and the ripening time of these fruits.

In contrast to the polyvoltine species of tropical fruit flies Rhagoletis cerasi has a diapause during the pupal stage. The diapause development lasts about 6 months and is terminated at temperatures between 0 and 4°C. However, the diapause affects the development of the various individuals of a population with a different intensity. Thus, a diapause-free strain of the species might be selected for rearing purposes. On the other hand Prokopy [8] has shown that the induction of the diapause in R. pomonella (Walsh) can be prevented by observing adequate temperatures and photoperiods. There is some hope that R. cerasi can be handled in the same manner. However, the diapause – which is a handicap from the rearing point of view – has the advantage that it enables one to stockpile pupae before the flying period.

In applying the sterile-insect technique the polygamy of the species is important.

EXPERIMENTAL WORK

1. Egg production and processing method

R. cerasi exhibits a peculiar oviposition behaviour. Certain stimuli must be present to trigger oviposition in the females: shape, size, surface and consistency of the oviposition medium have been found to be of crucial importance [5 - 7]. If these requirements are not met the females start to drop their eggs under increasing ovarian pressure. Like the olive fly, Dacus oleae (Gmel.), the cherry fruit fly does not oviposit into existing holes and lays in general only one egg into the cherries. A series of experiments were performed to investigate the best methods of producing the highest number of eggs per female and collecting and processing the eggs economically.

In one experiment the females had the alternative possibilities of either dropping their eggs through the wire-screening of the cage, of ovipositing in grape-berries or of laying their eggs in agar-balls (5% agar, 2 cm diam.) wrapped in Parafilm. Another experiment made use of the artificial oviposition device consisting of hollow wax domes, developed by Prokopy

TABLE I. COMPARISON OF DIFFERENT KINDS OF EGG COLLECTION

Experiment No.	Kind of egg collection	No. of eggs per female		Egg fertility (%)	No. of larvae per female
		Absolute	Relative		
1	Dropping	61.3	84.5	6.5 ± 7.3	4.5
1	Agar-agar balls	2.7	3.7	52.5 ± 16.8	1.4
1	Grapes	8.5	11.8	41.2 ± 17.1	3.5
1	Total	72.5 ± 8.2	100.0	- -	9.4
2	Wax domes	66.6 ± 11.2	-	85.7 ± 25.9	57.1

and Boller; the females push their ovipositors through the thin membrane of a special wax (Ceresin 1577,[1] 0.2 mm thick, 10 mm diam. and lay the eggs inside the domes where they can be rinsed off. We refer the reader to the relevant papers [6, 7] for specific data on the preparation and performance of these egging devices.

The results of these experiments, given in Table I, show clearly that egg fertility with wax domes is significantly higher compared to all other methods tested. Of special interest is the extremely low hatching rate of eggs dropped by the females.

A new type of oviposition cage has been developed at the Wädenswil laboratory [7] for mass-rearing purposes. Units of drum-shaped cages (64 cm diam.) containing 500 wax domes on a removable floor can be stacked one on top of the other forming oviposition towers which rotate on a vertical axle around light banks of strong mercury vapour lamps. The eggs are readily removed from the domes, without having to enter the rearing cage, by first lowering the jointed axle to a horizontal position and separating the cages and then flushing the inside of the domes with a water-jet. The water carrying the eggs flows into a funnel where the eggs are filtered out.

2. Feeding the larvae

In all feeding experiments wild flies were used for egg production. Only hatched larvae were transferred to the feeding substrates. Otherwise the egg mortality increased greatly.

The feeding substrates were prepared by mixing the solid substances and by dissolving the soluble ones in an adequate quantity of boiling tap water. When they were cool, both solutions were poured together. Since the geling properties of Gelgard change with the pH of the diet, half the amount of Gelgard and citric acid was added first and mixed with the other components and then the second half of both of them. About 18 - 20 g medium were normally provided per 100 larvae. The different feeding substrates are shown on Table II.

---

[1] Deutsche Erdöl AG, Hamburg, Federal Republic of Germany

TABLE II. COMPONENTS (% w/w) OF SOME FEEDING SUBSTRATES FOR LARVAE OF THE CHERRY FLY AND THEIR REARING QUALITIES

Food component	1 *	2 *	3 *	4 *	5 **
Wheat germs	0	0	0	0	5.0
Wheat germ diet [a]	11.5	11.5	10.6	0	0
Cellulose [b]	14.6	14.6	13.4	5.6	0
Paper pulp	0	0	0	0	20.0
Gelgard [c]	0	0	0	0	1.2
Peat	0	0	0	5.6	0
Powdered carrots	4.4	4.4	4.1	0	0
Cherries powdered and extracted by water	0	0	4.1	0	0
Brewer's yeast (or torula)	4.4	4.4	4.1	4.5	5.0
Sugar	4.4	4.4	0	4.5	5.0
Wesson's salt mixture [a]	(0.92) [e]	(0.92) [e]	(0.92) [e]	0	0.18
Vitamin diet fortification mixture [a]	2.7	2.7	2.7	0.22	0
Cholinchloride	(0.135) [d]	(0.135) [d]	(0.135) [d]	(0.135) [d]	0.07
Cholesterol	0	0	0	0	0.35
Ascorbic acid	(0.7) [d]	(0.7) [d]	(0.7) [d]	(0.7) [d]	0.1
l-Aspartic acid	0	0.0027	0	0	0
l-Glutamic acid	0	0.0018	0	0	0
β-Carotene	0	0	0	0.0034	0
Benzoic acid	0.055	0.055	0	0	0
Propionic acid	0	0	0.217	0.42	0
Citric acid	0	0	0	0	1.0
Nipagin M	0.11	0.11	0.102	0.26	0.12
Water	57.68	57.68	61.13	67.60	62.0
Total	100	100	100	100	100
pH	4.7	4.7	4.7	4.7	4.0
Mean yield of pupae per 100 larvae ± standard deviation	6.5 ± 3.3	11.1 ± 1.0	12.7 ± 3.4	15.5 ± 4.7	22.8 ± 4.8
Minimum time (days) to reach pupation ± standard deviation (days)	16.0 ± 5.0	16.1 ± 0.3	15.1 ± 1.7	15.7 ± 2.8	12.7 ± 0.7
Total number of tested larvae	1179	1094	1262	655	953
Number of replications	11	9	12	7	9

[a] Nutritional Biochemicals, Cleveland, Ohio, USA
[b] Schleicher u. Schüll No. 123, Fed. Rep. Germany
[c] Dow Chemical Int. Co., Midland, USA
[d] Contained in vitamin diet fortification mixture
[e] Contained in wheat germ diet

* Diets developed at Munich (Haisch)
** Diet developed at Wädenswil (Boller)

Wheat germs, cellulose, powdered carrots, paper pulp, peat, Gelgard and water are considered carrier substances. This does not exclude a nutritive function also. Carrier substances have to provide the larvae with the food components in adequate measure. The texture of the carrier substances largely influences the food consumption of the larvae and hence the development. It is very difficult to prepare a feeding substrate with a suitable texture, that retains this texture for at least 3 weeks. The carrier

substances in the substrates 1 - 3 do not sufficiently fulfil this precondition. Time and again some water had to be added to prevent the medium from becoming dry and hard. In this respect Gelgard and peat are effective: they bind a large amount of water and provide an adequate consistency.

The quality of the diets was judged by the speed of development of the larvae and the yield of pupae. Under natural conditions the development of the larvae up to pupation takes about 2 - 3 weeks.

The results of the rearing experiments are also shown in Table II. The main energy source for the larvae was sugar. According to experiments not described here, the development was prolonged by 4 or 5 days without additional sugar. We speak of 'additional sugar' because yeast, wheat germs and carrots also contain sugar.

The main protein source in all diets was yeast. According to previous experiences 4 to 8% yeast gave best results. Higher amounts of yeast were detrimental to the growth of larvae. This held also true if a certain part of this protein source is in the form of casein. Substrates 1 to 3 contained 3.2 or 3.0% casein in wheat germ diet. It can be assumed that this casein is unnecessary because the substrates 4 and 5 do not contain casein. In diet 2, l-aspartic and l-glutamic acid were used in addition to the protein amino acids because cherries contain relatively high amounts of these amino acids. The ℓ-configurations of these are of importance in the carbohydrate metabolism. But it is questionable whether an enrichment of these amino acids in a substrate of a real adequate composition also improves the yield of pupae as much as in the substrate 2 (or perhaps 3) compared with diet 1. Diet 3 contained cherry powder residue and therefore these compounds.

Knowledge about the mineral-salt requirements is still lacking. Concerning substrate 4, it seems that the addition of Wesson's salt mixture can be omitted. Also, the vitamins were added solely on the assumption that they are necessary and not already contained to a sufficient extent in the yeast. The same can be said of cholinchloride, cholesterol and β-carotene.

As already stated, an adequate texture is essential. It enables the larvae to make tunnels even in the earliest stage shortly after hatching, tunnels which are not filled with capillary water. There is no difficulty in obtaining such a consistency at the start of a feeding test, but after some days water evaporates, the substrates become hard, and the food consumption of the larvae decreases. This observation was made with substrates 1 to 3. Water was added repeatedly to prevent the diet from becoming dry and too hard. Substances with a high water capacity, e. g. agar-agar, are necessary for a permanently adequate texture. However, this is too expensive for mass rearing, so we tried substitutes. In diets 4 and 5 the wheat germ diet, the carrots and the cellulose are missing; finely sifted peat of Gelgard took over their function.

As important as the nutrients and bulking agents are the bacteriostats and fungistats. The growth of bacteria can normally without any difficulty be prevented by a pH-value below 4.5 and fungi can be controlled by parahydroxybenzoates, such as Nipagin M. However, it is difficult to suppress wild yeasts, which especially occur on sugar-containing diets such as those described. Yeasts produce alcohols, traces of which are detrimental to the larvae. They also form a mucous cover on the feeding media in which the larvae die. Benzoic acid is ineffective against the

observed yeasts. Since the bactericidal and fungicidal effect of benzoic acid can be obtained by other means, and since this acid is also toxic to larvae – at least to a certain degree – it can be substituted to advantage by other substances. Propionic acid, in the concentrations shown in Table II, hampers the growth of yeasts but may also be toxic to the larvae. The relatively high yield of larvae and the short development time with substrate 5 could be caused to a great extent by better and more suitable preservation means (as compared, for example, with substrate 4) in addition to a better texture.

3. Irradiation studies

At the Munich laboratory pupae were irradiated by gamma rays from a caesium-137 source (70 R/min) at the time when the first flies emerged. Emergence continued for 11 days. 50% of the flies could be observed after the 4th day and 75% at the 6th day. Immediately after emergence the sexes were separated. Every experimental unit held 50 pairs of flies, with one sex having been irradiated. The egg collection (according to Prokopy's method) was continued for the whole life span. The eggs could develop on a wet filter paper or gauze at a temperature of 23°C.

The results of the irradiation of male pupae showed that a 6-kR dose caused an egg mortality of more than 95% and 8 kR of 99% (Fig. 1). The fertility of the control was 67.3%, a relatively low value. It may be advantageous to correct all figures to a hatching rate of 100% in order to get a base for comparison with other figures. The corrected fertility rates of eggs are then

Radiation dose (kR):	0	2	4	6	8
% hatching:	100.0	19.4	9.0	5.7	1.7

It must be mentioned that at the fourth irradiation experiment a recovery of the males was observed by a statistically significant increase of egg hatching. It can be expressed by the partial regression between the number of hatching larvae per day and the age of the egg-laying females with the number of eggs laid as a constant variate. The calculation showed that the daily increase of hatching larvae was 0.5 larvae if the average number of hatching larvae is 100.

Under the experimental circumstances the egg production of irradiated females was as shown in Table III.

The radiation of female pupae with more than 2 kR caused obviously heavy damage to the ovaries. The egg production of females irradiated with dosages of more than 2 kR can therefore be neglected. The fact that the standard deviation is higher than the mean value indicates that the means are increased by a few extremely high values. Here egg production and even egg fertility are only rare cases. But because of the low egg production the data on egg fertility are not very informative.

In the laboratory at Wädenswil experiments were carried out to test the competitiveness of the irradiated males. There the dose rate of the ^{60}Co source was about 20 times as high as that of the source in Munich. Every population of the experiment had 10 females and 10 normal and 10 irradiated males. Irradiation doses of 0, 3, 5, 6 kR were chosen on the basis of the results of preliminary experiments in the laboratory at

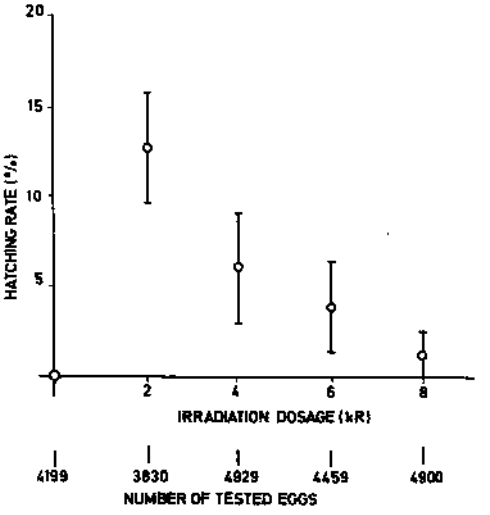


FIG. 1. Hatching rates of eggs after irradiation of the male parental pupae with increasing dosages of gamma rays. Control 67.3 ± 7.8%. (Average of 4 experiments).

TABLE III. EGG PRODUCTION OF IRRADIATED FEMALES

Radiation dose (kR):	0	2	4	6	8
Eggs per female per day	3.1 ± 1.77	0.05 ± 0.10	0.06 ± 0.10	0.06 ± 0.10	0.06 ± 0.10
Observed fertility of eggs (%)	67.3 ± 7.8	0.7 ± 3.8	0.0	2.1 ± 4.2	0.0
Fertility of eggs (%) corrected to 100% of control	100.0 ± 9.5	1.0 ± 4.6	0.0	3.1 ± 5.1	0.0

Munich. The irradiation times were 1 - 2, 3 - 4, 5 - 6 and 7 - 8 days before adult emergence. The eggs were collected many times.

The results (Table IV) showed that the irradiation time during the pupa stage between 1 and 8 days did not significantly affect the results. However, this observation does not confirm a lack of this effect, since the number of irradiated males was only 50% of the total number of males. But the dependence of the radiosensitivity on the development must certainly be considered in a different way compared with, for example, the Mediterranean fruit fly, since the cherry fruit fly has a post-diapausal development period of 21 - 26 days at 23°C, whereas the Mediterranean fruit fly develops in only 8 - 10 days.

To facilitate comparisons with other figures the observed figures were corrected to a hatching rate of the control of 100%. For practical purposes 90% was used because this figure may approach the natural situation.

TABLE IV. HATCHING RATES OF EGGS FROM A POPULATION CONSISTING OF NORMAL FEMALES AND MALES AND OF MALES IRRADIATED WITH DIFFERENT DOSES AT VARIOUS PUPAL STAGES (IRRADIATED MALES : NON-IRRADIATED MALES = 1 : 1)

Control 83.4 ± 1.1 (%)

Irradiation dose (kR)		Hatching rates (%)						
		Irradiation time in days before emerging				observed	corrected * to	
		1 - 2	3 - 4	5 - 6	7 - 8	1 - 8	90%	100%
3	a	63.5 ± 1.5	61.0 ± 2.0	61.3 ± 2.1	70.6 ± 4.2	62.6 ± 1.0	67.5	75.1
	b	1542	1389	964	455	4350	4350	4350
	c	3	3	2	1	-	-	-
4	a	50.2 ± 2.1	58.5 ± 2.8	52.8 ± 2.0	52.1 ± 2.6	53.0 ± 1.1	57.2	63.5
	b	1704	1097	598	1233	4632	4632	4632
	c	3	3	2	2	-	-	-
5	a	56.8 ± 1.4	50.7 ± 1.8	50.9 ± 2.8	58.8 ± 3.4	53.5 ± 1.6	57.7	64.1
	b	916	1452	1170	758	4296	4296	4296
	c	3	3	3	2	-	-	-

a = % hatching rate ± deviation from the average. The deviation was calculated as deviation from the regression of the number of hatching larvae on the number of eggs

b = number of tested eggs

c = number of replications

* = hatching rate of the eggs of the partly irradiated population corrected to a hatching rate of the eggs of the control population

Because of the relatively high fertility remaining in males irradiated with 3 kR, the fertility of the population was reduced from 100 to only 75.1% by adding irradiated males. If the irradiation dose was increased to 4 and 5 kR the hatching rate of eggs of the total population fell to 63.5% and 64.1%, respectively. The difference between these two figures is not significant.

If the competitiveness of irradiated males were not reduced by the irradiation the mean hatching rate would have to be 50%. Haisch [9] has developed a formula which allows one to calculate a factor which indicates the extent of the decreased vitality. It is 1.0 in the best case and can fall to 0.0. The formula, which is only correct for a single copulation, is:

$$e = \frac{q - f}{n\,(f - p)}$$

where e = competitiveness

q = hatching rate (%) of eggs of a normal population

p = hatching rate (%) of eggs of a population consisting of normal females and irradiated males

f = hatching rate (%) of eggs of a population consisting of normal pairs and irradiated males

$n = \frac{\text{number of irradiated males}}{\text{number of normal males}}$

In the experiment at the irradiation dosage of 5 kR the following values were taken:

q = 90 (%)
p = result of graphical interpolation of the figures of Fig. 1 and correction to 90 (%) hatching of control eggs = 6.5 (%)
f = 57.7 (%) (Table IV)
n = 1

Thus competitiveness of the males was decreased by irradiation at 5 kR from 1.0 to 0.63. The formula also enables one to calculate the number of irradiated males with which a normal population must be overwhelmed in order to suppress the hatching rate of the eggs of the mixed population to a certain level.

With the 5-kR irradiation and a desired fertility of 10% or 8%, n becomes 22.9 or 48.0 respectively, i.e. 23 or 48 irradiated males must be put to one normal pair of flies to reach these results.

The practical application of this calculation requires the knowledge of error possibilities. Combining the results of both our laboratories, using different irradiation sources, we have to take into consideration the different dosage rates which can affect the results — already noted by Rhode et al. [10] in irradiating Anastrepha ludens. However, there is some doubt whether the variation of the results due to the different dose rates and also due to the different relative biological effectiveness of the ^{60}Co and ^{137}Cs sources is higher than the variation caused by unknown factors within one experiment, as evidenced by Fig. 1.

A further point to be noted is that both sexes of R. cerasi mate many times under laboratory conditions. This does not have to be true in the field but the fact could affect our findings. It can be assumed that under laboratory conditions the normal and irradiated flies mix well together, but in the field the flying activity of irradiated flies could be lowered by irradiation damage or for other reasons. Large cage tests and small field tests are necessary to check this point. In further experiments the radiation dose must be raised in order to decrease the remaining fertility of irradiated flies.

When one considers how male competitiveness is already reduced by 5 kR, it seems doubtful whether the amount of fertility remaining in irradiated males can be further reduced without enormously lowering their vitality. But for information on this point further research is necessary.

REFERENCES

[1] BOLLER, E.F., HAISCH, A., RUSS, K., VALLO, V., Reports of the OILB working group on genetic control of the European cherry fruit fly, Rhagoletis cerasi L. I. Economic importance of the pest, the feasibility of genetic control and resulting research problems, Entomophaga 15 3 (1970) 305-313.

[2] FOOD AND AGRICULTURE ORGANIZATION, Production Yearbook 1966 20 (Rome, 1970) 171 - 172.

[3] WIESMANN, R., Das Wirtspflanzenproblem der Kirschenfliege, Landw. Jb. Schweiz 51 (1937) 1032-1044.

[4] THIEM, H., Über die Bedeutung der wilden Wirtspflanzen der Kirschfruchtfliege für die Verbreitung und Bekämpfung, Arb. physiol. angew. Ent. Berl. 6/7 (1939/40) 350-367.

[5] WIESMANN, R., Die Orientierung der Kirschfliege, Rhagoletis cerasi L., bei der Eiablage, Landw. Jb. Schweiz 51 (1937) 1080-1109.

[6] PROKOPY, R.J., BOLLER, E.F., Stimuli eliciting oviposition of European cherry fruit flies, Rhagoletis cerasi L., into inanimate objects, Entomologia exp. appl. (submitted for publication).

[7] PROKOPY, R.J., BOLLER, E.F., Artificial egging system for European cherry fruit fly, Rhagoletis cerasi, J. econ. Ent. 63 (1970) 1413-1417.

[8] PROKOPY, R.J., Influence of photoperiod, temperature and food on initiation of diapause in the apple maggot, Can. Ent. 100 (1968) 318-329.
[9] HAISCH, A., "Some observations on decreased vitality of irradiated Mediterranean fruit fly", Sterile-Male Technique for Control of Fruit Flies (Proc. Panel Vienna, 1969) IAEA, Vienna (1970) 71.
[10] RHODE, R.H., LOPEZ-D., F., EGUISA, F., TELICH-C., I., Effects of gamma radiation on the reproductive potential of the Mexican fruit fly, J. econ. Ent. 54 (1961) 202-208.

DISCUSSION

M. FRIED: The error term in Table IV seems very low. Is this the 95% significance level or does it represent something else?

A. HAISCH: The figures are deviations from means. The relation between the standard deviation and a confidence interval of, for instance, a 5% error probability is determined by the 't' value of that error probability and the degrees of freedom concerned.

STERILE-INSECT RELEASE METHOD AGAINST Rhagoletis cerasi L.

Preparatory ecological and behavioural studies

E.F. BOLLER
Swiss Federal Research Station for Arboriculture,
Viticulture and Horticulture,
Wädenswil, Switzerland
A. HAISCH
Institute for Soil Cultivation,
Plant Cultivation and Plant
Protection of the Federal State of Bavaria,
Munich, Federal Republic of Germany
and
R.J. PROKOPY
Department of Zoology, University of Texas,
Austin, Texas, United States of America

Abstract

STERILE-INSECT RELEASE METHOD AGAINST Rhagoletis cerasi L.: PREPARATORY ECOLOGICAL AND BEHAVIOURAL STUDIES.

Investigations concerning ecology and behaviour have been carried out and are still in progress in order to provide the necessary background information for a successful application of the sterile-insect release method. A long-term quantitative study of the population dynamics has been initiated. This work should facilitate the combination of the method with other means of control such as biotic mortality agents and trapping systems.

A study of colour-vision and reaction to various shapes of R. cerasi has led to the development of an effective visual throw-away trap that permits dispersion studies and experiments aiming at a strong reduction of the pest population. This fluorescent sticky-board is now in use in Switzerland, West Germany, Austria and the CSSR against R. cerasi and in Italy against Dacus oleae. Olfactory response investigations have been started with a new olfactometer in order to combine visual and chemical stimuli to an optimal trapping system.

Releases of marked flies and their recapture with the visual trap revealed a rather small cruising range of the flies (average 200 m, observed maximum 500 m) which is greatly influenced by the density of the fruit trees. Furthermore it was discovered that the orientation of the flies is mainly governed by the shape of the trees and not a host-specific odour. In consequence, as many flies could be recaptured on apple as on cherry trees. These findings suggest the combination of a grid system for release and a 'strategic release' in order to account for a heterogeneous distribution of the pest population within the release area.

The influence of irradiation and marking procedures on the flight capacity of R. cerasi is being studied by means of a 'flight mill' consisting of 10 low-friction rotors and a chart-recorder.

The field experiments have been carried out in isolated areas that have been selected as future sites for the first field experiments with the sterile-insect release method.

1. INTRODUCTION

The preceding paper in these Proceedings, by Haisch and Boller, covering some recent developments in the genetic control of the European cherry fruit fly, Rhagoletis cerasi L., dealt in general with the insect in

the laboratory. The present paper concerns the problems arising once the insect has left the laboratory and has been released in the field.

Investigators in Central Europe working actively on the cherry fruit fly problem have recognized that a sound knowledge of the ecology and the behaviour of the target species is of prime importance for a successful application of the sterile-insert release method [1]. Studies of the population dynamics of the pest were started in Switzerland in 1962 and in the CSSR in 1967 to find new solutions for the control of this pest and to combine suitable methods – such as genetic control, trapping systems, release of specific parasites, etc. – for a most efficient control program. This paper deals, however, only with those aspects of research in progress that have a direct bearing on the application of the sterile-insect release method.

2. PROBLEMS CONNECTED WITH THE RELEASE OF STERILE FLIES AND MONITORING THE ADULT POPULATIONS IN THE FIELD

Emphasis in our current investigations is put on the flight behaviour of the fly, its cruising range, its orientation toward the host-tree and the problems of marking and recapturing the flies in the field.

2.1. Trapping systems

Two basic stimuli are of special interest in the development of an efficient trapping system: visual and chemical stimuli that trigger certain responses in the cherry fruit flies seeking food sources. Up to 1968 only chemical stimuli such as food lures had been used – with doubtful success – to catch the adults in the field, and this situation greatly hampered dispersal and flight studies in the past. The lack of efficient attractants has been an incentive for investigations on olfactory response that started in 1969 at the Wädenswil laboratory. In the framework of an IBP-project (International Biological Program) on attractants for cherry fruit fly and olive fly we have developed a new olfactometer that meets the special requirements of our species. As we are still studying the fundamentals of the attracting principles in food baits, we decided to study at the same time the possibilities of exploiting visual stimuli to satisfy our immediate needs.

2.1.1. Studies concerning colour-vision of Rhagoletis cerasi

Stimulated by Prokopy's investigations on the apple maggot, R. pomonella [2], and his preliminary experiments with R. cerasi [3] in Poland, advantage was taken of his presence at the Wädenswil fruit fly laboratory to start colour experiments in 1969. We refer to a paper in preparation [4] for the details and summarize our investigations as follows.

On the basis of Prokopy's work we chose the purest yellow high-gloss paint we could find on the local market (Chromgelb 207)[1] as standard colour and mixed it with increasing amounts of red, blue, black and white paint.

[1] SAX-Farben, Urdorf/Switzerland.

TABLE I. INFLUENCE OF COLOUR ON TRAP EFFICIENCY
All visual traps 15 m × 20 m, coated with Bird Tanglefoot; relative figures

Colour	Index of attractiveness			
High-gloss paint				
Yellow (standard)	10	10	10	10
	Blue	Red	Black	White
Yellow + 0.5%	6.7	6.4	7.6	10
Yellow + 1.5%	3.7	6.0	3.8	10
Yellow + 5.0%	2.8	3.0	2.3	10
Yellow + 15%	0.7	0.7	0.4	8
Yellow + 45%	0.2	0.2	0.2	5
Fluorescent paint				
Day-Glo				
Saturn Yellow	16.7			
Signal Green	6.1			
Horizon Blue	0.8			
Fire Orange	0.4			
Aluminium foil	0			
McPhail traps				
Standard (4% NH_4HCO_3)	0.2			
Painted yellow	1.1			
Yellow + Bird Tanglefoot	5.2			

Masonite boards (15 cm × 20 cm × 0.5 cm) were painted with the various mixtures, then coated with a thin layer of Bird Tanglefoot[2] and hung in untreated cherry trees. The traps were replicated 5 times and tested in two different orchards. The results of this experiment are given in Table I.

It is evident that pure yellow attracts significantly more flies than any mixture with other colours except white. Even small additions of 0.5% of red, blue or black to yellow decrease the attractiveness of the traps to a considerable extent. Addition of white up to 5% had no significant effect but started to dilute the yellow colour too much at higher concentrations. Of special interest is the effect of fluorescent colours. The attractiveness of standard yellow could be increased by more than 60% by using day-light

[2] The Tanglefoot Company, 314 Straight Ave., Grand Rapids, Michigan 49502, USA.

fluorescent yellows (such as Day-Glo Saturn Yellow)[3] which increases the intensity of yellow by transforming light of short wave-lengths (u.v.) into light in the visible part of the spectrum. The results achieved with fluorescent orange, blue and green show clearly that it is not the fluorescence itself that is responsible for the efficiency of the trap nor the amount of sunlight reflected (highest reflection on white surfaces or mirrors) but the yellow colour. The fluorescent yellow board is about 80 times as attractive as the standard McPhail trap with a 4% solution of ammonium carbonate. Yellow boards have also given excellent results in Italy with Dacus oleae [5].

2.1.2. Various shapes of traps and their influence on catch

After yellow was found to attract most flies we proceeded to test various shapes of traps painted with standard yellow (Chromgelb 207). The experiments included spheres, cubes, cylinders, crossed boards and simple boards with diameters of 20 cm. Statistical analysis of the results showed no significant difference between the catches from each shape except from the cylinders, which showed a noticeably lower attractiveness.

Although the differences were not significant - probably because of the small numbers of replicates in the field - three-dimensional traps like spheres, cubes and crossed boards caught more flies than the two-dimensional boards (spheres average of 80.2 flies; crossed boards 71.8; cubes 66.8 against 43.0 on boards). No explanation is available for the poor performance of the cylinders (38.2). On spheres, cubes and to some extent on cylinders we could observe flies only on the lower half of the traps; this leads to the speculation that Trypetid species might show a similar organization of their eyes with regard to colour vision as, for example, some homoptera [6]. Another interesting observation concerned a significant increase of the attractiveness of McPhail traps when they were painted yellow. However, high catches were only achieved when the traps were coated with Bird Tanglefoot. This is an indication that flies land on the yellow traps attracted mainly by the visual component of the mixed visual and olfactory stimuli and apparently do not find the entrance to the traps very easily.

2.1.3. Development and application of an efficient disposable trap

Once the important features of an efficient visual cherry fruit fly trap had been recognized a cheap disposable trap was developed for mass-application in the field. For practical reasons white cardboards, 15 cm × 20 cm and 1 mm thick, were chosen as raw material rather than three-dimensional objects. The cardboards were sprayed with yellow fluorescent paint,[4] perforated with two holes, dipped for one second into hot liquefied Bird Tanglefoot and finally sandwiched between two plastic foils for transportation. A total of 4000 traps were produced in 1970 within a short time and applied in the field for dispersion studies, for

[3] Day-Glo Color Division, Switzer Bros., 4732 St. Clair Ave., Cleveland, Ohio 44103, USA, distributed in Switzerland by G. Labitzke Farbenfabrik, 8048 Zürich.

[4] Yellow Day-Glo 25644-498; G. Labitzke Farbenfabrik, 8048 Zürich, Switzerland.

measuring relative population densities in future release areas, and for direct control purposes in one of the three pilot communities. This latter experiment was carried out after we had made the rather unexpected observation that a local cherry fruit fly population was almost eliminated within a few days when visual traps were hung in each tree of a small cherry orchard. All trees of one zone of the community Hersberg (near Basel) that showed chronically a high infestation by the cherry fruit fly were supplied with traps at the beginning of the flight period. Up to 215 flies per tree were caught within a few days and were eliminated before oviposition took place. The average infestation of the crop could be reduced to 3.6% in the treated area compared with damages of 30 - 35% in nearby communities.

This new trap might be used in the future for reducing without insecticidal treatments the wild populations before the application of the sterile-insect release method; for the establishment of buffer-zones where natural barriers do not provide complete isolation; and for monitoring the development of wild and sterile fly populations during an eradication campaign. Investigations in progress aim at creating an even more efficient trapping system by combining visual and olfactory stimuli.

2.2. Marking techniques

One important aspect of a release program is the proper marking of released sterile insects. The requirements depend largely on the stage of the insect to be released. So far we have used the adult stage for dispersion studies but our present knowledge of the biology and behaviour of the flies points to the need to release the cherry fruit fly in the pupal stage and have the flies emerge in the field.

2.2.1. Methods used for marking adult flies

In the experiments carried out in 1969 we used exclusively fluorescent powders[5] to mark and to identify the recaptured flies. Pupae were placed in dishes and covered with a 2-cm layer of fine sand mixed with 0.5% of the respective marking powder [11]. Higher concentrations increased the mortality of the flies. At lower concentrations the proper tagging was not achieved. The flies emerged and crawled through the sand, picking up fluorescent powder with the protruded ptilinum. After a few days no marking substance could be detected on the body surface but the retracted ptilinum was very well marked. However, the examination of several thousand flies under the binocular and u.v.-lamp was very time-consuming, and mistakes could not be excluded because many flies became contaminated with the fluorescent paint when scratched from the sticky boards. So two of the present authors (Boller and Haisch) started to use the neutron-activation technique in 1970. Newly emerged flies were held for 2 - 3 days in the laboratory and were fed an aqueous solution of 20% sugar and 0.2% dysprosium chloride or europium chloride. Boller in his own experiment combined fluorescent powders with the rare-earth method in order to compare the reliability of the two approaches. The material

[5] Day-Glo Color Division, Switzer Bros., 4732 St. Clair Ave., Cleveland, Ohio 44103, USA.

from the dispersion experiment was processed by Haisch after the flies at Wädenswil had been examined for fluorescent markers. Both methods gave similar results, but in a few cases radioactive flies were observed which had not been detected before by means of fluorescence and vice versa. In conclusion we might say that the neutron-activation technique looks more promising when speed and automation of processing is concerned.

2.2.2. New requirements

The neutron-activation method as applied so far has the disadvantage that flies must be held in the laboratory for the marking process. Although we have not yet obtained complete knowledge of the behavioural changes of R. cerasi during the various time intervals of the adult stage, there are indications that the first few days after emergence are of crucial importance for the dispersion and the mating process in the field. The release of adult flies does not only mean additional costs for holding cages and food, but carries also the risk of wing damage during transportation and the disadvantage that the flies may be unable to adapt within a very short time to harsh field conditions. It might therefore be desirable to release the insects in the pupal stage, which calls for other marking methods. Investigations in progress are dealing with the possibility of incorporating dysprosium into pupae by soaking them in a dysprosium solution at various stages of diapause development. If pupae can be marked with rare earths there is no need for the hazardous and sometimes unreliable application of radioactive tracers. As for the application of fluorescent powders, it seems appropriate to study their potential negative side-effects on the flight performance of the flies in greater detail.

2.3. Influence of irradiation and marking methods on flight performance

Studies on the flight behaviour of irradiated and marked flies, started in June 1970, have yielded preliminary results only. The tests were conducted in the laboratory with a newly developed device ('flight mill') that consists of 10 low-friction rotors driven by individual flies suspended at the thorax. One rotation of the rotor equals a flight distance of 1.0 m. Rotations are recorded automatically for all 10 rotors simultaneously by a 10-channel chart-recorder and 10 mechanical counters. For details we refer to the paper in preparation [7]. Although the present data are not sufficient to justify a final conclusion we have observed that flies irradiated with 4000, 6000 and 8000 R show the same flight characteristics as untreated flies (total flight distance, flight speed, flight pattern). A total of 54 males flew an average of 2444 m and 54 females 3279 m during an experimental period of 6 hours. Whereas the males in the experiment were exhausted after 6 hours, the females had not yet reached exhaustion and will therefore have a much higher flight capacity than indicated by the figures above. Statistical analysis of the flight data showed no significant differences between the effects from the different doses. It is remarkable that the longest flight distance of 8760 m was achieved by a male irradiated with the highest dose of 8000 R.

However, a small experiment with only 10 males marked with a red fluorescent powder showed only an average flight distance of 474 m or 19.5% of the unmarked males. Their flight pattern was distinctly different

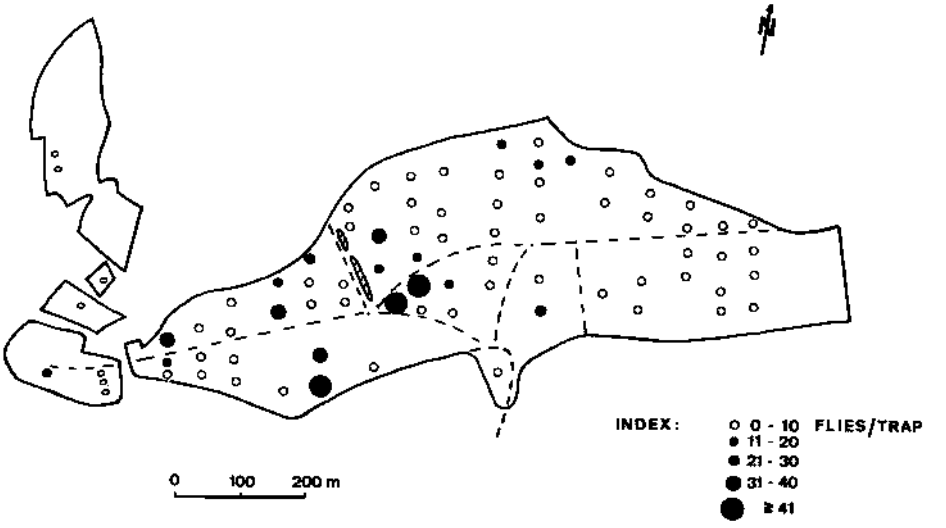


FIG. 1. Map of relative population densities of R. cerasi at Nuglar, 1970.

from the normal and demonstrates a fast increasing fatigue during the individual flights. It was interesting to observe that none of the 500 released red flies could be recaptured in the field whereas 4.5% of the yellow flies could be recovered. Since the red powder does not belong to the Day-Glo series generally used in our experiments and flies marked with yellow Day-Glo powder were not included in that test it is too early to make a general statement about the negative effects of fluorescent dusts as marking agents.

Summarizing these flight experiments we can say that irradiation of pupae up to 8000 R probably does not interfere with the flight behaviour of the flies although we observed a certain decrease in the mating frequency of flies irradiated with the highest dose of 8000 R. The possibility of negative side-effects influencing seriously the flight performance must be taken into account when fluorescent dyes as markers are considered.

2.4. Dispersion studies and density maps

Two dispersion studies were carried out in 1969 and 1970 in the community of Nuglar near Basel. This area is about 1300 m long and 500 m wide, contains 967 cherry trees, 32 apple trees and is completely surrounded and isolated by forest.

A total of 6000 marked flies were released in 1969 from a central releasing point and recovered by traps in concentric circles with radii increased by 100 m.

In 1970, 500 yellow and 500 red flies were released on the two opposite pheripheries of the area in order to study the barrier effect of the forest and the influence of wind directions. Nine trap lines – 100 m apart – with an equal trap density of 7 were established between the two release lines and served at the same time as a grid system for mapping the relative abundance of the wild population in the area (Fig. 1). Meteorological factors (temperature, wind direction and wind speed, hours of sunshine,

TABLE II. DISPERSION STUDIES 1969/1970 (R. cerasi L.)

Place: Nuglar/Switzerland

Release:	From central point (1969)	From periphery (1970)
No. of flies released	5958	529 Yellow (East) 537 Red (West)
No. of traps	262	71
Equal trap density	No	Yes
No. of flies recaptured	1124	24 Yellow 0 Red
Percentage of flies recaptured	18.9	4.5 0
Distances covered	Average number of flies per trap	
50 m	12.3 (0-100 m)	1.57
100 m		1.00
150 m	1.55 (101-199 m)	
200 m	0.73	0.57
300 m	0.21	0.28
400 m	0.25	0.0
500 m	0.11	0.0
600 - 1000 m	0.0	0.0

relative humidity and precipitation) were measured with a suitably equipped weather station in the centre of the experimental site. The results of the two dispersion studies are given in Table II.

The findings, confirming earlier reports about flight distances covered by the cherry fruit fly [8-10], indicate that most flies do not leave the 100-m perimeter around their place of origin as long as enough suitable host trees are available. Under these circumstances very few flies extend their cruising range up to 400 and 500 m. Despite the fact that cherry fruit flies might have an intrinsic flight capacity of more than 8000 m per day (as shown in the flight mill experiments), they usually fly short distances from tree to tree rather than long distances in non-stop flights. However, longer distances can be flown if cherry trees are separated by open fields but this seems to be the exception rather than the rule. In this regard it is interesting to see that the two flies recovered in 1969 at a distance of 500 m had to fly across two open fields with no opportunity to land in between.

2.5. Orientation toward host trees

The experiment carried out in 1969 reveals some other interesting aspects of the behaviour of the cherry fruit fly. Traps were hung not only in cherry trees but also in apple trees and in bushes along the margin of the forest. To our surprise we caught throughout the entire period of 6 weeks equal numbers of cherry fruit flies on apple and cherry trees. However, the portion of mature females increased faster on cherry trees than on apple trees although females with fully developed ovaries continued flying toward apple trees. No flies were ever caught on traps near the forest (beech; Fagus silvatica L.). This seems to abolish the earlier speculations that R. cerasi finds its host tree by means of a host-specific odour. We are inclined to believe that R. cerasi flies to any deciduous tree which has a similar silhouette to the cherry tree and often prefers the larger silhouettes. The orientation therefore seems to be rather visual than olfactory, leading the fly also to apple trees which are much richer in food sources than cherry trees. Flying to and fro the female sooner or later meets a suitable host tree with cherries in the optimal stage of ripeness for oviposition. Once arrived on the cherry tree there seems to be no accumulation of flies. The females, especially, fly again to apple and other deciduous trees in search of rich food sources. The behaviour of the male on the other hand is not yet fully understood. Observations in the laboratory and in the field indicate that males meet the opposite sex near or on the cherries. If this hypothesis holds true more males should be caught on cherry than on apple trees. This tendency could be observed in 1969 but needs further study.

3. CONCLUSIONS FOR FUTURE RELEASES

These results lead us to conclude that sterile cherry fruit flies should not be released from one central releasing point but dispersed over the entire area. A grid system for release with 50-m intervals should be considered for a satisfactory distribution. The quantity to be released depends on the density of the wild population in the respective grid (density maps). Optimal adaptation of the released material to field conditions can probably be achieved through releases in the pupal stage just before the emergence of the flies. Native populations can be reduced by heavy trapping in the previous year and potential channels of reinfestation (roads, open fields) can be surveyed and possibly blocked with visual traps.

ACKNOWLEDGEMENTS

The authors wish to thank Mr. Lüthi and Mr. Suter, of the Swiss Federal Institute of Technology, Zürich, who carried out part of the research for their diploma; they are also grateful to their technicians who assisted in many of the experiments.

REFERENCES

[1] BOLLER, E.F., HAISCH, A., RUSS, K., VALLO, V., Reports of the OILB working group on genetic control of the European cherry fruit fly, Rhagoletic cerasi L. 1. Economic importance of the pest, the feasibility of genetic control and resulting research problems, Entomophaga 15 (1970) (in press).

[2] PROKOPY, R.J., Sticky spheres for estimating apple maggot abundance, J. econ. Ent. 61 (1968) 1082-1085.

[3] PROKOPY, R.J., Orientation of apple maggot flies, Rhagoletis pomonella (Walsh), and European cherry fruit flies, Rhagoletis cerasi L. (Diptera: Trypetidae), to visual stimuli, XIIIth Int. Congr. Ent. Moscow, 1968.

[4] PROKOPY, R.J., BOLLER, E.F. (in preparation).

[5] CAVALLORO, R. (1969), personal communication.

[6] MOERICKE, V., Flughemmung und Fallreflexhaltung als Reaktion auf Gelbreiz bei Trialeurodes vaporariorum (Westwood), Z. PflKrankh. PflSchutz 73 1/2 (1966) 1-14.

[7] BOLLER, E.F., REMUND, U., SUTER, H., A modified flight mill for the study of the flight capacity of European cherry fruit flies, Rhagoletis cerasi L., J. econ. Ent. (in preparation).

[8] WIESMANN, R., Lebensgeschichte und Bekämpfung der Kirschenfliege. II. Mitteilung. Landw. Jb. Schweiz (1934) 281-338.

[9] PORTER, B.A., Recent advances in control of temperate-climate fruit flies, Proc. 10th Int. Congr. Ent. Montreal 1958, 3 (1958) 5-10.

[10] LESKI, R., Population studies of the cherry fruit fly, Rhagoletis cerasi L., Insect Ecology and the Sterile-Male Technique (Proc. Panel Vienna, 1967) IAEA, Vienna (1969) 1-7.

[11] NORRIS, K.R., A method of marking Calliphoridae (Diptera) during emergence from the puparium, Nature, Lond. 180 (1957) 1002.

DISCUSSION

L. MELLADO: The flight experiments show that the flies have a considerable flying capacity, whereas results of field experiments show that the flying range of the species is rather limited. Could you please comment on this fact?

E. BOLLER: We do not carry out our flight studies in the laboratory with the intention of extrapolating the results to field conditions. In the laboratory we compare the relative flight capacities of normal and of treated flies. However, the evident difference between flights by Rhagoletis cerasi of up to 8 km in the laboratory and the observed maximum cruising range of 500 m in the field can be explained by the limiting influence of high tree densities on dispersal.

GAMMA STERILIZATION OF THE MEDITERRANEAN FRUIT FLY

G. H. S. HOOPER
International Atomic Energy Agency,
Vienna

Abstract

GAMMA STERILIZATION OF THE MEDITERRANEAN FRUIT FLY.

The sterility of male C. capitata irradiated in the pupal stage two days before emergence with gamma doses of 1 - 13 krad was investigated. When plotted in arithmetic units the relationship between sterility (expressed as hatch of eggs from crosses of irradiated males and untreated females) and gamma dose is curvilinear. However, if gamma dose is plotted in logarithmic units and percent egg hatch in terms of the angular transformation, a linear relationship is obtained. At 11 krad male sterility was better than 90%. When males were irradiated 1, 2 or 3 days before emergence, the degree of sterility decreased but the competitiveness of the males increased as the time between irradiation and adult emergence decreased. No evidence of recovery of fertility in males irradiated with 5, 7 or 9 krad was found up to 26 days after irradiation. Females were more radiosensitive than males and 3 krad produced infecundity. The fecundity of untreated females was unaffected by matings with irradiated males.

INTRODUCTION

Gamma sterilization of the Mediterranean fruit fly, Ceratitis capitata (Wiedemann), has been studied in a number of laboratories (reviewed by Hooper [1]). In addition, field experiments to test the applicability of the sterile-insect release method for the control or eradication of C. capitata have been carried out in Hawaii, Tenerife, Spain, Nicaragua and Italy [2-7].

To provide information to support field experiments in Nicaragua and Italy in which the Joint FAO/IAEA Division of Atomic Energy in Food and Agriculture was co-operating, a program was started in 1969 at the Seibersdorf Laboratory to establish the optimal sterilizing dose for the Seibersdorf strain of C. capitata and to evaluate the effect of dosage on sexual competitiveness. This paper is the first of a series which will report the work carried out in the past 18 months and deals with the basic aspects of gamma-induced sterility.

MATERIALS AND METHODS

Larvae were reared on a diet based on wheat bran and yeast developed by Nadel [20] at 25 ± 2°C and 80 ± 5% r.h. Pupae and the test adults were maintained at 25 ± 1°C and 60 ± 5% r.h.

Irradiations were carried out in a ^{60}Co Gammacell, the dose rate of which decreased from 9.2 to 7.8 krad/min during the period of study. Pupae were irradiated in 120 ml polystyrene containers and the variance in dose received by the pupae was ± 5%.

Except where specifically stated, only flies which emerged 2 days (actually 40 - 48 hours) after irradiation were used, i.e. the flies had been irradiated 2 days before adult eclosion. The flies were sexed (within

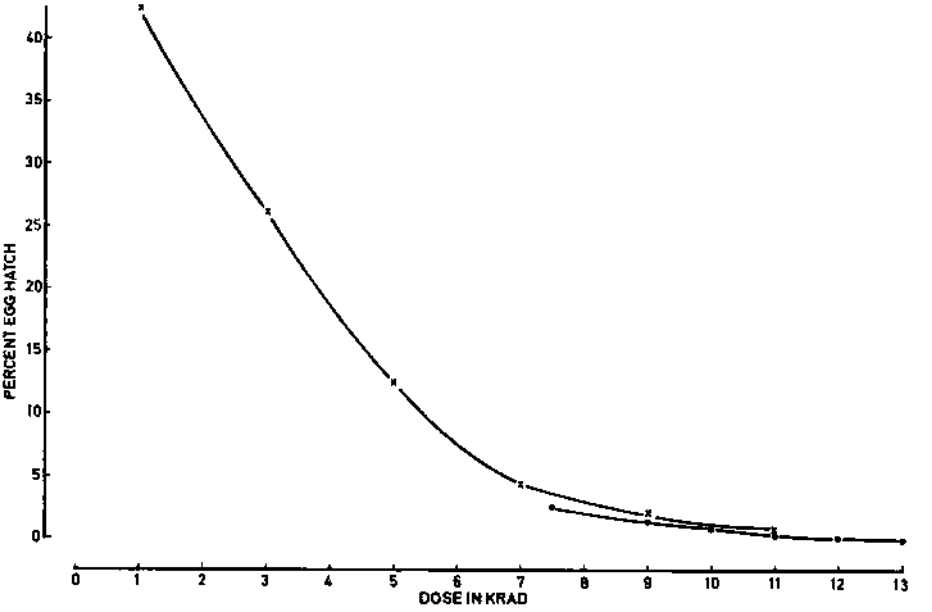


FIG. 1. Effect of increasing gamma dose given to male C. capitata on the hatch of eggs from the mating of these males with untreated females.

10 hours of emergence) and manipulated without anaesthesia by catching them in small glass vials. Unless otherwise stated, 25 males and 25 females were confined in 14 cm × 11 cm × 11 cm clear plastic cages designed by D. J. Nadel of this laboratory. The flies had access to water and 1:3 mixture of yeast hydrolysate and sugar. The front of the plastic cage was replaced by Terylene material (12 mesh per cm) through which the females oviposited, and the eggs fell to, and were collected on, moist black filter paper. Samples of approximately 150 eggs were taken 3 times per week over a 3-week period. The eggs were kept on moist filter paper in a Petri dish and after 5 days at 25°C the hatch was recorded.

To measure day-to-day variation, normally 5 replicates of each treatment were established, one per day. After angular transformation of the percent egg hatch data an analysis of variance was carried out and differences between means were evaluated by Duncan's multiple range test.

The degree of sterility induced in males was estimated from the hatch of eggs from matings of irradiated males and untreated females. Similarly, the effect of irradiation on females was studied by mating irradiated females with untreated males.

RESULTS

The relationship between gamma dose and male sterility (expressed as percent egg hatch adjusted for control hatch) found in two experiments is shown in Fig. 1. While a dose of 7 krad reduces egg hatch to 4.5%, a further 4-krad increment to a dose of 11 krad is required to lower egg

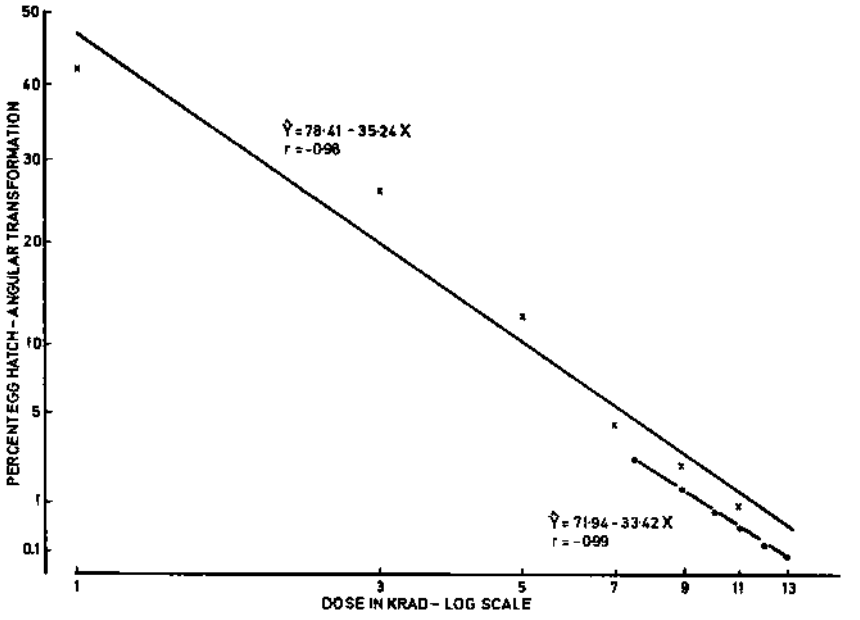


FIG. 2. The linear relationship which results when the data of Fig. 1 are plotted in transformed units. For each line the regression equation and the correlation coefficient (r) are shown.

hatch below 1%. Since the relationship between dose and male sterility is curvilinear when plotted in arithmetic units it is often difficult to compare results from different laboratories. However, if gamma dose is plotted in logarithmic units and percent egg hatch in terms of the angular transformation, a very good linear relationship is obtained (Fig. 2). While the regression lines for the two experiments (which were carried out several months apart) are not superimposed, the slopes of the lines are not significantly (P = 0.05) different. The data plotted in Figs 1 and 2 are mean egg hatch over a 3-week period. However, it should be noted that the egg hatch in the first week is appreciably higher than in the following 2 weeks (Table I). This phenomenon has been encountered repeatedly.

It had also been noted that the egg hatch of untreated females mated with untreated males decreased with time. An attempt was made to determine whether this decrease in egg viability was attributable to the male or the female. The experimental procedures and results are shown in Table II. Comparison of treatments A and B shows that after 21 days the egg hatch declined (90.2% to 80.5%). When 21-day-old previously mated females were mated with young, virgin males the egg hatch did not increase (treatment D). However, when 21-day-old previously mated males were mated with young, virgin females (treatment E), egg hatch was high (91.2%) and comparable with that of treatments A and C. Thus the data indicate that the decline in egg viability with time is attributable to the female and not the male. When the results of treatments F and G are compared with those of treatments A and B it is clear that females require the presence of males for longer than 5 days to ensure a high degree of egg viability for more than 21 days.

TABLE I. PERCENT EGG HATCH, CORRECTED FOR CONTROL HATCH, IN SUCCESSIVE WEEKS OF MATINGS BETWEEN UNTREATED FEMALES AND GAMMA-IRRADIATED MALES OF C. capitata

Dose (krad)	Corrected % egg hatch in week 1	2	3	3-week mean
3	41.6	17.6	18.1	26.0
5	18.0	10.4	7.9	12.5
7	5.7	3.8	3.2	4.3
9	2.7	1.8	1.9	2.2
11	1.1	0.8	0.6	0.9

TABLE II. PROCEDURES AND RESULTS OF AN EXPERIMENT TO DETERMINE WHICH SEX OF C. capitata IS RESPONSIBLE FOR THE DECLINE OF EGG HATCH WITH TIME

Mating procedure [a]	Mean % egg hatch
A. Newly emerged ♂ and ♀ mated. Egg hatch over first 21 days (6 replicates).	90.2
B. Surviving ♂ and ♀ of A after 21 days recombined. Egg hatch over next 16 days (4 replicates).	80.5
C. Newly emerged ♂ and ♀ mated. Egg hatch over first 21 days (6 replicates).	89.4
D. The 21-day-old ♀ from C mated with young, virgin ♂. Egg hatch over next 16 days (4 replicates).	79.8
E. The 21-day-old ♂ from C mated with young, virgin ♀. Egg hatch over next 16 days (4 replicates).	91.2
F. Newly emerged ♂ and ♀ mated. After 5 days ♂ removed. Egg hatch over first 21 days (4 replicates).	77.3
G. The 21-day-old ♀ from F maintained. Egg hatch over next 16 days (3 replicates).	57.7

[a] Each replicate consisted of 25 males and 25 females.

The main effect of gamma irradiation of females is infecundity. In an experiment employing only 2 replications females given 1 krad produced a normal number of eggs with normal egg hatch. At 3 krad and above no eggs were produced. However, this is not to say that females receiving more than 3 krad will never produce eggs; in other experiments a few eggs, which invariably did not hatch, have been produced by females which had received gamma doses as high as 7.5 - 13.0 krad.

TABLE III. FECUNDITY OF UNTREATED FEMALE C. capitata MATED WITH GAMMA-IRRADIATED MALES

Dose (krad)	Mean no. eggs/female/day[a] Week 1	Week 2	Week 3	Overall mean
0	14.50	31.89	27.89	24.76
7.5	19.11	28.03	27.83	24.99
9.0	20.47	32.74	29.95	27.72
10.0	20.43	31.21	27.21	26.24
12.0	11.98	24.62	29.32	21.96

[a] Means are based on 3 replicates per treatment and from an experimental period of 21 days.

TABLE IV. STERILITY AND COMPETITIVENESS OF MALE C. capitata IRRADIATED 1, 2 AND 3 DAYS BEFORE ADULT EMERGENCE

Dose (krad)	Cage population[A] I ♂ : U ♂ : U ♀	Corrected % egg hatch from males irradiated at indicated days before emergence[B] 1	2	3
5	25 : 0 : 25	14.0 a	12.7 a	9.4 b
7	25 : 0 : 25	6.7 a	7.6 a	5.3 b
9	25 : 0 : 25	3.9 a	3.1 ab	2.3 b
9	16 : 16 : 16	66.4 a	78.1 b	83.1 b

[A] I = irradiated; U = untreated.

[B] Means characterized by the same letter are not significantly different at the 5% level of probability. Comparison of means must be made only within each treatment.

The fecundity of untreated females mated with irradiated males was recorded over a 3-week period. The total number of eggs produced per day was adjusted for the number of females alive on that day and the data (Table III) clearly show that the fecundity of females was unaffected by the fertility of the males to which they were mated.

Table IV records the sterility induced in males given 5, 7 or 9-krad gamma radiation 24, 48 or 72 hours before emergence, i.e. when the males were -1, -2 or -3 days old respectively. A two-way analysis of variance showed that both gamma dose and age of male at time of irradiation significantly affected sterility. As the time between irradiation and adult emergence increased, the degree of sterility was higher. At no dose, however, did the sterility of males irradiated when -1 or -2 days old differ significantly, but at 5 and 7 krad males treated when -3 days old were significantly more sterile than males treated later in the pupal stage.

Since gamma irradiation affects sexual competitiveness as well as fertility of males, the competitiveness of males given 9 krad 1, 2 or 3 days before emergence was assessed by a 1:1:1 ratio experiment (16 irradiated

TABLE V. PROCEDURES AND RESULTS OF AN EXPERIMENT TO ASSESS THE PERMANENCE OF STERILITY IN MALE C. capitata IRRADIATED WITH 5, 7 or 9 krad.

Mating regime [a]	Mean % egg hatch [b] from crosses of untreated females and males irradiated with			
	0 krad	5 krad	7 krad	9 krad
A. Newly emerged males mated; after 5 days the ♂ were withdrawn.	94.0	16.2	9.3	3.6
B. The males from A were held in absence of ♀ for 7 days and then mated with young, virgin ♀. After 5 days the ♂ were withdrawn.	94.8	1.8	1.6	0.6
C. 12-day-old virgin ♂ were mated with young, virgin ♀. After 5 days the ♂ were withdrawn.	93.9	4.8	2.9	1.0

[a] In Regime A there were 10 replicates in order to ensure sufficient males for Regime B. In Regimes B and C there were 5 replicates. Each replicate consisted of 25 males and 25 females.

[b] The mean egg hatch data are based on 6 egg samples taken over a 14-day period.

males:16 untreated males:16 untreated females). When the appropriate level of male sterility is considered, the egg hatch expected if the irradiated males were fully competitive would be 51.9, 51.6 or 51.2% for males treated 1, 2 or 3 days before emergence respectively. Thus the data (Table IV) show that treatment with 9 krad decreases the competitiveness of the males. Whilst there was no significant difference in the competitiveness of males treated 2 days and 3 days before emergence, these males were significantly less competitive than males treated 1 day before emergence.

Although we have never encountered any evidence of recovery of fertility in irradiated males, a formal experiment was established to elucidate this point (Table V). The egg hatch of females mated with males which previously had been with females for 5 days and then kept isolated for 7 days (Regime B) was lower than the egg hatch of females to which these males had been mated first (Regime A). At the end of the experiment the males were 26 days old and even at the last egg assay, a low hatch was recorded. When males were kept virgin for 12 days and then mated to young, virgin females (Regime C) the egg hatch was again low. Thus there was no indication of recovery of fertility in males treated with 5, 7 or 9 krad for at least 26 days.

DISCUSSION

It has been established [8] that a more sexually competitive fly is obtained the closer the gamma irradiation is given to adult emergence. However, males emerging 2 days after irradiation have been used for most

of our experiments. This decision was taken because, for logistic reasons, irradiated flies shipped from this laboratory to the field release area in Italy [6,7] had to be irradiated 24 - 48 hours before expected adult emergence [9]. Thus our work was aimed primarily at obtaining radiobiological data of direct application to the flies actually used in the field experiments.

The data obtained on the relationship between gamma dose and the level of sterility induced in males are comparable with those obtained by other workers [10-14]. However, owing to the curvilinear dose-sterility relationship, difficulty was encountered in an earlier paper [1] in accurately comparing data reported by different laboratories. The finding that a linear relationship exists when the dose is expressed in logarithms and the percent egg hatch in terms of the angular transformation eliminates this problem. The data of Katiyar and Ramirez [12] also give a good linear relationship and the dose required for 95% male sterility is 6.8 krad while the corresponding figure from our work is 7.2 krad.

Female C. capitata are more radiosensitive than males. Females have been reported to become infecund at a gamma dose of 4 krad [13,15] and in our work 3 krad produced infecundity. Katiyar and Valerio [11] found that females given a dose of 7.5 krad one day before emergence did produce a few eggs and we have consistently noted a few eggs from females receiving up to 13 krad. However, in both these instances the eggs were non-viable whereas Steiner et al. [2] stated that at 8.5 krad females did produce some fertile eggs. While we found no reduction in the hatch of eggs from females given 1 krad, Feron [13] found that increasing the dose from 1 krad to 3 krad progressively reduced egg hatch.

In our work the fecundity of untreated females was unaffected by matings with irradiated males and the data of two other laboratories [11,13,15] allows the same conclusion. However, Lindquist [16], referring to the work of Steiner and Christenson [10], stated that female fecundity was decreased and the Spanish workers made similar observations [17]. We would not expect that mating with an irradiated male would reduce the fecundity of its untreated mate since virgin females of our strain of C. capitata produce as many (unviable) eggs as normally mated females.

Katiyar et al. [8] observed that the hatch of eggs from untreated males mated with untreated females declined over a 4-week period; this supports our findings. Our data also indicate that the decline is attributable to the female, and that when 21-day-old previously mated males are mated with young, virgin females normal egg hatch is obtained. Our data further suggest that females require access to males for longer than 5 days in order to ensure high egg fertility for up to 37 days. Even in the first 21 days the mean egg hatch was lower than that obtained when males were continuously available. Katiyar et al. [8] obtained rather different results; the egg hatch of females known to have mated only once and held without males was little different to that of females confined continuously with males.

The data of Katiyar and Valerio [11] show that above 5 krad there was little difference in the sterility of males irradiated 1, 2 or 3 days before emergence. However, at lower doses there was a tendency for sterility to increase as the time between irradiation and emergence increased. We were able to detect a significantly higher level of sterility in males irradiated 3 days before emergence than in males irradiated 1 or 2 days pre-emergence with 5 or 7 krad. With all doses (5, 7 and 9 krad) the

sterility of flies irradiated 3 days before emergence was greater than that of flies irradiated 1 day pre-emergence. The data presented also show that as the time of irradiation approached adult emergence the competitiveness of the flies increased. This conclusion is in agreement with the work of Katiyar et al. [8], which was based on a different approach, namely the ability of irradiated males to inseminate females.

The experiment to investigate the permanence of sterility induced in males by 5, 7 and 9 krad showed that regardless of whether the irradiated males were virgin or had mated, no recovery of fertility occurred up to 26 days after irradiation. In our other experiments we have never had any indication that the induced sterility was not permanent and Katiyar's group has not reported any loss of sterility. On the other hand, Steiner and his co-workers [2, 10] reported that with a dose of 8.4 krad or lower there occurred 'a substantial loss of sterility in males after 30 - 50 days'. However, these papers deal with two other Tephritid species as well as C. capitata and it is not clear whether the above conclusion is true of all three species. The histological work of Causse et al. [18] does not lend support to a re-population of the testes with sperm after irradiation. The apical region of testes of 4-day-old males which had received 4 or 5 krad two days before emergence was disorganized and consisted of de-membranated bundles of nuclei. If the situation in C. capitata is comparable with that in other Diptera which have been studied, one might expect that if there was to be replenishment of sperms from undamaged gonial cells it would have occurred about two weeks after irradiation [19].

ACKNOWLEDGEMENTS

The help of Mrs. M. Gallowitsch in the conduct of these experiments is gratefully acknowledged. Dr. A. Wakid also assisted with some of the work.

REFERENCES

[1] HOOPER, G. H. S., "Sterilization of the Mediterranean fruit fly: a review of laboratory data", in: Sterile-male Technique for Control of Fruit Flies (Proc. Panel Vienna, 1969) IAEA, Vienna (1970) 3-12.

[2] STEINER, L. F., MITCHELL, W. C., BAUMHOVER, A. H., Progress of fruit fly control by irradiation sterilization in Hawaii and the Marianas Islands, Int. J. appl. Radiat. Isotopes 13 (1962) 427-434.

[3] MELLADO, L., CABALLERO, F., ARROYO, M., JIMENEZ, A., Esayos sobre irradiación de Ceratitis capitata (Wied.) por el método de los "machos estériles" en la isla de Tenerife, Estacion de Fitopatologia Agricola de Madrid, Trabajos (Serie Fitopatologia) No. 399 (1966) pp. 29.

[4] MELLADO, L., NADEL, D.J., ARROYO, M., JIMENEZ, A., "Mediterranean fruit fly suppression experiment on the Spanish mainland in 1969", Short contribution to: Sterile-male Technique for control of Fruit Flies (Proc. Panel Vienna, 1969) IAEA, Vienna (1970) 91-92.

[5] RHODE, R. H., "Application of the sterile-male technique in Mediterranean fruit fly suppression: a follow-up experiment in Nicaragua", in: Sterile-male Technique for Control of Fruit Flies (Proc. Panel Vienna, 1969) IAEA, Vienna (1970) 43-50.

[6] NADEL, D.J., GUERRIERI, G., "Experiments on Mediterranean fruit fly control with the sterile male technique", Sterile Male Technique for Eradication or Control of Harmful Insects (Proc. Panel Vienna, 1968) IAEA, Vienna (1969) 97-105.

[7] MURTAS, I. D. de, CIRIO, U., GUERRIERI, G., ENKERLIN, D., 'An experiment to control the Mediterranean fruit fly on the island of Procida by the sterile insect technique", in: Sterile-male Technique for Control of Fruit Flies (Proc. Panel Vienna, 1969) IAEA, Vienna (1970) 59-70.

[8] KATIYAR, K.P., FERRER, F., VALERIO, J., In: The Application of Nuclear Energy to Agriculture, Triennial Report, 1 July 1963 - 30 June 1966 of the Inter-American Institute of Agricultural Sciences of the OAS, Turrialba, Costa Rica (1966) 45-60.

[9] HOOPER, G.H.S., NADEL, D.J., "The pre-release phase of the 1969 Mediterranean fruit fly suppression experiment in Italy", in: Sterile-male Technique for Control of Fruit Flies (Proc. Panel Vienna, 1969) IAEA, Vienna (1970) 51-58.

[10] STEINER, L.F., CHRISTENSON, L.D., Potential usefulness of the sterile release method in fruit fly eradication programs, Proc. Hawaiian Acad. Sci. 3 (1956) 17-18.

[11] KATIYAR, K.P., VALERIO, J., "Further studies on the possible use of sterile-male release technique in controlling or eradicating the Mediterranean fruit fly, Ceratitis capitata (Wied.) from Central America, Fifth Inter-American Symposium on the Peaceful Applications of Nuclear Energy, 9-13 March 1964 (1964) 197-202.

[12] KATIYAR, K.P., RAMIREZ, E., "Sterilization of the Mediterranean fruit fly and its application to fly eradication", in: The Application of Nuclear Energy to Agriculture, Triennial Report 1 July '66 - 30 June '69 for Contract AT(30-1)-2043 of Inter-American Institute of Agricultural Science of the OAS, Turrialba, Costa Rica (1969) 90-105.

[13] FERON, M., Stérilisation de la mouche Méditerranéenne des fruits, Ceratitis capitata (Wied.), par irradiation des pupes aux rayons gamma, Annls Épiphyt. 17 (1966) 229-239.

[14] SCHERNEY, F. von, HAISCH, A., Zur Zucht und Sterilisation von Schadinsekten, insbesondere der Mittelmeerfruchtfliege Ceratitis capitata (Wied.), Anz. Schädlingsk. 41 (1968) 7-14.

[15] KATIYAR, K.P., "Possibilities of eradication of the Mediterranean fruit fly Ceratitis capitata (Wied.) from Central America by gamma-irradiated males", Fourth Inter-American Symposium on the Peaceful Application of Nuclear Energy, 9-13 April 1962, Mexico City, 2 (1962) 211-217.

[16] LINDQUIST, A.W. (Ed)., Insect Population Control by the Sterile-Male Technique (Report Panel Vienna, 1962) Technical Reports Series No. 21, IAEA (1963) 28-29.

[17] ARROYO, M., JIMENEZ, A., MELLADO, L., CABALLERO, F., Aplicacion de isótopos radiactivas a la investigación de métodos sobre mucha biológica contra las plagas. III. Obtencion de "machos estériles" de Ceratitis capitata (Wied.), mediante la irradiacion de sus pupas con rayons gamma, Estacion de Fitopatologia Agricola de Madrid, Tabajos (Serie Fitopatologia) No. 390 (1965) 24.

[18] CAUSSE, R., FERON, M., PEREAU-LEROY, P., Effets comparés d'irradiations aux rayons gamma et aux neutrons sur les organes reproducteurs de la mouche Méditerranéenne des fruits Cetatitis capitata (Wied.) (Diptera: Trypetidae), Isotopes and Radiation in Entomology (Proc. Symp. Vienna, 1967) IAEA, Vienna (1968) 355-364.

[19] RIEMANN, J.G., THORSON, B.J., Comparison of effects of irradiation on the primary spermatogonia and mature sperm of three species of Diptera, Ann. ent. Soc. Am. 62 (1969) 613-617.

[20] NADEL, D.J., in: Advances in Insect Population Control by the Sterile-male Technique (La BRECQUE, G.C., and KELLER, J.C., Eds) Technical Reports Series No. 44, IAEA, Vienna (1965) 14-17.

DISCUSSION

B. A. G. SIGWALT: Mr. Taylor in his paper considered the possibility of mass rearing of Ceratitis capitata on a global scale by employing techniques involving a nitrogen atmosphere and chilling. Mr. Hooper, on the other hand, stressed that the results he was presenting applied to the strain reared in Vienna. Is there not a problem here?

G. H. S. HOOPER: On the basis of the doses of gamma radiation required to achieve certain levels of sterility in male Ceratitis capitata, there appears to be little difference between strains studied in several parts of the world. The only exception to this statement is to be found in work from the United Arab Republic.

However, I have no information on possible differences between various laboratory strains with respect, for example, to male competitiveness and longevity.

STERILITY PRINCIPLE FOR CONTROL OF INSECTS AFFECTING MAN AND ANIMALS

(Session III)

Chairman

J.W. WRIGHT (WHO)

Survey paper

MASS REARING OF TSETSE FLIES (Glossina spp.) Recent advances

T. A. M. NASH, A. M. JORDAN, M. A. TREWERN
Tsetse Research Laboratory, University of Bristol,
School of Veterinary Science,
Langford, Bristol, United Kingdom

Abstract

MASS REARING OF TSETSE FLIES (Glossina spp.): RECENT ADVANCES.

In this paper attention is largely confined to the work at Langford, Bristol and Maisons-Alfort, Paris; these are the only centres where tsetse flies are mass-reared. The importance of protecting colonies from contact with insecticide and other noxious substances is stressed, and mention made of likely sources of contamination. A brief account is given of the rearing techniques used at both centres with emphasis on recent innovations. The comparative efficacy of these techniques is illustrated with recent data for rates of insemination, survival, reproductivity, eclosion and pupal weight. Mention is made of experiments undertaken in Africa to test the viability of European laboratory-reared tsetse, marked and released in the field, with that of wild flies. Figures for a year's production at each centre are given, followed by figures based on Langford data which indicate the proportion of a colony which could be released weekly in the field. The paper aims to provide up-to-date information for those who may be contemplating the rearing of tsetse flies on a large scale in Africa.

There are only two centres where tsetse flies are reared on a scale which approaches relevance when viewed against the requirements envisaged for tsetse control by the use of sterile males: these are the Tsetse Research Laboratory, Langford, Bristol, and the Institut d'Élevage et de Médecine Vétérinaire des Pays Tropicaux, Maisons-Alfort, Paris. As the subject of this review is mass rearing, attention will be mainly directed to the work of these two centres.

INSECTICIDAL CONTAMINATION

Attempts to rear tsetse are doomed to failure unless stringent precautions are taken to exclude the introduction of insecticides, even in minute quantities. Examples of sources of contamination will be given.

The most difficult is the unidentified source, which can only be overcome by taking precautionary measures against postulated contamination. Three such incidents have occurred at Langford. The first was in the latter half of 1964 when the death rate among fertilized females rose from 41 to 53% for the first 30 days after fertilization and from 38 to 58% for the next 30 days; the start of this toxic period coincided with the dipping of the University sheep with dieldrin 50 m from the tsetse laboratory [1]. Precautions, which include the regular clipping and washing of the goats, have prevented any further large-scale contamination. In late 1965 an abnormal death rate was confined to flies in newly made-up cages; it was found that the woman who had the contract for re-netting the cages had

started doing the work on the sofa in her sister's house; the sister was a farmer's wife. Subsequently, all newly covered cages were hosed with water before use. The last incident, in 1968, was confined to flies which were fed on rabbit ears; longevity remained normal but reproduction virtually ceased. It was postulated that the long ears must have become contaminated with insecticide from a batch of contaminated hay. After the ears of all rabbits had been washed, tsetse in subsequently made-up cages reproduced normally, but the previously affected flies never regained their reproductivity. Chromatographic analyses of 7 affected flies showed a DDT level of 3.1 nanograms/fly as against only 1.3 ng/fly for 7 unaffected control flies which had been fed on goats. The ears of all rabbits are now washed at monthly intervals. A recent analysis of Langford goat-fed flies showed a DDT level of only 0.06 ng/fly, which suggests that the precautionary measures taken have been very effective.

To turn to more concrete sources of contamination; newly purchased animals should be washed in hot water with soft soap (Sapo Mollis, B.P.), and test flies should be fed off them before they become part of the fly-feeding stock. The animal house should be carefully supervised, more especially if it holds animals used by various departments. In July 1965, the Lisbon tsetse colony was seriously depleted by the unauthorized spraying with dieldrin of the guinea pig room, to eliminate cockroaches. Three days after application the room was thoroughly hosed down and the guinea pigs replaced. By January 1966 the G. morsitans colony had decreased from 2216 adults to about 200 [2]. In Antwerp a decrease in the G. morsitans colony was associated with the hanging of a Vapona strip (DDVP, dichlorvos) from the ceiling of the small animal room. It was suggested that the insecticide was taken up from the atmosphere in the greasy layers of guinea pigs' skin [3]. Even though Vapona strips are banned from animal and tsetse breeding rooms, their presence in the same building can be dangerous. Thus one day at Maisons-Alfort mortality among reproducing females rose to over 10%; the cause was traced to Vapona strips which had been placed in adjacent offices [4].

There is no need to use insecticides to control house and stable flies in laboratories. A trap which electrocutes flies (including tsetse) which have been attracted by ultra violet light is on the market. Three years ago one of these traps was installed in the Langford goat house and another in the main tsetse handling room; successful control was established in both rooms. A number of these traps were later installed in a new veterinary post-mortem unit, built within 8 m of the tsetse laboratory goat house; the pathologists find these traps far more effective than the aerosols and Vapona strips which they had used in their old premises.

Mention must be made of the danger inherent in the decoration of tsetse breeding rooms, a danger that may be due to the inclusion of DDT or to the solvent used in the paint. Itard [5] records that despite airing his fly-breeding room for 4 days after repainting, an abnormally high mortality, followed by reduced productivity, occurred in his G. tachinoides colony after it had been put back. Before the Langford breeding rooms were redecorated in 1968, the chief chemist of a large paint manufacturing firm was consulted. He advised the testing of three insecticide-free paints by placing cages of tsetse in newly painted boxes. Only one of the paints was somewhat toxic. The rooms were redecorated, one by one, by the scientific staff, without abnormal mortality among the flies.

Materials which may contain formaldehyde such as varnishes and plywood should be avoided [6].

The realization that stringent precautions against insecticidal contamination are essential is probably the greatest advance that has been made in tsetse breeding in recent years. Schemes to rear tsetse on a massive scale in Africa will fail unless the precautions mentioned above are observed; in addition, the tsetse rearing units should be remote from laboratories, stores or vehicles concerned with insecticides, and exchange visits by staff should be prohibited if working clothes are worn.

SOME DIFFERENCES IN POLICY BETWEEN LANGFORD AND MAISONS-ALFORT

Colonies of G. morsitans, originating from Rhodesia, and of G. austeni from Zanzibar are kept at both centres; the French G. austeni colony is an offshoot from the Langford colony. In addition, a large colony of G. tachinoides from Chad and a less well-established colony of G. fuscipes from the Central African Republic are maintained at Maisons-Alfort.

At Langford the policy is to vary the size of the stock of each species held to comply with the research needs of many other workers, whereas at Maisons-Alfort the intention is to hold the stock at 2000 females for each of four species. At both centres the total stock of female tsetse flies is currently about 7000.

The biggest difference in method is that since January 1967 all tsetse flies at Maisons-Alfort have been fed on the ears of lop-eared rabbits, using the technique devised by Nash, Jordan and Boyle [7], wheras at Langford only 11% of the G. morsitans and 21% of the G. austeni are fed on this host, the rest being fed on the flanks of goats. At Langford preference has been given to the goat as a hardy animal, readily available in many parts of Africa, which can feed far more flies in a given time than the rabbit, even though the latter is a better host judging from fly performance. The policy is to rely on two dissimilar host species lest one is decimated by a specific disease, or one animal house becomes contaminated by insecticide.

A BRIEF COMPARISON OF TECHNIQUES EMPLOYED AT LANGFORD AND MAISONS-ALFORT

Maintenance climate

Langford. All adults are kept at 25°C, with 60-70% relative humidity for G. morsitans and 70-80% r.h. for G. austeni; all pupae are kept on the bottom shelves where the temperature is 23.5°C [8].

Maisons-Alfort. Adults and pupae of all species are kept at 25°C; adults over 10 days old are kept at 70-75% r.h., but younger flies and pupae at 75-80% r.h.

Fertilization

Langford. A fed, 3-day-old female is put in a 2.5 cm × 7.8 cm tube; a male at least 15 days old is added (a male is rarely used more than twice). The pairs are left together for 24 hours [8]. The insemination rate for 740 G. austeni and 427 G. morsitans dissected was in each case 98.8%.

Maisons-Alfort. Ten males, 7-10 days old, are put in a cage with ten females, 3-4 days old; they are left together for 4-6 days [9]. No insemination rate data have been published.

Adult maintenance

Cages

Langford. Tsetse are kept in considerably modified Roubaud-type cages: a stainless steel wire frame, with a closed end fitted with a cork-hole, is covered with black Terylene netting having a 2.5-3.0 mm mesh through which the larvae can escape [10]. Two sizes are used. The large size, 25.4 cm × 12.7 cm × 5.1 cm deep, holds 25 goat-fed female tsetse; the small size, 15 cm × 8.5 cm × 5 cm deep, is now stocked with 15 rabbit-fed females instead of 10.

Maisons-Alfort. The cage type is basically similar to the above but made with a plasticized metal frame, with a net bag instead of a closed end; the dimensions are 14 cm × 8.5 cm × 5 cm [9]. The cage is stocked with 30 rabbit-fed flies [11].

Cage storage

Maisons-Alfort. An ingenious metal rack has been devised to hold 10 small-sized cages containing 300 female flies. The cages, standing on a narrow side, are placed one behind the other down the length of the rack; below is a sloping gutter or chute; the larvae fall into the chute, roll down to the front of the rack and drop into a small tray lined with tissue paper. Here they pupate and are collected daily. The subsequent emergence rate remains at around 92% [11].

Langford. Impressed by the economy in space and the speed of pupal collection when using Itard and Gruvel's rack, their basic idea has been adopted, but the design is different. The cages are arranged as described above. Running the length of the rack are two metal rods, across which the cages stand; below are 2 metal sheets which slope downwards at an angle of 15° to produce a median slot, 14 mm wide; the larvae fall through the slot into a tray which runs the full length of the rack. The median slot is protected by a metal 'faecal catcher', which prevents contamination of the pupae. Each rack holds 250 goat-fed flies kept in the larger type of cage. The rack is made of half-hard sheet aluminium, 1.2 mm thick, which is suitable for bending in a folding press. The subsequent emergence is higher than 92%, but this is probably due to the factors referred to in the next sub-section.

Possible advantages of the Langford design are that the racks can be made cheaply in an institute's workshop instead of by an engineering

company; the larvae do not fall into a chute contaminated by the liquid faeces of the adults and the vertical space required for a loaded rack is much less as the back need not be higher than the front to produce the slope.

Pupal maintenance

Langford. The collection of pupae is poured daily onto dry sand in a 30.5 cm × 25.5 cm × 5.0 cm deep enamel tray, until the total has reached about 2500 pupae. Several days before emergence is due, the tray is placed in an emergence cage 30.5 cm × 30.5 cm × 38.0 cm high: the cage has a Perspex top and black netting sides, one of which is a sleeve to facilitate the collection of young flies: an illumination of 7 lux on the soil surface is provided for 12 hours daily. Previously, G. austeni pupae were kept in pots, but 10.9% of emergences failed to develop their wings, giving an effective emergence rate of only 86.9%; comparable figures after using the new technique were 0.7% and 98.0% from 11 400 pupae. The cause of the virtual elimination of 'crippling' is that, given space and light, the first hatched flies climb up the sides of the cage and do not disturb those that are crawling out of their pupal cases [8].

More recent results from 16 600 G. austeni pupae gave an effective emergence rate of 98.5%. The comparable figure from 8000 G. morsitans pupae was 96.0%; 98.9% can be achieved if sand is put in the tray, but this increases labour.

Maisons-Alfort. Pupae are collected daily and are kept at the rate of 30-35 per Borrel tube, until emergence. (These are ordinary glass tubes believed to be about 3.5 cm in diameter and 8 cm high.) The following emergence rates have been recorded: G. austeni 89-93%: G. morsitans 93%: G. tachinoides 90-94%: G. fuscipes over 90% [9]. These are believed to be overall rates from which no deduction has been made for flies that fail to develop their wings.

Fly feeding and performance

Using rabbits as hosts

Although rabbit-fed flies produce only 15% of the Langford pupal output, this host will be dealt with first since it allows a direct comparison with Maisons-Alfort.

The fly feeding method has been described [7]. Briefly, a lop-eared rabbit is placed in a close-fitting box provided with two ear-rests. A soft pad, provided with elastic straps, is placed on the rest, the ear is laid on the pad, the cage placed on top of the ear and the whole secured with the straps. The wooden end to the box has been replaced by rubber sheeting to reduce the risk of a compression fracture of the spine if the rabbit kicks backwards.

Langford. Three groups of rabbits are used; each group is brought in for fly feeding on every 3rd day – or 4th day when a Sunday intervenes. Initially, two cages, each holding up to 10 female flies, were applied to each ear for 15 min. The number of flies applied to a rabbit did not exceed 160/day; about half of the flies would feed [12]. Currently 15 females are kept per cage, and the feeding time is reduced to 10 min; this economy does not appear to have lowered productivity or pupal weight.

TABLE I. FEMALE PERFORMANCE USING RABBITS AT LANGFORD

	Reproductivity during life-span		Survival by day 60 after	Mean pupal weight Pupae 0-24 h old	
	No. ♀♀	Mean pupae/♀	fertilization[a]	No. pupae	Weight
G. austeni	1171	14.2	88%	4708	23.8 mg
G. morsitans	885	10.6	95%	4293	30.7 mg

[a] 'Infantile mortality' by completion of fertilization on the 4th day of life is 3-4%; hence these rates should be reduced accordingly to equate to those given in Table II for Maisons-Alfort.

Great caution is exercised at Langford before introducing economies in fly feeding lest the quality of stock be impaired. Since tsetse nutrition is based upon protein, mainly from haemoglobin, a fall in this level could reduce fly performance and pupal weight. The 'challenge' per rabbit, in a team of three, was raised from 160 to 400-450 female G. austeni, whose performance was recorded. Two of the rabbits were alive after 380 days, but one died from unrelated causes after 310 days. Blood pictures were taken at long intervals, lest the actual bleeding falsify the picture. The 6 to 8 pictures for each rabbit showed that in no instance was the haemoglobin level ever as high as it had been originally; it fluctuated at levels which were often appreciably lower. Towards the end of the experiment the mean pupal weight and survival at day 60 were above average, but the mean reproductivity was below. A similar experiment was undertaken with G. morsitans in which the 'challenge' was raised to 270-300 females applied; survival was average and pupal weight and reproductivity slightly above. It was impossible to obtain a full series of blood pictures, but it seems that 270-300 G. morsitans applied to rabbits on feeding days is acceptable to both rabbit and tsetse, whereas 400-450 G. austeni is near the limit as it seems to lower the haemoglobin level and reduce productivity.

The average female performance from tests started between April 1968 and October 1969 is as shown in Table I.

Maisons-Alfort. Fly performance data, presented in a form comparable to that given for Langford, are only available for 1968 [9, 13]; these are given in Table II. However, it is known from personal communications from Itard of June 1970 that major changes in technique have been made, some of them only recently.

All flies are offered food daily, Sundays excepted, for 3-4 min. Originally, each rabbit was brought in daily for one week and then rested for 3 weeks; about 1000 flies were applied daily, in cages holding 15-20 flies [13]. In November 1968, each group of 6 rabbits was brought in every 5th or 6th day and over 1200 flies were applied to each rabbit; each cage then held 20-30 flies [9]. These changes will have speeded up fly feeding, but do not seem to have improved female fly performance or pupal weight [Itard, private communication]. Since January 1970, a group of 8 rabbits has been used once a week, when about 1400 flies are applied to each beast; each cage now holds 30-38 flies. Rabbit mortality is very

TABLE II. FEMALE PERFORMANCE USING RABBITS AT MAISONS-ALFORT

	Reproductivity during life-span		Survival by day 60 after emergence	Mean pupal weight Pupae 0-24 h old
	No. ♀♀	Mean pupae/♀		
G. austeni	279	8.5	77%	23.3 mg
G. morsitans	888	3.8	71%	26.4 mg
G. tachinoides	100	7.0	68%	16.2 mg
G. fuscipes	648[a]	5.2[a]		30.9 mg

[a] From figures for year ended June 1969 [Itard, personal communication].

TABLE III. AVERAGE FEMALE PERFORMANCE USING GOATS

	Reproductivity during life-span		Survival by day 60 after fertilization	Mean pupal weight Pupae 0-24 h old	
	No. ♀♀	Mean pupae/♀		No. pupae	Weight
G. austeni	2750	9.7	87%	2871	23.6 mg
G. morsitans	8000	7.5	80%	8867	29.6 mg

low, and there is no weight loss [Itard, private communication], but no mention is made of the blood picture. The races of lop-eared rabbits used at Maisons-Alfort are bigger than those used at Langford. For example, adults of their Bouscat race weigh 5-7 kg [9], whereas the mean weight of the Langford rabbits is 3.6 kg, the largest weighing only 5 kg.

The figures given in Table II for female performance at Maisons-Alfort were collected in 1968 [9].

Using goats as hosts at Langford

The fly-feeding method has been described in detail [14], as have some subsequent modifications [8]. Goats are mounted on trolleys. Six of the large-size cages are strapped onto the flanks of each fully grown goat, enabling up to 600 flies to be applied in an hour. Cages are changed at 15-min intervals. The same group of goats is brought in on every 3rd day – or 4th day if a Sunday intervenes. (Four goats are still in regular use after 7 years of this regimen.) A total of 700 flies can be safely applied to a goat on a feeding day; a current experiment which has run for 9 months suggests that 1000 flies may be safe. In the case of goats, which weigh up to 73 kg, excess fly feeding would be most unlikely to induce anaemia but it might thicken the hide and so inhibit probing. The average female performance from tests started between April 1968 and October 1969 is given in Table III. The mean weight recently obtained for 3000 G. morsitans pupae from the rack-maintained females was 30.1 mg.

TABLE IV. PRODUCTIVITY OF THE COLONIES

	Mean ♀ stock [a]	Total pupae deposited	Pupae and adults	
			Given away	Destroyed [b]
LANGFORD, 1.6.69 - 31.5.70				
G. austeni	2 906	76 599	41 908	9 013
G. morsitans	3 543	91 009	51 879	7 991
Totals:	6 449	167 608	93 787	17 004
MAISONS-ALFORT, 14.12.68 - 8.12.69 [c]				
G. austeni	2 178	54 238	31 322	
G. morsitans	2 025	36 546	13 087	
G. tachinoides	2 458	56 415	24 348	
G. fuscipes	475	5 808	53	
Totals:	7 136	153 007	68 810	

[a] Includes virgin females. [b] Adults produced in excess of demand. [c] Personal communication from Itard.

Occasionally a goat becomes unsuitable as a host in that many flies fail to obtain blood from their probings; recognition of this state is important. At about 4-monthly intervals, each goat is tested for host suitability, an index being obtained from the proportion of previously unfed, 2-day-old male flies which feed off the beast. Unsuitability may be due to sensitization, thickening and hardening of the skin or poor configuration due to old age or recent parturition [8]. Since the policy was introduced of breeding from the goats used for fly feeding, challenging the kids with 10 fly bites within 24 hours of birth and thereafter slowly raising the challenge, sensitization and its associated ill effects have ceased to present a problem; it would seem that immunological tolerance against the saliva of tsetse may have been induced in the Langford herd [15]. It is essential to good fly feeding that the goats be close-clipped at fortnightly intervals; washing at 2-monthly intervals removes dried tsetse faeces. In addition, these measures guard against a slow build-up of insecticidal contamination.

VIABILITY IN THE FIELD, OF LABORATORY-REARED TSETSE

The concept of tsetse control by releasing large numbers of sterile males is dependent on the assumption that laboratory-reared males will be fully viable in the field.

Langford. In April and May 1969, 3000 pupae of G. morsitans were sent to Rhodesia for testing. When males of the Langford colony were released into their original field habitat 2 years after colonization, they survived and dispersed as well as the native flies which served as controls [16]. No differences were detected in mating studies conducted in the laboratory [D. A. Dame, personal communication].

Maisons-Alfort. During 1969, 4370 adult and 5608 pupae of G. tachinoides were sent to Fort Lamy for field release after marking. The results are not yet available, but several flies were recaptured more than 15 days after release [Itard, personal communication].

PRODUCTIVITY OF THE COLONIES

Data for the productivity of the tsetse colonies maintained at Langford and Maisons-Alfort are given in Table IV. These data indicate that the somewhat smaller Langford stock of females is more productive than the larger stock kept at Maisons-Alfort; this was to be expected from the data for fly performance given earlier. It must be pointed out, however, that Itard and his colleagues are more interested in producing sufficient material for their research by the easiest method rather than in devising techniques to exploit the maximal reproductive potential of tsetse flies [9].

The ultimate longevity of G. morsitans is considerably less than that of G. austeni, a factor which contributes to its lower productivity as indicated earlier in Tables I-III. Output is, however, not as greatly reduced as might be expected from the figures shown because in both species early survival is good and, since there are more young flies in a colony than old, it is the young females which make the major contribution to the output. Using methods already described [17, 18], it has been found from the

Langford data that, depending on the host used and whether unsexed pupae or adult males were to be released, the weekly surplus of G. austeni males could range from 21 to 30% of the colony size; comparable figures for G. morsitans are 18 to 25%.

ACKNOWLEDGEMENT

All the work at the Langford centre, described above, has been financed by the Ministry of Overseas Development.

REFERENCES

[1] NASH, T.A.M., KERNAGHAN, R.J., BOYLE, J.A., The large-scale rearing of Glossina austeni (Newst.) in the laboratory I. - The use of pregnant and non-pregnant goats as hosts, Ann. trop. Med. Parasit. 60 1 (1966) 39.

[2] FRAGA DE AZEVEDO, J., PINHÃO, R. DA C., A cultura da mosca tsétsé em Lisboa. Um acidente por insecticida, Anais Inst. Med. trop., Lisb. 23 1/2 (1966) 11.

[3] VAN DER VLOEDT, A.M.V., EVENS, F.M.J.C., CALLENS, G.M.J., Rearing of Glossina morsitans II. - Development of a colony of Glossina morsitans and "vapona strip" influence, Annls Soc. belge Méd. trop. 48 2 (1968) 165.

[4] ITARD, J., MAILLOT, L., BRUNET, J., GIRET, M., Observations sur un élevage de Glossina tachinoides West., après adoption du lapin comme animal-hôte, Rev. Elev. Méd. vét. Pays trop. 21 3 (1968) 387.

[5] ITARD, J., "Résultats récents d'un élevage de Glossina tachinoides West. entrepris à Maisons-Alfort", Control of Livestock Insect Pests by the Sterile-male Technique (Proc. Panel Vienna, 1967) IAEA, Vienna (1968) 71.

[6] NASH, T.A.M., KERNAGHAN, R.J., Danger of spraying wooden insect cages with melamine resins, Nature, Lond. 203 4950 (1964) 1195.

[7] NASH, T.A.M., JORDAN, A.M., BOYLE, J.A., A promising method for rearing Glossina austeni (Newst.) on a small scale, based on the use of rabbits' ears for feeding, Trans. R. Soc. trop. Med. Hyg. 60 2 (1966) 183.

[8] NASH, T.A.M., JORDAN, A.M., BOYLE, J.A., The large-scale rearing of Glossina austeni Newst. in the laboratory IV. - The final technique, Ann. trop. Med. Parasit. 62 3 (1968) 336.

[9] ITARD, J., MAILLOT, L., Les élevages de glossines à Maisons-Alfort (France), Proc. First Symp. Tsetse Fly Breeding under Laboratory Conditions, Lisbon 1969 (in press).

[10] NASH, T.A.M., JORDAN, A.M., BOYLE, J.A., The large-scale rearing of Glossina austeni (Newst.) in the laboratory III. - Confirmation of the value of pregnant goats as hosts, Ann. trop. Med. Parasit. 60 4 (1966) 469.

[11] ITARD, J., GRUVEL, J., Description d'un appareil destiné au stockage des femelles de Glossines et à la récolte des pupes, Rev. Elev. Méd. vét. Pays trop. 22 2 (1969) 289.

[12] JORDAN, A.M., NASH, T.A.M., BOYLE, J.A., The rearing of Glossina austeni Newst. with lop-eared rabbits as hosts I. - Efficacy of the method, Ann. trop. Med. Parasit. 61 2 (1967) 182.

[13] ITARD, J., MAILLOT, L., Elevages de glossines à Maisons-Alfort: résultats - intoxication accidentelle par insecticide, Proc. XIIth Meeting ISCTR Bangui 1968 (in press).

[14] NASH, T.A.M., KERNAGHAN, R.J., The feeding of haematophagous insects on goats and sheep: techniques for host restraint and cage application, Ann. trop. Med. Parasit. 58 2 (1964) 168.

[15] NASH, T.A.M., Possible induction in goats of immunological tolerance against the saliva of tsetse flies (Glossina spp.), Trop. anim. Hlth. Prod. (in press).

[16] DAME, D.A., SCHMIDT, C.H., The sterile-male technique against tsetse flies, Glossina spp., Bull. ent. Soc. Am. 16 1 (1970) 24.

[17] JORDAN, A.M., CURTIS, C.F., Productivity of Glossina austeni Newst. maintained on lop-eared rabbits, Bull. ent. Res. 58 2 (1968) 399.

[18] CURTIS, C.F., JORDAN, A.M., Calculations of the productivity of Glossina austeni Newst. maintained on goats and on lop-eared rabbits, Bull. ent. Res. 59 4 (1970) 651.

DISCUSSION

Rachel GALUN: Would you care to give a rough estimate of the cost of an adult fly produced by your technique?

A.M. JORDAN: At Langford we have not made any estimates of production costs. Under our present system it would be impossible to differentiate between production for research purposes and production for disposal. Costs could be reduced if all research were discontinued. Costs would also depend very largely on where mass rearing was undertaken. Labour, the availability and cost of obtaining and maintaining host animals and building costs all show much variation from place to place.

A. MEWS: As one of the small breeders of tsetse flies that Mr. Jordan referred to in his excellent paper, perhaps I might make one or two comments. To speed up handling, the flies are immobilized by cooling them in a stream of air at 4°C in a converted deep-freeze cabinet. The flies become immobilized within two or three minutes and recover within about one minute of being removed from the cabinet.

To save time we mass-mate Glossina morsitans. The males are cooled and placed in a special tube with funnel attached. They are allowed to recover for about half an hour and are then introduced into the female cage in the proportion of 12 males to 10 females. The sexes are separated after 3-4 days, again by cooling the flies, two males being left in the female cage to ensure complete mating. Of 56 10-day-old female flies dissected so far, none were found to have empty spermathecae.

A.M. JORDAN: When Glossina austeni was mass-mated at Langford only about 88% of the females were found to be inseminated. This compares with 98.8% when the flies are mated individually in tubes. This means that some 10% of unproductive flies would have to be kept if mass-mating were employed. Individual mating also means that the flies only have to be sexed once, when young flies are collected from the emergence cages. With two flies per tube at the end of the mating period, it is only necessary to determine the sex of one individual and thus only half the flies have to be sexed at this time.

E.D. OFFORI: As a prospective breeder, I find your paper most informative.

In view of the difficulty of obtaining lop-eared rabbits in Africa, I wonder whether you could comment on the possibility of using the West African dwarf goat for feeding tsetse flies in the laboratory.

A.M. JORDAN: The West African dwarf goat has been used for feeding Glossina in Nigeria. I see no reason why, with good husbandry, it should not prove to be a satisfactory host. It is a smaller breed than the British breeds we use at Langford, and this would probably mean that only two cages of flies should be applied to each flank, compared with the three we can apply at Langford.

D.G. CAMPION: Since sub-lethal doses of insecticides act as reproduction inhibitors, is there any evidence of such an effect in the field after insecticide has been applied and, if so, which insecticide was the most effective?

A.M. JORDAN: Sub-lethal doses of insecticides have been shown to inhibit the reproduction of Glossina in the laboratory, but not, as far as I know, in the field. I see no reason, however, why the phenomenon should not occur in the field.

J. ITARD: May I comment further on a few of the points that have been raised.

Firstly, as regards the use of cooling for anaesthetizing the flies for sexing, the use of CO_2 might possibly offer advantages over this method.

Secondly, with respect to the feeding of flies on lop-eared rabbits, the rabbits currently used as hosts at Maisons-Alfort are of the Bouscat breed. They are not lop-eared, but they do have ears that are 15 - 20 cm long, and they have more resistance than the lop-eared variety. They have been used in the Central African Republic and crossed with a local breed.

Finally, I should like to mention that at Maisons-Alfort we use the mass-mating method. Mating takes place in cages containing 10 females 3 - 4 days old and 10 males 7 - 10 days old. The pairs are separated 4 - 6 days after mating. This method results in a fecundation rate of 90 to 95%. It is used in preference to the individual mating method applied at Langford because four different species are reared at Maisons-Alfort and the available space is limited.

J. W. WRIGHT (Chairman): I should like to ask Mr. Jordan whether long laboratory colonization might not result in the selection of a strain that will not be able to survive in nature.

A. M. JORDAN: That is possible. The position of the Langford colony of Glossina morsitans has recently been investigated in two ways, in co-operation with Mr. Dame and his colleagues in Rhodesia. He released and marked some 3000 Glossina morsitans which had been bred at Langford. This stock had originated in the same part of Rhodesia and had been colonized for two years. The insects were put into the field as pupae. Recapture of marked flies showed that the laboratory-reared insects survived as well and travelled as far as wild flies. Their ability to compete for mates in the field was not determined. Mr. Dame also sent fresh supplies of wild pupae to Langford, where we undertook tests on the performance of all possible crosses between flies of Langford and Rhodesian origins. On the basis of these, we concluded that the Langford colony was performing neither abnormally badly, which would have suggested that it was suffering from the adverse effects of inbreeding, nor abnormally well, which would have suggested adaptation to the laboratory.

J. W. WRIGHT: I have one more question. Is artificial insemination of the tsetse fly possible?

A. M. JORDAN: It has not been attempted as far as I know.

TECHNIQUES DE MARQUAGE DES GLOSSINES AU MOYEN DE RADIOISOTOPES

D. CUISANCE, J. ITARD
Institut d' élevage et de médecine vétérinaire
des pays tropicaux,
Maisons-Alfort, France

Abstract — Résumé

TECHNIQUES FOR LABELLING TSETSE FLIES WITH RADIOISOTOPES.

Four radioactive substances injected intravenously into the rabbit, which was used as host, were tested for labelling tsetse flies (Diptera-Muscidae), which are strictly hematophagous and which are the vectors of African trypanosomiasis. The purpose of the tests was to study the dynamics of populations in the field. The radioisotopes studied were: chromium-51, iron-59, cobalt-57 and zinc-65. The iron-59 and chromium-51, in particular, were chosen because of their affinity for the formed elements of blood. Chromium-51 (half-life: 27.8 days) has a biological half-life of 2 to 3 days in the tsetse fly. This radionuclide did not reappear in the progeny. Maximum elimination of the chromium-51 took place on the second and third days after feeding on the labelled rabbit. Iron-59 (half-life: 44.5 days) has a biological half-life of 2 to 3 days in the male tsetse fly, but the counting rate is higher than that for the controls over a period of more than 20 days. The average biological half-life is 24 days in fertilized females; it is dependent on the egg-laying. The radionuclide appears in the progeny; strictly speaking, it is fixed on the nymph, the puparium showing only very slight radioactivity. Cobalt-57 (half-life: 267 days) has a biological half-life of 36 hours in the male tsetse fly and of 3 to 4 days in the female. The radionuclide was not found in the progeny. The radioactivity of the excrement increases very rapidly between the third and seventh days. The quantities of zinc-65 (half-life: 245 days) at disposal were not enough to make a complete study. However, the first results indicate that the duration of labelling in the tsetse fly is at least 15 days after several radioactive meals and that the radioisotope reappears in the progeny.

TECHNIQUES DE MARQUAGE DES GLOSSINES AU MOYEN DE RADIOISOTOPES.

Quatre corps radioactifs injectés, par voie intraveineuse, au lapin utilisé comme animal hôte, ont été expérimentés pour le marquage des glossines (Diptera-Muscidae), insectes strictement hématophages, vecteurs des trypanosomiases africaines, dans le but d'étudier, sur le terrain, la dynamique des populations. Les radioisotopes étudiés sont: le chrome-51, le fer-59, le cobalt-57 et le zinc-65. Le fer-59 et le chrome-51, en particulier, ont été choisis pour leurs affinités pour les éléments figurés du sang. Le chrome-51 (période: 27,8 jours) a une période biologique, chez la glossine, de 2 à 3 jours. Ce radioélément n'a pas été retrouvé dans la descendance. L'élimination du chrome-51 est maximale les deuxième et troisième jours après le repas sur le lapin marqué. Le fer-59 (période: 44,5 jours) a une période biologique de 2 à 3 jours chez les glossines mâles, mais le taux de comptage est plus élevé que celui des témoins pendant plus de 20 jours. La période biologique est de 24 jours en moyenne chez les femelles fécondées; elle est conditionnée par la ponte. Le radioélément se retrouve dans la descendance; il est fixé sur la nymphe sensu stricto, le puparium ne présentant qu'une très faible radioactivité. Le cobalt-57 (période: 267 jours) a une période biologique de 36 heures chez les glossines mâles et de 3 à 4 jours chez les glossines femelles. Le radioélément n'a pas été retrouvé dans la descendance. La radioactivité des excréments croît très rapidement entre les troisième et septième jours. Les quantités de zinc-65 (période: 245 jours) dont on disposait n'ont pas permis d'effectuer une étude complète. Cependant, les premiers résultats obtenus indiquent que la durée de marquage chez la glossine est d'au moins quinze jours après plusieurs repas radioactifs et que le radioisotope se retrouve dans la descendance.

INTRODUCTION

Le projet de lutte contre les glossines par la méthode du lâcher de mâles stériles qui doit être réalisé en République Centrafricaine prévoit que les mâles stériles seront marqués par une technique aussi inoffensive

que possible afin de pouvoir étudier leur comportement sur le terrain [1]. C'est dans le cadre de ce projet qu'ont été réalisés les essais de marquage radioactif qui font l'objet de la présente communication. Ces études ont été effectuées à la Division de biologie (Secteur entomologie) de l'Etablissement d'Ispra (Italie) du Centre Commun de Recherches EURATOM.

Les insectes utilisés appartiennent à l'espèce Glossina austeni Newstead, provenant de l'élevage réalisé au laboratoire d'entomologie de l'IEMVT à Maisons-Alfort (France). Ces insectes ont été expédiés à Ispra soit à l'état adulte, en boîte isotherme, soit à l'état pupal. Une petite salle climatisée munie d'un évaporateur et d'un radiateur électrique a été installée dans les locaux du Service de biologie d'Ispra pour le maintien de ces insectes.

Des lapins de race «fauve de Bourgogne» ont été maintenus individuellement dans des cages à métabolisme, placées dans un couloir donnant accès à la salle de glossines.

1. TECHNIQUE

Les glossines en élevage sont maintenues dans des cages de type Roubaud identiques à celles utilisées à Maisons-Alfort [2]. Elles sont nourries chaque jour sur les oreilles de deux lapins servant uniquement à l'alimentation des mouches normales.

Deux autres lapins sont utilisés pour nourrir les glossines marquées, afin d'éviter une contamination éventuelle des lapins servant à la nourriture des mouches normales.

Pour le déroulement des expériences, on a constitué des lots de dix mouches maintenues, individuellement, dans une petite boîte de plastique, sans couvercle et à fond mobile, permettant de changer tous les jours le papier «Joseph» qui en garni le fond, afin de recueillir les excréments. La boîte est recouverte d'un carré de tulle à mailles moyennement fines.

Les glossines marquées sont transportées jusqu'à la salle de comptage dans une petite mallette isotherme. Elles sont placées, individuellement, dans un tube de comptage et immobilisées avec un peu de coton afin d'éviter les erreurs de géométrie. Le tube ne sert qu'une fois. Les excréments déposés sur le papier Joseph sont également soumis au comptage.

Les lapins marqués sont maintenus à l'écart des autres lapins, dans des cages à métabolisme auxquelles ont été adjoints une petite citerne destinée à recueillir les urines radioactives et un récipient métallique pour la récolte des excréments. Tous les deux jours 1 ml de sang des lapins marqués est soumis au comptage.

Quatre radioisotopes ont été expérimentés: le ^{51}Cr (période = 27,8 jours); le ^{59}Fe (période = 44,5 jours); le ^{57}Co (période = 267 jours); et le ^{65}Zn (période = 245 jours).

Les raisons ayant dicté ce choix sont les suivantes : les glossines étant strictement hématophages et ayant une longévité nettement supérieure à un mois (certaines femelles vivent plus de 200 jours), les radioisotopes devaient avoir une période physique d'au moins 30 jours avec une période biologique aussi longue que possible; ils devaient être émetteurs de rayons γ à grande énergie, afin d'en rendre la détection facile; ils ne devaient pas être toxiques pour le lapin donneur de sang et pour la glossine; ils devaient enfin présenter, si possible, des affinités pour les éléments sanguins.

Nous n'avons pas expérimenté le ^{32}P, ce corps ayant déjà été utilisé par Azevedo [3] qui ne lui trouve pas des propriétés très intéressantes.

Les radioisotopes ont été utilisés en solution et injectés dans la veine marginale de l'oreille gauche du lapin, les glossines étant ensuite nourries sur son oreille droite, à des intervalles de temps variables.

Les comptages ont été effectués par un analyseur multicanal RCL de 256 canaux comprenant:

a) une sonde à cristal d'iodure de sodium activé au thallium, de 3 pouces sur 3 pouces, à puits de 18 mm de diamètre sur 51 mm de profondeur. Cette sonde donne, pour le pic du ^{137}Cs une résolution de 7,4%. Elle est enfermée dans un château de plomb de 10 cm d'épaisseur, garni à l'intérieur d'une plaque de cadmium absorbant les rayonnements du plomb, et d'une plaque de cuivre absorbant les rayonnements du cadmium. La sonde ne perçoit ainsi aucun rayonnement extérieur ou provenant de l'enceinte.

b) un stabilisateur du zéro et un stabilisateur de pic.

c) une imprimante Hewlett Packard.

2. CHROME-51

2.1. Dilution, étalonnage

Le ^{51}Cr utilisé provient du centre radiochimique d'Amersham (Angleterre). Il est présenté en solution de chromate de sodium, en flacon type pénicilline de 5 ml correspondant à 5 mCi. Cette solution a été diluée dans 29 ml de solvant auxquels fut ajouté 1 ml de pénicilline (100 000 U. I.[1]). Le flacon d'origine a été rincé ensuite trois fois avec 5 ml de solvant, si bien que la solution commerciale radioactive fut finalement diluée dans 45 ml de solvant; 1 ml de cette solution finale correspond à 0,1 mCi.

Un échantillon de cette solution, dilué de façon à obtenir 0,1 μCi par ml, a été soumis au comptage, pendant 15 h, sur 23 canaux définis pour le pic du ^{51}Cr (320 keV). Le comptage a été de 25 048 coups, soit 27,83 coups par minute.

2.2. Injection au lapin

Elle a été pratiquée le jour où la solution possédait, suivant les tables de décroissance, une activité de 5 mCi. Le volume sanguin du lapin est en moyenne de 61,6 ml par kg vif. Le lapin (numéro 1) utilisé pesait 3,2 kg, ce qui correspond à un volume sanguin de 197,12 ml, soit pratiquement 200 ml. 1 ml de solution ayant été injecté dans la veine marginale de l'oreille gauche, le lapin a reçu 100 μCi. Une glossine absorbant 0,2 à 0,3 ml de sang, absorbera donc théoriquement environ 0,1 μCi. Aucun trouble n'a été constaté chez ce lapin après l'injection.

Un autre lapin pesant 3,25 kg (numéro 2) reçoit, le lendemain, 1 mCi de la solution de ^{51}Cr, puis, 35 jours plus tard, à nouveau 1,5 mCi.

[1] Unités Internationales.

TABLEAU I. TAUX DE COMPTAGE DE CINQ GLOSSINES NOURRIES 3 MIN APRES L'INJECTION RADIOACTIVE DE ^{51}Cr AU LAPIN
Nombre de coups pour 5 min

Glossines	Nourries 3 min après l'injection au lapin
1	3809
2	3444
3	1978
4	2917
5	3969
	m = 3223
	sm = 361

m = moyenne
sm = écart type

TABLEAU II. TAUX DE COMPTAGE DE DIX GLOSSINES NOURRIES 1 H APRES L'INJECTION RADIOACTIVE DE ^{51}Cr AU LAPIN
Nombre de coups pour 5 min

Glossines	Nourries 1 h après l'injection au lapin
1	6678
2	6191
3	5246
4	6805
5	4928
6	5520
7	5340
8	3970
9	4426
10	6023
	m = 5512,7
	sm = 293,7

m = moyenne
sm = écart type

2.3. Marquage des glossines

Quinze glossines mâles, vierges, à jeun depuis 24 h, sont placées sur l'oreille droite du lapin numéro 1, trois minutes après l'injection. Cinq de ces glossines, bien gorgées, ont été soumises individuellement au comptage (tableau I), pendant 5 min. La radioactivité est faible.

TABLEAU III. TAUX DE COMPTAGE DE CINQ GLOSSINES MALES (1 à 5) ET CINQ GLOSSINES FEMELLES VIERGES (6 à 10) NOURRIES 15 MIN ET 30 MIN APRES L'INJECTION RADIOACTIVE DE ^{51}Cr AU LAPIN

	Témoins	Coups en 5 min	Glossines	Dates des comptages (coups/min)							
				16.12.69	17.12	18.12	19.12	20.12	22.12	23.12	3.1.70
♂ vierges	1	183	1	2413	2201	677	137	60	51	51	34
	2	188	2	2816	1605	592	329	131	67	67	49
	3	177	3	2262	1814	571	434	184	68	55	51
	4	161	4	1811	1820	1465	343	126	61	73	63
	5	183	5	3796	2994	1365	625	251	69	57	50
♀ vierges	6	160	6	5039	4402	1723	293	91	64	53	46
	7	179	7	2443	2397	2183	1727	685	97	61	40
	8	198	8	3348	3167	2412	881	132	44	52	41
	9	178	9	4373	4281	3641	2864	1643	815	155	81
	10	188	10	4004	3477	1418	252	45	46	56	33
	m = 179,5		m = 3230		2815	1604	788	334	138	68	48,8
	sm = 3,69		sm = 332		321	302	218	598	88	9	4,45
	Excréments témoins	173	Excréments glossines		1565	7747	4721	3255	785	590	39

m = moyenne sm = écart type

TABLEAU IV. TAUX DE COMPTAGE DE DIX GLOSSINES FEMELLES FECONDEES NOURRIES 35 J APRES L'INJECTION RADIOACTIVE DE ^{51}Cr AU LAPIN
(chiffres soulignés = taux de comptage des pupes produites)
Nombre de coups pour 1 min

	Témoins pupes		Gloss. pupes	Date 22.1.70	23	26	28	30	2.2.70	4	6	9	11	13
♀ Accouplées	1	35	1	3028	69	53	40	43	55 <u>34</u>	29	46	32 <u>38</u>	36	26
	2	28	2	1597	1158	864	395	53	43	51	33	28	37	37 <u>29</u>
	3	27	3	2030	294	81	30	37 <u>38</u>	30	39	46 <u>34</u>	24	41	25
	4	30	4	2520	583	33 <u>31</u>	37	35	49	44 <u>27</u>	31	39		
	5	30	5	1539	121	40 <u>39</u>	53	40	31	35	36	35		
	6	36	6	3885	498	147	127	107	94	80	86	65	78	67
	7	22	7	3036	432	344	291	235	224	217	173	198	156	143
	8	30	8	2804	1003	549	506	210	201	158	153	143	135	112
	9	40	9	2474	38	51	31	35	41	45	39	36	43	46
	10	29	10	1194	840	194	44	48	39	36	21	32	34	30 <u>28</u>
	m = 30,7			2410,7	497,6	235,6	155,4	84,3	80,7	73,4	66,4	63,2	70,00	60,75
	sm = 1,61			261,7	124,8	87,2	55,8	24,0	22,7	20,0	17,0	18,6	17,3	15,6

m = moyenne sm = écart type

Quinze autres glossines sont mises à nouveau sur l'oreille droite du lapin numéro 1 une heure après l'injection. Dix d'entre elles, bien gorgées, sont soumises au comptage pendant 5 min. Les taux sont nettement plus élevés (tableau II).

Dix mâles vierges, à jeun depuis 24 h, sont placés sur l'oreille droite du lapin numéro 2, 15 min après l'inoculation. Dix femelles vierges, à jeun, sont placées sur cette oreille 30 min après l'injection. Cinq mâles et cinq femelles, bien gorgés, sont conservés pour les comptages journaliers. Ces glossines n'ont absorbé qu'un seul repas radioactif sur un lapin marqué par 1 mCi de ^{51}Cr. Dix autres glossines (5 mâles et 5 femelles), n'ayant absorbé aucun repas radioactif, constituent le lot témoin (tableau III).

Enfin dix femelles fécondées depuis 48 h ont été nourries sur le lapin numéro 2, trente-cinq jours après. Ces femelles ont été soumises, ainsi que les pupes produites, à des comptages réguliers (tableau IV).

2.4. Résultats

a) Les lapins n'ont présenté aucun symptôme d'intolérance et ont bien supporté les injections de 100 μCi et 1 mCi. L'autopsie du lapin numéro 2 n'a révélé aucune lésion.

b) La période biologique du ^{51}Cr dans le sang des lapins est d'environ 5 à 6 j. L'élimination est rapide, ce qui a été confirmé par les comptages effectués sur l'urine et les excréments. L'urine est fortement radioactive pendant les premiers jours qui suivent l'injection.

c) Chez les glossines, les femelles ont une plus forte radioactivité que les mâles, du fait que la quantité de sang absorbée est deux fois plus importante.

d) La période biologique du ^{51}Cr chez les glossines, mâles ou femelles, est de 2 à 3 j. Les femelles fécondées présentent une élimination identique à celle des mâles et des femelles vierges. Les pupes pondues ne sont pas radioactives. L'élimination du ^{51}Cr dans les excréments des glossines croît très rapidement les deuxième et troisième jours, période correspondant à la perte maximale chez les glossines. Sept jours après le marquage, il est pratiquement impossible de distinguer une glossine marquée d'une glossine témoin. Un test de T pratiqué à cette période n'est pas significatif.

e) Les glossines femelles inséminées ont une fécondité normale. Aucune mortalité n'a été enregistrée chez les glossines marquées.

3. FER-59

3.1. Présentation, étalonnage

Le ^{59}Fe provient également des laboratoires d'Amersham. Il est présenté en solution de citrate de fer, dans des flacons type pénicilline de 10 ml contenant 112 μg de fer, représentant une activité de 1 mCi. L'activité au jour de l'injection est de 1, 4 mCi pour 20 ml. Il n'a pas été effectué de dilution.

Quelques gouttes de la solution de rinçage du flacon d'origine sont soumises au comptage. Les deux pics correspondants aux plus hautes

TABLEAU V. TAUX DE COMPTAGE DE CINQ GLOSSINES MALES (1 à 5) ET CINQ GLOSSINES FEMELLES FECONDEES (6 à 10) NOURRIES 30 MIN APRES L'INJECTION RADIOACTIVE DE ^{59}Fe AU LAPIN
(chiffres soulignés = taux de comptage des pupes produites)
Durée de chaque comptage = 1 min

	Témoins gloss.	Coups en 5 min	Glossines	Date 7.1.70	8.1	9.1	10.1	12.1	13.1	14.1	15.1	16.1	19.1	21.1
♂	1	103	1	43 746	34 667	15 418	8 279	4 735	4 471	4 392	4 395	4 346	4 133	3 958
♂	2	95	2	17 809	15 880	8 545	6 239	5 885	5 861	5 729	5 568	5 404	5 262	5 060
♂	3	115	3	17 197	14 706	9 325	8 508	7 234	7 145	6 867	6 805	6 550	6 279	6 056
♂	4	93	4	23 556	18 336	12 987	12 474	11 500	11 082	10 945	10 661	10 705	9 955	9 685
♂	5	99	5	12 776	9 930	7 884	7 281	6 705	6 546	6 293	6 232	6 256	5 794	5 780
♀	6	84	6	54 713	53 733	52 343	50 430	49 045	46 465	45 918	45 638	45 103	42 959	41 831
♀	7	96	7	59 479	57 550	57 333	56 681	54 076	51 976	49 993	49 155	48 719	46 261	31 513 13 545
♀	8	98	8	64 210	60 931	59 189	57 736	55 539	53 851	52 519	51 860	51 873	48 835	37 315 10 714
♀	9	110	9	30 095	30 013	29 473	29 355	28 041	26 618	26 526	25 618	19 418 6 250	18 485	17 621
♀	10	90	10	28 874	28 221	27 270	26 576	25 384	24 365	23 976	23 713	23 675	22 665	22 117
m = 98,3				35 245,5	32 416,7	27 976,7	26 355,9	24 814,4	23 841	23 315,8	22 963,5	22 204,9	21 062,8	18 093,6
sm = 2,89				5 981	597,2	6 625	6 753	6 649,6	6 375	6 211	4 196,8	6 105,8	5 783,5	4 557,3
Excréments glossines					15 598	32 996	9 052	4 166	407	727	841	51	203	92
Sang du lapin				1 196 844	931 532	1 217 654	1 623 162	1 505 037	1 610 264	1 632 014	1 592 283	1 400 740	1 614 529	1 641 005

TABLEAU V, suite.

Date												Témoins pupes	Coups en 1 min
23.1	26.1	28.1	30.1	2.2	4.2	6.2	9.2	11.2	13.2	16.2	18.2		
3773	3684	3536	3549	3184	3227	3233	3109	2956	2871	2663	2600	1	25
4858	4730	4581	4631	4244	4015	3967	3758	3620	3529	3375	3092	2	21
5893	5713	5510	5409	5043	4831	4778	4566	4459	4197	3934	3910	3	19
9428	9267	8639	8559	7947	7846	4808	7137	6710	6683	6529	6204	4	23
5347	5127	5054	4885	4699	4396	4420	4165	4198	3919	3661	3695	5	31
40099	38393	37863	36384	29532 5842	28013	27386	24664	23405	22669	21463	21001	6	27
30518	29104	28424	22302 5144	21603	20638	20171	16438 2684	15782	15231	14549	14168	7	44
36090	34198	33043	26058 6435	24880	23732	23833	19853 2565	18803	18700	17416	15802	8	22
16863	16418	15917	15222	14517	12439 1777	12170	11564	11176	10611	8939	8593	9	26
16266 4913	15635	15133	15153	10982 3165	10578	10168	9696	8377 1032	8048	7676	7678	10	26
16913,5	16226,9	15770	14215,2	12663,1	11971,5	11793,4	10495	9948,6	9645,8	9020,5	8674,5	m = 26,4	
4375	4155,8	4075,6	3500	3028,8	2865,7	2820,7	2386,5	2474,1	2214,8	2085,2	1989	sm = 2,2	
155	104	154	69	63	97	65	1719	38	63	46	31		
1631740	1513232	1467441	1514872	1446143	1420977	1298746	1290954	1303422	1195760	autopsie			

m = moyenne

sm = écart type

TABLEAU VI. TAUX DE COMPTAGE DE CINQ GLOSSINES MALES AGEES DE 45 J, NOURRIES 17 J APRES L'INJECTION RADIOACTIVE DE ^{59}Fe AU LAPIN
(plusieurs repas radioactifs)
Nombre de coups pour 1 min

♂	Nombre de repas radioactifs pris	Date									
		24.1	26.1	28.1	30.1	2.2	4.2	9.2	11.2	13.2	18.2
1	5	40 794 *	13 646 *	21 623 **	15 523 *	974	245	205	209	214	195
2	4	25 105 *	14 866 *	19 029 *	3 083	7 172 *	528	266	265	276	256
3	4	24 510 *	2 979	27 073 **	4 575	8 421 *	616	203	199	171	155
4	4	26 878 *	25 342	74 921 **	65 695	43 032 *	32 742	21 802	14 263	11 563	5977
5	4	30 654 *	5 020	17 049 *	1 926	8 716 **	1 954	174	186	173	150
Excréments glossines			88 990	29 850	53 886	59 288	17 944	5 435	3 728	870	2200

* Repas radioactifs

énergies (1100 et 1290 keV) sont stabilisés sur les canaux 154 et 181 du spectromètre. Le bruit de fond, en 15 h, est de 18,18 coups/min.

3.2. Injection au lapin

20 ml de la solution de citrate de ^{59}Fe (1,4 mCi) sont injectés dans la veine marginale de l'oreille gauche d'un lapin pesant 3 kg. Le lapin n'a présenté aucun symptôme d'intolérance, si ce n'est une dépigmentation des poils au niveau du chanfrein, autour des yeux, sur les joues, dans les jours suivants.

3.3. Marquage des glossines

25 min après l'injection, vingt glossines, mâles et femelles accouplés depuis 36 h, sont placées sur l'oreille droite. 5 mâles et 5 femelles bien gorgés sont retenus et observés tout au long de l'expérience. Leur radioactivité est très forte et les temps de comptage de 1 min sont très suffisants. Ces glossines sont soumises au comptage tous les jours pendant la première semaine, puis tous les deux jours pendant les semaines suivantes (tableau V).

Dix-sept jours après l'injection au lapin, dont le sang manifestait toujours une activité très intense, 15 mâles âgés de 45 j, à jeun depuis 48 h et accouplés avec un nombre identique de femelles sont placés sur l'oreille du lapin. Cinq mâles bien gorgés sont isolés, soumis au comptage, et nourris, tous les jours, sur le lapin actif, jusqu'à ce qu'ils aient pris chacun au moins quatre repas radioactifs (tableau VI). Cinq femelles âgées de 45 j également bien gorgées ont été isolées afin de suivre leur ponte. Ces femelles (qui n'ont pris qu'un repas radioactif) et les pupes produites sont régulièrement soumises au comptage (tableau VII).

Enfin 10 mâles venant d'éclore ont pris leur premier repas sur ce même lapin, 28 j après l'injection. Cinq de ces mâles, bien gorgés, ont été isolés et soumis au comptage (tableau VIII).

3.4. Résultats

a) Le lapin, qui a reçu 1,4 mCi de ^{59}Fe, n'a présenté aucun symptôme d'intolérance, à l'exception d'une dépigmentation des poils au niveau de la tête. Deux examens de sang (numération et formule leucocytaire), pratiqués à trois semaines d'intervalle, n'ont révélé qu'une légère lymphocytose et une neutropénie. Huit jours après l'injection radioactive, 2 ml de sang ont été prélevés, héparinés et centrifugés. Le comptage du culot de centrifugation d'une part, et du sérum surnageant d'autre part, a montré que la radioactivité est localisée aux hématies, le sérum n'étant que très faiblement radioactif. L'autopsie, pratiquée en fin d'expérience n'a révélé aucune lésion. Les cendres présentent une radioactivité très élevée dans le sang, importante dans le foie, les reins et au niveau des oreilles.

TABLEAU VII. TAUX DE COMPTAGE DE CINQ GLOSSINES FEMELLES AGEES DE 45 J NOURRIES 17 J APRES L'INJECTION RADIOACTIVE DE ^{59}Fe AU LAPIN
(chiffres soulignés = taux de comptage des pupes produites)
Nombre de coups pour 1 min. Témoins pupes: 26,4 ± 2,23

Gloss. ♀ pupes	Date										
	24.1	26.1	28.1	30.1	2.2	4.2	6.2	9.2	11.2	13.2	16.2
1	65130	55805	48610	24934	2347	1540	1213	1028	1059	1026	854
2	33628	24303	3042	293	268	280	264	202 69	199	174	189
3	58716	57818	26944	3275	801	627	677	612	537	548	515
4	49004	21744	17914	7497	519	456	464	407	344 79	325	315
5	36230	35474	25104	5305	1137	1049	820	710 62	692	591	514
m	48541,6	39028	30403	8260,8	1014,4	790,4	687,6	591,8	566,2	532,8	477,4
sm	6152,4	7645,5	7407,1	4345,7	364,21	227,3	162	140	149,3	145,1	113,2
Excréments		26000	39718	58268	18385	581	259	182	60	66	58

m = moyenne sm = écart type

TABLEAU VIII. TAUX DE COMPTAGE DE CINQ GLOSSINES MALES AGEES DE UN JOUR, NOURRIES 28 J APRES L'INJECTION RADIOACTIVE DE ^{59}Fe AU LAPIN

Nombre de coups pour 1 min

♂	Date					
	4.2	6.2	9.2	11.2	13.2	18.2
1	29113	1851	270	260	256	266
2	20796	11103	278	272	228	217
3	27563	5098	455	360	morte	"
4	26243	6405	310	270	274	253
5	22283	22316	867	302	277	256
m	25199,6	9354,6	436	292,8	258,7	248
sm	1583,6	3574,6	113,1	18,2	11,2	10,7
Excréments	3905		28789	440	1315	33

m = moyenne sm = écart type

TABLEAU IX. TAUX DE COMPTAGE DES PUPES PRODUITES PAR LES GLOSSINES FEMELLES MARQUEES AU ^{59}Fe

Nombre de coups pour 1 min

n° de la ♀	Jour de la ponte de la pupe	Age de la pupe le jour de la dissection (j)	Radioactivité de la pupe le jour de sa ponte	Radioactivité de la nymphe	Radioactivité du puparium
6	2.2.70	11	5842	4368	176
7	21.1.70	23	13545	9486	139
	9.2.70	1	1447	1061	194
8	21.1.70	23	10714	7258	97
	18.2.70	9	2684	2167	54
9	4.2.70	9	1777	775	291
	16.2.70	2	1419	1307	106
10	23.1.70	21	4913	3572	70
	11.2.70	6	1032	745	103
					Témoins pupes: 26,4 coups/min ± 2,23
9	16.1.70	adulte	6250	3968	61

TABLEAU X. TAUX DE COMPTAGE DE CINQ GLOSSINES MALES (1 à 5) ET CINQ GLOSSINES FEMELLES (6 à 10) NOURRIES 25 MIN APRES L'INJECTION RADIOACTIVE DE ^{57}Co AU LAPIN

(chiffres soulignés: taux de comptage des pupes produites)

Nombre de coups pour 1 min

	Témoins gloss.		Gloss. pupes	Date 13.1.70	14.1	15.1	16.1	19.1	21.1	23.1	26.1	28.1	30.1	Témoins pupes	
♂	1	40	1	462	271	93	46	45	30	39	49	35	40	1	36
♂	2	35	2	477	255	91	43	37	41	35	31	42	45	2	58
♂	3	36	3	517	294	94	40	43	43	51	29	42	40	3	33
♂	4	31	4	560	369	170	75	65	35	46	53	35	36	4	31
♂	5	41	5	629	532	170	72	53	31	31	41	34	37	5	35
♀	6	41	6	1250	1237	1271	1133	214	112	78	57	65	67	6	36
♀	7	44	7	1549	1459	1594	1131	339	103	58	49	55	49	7	40
♀	8	39	8	705	718	679	612	98	78	61	54	77	49	8	39
♀	9	35	9	487	481	353	176	73	53	44	61	63	51	9	38
♀	10	37	10	1209	1256	707	339	54	47	31 43	44	54	37	10	36
	m	37,8		784,5	687,2	522,2	366,7	102,1	57,3	48,6	46,8	49,2	45,1	m	38,2
	sm	1,24		125,9	145,6	170	139,7	31	9,45	4,42	3,35	4,50	3	sm	2,35
	Excréments glossines				618	1363	1318	1516	355	106	59	47	40		
	Sang lapin			36783	7572	4452	4022	2157	1573	1273	958	844	708		

m = moyenne sm = écart type

b) La période biologique dans le sang du lapin est de plusieurs semaines. Le ^{59}Fe se fixe dans le sang et n'en disparaît que très lentement.

c) Chez les glossines nourries 30 min après l'injection, la quantité de ^{59}Fe absorbée et sa période biologique varie en fonction du sexe (tableau V).

La femelle absorbe une quantité de sang nettement plus importante que le mâle et présente par la suite une plus forte radioactivité.

L'élimination est plus brutale chez les mâles, pendant les trois premiers jours suivant l'injection et s'atténue ensuite en un long plateau à pente faible. La période biologique est de 2 à 3 j, mais le marquage demeure bien décelable pendant très longtemps (plus de 6 semaines).

Chez les femelles, l'élimination est très lente et présente, dès le premier jour, l'aspect d'un plateau à pente faible. La période effective est longue, 24 j en moyenne. Sa durée est conditionnée par la ponte. Les femelles fécondées produisent des pupes possédant un taux relativement élevé de radioactivité. Cette radioactivité se retrouve dans les pupes successivement pondues par une même femelle, celle-ci éliminant ainsi à chaque ponte une certaine quantité de sa radioactivité originelle, ce qui se traduit sur les graphiques par une courbe significative en escalier. Les pupes ont été disséquées; la nymphe et le puparium ont été soumis séparément au comptage. On a ainsi constaté que la quasi-totalité de la radioactivité provient de la nymphe; le puparium n'est que très faiblement radioactif. Avant la fin de l'expérimentation, une des pupes a donné naissance à un adulte mâle. La radioactivité était localisée presque exclusivement dans le corps de l'adulte (tableau IX).

d) Chez les glossines nourries 17 j et 28 j après l'injection radioactive au lapin, l'élimination du ^{59}Fe est très rapide. Chez les mâles (âgés de 45 j), nourris plusieurs fois sur le lapin radioactif, la radioactivité s'élève au moment du repas, mais il n'y a pas accumulation et, 24 h après le dernier repas, la radioactivité est très faible. L'âge des mouches n'est pas en cause, puisque les mâles nourris le jour de l'éclosion sur le lapin radioactif, 28 j après l'injection, présentent une élimination identique à celle des insectes âgés.

Chez les femelles âgées de 45 j et fécondées, l'élimination est un peu moins rapide que chez les mâles, mais nettement plus importante que chez les mouches nourries 30 min après l'injection. Les pupes produites sont très faiblement marquées.

On peut penser que le ^{59}Fe injecté dans le torrent circulatoire du lapin reste libre pendant quelque temps, cette durée étant inférieure à 8 j, puis se fixe sur les hématies. Les glossines assimileraient bien le fer libre, mais ne pourraient plus le métaboliser lorsqu'il est fixé sur les hématies.

Des expériences actuellement en cours doivent permettre d'éclaircir ce point et de préciser à quel moment, après l'injection, la glossine assimile le mieux le fer libre et la quantité maximale qu'elle peut alors fixer.

e) Outre les comptages effectués au spectromètre gamma 256 canaux, un appareil portatif type G-M a été utilisé, dans l'optique d'une recherche, sur le terrain, de glossines marquées et lâchées. L'appareil utilisé est un analyseur muni d'une sonde à cristal et d'un photomultiplicateur (Ludlum Model 16 Analyseur). Cet appareil, de faible volume et aisément transportable, a permis de déceler une radioactivité chez les glossines 6 semaines après le marquage, les femelles se distinguant nettement des mâles en raison de leur radioactivité plus importante.

4. COBALT-57

4.1. Présentation, étalonnage

Il est délivré par le laboratoire d'Amersham sous forme de cyanocobalamine ou vitamine B12 marquée au ^{57}Co, en solution sous un volume de 10 ml correspondant à 100 µg de cobalt et à une activité de 10 µCi. Aucune dilution n'a été faite en raison de la faible activité.

Le pic à 136 keV et une partie du pic à 123 keV ont été retenus pour le comptage. Le pic à 136 keV est stabilisé sur le canal 137 et le comptage se fait entre les canaux 24 et 48.

Le bruit de fond est de 36,48 coups/min.

4.2. Injection au lapin

La solution est injectée en une fois à la veine marginale de l'oreille gauche, ainsi que 5 ml de liquide physiologique ayant servi au rinçage du flacon d'origine. Aucun symptôme n'a été observé après l'injection. En fin d'expérience le lapin a été saigné et autopsié. Le sang et les différents organes, desséchés à l'étuve pendant 72 h puis incinérés à 450°C, ont montré après comptage une fixation du cobalt surtout importante dans le foie et les reins.

4.3. Marquage des glossines

Vingt glossines mâles et femelles, à jeun depuis 48 h et accouplées pendant 24 h ont été placées sur l'oreille droite du lapin 25 min après la fin de l'injection. 5 mâles et 5 femelles bien gorgés sont retenus et soumis au comptage (tableau X).

4.4. Résultats

a) Le lapin n'a présenté aucun trouble durant le mois qui a suivi l'injection. L'autopsie n'a révélé aucune lésion macroscopique.

b) La période biologique est, chez le lapin, inférieure à 24 h. La disparition du cobalt, dans le sang, est très rapide (tableau X).

c) La période biologique, chez les glossines, est de 36 h en moyenne chez le mâle et de 3 à 4 j chez la femelle. La durée de marquage, après comptage de 1 min, est de 3 à 5 j pour les mâles et de 10 à 15 j au maximum pour les femelles. La décroissance biologique du ^{57}Co se traduit par une élévation rapide de la radioactivité des excréments, principalement entre le troisième et le septième jour.

d) Une seule pupe a été pondue. Elle n'était pas radioactive.

5. ZINC-65

5.1. Présentation, étalonnage

1 ml d'une solution chlorhydrique de ^{65}Zn, d'une activité de 100 µCi, cédé par le Service de biochimie organique, a été dilué dans 6 ml de sérum physiologique auquel a été ajouté 1 ml de pénicilline (100 000 U. I.). Il

s'est produit alors un floculat important. Après décantation, le liquide surnageant, dans lequel l'activité était concentrée, a été injecté au lapin.

Le pic à 1140 keV a été stabilisé sur le canal 159 et le comptage se fait entre les canaux 148 et 170.

Le bruit de fond est de 9,19 coups/min.

5.2. Injection au lapin

La solution a été injectée, dans la veine marginale de l'oreille gauche d'un lapin pesant 2,9 kg. L'animal a manifesté quelques symptômes d'intolérance: agitation, tachycardie, polypnée.

5.3. Marquage des glossines

Vingt glossines accouplées depuis 48 h et à jeun depuis 24 h ont été placées sur l'oreille droite du lapin. 5 mâles et 5 femelles bien gorgés sont retenus et soumis au comptage. La radioactivité étant faible, ces mouches ont été à nouveau nourries sur le lapin marqué pendant 6 j, au cours desquels elles ont pris deux à trois repas radioactifs.

5.4. Résultats

a) Il faut tout d'abord signaler que l'étude de ce corps radioactif a été entreprise fortuitement. Les délais de commande du ^{65}Zn sous forme injectable étant très longs, nous n'avons pu avoir à notre disposition que la solution chlorhydrique que le Service de chimie a bien voulu nous céder. Cette solution, non physiologique, explique vraisemblablement les symptômes d'intolérance manifestés par le lapin. Dans les jours suivants l'injection sont apparues des nécroses vasculaires des extrémités (queue, oreille) qui ont nécessité l'euthanasie du lapin. Cependant la seule lésion relevée à l'autopsie consistait en un foyer de nécrose sur le lobe diaphragmatique du poumon droit. Le ^{65}Zn était fixé en grande partie dans le foie, la rate, les reins et au niveau de l'oreille gauche.

b) La période biologique dans le sang du lapin est inférieure à 24 h.

c) Les taux de comptage chez les glossines et les pupes produites sont très faibles. Cependant le marquage, chez la glossine adulte, semble persister au moins 15 j après plusieurs repas radioactifs.

d) Deux femelles ont produit chacune plusieurs pupes. Celles-ci, par comparaison avec les pupes témoins, apparaissent radioactives (tableau XI).

CONCLUSIONS

Des quatre corps radioactifs utilisés, le ^{59}Fe semble être le plus intéressant pour le marquage des glossines. Ce corps est bien supporté par le lapin donneur de sang, chez lequel il persiste longtemps. Chez les glossines nourries 30 min après l'injection radioactive, la radioactivité persiste pendant une longue période à des taux de comptage suffisamment élevés pour être perçus par un appareil de détection portatif type G-M.

TABLEAU XI. TAUX DE COMPTAGE DE CINQ GLOSSINES MALES (1 à 5) ET CINQ GLOSSINES FEMELLES (6 à 10) NOURRIES PENDANT SIX JOURS SUR LE LAPIN AU ^{65}Zn
(chiffres soulignés: taux de comptage des pupes produites)
Nombre de coups pour 1 min

	Témoins glos.		Glos. pupes	Date 9.1.70	10	12	13	14	15	16	19	21	23	26	28	30	2.2	4	6	9	11	13	18	Témoins pupes	
							← repas radioactifs →																		
♂	1	7	1	49	56	24	17	23	15	21	21	morte	"	"	"	"	"	"	"	"	"	"	"	1	10
	2	16	2	86	70	31	27	18	36	32	31	21	32	21	26	32	24	18	23	14	16	17	14	2	13
	3	10	3	79	52	30	33	26	25	29	43	30	40	31	43	35	22	30	21	24	24	23	23	3	8
	4	10	4	72	55	25	18	30	28	28	37	21	32	25	22	28	21	31	21	13	18	16	11	4	16
	5	8	5	75	70	24	9	20	27	20	32	27	19	26	34	23	47	25	22	9	19	11	17	5	8
♀	6	11	6	210	133	133	114	105	60 98	65	77	65	35 41	38	35	39	47 22	35	32	17	18 13	21	16	6	7
	7	12	7	186	152	158	143	144	85 77	84	77	75	86	37 44	56	42	36 27	26	53	26	34	16 12	23	7	10
	8	15	8	134	111	88	95	86	75	88	96	63	64	64	84	37	36	25	40	27	28	22	21	8	9
	9	9	9	111	86	75	57	92	80	90	88	64	48	60	49	50	41	29	39	25	18	21	15	9	5
	10	6	10	67	48	41	49	48	55	42	33	28	36	21	27	33	24	17	21	19	14	20	14	10	15
	m	10,3		106,9	83,3	62,9	56,2	58,8	48,6	49,9	53,4	43,7	43,5	35,8	38,4	35,4	30,4	26,2	30,2	18,3	21	18,5	17,1	m	9,9
	sm			17	11,6	15,2	14,5	14,1	8,1	9,1	8,7	7,41	6,7	5,9	4,1	2,6	3,2	1,9	3,8	2,1	2,03	4	1,42	sm	3,6
	Excréments glossines				78	178	32	14	31	30	73	39	36	26	38	33	29	30	21	13	21	11	18		
	Sang lapin marqué			1952	427	455	376	333	324	302	297	autopsie													

m = moyenne sm = écart type

La descendance est également radioactive et il est possible, bien que nous ne puissions être très affirmatifs en l'état actuel de nos recherches, qu'il y ait marquage du sperme chez le mâle.

Des recherches actuellement en cours doivent nous permettre de préciser certains points soulevés au cours de cette étude, tels que le moment de la fixation de ce corps sur les hématies du lapin, l'état sous lequel il est le plus facilement métabolisé par les glossines, son action sur la descendance (développement de l'embryon, fécondité ultérieure de l'imago qui en est issu).

Le ^{65}Zn semble présenter des propriétés également intéressantes pour le marquage des glossines. L'étude de ce corps, que nous avons pu obtenir sous forme injectable, est actuellement poursuivie.

Le ^{51}Cr et le ^{57}Co ont une vitesse d'élimination trop rapide pour présenter un intérêt comme marqueur chez les glossines.

REMERCIEMENTS

Nous adressons nos plus vifs remerciements aux Services EURATOM et plus particulièrement aux Services de l'Etablissement d'Ispra du Centre Commun de Recherches EURATOM, qui nous ont permis d'effectuer ces recherches dans les laboratoires de cet organisme. Nous exprimons toute notre gratitude et notre reconnaissance à Monsieur R. Cavalloro, Chef du Secteur d'entomologie d'Ispra, qui a bien voulu nous accueillir dans son laboratoire et à Messieurs Myttenaere et Lepers qui nous ont largement fait profiter de leur expérience.

REFERENCES

[1] FINELLE, P., Lutte contre les Glossines par la méthode du mâle stérile – Colloque O.C.A.M. sur l'Élevage, Fort-Lamy, 8-13 Décembre 1969.

[2] ITARD, J., MAILLOT, L., Observations sur un élevage de Glossina tachinoides West., après adoption du lapin comme animal hôte, Rev. Élev. Méd. vét. Pays trop. 21 3 (1968) 387.

[3] AZEVEDO, J. Fraga de, CARVAO GOMES, F.A., PINHAO, R., Biological studies carried out with Glossina morsitans colony of Lisbon. IV – Utilisation of the ^{32}P and the ^{60}Co in the study of biology of the Glossina morsitans, Anais Esc. nac. Saúde publ. e Med. trop. 2 (1968) 61.

DISCUSSION

G. BORGHI: The absorption of ^{32}P by Culex pipiens observed in work at Recife resulted in differences between the radioactivity of males and females similar to those noted by Mr. Itard. We interpreted the phenomenon as being due to the fact that the females of Culex pipiens are twice as large as the males. Moreover, an anomalous maximum in the radioactivity of the males was observed 2-3 days after the absorption of ^{32}P.

J. ITARD: Male and female tsetse flies are the same size, but the females do absorb twice as much blood as the males and it would appear that there are accumulations of radioisotopes (^{59}Fe in particular) in the uterine glands, which provide food for the larvae. We have not observed

in the case of the isotopes used by us (Cr, Co, Fe and Zn) any maximum activity in the males 2-3 days after the radioactive meal.

A. M. JORDAN: Did any flies that had received one radioactive meal and a number of subsequent normal meals show detectable radioactivity?

J. ITARD: Yes. Flies that had taken a single radioactive meal and had then been fed on unlabelled rabbit exhibited a clearly detectable radioactivity for several weeks. These were flies that had been fed 30 minutes after injection of the rabbit with ^{59}Fe.

LIFE TABLE STUDIES OF *Aedes albopictus* (SKUSE)*

KAI-LOK CHAN
Vector Control and Research Branch,
Ministry of Health,
Singapore

Abstract

LIFE TABLE STUDIES OF *Aedes albopictus* (SKUSE).

Aedes albopictus has been found to be involved in the transmission of dengue haemorrhagic fever in some countries in South-east Asia. A study was made to investigate certain aspects of its population dynamics to determine its relationship to the epidemiology of the disease and for control purposes.

Complete life histories of the species were carried out under both field and laboratory conditions. Under field conditions, using painted condensed milk tins provided with bristol boards to serve as ovitraps, the mean duration of life cycle from oviposition to adult emergence was 19.2 days with a range of 10 - 46 days. The mortality rates at each stage were as follows: egg 9.9%, 1st instar 66%, 2nd instar 20.4%, 3rd instar 20.3%, 4th instar 31.1% and pupa 1.0%, giving a total mortality of 89.9%.

Laboratory experiments using fixed numbers of larvae with varying amounts of food showed similar increasing mortalities in the larval stages under suboptimal levels of food (60% wheat + 30% milk powder + 10% yeast) supplied. Mortality was less than 5% above 0.02 mg of food/larva/ml water. Below this level of food, mortality increased hyperbolically until it reached nearly 100% at about 0.004 mg/larva/ml. The life cycle duration was shortest at 0.02 mg/larva/ml, not exceeding 10 days, but increased rapidly with decreasing food.

Other aspects of the life budget of *A. albopictus* studied included sex ratio, fecundity, gonotrophic cycle, adult longevity and adult survival rate in nature.

1. INTRODUCTION

Aedes albopictus (Skuse) had been implicated in dengue haemorrhagic fever transmission in Singapore [1] and in Thailand [2] in addition to *Aedes aegypti* (L.) which is the primary vector [3, 4]. In Singapore, *A. albopictus* is one of the three commonest and most abundant peridomestic mosquitoes [5]. Since it is largely anthropophilous and bites persistently, it is also a major nuisance and a chief cause of public complaints.

Unlike *A. aegypti*, which has been widely studied, especially by the WHO Aedes Research Unit in Bangkok and by Chan et al. [6-8] and Ho et al. [9] in Singapore in recent years, *A. albopictus* has been little studied. For this reason the life budget aspect of its population dynamics is studied so that more effective control measures by integrated or genetical methods could be developed.

2. METHODS

2.1. Life budget

The life budget of *A. albopictus* was determined under both field and laboratory conditions.

* This research project was supported by a medical research grant from the Ministry of Health, Singapore.

In the field, black condensed milk tins, one of its most preferred habitats [7], were used as ovitraps. Bristol boards, each measuring 1 in. × 4½ in. × 1/8 in. were inserted into each tin to serve as the oviposition substrate or paddle. The ovitraps were placed at ground level in suitable shaded sites, e.g. under bushes and banana clumps, in the compound of the Vector Control & Research Branch within the Airport area where A. aegypti was not known to occur. The ovitraps were examined daily and when eggs were found, they were counted with as little disturbance as possible. The ovitraps were then covered securely with a fine wire gauze and returned to their original positions. No food was added. They were examined daily for development and mortality until complete adult emergence.

In the laboratory, eggs collected from a colony were hatched and varying numbers of larvae (10, 20, 40, 60, 80) were reared in ceramic rice bowls filled with 170 ml of water and supplied with 60 mg of food comprising 60% wheat, 30% milk powder and 10% yeast, thoroughly mixed with a few drops of water to form a paste. In another series of experiments, 40 newly hatched larvae were fed on varying amounts of food. Both series of experiments were carried out simultaneously and each series had five replicates.

As in the field tins, the larvae in each bowl were examined daily for development and mortality until complete emergence.

2.2. Sex ratio

Adults emerged from eggs laid by females from the laboratory colony and used for life table and competition experiments (with A. aegypti [8]) were sexed and counted.

2.3. Fecundity and gonotrophic cycle

Gonotrophic cycle used here is the time interval between blood feeding and oviposition.

Two-day-old females and four-day-old males kept on a 10% sugar solution in a 1-ft^3 cage, were fed on man (5 hosts) and a similar cage of females fed on rabbit (3 hosts). After feeding, individual females were isolated, each in a flat-bottomed 1 in. diam. × 3 in. high cylindrical tube for oviposition. The top open end of the tube was covered with a fine gauze onto which water could be dropped on the second day after feeding; the water then drops onto a moist cotton pad placed at the bottom of the tube, covered with a piece of filter paper.

Three separate batches of females were experimented on, to determine the fecundity in the 1st, 2nd and 3rd gonotrophic cycles respectively. The females were dissected to determine the number of eggs retained at the end of each of the three gonotrophic cycles. For the 2nd and 3rd cycles, females were removed into new oviposition tubes described above and re-fed after the 1st and 2nd cycles respectively.

Daily examination for eggs was done at half-hourly intervals between 8.30 a.m. and 5 p.m. and when eggs were laid, they were counted and the time of oviposition recorded. For the calculation of duration of gonotrophic cycle, only females actually seen laying eggs between 8.30 a.m. and 5 p.m. were counted. Those which laid outside this period were discarded.

2.4. Adult longevity

Adult female longevity in nature was determined from ovary dissections of field populations caught in houses and in the open using human baits, from September 1966 to March 1968.

Four human bait traps were set up in the open in a homogeneous shophouse-cum-resident area in Geylang at the following localities: Lorong 32, Jalan Satu, Kallang Clinic and Lorong 13. Two men at each station, one acting as bait with limbs and body exposed and the other as catcher with aspirator and torchlight, caught all mosquitoes between 5.30 p.m. and 10 p.m.[1] on alternate days throughout the period. The catching time was also intended for collecting Culex fatigans (Wied.) but covered the peak biting time of A. albopictus (unpublished data).

For the house catches, 20 shop-cum-resident houses in the same Geylang area were used as collecting stations. Two trained catchers, supervised by a senior officer, caught all resting and biting mosquitoes in each house in 15 min, using sweep-nets, WHO aspirators and torchlights. Catches were made once a week in the morning.

2.5. Adult survival rate

The adult female survival rate used here denotes the probability of survival through one day in nature based on dissections of resting and biting populations caught inside houses and on biting populations caught outside premises. The physiological and calendar ages of the females were determined by Detinova's method of counting the number of follicular dilatations [10].

3. RESULTS

3.1. Life cycle duration and mortality rates

Under field conditions, an average of 40.5 eggs were laid in each tin with a range of 8 - 128 eggs per tin (Table I). The majority (68.2%) were laid on the under surface of the paddles just above the water level, with 12.7% laid on the upper surface and side edges. Only 10.6% and 8.5% respectively were laid on the water surface and on the sides of the tins above the water level.

The duration of life cycle, from oviposition to adult emergence, averaged 19.3 days (Table I). Generation survival of the species represented 10.7% and total mortality 89.3%. Mortality was highest in the 4th larval instar and lowest in the pupal stage.

Under laboratory conditions, similar mortality rates were obtained, whether by supplying fixed amounts of food to varying numbers of larvae (Table I) or by supplying varying amounts of food to fixed numbers of larvae (Fig. 1). In both groups, food supplied was suboptimal to near optimal. An optimum amount of food is that which would result in the shortest life cycle duration and minimum mortality.

---

[1] On most nights some A. albopictus females continued to bite after dusk at about 7 p.m.

TABLE I. LIFE CYCLE DURATION, SURVIVAL AND MORTALITY RATES OF Aedes albopictus UNDER FIELD AND LABORATORY CONDITIONS

Condition	a	b	Mean[c] (range) duration of life cycle (days)	% Adult emergence			Mortality (%)						
				Male	Female	Total	Egg	1st	2nd	3rd	4th	Pupa	Total (range)
Field	25	40.5 (8-128)	19.3 (10-46)	4.0	6.7	10.7	9.9	6.6	20.4	20.3	31.1	1.0	89.3 (85.1-93.7)
Laboratory	10	0.035	6.1 (5-7)	62.5	35.0	97.5	-	0.0	0.0	0.0	0.0	2.5	2.5 (0-10)
	20	0.018	6.7 (6-12)	60.0	33.8	93.8	-	0.0	0.0	0.0	3.7	2.5	6.2 (0-10)
	40	0.009	8.7 (6-35)	38.7	14.4	53.1	-	0.0	0.0	1.9	34.4	10.6	46.9 (42.5-60)
	60	0.006	20.0 (5-41)	2.5	2.9	5.4	-	0.0	0.0	8.3	84.6	1.7	94.6 (91.7-96.7)
	80	0.004	32.4 (12-60)	0.0	1.6	1.6	-	0.0	0.0	15.3	82.5	0.6	98.4 (96.3-100)

[a] Number of tins (field); number of larvae introduced per bowl (laboratory).
[b] Mean (range) number of eggs laid per tin (field); food supplied (mg/larva/ml) (laboratory).
[c] From oviposition to adult emergence (field); from egg hatching to adult emergence (laboratory).

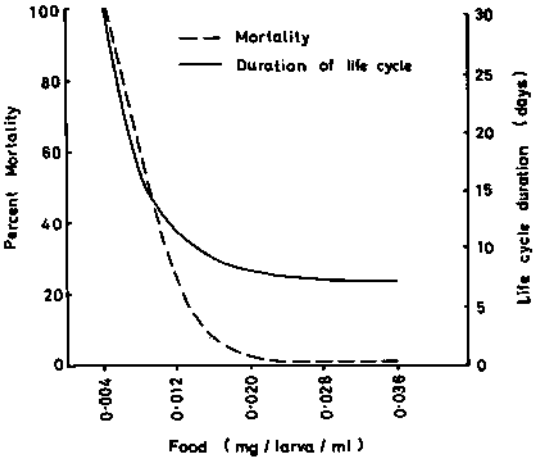


FIG. 1. Effect of food supply on duration of life cycle and mortality in Aedes albopictus.

The life cycle duration increased rapidly with decrease in food supply and decreased rapidly with increase in food supply (Fig. 1). At 0.035 mg/larva/ml, the mean life cycle duration from egg hatching to adult emergence was 6.1 days while at 0.004 mg/larva/ml, it was 32.4 days. At these same levels of food supply, the adult emergence was respectively 97.5% and 1.6%. Mortality was less than 5% above 0.02 mg/larva/ml. Below this level, mortality increased rapidly until it reached nearly 100% at about 0.004 mg/larva/ml. Mortality in the immature stages began earlier at low than at high food levels and in each larval instar it increased proportionately with decreasing food supply. In the pupa, however, there was no proportionate increase in mortality with decreasing food supply.

3.2. Sex ratio

The number of males and females emerging from 114 samples of randomly collected eggs laid by a laboratory colony was respectively 2138 and 1748, thus giving a sex ratio of 1.22 males to 1 female.

3.3. Fecundity

More eggs were laid on rabbit than on human blood (Table II) and the number of eggs decreased with each subsequent oviposition. The majority of females laid eggs readily in confinement. Only a few retained eggs.

3.4. Gonotrophic cycle

The mean duration of the gonotrophic cycle was 2.9 days (Table III). The shortest time taken for the gonotrophic cycle was two days. This is the time required from feeding through egg development and maturation to oviposition, almost without delay. The time taken to oviposit after egg

TABLE II. FECUNDITY OF *Aedes albopictus* FED ON HUMAN AND RABBIT BLOOD

Host	Egg batch	Number of females experimented	Number of eggs laid		Number of females retaining eggs	Number of eggs retained
			Mean	Range		
Man	1st	52	88.3	42-125	4	29, 56, 72, 1
	2nd	37	67.1	48-92	3	16, 4, 2
	3rd	22	58.5	41-74	2	56, 1
Rabbit	1st	38	122	72-203	3	23, 66, 77
	2nd	16	117.5	63-167	2	64, 65

TABLE III. GONOTROPHIC CYCLE OF *Aedes albopictus* FED ON HUMAN BLOOD

Gonotrophic cycle	Number of specimens experimented	Average (range) duration[a] of gonotrophic cycle	
		Hours	Days
1st	36	59.2 (46.5-73.5)	2.5 (1.9-3.1)
2nd	20	76.3 (50.2-147.9)	3.2 (2.1-6.2)
3rd	16	73.5 (51.0-116.8)	3.1 (2.1-4.9)

[a] From feeding to oviposition to the nearest half hour.

TABLE IV. FEMALE OVIPOSITION RHYTHM AFTER EMERGENCE

Oviposition rhythm	Day	
	Without rest[a]	With 1 day rest
1. Emergence.....	0	0
2. 1st blood meal/Mate or Mate/1st blood meal.....	2	2
3. 1st oviposition.....	5	5
4. Rest/2nd blood meal.....	5	6
5. 2nd oviposition.....	8	9
6. Rest/3rd blood meal.....	8	10
7. 3rd oviposition.....	11	13
and so on.		

[a] Or with less than 1 day's rest.

maturation, however, may be prolonged for an additional 4.2 days in confinement. The gonotrophic cycles appeared to be slightly lengthened after the first oviposition.

Some females would readily feed again soon after egg laying while others did not feed again until one day later. The majority of females began taking their first blood meal on the second day after emergence. Mating occurred just before or soon after the first blood meal. In some cases, mating was attempted while the female was feeding.

Taking a mean of three days for the gonotrophic cycle, the female oviposition rhythm after emergence would be as shown in Table IV.

3.5. Longevity and survival rate in nature

The physiological age of the females was determined by the number of follicular dilatations and the calendar age calculated from the duration of the gonotrophic cycle as illustrated in section 3.4.

In both the human bait and house catches, the oldest females caught over the period of 19 months were those which had laid three batches of eggs (Table V), i.e. between 11 and 13 days old. About half (51.3%) inside houses and 43.7% at human baits were nullipars. Ninety five percent of females caught at human bait and 91% caught inside houses were less than two gonocycles old, thus showing that few A. albopictus females ever lived to beyond two or three gonotrophic cycles. There were more fed and gravid females indoors than outdoors, but a larger proportion of older females existed outdoors (Table V). The proportion parous was slightly higher for females caught outdoors at bait than for those caught inside houses. The mean monthly proportion parous caught outdoors and the mean monthly density of the mosquito (females) were directly correlated (unpublished data).

TABLE V. PROPORTION PAROUS AND PROBABILITY OF DAILY SURVIVAL OF A. Albopictus CAUGHT ON HUMAN BAITS OUTDOORS AND IN HOUSES INDOORS, SEPTEMBER 1966 - MARCH 1968, IN GEYLANG

Collection method	Number caught	Number of females per man-h	Female condition (%)			Percent with sperms	Parous rate	Parity (%)			
			Unfed	Fed	Gravid			N	1	2	3
Human bait (outdoor)	546	0.40 (0.16 - 0.92)[a]	33.5	59.5	7.0	72.6	0.56 (0.83 - 0.31)[a]	43.7	51.8	4.3	0.2
House catches (indoor)	85	0.27 (0.20 - 0.40)[a]	16.7	67.9	15.4	60.3	0.49 (1.0 - 0.0)[a]	51.3	39.7	7.7	1.3

p ($2\frac{1}{2}$-day cycle) = 0.77; p (3-day cycle) = 0.81

[a] Range. $p = \sqrt[n]{\text{parous rate}}$, where n = gonotrophic cycle (in days)

4. DISCUSSION

The life cycle of A. albopictus had been studied by a number of workers under laboratory conditions [11-15]. Whilst all these authors had recognized that the duration of life cycle, generally between one and two weeks under laboratory conditions, is dependent on temperature, none of them had shown, as in the present paper, that it is also dependent on the amount of food available to the larvae. This study shows that in nature the life cycle averages longer than under laboratory conditions. The duration varies widely (10-46 days), averaging about three weeks, thus indicating that in nature the amount of food available in containers is suboptimal or that there is usually overcrowding of larvae. Resulting mortality is therefore higher than under laboratory conditions. Temperature is not considered important enough to significantly affect the duration since it is very constant in Singapore. The mean daily temperature for the four years 1966-1969 showed a variation of only 2.5 deg C, from 25.2° C to 27.7° C.

There had been very few studies on the fecundity of A. albopictus. Galliard [16] found that the number of eggs laid varied from 30 to 50 under laboratory conditions and the total number per female in her life time reached 950. Del Rosario [15] recorded an average of 46 eggs per female with a range of 6 - 124 when fed on human blood. Both authors did not include eggs retained after each oviposition. The actual fecundity of the mosquito was therefore not determined by both authors. The present study shows that, like most other mosquitoes, A. albopictus lays fewer eggs after each succeeding gonotrophic cycle and that human blood is not the most productive since the blood of at least one other host (rabbit) is more productive. However, Del Rosario [15] has shown that A. albopictus prefers human blood to that of rabbit, mice or chicken. In Singapore, the precipitin test was nearly always positive for human blood [17], thus indicating that man is probably its normal host.

The present study agrees with earlier findings [14, 15] that the gonotrophic cycle is about 3 days. There was considerable difference in time between individuals in the laying of eggs in all the three gonotrophic cycles. This difference, however, is due to the time taken to oviposit after egg maturation which may vary even in the same individual in confinement, and not to the actual duration taken for the eggs to develop and mature. The latter is controlled by temperature and relative humidity [10] whilst the retention of eggs after maturation is probably related to the neurosecretory state of the female and the nature of the ovipositing substrate.

In the laboratory, A. albopictus females had been kept alive for up to 82 days on a sugar diet and up to 61 days on human blood at 26° C and 85% r.h. [15]. In nature, however, this duration is probably never reached.

In the field, Fisher and Ford's [18] deterministic model and Jolly's [19] stochastic model had been used for estimating population size, survival rates and expectation of infective life, based on mark-release-recapture experiments, by Sheppard et al. [20] in their study on the population dynamics of A. aegypti in Bangkok. These methods were not employed for A. albopictus in this study. A simpler method was used, based on the number of follicular dilatations [10] and on graphed values for expectation of infective life and probabilities of daily survival derived from parous rates [21].

TABLE VI. LIFE TABLE OF Aedes albopictus IN NATURE, SINGAPORE

Age interval (x)	Number alive at beginning of x (lx)	Number dying during x (dx)	dx as % of original lx i.e. as % of 88 (100 qx)
Egg	88	8.7	9.9
Larva			
1st	79.3	5.8	6.6
2nd	73.5	17.9	20.4
3rd	55.5	17.9	20.3
4th	37.6	27.4	31.1
Pupa	10.2	0.9	1.0
	Total:	78.6	89.3
Adult	9.4	-	10.7 survival

Parameters:

Generation survival = 9.4 or 10.7%

Generation mortality = 78.6 or 89.3%

p (proportion surviving through one day) = 0.77 - 0.81

Number of females surviving through one day = 3.34

Number of females surviving through 11 or more days = 0.025

Expectation of infective life (in days)

$= \frac{p^n}{-\log_e p}$ where n = 10, the value (in days) of the incubation period of dengue virus

= 0.4 (0.3 - 0.6)

From all the above data collected, it would now be possible to (1) construct a life table of A. albopictus as shown in Table VI, (2) relate the epidemiology of dengue diseases transmitted by the species to its population dynamics, and (3) estimate from known parameters, the number of sterilized males that would have to be released in a large-scale control program using the sterile-male technique.

The life table presented in Table VI is preliminary since at least one group of mortality factors were not taken into account, namely predation in nature especially by Toxorhynchites. In Singapore, predation of A. albopictus by T. splendens in rural areas is highly significant and may account for up to 100% mortality of the prey species for several generations in containers where the predator species occurs [22]. In the city, however, the predator species is very uncommon [22].

Table VI shows that from a pair of mosquitoes of one generation, at an average fecundity of 88 eggs per female per batch, an average of 9.4 adults would result in the next generation. This represents a probability of survival through the aquatic stages of 0.11 or 10.7%. The probability of

survival from egg to successful adult emergence constitutes one of the most important mechanisms for the natural regulation of the adult densities of all mosquito vector species.

The important parameters in terms of the reproductive ability of the mosquito population and hence its relation to disease transmission, are:

(1) the probability of daily survival of the adult female;
(2) the average number of eggs in a batch;
(3) the length of the aquatic cycle in days;
(4) the probability of survival of an egg to successful adult emergence; and
(5) the oviposition rhythm, as expressed by the average times between emergence and first oviposition and between successive ovipositions.

The values of these parameters for A. albopictus are respectively (1) 0.77-0.81, (2) 88, (3) 19.3, (4) 0.11, (5) 5/ $2\frac{1}{2}$, 3, 4 (the first oviposition occurs at day 5 and the following ovipositions every $2\frac{1}{2}$ or 3 or 4 days).

From these parameters, it is possible to relate the mosquito population density to transmission of dengue and dengue haemorrhagic fever. It is known that the incubation period for most viral and protozoal infective agents is about 10 days under tropical conditions. All vector females of 10 days old and above, therefore, are potentially dangerous as they would transmit the agent if the disease reservoir is present. It is important, therefore, to calculate the number of females that are 10 or more days old at any known density level of the mosquito population. This can be calculated as follows (for convenience the age of females capable of transmitting disease is here taken as 11 or more days or three or more gonotrophic cycles (see section 3.4)):

Number of females emerging from one batch of 88 eggs = 4.23
Proportion surviving through one day = 0.79
∴ Number of females surviving one day = $0.79 \times 4.23 = 3.34$
Number of females surviving 11 or more days or three or more gonotrophic cycles (see section 3.4 and Table V)

$= \frac{0.2}{100} \times 3.34$ to $\frac{1.3}{100} \times 3.34$

= 0.0067 to 0.0434 = 0.025 on the average.

Since a single female mosquito of one generation would give rise to 0.025 females capable of surviving through 11 days in the next generation at a density of 0.34/man-h (average of 0.40/man-h and 0.27/man-h, Table V), there would be 0.025×0.34 or 0.0085 female mosquitoes surviving the critical period of 10 days and above arising from the single female of the previous generation.

Since 0.0085 females would survive 11 days and above at a density of 0.34/man-h, one female surviving this period would require a density of 40 females per man-hour. This density, 40/man-h, would therefore be the minimum threshold level for transmission of dengue viruses in the Geylang area of Singapore by one single female 11 days old and above. It should be noted that A. albopictus is not uniformly distributed in Singapore but shows focal breeding [6]. Its density therefore varies from locality to locality.

The expectation of infective life of the mosquito for a virus incubation period of 10 days at the prescribed range of proportion surviving through one day (0.77-0.81) would be between 0.3 and 0.6 days with a mean

of 0.4 days based on data graphed in Ref. [21]. This means that a single female mosquito at this infective age would be able to transmit dengue viruses for only about 10 hours at the time of blood feeding on the 11th day.

In an eradication program using the sterile-male technique, daily swamping every breeding place with very large numbers of sterile males either through sterile pupae or by direct release of sterile males would be necessary in order to reduce to a low level the probability that a virgin female would mate with its own wild fertile male. For this, 'factory' eggs must be mass-produced in enormous numbers, a technique which is already available [23, 24]. The number of sterile individuals, whether pupae or adults, must be related to the number of normal eggs, larvae, pupae or adults found in nature where the release is to be made. This could be achieved directly by estimating the number of larvae of a particular instar, allowing for previous mortality and non-hatching of eggs (as the life table enables) or indirectly by the use of a computer into which are fed the relevant parameters. In the second method, it would also be possible to estimate the duration of treatment necessary for eradication of the mosquito, as is being done at the London Ross Institute of Tropical Hygiene by Dr. C. B. Cuellar.

5. CONCLUSION

This paper provides the groundwork for assessment of the possibility of controlling A. albopictus by the sterile-male technique and for further studies on the population dynamics of the mosquito in those aspects that would lend themselves to the control of this species by the sterile-male technique. Additional data on the biology of its natural populations in S.E. Asia should be collected, especially on its population size and dispersal rates, before actual releases of sterile males into the environment is attempted.

ACKNOWLEDGEMENTS

The technical assistance of Miss Low Sue Bee, Miss Tay Siew Khuan and Mr. Seah Seng Tee is gratefully acknowledged. My sincere thanks are also due to Dr. H. Guan Lim, Director of Medical Services, Ministry of Health, Singapore, for making available to me the medical research grant.

REFERENCES

[1] CHAN, Y.C., LIM, K.A., HO, B.C., Recent epidemics of haemorrhagic fever in Singapore, Jap. J. med. Sci. Biol. 20 (1966) 81.

[2] GOULD, D.J., YUILL, T.M., MOUSA, M.A., SIMASATHIEN, P., RUTLEDGE, L.C., An insular outbreak of dengue haemorrhagic fever. III. Identification of vectors and observations on vector ecology. Am. J. trop. Med. Hyg. 17 (1968) 609.

[3] RUDNICK, A., CHAN, Y.C., Dengue type 2 virus naturally infected Aedes albopictus mosquitoes in Singapore. Science, N.Y. 149 (1965) 638.

[4] RUDNICK, A., Dengue viruses isolated from mosquitoes in Singapore, 1960-61. Bull. Wld Hlth Org. 35 (1966) 63.

[5] CHAN, K.L., Pilot mosquito control study at Geylang, Singapore, Singapore Publ. Hlth Bull. 1 (1967) 52.
[6] CHAN, K.L., HO, B.C., CHAN, Y.C., Aedes aegypti (L.) and Aedes albopictus (Skuse) in Singapore: 1. Distribution and density, Bull. Wld Hlth Org. (in press).
[7] CHAN, K.L., HO, B.C., CHAN, Y.C., Aedes aegypti (L.) and Aedes albopictus (Skuse) in Singapore: 2. Larval habitats, Bull. Wld Hlth Org. (in press).
[8] CHAN, K.L., HO, B.C., CHAN, Y.C., Aedes aegypti (L.) and Aedes albopictus (Skuse) in Singapore: 3. Competition between species, Bull. Wld Hlth Org. (in press).
[9] HO, B.C., CHAN, K.L., CHAN, Y.C., Aedes aegypti (L.) and Aedes albopictus (Skuse) in Singapore: 4. Population fluctuations, Bull. Wld Hlth Org. (in press).
[10] DETINOVA, T.S., Age-grouping methods in Diptera of medical importance, WHO Monograph Series No.47, WHO, Geneva (1962).
[11] SEN, S.K., Experiments on the transmission of rinderpest by means of insects, Mem. Dept. Agric. India, Ent. Serv. 9 (1926) 59.
[12] TSENG, S., WU, L., An ecological study of mosquitoes in Wuhan area, Bull. ent. Res. 42 (1952) 527.
[13] ISHII, N., NAKAYAMA, A., ISHII, Y., Biological observations on the mosquito, Aedes albopictus, Yokohoma med. Bull. 5 (1954) 275.
[14] HALCROW, J.G., Notes on a laboratory colony of Aedes (Stegomyia) albopictus (Skuse) and its distribution in Mauritius, Proc. R. ent. Soc. Lond. (A), 30 (1955) 40.
[15] DEL ROSARIO, A., Studies on the biology of Philippine mosquitoes. II. Observations on the life and behaviour of Aedes albopictus (Skuse) in the laboratory, Philipp. J. Sci. 92 (1963) 89.
[16] GALLIARD, H., Recherches sur la biologie des culicides à Hanoi (Tonkin-Nord-Vietnam). I. Développement larvaire comparatif de différentes souches d'Aedes albopictus, Aedes aegypti, Culex fatigans et Armigeres obturbans, Annls Parasit. hum. comp. 53 (1958) 131.
[17] COLLESS, D.H., Studies in the systematics and biology of the Culicine mosquitoes of Singapore, Ph.D dissertation, Univ. of Malaya (1956).
[18] FISHER, R.A., FORD, E.B., The spread of a gene in natural conditions in a colony of the moth Panaxia dominula L., Heredity, Lond. 1 (1947) 143.
[19] JOLLY, G.M., Explicit estimates from capture-recapture data with both death and immigration - stochastic model, Biometrika 52 (1965) 225.
[20] SHEPPARD, P.M., MACDONALD, W.W., TONN, R.J., GRAB, B., The dynamics of an adult population of Aedes aegypti in relation to dengue haemorrhagic fever in Bangkok, J. animal Ecol. 38 (1969) 661.
[21] GARRETT-JONES, C., GRAB, B., The assessment of insecticidal impact on the malaria mosquito's vectorial capacity, from data on the proportion of parous females, Bull. Wld Hlth Org. 31 (1964) 171.
[22] CHAN, K.L., Observations on Toxorhynchites splendens (Wiedemann) (Diptera: Culicidae) in Singapore, Mosquito News 28 (1968) 91.
[23] MORLAN, H.B., HAYES, R.O., SCHOOF, H.F., Methods for mass rearing of Aedes aegypti, Publ. Hlth Rep., P.H.S. U.S.D.H. Education & Welfare 78 (1963) 711.
[24] FAY, R.W., McCRAY, E.M. Jr., KILPATRICK, J.W., Mass production of sterilized male Aedes aegypti, Mosquito News 23 (1963) 210.

DISCUSSION

R. PAL: You have indicated that three gonotrophic cycles are required before transmission can start. This takes about 11 days, and with the survival rate of 0.4 after 11 days the transmission potential is indeed extremely low. It would seem that two gonotrophic cycles should be sufficient for transmission under actual field conditions.

Kai-Lok CHAN: That is true. The incubation period of the virus under normal tropical conditions is about 10 days. For transmission of the dengue virus, a female mosquito must therefore be at least 10 days old. Where there is a mean value of three days for the gonotrophic cycle and the first oviposition occurs on day 5 after emergence, the female mosquito can begin transmitting the virus on day 10 after the first blood meal, i.e. after the second oviposition and before the third oviposition,

which would occur on day 11. The female would then have a total of 24 + 10 = 34 hours for transmitting the virus.

Rachel GALUN: Are three gonotrophic cycles necessary for the transmission because the length of time necessary for these cycles coincides with extrinsic incubation period of the virus?

Kai-Lok CHAN: The answer to this in fact emerges from the remarks Mr. Pal and I myself have just made. Transmission of the virus can begin on about day 10 after the first blood meal (assuming a patient's blood is taken). On the basis of a three-day gonocycle, at least three gonotrophic cycles will be required before the female can transmit the virus. On the basis of a two-day cycle, only two gonocycles will be required.

Rachel GALUN: Is the higher egg productivity of females fed on rabbit blood as compared with that of females fed on human blood due to a difference in the size of the meal on the two hosts?

Kai-Lok CHAN: No measurements of the size of the blood meal were carried out, but in both cases females were fed to repletion, and it may be assumed that amounts consumed were approximately the same, i.e. about twice the body weight of the mosquito. The differences in egg productivity would probably be due to differences in the blood of the two hosts, for example in the proportion of protein-producing constituents.

POSSIBLE USE OF THE STERILE-MALE TECHNIQUE FOR ERADICATION OF THE HORN FLY

S.E. KUNZ*, J.L. ESCHLE†
Entomology Research Division, Agricultural Research Service,
United States Department of Agriculture,
Texas, United States of America

Abstract

POSSIBLE USE OF THE STERILE-MALE TECHNIQUE FOR ERADICATION OF THE HORN FLY.

The horn fly, Haematobia irritans (Linnaeus), is an obligate, bloodsucking parasite of major importance on cattle in North America. Field studies on the biology and habits of this species have shown that an integrated eradication program utilizing the sterile-male technique might be possible.

Mass-rearing procedures have been developed which could at present supply about 350 000 adults/week at a cost of $1 per 1000 flies. Studies are being made to improve the larval rearing media and adult blood diet in an effort to increase reproduction of colony flies, minimize fluctuations in production, and reduce cost.

Horn fly pupae can be completely sterilized with 2500 rad from a cobalt-60 source with no apparent reduction in adult emergence or survival. However, sexual competitiveness of the sterile males appears to be reduced, perhaps by as much as 50%. Similar results have been obtained with the chemosterilant tepa. Before a release program can be initiated, a method of producing a more competitive sterile male will need to be developed.

In the geographical area studies, there is a period of low adult emergence in the life cycle of the horn fly during which the species would be vulnerable to an eradication attempt. It appears that the sterile-male method would probably be most effective if it were integrated with a chemical control program. The release of sterile, insecticide-resistant flies, the application of late-season chemical control to reduce the overwintering population of normal flies, the introduction of a non-diapausing strain of flies, and the release of a biological control agent, could potentially be used in conjunction with the sterile-male technique.

INTRODUCTION

The horn fly, Haematobia irritans (L.), an obligate bloodsucking parasite of cattle, is distributed throughout the New World from Venezuela to Canada and in the Old World from North Africa to Lapland [1]. In the United States, it is cosmopolitan in distribution and is one of the major insect pests of cattle.

In the absence of control and with favourable weather, extremely large populations of these flies may build up in almost any location where cattle are produced; infestations of 2000 to 3000 flies per animal are not uncommon in the humid areas of the southern and southeastern United States. The adults remain on the host day and night, and the females leave for only a few minutes at a time to deposit eggs on freshly voided cattle faeces. Both males and females take several blood meals a day.

The constant presence and feeding of these flies irritate and annoy the host animals so that they cannot feed or rest properly. Since heavily infested cattle may graze little, they are thus slow to gain weight or may

* Livestock Insects Investigations Laboratory, College Station, Texas 78040, USA
† Livestock Insects Investigations Laboratory, Kerrville, Texas 78028, USA

even lose it. We have observed cattle stampeding through brush in attempts to rid themselves of large numbers of flies. Also, infested range cattle constantly swing their heads in attempts to dislodge the flies.

Accurate estimates of the losses caused by the horn fly are difficult, but the evidence indicates that the constant stress on the host animals can be a significant factor in the production efficiency of a beef or dairy cattle herd. One figure suggests [2] that horn flies cause losses of $179 million per year in the United States. The only known role of the horn fly as a vector of a disease or parasite is its association with the filaria worm Stephanofilaria stilesi Chitwood [3].

An extensive literature concerns the horn fly, particularly conventional methods of control, but much remains to be learned of its life history and habits. However, our available information does indicate that the horn fly might be a good candidate for an integrated eradication program. A concerted effort is therefore being made by entomologists of the U.S. Department of Agriculture, primarily at Kerrville, Texas, to find the answers to the many questions about rearing, sterilization, field biology, and ecology which are needed to determine whether such a program is feasible.

1. MASS REARING

Laboratory colonies must be established before investigators can study the life history and biology of an insect and develop the mass-rearing techniques required for release programs. However, the initial efforts to colonize the horn fly were unsuccessful. Glaser [4, 5] and McLintock and Depner [6] reported that larvae could easily be reared to adults and adults could be maintained on defibrinated or citrated bovine blood, but the females did not produce eggs. Therefore a permanent colony of horn flies was maintained in the laboratory by using adult cattle as hosts for the adult stages; the immature stages were reared on the faeces of the animal [7].

Harris [8] established the first laboratory colony of horn flies that reproduced without contact with a host animal. He began with the theory that micro-organisms developing in citrated bovine blood were detrimental to the flies or that the laboratory diet of bovine blood was deficient in the nutrients required for egg production. His colony was established by feeding the adult flies a diet of citrated beef blood to which antibiotics and a saline extract of beef muscle had been added. Then Harris et al.[9] developed an artificial larval rearing medium which contained no bovine faeces but which compared favourably with faeces as a medium for rearing horn fly larvae.

Therefore, since 1965, the basic rearing techniques developed by Harris have been used, with various modifications, in an effort to develop a mass-rearing technique for the horn fly. The major modifications have been the addition of bovine faeces to the artificial larval medium and the exclusion of the beef extract from the adult diet. The results of these changes were first reported by Schmidt et al.[10], who also reported later modifications [11].

The large-scale colony is at present being maintained as follows. Adults are fed a diet of beef blood obtained from abattoir-slaughtered cattle. Sodium citrate is added as the anticoagulant, and kanamycin sul-

phate and nystatin are added to retard development of micro-organisms. Flies are fed at 08.00 and 16.00 hours daily by placing cotton pads soaked with the diet on the tops of the screened cages. Eggs dropped by females fall through the screened bottom of the cages onto a wet cotton pad covered with cloth. The eggs are washed from the pad, measured in a graduated tube (about 8000 eggs per cm^3), and then transferred to the larval rearing medium which consists of the following:

	Parts by wt (g)
Ground sugar cane pulp	266
Whole wheat flour	48
Fish meal (60% protein)	36
Sodium bicarbonate (baking soda)	6
Faeces (from bovine fed only alfalfa)	545
Distilled water	1300

The dry ingredients are mixed, the faeces and water are blended together in a slurry, and the slurry is thoroughly hand-mixed into the dry ingredients. (About 450 g of this medium is required to produce 1000 pupae.) Six days after egging, the pupae are separated from the larval medium by flotation and dried in a rotary-drum dryer [12]. Adults are allowed to emerge directly into holding cages. Larvae are maintained at a room temperature of 80°F; pupae and adults are held at 90°F and 50-55% relative humidity.

Using these methods, we maintain a breeding colony of about 21 000 adults so that an excess of 120 000 adults per week is available for research purposes. Moreover, it is estimated that, with the present facilities, a maximum colony of 63 000 breeding adults could be maintained which would supply in excess of 350 000 adults per week for a sterile-male release program at a cost of about $1.00 per 1000 flies.

Thus, during periods of peak production, the present techniques will supply large numbers of horn flies, and capacity appears to be limited only by available space and labour. However, several problems must be solved before a sufficient number of flies could be produced consistently, a necessity for a sterile-male release program.

For example, the reproductive capacity of the colony flies must be improved. At present, the average number of eggs per female per day is about 8, though female horn flies have the capacity to mature an average of 20 eggs at a time and to lay as many as 14 at one time in the field [13]. Because the first laboratory-reared horn flies did not produce eggs until antibiotics were added to the blood diet, other improvements in adult nutrition might result in a further increase in egg production. However, the role that antibiotics play in the blood diet is not clearly understood. In a preliminary study, no difference could be detected in either adult longevity or egg production when flies fed blood diets containing antibiotics were compared with flies fed untreated diets. Through selection pressure in the laboratory, the colony flies may have developed resistance to the micro-organisms in the blood diet or have become otherwise adapted. If there is a nutritional deficiency, it may manifest itself only after several generations of feeding on untreated blood.

Larval rearing efficiency must also be improved. At present, only about 25% of the eggs that are planted on larval medium produce adult

flies. The egg hatch averages about 60%, and adult emergence from the pupae about 80%. Therefore, though it would, of course, be desirable to increase egg hatch and adult emergence, the major problem appears to be the survival of larvae: only about 30% of the eggs planted reach the pupal stage. Harris et al. [9] reported that larvae could be reared satisfactorily in a medium containing no bovine faeces, but results of attempts to rear larvae in the large-scale colony on the medium without faeces have generally been unsatisfactory. Production is low, the pupae are comparatively small, and the results are generally highly variable. In the laboratory colony, pure faeces have proved superior to the artificial medium alone, but the best results in mass rearing have been obtained with a combination of artificial medium plus faeces. Studies indicate that the artificial medium undergoes decomposition, which results in a temperature cycle with a relatively high peak (40 - 44°C), but when the medium contains faeces, the temperature begins to rise about 10 h earlier, and the peak is about 6 deg C lower. Pure faeces do not exhibit such a rise in temperature. Also, Pseudomonas spp., characterized by the presence of a green pigment, readily develop in artificial medium that does not contain faeces, and these bacteria completely inhibit development of horn fly larvae. However, the detrimental effects of Pseudomonas appear to be reduced by the addition of faeces. Studies are therefore being made to determine the effect on larval development of temperature, micro-organisms, decomposition products, and other factors associated with the artificial medium. We are also attempting to determine what the larvae are feeding on and how the faeces increase the quality of the artificial media. In addition, efforts are being made to isolate micro-organisms that might provide the essential nutrients for growth of horn fly larvae and at the same time either eliminate the effect of detrimental micro-organisms, such as Pseudomonas spp., or prevent their development in the media.

Another serious problem that has plagued the horn fly colony since its beginning is the recurrence of sudden and unexplained dips in production that usually occur during the winter months. During these periods, egg production and hatch are usually low, adult longevity is reduced, and mortality of larvae and pupae is high. In general, the colony seems to suffer a loss of vigour. An apparently similar problem was encountered in early attempts to rear larvae of the house fly, Musca domestica L., in horse manure: it was impossible to maintain laboratory colonies from mid-December to mid-April [5, 14, 15]. In that case, the difficulty was apparently resolved by adding autoclaved yeast cells to the horse manure, which suggests a nutritional deficiency. Perhaps with horn flies, a physiological change in the bovine host or in the microflora of the faeces during the winter causes a nutritional deficiency in the larval stage which results in a loss of vigour in the adults and a subsequent decrease in production. Hopefully, improvements in the adult diet and in the larval rearing techniques will result in an increase in reproductive capacity and overall vigour of the flies.

2. STERILIZATION OF HORN FLIES

A basic requirement of the sterile-male technique is that irreversible sterility be induced in the insect to be released without destroying mating competitiveness [16].

In studies at Kerrville, a ^{60}Co source was used to treat horn fly pupae with 2500 rad of gamma irradiation [17]. When pupae were irradiated at 102, 126, or 135 h after they began pupation, there was no detectable reduction in adult emergence or survival, males and females were completely sterile (determined by egg hatch), and there was no recovery of fertility, even after the 10th or 11th day of adult life.

In preliminary studies of competitiveness, when sterile colony males, non-sterile colony males, and non-sterile colony females were caged in certain ratios, the number of sterile eggs laid by the females was about one-half that expected. This result seems to indicate that the dose used (2500 rad) seriously reduced the competitiveness of the sterile males [17]. Perhaps a lower dose level will help solve the problem. However, currently available data indicate that about 1% of the eggs laid by untreated females mating with males from pupae irradiated with 2000 rad will be fertile [17]. Moreover, females from irradiated pupae mating with normal males, though they were completely sterile when they began producing eggs, recovered some fertility toward the end of the egging period. If the lower dose of irradiation results in a highly competitive sterile male, a low level of fertility might be tolerated in a release program, but it will depend largely on the number of sterile males necessary to produce a downward trend in the natural population and the number of adult flies that can be economically tolerated on cattle. Also, with a low level of egg hatch (reduced dose of irradiation), it is possible that no progeny will develop.

Other studies at Kerrville have indicated that sterility can be induced in horn flies by the use of chemosterilants. Topical applications of 0.05% tepa to the adult flies or 5 - 10 ppm fed in the adult diet produced complete sterility in adults [18]. However, the reduction in mating competitiveness of the sterile males was similar to that observed in males sterilized with gamma irradiation.

However, mating competitiveness might not be a problem if improvements in the mass-rearing method resulted in a more vigorous colony male. Probably the detrimental effects of irradiation are more severe when the flies are naturally low in vigour at the time of treatment.

Meanwhile, studies of the emergence of adult horn flies from pupae [19] have pointed to a simple way to separate the sexes for selective irradiation, a desirable objective. In this study, 61% of the flies that emerged during the first day were females; 95% of those that emerged the second day were males. If this pattern proves to be a consistent characteristic of the colony flies, then the flies emerging first, primarily females, could be returned to the colony, and the remaining pupae, primarily males, could be irradiated for release.

3. FIELD BIOLOGY AND ECOLOGY

To implement a sterile-release program, we need as much information as possible about the population dynamics of an insect pest species. Especially, quantitative data on the density of the natural population at the low levels in the population cycle are needed. Also, rates of natural increase must be known to provide a guide in determining the rate of release necessary to overwhelm a natural population with sterile insects.

TABLE I. EMERGENCE OF HORN FLIES AND OF ALL OTHER INSECTS FROM UNDISTURBED MANURE PATS IN THE FIELD IN CENTRAL TEXAS

Species	Average number of insects emerging per pat when pat was covered at indicated time after being dropped					
	5 min	30 min	2 h	4 h	6 h	24 h
Horn fly	46.5	27.7	11.7	9.3	4.1	4.6
Other insects	7.4	20.4	23.3	28.7	46.6	41.7

We therefore initiated a field study in 1968 to determine how the ovipositional habits and production of the horn fly were affected by conditions in central Texas (30° latitude, 99° longitude) [20]. At various periods throughout the 24-h day, individual manure pats were covered with emergence traps within 3 - 5 min of defaecation of the host animals to eliminate as many as possible of the parasites, predators, and other arthropod species competing for space and food. By visual observations and by subsequent adult emergence, we established that the horn fly females oviposited at any time during a 24-h day if fresh faeces were present. In these studies, the average production of horn flies per pat ranged from 29.9 (08.00 - 10.00 h) to 99.0 (02.00 - 04.00 h), and the largest number of flies collected from a single pat was 475. This, then, was the reproductive potential of an individual manure pat. Similar results were reported by Sanders and Dobson [21]. Also, the potential rate of production (obtained from the average total production of horn flies from protected pats for 22 weeks during the peak period of production, regardless of time of day) was 66.5 adults per pat. This rate may be less in many regions in the USA, but in some it may be low. Also, it should be emphasized that the averages indicate the production potential, not actual production in the field, because the competitors were excluded.

Horn-fly production in more normal conditions was tested in 1968 by leaving freshly voided manure pats exposed for various periods and then covering them with emergence traps [22]. Results, summarized in Table I, indicate that during most of the daylight hours arthropod competitors became established within the first 6 h after defaecation in manure on which horn fly eggs were deposited.

We therefore devoted May to October of 1969 to a study to determine the gross effects of competition on the actual production of horn flies exposed to the same animal hosts in the same test area. Manure pats 0 - 24 h old were marked and left exposed to the environment for 7 days. After 7 days, the pats were covered with emergence traps, and flies were collected as in 1968. The patterns of production were similar to those noted in the 1968 pilot study. Also, gross competition did play an important role: it reduced the production of horn flies almost 10-fold. The average production in 1969 was 6.64 flies per pat compared with 66.75 flies per pat in 1968 when competition was eliminated.

TABLE II. AVERAGE EMERGENCE OF HORN FLIES FROM UNDISTURBED MANURE PATS AND ESTIMATED NUMBER OF FLIES PER ANIMAL IN THE FIELD IN CENTRAL TEXAS

Month	Average number of flies	
	Range per pat	Range per animal
1969		
June	0.8 - 2.7	-
July	7.3	430
August	3.2 - 18.7	980 - 1000
September	1.1 - 33.5	1000 - 1420
October	0.3 - 1.1	300 - 1000
November	0 - 0.1	50 - 500
December	0 - 0.3	25 - 75
1970		
January	0	0 - 1
February	0 - 0.1	0 - 15
March	0.1	20 - 90
April	0.1 - 1.1	100 - 1000
May	1 - 1.1	650

4. POSSIBLE APPLICATIONS

Efforts to estimate numbers of adult horn flies on host animals have been, for the most part, unsatisfactory because when the numbers exceed 200 - 300 per animal, it becomes almost impossible to make accurate counts. However, if the number of horn flies emerging from manure pats can be used as an estimate of the trend of natural populations, the data from field studies indicate that 1 January to 1 April is the lowest point in the natural population cycle in the south Texas study area (Table II). Therefore, any large-scale program in which sterile males are used to attack the horn-fly population in this area should be initiated during this 3-month period.

With conditions such as those in the study area where emergence of adult flies was apparently reduced to zero in January, a sterile-male release of 5 - 10 flies per animal begun 1 January and continued throughout the winter and spring might eliminate the normal build-up of adult horn flies on the animals. Then, even if low-level releases were required

TABLE III. HYPOTHETICAL MODEL

Generation	Theoretical number of adult horn flies per 100 cattle per day in south Texas study area			
	Number of fertile flies[a]	Number of sterile flies released	Ratio of sterile to fertile flies	Number of flies reproducing
Parent	2000	18 000	9 : 1	200
F_1	1000	18 000	18 : 1	53
F_2	265	18 000	68 : 1	4
F_3	20	18 000	900 : 1	0

[a]Assuming an initial average emergence of 2 flies per pat per day from 1000 pats.

throughout the summer, the numbers necessary would probably not be detrimental to the cattle. In other geographical areas, where winter emergence would probably not be reduced to zero, it would probably be necessary to utilize an integrated approach. For example, if, at the low point of the population cycle, there were as many as 2 flies per pat emerging daily, we could use this number to estimate the number of sterile flies that would have to be released during a given period. It has been observed that cattle defaecate an average of 10-12 times per 24-h period. Assuming 10 defaecations per day per cow, a herd of 100 cattle in an isolated situation and an average 2 flies per pat, an average 2000 adults should be added to the natural population of the area each day.

It was estimated by Knipling [16] that a 5-fold increase per generation is a valid estimate for most well-established insect species. Actually, because of the limited number of breeding sites available to the horn fly, which oviposits only on freshly dropped faeces, the natural rate of increase of this species may be less than that. However, with an assumed 5-fold rate of increase, the hypothetical model shown in Table III can be constructed based on the theories proposed by Knipling [16]. According to the model, when 90% of the population of the first generation consists of sterile flies, the population would theoretically begin a downward trend and would be eliminated by the F_3 generation.

The release of 18 000 flies per day (an average 180 per animal) for only a day or two would obviously result in a population of sterile flies on the cattle which would far surpass numbers that are usually considered damaging. Therefore, sustained releases at this level would certainly be unacceptable to cattle producers. Perhaps the natural rate of increase will be found to be less than 5-fold, in which instance fewer sterile flies would be required. Or, if the procedure for selectively sterilizing males (discussed in section 2) proves practical, fewer total sterile flies would

be required. For example, if 90% of the sterile flies were males, only 10 000 per day rather than 18 000 would have to be released to obtain the desired 9:1 ratio. However, if the estimated emergence of 2 flies per manure pat per day were occurring when the releases were begun, then the natural population would apparently have to be reduced through conventional means before releases would be practical.

One characteristic that makes the horn fly especially suited to an integrated program is its susceptibility to control with insecticides. Many of those that are available for use can reduce the number of adult horn flies to levels that are essentially undetectable. Therefore, if a herd is isolated so that no flies can migrate into the area, elimination of horn flies from the herd might be possible with insecticides alone. This level of control can probably be achieved quite economically with a maximum of 2 - 4 applications made at 10- to 14-day intervals, even if the population was high. Therefore, at almost any time of the year, we should be able to suppress horn-fly populations to low enough levels so that a sterile-male release program could be initiated. (Insecticides would also be very effective as an emergency control in the event of a temporary lapse in a release program.)

One possibility might be the simultaneous use of an insecticide to reduce the natural population and the release of sterile insects that are highly resistant to that insecticide. For example, a laboratory strain of horn flies is at present being maintained at the Kerrville laboratory which exhibits more than a 400X resistance to ronnel [23]. Then, from the hypothetical model, if ronnel were used to reduce the number of adults on the herd of 100 cattle by 90% and if ronnel-resistant sterile flies were released at the same time, the number of sterile flies required would drop from 18000 per day (180 per animal) to 1800 (18 flies per animal per day), a rate that would be much more realistic from the standpoint of damage to the cattle.

The application of two or more properly timed treatments with insecticide to cattle in late summer and early fall might result in an even more efficient integrated control program. Studies indicate that diapause in horn flies is controlled by an interaction between the effect of photoperiod on the adult fly and the effect of temperature during subsequent larval development [24, 25]. Therefore, adults found on the cattle in the late autumn are apparently the parents of the diapausing generation(s) responsible for the spring build-up in areas where diapause is necessary for survival over the winter. Then the use of insecticides to control the adults found on cattle in autumn would probably severely reduce, if not eliminate, the overwintering population. In southern areas of the horn fly range where some emergence occurs throughout the year, sterile flies could be released at low level (2 - 5 per animal per day) throughout the winter and spring months to eliminate the few remaining flies. In the northern regions, releases of sterile flies might not be necessary until early spring just before the beginning of emergence of the native flies. Even if total eradication of the flies in an area could not be accomplished, a sustained low-level release of sterile flies throughout the year might keep the native population in check, once it had been suppressed to a low level by conventional means. The constant presence of 25 - 50 sterile horn flies on animals throughout the year would probably not be detrimental. Also, this type of constant pressure on the native population might cost

no more than is presently being spent on conventional control which is much less efficient.

The introduction of biological control agents might also play an important role in an integrated control program. Although we cannot yet be specific about which species are the most important, the field trials mentioned in section 3 demonstrated clearly that competition plays an important part in the number of horn flies produced in manure pats. If the native competitor species causes a 10-fold reduction, an introduced species might cause a greater reduction and could thus greatly facilitate the chemical and/or sterile-release program. The possibility of introducing African species of dung beetles (Scarabaeidae) into the USA is therefore being investigated. Such an introduced species could destroy the manure pats and cause a significant additional reduction in the populations of horn flies [26].

5. OTHER FACTORS ASSOCIATED WITH HORN FLY STERILE-RELEASE PROGRAM

In the hypothetical model discussed, isolation of the host animals was assumed, but we lack knowledge about the migration of flies from adjacent areas or herds. This lack makes it difficult to estimate the number of sterile males needed in larger releases. Some efforts have been made to determine the pattern of movement of horn flies [27-29], but little is actually known. Possibly the newly emerged fly migrates extensively in search of its initial host (animal-to-animal migration within herds would be normal because the animals come in close proximity to each other), but, unless the flies become overcrowded, there is no need for movement because the animal provides the horn fly with its food supply and oviposition medium. This behaviour is an advantage to the sterile-male release of horn flies since one prerequisite of a successful program is the easy location by the male of its female partner. Because the horn fly is an obligate, host-specific parasite on cattle and remains there day and night, no extensive searching for the female is needed once the sterile male has found its host – the female will also be there.

Certainly, the release of bloodsucking parasites, sterile or not, will add to the annoyance and destruction caused by the native population. However, if integrated programs are used, this added annoyance should not pose an important problem. Indeed, if an integrated sterile-release program were effective, the number of naturally occurring flies would be so reduced that the total population, including sterile flies, would probably be lower than the normal population, especially in the southern areas of the USA.

It has been suggested that with successful control or eradication of a species, another species might increase to fill the vacated ecological niche [30]. However, the only other cattle parasite that might possibly become a problem would be the face fly, Musca autumnalis De Geer, which also breeds in cattle manure on the range. The extent of competition of the horn fly with the face fly in areas where both species occur is unknown.

The horn fly does not appear to be isolated in its range, either by topography or climate. Therefore, the eventual eradication of this pest from North and Central America would require a highly co-ordinated

international effort. We are confident, however, that many of the problems of population dynamics and mass rearing can be solved. Increased efficiency should allow us to produce horn flies at a cost below the present $1.00 per 1000, but even if this cost could not be reduced, the use of sterile flies during the final stages of an integrated program might be less expensive and more efficient than insecticides as a method of eliminating the few remaining insects [16]. In addition, an integrated eradication program similar to that suggested would, in the long run, be much more economical than the present conventional methods being used.

REFERENCES

[1] McLINTOCK, J., DEPNER, K.R., A review of the life-history and habits of the horn fly, Siphona irritans (L.) (Diptera: Muscidae), Can. Ent. 86 (1954) 20.

[2] USDA, ARS. Losses in Agriculture, p.82. Agr. Handbook No.291 (1965).

[3] HIBLER, C.P., Development of Stephanofilaria stilesi in the horn fly, J. Parasitol. 52 5 (1966) 890.

[4] GLASER, R.W., The effect of food on longevity and reproduction in flies. J. exp. Zool. 38 (1923) 383.

[5] GLASER, R.W., Rearing flies for experimental purposes with biological notes, J. econ. Ent. 17 (1924) 486.

[6] McLINTOCK, J., DEPNER, K.R., Preliminary attempts to establish a laboratory colony of the horn fly, Siphona irritans (L.) (Diptera: Muscidae), J. Parasitol. 43 (1957) 209.

[7] DEPNER, K.R., Continuous propagation of the horn fly, Haematobia irritans (L.) (Diptera: Muscidae), Can. Ent. 94 8 (1962) 893.

[8] HARRIS, R.L., Laboratory colonization of the horn fly, Haematobia irritans (L.), Nature, Lond. 196 4850 (1962) 191.

[9] HARRIS, R.L., FRAZAR, E.D., GROSSMAN, P.D., Artificial media for rearing larvae of horn flies, J. econ. Ent. 60 3 (1967) 891.

[10] SCHMIDT, C.D., HARRIS, R.L., HOFFMAN, R.A., Mass rearing of the horn fly, Haematobia irritans (Diptera: Muscidae), in the laboratory, Ann. ent. Soc. Am. 60 3 (1967) 508.

[11] SCHMIDT, C.D., HARRIS, R.L., HOFFMAN, R.A., New techniques for rearing horn flies at Kerrville, 1967, Ann. ent. Soc. Am. 61 4 (1968) 1045.

[12] HOFFMAN, R.A., SCHMIDT, C.D., A Tumbler Dryer for Fly Pupae, USDA ARS-33-128 (1968).

[13] BRUCE, W.G., The History and Biology of the Horn Fly, Haematobia irritans (Linnaeus); with Comments on Control, N. Carolina Agr. Exp. Sta. Tech. Bull. 157 (1964).

[14] GRADY, A.G., Studies in breeding insects throughout the year for insecticide tests, J. econ. Ent. 21 (1928) 598.

[15] GLASER, R.W., Note on the continuous breeding of Musca domestica, J. econ. Ent. 20 (1927) 432.

[16] KNIPLING, E.F., The Potential Role of the Sterility Method for Insect Population Control with Special Reference to Combining This Method with Conventional Methods, USDA ARS-33-98 (1964).

[17] CHAMBERLAIN, W.F., Entomology Research Division, Agr. Res. Serv., USDA, Kerrville, Texas, personal communication.

[18] HARRIS, R.L., FRAZAR, E.D., Chemosterilization of adult horn flies, J. econ. Ent. 59 5 (1966) 1171.

[19] HARRIS, R.L., MILLER, J.A., FRAZAR, E.D., Eclosion rhythms of horn flies under laboratory conditions, Ann. ent. Soc. Am. (in preparation).

[20] KUNZ, S.E., BLUME, R.R., HOGAN, B.F., MATTER, J.J., Biological and ecological investigations of horn flies in central Texas: Influence of time of manure deposition on oviposition, J. econ. Ent. (1970) (in press).

[21] SANDERS, D.P., DOBSON, R.C., Contributions to the biology of the horn fly, J. econ. Ent. 62 6 (1969) 1362.

[22] BLUME, R.R., KUNZ, S.E., HOGAN, B.F., MATTER, J.J., Biological and ecological investigations of horn flies in central Texas: Influence of other insects in cattle manure, J. econ. Ent. (1970) (in press).

[23] HARRIS, R.L., FRAZAR, E.D., GRAHAM, O.H., Resistance to ronnel in a strain of horn flies, J. econ. Ent. 59 2 (1966) 387.

[24] DEPNER, K.R., The effect of temperature on development and diapause of the horn fly, Siphona irritans (L.) (Diptera: Muscidae), Can. Ent. 93 10 (1961) 855.

[25] WRIGHT, J.E., Diapause in the horn fly, Haematobia irritans: a preliminary study, Ann. ent. Soc. Am. (in press).
[26] BORNEMISSZA, G.F., Could dung eating insects improve our pastures, J. Australian Inst. agr. Sci. 26 1 (1960) 54.
[27] EDDY, W.G., ROTH, A.R., PLAPP, F.W., Jr., Studies on the flight habits of some marked insects, J. econ. Ent. 55 (1962) 603.
[28] TUGWELL, P., BURNS, E.C., WITHERSPOON, B., Notes on the flight behavior of the horn fly, Haematobia irritans (L.) (Diptera: Muscidae), J. Kansas ent. Soc. 39 4 (1966) 561.
[29] HOELSCHER, C.E., COMBS, R.L., Jr., BRAZZEL, J.R., Horn fly dispersal, J. econ. Ent. 61 2 (1968) 370.
[30] PROVERBS, M.D., Induced sterilization and control of insects, Ann. Rev. Ent. 14 (1969) 81.

DISCUSSION

L.E. LaCHANCE: The program outlined by Mr. Kunz for the control of the horn fly is indeed most intriguing. I would certainly foresee a high probability of success. I would, however, have considerable reservations about using a combination of gamma rays and chemosterilant to produce a competitive sterile fly; I believe all possible modifications of irradiation alone or chemical treatment alone should be considered, such as irradiation in nitrogen as described by Mr. Hooper in his paper (IAEA-SM-138/45). There are also many other modifications of radiation treatment which might increase male competitiveness.

S.E. KUNZ: I would agree with your observations. As was pointed out several times in Sessions I and II, we may be better off by accepting a sterility of something less than 100% if we can provide a fly that is more competitive.

It may also be that if we can develop a colony of flies more vigorous than the present one, we will have a higher degree of competitiveness when flies are treated at the doses mentioned.

J.W. WRIGHT (Chairman): Has anything been done to introduce genetic factors into the laboratory colony of horn fly which would perhaps give it more competitiveness and activity, as has been done in the case of other insects?

S.E. KUNZ: Yes, studies are being conducted now in which wild flies are being introduced into the colony. I do not, however, have any results of these studies to pass on at the moment.

C.T. LEWIS: In Texas, to what extent are dung beetles a factor in limiting the population of horn flies by breaking down the manure pats before the larvae can develop?

Also, can you comment further on the possible value of introduced species?

S.E. KUNZ: The competition of all other species inhabiting the manure pat is apparently responsible for about a tenfold decrease, as measured by the emergence of flies from pats left exposed to the environment and those that were protected from these competing species. What part the native dung beetles play is not known.

The native dung beetles inhabit the manure, but they do not break up the pat as do the African species being used in Australia to help control the buffalo fly.

If the species being considered for introduction are going to act as they do in Australia, they should greatly reduce horn fly production, as these beetles completely break up the manure pat, making it unsuitable for horn fly development.

STERILITY AND CHAGAS' DISEASE VECTOR CONTROL

J.C. GÓMEZ-NÚÑEZ
División de Endemias Rurales,
Dirección de Malariología,
Ministerio de Sanidad,
Maracay, Venezuela

Abstract

STERILITY AND CHAGAS' DISEASE VECTOR CONTROL.

A review is made of results obtained from the application of ionizing radiations and chemosterilants in laboratory studies related to the control of Rhodnius prolixus (Stål) in Venezuela. This vector of Chagas' disease was chosen not only by its local importance but also because its ecological characteristics were considered appropriate for the sterile-male technique.

Because of the damage caused by the radiation required for complete sterility the use of both X- and gamma-radiations failed to produce sterile males able to compete with normal ones. However, males exposed to a dose causing 75% sterility had their activity increased and mortality among progeny from normal females, mated with irradiated males, was significantly higher than in normal nymphs. These males were tested at varying ratios to normal males in the laboratory and in all cases the initial group fertility reduction was followed by a recovery.

Similar tests with chemosterilants produced less undesirable results but recovery of fertility and certain manipulation difficulties indicate that the release of laboratory-treated males in the field would be unfeasible. The same results, though, suggested another approach based on the direct use of chemosterilants within houses infested by vectors of Chagas' disease. Although the work is still at an experimental stage, preliminary results from laboratory studies indicate that chemosterilants placed in a container to prevent human contact could provide an effective control method not only for Rhodnius prolixus but for other house-infesting vectors.

INTRODUCTION

Chagas' disease has been the subject of considerable attention in the neo-tropical region. Its pathogen, Trypanosoma cruzi, a protozoan with reservoirs in mammals, is mainly transmitted by Reduviidae bugs and may ultimately invade myocardium cells. An indication of the disease's importance as a public health hazard can be obtained from data gathered in Venezuela indicating that about 4% of the population shows electrocardiographic signs of heart damage specific to this disease.

At present there is no practical means of treating the disease and the main preventive effort is directed towards the elimination or control of vectors responsible for the human infection.

In Venezuela Rhodnius prolixus (Stål) is paramount in this role. It has three known habitats: certain palm trees, bird nests and human dwellings of the more primitive kinds. Currently applied control measures include the use of insecticides and the execution of rural housing programs. The high cost of both measures, R. prolixus being resistant to most inexpensive insecticides, has made imperative intensive biological and ecological studies of the vector in search of new or improved control methods.

The effect of induced sterility in males has been included in these studies and this paper reviews and compares results obtained from the application of ionizing radiations [1, 2] and metepa, tris 1-(2-methyl-aziridinyl) phosphine oxide, the first of a series of chemosterilants programmed to be tested [3].

MATERIAL AND METHODS

The insects used came from colonies kept according to standard local procedures [4] where adult output is of equal morphological development and physiological age and thus biologically comparable. Samples, varying in size depending on the parameter studied, were kept in 3900-ml glass jars at a constant temperature of 28 ± 1°C and a relative humidity ranging from 52 to 70%. Hen blood was used as food and a feeding opportunity of two hours per jar was given every 14 days. Sexes were separated during the fifth nymphal stage to prevent unprogrammed matings. Except for tests to determine effects caused by alternating the sequence of feeding and sterilization, insects were exposed to radiations or metepa after feeding and emergence to adult. Other morphological stages were previously tested but results were discouraging [1, 5].

Two sources of ionizing radiations were used: A 200-kW 20-mA X-ray emitter [1] and a ^{60}Co 500-Ci unit for gamma radiation [2]. Dosage was measured by ionizing chamber and chemical means. Metepa was applied in an acetone solution on the thorax by a microdosifier [3].

Fertility, oviposition and longevity were determined by daily and weekly direct observations. Male mating frequency was measured by tests consisting of ten pairs, male and virgin female, placed in individual containers, and by observing, directly and by egg fertilization, the number of matings in one hour, and the time required to complete each mating.

Relative activity was determined by direct observation of the insect's movement and by the use of a photo-cell counting device.[1]

Mating competitiveness of treated males, when challenged by the presence of normal males, was evaluated by observing the percentage of eggs hatching from jars containing normal males:treated males:normal females in ratios: 10:0:10, 9:1:10, 7:3:10, 5:5:10, 3:7:10, 1:9:10 and 0:10:10.

A comparison was then made of observed and expected values. Expected values were graphically calculated by drawing a straight line connecting the observed hatching percentage from jars containing only normal males (10:0:10) to that from jars with only treated males (0:10:10) and using as a baseline a scale proportional to the ratios.

The connecting straight line was assumed to represent the expected percentage of eggs hatching at any normal male:treated male ratios if both were equally competitive. Values above the connecting line indicate more eggs hatching than expected, and thus a dominance by normal males, and values below the line indicate greater influence by treated males.

The effect of time was noted by repeating tests every 2 weeks using the same treated specimens.

---

[1] Throughout this paper the word 'activity' is used in the sense given here, except in the term 'mating activity'.

TABLE I. EFFECTS OF IONIZING RADIATION ON MALE R. prolixus

Parameter		Ionizing radiation (kR): Control	2.5	5.0	10.0	20.0	40.0
% Fertility		91.1	58.0	30.5	7.9	0.6	0.0
Induced oviposition: eggs/female/wk		10.9	14.4	14.3	13.4	2.8	-
% Males mating in one hour		40.0	-	60.0	50.0	-	-
Relative activity		100	106	119	87	21	0
Mean longevity (days)		117	98	96	58	36	11
% F_1 nymphal mortality		45.2	83.8	92.4	91.5	-	-
Relative mating competitiveness							
1:1 ratio treated male: normal male							
% Hatching:	Expected value	92.0	74.5	64.1	51.0	-	-
	Observed value	92.0	78.1	68.3	56.4	-	-
	Difference	0.0	+3.6	+4.2	+5.4	-	-

All control groups were similarly handled and kept as the test groups. The significance of the differences was checked by chi-square and T-distribution tests and based on a 5% level.

More detailed descriptions of material and methods are given in the references.

RESULTS

Although tests with male R. prolixus exposed to either X- or gamma-radiation produced similar results, a not significant trend in fertility, activity and longevity changes suggested that X-radiation caused greater damage than gamma-radiation for the same level of sterility [2].

A summary of mean values obtained through the use of both radiations is given in Table I and Fig.1 and except for those related to longevity they represent the average effects observed during a 4-week post-irradiation period; this being longer than the expected adult R. prolixus survival in the field [6].

Exposure to 20 kR inhibited almost all fertility and to 40 kR produced completely sterile but, because of excessive damage, non-functional males. Irradiation before feeding caused more relative sterility but also more unwanted damage.

Specimens not fed after exposure to 10 or more kR survived longer than those fed regularly after similar irradiation. Sterility increased in direct relation to the number of matings accomplished.

Induced oviposition, resulting from normal females mated to irradiated males, was increased by exposures up to 10 kR and inhibited by more radiation. Insect activity and mating frequency followed a similar pattern,

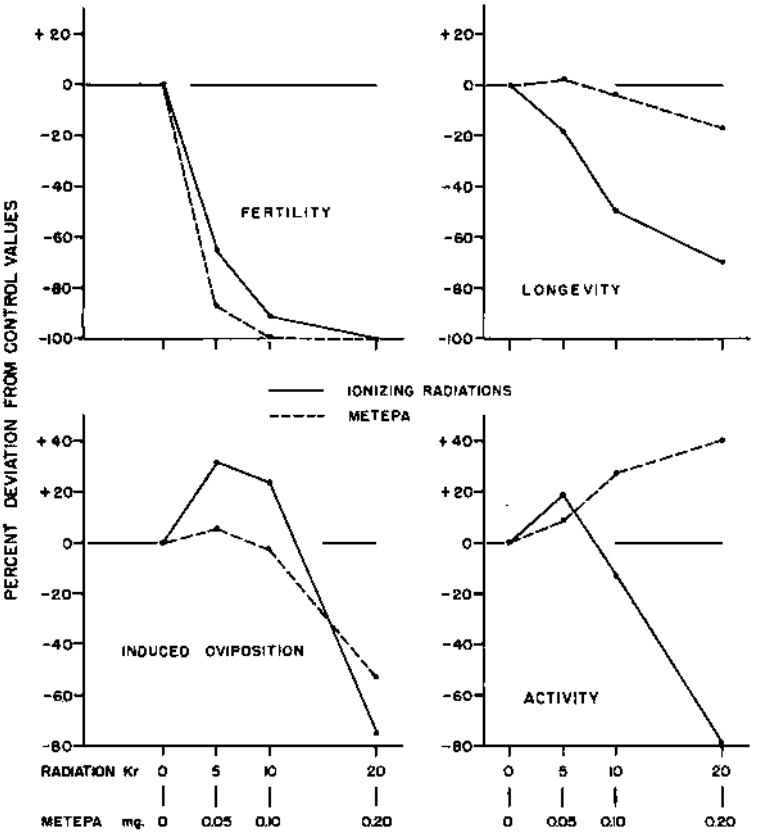


FIG. 1. Effects of ionizing radiations and metepa on R. prolixus.

but generally the mating process lasted longer in irradiated males – twice as long at 10 kR – than in normal controls. Longevity was significantly reduced by exposures exceeding 5 kR.

In all cases sterility increased with time at an average weekly rate of 8% for X- and 5% for gamma-radiation; but this was associated with decreased activity and increased mortality.

A residual effect of radiation was noted in nymphs descendent from irradiated males and normal females. Nymphal mortality, accumulative throughout the five morphological stages, increased according to male parent exposure and was double that of controls at 10 kR.

As lower or higher exposures produced less favourable results, either because of insufficient sterility or excessive radiation damage, mating competitiveness values are given only for the groups exposed to 5 and 10 kR. Differences between observed and expected percentage of eggs hatching at a 5:5:10 ratio are shown in Table I and results from all ratios in Fig.2. The similarity of the observed to the expected values suggests that males exposed to 5 kR are more competitive than those exposed to 10 kR although the latter, being less fertile, are capable of a more

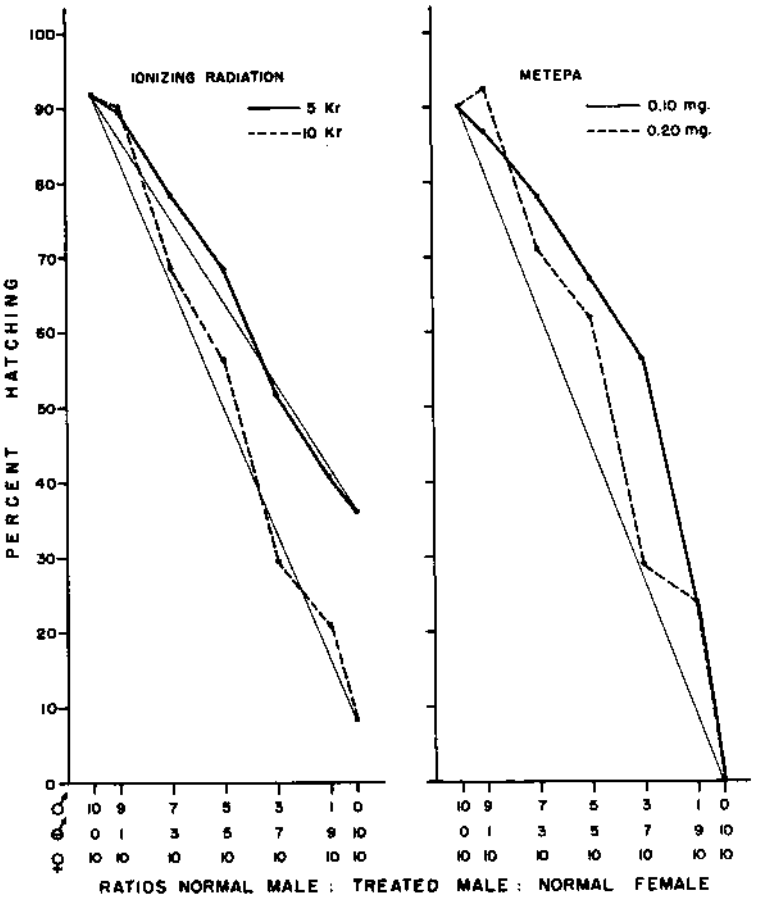


FIG.2. Comparative competitiveness of sterile male R. prolixus.

marked percent hatch reduction. At the end of the 4 weeks of observation average percentage of eggs hatching in the two competitiveness tests was 20% greater than at the start; but the sterility of irradiated males also increased 20% during the same period.

Results from preliminary studies of male R. prolixus sterilization by metepa are given in Table II and Fig.1. Since metepa was diluted in acetone, normal males and males treated with acetone alone were compared to determine any significant difference and none was found.

Complete sterility was approached with 0.10 mg of metepa per male and induced oviposition became significantly lower with larger doses. Male mating frequency increased proportionally with doses up to 0.10 mg but was inhibited by larger ones. Activity progressively increased in direct proportion to dosage.

Contact with metepa adversely affected longevity but its reduction was significant only in specimens treated with 0.40 mg.

TABLE II. EFFECTS OF METEPA ON MALE R. prolixus

Parameter		Control	Metepa dose (mg): 0.05	0.10	0.20	0.40
% Fertility		92.5	11.7	1.2	0.0	0.0
Induced oviposition: eggs/female/wk		11.4	12.0	11.1	5.4	5.0
% Males mating in one hour		40	57	76	43	34
Relative activity		100	109	128	140	202
Mean longevity (days)		73.0	74.5	70.0	61.0	45.5
Relative mating competitiveness						
1:1 ratio treated male: normal male						
% Hatching:	Expected value	90.0	-	45.0	45.0	-
	Observed value	90.0	-	67.0	62.0	-
	Difference	0.0	-	+22.0	+17.0	-

Male competitiveness (Table II and Fig.2) was adequate only with 0.10 and 0.20 mg treatments. Lower doses did not sufficiently reduce fertility and higher ones inhibited mating.

Fertility recovered at a general average weekly rate of 3% but males treated with 0.10 mg or less were again fully fertile 5 weeks after treatment. On the other hand the oviposition inhibition caused by treated males on normal females remained throughout the 11 weeks of observation.

The increase in mating frequency noted during the first 4 weeks after treatment subsided after 5 weeks and became half the control value at the end of 11 weeks.

The sequence of treatment in relation to feeding and variation in insect age produced no significant differences in the results.

DISCUSSION

Effects of ionizing radiations on male R. prolixus were characterized by the inhibition of fertility and longevity in direct proportion to exposure, and by changes in the insect's activity. Fertility was furthermore reduced by the number of matings and time elapsed after exposure; this suggests that radiation, besides the effect on the whole organism, acted on the reproductive system by affecting mainly spermatogonia and not spermatozoa. This assumption was verified by the observation, shortly after irradiation, of damaged apex germ cells coinciding with apparently normal active spermatozoa in specimens exposed to 10 and 20 kR, and the disappearance of that activity with time and consecutive matings [1, 2].

Evaluation of fertility changes requires caution since inhibition of mating capability may be mistaken for sterility. However, in R. prolixus,

where by mating the male stimulates female oviposition, the number of eggs deposited during a fixed time period is a suitable indicator of successful copulation. The observed rise in induced oviposition caused by males exposed to 2.5, 5.0 and 10.0 kR was in accord with the similar rise in mating frequency, and with the increase in activity observed in those males. Consequently, since the low fertility of males exposed to 20 and 40 kR coincided with a low capacity to induce oviposition, it was assumed that instead of actual sterility what the results reflected was mainly a loss of mating capability. This was supported by the lack of activity noticed in those males. The correlation between induced oviposition, mating frequency, and activity resulting from exposing males to 2.5 and 5 kR could have suggested that mating frequency was stimulated by the increase of activity, which favours the chances of both sexes meeting. However, since males exposed to 10 kR showed a higher frequency of mating but less activity than the controls, changes in these two parameters were probably somewhat independent manifestations of an overall physiological stimulus, increasingly triggered by exposures up to 5 kR and continuing from 5 to 10 kR but then subdued by the damage from higher exposures.

Longevity was greater in R. prolixus fed routinely after irradiation; however, when exposure exceeded 10 kR, specimens kept starved survived longer. This may have been motivated by the increase in cellular activity produced in R. prolixus by feeding [7] and that this activity in turn makes evident latent cell damage [8]. Thus the appearance of the excessive damage produced by exposures to 10 or more kR was accelerated in fed specimens and caused them to die earlier than starved ones. The same process could have been responsible for the significantly greater sterility noted in males fed after irradiation when compared to those fed before it [2].

Mortality in descendants from irradiated males was high in the 2.5 to 10 kR groups and complete in the one exposed to 20 kR. Death usually occurred during hatching and was characterized by the nymphs' inability to emerge through the operculum.

Concerning the results from irradiated male - normal male mating competitiveness tests, males exposed to 10 kR reduced the percentage of eggs hatching more than those subjected to the other exposures used in the study. However, males exposed to 5 kR, although less sterile, did not have their activity and longevity so adversely affected and were able to compete more efficiently with normal males.

Regarding the metepa tests [3], an individual dose of 0.10 mg caused almost complete sterility in adult male R. prolixus. However, fertility started to recover 5 weeks after treatment and although larger doses retarded this recovery it was at the expense of more unwanted damage to the insect.

Metepa's action apparently was directed mainly towards the spermatozoa and if the gonads were also affected it was in a reversible manner.

The effect of induced oviposition probably was caused by two different actions: by inhibition of mating frequency as noted in males treated with 0.40 mg and by the possibility of metepa being carried in the sperm and thereby affecting the normal female's gonads in a similar manner as in metepa-treated house flies [9]. This was suggested by the inconsistency of normal mating activity along with the significantly lower induced oviposition observed in 0.20-mg-treated males.

Mating frequency followed the increase in activity up to and including a dose of 0.10 mg. With higher doses activity continued its increase but mating frequency declined. Insects treated with 0.40 mg showed a marked restlessness and apparently this interfered to some extent with mating. This effect of metepa on the behaviour of R. prolixus indicated that it may have acted as an activity stimulant proportional to the dose applied.

Longevity was significantly reduced only in the group treated with 0.40 mg of metepa, but the insects remained active until death.

Results from mating competitiveness tests showed males treated with doses of 0.10 and 0.20 mg to be the most successful in reducing the percentage of eggs hatching, but they were not equally competitive with the untreated males. Comparison of the two groups indicated that those treated with 0.10 mg, although as sterile as and more sexually active than the 0.20 mg group, were the less efficient in affecting reproduction. The difference could have been motivated by the faster recovery of fertility in the 0.10 mg group and by the assumed passing of metepa to the female by males treated with 0.20 mg. If this actually occurred, then males from the 0.20 mg groups had the added advantage of neutralizing, by the damage caused to the female's reproductive system, any subsequent mating with normal males.

Comparing results from the ionizing radiation test with those from the metepa tests (Fig.1) it could be concluded, from all parameters studied, that metepa is the most suitable for R. prolixus sterilization. The main differences between the two agents were found not so much in the results as in the means of causing them. Metepa-induced sterilization, apparently by affecting mainly the spermatozoa, was reversible and generally the effects were confined to the reproductive system, thus causing less damage to the insect. Conversely, ionizing radiations, by affecting mainly the spermatogenesis, caused an irreversible sterility which augmented with time; effects were also generalized and greater damage was inflicted upon the insect. The observations with regard to the difference in the extent of damage are supported by the effects on longevity and by the influence that the sequence of feeding and irradiation had on the results from ionizing radiations but not on those from the metepa tests.

Differences in the relationship between induced oviposition, mating frequency and activity suggest that although up to a treatment level results were similar, metepa and ionizing radiations acted in a different manner. Both promoted frequency of mating at lower doses and then hindered it with higher ones: ionizing radiations hindered it by general damage and immobility, and metepa apparently hindered it by excessive activity.

Induced oviposition in the groups exposed to radiation was related to mating frequency, as in normal R. prolixus, and the female's egg-producing capacity was not affected by the male's exposure to radiations. Induced oviposition, in the metepa groups treated with 0.20 mg or more, lost its correlation to mating frequency and the normal female was irreversibly affected by the male's treatment.

The advantage of metepa over ionizing radiations, as a means for applying the sterile-male technique to R. prolixus control, diminishes when a comparison is made of the response of the respectively treated males to the presence of normal males (Fig.2). Although males treated with 0.20 mg of metepa, by being completely sterile, were able to reduce the percentage of eggs hatching to a lower level than males exposed to

10 kR, the latter produced results nearer to those expected, indicating better competitiveness. That males exposed to 10 kR were more efficient competitors than males treated with 0.20 mg of metepa could have resulted from a shorter time required for mating, irreversible sterility and lack of excessive restlessness. Although from the studies it was concluded that neither sterilizing agent could be successfully used for the application of the sterile-male technique to R. prolixus control, certain characteristics of metepa suggested the possibility of another approach based on the placement of the sterilizing agent in special containers to avoid human and other vertebrate contact, within houses infested by R. prolixus [10]. Preliminary laboratory tests using metepa-impregnated plastic strips have given results which indicate that a short contact with strips dipped in a 25% solution sterilized both nymphs and adults, and also that metepa acts as a slight attractant to R. prolixus. Field trials were made in houses, with varying degrees of infestation, to determine the probability of any individual R. prolixus passing through a strip container [11], and results gave the probability of sterilization of all R. prolixus in a house as approaching 1.0 during a period of 29 days. Since R. prolixus needs a minimum of 83 days to complete its life cycle, the 29-day period theoretically implied that no R. prolixus could complete its life cycle without coming into contact with the sterilant. Because they tend to repel R. prolixus common insecticides if used in this method would prolong the period required for "P" to approach 1.0.

Although the studies reviewed did not produce favourable results in relation to the sterile-male technique, they did promote intensive research programs dealing with the biology and ecology of R. prolixus and other vectors of Chagas' disease. Furthermore the experiments with chemical sterilants have opened up new possibilities for control of these vectors and have also stimulated the search for general attractants and more persistent sterilizing agents for use in the future work.

REFERENCES

[1] GOMEZ-NUÑEZ, J.C., GALLIMORE, J.C., FERNANDEZ, J., GROSS, A., Acta cient. venez. 13 2 (1962) 46.
[2] GOMEZ-NUÑEZ, J.C., GROSS, A., MACHADO, C., Acta cient. venez. 15 3 (1964) 97.
[3] FELICIANGELI, D., División de Endemias Rurales, Dirección de Malariología, Maracay, Venezuela (in preparation).
[4] GOMEZ-NUÑEZ, J.C., Bull. Wld Hlth Org. 31 (1964) 565.
[5] BALDWIN, W.F., SHAVER, E.L., Can. J. Zool. 41 (1963) 637.
[6] GOMEZ-NUÑEZ, J.C., J. med. Ent. 6 1 (1969) 83.
[7] WIGGLESWORTH, V.B., J. exp. Biol. 14 (1937) 364.
[8] BALDWIN, W.F., SALTHOUSE, T.N., Radiat. Res. 10 (1959) 397.
[9] CHANG, S.C., BORKOVER, A.B., WOODS, C.W., J. econ. Ent. 59 (1966) 937.
[10] FELICIANGELI, D., GOMEZ-NUÑEZ, J.C., Proceedings XIX Meeting Asc. Venez. Avance de la Ciencia (1969) 239.
[11] GOMEZ-NUÑEZ, J.C., Acta cient. venez. 16 1 (1965) 26.

DISCUSSION

R.U. CARCAVALLO: Just to ensure that we are applying the same criteria, might I ask you how you define 'sterility' and 'recovery of fertility'?

J.C. GÓMEZ-NÚÑEZ: Sterility was taken to be the elimination of eclosion from eggs produced by females mated with treated males. Recovery

was signified by the occurrence of eclosion from the eggs of females mated subsequently with these males.

R.C. CARCAVALLO: As it is perhaps interesting to compare Rhodnius prolixus and Triatoma infestans, I should like to mention that bugs of the latter species move their antennae before copulation and before eating, but we have not established whether this behaviour is affected by the presence of the other sex.

The greater activity of Triatoma infestans and the greater number of matings could be due to stimulus of the interstitial cells of the testicle.

J.C. GÓMEZ-NÚÑEZ: We have found that the movement of the antennae of Rhodnius prolixus is specifically related to the presence of the opposite sex.

K.S. RAI: What is the possible explanation for the increased mating ability per hour and the increased activity that you observed in both irradiated and chemically treated insects?

J.C. GÓMEZ-NÚÑEZ: We do not have any specific data with respect to chemically treated specimens at the moment. However, the activity increase in irradiated Rhodnius prolixus up to a certain exposure dose is apparently the result of a general physiological stimulus generated at cell level. Oxygen consumption by irradiated immobilized specimens follows the same pattern as the activity changes.

RACHEL GALUN: Seeing that the treated, released male will feed on humans, why do you consider that this insect should be controlled by the sterile-male technique?

J.C. GÓMEZ-NÚÑEZ: No household release was contemplated; the technique could have been applied in infested areas remote from human dwellings.

RACHEL GALUN: Did you try to rear this insect by artificial feeding?

J.C. GÓMEZ-NÚÑEZ: Yes, but results were not satisfactory.

D.G. CAMPION: We have also found that there is increased mating of the moth Diparopsis castanea after the application of certain chemosterilants. This was associated with increased levels of catecholamines, a major component of which was noradrenaline.

D.W. WALKER: What are the specific courtship signals of this species? Can they be used to attract males in a natural habitat?

J.C. GÓMEZ-NÚÑEZ: I think Mr. Baldwin has more experience in this subject than I.

W.F. BALDWIN: In answer to Mr. Walker's question, we have not been able, in studies of sexual pheromones in Rhodnius prolixus, to detect any signs of sexual excitement in the male insects. It is always possible to induce mating by shaking a fed male with a fed female in a vial, but males do not react to the presence of a female, other than by the act of mating.

I should like to put a question of my own, if I may. Dr. Gómez-Núñez has stated that in houses all Rhodnius prolixus can be expected to come in contact with a chemosterilant at some time during their life cycle. My question is: will a sterile adult male result from contact of the insect with the chemosterilant during the second or third instar stage of development?

J.C. GÓMEZ-NÚÑEZ: The doses currently administered may even cause death in the early nymphal stages.

WHO program

R. PAL: I should like to take this opportunity to describe very briefly the World Health Organization program of work on the genetic control of insects of importance from a public health point of view. Before I mention the actual work in progress I shall outline how this program is being conducted by WHO.

WHO established its own field research units, and one of them, located at New Delhi, is responsible for carrying out work on the genetic control of Culex pipiens fatigans Wiedemann, Aedes aegypti and Anopheles stephensi. This unit is working in very close co-operation with the WHO International Reference Centres and collaborating laboratories.

WHO has established four International Reference Centres, concerned respectively with work on Culex pipiens fatigans Wiedemann (under the direction of Professor Laven, Mainz, Germany), Aedes aegypti (under the direction of Professors Craig and Rai, University of Notre Dame, Indiana, USA), Anopheles (under the direction of Dr. Davidson, London, UK) and house flies (under the direction of Professor Milani, Pavia, Italy). I am happy to see that three of them are present at this Symposium and will be telling you about their work themselves.

Then there are the collaborating laboratories and scientists. Their activities include work on reduviid bugs, Simulium and ticks; I am pleased to see Drs. Gómez-Núñez, Carcavallo and Galun here.

Now let me say a few words about the actual work. Studies on the following species are in various stages of completion:

Culex pipiens fatigans Wiedemann	- Vector of filariasis
Aedes aegypti	- Vector of arboviruses
Anopheles gambiae	- Chief malaria vector in Africa
Anopheles stephensi	- An important malaria vector in South-East Asia and the Middle East
Tsetse flies	- Vector of trypanosomiasis
Reduviid bugs	- Chagas' disease
Simulium	- Onchocercosis
Ticks	- Relapsing fever.

Our major effort is being devoted to work on culicine mosquitoes. WHO has established a large unit at Delhi in collaboration with the United States Public Health Service and the Indian Council of Medical Research. Three international and 20 Indian scientists of high standing have been appointed. At its peak activity, the unit will employ about 180 persons and will have a budget of about 2.8 million dollars over the 7-year period. The objective of the unit is to demonstrate the operational and economic feasibility of the genetic control of Culex pipiens fatigans Wiedemann and Aedes aegypti by employing any of the known genetic mechanisms, i.e. the sterile-male technique, cytoplasmic incompatibility, translocations, etc. The detailed plan of this unit, which started operation in 1969, has been prepared on the basis of the PERT system; a few copies of the plan are available for distribution.

The practical problems which must be solved before releases can be made include a determination of whether the dispersal, survival and mating behaviour of released males under normal conditions are adequate to make them competitive with normal males, the development of a method for determining how many males must be released per unit area, the development of inexpensive procedures for rearing millions of mosquitoes, the separation of males from females, etc. Some of the mechanisms will be discussed in Session VII and I shall not go into the details of these at present. However, I would make three points.

Firstly, considerable emphasis is being placed in this unit on ecological studies, particularly on population dynamics, dispersion and mating habits. Secondly, attention is being devoted to mass-rearing techniques and the release pattern, etc. Last, but not least, stress is being laid on integrated control, as it would be necessary to use insecticides to reduce the population before releases are made.

As was mentioned earlier, the question of an ecological vacuum is also being borne in mind in case it should be necessary to replace the species.

The work on Anopheles mosquitoes concerns Anopheles gambiae and Anopheles stephensi.

Anopheles gambiae is a special complex of five sibling species, and crosses between them produce hybrid sterile males but normal females. In certain crosses, the sex ratio is distorted and only males are produced. In the laboratory, when hybrid sterile males were released in a cage with normal males and females, the sterile males were competitive and females produced sterile eggs. The idea was to obtain the eggs of hybrid sterile males on filter paper and release them in the breeding sites. This was done in the field by Davidson and others, but owing to heavy natural mortality pupae were released instead. The experiment was not successful as the males produced from a cross between species B and melas did not mate with species A found in the experimental village of Pela, Bobo-Dioulasso, Upper Volta.

Further studies by Dr. Davidson have shown that hybrid sterile males give better results with either of the two species crossed, but whether hybrid males will mate in nature has yet to be ascertained.

With respect to Anopheles stephensi, efforts are being made to discover some genetic mechanisms. The species is known to consist of two races.

In addition to the activities in connection with the species mentioned above, work is also being carried out on tsetse flies by IAEA and the United States Department of Agriculture, and as you have heard this morning, a UNDP project for Zambia is under consideration.

Dr. Gómez-Núñez has already described results of his investigations on reduviid bugs. I hope Dr. Galun will have an opportunity to describe her work on ticks. Work on Simulium has just been started.

WHO has initiated a vector genetics information service and arranged the course on vector genetics at the University of Notre Dame. It is very gratifying to see that eight of the participants in this course are present here. WHO is placing increasing emphasis on the development of non-chemical methods of control and is working closely with IAEA and FAO.

J.C. GÓMEZ-NÚÑEZ: Experience with vectors of Chagas' disease has shown that replacement of one vector by another is not an uncommon event. Specifically, in Venezuela, the insecticide operations against Rhodnius prolixus apparently also eliminate a limiting factor that keeps Triatoma maculata out of doors, and this species is replacing Rhodnius prolixus as the main indoor vector in some areas of the country. I have made this comment to emphasize the importance of ecological studies before control measures.

K.S. RAI: We have recently induced with radiation several autosomal and sex-linked translocations in Aedes aegypti. We are studying the basic genetics and the population control potential of these translocations. To evaluate theoretically the comparative effectiveness of the autosomal and sex-linked translocations, we have undertaken computer simulations using a model kindly provided by Mr. Max Whitten. Although the sex-linked translocations that we have studied in detail to date involved the male-determining chromosome (T_M), through crossing over we have established lines in which the female-determining chromosome (T_m) is translocated. Our computer simulations have indicated that the use of T_m (involving the female-determining chromosome) translocations is more effective for control purposes than the use of T_M translocations.

We have just completed work designed to test the effectiveness of these translocations in laboratory population cages, with encouraging results. More recently, we have set up field population cages in collaboration with Mr. Weidhaas and Mr. Seawright and are awaiting the results of these experiments.

In summary, and basing myself on the work we have done to date, I feel that the chromosomal translocations should provide an effective method for the genetic control of insects. The use of this method for Aedes aegypti control will be field-tested by the World Health Organization field unit in India.

H. LAVEN: We are currently conducting a field experiment with translocation heterozygotes to combat Culex pipiens in southern France. It is supported by the Entente Interdépartementale de la Demoustication. After solving several technical difficulties we started to release semi-sterile males (50-60% sterility) into an isolated natural population at an average ratio of 5-6 translocation males to one normal wild male. An experiment of this kind passes through three stages. In the initial stage evidence has to be obtained as to whether copulation between the released males and the wild females takes place. This evidence was found in due time. In the second stage we have tried to replace the normal males by translocation males. When I left France last week 80% of the male field population consisted of translocation males. I have no doubt that we can reach the saturation point, that is the total elimination of normal males. In this case we have a population with a reduced reproductive potential, depending on the percentage of lethality imposed by the translocation. What will happen to such an isolated population is open to speculation and is a matter for mathematical model builders.

M.J. WHITTEN: I suppose that as one of the model builders just referred to, I should comment on the statement. Genetic theory would predict that it is most unlikely that realistic conditions will be found to allow replacement of field males not containing any translocation by

laboratory-reared males carrying a translocation which is secured to the male-determining element. At least, this replacement should not be possible in populations of any significant size, where stochastic elements are unimportant. I do not believe Mr. Laven has any evidence to contradict this prediction, nor do I anticipate that he will provide us with such evidence for large populations. In any case, the truth or falsity of this prediction is not really important because other types of chromosome rearrangement exist which should be much easier to fix in natural populations and which have the same impact as male-limited translocations on population size. I will describe these in my paper later in the Symposium (IAEA-SM-138/8).

CHEMOSTERILIZATION AND REPRODUCTIVE PHYSIOLOGY

(Session IV)

Chairman

Rachel GALUN (Israel)

Survey paper

EFFECTS OF CHEMOSTERILANTS ON REPRODUCTIVE ORGANS AND EMBRYOGENESIS IN INSECTS

V. LANDA, S. MATOLÍN
Institute of Entomology,
Czechoslovak Academy of Sciences,
Prague, Czechoslovak Socialist Republic

Abstract

EFFECTS OF CHEMOSTERILANTS ON REPRODUCTIVE ORGANS AND EMBRYOGENESIS IN INSECTS.

The use of chemosterilants in practice is limited by insufficient choice of suitable compounds which would be non-toxic or mutagenic to vertebrates. In testing new compounds data on the effect of chemosterilants on reproductive organs and embryogenesis are required. The present knowledge of the following topics is discussed: (1) the effects of chemosterilants on ovaries and oogenesis, (2) the effects of chemosterilants on testes and spermatogenesis, (3) the effects of chemosterilants on embryogenesis, (4) sterility effects of compounds with juvenile hormone activity. Chemosterilants affect in specific ways panoistic, telotrophic and polytrophic ovaries. With males, in addition to the induction of dominant lethal events, greater attention should be given to the changes in spermatogenesis itself, to the effect on the secretion of accessory glands and to the activation of sperm. The embryology is affected in certain well defined ways: at the inital phase of development of the embryo in cleavage division (alkylating agents), in advanced organogenesis (antimetabolites), or before hatching (furyltriazine). Compounds with juvenile hormone activity as chemosterilants are promising. They are directly transferred by copulation and affect the embryogenesis in a specific way. Non-segmented, apodal, asymmetric and miniature embryos are formed. No autolysis takes place; the deformed embryos survive for quite a long time.

INTRODUCTION

Thanks to comprehensive publications (Bořkovec, 1966; La Brecque & Smith, 1968) and general and special reviews (in recent years e.g. Bořkovec, 1968; Knipling et al., 1968; Ascher, 1969) we have quite a lot of information on chemosterilization as well as the research accomplished. Nevertheless, chemosterilants have not been employed to a desirable extent, particularly where their prospects are most promising, i.e. in long-term autochemosterilization in natural populations. This is especially due to the still insufficient choice of suitable compounds, i.e. compounds which would have specific effects, would not be toxic and would have no mutagenic or carcinogenetic effects on other animals, in particular vertebrates.

The following aspects give a quite reliable picture of the effects of individual compounds in routine screening: fertility, hatchability, pupation, and perhaps also the emergence of adults and their sex ratio. But if we are to progress efficiently in seeking new chemosterilants and to be able to consider applying them in practice, we have to know more about their effects on the reproductive organs and on the development of individual insect species.

In this paper we shall attempt to present a survey of present knowledge in this field. We have based it on the excellent review given by LaChance et al. (1968) and on the report and critical appreciation of the problem by Saxena (1969). We make use of data from publications dealing with the topic as well as results obtained in the Institute of Entomology, Czechoslovak Academy of Sciences. The paper is divided into the following sections: 1. Effects of chemosterilants on ovaries and oogenesis; 2. Effects of chemosterilants on testes and spermatogenesis; 3. Effects of chemosterilants on embryogenesis; 4. Sterilizing effects of compounds with juvenile hormone activity.

1. EFFECTS OF CHEMOSTERILANTS ON OVARIES AND OOGENESIS

Most of the research has been done on flies (Musca domestica L., Cochliomyia hominivorax (Coquerel), Drosophila melanogaster Meigen, Aedes aegypti (L.) and others), on species with polytrophic, most perfectly evolved ovarioles. Destruction of egg chambers and changes taking place during their degeneration in Musca domestica after treatment with tepa, metepa and apholate have been described by Morgan & LaBrecque (1962, 1964 a, b), after hempa by Morgan (1967), after tretamine, methylmethanesulphonate and hydroxyurea by Kissam, Wilson & Hays (1967), after thiotepa, metepa, tepa and apholate by Combiesco et al. (1969), after p, p-bis (1-aziridinyl)-N-methylphosphinic amide and p, p-bis (1-aziridinyl)-N-(3-methoxypropyl) phosphinothionic amide by Wilson & Hays (1969). Complete degeneration of egg chambers through irregular clusters of chromatin has been observed in Drosophila melanogaster after treatment with apholate by Cantwell & Henneberry (1963), in Anopheles labranchiae Van Thiel after tepa by D'Alessandro et al. (1966). LaChance & Leverich (1968a) found that in Cochliomyia hominivorax alkylating agents affect most the endomitosis of nutritive cells, which takes place immediately after emergence. Thus all the authors have observed that the main impact is directed on egg chambers during vitellogenesis, and a slightly weaker effect on the germarium.

The effects of several tens of compounds from the groups of alkylating agents, antimetabolites, steroides, and other chemicals on Musca domestica have been studied in the Institute of Entomology of the Czechoslovak Academy of Sciences. Some of the results have been published (Landa & Řežábová, 1965; Řežábová & Landa, 1967; Řežábová & Landa, 1968; Bennett-Řežábová et al., 1968; Landa, 1970); Matolín & Landa, 1970).

Changes induced by a dose of 4 - 8 μg of metepa can serve as an example. The division of oogonia in the germarium is affected in the upper zone. In the bottom one it prevents oocytes and trophocytes from growing and, in particular, it inhibits multiplication of prefollicular cells and formation of the follicular sheath of the third egg chamber. The main effect is specifically directed on the egg chambers, including also the first, oldest one. If metepa is applied before the vitellogenesis, it affects the endomitosis of trophocytes. However, it attacks most heavily the follicular epithelium. Nucleoli and then nuclei duplicate and a rapid tumour-producing proliferation takes place. A quick degeneration follows, during which the contents of the egg chamber are resorbed. The changes are also anatomically apparent. The chambers become milky, opaque,

of an irregular shape, and they burst easily. The steroids and thalidomide have a somewhat different effect – after their application the cellular membranes of the follicular epithelium are decomposed.

The changes in the follicular epithelium have been studied also biochemically and from the viewpoint of metabolic changes. The formation of the tumour is accompanied by a multiple growth of the DNA contents and by an increase in the contents of RNA. The proteosynthesis is suppressed. Autoradiographic studies confirm an increased incorporation of precursors of the nucleic acids before the proliferation begins, and the activity of proliferating nuclei (Řežábová, 1968).

The study of mitochondria isolated from normal as well as treated egg chambers has shown the specific effects of the chemosterilants applied. Oxygen consumption increases, depending on the phase of development (stage of vitellogenesis). Mitochondria isolated from treated ovaries show a higher metabolic activity, even before the effect of the chemosterilants on the egg chambers appears in their morphology (Bennett-Řežábová & Turner, 1970).

No detailed data on the effects of chemosterilants on telotrophic and panoistic ovaries have been published. We shall therefore use results obtained in the Institute of Entomology of the Czechoslovak Academy of Sciences.

Changes in telotrophic ovaries after the administration of tepa, metepa and apholate were studied in the beetles Hylobius abietis (L.), Acanthoscelides obtectus Say and Trogoderma granarium Everts (Landa et al. 1968; Ondráček & Matolín, 1970). The chemosterilants affected the upper zone of the germarium where the division of trophocytes had been blocked. The number of nuclei in the third zone decreased. Secretion was limited but not blocked; the core remained. The strongest influence was concentrated on the fourth zone, where young oocytes grow and nuclei of the prefollicular cells are divided. The primary oocytes develop irregularly, but yet they grow, partly at least, because they are connected with the trophic core. However, the prefollicular cells, or their nuclei, are entirely decimated, so that the descending oocytes are not enveloped by the follicular epithelium and are not separated; the previtellarium and vitellarium do not grow longer owing to the lack of follicular cells; and the oocytes are compressed and finally degenerate. If the vitellogenesis has already begun at the time of application, it is generally completed; if not, resorption takes place, beginning with the first egg. After only one application regeneration may occur after a certain time, and the development continues with some anomalies. Similar results were obtained on Pyrrhocoris apterus, where the formation of compound eggs was experimentally induced with 6-azauridine.

Changes in panoistic ovaries due to the effect of metepa were studied in the firebrat Thermobia domestica (Pack.). Metepa quickly and intensely affects all dividing cells as well as the nuclei of cells with increased proteosynthesis. Already on the third day the division of oogonia is inhibited; transitory stages of young oocytes perish and are resorbed. Degenerating young oocytes appear in the germarium as white droplets. Also the nuclei of the prefollicular cells stop dividing and degenerative pycnosis takes place. In the previtellarium the nuclei of primary cells undergo changes leading to their dissolution, vacuolization of cytoplasm, disintegration and, finally, to the resorption of the whole previtellarium.

The total effect of metepa on the ovarioles appears within 7 - 10 days in the reduction of the germarium to a mere 'cap' with a few cells, and in the shortening or complete resorption of the previtellarium and vitellarium. The application does not affect moulting at all. One dose is enough to block the development of ovarioles for about 40 days (three to four instars). Afterwards a partial regeneration takes place. Of course the development is very irregular.

The study of the effects of chemosterilants on different types of ovarioles provides some opportunity of discovering the principles of activity of individual compounds. Also the application of these compounds on resistant strains is likely to produce promising new information (Abasa, 1968; Turner & Maheswary, 1969). The effect of chemosterilants on the endocrine system will require special attention; the proportion of their direct and indirect effects will have to be ascertained.

2. EFFECTS OF CHEMOSTERILANTS ON TESTES AND SPERMATOGENESIS

Our knowledge of the effect of chemosterilants on testes and spermatogenesis is much less complete than on ovaries and oogenesis. The classical sterile-male technique is based on the sterilization of males by irradiation, which causes the rise of dominant lethal mutations. The earliest work on chemosterilants was also aimed primarily in this direction, and the most extensive studies on the effect of chemosterilants on spermatogenesis were mainly concerned with dominant lethal events. Mutagens affect either entire chromosomes or individual chromatids. Changes are not usually found on sperm, but most often they are evident in anomalies in the division of the zygote in the fertilized egg. Dominant lethal mutations in Cochliomyia hominivorax were described in detail by LaChance & Riemann (1964) and LaChance & Crystal (1965). Information on and problems connected with dominant lethal mutations produced in insects by irradiation as well as sterilants have been presented by LaChance (1967), who includes a detailed bibliography comprising also works which dealt with this topic long before the era of chemosterilants.

Of course chemosterilants affect testes as well as spermatogenesis and the vitality of ripe sperm. Because the practical application of strong mutagens is out of the question, the possibilities of using aspermia or inactivation of sperm for sterilization have lately been considered. In general, we can say that spermatogenesis is affected in a lesser degree than oogenesis. This conclusion is connected also with the fact that in females chemosterilants have a marked effect on that phase of oogenesis which has no counterpart in males. Complete aspermia is an extreme manifestation of the influence of chemosterilants on spermatogenesis, but it does not occur very often. It follows from papers published so far (Cantwell & Henneberry, 1963 – apholate on Drosophila melanogaster; Keiser et al. 1965 – eight compounds on Dacus cucurbitae Coquillett, Dacus dorsalis Hendel, Ceratitis capitata (Wiedemann)) that spermatogonia undergoing division are affected the most; somewhat less affected are young spermatocytes, and least of all the stages of spermateliosis. With the destruction of cells the whole testes become smaller; this has often been reported (Lindquist et al., 1964 – apholate on Anthonomus

grandis Boheman; Schwarz, 1965 – metepa and tepa on Hippelates pusio Loew). In contrast, other authors did not observe any changes (Rai, 1964 – apholate on Aedes aegypti (L.); Outram & Campion, 1967 – tepa on Diparopsis castanea (Hmps)). In newly emerged males sperm would be fully developed; the frequency of younger stages of spermatogenesis greatly varies with individual species. So clearly the same chemosterilant can cause a different reaction in different species.

Hamilton & Sutter (1969) report the effect of apholate on accessory glands in Diabrotica undecimpunctata Howardii Barber. The accessory glands degenerate and the transfer of sperm during copulation is blocked. Seeing that the filling of accessory glands corresponds to a large extent to vitellogenesis in females, we can expect promising results from this line of research.

The induction of dominant lethal mutations and the effect of chemosterilants on spermatogenesis is often followed by lowered vitality of sperm, which may lead to its immotility or even mortality (Whiting & von Borstel, 1954 – nitrogen mustard on Habrobracon juglandis Ashmead; LaChance & Leverich, 1968b – nine chemosterilants on Habrobracon juglandis). Ascher & Avdat (1967) and especially Ascher et al. (1968) studied the degree of motility of sperm in spermathecae of Musca domestica females sterilized with Brestan and Tinicide. They found that the degree of fertility is in direct relation to the degree of motility of sperm. The activation of sperm is a complex problem that will require further detailed study (Landa (1960), for example, observed a complicated process of activation in the spermatophore of Melolontha melolontha (L.)).

Our preliminary study of the effects of chemosterilants on male Hylobius abietis and Melolontha melolontha has generally confirmed the published results. Metepa, 6-azauridin and especially hempa applied in the course of spermatogenesis inhibit the division of spermatocytes and cause their degeneration. Spermateliosis is not morphologically affected. An extensive study of the effect of chemosterilants on spermatogenesis, on the secretion of accessory glands and the activation of sperms has been launched. The objects are Musca domestica, Melolontha melolontha, Apis mellifera L. and Laspeyresia pomonella (L.).

3. EFFECTS OF CHEMOSTERILANTS ON EMBRYOGENESIS

Detailed data on the effects of chemosterilants on embryogenesis were obtained from the study of Muśca domestica, Cochliomyia hominivorax and Habrobracon juglandis.

Embryogenesis is the process most affected and the development of the embryo is usually inhibited in the initial phase. This is due to dominant lethal mutations causing the death of the zygote. Dominant lethal mutations have been induced by a number of compounds (Whiting & von Borstel, 1954; von Borstel, 1955; Atwood et al., 1956). The dominant lethal mutation causing sterility may be brought into the zygote by the male (sperm) as well as female (nucleus of the oocyte) (Sonnenblick & Henshaw, 1941). The development of the embryo is most often inhibited at the early stage of a few cleavage nuclei before the formation of blastoderm (LaChance & Riemann, 1964; LaChance & Crystal, 1965; Smittle,

Schmitt & Burden, 1966; LaChance, 1967; Valcovic & Grosch, 1968). As for cytology, the disturbance of cleavage division is manifested in chromosome bridges. Chemosterilants from the group of alkylating agents appear to be the most effective chromosome breakers. In a biochemical respect this damage can be considered as the inhibition of the synthesis of nucleic acids (Kilgore & Painter, 1964; Chamberlain & Barrett, 1968).

Development may also be inhibited at a later stage. This happens most often when antimetabolites have been administered. Reciprocal translocations are probably the cause, or deletion of local character, when a certain gene asserts itself only at an advanced stage of development. However, it may just as well be a case of a direct effect on egg metabolism, e.g. 5-fluorouracil influences development by replacing the normal metabolite uracil in RNA (Kilgore & Painter, 1966).

Finally, some compounds cause the mortality of larvae rather than embryos (Morgan & La Brecque, 1964b).

In our experiments we tried to ascertain the effects of chemosterilants on development of the embryos of Musca domestica, Acanthoscelides obtectus and Pyrrhocoris apterus (L.) (Matolín, 1969; Matolín & Landa, 1970, Ondráček & Matolín, 1970). When alkylating agents (apholate, tepa) had been applied, development was inhibited at the stage of several cleavage nuclei, so that blastoderm was not formed. Hempa had the same effect. In all cases where development was inhibited at its initial stages an immediate rapid autolysis took place; the egg content disintegrated into numerous granules of various sizes. After the application of antimetabolites (6-azauridine) the development of some eggs was inhibited at the initial stage, but most often death occurred only in advanced organogenesis. The effect of furyltriazine on embryogenesis is remarkable. It allows the embryo to complete its development, and the larva dies only before hatching, during the hatching, or in the course of postembryonic development. Pupation is low. Seeing that furyltriazine acts in this way even when administered at a later stage of oogenesis, we may assume that it affects egg metabolism.

The results show that chemosterilants affect embryogenesis in certain well defined ways. Further work on chemosterilants in relation to embryogenesis are clearly desirable.

4. STERILIZING EFFECTS OF COMPOUNDS WITH JUVENILE HORMONE ACTIVITY

It has been discovered in recent years that a number of chemicals with juvenile hormone activity have sterilizing effects. These compounds have a number of excellent properties: they are not toxic, they should have no mutagenic effects, their influence is specific and they are directly transferred by copulation. Some results were published (Sláma & Williams, 1966 – Pyrrhocoris apterus; Riddiford & Williams, 1967 – Hyalophora cecropia, Antherea pernyi Guérin; Novák, 1969 – Schistocerca gregaria Forskal), but most of the new findings are not yet in print. Compounds of this type do not inhibit the development of ovaries, rather they accelerate oogenesis. But either directly or through copulation with treated males they penetrate eggs and even in negligible doses they affect embryogenesis.

An extraordinarily powerful chemosterilizing effect of 3, 7, 11-trimethyl-7, 11-dihydrodichloro-2-dodecenic acid on adult Pyrrhocoris apterus has been discovered (Masner et al., 1968; Matolín, 1970). The compound administered to females in a dose of 10 μg induces complete sterility throughout the reproductive cycle. If it is transferred by copulation with treated males, the percentage of sterility depends on the dose and time when males were treated before copulation (Masner et al., 1970). Embryonic development proceeds in a normal way until the blastoderm stage. It is inhibited at the time of RNA synthesis and differentiation of the embryo. Non-segmented, apodal, asymmetric and miniature embryos are formed. In contrast to what happens with alkylating agents, immediate autolysis does not take place, the deformed embryos surviving for quite a long time.

It is seen that compounds with juvenile hormone activity affect embryogenesis in a manner different from those chemosterilants mentioned in section 3. An important result was obtained by exposure of Thermobia domestica eggs to vapours of 3, 7, 11-trimethyl-7, 11-dihydrodichloro-2-dodecenic acid. Embryogenesis was inhibited and albinotic forms were obtained. This phenomenon needs to be studied further.

The present review is certainly not exhaustive, but aims at summarizing the present techniques with a view to ensuring that future work on these lines will be effective.

REFERENCES

ABASA, R.O. (1968). An apholate-resistant strain of house fly, Musca domestica. II. Ovarian growth and oogenesis, Ann. ent. Soc. Am. 61, 6: 1351-54.

ASCHER, K.R.S., AVDAT, N. (1967). Sterilizing the male housefly with m-xylohydroquinone, Int. Pest Control 9, 2: 8-13.

ASCHER, K.R.S., MEISNER, J., NISSIM, S., (1968). The effects of fentins on the fertility of the male housefly, World Rev. Pest Control 7: 84-96.

ASCHER, K.R.S. (1969). Insect pest control by chemosterilization and other advanced methods (antifeedants, microbical pesticides etc). Cong. Int. Antiparasitaires, Milan, 4: 1-22.

ATWOOD, K.C., VON BORSTEL, R.C., WHITING, A.R. (1956). An influence of ploidy on the time of expression of dominant lethal mutations in Habrobracon, Genetics 41: 804-13.

BENNETT-ŘEŽÁBOVÁ, B., TURNER, R.B. (1970). Effect of chemosterilants in vivo and in vitro on respiration of ovarian mitochondria of the house fly, Musca domestica L. Acta ent. bohemoslov. 67: 65-69.

BENNETT-ŘEŽÁBOVÁ, B., LANDA, V., TURNER, R.B. (1968). Metabolic changes in the ovaries of the house fly, Musca domestica L., after the administration of aziridine chemosterilants. Proc. XIIIth Int. Congr. Ent. Moscow (in press).

BOŘKOVEC, A.B. (1966). Insect chemosterilants. Advances in Pest Control Res. 7: 1-143.

BOŘKOVEC, A.B. (1968). "Chemosterilants in Entomology and the Sterile-Male Technique", in: Isotopes and Radiation in Entomology (Proc. Symp. Vienna, 1967) IAEA, Vienna 201-8.

CANTWELL, G.E., HENNEBERRY, T.J. (1963). The effects of gamma radiation and apholate on the reproductive tissues of Drosophila melanogaster, J. Insect Path. 5: 251-64.

CHAMBERLAIN, W.F., BARRETT, C.C. (1968). Incorporation of tritiated thymidine into the ovarian DNA of stable flies: effects of treatment with apholate, Nature, Lond. 218: 471-72.

COMBIESCO, I., DUPORT, M., ENESCO, A. (1969). Études sur la stérilisation de l'espèce Musca domestica L. avec divers chimiostérilisants, Congr. Int. Antiparasitaires, Milan, 9: 1-8.

D'ALESSANDRO, G., BRUNO-SMIRAGLIA, C., LAVAGNINO, A. (1966). Saggi di chemosterilizzazione con tepa su Anopheles labranchiae, Riv. Malar. 45: 39-49.

HAMILTON, E.W., SUTTER, G.R. (1969). Chemosterilizing southern corn rootworm beetles with apholate, J. econ. Ent. 62, 6: 1285-88.

KEISER, I., STEINER, L.F., KAMASAKI, H. (1965). Effect of chemosterilants against the oriental fruit fly, melon fly, and Mediterranean fruit fly, J. econ. Ent. 58: 682-685.

KILGORE, W.W., PAINTER, R.R. (1964). Effect of the chemosterilant apholate on the synthesis of cellular components in developing housefly eggs, Biochem. J. 92: 353-57.

KILGORE, W.W., PAINTER, R.R. (1966). Insect chemosterilants: Incorporation of 5-fluorouracil into house fly eggs. J. econ. Ent. 58, 3: 746-47.

KISSAM, J.B., WILSON, J.A., HAYS, S.B. (1967). Selective effects of certain anti-fertility compounds on the house fly as shown by reciprocal crosses and histological sectioning, J. econ. Ent. 60: 4: 1130-35.

KNIPLING, E.F., LAVEN, H., CRAIG, G.B., PAL, R., KITZMILLER, J.B., SMITH, C.N., BROWN, A.W.A. (1968). Genetic control of insects of public health importance, Bull. Wld Hlth Org. 38: 421-38.

LaCHANCE, L.E., RIEMANN, J.G. (1964). Cytogenetic investigations on radiation and chemically induced dominant lethal mutations in oöcytes and sperm of the screw-worm fly, Mutation Res. 1: 318-33.

LaCHANCE, L.E., CRYSTAL, M.M. (1965). Induction of dominant lethal mutations in insect oocytes and sperm by gamma rays and an alkylating agent: dose-response and joint action studies, Genetics 51: 699-708.

LaCHANCE, L.E. (1967). "The induction of dominant lethal mutations in insects by ionizing radiation and chemicals as related to the sterile-male technique of insect control", in: WRIGHT & PAL, Genetics of Insect Vectors of Diseases, Amsterdam: 617-650.

LaCHANCE, L.E., NORTH, D.T., KLASSEN, W. (1968). "Cytogenetic and cellular basis of chemically induced sterility in insects", in: LaBRECQUE & SMITH, Principles of Insect Chemosterilization, Meredith Corp. ACC, New York: 100-46.

LaCHANCE, L.E., LEVERICH, A.P. (1968a). Cytology of oogenesis in chemosterilized screw-worm flies, Cochliomyia hominivorax, as related to endomitosis in nurse cells. Ann. ent. Soc. Am. 61, 5: 1188-97.

LaCHANCE, L.E., LEVERICH, A.P. (1968b). Chemosterilant studies on bracon (Hymenoptera: Braconidae) sperm. I. Sperm inactivation and dominant lethal muations, Ann. ent. Soc. Am. 61, 1: 164-73.

LANDA, V. (1960). Origin, development and function of the spermatophore in cockchafer (Melolontha melolontha L.), Acta Soc. Ent. Čechoslov. 57: 297-316.

LANDA, V., ŘEŽÁBOVÁ, B. The effect of chemosterilants on the development of reproductive organs in insects, Proc. XIIth Int. Congr. Ent. 1964, London: 516-17.

LANDA, V., NOVÁK, V., ŘEŽÁBOVÁ, B. (1968). The possibility of applying chemosterilants to the control of some forest pests, Proc. XIIIth Int. Congr. Ent. Moscow (in press).

LANDA, V. (1970). L'influence des stimuli externes sur la gammetogenèse des insectes (The effects of some chemosterilants on the reproductive organs of insects), Coll. int. CNRS, Tours: 411-420.

LINDQUIST, D.A., GORZYCKI, L.J., MAYER, M.S., SCALES, A.L., DAVICH, T.B. (1964). Laboratory studies on sterilization of the boll weevil with apholate. J. econ. Ent. 57: 745-50.

MASNER, P., SLÁMA, K., LANDA, V. (1968). Sexually spread insect sterility induced by the analogues of juvenile hormone, Nature, Lond. 219: 395-96.

MASNER, P., SLÁMA, K., ŽĎÁREK, J., LANDA, V. (1969). Natural and synthetic materials with insect hormone activity. X. A method of sexually spread insect sterility, J. econ. Ent. 63, 3: 706-10.

MATOLÍN, S. (1969). The effect of chemosterilants on the embryonic development of Musca domestica L., Acta ent. bohemoslov. 66, 2: 65-9.

MATOLÍN, S. (1970). Effects of a juvenile hormone analogue on embryogenesis in Pyrrhocoris apterus L., Acta ent. bohemoslov. 67: 9-12.

MATOLÍN, S., LANDA, V. (1970). The effects of furyltriazine on oogenesis and embryogenesis in Musca domestica L., Acta ent. bohemoslov.(in press).

MORGAN, P.B., LaBREQUE, G.C. (1962). The effect of apholate on the ovarian development of house flies, J. econ. Ent. 55: 626-8.

MORGAN, P.B., LaBRECQUE, G.C. (1964a). Studies of the effect of meat exposed to gamma radiation or chemosterilants on the reproductive capacity of a blow fly Phaenica cuprina (Wiedemann), Fla. Entomol. 47, 1: 31-3.

MORGAN, P.B., LaBRECQUE, G.C. (1964b). Effect of tepa and metepa on ovarian development of house flies, J. econ. Ent. 57, 6: 896-99.

MORGAN, P.B. (1967). Effects of hempa on the ovarian development of house flies (Musca domestica L.). Ann. ent. Soc. Am. 60: 812-818.

NOVÁK, V.J.A. (1969). Morphogenetic analysis of the effects of juvenile hormone analogues and other morphogenetically active substances on embryos of Schistocerca gregaria (Forskål), J. Embryol. exp. Morph. 21, 1: 1-21.

ONDRÁČEK, J., MATOLÍN, S. (1970). Effects of the chemosterilant tepa on the female reproductive system and embryogenesis of Acanthoscelides obtectus Say (bean beetle), Acta ent. bohemoslov. (in press).

OUTRAM, J., CAMPION, D.G. (1967). Morphology of the male reproductive system and spermatophore of the red bollworm, Diparopsis castanea, and the possible effect of the chemosterilant tepa on the gross structure, Ann. appl. Biol. 60: 439-44.

SLÁMA, K., WILLIAMS, C.M. (1966). "Paper factor" as an inhibitor of the embryonic development of the European bug, Pyrrhocoris apterus, Nature, Lond. 210: 329-30.

SMITTLE, B.J., SCHMITT, J.B., BURDEN, G.S. (1966). Effect of tepa on the reproductive organs and embryogeny of the german cockroach, J. econ. Ent. 59: 1419-23.

RAI, K.S. (1964). Cytogenetic effects of chemosterilants in mosquitoes. I. Apholate-induced aberrations in the somatic chromosomes of Aedes aegypti L. Cytologia 29, 3: 346-53.

RIDDIFORD, L.M., WILLIAMS, C.M. (1967). The effects of juvenile hormone analogues on the embryonic development of silkworms, Proc. natn. Acad. Sci. USA 57: 349-352.

ŘEŽÁBOVÁ, B., LANDA, V. (1967). Effect of 6-azauridine on the development of the ovaries in the house fly, Musca domestica L. (Diptera), Acta ent. bohemoslov. 64: 344-51.

ŘEŽÁBOVÁ, B. (1968). Changes in the metabolism of nucleic acids in the ovaries of the house fly, Musca domestica, after application of chemosterilants, Acta ent. bohemoslov. 65, 5: 331-40.

ŘEŽÁBOVÁ, B., LANDA, V. (1968). Effects of thalidomide on the development of the house fly Musca domestica L., Acta ent. bohemoslov. 65, 3: 212-15.

SAXENA, S.C. (1969). Some controversial aspects of insect chemosterilization, Congr. Int. Antiparasitaires, Milan, 36: 1-9.

SCHWARTZ, P.H., Jr. (1965). Effects of apholate, metepa, and tepa on reproductive tissues of Hippelates pusio, J. Invert. Path. 7, 2: 148-51.

SONNENBLICK, B.P., HENSHAW, P.S. (1941). Influence on development of certain dominant lethals induced by X-rays in Drosophila germ cells, Proc. Soc. exp. Biol. Med. 48: 74-9.

TURNER, R.B., MAHESWARY, N.P. (1969). Biochemical effects of apholate on mosquito ovarian tissue, Ann. ent. Soc. Am. 62, 5: 1091-95.

VALCOVIC, L.R., GROSCH, D.S. (1968). Apholate-induced sterility in Bracon hebetor, J. econ. Ent. 61, 6: 1514-17.

VON BORSTEL, R.C. (1955). Differential response of meiotic stages in Habrobracon eggs to nitrogen mustards, Genetics 40: 107-116.

WHITING, A., VON BORSTEL, R.C. (1954). Dominant lethal and inactivation effects of nitrogen mustard on Habrobracon sperm, Genetics 39: 317-25.

WILSON, J.A., HAYS, S.B. (1969). Histological changes in the gonad and reproductive behaviour of house flies following treatment with chemosterilants p, p-bis(1-aziridinyl)-N-methylphosphinic amide and p, p-bis(1-aziridinyl)-N-(3-methoxyprophyl)-phosphinothioic amide, J. econ. Ent. 62, 3: 690-1.

DISCUSSION

D. S. GROSCH: Do you apply the hormone to the exterior of the insect – i.e. was the method used topical application?

V. LANDA: Yes, the substance with juvenile hormone activity was applied topically.

D. S. GROSCH: What solvent do you use?

V. LANDA: The compound was used as such or in an acetone solution.

A. ECONOMOPOULOS: Do you have any information on male competitiveness or general sexual behaviour after the accessory glands have been damaged by chemosterilants?

V. LANDA: At the Institute of Entomology in Prague we have been studying the changes in the general sexual behaviour of Musca domestica L. and Pyrrhocoris apterus L. after the application of alkylating agents and compounds with juvenile hormone activity. This study was not really connected with that on the accessory glands. Some time ago we started the experiments on the accessory glands of Melolontha melolontha. The results will be published within the next year.

STUDIES OF COMPETITIVENESS, CHEMOSTERILANT PERSISTENCE AND SPERM STRUCTURE IN TREATED RED BOLLWORMS, Diparopsis castanea (HMPS.)

D.G. CAMPION
Tropical Pesticides Research Headquarters and Information Unit,
Ministry of Overseas Development, London
and
C.T. LEWIS
Department of Zoology and Applied Entomology,
Imperial College, London,
United Kingdom

Abstract

STUDIES OF COMPETITIVENESS, CHEMOSTERILANT PERSISTENCE AND SPERM STRUCTURE IN TREATED RED BOLLWORMS, Diparopsis castanea (HMPS.).

In tests of chemosterilants applied by injection to adult moths Diparopsis castanea (Hmps.) obtained from pupae shipped from Malawi, the male sterility indices (LD_{50}/SD_{50}) for apholate, tepa and metepa were 24, 17.9 and 11.5 respectively. Both hempa and triphenyl tin acetate had low sterility indices of 1.4 and -7.7. The female sterility index of tepa was 4.2. Tepa treatment of female moths significantly reduced the oviposition rate. When male moths were treated, repetitive mating tests showed that tepa had no significantly adverse effect on mating frequency applied at the SD_{95} level, whereas apholate-treated males at such a dose level mated significantly less frequently. The sterilizing effect of tepa on males was permanent throughout a 6-day period, virtually the adult life-span of the moth at 27°C. Topical treatment of male moths with tepa gave a sterility index of 34.6. At the SD_{95} they were fully competitive.

A rapid rate of tepa degradation occurred after injection, half-life values ranging from 14.3 h at 27°C to 18.6 h at 15°C. After topical application, tepa degradation occurred more slowly, reaching the 50% level after 45.6 h at 27°C, 72.3 h at 20°C and 143.3 h at 15°C. The results were related to the relatively slow rate of absorption from acetone solutions. Potential environmental contamination hazards are considered.

No obvious damage to the ultra-structure of the sperm of Diparopsis was observed after treatment of the male moth with injected doses of tepa in excess of the sterilizing level.

INTRODUCTION

The red bollworm Diparopsis castanea (Hmps.) is an important pest of cotton in Central Africa. It spends most of its larval life inside the cotton boll and is therefore particularly difficult to control by the use of insecticides. There is, however, a possibility of controlling this insect by either the mass-release of sterilized insects or by attracting the natural population to some form of bait-station.

Previous work has shown that the adult moth can be sterilized by several aziridine chemosterilants. Weak sterilizing activity was also shown by other classes of chemosterilants such as phosphoramides, s-triazines and organo-metals [1-3]. This paper presents the results of further laboratory investigations to gain more quantitative information on the sterilizing and toxic effects of those chemosterilants having appreciable

activity against Diparopsis. Since the aziridines present a possible mutagenic hazard to man and higher animals, the rate of breakdown following topical and injection application of an active chemosterilant, tepa, was studied. Preliminary observations on the complex sperm ultrastructure and its possible susceptibility to damage by chemosterilants are also reported.

MATERIALS AND METHODS

Standard test procedure

The insects were obtained from pupae collected in the field and shipped from Malawi. Graded concentrations of the chemosterilants were applied to one-day-old moths of specified sex to determine the sterility and toxicity caused by known dosages. Solutions of the chemosterilants dissolved in 1 μl of distilled water or acetone were injected through the dorsal surface of the thorax or applied topically to the ventral abdominal surface, by means of a microdrop applicator fitted with an all-glass syringe equipped with No. 27 gauge hypodermic needles. Before treatment the moths were immobilized by momentary exposure to carbon dioxide.

Sterilizing effects

To determine the sterilizing effects, the insects were treated in the afternoon and mated the same evening to untreated insects of the opposite sex in disposable plastic cups ($3\frac{3}{4}$ in. $\times$ $2\frac{3}{4}$ in.) partially lined with blotting paper, with one pair of moths in each cup. Plastic Petri dishes were used as lids. The cups were kept at 27°C and 70% r.h. with a 12 h photoperiod under artificial lights for 6 days. The blotting paper on which the eggs were laid was replaced each day and each batch of eggs incubated separately for 6 days at 27°C and 70% r.h. to determine whether or not sterility had been induced. At the end of the 6-day test period the females were examined for spermatophores as proof of mating and eggs from unmated females were discarded. In each replicate test 25-30 insects were treated, while a control replicate treated with solvent only was included in each test.

Mortality tests

After similar treatments of one-day-old moths, they were held in groups of 25-30 insects in plastic sandwich boxes (11 in. $\times$ 6 in. $\times$ $3\frac{1}{2}$ in.) for a period of 4 days under the same temperature, humidity and light conditions as the insects tested for sterility. Corrections for control mortality and sterility were made by Abbot's formula [4]. Dosage/mortality and dosage/sterility data were analysed by means of probit analysis [5]. The significance of the reduction in the rate of oviposition as a result of treatment was assessed by the Mann-Whitney U test.

Repetitive mating tests. In the repetitive mating tests, each treated male was confined individually to a mating container into which was introduced, on six successive nights, a fresh one-day-old virgin female

which was removed each morning to glass specimen-tubes (2 in. ×1 in.) lined with blotting paper and the eggs laid were assessed in the standard manner. In each replicate test 33-39 insects were treated.

Permanence of sterility. The permanence of the sterilizing effect was assessed by mating groups of 25 treated males with one- to three-day-old virgin females on six successive days post-treatment.

Competitive mating tests. The standard disposable cartons were again used as mating containers. Treatments occurred in the afternoon and each treated male was introduced in a separate carton together with one untreated female. The insects remained together for the whole test period of 6 days. Daily collections of eggs were made in the standard manner. In each replicate test 39 to 99 insects were treated.

Assay of residual tepa. Twenty-five to thirty-five male moths 2-4 days old were treated with 10 μg of tepa either in 1-μl amounts of distilled water by injection or 1-μl amounts of acetone by topical application. They were held for varying lengths of time at 27°C and 70% r.h., 20°C and 47% r.h., and 15°C and 40% r.h. under continuous light in plastic sandwich boxes (11 in. × 6 in. × 3½ in.). Before extraction dead moths were discarded and samples of 25 moths were homogenized for 5 min in a high-speed homogenizer in 50 ml of chloroform. To determine the amounts of tepa in the cuticular layer, whole moths were shaken in chloroform for 5 min. The mixtures were then shaken with anhydrous sodium sulphate to remove water and then filtered. After the volume of filtrate had been noted, it was evaporated to dryness at 40-50°C in a rotary evaporator. The residue was dissolved in acetone with 1 ml of solvent for every 2 ml of chloroform extract obtained. The solution was stored in a deep-freeze until ready for analysis. Each determination was replicated three times.

Tepa was analysed by the method of Epstein et al. [6]. A standard curve was obtained for known quantities of the chemosterilant. For residue analysis, 2-3 ml of the acetone extracts contained in 10-ml calibrated tubes was evaporated to dryness in a stream of air. To each tube was added 3 ml distilled water, 1 ml pH 4 buffer and 1 ml of the γ-(4-nitrobenzyl) pyridine reagent. The mixtures were heated for 20 min in a boiling water bath and then cooled in an ice water bath. To each tube was added in quick succession 4 ml of acetone, 1 ml of 1M aqueous potassium carbonate and distilled water to make the volume up to 10 ml. To avoid turbidity Hyflo Super-cel was added and after filtration the colour intensity immediately measured at 600 mμ on a spectrophotometer using 1-cm cells of 3 ml capacity. Speed was essential at this stage since the blue colour formed was stable for only 30 min. The residue values obtained were corrected for the apparent residues found in control samples. Each sample was assayed at two dilutions and the mean value recorded.

Sperm preparations. Observations under the light microscope were made of sperm dissected from female spermathecae into insect Ringers solution and measurements of sperm length noted by means of camera lucida drawings. For transmission electron microscopy, testes were

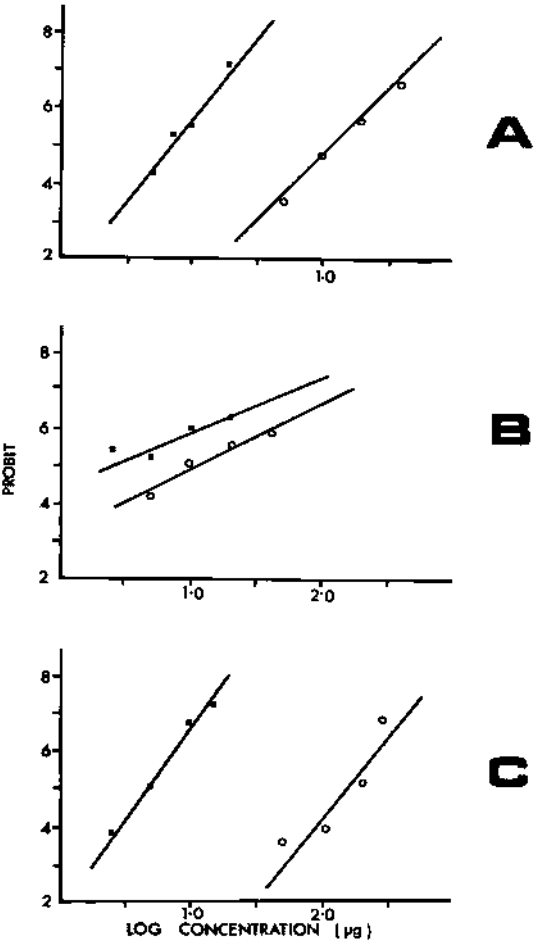


FIG. 1. Regression lines for the calculation of sterility indices after tepa treatment. (A) Injection treatment of male moths, (B) injection treatment of female moths, (C) topical treatment of male moths.
■ = sterility values
○ = mortality values.

fixed at 0-4°C for 2 h in phosphate-buffered 2.5% glutaraldehyde, post-fixed for 1-2 h with 2% buffered osmium tetroxide, dehydrated in alcohol and embedded in Araldite. Sections were taken with a Reichert ultramicrotome and double-stained with uranyl acetate and lead citrate. They were examined in a JEM 7 electron microscope. For scanning electron microscopy, squashes of fresh testes were air dried or fixed in glutaraldehyde coated in vacuo with 500 Å of gold/palladium, and examined in a Cambridge Instruments Stereoscan electron microscope.

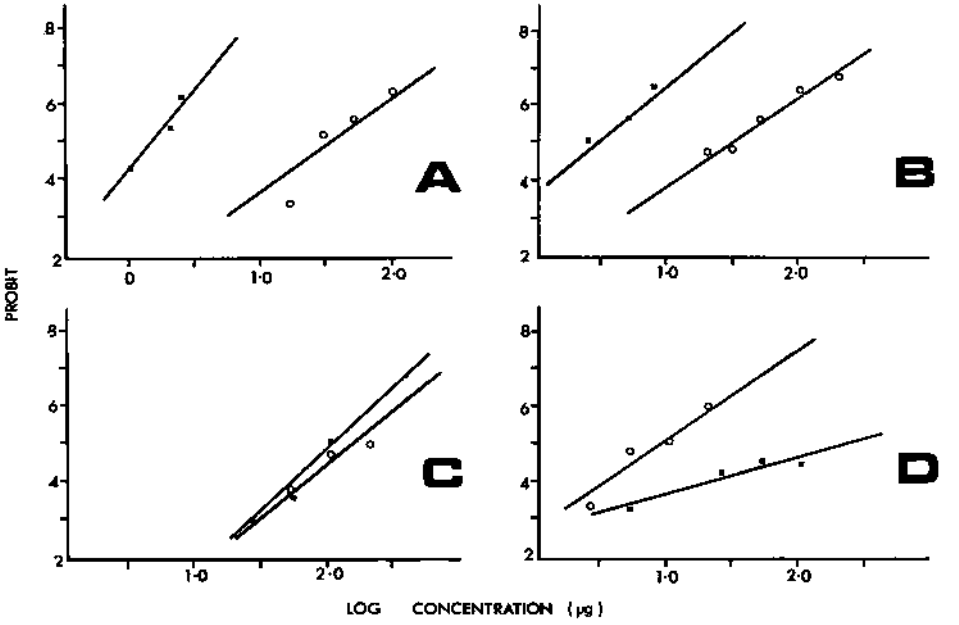


FIG. 2. Regression lines for the calculation of sterility indices for male moths after injection with (A) apholate, (B) metepa, (C) hempa and (D) triphenyl tin acetate.
■ = sterility values.
○ = mortality values.

RESULTS

Treatment of adult moths by injection; primary evaluation

The regression lines obtained (Figs 1 and 2) showed that in all instances a linear relationship existed between the concentration of chemosterilant (log x) and the percentage sterility (probit y). The regression equations and ED_{50} values are shown in Table I. A comparison of the heterogeneity (χ^2) about the sterility and mortality regression lines fitted with and without the constraint of parallelism gave no evidence that they were not parallel in the case of tepa, apholate, metepa and hempa. This made it possible to calculate the ratio between sterilizing and toxic doses of the applied chemosterilants (Table II). This ratio will be termed the sterility index, by analogy with the term 'therapeutic index' as used in pharmacology. The regression lines for triphenyl tin acetate were not parallel so that the relative potency shown in Table II is at the ED_{50} level only.

In males the greatest sterility index of 24.0 was shown by apholate followed by tepa with 17.9 and metepa with 11.5. For these substances, the calculated 50% sterilizing doses (SD_{50}) were 1.5, 0.73 and 2.7 µg respectively. A very low sterility index of 1.4 was obtained with hempa and a negative value of -7.7 for triphenyl tin acetate. This showed that complete sterility could only be achieved at doses with considerable toxic side-effects, a result which precludes their use as practical chemosterilants against Diparopsis.

TABLE I. STERILITY AND MORTALITY REGRESSION EQUATIONS AND CALCULATED ED_{50} OF CERTAIN CHEMOSTERILANTS AGAINST Diparopsis MOTHS (A) WHEN APPLIED BY INJECTION, (B) WHEN TEPA APPLIED TOPICALLY TO MALE MOTHS

Chemosterilant	Sex treated	Sterility				Mortality			
		Regression equation	log factor	ED_{50} (μg)	95% fiducial limits	Regression equation	log factor	ED_{50} (μg)	95% fiducial limits
A. Tepa	Males	Y = 1.17 + 4.47x	10	0.73	0.67 - 0.78	Y = 1.40 + 3.37x	1	11.7	8.6 - 15.9
Tepa	Females	Y = 4.42 + 1.42x	1	2.56	1.83 - 3.58	Y = 3.26 + 1.63x	1	11.7	8.0 - 17.1
Apholate	Males	Y = -0.13 + 4.44x	10	1.5	1.44 - 1.66	Y = 1.39 + 2.41x	1	31.5	20.1 - 49.3
Metepa	Males	Y = 3.68 + 2.93x	1	2.8	2.10 - 3.77	Y = 1.65 + 2.28x	1	29.5	21.3 - 40.8
Hempa	Males	Y = -1.10 + 3.04x	1	101.5	77.8 - 132.6	Y = 0.76 + 2.67x	1	143.6	112.1 - 184.0
Triphenyl tin acetate	Males	Y = 2.76 + 1.01x	10	16.1	4.90 - 53.20	Y = 2.84 + 2.33x	1	8.5	5.9 - 12.2
B. Tepa	Males	Y = 1.77 + 4.78x	1	4.74	4.70 - 4.78	Y = 0.47 + 2.23x	1	105.4	81.3 - 143.0

TABLE II. RATIO OF STERILE AND LETHAL DOSES (≡ STERILITY INDEX) OF (A) CERTAIN CHEMOSTERILANTS APPLIED TO Diparopsis castanea ADULTS BY INJECTION, (B) TEPA APPLIED TOPICALLY TO MALE MOTHS

	Chemosterilant	Sex treated	Sterility index	95% fiducial limits	χ^2 for parallelism of regression lines
A.	Tepa	Males	17.9	10.1 - 30.7	1.05
	Tepa	Females	4.2	2.8 - 6.4	0.24
	Apholate	Males	24.0	13.1 - 43.7	3.75
	Metepa	Males	11.5	6.6 - 19.9	1.58
	Hempa	Males	1.4	0.1 - 1.9	0.22
	Triphenyl tin acetate	Males	- 7.7 [a]		4.43
B.	Tepa	Males	34.6	28.10-45.90	0.73

[a] For ED_{50} values only.

TABLE III. EFFECTS OF TEPA INJECTIONS ON THE MATING OF Diparopsis

Dose (μg)	Mating as percentage of control mating [a]	
	Treated females × normal males [b]	Treated males × normal females [b]
80	0	0
40	19.5	24.9
20	75.6	37.5
10	81.0	85.0
5	48.7	60.0
2.5	-	106

[a] Minimal mortality at all dose levels for the 2-day mating period.
[b] Data based on 20 replicates at each concentration.
SD_{95} for female moths 30.9 μg; SD_{95} for male moths 1.3 μg.

A low sterility index of 4.2 was obtained for tepa-treated females, which again suggests that mating would be adversely affected at doses causing complete sterility. This conclusion was confirmed by mating experiments (Table III). Treatment of females with tepa also caused a significant reduction in the rate of oviposition (Table IV).

TABLE IV. EFFECT OF TEPA TREATMENT BY INJECTION ON THE RATE OF OVIPOSITION IN Diparopsis FEMALES MATED WITH UNTREATED MALES (4-DAY OVIPOSITION PERIOD)

Dose (μg)	No. of mated females	No. of eggs	Mean No. of eggs per female	U value	Significance of oviposition reduction compared to control p
40	2	7	3.5	2	0.025
20	14	369	26.6	43	0.01
10	21	486	26.4	142	0.006
5	29	1109	38.2	239	0.42
25	3	122	40.6	23	
C	16	659	41.2		

TABLE V. MATING FREQUENCY AND MEAN MORTALITY OF MALE Diparopsis castanea NOTED IN REPETITIVE MATING TESTS AFTER TREATMENT BY INJECTION WITH ESTIMATED ED_{95} STERILIZING DOSES OF TEPA AND APHOLATE

Treatment	No. of males treated	Mean mortality in days	Mean No. matings per male	Mating frequencies				χ^2 values for heterogeneity for mating frequency	p
				X0	X1	X2	X3		
Control	37	4.6	1.2	9	15	11	2	0.5	> 0.05
Tepa	33	4.0	1.0	9	15	8	1	19.0	< 0.001
Apholate	39	3.1	0.5	21	17	1	0		

TABLE VI. PERMANENCE OF STERILITY OF MALES OF Diparopsis castanea INJECTED WITH TEPA AT ED_{95} STERILITY LEVEL (25 MALES PER TREATMENT)

Time of mating in days post-treatment	No. mating with 1-day-old females	Total eggs laid	No. hatching	% hatch
1	13	1401	3	0.2
2	14	1542	0	0
3	9	695	0	0
4	4	352	24	7
5	6	346	12	3.5
6	4	330	0	0
	Total:	4666	39	0.84

Treatment of adults by injection; evaluation of sexual vigour and permanence of the sterility effect

The relative merits of the two most suitable chemosterilants, apholate and tepa, were evaluated by repetitive mating tests. The results shown in Table V clearly indicate tepa to be the most effective chemosterilant. The mating frequency approximated closely to the control mating frequency ($\chi^2 = 0.5$), whereas the mating frequency after apholate treatment was reduced ($\chi^2 = 19$). The results show also the mating potential of the male moth. Although some insects mated three times, the mean number of control matings per male was one since many males did not mate at all.

Sterility induced in male moths by tepa injection at the SD_{95} level was permanent. No significant recovery of fertility occurred throughout the life of the adult moth (Table VI).

Tepa treatment of adult moths by topical application

The regression lines obtained following topical application of graded concentrations of tepa again showed that a linear relationship existed between doses of chemosterilant and percentage mortality and sterility (Table IB). The regression lines were parallel ($\chi^2 = 0.73$) and therefore a sterility index of 34.6 was calculated with an SD_{50} value of 4.74 μg.

The results of competitive mating tests are shown in Table VII. The frequency of sterile mating compared with fertile mating ($\chi^2 = 3.65$) gave no evidence for significant heterogeneity and it was concluded that mating was not adversely affected as a result of treatment. A comparison of the mean fecundity of females mated with sterile males compared with those mated with fertile males ($\chi^2 = 10.95$) indicated significant heterogeneity at the 1% level. This suggested a secondary reduction in oviposition, perhaps caused by the transmission of a proportion of the chemosterilant during copulation. A comparison of the competitiveness of

TABLE VII. COMPETITIVENESS[a] OF MALE Diparopsis TREATED TOPICALLY WITH 15 μg OF TEPA

No. of replicates	Total eggs from fertile matings	Total eggs from sterile matings	Mean eggs per fertile mating [b]	Mean eggs per sterile mating [b]	Total matings	No. fertile	No. sterile	% sterile mating	
								Expected [c]	Actual
99	2690	1110	104	62	44	26	18	50	40.9
97	1993	559	71	40	42	28	14	50	33.3
91	2067	850	115	57	34	18	15	50	45.5
73	2699	686	117	98	31	23	7	50	23.3
39	1223	758	153	126	14	8	6	50	42.8

[a] In all replicates one treated male, one normal male and one normal female were enclosed together. Matings were confirmed by dissection of females for spermatophores at the end of the test period.

[b] Comparison of mean fecundity of females mated with sterile or fertile females, $\chi^2 = 10.95$, indicating significant heterogeneity of 1% level.

[c] Frequency of sterile matings compared with fertile mating, $\chi^2 = 3.65$, indicating no evidence for significant heterogeneity.

TABLE VIII. COMPETITIVENESS OF MALE Diparopsis ON SUCCESSIVE DAYS FOLLOWING TOPICAL TREATMENT WITH 15 μg TEPA

Days post-treatment when mated	Total matings	Fertile matings	Sterile matings	% sterile matings	
				Expected[a]	Actual
1	38	31	7	50	18.6
2	41	27	14	50	34.1
3	31	16	15	50	48.4
4	26	15	11	50	42.4

[a] Frequency of sterile mating compared with fertile mating, $\chi^2 = 7.81$, which indicates heterogeneity at 5% level.

sterile and fertile males when mated on successive days post-treatment is shown in Table VIII. The results suggested that shortly after treatment some incapacitation of sterilized males occurred, although the insects soon completely recovered.

Tepa residue analysis

The results showing the varying rate of tepa degradation at 15, 20 and 27°C after injection and topical application, taking the mean of the three replicates, are given in Table IX. By plotting the log of the residual tepa (μg/moth) against time, a series of regression lines were obtained and by analysis of variance shown to be linear in all instances (Fig. 3). From the regression equations (Table X) the time in hours when 50% of the chemosterilant had been degraded after the various treatments was calculated. A rapid and very similar rate of decomposition occurred after all injection applications, the half-life values ranging from 14.3 h at 27°C to 18.6 h at 15°C. After topical application tepa decomposition occurred much more slowly, reaching the 50% level after 45.6 h at 27°C, 72.3 h at 20°C and 143.3 h at 15°C. The rate of absorption of tepa at 20°C from a 10-μg topical dose was followed by analysing a series of washings of intact treated insects. The half-life value at this temperature was 48.6 h.

The rate of decomposition of tepa applied to topically treated insects was lower than the rate of absorption (Fig. 4).

Structure of Diparopsis sperm and some preliminary observations on the action of tepa

The mobility of sperm is most evident after mating has taken place and can readily be observed under the light microscope. When tepa is injected into males at doses just sufficient to induce sterility the sperm when transferred in copulation to the female appears to be normally active. However, at much higher doses agglutination of transferred spermatozoa in the spermatheca was observed. Thus, while the primary sterilizing action

TABLE IX. RESIDUES OF TEPA IN μg (MEAN OF 3 REPLICATES)

Interval post-treatment (hours)	(A) Extraction of homogenized insects						(B) From surface washes of whole moths after topical application
	Topical application			Injection application			
	15°C	20°C	27°C	15°C	20°C	27°C	20°C
0	10.7	11.0	10.4	8.5	9.2	9.4	8.5
3	8.6	11.3	10.6	7.5	7.3	8.2	-
6	7.6	8.2	10.9	6.5	4.4	6.9	8.0
12	7.2	8.0	8.1	5.0	2.7	3.4	-
24	7.4	5.7	6.7	3.0	2.8	1.7	5.6
48	6.1	5.2	5.7	1.3	0.6	1.0	4.0
72	6.0	3.8	3.9				2.6
96	5.5	4.4	2.3				2.4

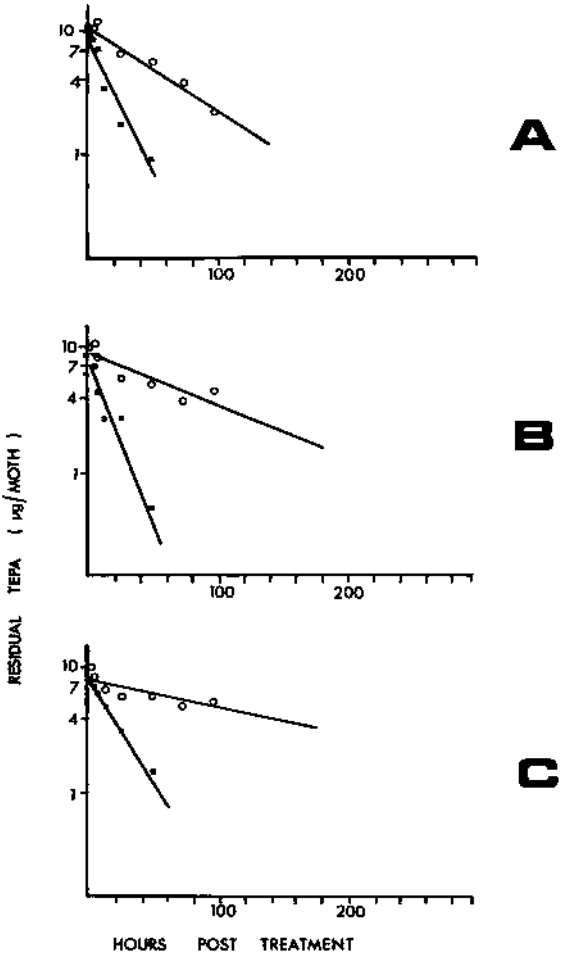


FIG.3. Rate of tepa degradation in male moths after injection and topical application.
(A) 27°C, (B) 20°C, (C) 15°C.
■ = injection application
○ = topical application.

of aziridines no doubt concerns the chromatin, the possibility of secondary effects upon the highly organized locomotory apparatus of spermatozoa cannot be entirely eliminated, especially at higher doses.

In the course of an investigation of the fine structure of Diparopsis spermatozoa, the opportunity was therefore taken to look for possible structural abnormalities in sperm from tepa-treated males.

Diparopsis sperm have a mean length of 450 μ and a diameter varying between 0.2 and 0.5 μ. The head possesses a short acrosome which projects from the anterior end. The chromatin stains intensely and associated with the nucleus is a tubular structure, also noted by Yasuzumi and Oura [7] in Bombyx mori. From its size and position in Diparopsis this may possibly be associated with the acrosome.

TABLE X. TEPA DEGRADATION IN MALE Diparopsis. SUMMARY TABLE TO SHOW EVIDENCE FOR LINEARITY OF REGRESSION LINES, REGRESSION EQUATIONS AND 50% BREAKDOWN TIME

Temperature °C	Method of treatment	Method of extraction	Mean squares		F ratio	Regression equation	50% breakdown time (hours)
			Regression variance	Within dose variance			
15	injection	homogenization	0.0013	0.0079	0.16	y = 0.923 - 0.016x	18.6
20	"	"	0.0540	0.0221	2.44	y = 0.892 - 0.026x	19.3
27	"	"	0.0124	0.0323	0.28	y = 0.904 - 0.021x	14.3
15	topical	"	0.0120	0.0214	0.48	y = 0.843 - 0.0021x	143.3
20	"	"	0.0056	0.0251	4.64	y = 0.957 - 0.0043x	72.3
27	"	"	0.0077	0.0860	0.90	y = 1.002 - 0.0066x	45.6
20	"	surface wash of whole insects	0.0050	0.0044	1.12	y = 0.9121 - 0.0064x	48.6

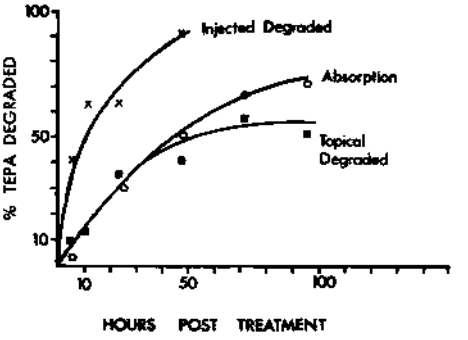


FIG.4. Total degradation of tepa in male moths after topical and injection methods of application compared with the rate of chemosterilant absorption through the cuticle.

Surrounding the posterior head and the middle regions is a radial mantle which takes the form of a series of petaloid structures. These structures, termed 'appendices laciniae' by André [8, 9], who first described them in sperm of Pieris and Macroglossum, arise from the cell membrane and are finely striated transversely. Phillips [10] has observed in several lepidopteran species that these radial appendices are greatly reduced or lost from sperm in the ejaculatory duct, with the exception of one appendage which is recognizably different in having a reticular structure. This reticular appendage is also found in Diparopsis.

Easily the largest organelles of the spermatozoon are the paired elongate mitochondrial structures which have conspicuous, regular clefts in their outer zone (Fig. 5). These giant mitochondrial structures are derived during spermatogenesis from the aggregation of mitochondria into nebenkern, the material of which reorganizes itself and elongates along the developing flagellum (see the review of Phillips [10]).

In Diparopsis, the rod-like mitochondrial derivatives are of unequal size and run alongside the flagellum for much of its length. In the terminal region the mitochondrial rods are tapered off, leaving the bare flagellum (Fig. 6). The flagellum possesses the familiar organization of two central fibrils with nine outer doublets. An additional nine single fibrils which stain more intensely are closely associated with the outer ring of doublets.

When whole air-dried spermatozoa are observed in the scanning electron microscope, the delicate radial appendages cannot be seen. Probably they have collapsed in the process of drying. Longitudinal divisions between the two mitochondrial rods and the flagellum are evident. If the preparation is shadow cast, regular transverse corrugations are revealed which may represent a close spiral organization of the mitochondrial cortex or the collapsed radial appendages (Fig. 7). Such a spiral organization cannot be deduced from the thin sections by transmission electron microscopy.

No evidence of damage to the delicate ultrastructure of the spermatozoa was observed three days after the injection of males with 10 μg of tepa, a treatment considerably in excess of the minimum sterilizing dose. Observations on agglutinated sperm from the spermatheca have yet to be completed.

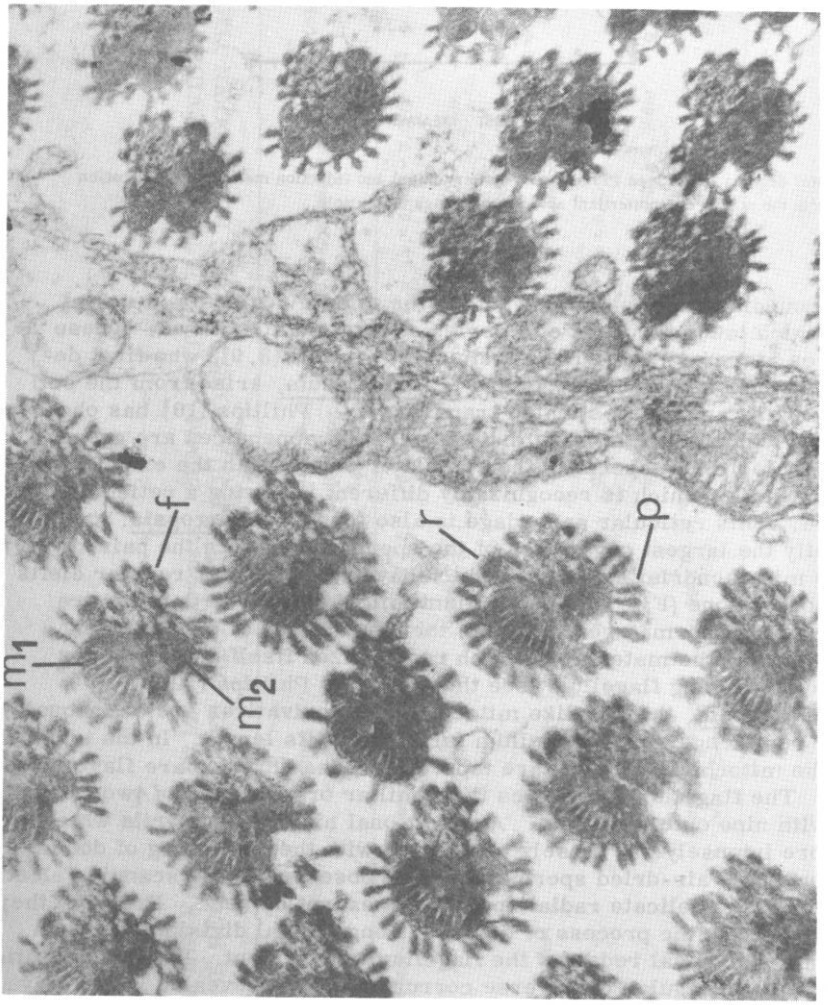


FIG. 5. Transverse section through spermatozoa of two adjacent follicular cysts, showing the two mitochondrial derivatives (m_1, m_2), petaloid structures of the radial mantle (p), the reticular appendage (r) and the axial filament (f). × 33 300

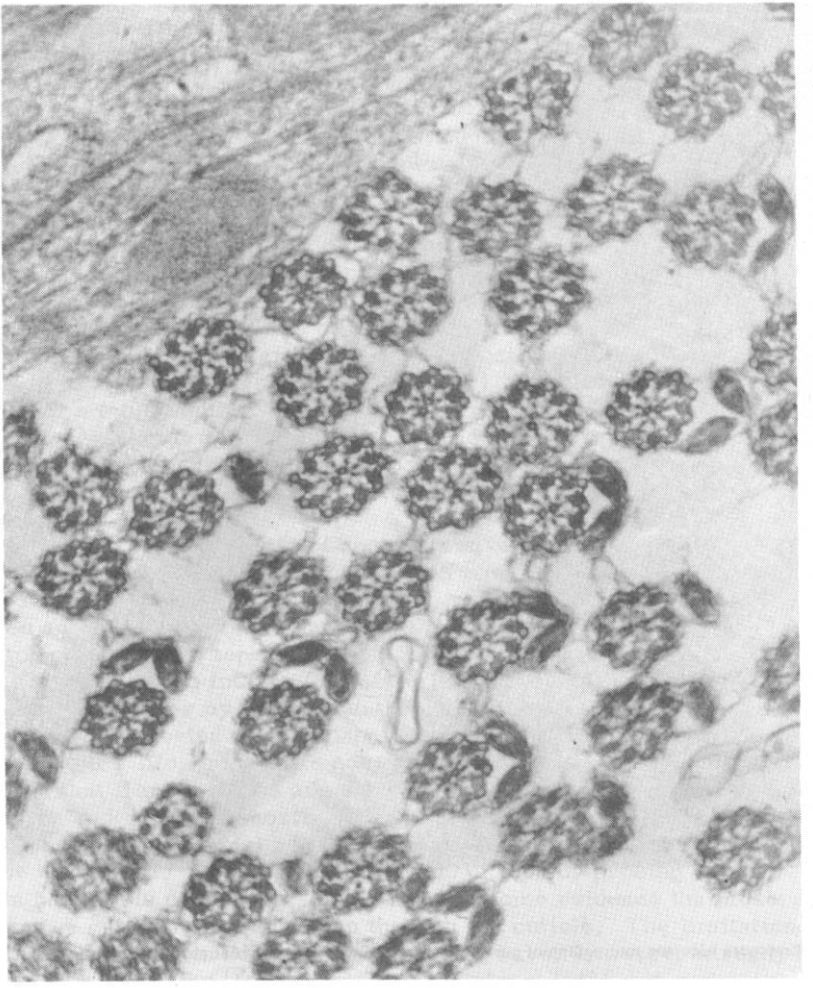


FIG. 6. Transverse section of spermatozoa showing the flagellum in the posterior region where the mitochondrial derivatives terminate. × 59 800

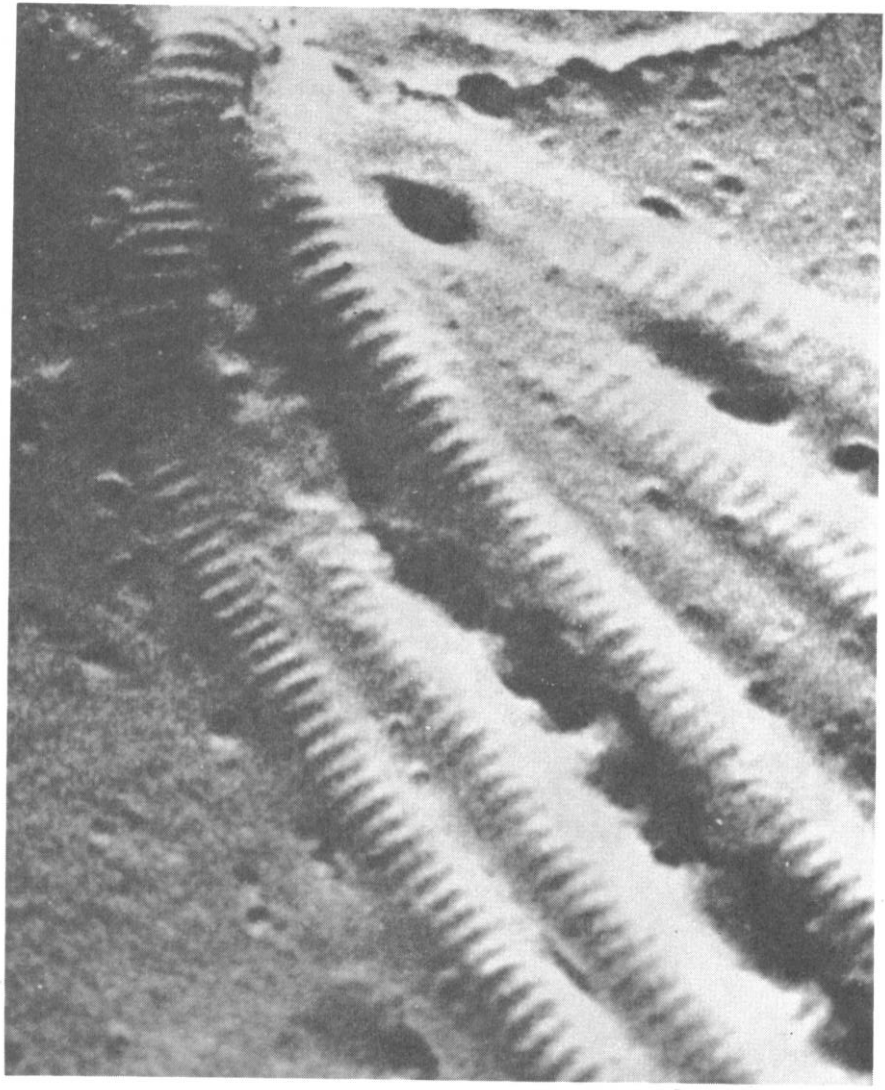


FIG.7. Stereoscan electron micrograph of part of sperm bundle showing regular transverse corrugations. × 41 200

It should be observed that the spermatozoa of Diparopsis are all fully formed when the adult emerges from the pupal stage. Thus tepa applied to the young adult cannot affect sperm development, only mature sperm structures. For the same reason, in this species, sterilized males are unlikely to recover fertility later in adult life.

DISCUSSION

To be of practical value a chemosterilant should not only be effective at very low levels, but also exhibit a wide margin between sterilizing and lethal doses. In addition the sterile insects must be competitive with those of the natural population and remain sterile throughout adult life. Finally, the rate of breakdown of the applied chemosterilant should be rapid to minimize possible environmental contamination.

The experiments described in this paper were designed to test whether these criteria could be satisfied in work on Diparopsis, as a prelude to field evaluation. When efficacy was expressed as the ratio between sterilizing and lethal doses, it was shown that apholate and tepa were significantly better candidates than metepa, hempa and triphenyl tin acetate.

The repetitive mating tests, however, clearly indicated tepa to be the more effective chemosterilant against male moths. Against female moths, on the other hand, the low sterility index of tepa indicated that sterility could only be achieved at a dose that would reduce mating efficiency. The competitiveness of male Diparopsis treated topically suggests that the release of sterile mass-reared or mass-collected insects would be feasible, although the females should be separated and eliminated.

The rate of tepa degradation after injection into male Diparopsis was rapid, as was that noted by Cox et al. [11], after oral application to the fall army worm moth, Spodoptera frugiperda, when estimated radiometrically. A slower rate of tepa degradation after topical application to the codling moth, Carpocapsa pomonella, was observed by Maitlen and McDonough [12] when the treated moths were maintained in outdoor cages.

In Diparopsis the rate of degradation of topically applied tepa was appreciably slower than that of injected tepa. Thus at 20°C, 50% of a dose of 10 μg was metabolized in 19.3 h when injected, but in 72.3 h when applied topically. It is evident from Fig. 4 that the longer persistence of topically applied tepa is almost entirely due to its relatively slow rate of penetration into Diparopsis. The steady recruitment of applied tepa to the tissues by absorption through the cuticle may conceivably be of value in a species in which spermatozoa continue to mature for some days in adult life, but this is not the case in Diparopsis. It may be concluded that if male moths are sterilized by topical treatments of tepa in acetone and released shortly afterwards (necessary because of their short life) then some residual active tepa will be present on the cuticle at the time of release. Possibly the ingestion of tepa by probing would be a less hazardous procedure, since there is some evidence that absorption from the gut is more rapid than through the cuticle. The limitations of inducing sterility in Diparopsis by probing have, however, already been described (Campion [14], Campion and Outram [15]) and such a method does not seem to be of practical value. Topical treatments are, therefore, necessary.

Two factors operate to reduce the brief hazard that residues of topically applied tepa may represent. The first is that the combined effects of absorption and metabolism exhibit a high temperature coefficient (approximately 2.6 for topical treatments, compared with a Q_{10} of 1.3 for injected treatments). Typical meteorological data from the cotton growing areas of Central Africa where Diparopsis is prevalent

were reported by Tunstall, Sweeney and Rose [13]. The mean temperature in Makanga, Malawi, during the 1956/7 cotton-growing season, for example, was 28.5°C; in Gatooma, Rhodesia, it was 22.5°C. It is clear that at high temperatures approaching 30°C, a more rapid breakdown of tepa would occur, following the release of moths sterilized by some form of topical application, although at lower temperatures a much greater persistence would be expected.

The second factor is that Diparopsis is restricted to the cotton plant and is not found near food crops. Thus the release of tepa-sterilized moths is unlikely to have harmful effects on man or other mammals. Nevertheless, it is clearly desirable to ensure that virtually no traces of residual tepa enter the environment. It would certainly be worth while investigating the possibilities of improved formulation of tepa, e.g. by the use of oily solvents which, by diffusing over the whole insect, may promote faster penetration of the sterilant (cf. Lewis [16]). By such means a lower effective dose, a more rapid loss of tepa by metabolism and a consequent reduction in environmental contamination may be achieved. This possibility is being investigated.

In spite of the observed agglutination of spermatozoa at very high doses, there was no evidence of secondary damage to the delicate ultrastructure at doses of tepa in excess of the minimum sterilizing dose. Damage to sperm structure may occur at even higher dose levels of the chemosterilant, where insect mortality is high, but because of the greatly reduced mating at these levels is unlikely to be of significance.

ACKNOWLEDGEMENTS

The authors are grateful to the Agricultural Research Council of Malawi for the supply of Diparopsis pupae, to Dr. A.B. Borkovec of the U.S. Department of Agriculture for the chemosterilants and to Mr. R.H. Williams for skilful assistance.

One of the authors (DGC) wishes to thank Professor T.R.E. Southwood for working facilities provided at Imperial College Field Station, Silwood Park, Ascot.

REFERENCES

[1] CAMPION, D.G., Nature, Lond. 214 (1967) 1031.
[2] CAMPION, D.G., XIII Int. Congr. Ent. Moscow (1968).
[3] CAMPION, D.G., Cott. Gr. Rev. 47 (1970) 135.
[4] ABBOT, W.S., J. econ. Ent. 18 (1925) 265.
[5] FINNEY, D.J., Probit Analysis, Cambridge Univ. Press (1952).
[6] EPSTEIN, J.S. et al., Anal. Chem. 27 (1955) 1435.
[7] YASUZUMI, G., OURA, C., Z. Zellf. mikr. Anat. 67 (1965) 502.
[8] ANDRÉ, J., Ann. Sci. Nat. Zool. Biol. Animale 1 (1959) 283.
[9] ANDRÉ, J., J. Ultrastruct. Res. Suppl. 3 (1962).
[10] PHILLIPS, D.M., J. Cell. Biol. 44 (1970) 243.
[11] COX, H.C. et al., J. econ. Ent. 60 (1967) 1111.
[12] MAITLEN, J.C., McDONOUGH, J.M., J. econ. Ent. 60 (1967) 1391.
[13] TUNSTALL, J.P. et al., Cotton Pest Res. Scheme C. Africa Annu. Rpt. 1956/57 (1958).
[14] CAMPION, D.G., Pest Articles & News Summaries 15 (1969) 535.
[15] CAMPION, D.G., OUTRAM, I., Ann. appl. Biol. 60 (1967) 191.
[16] LEWIS, C.T., in: Radiation and Radioisotopes applied to Insects of Agricultural Importance (Proc. Symp. Athens, 1963) IAEA, Vienna (1963) 135.

ETUDE A L'AIDE D'UNE TECHNIQUE D'IRRADIATION AUX RAYONS GAMMA DE LA MIGRATION ET DE L'UTILISATION DES SPERMATOZOIDES CHEZ LA BRUCHE DU HARICOT, Acanthoscelides obtectus (SAY)

J. HUIGNARD
Laboratoire d'écologie et de biocoenotique expérimentales,
Université de Tours,
Tours, France

Abstract — Résumé

STUDY, USING THE GAMMA IRRADIATION TECHNIQUE, OF THE MIGRATION AND UTILIZATION OF SPERMATOZOA IN THE BEAN WEEVIL, Acanthoscelides obtectus (SAY).

Males of Acanthoscelides obtectus were irradiated with gamma rays from a cobalt-60 bomb; the dose used was 9000 rad. These males copulate normally, depositing into the copulatory bursa of non-irradiated females a spermatophore containing all the paragonial secretions and spermatozoa which migrate actively towards the spermatheca. The females have a normal fecundity, but their fertility is nil; the embryonic development of the eggs laid stops at a very early stage. The successive use of sterile males and normal males provides a means of studying the migration and utilization of spermatozoa in the same female after two copulations. The spermatozoa deposited during the second copulation, whether by a normal male or by an irradiated male, all migrate into the spermatheca, but they are only partially utilized to fertilize the ovocytes; in all cases the spermatozoa deposited during the first copulation have a predominant role. It is probable that the mixing of the spermatozoa introduced into and stored in the spermatheca after two successive copulations would be only partial, even when egg laying is halted for several days due to the absence of the bean seeds. The use of sterile males in the control of Acanthoscelides obtectus will be effective only if the females encountered are virgin. This would seem to be fairly rare under natural conditions; when the density of insects is high the females mate a few hours after birth. The sterile males will therefore have to be introduced into the contaminated seed store before the adults emerge, but the effectiveness of this method of control may be diminished.

ETUDE A L'AIDE D'UNE TECHNIQUE D'IRRADIATION AUX RAYONS GAMMA DE LA MIGRATION ET DE L'UTILISATION DES SPERMATOZOIDES CHEZ LA BRUCHE DU HARICOT, Acanthoscelides obtectus (SAY).

Les mâles d'Acanthoscelides obtectus sont soumis à une irradiation de rayons gamma émis par une bombe au cobalt-60; la dose utilisée est de 9000 rad. Ces mâles copulent normalement; ils émettent dans la bourse copulatrice de femelles non irradiées un spermatophore contenant toutes les sécrétions élaborées par les paragonies, et des spermatozoïdes qui migrent activement vers la spermathèque. Les femelles ont une fécondité normale, mais leur fertilité est nulle; le développement embryonnaire des œufs émis s'arrêtant à un stade très précoce. L'emploi successif des mâles stériles et de mâles normaux permet d'étudier la migration et l'utilisation des spermatozoïdes, chez une même femelle, après deux copulations. Les spermatozoïdes, émis lors du second accouplement par un mâle normal ou un mâle irradié, migrent tous dans la spermathèque, mais ne sont que partiellement utilisés pour féconder les ovocytes; dans tous les cas, les spermatozoïdes émis lors de la première copulation ont un rôle prédominant. Il est probable que le mélange des spermatozoïdes introduits et stockés dans la spermathèque après deux copulations successives ne soit que partiel, même lorsque l'émission des œufs est arrêtée, par suite de l'absence de graines de haricot, pendant plusieurs jours. L'utilisation de mâles stériles dans la lutte contre Acanthoscelides obtectus ne sera efficace que si les femelles rencontrées sont vierges. Ceci semble assez rare dans les conditions naturelles, lorsque la densité d'insectes est élevée, les femelles s'accouplant quelques heures après leur naissance. Les mâles stériles devront donc être introduits dans le stock de grains contaminé avant la sortie des adultes, mais l'efficacité de cette méthode de lutte peut être diminuée.

INTRODUCTION

Chez Acanthoscelides obtectus, lorsque deux mâles copulent successivement avec une même femelle, deux spermatophores peuvent être déposés dans la bourse copulatrice. Après chaque accouplement, il y a stimulation nette de l'ovogenèse [1], mais la migration, puis l'utilisation des spermatozoïdes provenant de la seconde copulation n'ont pas été étudiées jusqu'à présent.

Un certain nombre de recherches de ce type ont été entreprises chez les insectes en utilisant successivement des mâles rendus stériles par une irradiation aux rayons X ou aux rayons gamma, puis des mâles normaux.

Les spermatozoïdes émis lors de la première copulation jouent un rôle prédominant au moment de la fertilisation des ovocytes chez Glossina austeni [2], et chez Heliothis virescens [3]. En étudiant Cylas formicarius Walker [4] constate que le premier accouplement est seul efficace. Chez d'autres insectes, tels que Trichophisia ni, [5], Carpocapsa pomonella [6], Epilachna varivestis [7], les spermatozoïdes émis lors de la seconde copulation jouent un rôle prédominant, ou deviennent seuls efficaces au moment de la fertilisation des ovocytes. Lefevre et Jonsson [8], en étudiant la drosophile, pensent que les spermatozoïdes émis lors de la seconde copulation provoquent le déplacement d'une quantité plus ou moins importante de spermatozoïdes déjà présents dans la spermathèque et jouent de ce fait un rôle prédominant au moment de la fertilisation des ovocytes. Chez Musca domestica [9] et Anopheles gambiae [10] le mélange des spermatozoïdes, provenant des deux copulations, serait total et leur utilisation se ferait au hasard.

L'influence des spermatozoïdes émis lors de la seconde copulation varie donc suivant les insectes. Au cours des expériences, nous étudierons la fertilité des femelles d'Acanthoscelides obtectus, mise en présence de mâles normaux puis de mâles rendus stériles par une irradiation aux rayons gamma (ou inversement).

1. METHODES

Les bruches utilisées au cours des diverses expériences appartiennent à la lignée II, sélectionnée par Labeyrie [11]. Les femelles dont la fécondité est élevée ne pondent qu'en présence de graines de haricot (stimulus indispensable).

Les mâles sont séparés en deux lots, placés dans des conditions identiques dès leur naissance.

Premier lot: Les mâles sont soumis, à l'âge de 3 jours, à une irradiation de rayons gamma émis par une bombe au cobalt-60. La dose utilisée est de 9000 rad; après ce traitement, comme l'ont constaté Pesson [12], Cavalloro et Bonfanti [13], tous les mâles sont stériles.

Deuxième lot: Tous les mâles sont normaux.

Tous les insectes utilisés au début des expériences sont âgés de 4 jours.

L'étude histologique des spermatophores prélevés après les diverses copulations est faite sur coupes après fixation au Bouin, puis coloration à l'Azan.

2. EFFETS DE L'IRRADIATION SUR L'ACTIVITE REPRODUCTRICE DES MALES

Lorsque les mâles irradiés mis en présence de femelles vierges normales copulent, un spermatophore contenant les diverses secrétions mâles élaborées par les paragonies et les spermatozoïdes est déposé dans la bourse copulatrice de ces femelles. Les spermatozoïdes irradiés sont activés dans le spermatophore, à la fin de la copulation, puis migrent dans la spermathèque. La migration des spermatozoïdes est achevée, deux heures après la copulation, aussi bien chez les femelles ayant copulé avec un mâle normal, que chez les femelles ayant copulé avec un mâle irradié. L'irradiation des spermatozoïdes par les rayons gamma ne modifie donc pas leur activité.

La fécondité et la production ovarienne des femelles, isolées après une seule copulation avec un mâle irradié et placées en présence continue de graines de haricot (stimulus nécessaire à la ponte) sont identiques à celles des femelles témoins ayant copulé avec un mâle non traité. Les différences observées (tableau 1) ne sont pas significatives. L'introduction du spermatophore émis par le mâle irradié a donc normalement stimulé l'ovogenèse comme cela a été mis en évidence chez Acanthoscelides obtectus [1]. La fertilité, cependant, est nulle; le développement embryonnaire des œufs pondus s'arrête à un stade très précoce; il y a donc eu fertilisation des ovocytes par les spermatozoïdes irradiés. Grosch [14] pense que l'irradiation provoque des aberrations chromosomiques, au niveau des spermatozoïdes, et l'œuf fertilisé par ceux-ci ne peut se développer.

Ce phénomène nous permet d'étudier la migration puis l'utilisation des spermatozoïdes provenant de deux copulations successives chez des femelles mise en présence d'un mâle normal et d'un mâle irradié. Les œufs ayant un développement embryonnaire complet ne peuvent être fertilisés, dans ces conditions, que par des spermatozoïdes non irradiés.

TABLEAU I. EFFETS DE L'IRRADIATION SUR LE POUVOIR FECONDANT DES MALES

	Femelles témoins (n = 74)	Femelles mises avec mâles irradiés (n = 90)	Valeur de t	Signification du test t
Fécondité moyenne	57,2	57,5	0,14	-
Fertilité moyenne	51,7	0	-	+
Production ovarienne moyenne	59,2	59,4	0,12	-

3. UTILISATION DE MALES IRRADIES ET DE MALES NORMAUX AU COURS D'EXPERIENCES DE COPULATIONS SUCCESSIVES AVEC DES FEMELLES NON IRRADIEES

3.1. Description des expériences réalisées

Les femelles de la lignée II sont mises en présence d'un premier mâle au début de l'expérience, puis d'un second mâle quatre jours plus tard. Après chaque copulation le mâle est retiré et les femelles sont isolées dans des boîtes d'élevage. Durant les six premiers jours les femelles privées de graines de haricot n'émettent aucun œuf. Le sixième jour les femelles sont mises en présence de graines de haricot et commencent à pondre.

Comme il n'y a eu aucun œuf émis jusqu'au sixième jour, on peut supposer, qu'au début de la période de ponte, la spermathèque contient des spermatozoïdes, émis lors des deux copulations en quantités équivalentes. A ce moment, si le mélange était total, à l'intérieur de la spermathèque, tous les spermatozoïdes auraient autant de chances de fertiliser les ovocytes.

Les expériences sont réalisées sur trois lots d'insectes, de la même génération, placés dans des conditions rigoureusement identiques, pour limiter au maximum la variabilité (fig. 1).

Expérience 1 (témoins): Les femelles sont mises en présence de deux mâles normaux.

Expérience 2: Les femelles sont mises en présence d'un mâle normal puis d'un mâle irradié.

Expérience 3: Les femelles sont mises en présence d'un mâle irradié puis d'un mâle normal.

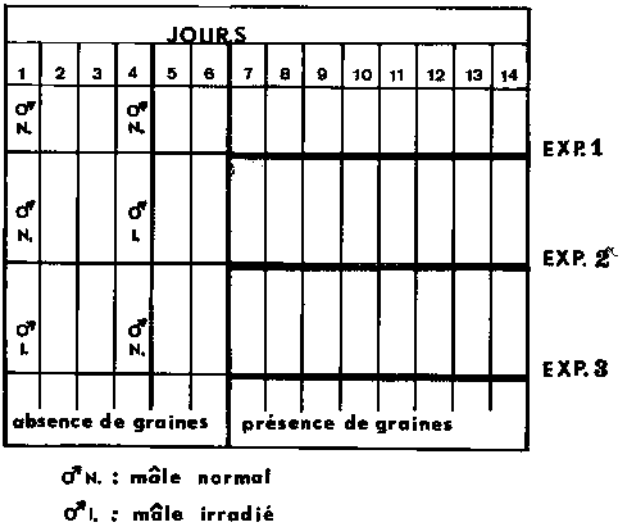


FIG. 1. Schéma des trois expériences.

3.2. Résultats obtenus au cours de l'expérience témoin

La fécondité élevée, le septième jour, juste après l'introduction des stimulus de ponte, décroît progressivement au cours de l'expérience.

En étudiant quotidiennement l'ensemble des femelles pondeuses, on constate que le rapport r entre la fertilité et la fécondité varie peu durant les dix premiers jours de l'expérience; 10 à 15% des œufs émis ne sont pas fertilisés.

Durant les derniers jours de l'expérience, le rapport r diminue, le taux d'œufs non fertilisés augmente, mais cette variation est peu significative étant donné la fécondité individuelle de chaque femelle, très réduite à ce moment (tableau II).

Utilisation des résultats de l'expérience témoin dans le calcul de la fertilité théorique

Au cours des expériences utilisant successivement un mâle irradié et un mâle normal nous ne pouvons pas comparer les fécondités et les fertilités des femelles, car 10 à 15% des œufs n'ont pas été fertilisés et il est difficile de les distinguer des œufs ayant eu un développement embryonnaire avorté à cause des spermatozoïdes irradiés. Cependant, les femelles témoins peuvent nous servir de points de comparaison; nous pouvons supposer que la valeur quotidienne du rapport r serait susceptible de varier dans des proportions identiques si tous les mâles mis en présence des femelles lors des expériences 2 et 3 étaient normaux.

Dans ces conditions on peut déterminer quotidiennement, compte tenu de la fécondité de l'ensemble des femelles, la fertilité théorique. La valeur de la fertilité théorique serait celle obtenue si tous les mâles mis en présence des femelles, lors des expériences 2 et 3, n'avaient subi aucune irradiation.

Cette fertilité théorique (f_{th}) pour le jour n sera

$$f_{th} = F_n \times r_n$$

F_n = fécondité des femelles en expérience le jour n

r_n = rapport $\frac{\text{fertilité}}{\text{fécondité}}$ trouvé chez les femelles témoins le jour n

La fertilité théorique nous servira de valeur de référence; la différence obtenue, le jour n, entre la fertilité théorique et la fertilité expérimentale, ne pourra être due qu'à l'influence des spermatozoïdes irradiés.

3.3. Résultats obtenus lors de l'expérience 2

En étudiant le tableau III, nous constatons que la fertilité expérimentale est nettement différente de la fertilité théorique; la valeur du X^2 (282) étant très supérieure à la valeur limite (18,48 pour 7 D.L.) pour un coefficient de sécurité de 99%.

Les spermatozoïdes irradiés issus de la seconde copulation migrent donc dans la spermathèque et sont utilisés pour la fertilisation des ovocytes.

TABLEAU II. RESULTATS DE L'EXPERIENCE 1. FEMELLES AVEC DEUX MALES NORMAUX

Jours	J.7	J.8	J.9	J.10	J.11	J.12	J.13	J.14
Nombre de femelles pondeuses	37	37	37	37	36	35	29	23
Fécondité	1168	452	391	317	291	262	190	132
Fertilité	1021	382	308	241	237	178	131	85
Rapport r	0,87	0,84	0,78	0,76	0,81	0,67	0,68	0,64

TABLEAU III. RESULTATS DE L'EXPERIENCE 2. FEMELLES AVEC MALES NORMAUX, PUIS IRRADIES

Jours	J.7	J.8	J.9	J.10	J.11	J.12	J.13	J.14
Nombre de femelles pondeuses	45	42	44	44	41	41	43	29
Fécondité	1417	575	565	429	323	283	290	130
Fertilité expérimentale	943	355	320	242	175	128	135	54
Fertilité théorique	1277	485	445	324	263	192	199	83
Rapport I_2 $\frac{\text{Fert. exp.}}{\text{Fert. th.}}$	0,74	0,73	0,71	0,74	0,66	0,66	0,67	0,65

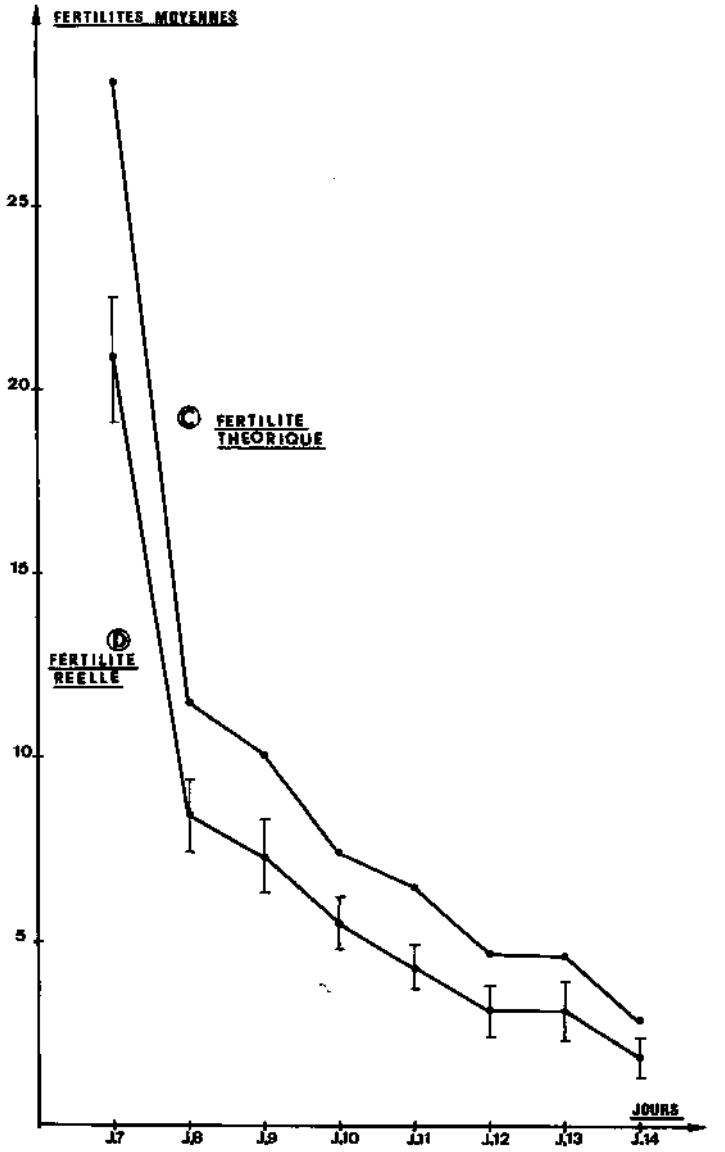


FIG. 2. Etude quotidienne de la fertilité théorique moyenne (C) et de la fertilité moyenne des femelles au cours de l'expérience (D). (Moyenne expérimentale $m \pm t \times sm$ pour un coefficient de sécurité de 95%; $t = 1,96$ dans les conditions de l'expérience.)

TABLEAU IV. RESULTATS DE L'EXPERIENCE 3. FEMELLES AVEC MALES IRRADIES, PUIS NORMAUX

Jours	J.7	J.8	J.9	J.10	J.11	J.12	J.13	J.14
Nombre de femelles pondeuses	39	39	39	39	37	36	26	12
Fécondité	1365	559	391	319	268	220	124	37
Fertilité expérimentale	466	158	111	82	76	50	27	5
Fertilité théorique	1230	472	308	241	218	149	85	15
Valeur de I_3 $\frac{\text{Fert. exp.}}{\text{Fert. th.}}$	0,37	0,33	0,36	0,34	0,34	0,33	0,31	0,33

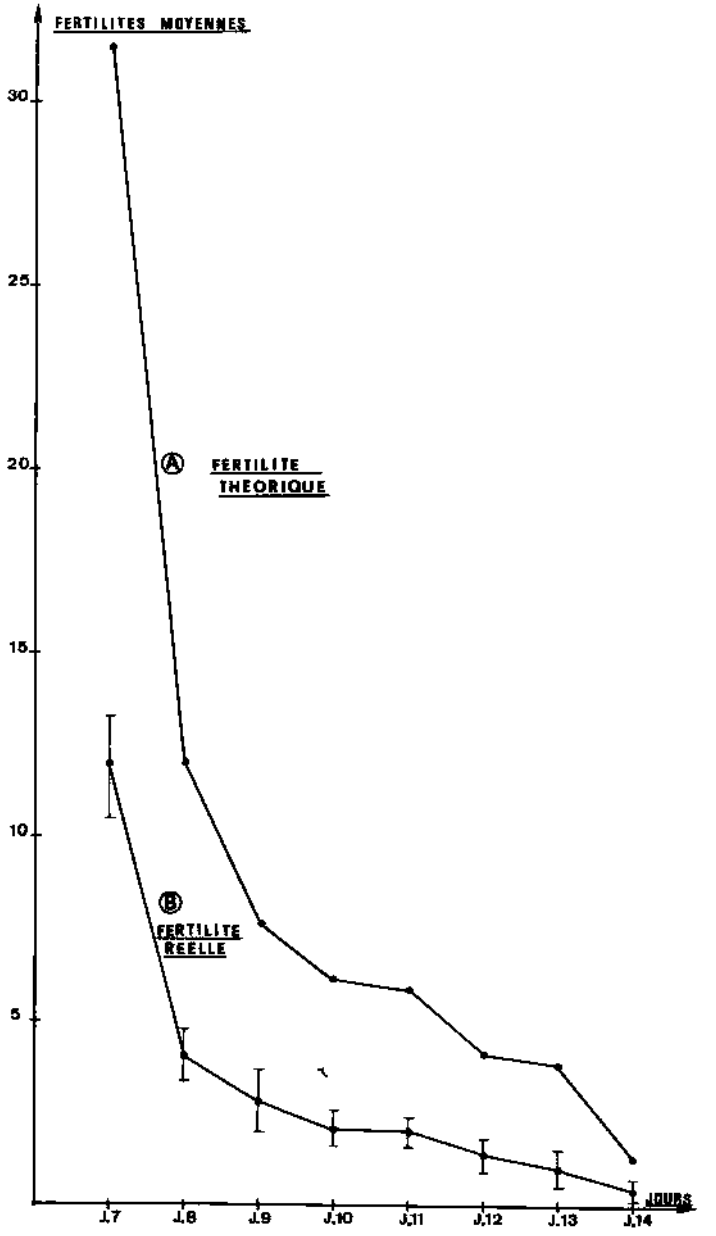


FIG. 3. Etude quotidienne de la fertilité théorique moyenne (A) et de la fertilité moyenne des femelles au cours de l'expérience (B). (Moyenne expérimentale $m \pm t \times sm$ pour un coefficient de sécurité de 95%; $t = 1,96$ dans les conditions de l'expérience.)

L'étude du rapport I_2 entre la fertilité expérimentale et la fertilité théorique, relativement constant pendant toute la durée de l'expérience, montre que les spermatozoïdes non irradiés, issus de la première copulation, jouent un rôle prédominant dans la fertilisation des ovocytes.

L'étude quotidienne de la fertilité théorique moyenne et de la fertilité moyenne des femelles au cours de l'expérience (fig. 2) confirme ce résultat; 75% des œufs pondus sont fertilisés par les spermatozoïdes émis par le mâle normal.

3.4. Résultats obtenus lors de l'expérience 3

Nous constatons également que la fertilité expérimentale est très différente de la fertilité théorique, la valeur du X^2 (1108) étant très supérieure à la valeur limite (18,48 pour 7 D.L.) pour un coefficient de sécurité de 99%. Les spermatozoïdes normaux, émis lors de la seconde copulation pénètrent donc dans la spermathèque, mais ne jouent pas un rôle prédominant dans la fertilisation des ovocytes puisque le rapport I_3 reste voisin de 0,35 durant toute l'expérience (tableau IV).

35% des œufs pondus ont donc été fertilisés par les spermatozoïdes émis par le mâle normal durant la seconde copulation; la figure 3 montre bien la différence existant au cours de l'expérience entre la fertilité des femelles et la fertilité théorique.

3.5. Interprétation des résultats

Ces expériences montrent qu'une irradiation de 9000 rad ne modifie ni l'activité des spermatozoïdes irradiés, ni leur aptitude à fertiliser les ovocytes chez Acanthoscelides obtectus. Dans tous les cas, les spermatozoïdes émis lors de la première copulation, qu'ils soient normaux ou irradiés, jouent un rôle prédominant dans la fertilisation des ovocytes.

L'étude de l'évolution journalière du rapport I_2 et du rapport $1-I_3$ (correspondant aux œufs fertilisés par les spermatozoïdes irradiés), montre que les deux courbes obtenues se superposent (fig. 4).

On doit admettre dans ces conditions, que 65 à 75% des œufs pondus par les femelles ont été fertilisés par les spermatozoïdes, déposés dans la spermathèque lors de la première copulation.

Curtis [2] observe le même phénomène chez Glossina austeni, bien que les spermatozoïdes normaux et irradiés déposés dans la spermathèque aient également la même valeur compétitive.

Plusieurs hypothèses peuvent être envisagées pour expliquer ce phénomène.

1) Etant donné l'état de réplétion de la spermathèque, après le premier accouplement, on peut supposer que la migration des spermatozoïdes émis lors de la seconde copulation ne soit que partielle. La plus grande partie des spermatozoïdes, émis dans ces conditions, demeurait à l'intérieur du second spermatophore.

La spermathèque pourrait donc contenir un plus grand nombre de spermatozoïdes provenant du premier accouplement et ceux-ci auraient de plus grandes chances de fertiliser les ovocytes. En étudiant sur coupes histologiques des spermatophores prélevés vingt, quarante puis soixante minutes après la seconde copulation, on constate que la migration des

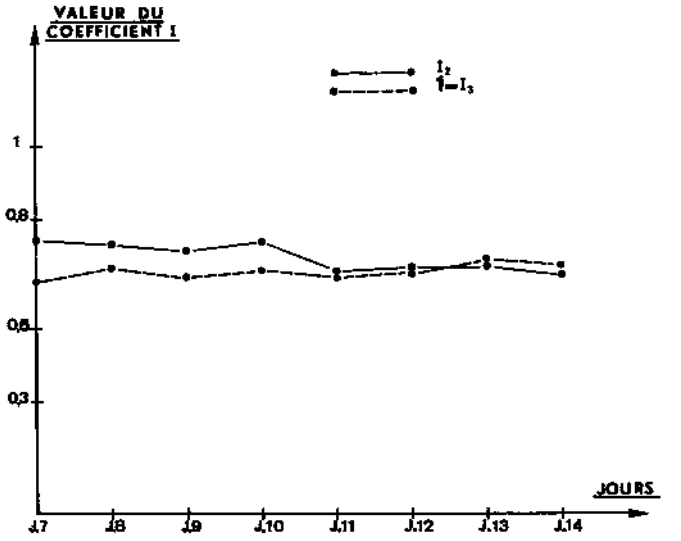


FIG. 4. Etude quotidienne de l'évolution du rapport I_2 (expérience 2) et du rapport $1 - I_3$ (expérience 3).

spermatozoïdes est totale. On ne trouve pratiquement plus de spermatozoïdes dans le deuxième spermatophore déposé à l'intérieur de la bourse copulatrice de la femelle ni dans les voies génitales soixante minutes après l'accouplement.

Cette hypothèse ne peut donc être admise; la spermathèque, au début de la période de ponte, doit sans doute contenir des quantités probablement équivalentes de spermatozoïdes émis par les deux mâles successifs.

2) On peut supposer que le mélange des spermatozoïdes provenant des deux copulations ne soit que partiel, les spermatozoïdes issus de la première copulation restant localisés dans la région du ductus receptaculi. Etant donné l'état de réplétion de la spermathèque, les spermatozoïdes en rétention sont en effet peu mobiles, et on peut concevoir dans ces conditions que le mélange se fasse difficilement. Flint et Kressin arrivent à la même conclusion chez Heliothis virescens [3], la première copulation joue également un rôle prédominant et le mélange des spermatozoïdes à l'intérieur de la spermathèque ne serait que partiel.

Chez la drosophile, Lefevre et Jonsson [8] pensent qu'il existerait une circulation continue des spermatozoïdes du réceptacle vers l'oviducte, et, le réaccouplement provoquerait un déplacement en plus ou moins grande proportion des spermatozoïdes déjà présents dans la spermathèque.

Gugler, Kaplan et Kidd [15] ont d'ailleurs constaté, par autoradiographie, que des spermatozoïdes marqués avec de la désoxycytidine tritiée, émis lors de la seconde copulation, sont beaucoup plus abondants dans la spermathèque, que les spermatozoïdes non marqués provenant du premier accouplement. Ils ont donc plus de chances de fertiliser les ovocytes.

L'hypothèse d'un déplacement des spermatozoïdes, après la seconde copulation, peut être envisagée chez Acanthoscelides obtectus. L'arrivée de nouveaux spermatozoïdes dans la spermathèque provoquerait un déplacement du sperme déjà présent, puis à la fin de la migration, un rassemblement des spermatozoïdes émis par le premier mâle, dans la région du

ductus receptaculi. La densité des spermatozoïdes restant élevée, à l'intérieur de la spermathèque, et leur mobilité réduite, on peut très bien concevoir que le mélange des spermatozoïdes reste partiel pendant toute la durée de l'expérience.

Une étude autoradiographique, à l'aide de spermatozoïdes marqués par un isotope radioactif, devra être entreprise pour vérifier cette hypothèse.

4. UTILISATION DES MALES IRRADIES DANS LA LUTTE BIOLOGIQUE CONTRE Acanthoscelides obtectus

L'irradiation des mâles par les rayons gamma, à la dose de 9000 rad, ne semble pas modifier leur comportement sexuel.

Des femelles vierges de la lignée II sont mises simultanément en présence d'un mâle vierge irradié, et d'un mâle vierge normal. Ces mâles âgés de 4 jours, proviennent de la même génération de bruches sélectionnées. Les femelles sont laissées en présence des mâles durant six heures, puis sont isolées dans des boîtes d'élevage contenant des graines de haricot. Pendant six heures, 95% des femelles ne s'accouplent qu'avec un seul mâle; leur bourse copulatrice ne contient d'ailleurs qu'un seul spermatophore.

Résultats de l'expérience: 182 femelles sont étudiées:

- 96 pondent des œufs fertiles et se sont donc accouplées avec un mâle normal
- 86 pondent des œufs dont le développement embryonnaire avorte

et ont donc copulé avec un mâle irradié.

Si l'accouplement se faisait au hasard chaque mâle aurait autant de chances de copuler avec la femelle. 91 mâles normaux et 91 mâles irradiés devraient donc copuler avec les 182 femelles.

Comparons les valeurs théoriques et expérimentales; le X^2 (0,54) est nettement inférieur à la valeur limite (6,64 pour 1 D.L.) pour un coefficient de sécurité de 99%.

L'accouplement se fait donc au hasard et le mâle irradié a autant de chances de rencontrer la femelle que le mâle normal, dans les conditions de l'expérience. Les mâles stériles introduits dans une population de bruches auront donc le même comportement sexuel que les mâles normaux. Cependant, ils ne seront vraiment efficaces dans la lutte biologique que s'ils s'accouplent avec des femelles vierges, puisque la première copulation joue un rôle prédominant. Or, dans les lieux de stockage où les graines sont contaminées la densité d'insectes est très élevée et les femelles s'accouplent généralement quelques heures après leur naissance.

Les mâles stériles devront donc être introduits dans le stock de grains contaminé avant la naissance des adultes. Ils entreront en compétition avec les jeunes mâles normaux, nés en même temps que les femelles; l'efficacité de la lutte à l'aide des mâles irradiés contre les pullulations de bruches du haricot risque, dans ces conditions, d'être diminuée.

CONCLUSIONS

Les mâles d'Acanthoscelides obtectus sont soumis à une irradiation de rayons gamma émis par une bombe au cobalt-60; la dose utilisée est de 9000 rad. Ces mâles copulent normalement; ils émettent dans la bourse

copulatrice de femelles non irradiées un spermatophore contenant toutes les sécrétions élaborées par les paragonies, et des spermatozoïdes qui migrent activement vers la spermathèque. Les femelles ont une fécondité normale, mais leur fertilité est nulle, le développement embryonnaire des œufs émis s'arrêtant à un stade très précoce.

L'emploi successif des mâles stériles et de mâles normaux permet d'étudier la migration et l'utilisation des spermatozoïdes, chez une même femelle, après deux copulations. Les spermatozoïdes, émis lors du second accouplement, par un mâle normal ou un mâle irradié, migrent tous dans la spermathèque, mais ne sont que partiellement utilisés pour féconder les ovocytes; dans tous les cas, les spermatozoïdes émis lors de la première copulation ont un rôle prédominant. Il est probable que le mélange des spermatozoïdes introduits et stockés dans la spermathèque, après deux copulations successives, ne soit que partiel, même lorsque l'émission des œufs est arrêtée, par suite de l'absence de graines de haricot, pendant plusieurs jours.

L'utilisation de mâles stériles, dans la lutte contre Acanthoscelides obtectus, ne sera efficace que si les femelles rencontrées sont vierges. Ceci semble assez rare dans les conditions naturelles, lorsque la densité d'insectes est élevée les femelles s'accouplant quelques heures après leur naissance. Les mâles stériles devront donc être introduits dans le stock de grains contaminé avant la sortie des adultes, mais l'efficacité de cette méthode de lutte peut être diminuée.

REFERENCES

[1] HUIGNARD, J., C.r. hebd. Séanc. Acad. Sci., Paris 268 (1969) 2938.
[2] CURTIS, C.F., J. Insect. Physiol. 14 (1968) 1365.
[3] FLINT, H.M., KRESSIN, E.L., J. econ. Ent. 64 (1968) 473.
[4] WALKER, J.R., J. econ. Ent. 59 (1966) 1206.
[5] NORTH, D.T., HOLT, G.G., Isotopes and Radiation in Ent., Vienne (1967) 391-403.
[6] HATAWAY, D.O., J. econ. Ent. 59 (1966) 35.
[7] HENNEBERRY, T.J., SMITH, F.F., McGOVERN, W.L., J. econ. Ent. 57 (1964) 813.
[8] LEFEVRE, G., JONSSON, J.R., Genetics 47 (1962) 1719.
[9] ZINGRONE, L.D., BRUCE, W.N., DECKER, G.C., J. econ. Ent. 52 (1959) 236.
[10] GOMA, L.K.H., Nature, Lond. 197 (1963) 99.
[11] LABEYRIE, V., Soc. Biol. Poitiers 162 12 (1968) 2203.
[12] PESSON, P., Industr. alimen. agr. 80 (1963) 211.
[13] CAVALLORO, R., BONFANTI, G., Boll. Zool. II 8 (1967).
[14] GROSCH, D.S., Bios. 36 2 (1965) 55-62.
[15] GUGLER, H.D., KAPLAN, W.D., KIDD, K., Drosoph. Inf. Serv. 40 (1965) 65.

DISCUSSION

L.E. La CHANCE: In your double-mating studies you showed that sperm from irradiated males was utilized by females as well as that from normal males, but that the sperm from the first mating was predominantly utilized, regardless of whether the male was treated or not. Why then do you conclude that the sterile-male technique would require excessive numbers of released males, i.e. more than would normally be required?

J. HUIGNARD: The young males are usually more competitive than the irradiated males introduced into the contaminated stored grain, which are of course older. The sterile males could no doubt mate with a certain number of females, but they risk being ousted by the new-born males. That is why I think that the sterile-male method must have a reduced effectiveness unless a very large number of irradiated males are introduced.

M.E. TZANAKAKIS: Was the duration of the first mating of the female the same as that of the second?

J. HUIGNARD: Yes, the second mating lasts as long as the first, about seven minutes.

THE RESPONSE OF THE FEMALE ARTHROPOD'S REPRODUCTIVE SYSTEM TO RADIATION AND CHEMICAL AGENTS

D. S. GROSCH
North Carolina State University,
Raleigh, N.C., United States of America

Abstract

THE RESPONSE OF THE FEMALE ARTHROPOD'S REPRODUCTIVE SYSTEM TO RADIATION AND CHEMICAL AGENTS.

This paper proposes more utilization of altered female reproductive performance when devising new approaches to population collapse of pests. It discusses results obtained and methods of analysing the mode of action of a variety of chemical agents in addition to X and gamma rays.

An extremely important feature which determines whether a population will survive or collapse in response to a deleterious influence is the maximum number of offspring per female equal to the number of functional eggs produced. Fecundity data can be compared with the Wallace diagram of expectations from induced dominant and recessive mutational contribution to extinction.

Females in which ovaries failed to develop, or in which the ovaries have been destroyed may serve as the optimum siphon for sperm from a population's males provided females remain attractive and receptive. Peak vulnerability to destructive agents usually occurs during pre-imaginal development. But in some species where internal transformation lags behind external appearance, the opportunity for complete destruction exists in so-called adults.

The alternative situation is one in which the ovaries are fully mature. Two main categories are met here: (1) with oocytes already differentiated to provide for 1/3 or more of the lifetime egg deposit, or (2) with a minor portion of the potential eggs differentiated to oocytes but the major portion as undifferentiated gonial or stem cells. Because of features uniquely suited to such investigations, most of the truly analytical experiments revealing differences in the responses of cell types have been performed in Bracon, but the analysis can be extended to other species. Daily egg production records over an appreciable period are required. Then average performance for the most appropriate period of time is plotted. Also plotted is cumulative egg production. Characteristic curves distinguish between agents which cause chromosomal damage (radiations, alkylating agents) and those which interfere with nurse cell function (antimetabolites). A quite different response is obtained with agents which attack somatic tissues (toxic metals, chlorinated hydrocarbons). In conjunction with oviposition studies, information on egg hatchability provides important corroborative evidence. The analysis should not stop with scoring the unhatched eggs. A determination of stage of embryonic death may provide conclusive evidence on mode of action. The failure to complete cleavage and set up a blastoderm can be identified and distinguished from all other types of moribund conditions or stages of death. This feature is characteristic of potent chemosterilants which damage chromosomes.

1. INTRODUCTION

Although a few scattered references indicate that the sterilized female can play a positive role in a control program [1,2], the female has been the neglected sex. Two factors have contributed to the situation. Muller's classic techniques for detecting mutation employed treated males, and credit for the U.S.D.A. success in controlling the screw-worm has been attributed to the males released. This paper proposes greater utilization of altered female reproductive performance when devising new approaches to the collapse of pest populations. Auspiciously the active competitiveness necessary for males to carry genetic defects into a population is no problem when females are used in species where her role in mating is a passive one. She needs to be merely attractive and receptive to the male.

The consequences of introducing female sterility genes into a natural population has already been discussed (although briefly) at an I.A.E.A. symposium [1]. An extreme type of female sterility will be described below. In addition, the kinds of responses to chemical agents and radiations will be identified, and methods of analyzing their modes of action will be explained.

2. SIGNIFICANCE OF FECUNDITY

In our desire for means of rapid eradication we tend to lose sight of the important biological feature which determines whether a population will survive or collapse in response to a deleterious influence. This is the maximum number of offspring per female, in turn limited by the number of functional eggs produced. This paper directs attention to this subject.

Although a number of factors are involved in evaluating the damage from a mutagenic and cytotoxic agent, Chamberlain [3] found a composite evaluation possible if he employed a formula in which the important terms represent the number of viable eggs per female in the control and the treated group; furthermore, when the amount of dominant lethality has been determined from dose vs. effect experiments, it is possible to construct survival-extinction curves which show the average number of eggs each female must produce if the population to which she belongs is to persist. Laboratory experiments with Drosophila populations verified the predictive success of this theoretical treatment [4]. For genes with specific deleterious effects only when homozygous, a similar estimate is possible. Again it devolves to the number of eggs a female must produce in order to maintain heterotic systems involving a given number of independent gene loci [5].

Many insect types fall within the Drosophila fecundity range of 300 to 500 eggs per female. On the other hand, Anopheles mosquitoes which survive long enough to lay 10 batches of eggs can produce 2500 eggs each [6]. This impressive figure provides a reproductive potential equivalent to that of the primitive arthropod, Artemia salina. Nevertheless, because so much of this potential is a reserve used to buffer drastic shifts in environmental influences, only a fractional reduction in fecundity creates a situation precarious for the survival of Artemia populations [7].

3. COMPLETE ABSENCE OF OVARIES

The ultimate state of infecundity is exhibited by females lacking functional ovaries. Females in which ovaries failed to develop, or in which ovaries have been destroyed could serve as the optimum type of siphon for sperm from males of an unwanted species.

The parasoid wasp Bracon hebetor is useful in biological control of Lepidopteran pests. As such, fecundity is desired. Our investigations are presented to exemplify extreme cases usually considered undesirable. Typically these are identified by a failure to oviposit despite avid feeding on the host caterpillar. Because of the parthenogenetic production of normal males, mating is not obligatory for oviposition. Upon dissection of this type of female, no ovarioles are evident but all other components of the reproductive system are present. These comprise the spermatheca and spermathecal glands, the lubricating gland, the poison glands and reservoir, the sting and all associated tubes and ducts. The condition can be the final stage of ovariole deterioration following exposure to a potent alkylating agent, or it may be caused by true gene mutation. One such case appeared several years ago in an inbred laboratory line. Easily maintained in the heterozygous condition, it differs morphologically from induced examples by the absence of the groups

of tracheae ordinarily serving the ovarioles. Following the destruction of formed ovarioles, obvious bundles of tracheoles are found close to the distal end of the oviduct. The presence of sperm in the spermathecae have provided evidence that such types attract mates and allow mating.

4. DESTRUCTION OF OVARIOLE CONTENTS

Tissues of the ovariole sheath are insensitive to ionizing radiation. The intact tubes filled with degenerating nurse cells and cytolytic debris characterize the ovaries in Bracon [8], Drosophila [9], and Callitroga [10] within a week after 5000 R or more. Even more comprehensive destruction can result from irradiating pre-imaginal insects. A most impressive example was obtained by X-raying Bracon pupae. Instead of ovarioles, the adult structures were short, horn-like sacs filled with acellular debris [11].

In general, peak vulnerability of the ovariole contents occurs before the imaginal stage. But in some species where internal transformation lags behind external appearance, immature ovaries are still available during the first few days of adulthood (flies) or until special nutritional requirements are met for maturation (mosquitoes). Thus the opportunity for producing females without ovaries exists in so-called adults. Whether formed material is destroyed or whether the development of the ovary is halted is secondary to the main purpose of producing females which lack ovaries. Aminipterin, nitrogen mustard, and colchicine, three quite different types of agents, have accomplished this in houseflies [12] by interfering with growth of the ovary, each agent in its own fashion.

5. A SOLUTION OF THE PROBLEM OF DIFFERENTIATED OOCYTES

Insects which develop fully matured ovarioles before adulthood fall into two main categories:- (1) those in which sufficient oocytes are already differentiated to provide a third or more of the lifetime egg deposit, or (2) those with only a minor portion of the potential eggs differentiated to oocytes. The former appears to pose more of a problem in insect control because differentiated oocytes in certain stages of meiotic prophase are surprisingly radioresistant. A further complication is added by the possibility of parthenogenetic development of the sperm nucleus in heavily irradiated cytoplasm [13]. Once the follicle-enclosed, trophocyte-nourished oocyte is fully developed it tends to produce an ovum despite massive doses of radiation. While radiation alone cannot halt egg production completely it can do so in combination with a carefully selected antimetabolite as demonstrated in the following experiment.

Figure 1 presents the average egg production of 6 samples of 10 female braconids each. Five of the samples ingested a single meal of 6-diazo-5-oxo-L-norleucine (DON) from a dilution series prepared by dissolving 10 mg of DON in 25,50,100,200, and 400 ml of saturated sugar solution. As shown, no eggs were deposited by females fed the two higher concentrations until the fourth and fifth days respectively. Subsequently egg production climbed quickly to control levels.

To determine whether this pattern of oviposition does indeed result from cell destruction, additional samples of 30 wasps were fed the higher concentrations. Of these samples five females were dissected each day. Within 24 hours only diffuse granular debris remained in the egg accumulating region of the ovarioles. Subsequently within 2 to 3 days deterioration of the entire oocyte sequence became evident. Except for the oogonial masses in the blind ends of the tubes, no formed elements persisted within the sheaths.

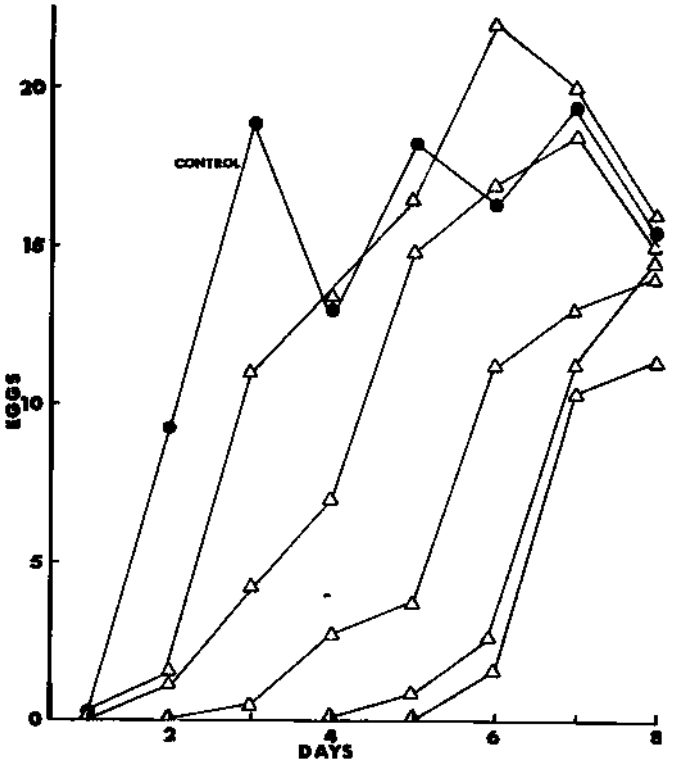


FIG. 1. The average number of eggs deposited per day by samples of braconid wasps (Bracon hebetor Say) fed a single meal from one of a series of 6-diazo-5-oxo-norleucine (DON) solutions prepared in saturated sugar water. The DON solutions ranged from 0.0002 to 0.004%. The most dilute solution decreased egg production for only the first three days. Females fed the most concentrated solution laid no eggs until the 6th day and required three additional days to attain the control level. No significant differences from control values were obtained after the 9th day for any of the samples.

Repopulation of the ovarioles dominated the histological aspect on the fourth and fifth days. A complete polytrophic sequence filled the ovarioles before oviposition began again. The pattern of response to DON is in direct contrast to that observed after delivery of X or gamma rays. After irradiation intra-ovariole debris is slower to appear and it is the oogonia of the distal end of the ovariole which are destroyed [8]. Therefore if completely infecund females are desired, the two agents complement each other exactly. Figure 2 shows what happens when a radiation dose which halts egg production after five days is combined with a DON dose which eliminates egg production for the first five days. A group of females was produced which never laid any eggs. The fecundity of their sisters is shown by the control data plotted as well as by the impressive oviposition of the DON group after the eighth day.

6. REVEALING DIFFERENCES IN CELL TYPE SENSITIVITY

Now we will consider situations in which only part of the ovariole contents are destroyed. A decrease in the total number of eggs deposited by a group of females indicates nothing more than a deleteriousness of the

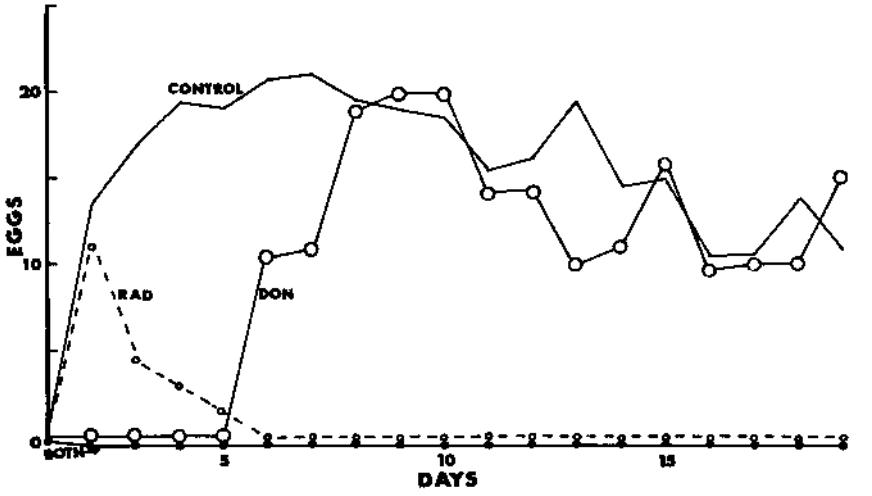


FIG. 2. Average daily egg production for three samples of treated braconids contrasted with control values:- after 6000 R of ^{60}Co gamma rays (designated RAD), after a single meal of 0.004% DON (designated DON), or both treatments (designated BOTH). All treatments were at time zero.

agent used. It may even reflect damage to somatic organ systems rather than destruction of the germ line. Experiments to reveal differences in the responses of the gonad's cell types require egg-production records per unit time. The entire period of time covered by such records must be long enough to include eggs derived from the most primitive type of precursor cell as well as the most highly differentiated kind of oocyte. In addition, the females of the groups compared must possess an equivalent number of functional ovaries, and only fecund females are considered.

LaChance's laboratory applied this approach to Cochliomyia hominivorax [10,14,15], accomplishing the task for a pest in which each ovary contains more than 100 ovarioles, each of which can produce an egg for each egg mass. Perhaps it is the labor more than any other factor which explains a sparse literature. Be that as it may, by sequencing the deposits and correlating them with a cytological study of ovariole contents, LaChance succeeded in determining the comparative radiosensitivity of cell types. At the same time he established the vulnerability of trophocyte endomitosis in the screwworm, a substantiation of earlier reports on Bracon [8]. Furthermore, certain Drosophila data provide additional corroboration when regrouped. Particularly interesting are the egg deposits of the 3000 R experiment by Abrahamson and Herskowitz [16]. When pooled by days instead of shorter periods a decline during the first week becomes obvious for females receiving the acute dose. In contrast fractionated delivery of the same dose has little effect. Unfortunately no one has made the 30 to 40 day study of oviposition rate in treated Drosophila as recommended nearly 40 years ago [17].

In contrast to many other insects considered, Habrobracon's modest egg production from only two synchronized ovarioles per ovary has proved uniquely suitable for analytical experiments. Five distinct types of modification in the wasp's oviposition pattern were identified previously [18]. Briefly these are (I) the family of two-humped curves obtained with low and moderate radiation exposures. The interposed valley attests to the vulnerability of transitional cells in mitosis [19]. Recently similar curves have

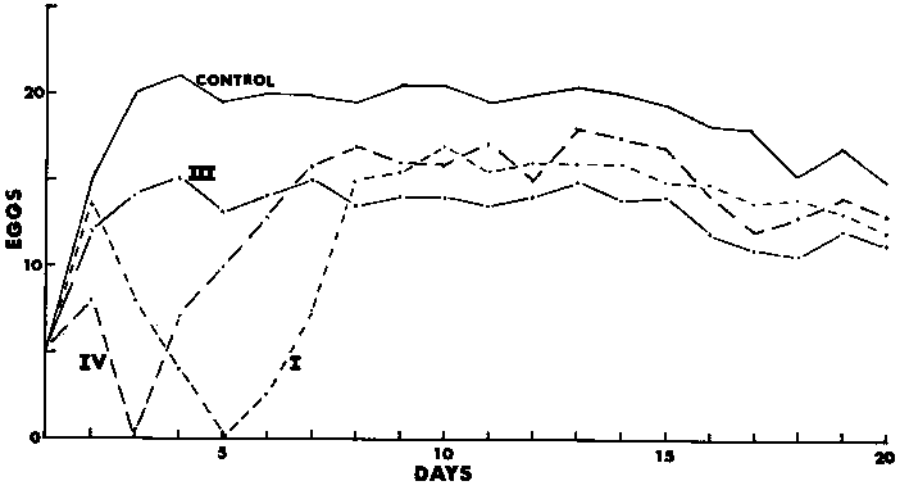


FIG. 3. A comparison of different types of egg production curves which result in 100 egg deficits from control totals. The Roman numerals refer to a classification of curve types presented in the text. For the sake of figure clarity, curve type VI is not drawn here but the DON curve of Fig. 2 can serve as an example.

been obtained with a potent alkylating chemosterilant, apholate [20]. Higher doses of radiation destroy the oogonia. This kind of response, designated II, was shown on Figure 2. A third situation (III), a constant deficit in eggs every day seems to be characteristic of somatic debility. This has been produced by Fourth Period cation poisoning, classic organic enzyme inhibitors, and recently by heptachlor, a chlorinated hydrocarbon insecticide [21]. Completely different organ systems may be attacked by the different kinds of agents. The fourth kind of pattern (IV) is induced by antimetabolites which cause a valley to develop two to three days earlier than a radiation induced low point, i.e. a low by the third day [22]. Still another type of curve (V) has been obtained with agents which temporarily inhibit mitosis [23]. In this case there is a compensatory deposit of eggs which makes up for earlier deficits. The present report of DON destruction of oocytes adds a sixth kind of curve (VI) in which egg production stays at zero for the first five days and then rises to control levels. A lower total number of eggs deposited may be due to any one of at least four kinds of modifications in egg production. Figure 3 demonstrates how a 100 egg deficit might result.

Whereas a plot of the day-to-day average serves to reveal differences in the sensitivity of the cell types by demonstrable peaks and valleys, plotting the cumulative total enables a rate comparison, reflected in the slopes of the lines. Also this kind of plot opens the door to statistical analysis via the methods of linear regression. For Bracon the approach has been helpful in analyzing the reproductive performance of females from Biosatellite experiments [24]. The typical radiation response shows an obvious inflection and lower rate of egg deposit from days 4 to 10; subsequently, the rate returns nearly to control levels. Figure 4 shows data plotted after groups of females were subjected to one of several agents. The response is quite different from that to irradiation. The daily deficit for wasps poisoned with Ni^{++} or with arsenite is reflected in a lower rate of deposit, and hence divergence from the control line. Methotrexate, a folic acid antagonist, induces a very low rate of egg production until after the sixth day, although subsequently in the second week an appreciable slope is apparent.

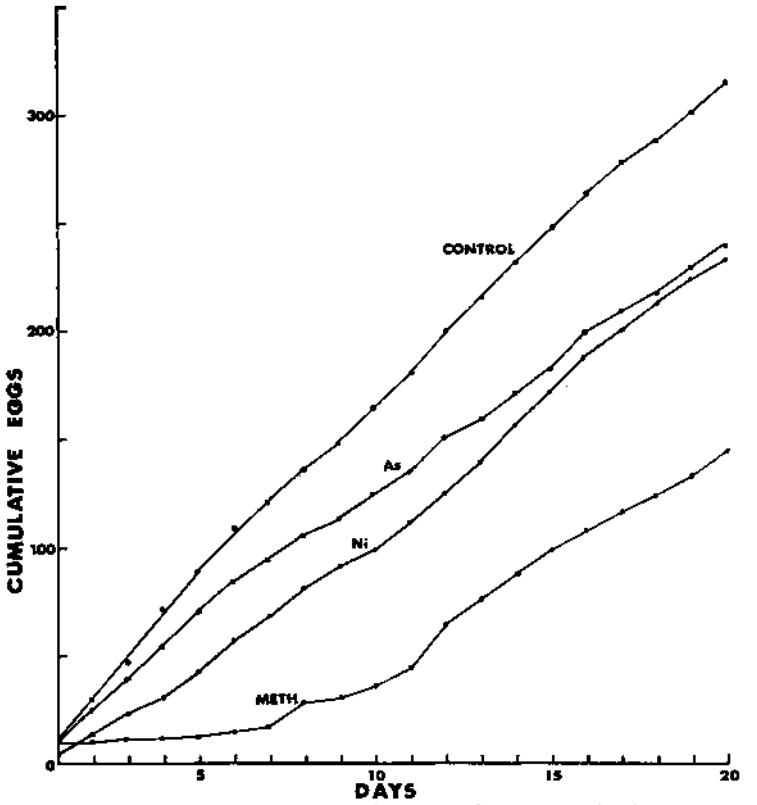


FIG. 4. Cumulative egg production. Each line plots the average for a sample of 20 braconids. "As" designates the results from a single meal of 0.005% sodium arsenite. "Ni" indicates results after an 0.001% $NiSO_4$ feeding. "Meth" identifies results after a 0.07% methotrexate feeding. All solutions were in sugar water presented at time zero.

TABLE I. THE PERCENTAGE OF EGGS WHICH HATCHED AFTER DEPOSIT BY BRACON FEMALES FED CERTAIN CHEMICAL AGENTS OR IRRADIATED

Cell Type From Which Derived:	Oocytes	Transitional	Oogonia	Oogonia (Senility)
Deposited on Days:	1 - 5	6 - 10	11 - 15	16 - 20
Control	98.1	95.6	83.4	78.5
DON 0.004%	-	91.5	89.9	87.7
$NaAsO_2$ 0.005%	91.2	93.8	89.3	78.5
$NiSO_4$ 0.001%	68.2	81.5	89.7	82.5
2000 R Co-60	75.3	67.6	80.1	58.9

7. CORROBORATION FROM HATCHABILITY AND STAGE OF DEATH

Information on egg hatchability and the stage achieved before death of the embryo is important for establishing an agent's mode of action. Most significant as an indication of inducers of chromosome aberration and gross nuclear damage is poor hatchability in which a majority of embryos died in what Von Borstel's laboratory has called Stage 1 Death [25].

Not every chemical agent which interferes with egg production induces damage in genetic mechanisms. As evidence of this, Table 1 presents hatchability summarized by five day periods to correspond to the cell type in the ovaries at the time of treatment. This recognizes that eggs destined to be deposited on a particular day were in one of the precursory stages on the initial day of an experiment.

An additional category, Days 16-20, is distinguished on the basis of age of the mothers. B. hebetor females typically enter their senile decline on the 15th day, as is reflected in the decreased hatchability of control groups of eggs.

Neither DON nor $NaAsO_2$ lowered hatchability impressively. An initial low hatchability, such as that produced by Ni^{++} seems characteristic of toxic fourth period cations (unpublished data), evidently due to their incorporation into the oocytes. For contrast, data from a radiation experiment is shown. Egg hatchability is impressively lower throughout the reproductive period of the irradiated females.

A further difference in mode of action is revealed when a classification of the stages of death is considered. After 2000 R of Co-60 gamma rays a majority (68%) of the embryos were classifiable as Stage 1 Deaths. On the other hand, this type of embryonic death was not obtained as a response to the chemical agents of Table 1. The rare cases seen did not even amount to control proportions of 6% during the first five days. With maternal aging this decreased to 2% for the senile period. In other words, except in the radiation experiment, embryo deaths occurred late in development. Stage 1 Death results from an inability to accomplish cleavage. Instead of a cleared egg periphery representing blastoderm formation, Stage 1 Death is a non-homogeneous, cloudy white moribund state. As the most frequent kind of radiation-induced dominant lethality, something equivalent to the braconid example presumably characterizes the zygotic deaths inherent in the screwworm "sterility" approach. Bracon merely provides exceptionally suitable material for detecting deviations from the normal course of development. The thin chorion is so transparent that a microscopist can see exactly what is happening inside an egg without recourse to microtechnique.

8. RESPONSE TO THE INHIBITION OF DNA SYNTHESIS

More subtle responses in cell type sensitivity than those yielding deficits of 100 eggs or more can be detected by the careful sequencing of egg production. An extremely important matter in our understanding of the operation of the polytrophic ovariole is the time of DNA synthesis.

Beginning with tests of FUDR by Dr. Roger H. Smith (M.S. Thesis North Carolina State University, 1962), experiments subjecting braconid females to a variety of DNA inhibitors have been performed. Several herbicides including propanil seemed ineffective, as well as the antibiotic novobiocin. Perhaps some of these compounds need to be reconsidered from the perspective of recent experiments with hydroxyurea.

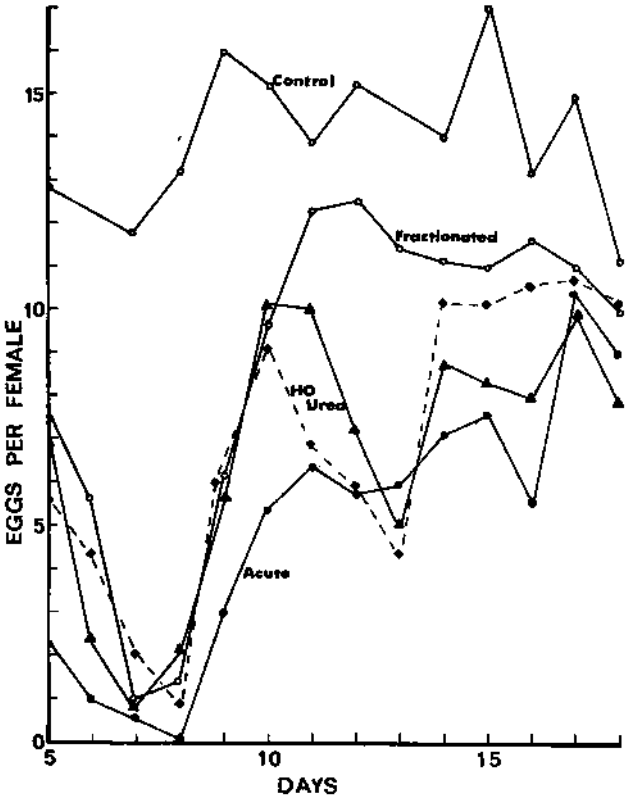


FIG. 5. Contrasts between the daily average number of eggs produced by three groups of adult Habrobracon females after exposure to two 2500-R doses of gamma rays separated by 4 hours. Two of the groups were injected with hydroxyurea: 0.1 M designated by squares, 0.01 M designated by triangles. For comparison, results are plotted after the acute dose of 5000 R delivered in two minutes. Untreated control data are also shown.

Hydroxyurea is the currently preferred means of synchronizing mammalian tissue cultures because it kills cells in S phase (26). Its cytocidal effectiveness has been demonstrated in vivo on neoplasms and on a wide variety of normal cell types including bacteria, protozoa, plant roots, invertebrate and vertebrate embryos, and mammalian bone marrow (27). Now cell-destruction experiments with Habrobracon have identified a particular group of cells in the ovariole germarium which are sensitive to hydroxyurea.

The most elucidative type of experiment employed two radiation exposures separated by a four-hour period, during which an injection had assured presence of hydroxyurea in the female abdomen. Cs 137 provided the gamma rays. Figure 5 shows how similar oviposition by two injected groups is to that of an uninjected group until the tenth day after the fractionated dose of radiation. Then hydroxyurea caused a sharp drop. On the 13th day fewer eggs were deposited than by wasps given the acute dose of 5000 R in two minutes. Hydroxyurea alone produces a slight decrease from the control level of egg production between 12 and 14 days after injection. The more pronounced effect in the traditional test for radiation recovery suggests that hydroxyurea either interferes with chromosomal rejoining mechanisms or promotes the formation of actual lesions at provisional sites of damage.

9. APPLICABILITY STATEMENT

The primary purpose here has been to contribute to our understanding of the insect's reproductive physiology. Nevertheless, in addition to eliminating the necessity of sexing pupae before release, as has been the standard procedure in manipulating screwworm populations, alternative uses of treated females suggest themselves. The incorporation of infecundity genes, as well as other cytogenetic features in complex stocks derived for insect control could enhance the effectiveness of the method, provided appearance of the defect is devised for subsequent generations. This is the delayed or "time-bomb" approach. Quite different in concept is the direct killing of the "booby-trap" technique, wherein infecund females from a pesticide resistant strain are coated with an agent which kills susceptible males during mating attempts (2). The ingenuity of genetically trained entomologists may be expected to provide additional applications.

10. SUMMARY AND CONCLUSIONS

After discussing the significance of female fecundity in survival and extinction situations, extreme types of infecundity were described, as well as methods for the destruction of the ovariole contents. Mere decrease in numbers of eggs deposited is completely inadequate for making a decision concerning the mode of action of a tested chemical agent. For a rational understanding of an agent's potency and limitations there is a dearth of information. Data is needed on sequenced egg deposits and the effective lethal phase in the embryos. Events even as subtle as the S period preceding the mitotic activity of the cystoblast can be revealed. This area of biology is relatively unexploited both from the standpoint of investigation and application.

ACKNOWLEDGMENT

Support for studying the mode of action of chemical agents upon reproductive performance is provided by U.S.P.H.S. grant ES-00044, Division of Environmental Engineering and Food Protection.

REFERENCES

[1] VON BORSTEL, R. C., BUZZATI-TRAVERSO, A. A., I.A.E.A. Symp. Radioisotopes and Radiation in Entomology, Vienna, 1962, p. 276.

[2] WHITTEN, M. Concepts of Pest Management, N. C. State Univ. Conference, (1970) 119.

[3] CHAMBERLAIN, W. F., J. Econ. Ent. 55 (1962) 240.

[4] WALLACE, B., DOBZHANSKY, Th., Radiation Genes and Man, Holt, Rinehart & Winston, N. Y. (1959).

[5] WALLACE, B., Statistical Genetics and Plant Breeding. NAS-NRC 982 (1963) 3.

[6] CLEMENTS, A. N., The Physiology of Mosquitoes, MacMillan Co., N. Y. (1963)

[7] GROSCH, D. S., Biol. Bull. 131 (1966) 261.

[8] GROSCH, D. S., SULLIVAN, R. L., Radiation Research 1 (1954) 294.

[9] KING, R. C., Exp. Cell Research 13 (1957) 545.

[10] LACHANCE, L. E., Int. J. Rad. Biol. 7 (1963) 321.

[11] ERDMAN, H. E., Int. J. Rad. Biol. 3 (1961) 183.

[12] MITLIN, N., BUTT, B. A., SHORTINO, T. J., Physiol. Zool. 30 (1957) 133.

[13] WHITING, A. R., Radiation Research 2 (1955) 71.

[14] LACHANCE, L. E., LEVERICH, A. P., Genetics 47 (1962) 721.

[15] LACHANCE, L. E., BRUNS, S. B., Biol. Bull. 124 (1963) 65.

[16] ABRAHAMSON, S. and HERSKOWITZ, I. H., Genetics 42 (1957) 405.

[17] SHAPIRO, H., Biol. Bull. 63 (1932) 456.

[18] GROSCH, D. S., Proc. XI Int. Cong. Genetics I (1963) 73.

[19] GROSCH, D. S., Bios. 34 (1965) 55.

[20] VALCOVIC, L. R., GROSCH, D. S., J. Econ. Ent. 61 (1968) 1514.

[21] GROSCH, D. S., J. Econ. Ent. 63 (1970) 1349.

[22] GROSCH, D. S., Science 141 (1963) 732.

[23] GROSCH, D. S., Annals Ent. Soc. America 52 (1959) 294.

[24] GROSCH, D. S., Mutation Research 9 (1970) 91.

[25] VON BORSTEL, R. C., REKEMEYER, M. L., Genetics 44 (1959) 1053.

[26] SINCLAIR, W. K., Science 150 (1965) 1729.

[27] THURMAN, W. G., (Ed.) Symposium on Hydroxyurea. Cancer Chemotherapy Rep. 40(1964) 75 pp.

DISCUSSION

L. E. LaCHANCE: Would you care to comment on the mode of action of DON on mature oocytes?

D. S. GROSCH: Our first concern was to make sure that the large, highly-differentiated oocytes were indeed induced to degenerate. Our observations on oosorption are given in the text of the paper. Since synthesis and the incorporation of stored materials is practically complete, it is difficult to see how an antimetabolite could have any direct influence on the structure. Indirect mechanisms, including hormonal controls, should be investigated. Furthermore, it should be noted that these were feeding experiments, for which partial starvation is a necessary preliminary, and one that interferes with any accumulation of stored metaphase eggs.

R. PAL: Some of the work carried out by Soviet research workers in connection with the development of resistance to insecticides has shown

that DDT affects oogenesis in house flies. Perhaps Mr. Grosch would like to comment on these findings.

D. S. GROSCH: The literature on this subject appears to fall into two categories. Firstly, there are observations on populations subjected to DDT for more than one generation, where the very powerful genetic influence of selection has had an opportunity to act. Secondly, there is information on the exposure of flies with immature ovaries. In this case DDT acts upon a developmental situation which depends upon the functioning of the somatic metabolism. Dr. Beard of the Connecticut Experiment Station (USA) has published some observations on this matter.

The decreased average egg production shown in the figures reflects poor performance by poisoned females. The somatic fitness of the mother is easily influenced by pesticide.

CHEMOSTERILANT EFFECTS ON THE LARVAL POSTEMBRYONIC DEVELOPMENT OF THE STABLE FLY, Stomoxys calcitrans (L.)

J. S. BADMIN
Imperial College Field Station,
Ashurst Lodge, Sunninghill, Berks,
United Kingdom

Abstract

CHEMOSTERILANT EFFECTS ON THE LARVAL POSTEMBRYONIC DEVELOPMENT OF THE STABLE FLY, Stomoxys calcitrans (L.).

Histological aspects of the action of tepa on the postembryonic developmental system of the stable fly have been investigated.

Larvae were more resistant than adults to the toxic effects of tepa altough their sensitivity varied with age, older individuals being less affected. Treated larvae pupated later than controls and over longer periods and these pupae were often partially tanned or malformed.

The larval anatomy of Stomoxys calcitrans (L.) is described with special reference to the organization of the major developmental discs. An overall reduction in the size of these primordia was observed following topical treatment of the larvae, this effect lessening with later application.

Some of the factors influencing normal growth and differentiation of the eye-antennal discs are reported in detail. The four main effects of chemosterilant damage to this disc are: (1) A reduction in the rate of anlagen growth; (2) Abnormal differential growth of the disc; (3) I and II instar treated larvae emerged as eyeless and aristapedia mutants; (4) Late III instar treated larvae emerged as normal adults with defective eyes. This last damage was confined to a vertical band of abnormally arranged facets on the cornea. Successively later treatments with tepa caused this pattern of damage to extend anteriorly across the eye. Factors which may influence this process, including possible hormonal control, are discussed.

INTRODUCTION

Population control by the release of partially or completely sterile insects has received an increasing amount of attention during recent years following the successful eradication of the screw-worm, Cochliomyia hominivorax (Coquerel) from the West Indian island of Curaçao and from the United States of America. The major characteristics of chemosterilants which have absorbed the interests of workers in this field are the induction of sterility in the adult male and the inhibition of ovarian development in the female. A survey of the literature shows that little attention has been devoted to their action on other stages of development.

Many compounds which are effective adult sterilants have revealed low sterilizing, toxic properties when applied to larvae of the house-fly, Musca domestica L. [1] and the screw-worm fly C. hominivorax (Coquerel) [2].

In the few investigations that have been reported, the following types of cellular damage caused by chemosterilant applied to the larval stage have been observed: delayed induction of sterility and interference with gonadial development in Culex pipiens quinquefasciatus Say by apholate [3]; induction of chromosomal aberrations, including fragmentation and stickiness [4], and delayed breakdown of nerve fibres in the adult brain and degeneration

of the mid gut epithelium in Aedes aegypti (L.) by apholate [5]; a lower rate of chemosterilant breakdown than that exhibited by adult Culex tarsalis Coquillet [6] and interference with postembryonic development of the codling moth Carpocapsa pomonella (L.) by tepa [7].

An advantage of studying the effects of chemosterilants on the larval stage is that many developmental systems can be observed simultaneously in the one insect. In Diptera a large proportion of the external features of the adult arise from the embryonic hypoderm as discrete groups of actively dividing cells and continue their development as imaginal discs throughout the remaining larval period.

In the present study several effects of tepa (tris (1-aziridinyl) phosphine oxide) on postembryonic development of the eye-antennal disc of the stable fly, Stomoxys calcitrans (L.), are described. For comparison, complete sterility of the adult fly has been achieved by topical application of 3.7 μg tepa [8].

TECHNIQUES

Eggs of S. calcitrans deposited over a one-hour period were transferred to dishes containing the culture medium (50 larvae per 50 g food) and maintained at 27°C, 70% r.h. Hopkins' method [9] of culture was followed.

Under these conditions, with variations in hatching time and variable rates of larval development, both the first and second instar lasted approximately 24 hours and the entire larval period covered 10 - 12 days.

Under experimental conditions 0.5 μl of tepa : acetone solution from a micro drop applicator were applied topically to individual larvae of the required age, which were then returned to the dishes after a holding period of 10 min. Owing to the small size of both first and second instars it was found easier to mix a measured amount of aqueous tepa solution, ranging from 0.1 - 100 ppm, into the culture medium than to apply it topically.

TOXICITY

Larval and pupal LD_{50}s for tepa treatments applied during each instar of S. calcitrans are represented in Table I. The results show an increasing tolerance during the early stages of larval development from 6 μg (first instar), 9 μg (second instar), to 34.5 μg midway through the final larval stadium. The remaining period of larval growth up to the time of pupation shows a decline in tolerance to 17 μg.

Between 0.5 and 100 μg tepa, duration of the larval period was extended in direct proportion to the dose applied, as shown by Fig. 1. Further evidence for a decline in tolerance before pupation was given by measurements of the amount of chemosterilant required to extend the larval period by 50%. Mid-third instar larvae required 7.1 μg tepa, while a similar delay in pupation of a later stage of this instar needed 1.15 μg.

In all these experiments there were considerable numbers of partially tanned or mis-shapen puparia especially at high doses. The frequency of occurrence of these distorted specimens also increased directly with the amount of chemosterilant used.

TABLE I. LD_{50} OF Stomoxys DURING SUCCESSIVE LARVAL INSTARS EXPRESSED IN μg, AFTER TOPICAL TREATMENT WITH TEPA

Stage of application	Larval mortality	Pupal mortality
Instar I	2 - 5.85	1 - 2.5
II	3 - 9.1	1 - 3.0
III day 1	15	0.19
day 3	34.5	3.3
day 5	15.1	2.17
day 7	17.3	1.37

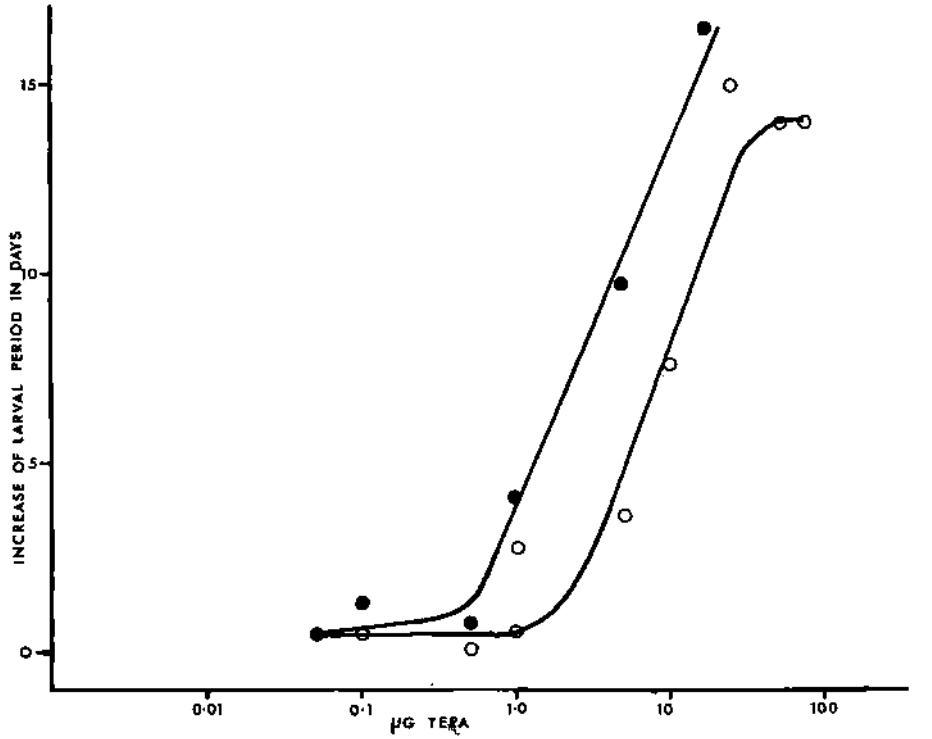


FIG. 1. Mean increase in the larval period of Stomoxys calcitrans (L.) after topical application of tepa.
○ applied on day 5
● applied on day 11

DEVELOPMENT OF THE CEPHALIC DISC

Overall growth of the cephalic or frontal disc during postembryonic development was studied by measuring the relative increase in area of dissected discs under a stereoscopic microscope with an ocular micrometer. Usually five specimens were measured from each of three replicates at any one dose.

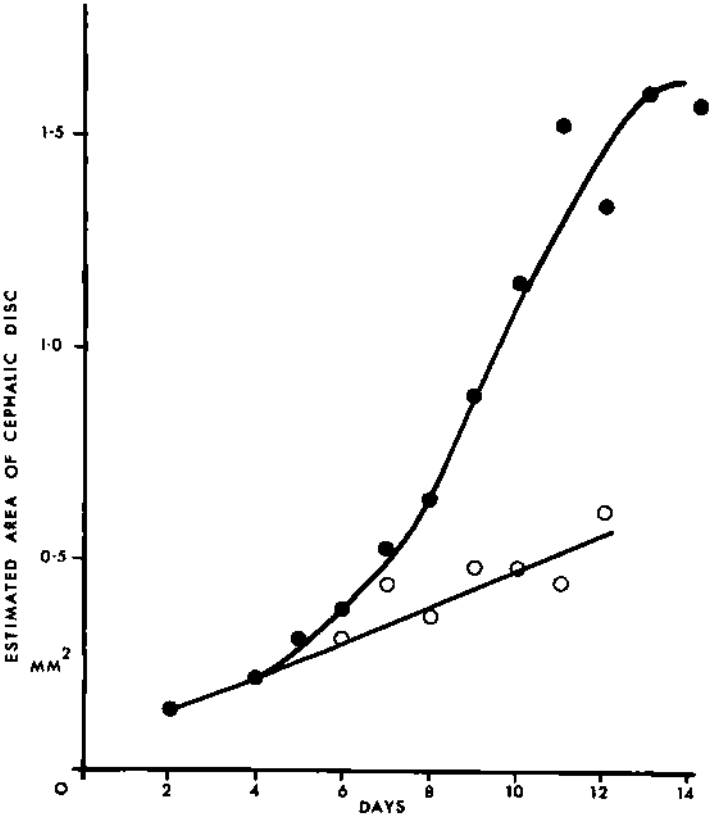


FIG. 2. Increase in relative cross-sectional area of the cephalic disc during larval life, each point representing the mean of five measurements. Disc area was calculated from the product of the diameters of the two major axes.
● control
○ 10 μg

On hatching, the rudiments of the cephalic disc in S. calcitrans are well established. Growth is slow at first, but during the second instar the disc begins to alter shape into a small proximal and broad distal section and these areas subsequently differentiate into the antennal and eye regions respectively. Development within the final larval instar is characterized by an accelerating growth phase and the initiation of ommatidial differentiation.

Topical application of 10 μg tepa at the beginning of the third instar resulted in a considerable suppression of growth as shown by Fig. 2. Up to the time of pupation in the control dishes, growth of the treated discs was slow but otherwise apparently normal. Later, especially after first and second instar treatments, imaginal discs developed enlarged antennal or optic regions which may have been capable of further differentiation. Above 10 μg tepa the frontal disc failed to differentiate correctly, its rigid structure collapsed and a darkened mass of cells appeared in its centre. Sections of this material revealed severe destruction and disorientation of the imaginal cells.

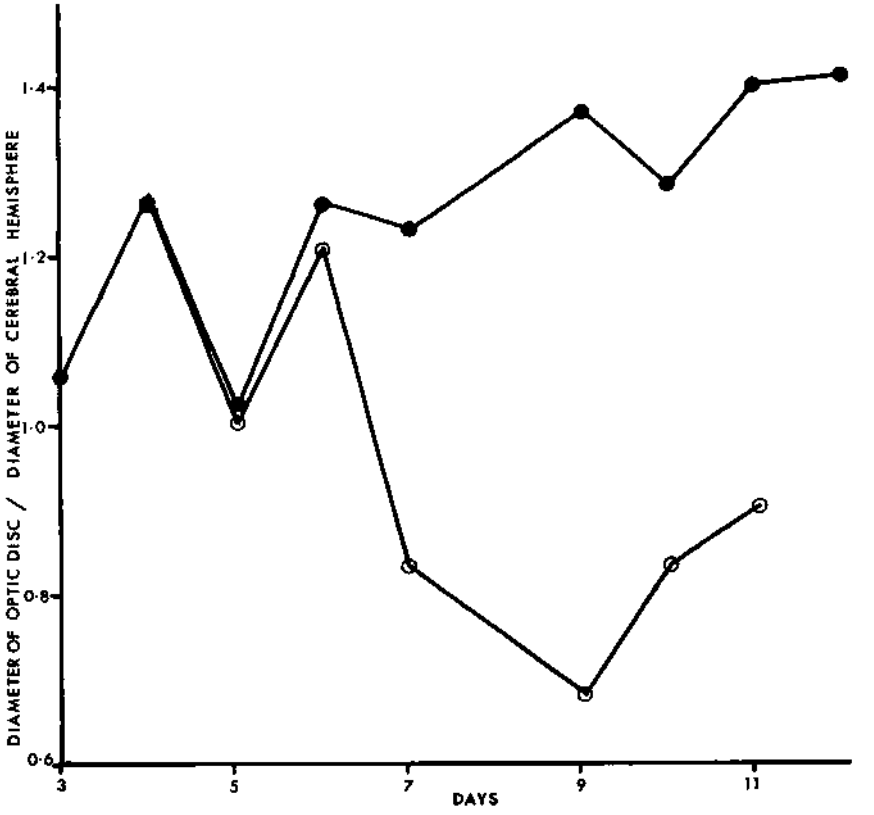


FIG. 3. Effect of tepa on the relationship between diameters of the larval brain and its associated eye disc during the third instar.
● control
○ 10 μg

Although the decrease in diameter of the cerebral lobe was small in comparison with the marked changes which occurred in the growth of the cephalic disc, the changes in the relative sizes of the two resulted in the optic anlagen being stretched across the surface of the brain (Fig. 3). The structural collapse of the frontal disc and its failure to differentiate may be a consequence of the direct action of tepa, or a combined effect of direct action coupled with the external stresses caused by differential growth of the surrounding tissues.

SENSITIVITY TO CHEMOSTERILANT

The sensitivity of the frontal disc in Stomoxys as measured by the amount of subsequent growth following chemosterilant treatment is depicted in Fig. 4. A median inhibitory dose of 1.58 μg during the third instar indicates an organ which is extremely sensitive to tepa. The effect of tepa

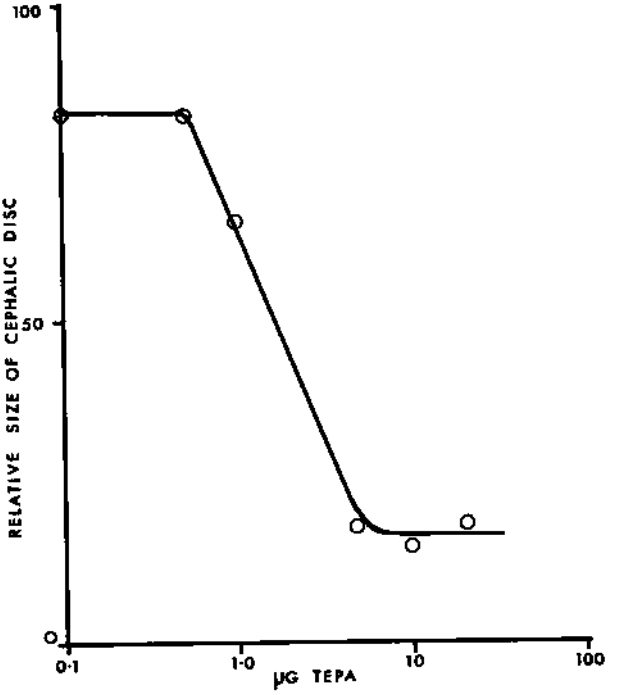


FIG. 4. Effect of tepa applied at the beginning of the third instar on the size of the cephalic disc. Each point represents the mean of ten measurements.

on the size of the disc during different stages of the final instar is depicted in Fig. 5. The results emphasize an increase in the final disc area following successively later applications and suggest a decline in sensitivity.

ADULTS DERIVED FROM I AND II INSTAR EXPERIMENTS

As development proceeds, so individual cells of an imaginal disc become progressively more determined into their prospective roles. By applying tepa to different stages of this process of determination one can vary the morphology of the resulting adult.

With first and second instar treatments, although considerable mortality occurred at doses greater than 10 ppm (Table II), survivors often emerged with mis-shapen antennae or reduced eyes. The exact transformation of prospective antennal material into leg material noted by Bodenstein and Abdel-Malek [10] in Drosophila using nitrogen mustard was not observed in tepa-treated Stomoxys. Variations in the relative size of the arista and its parent segment, enlargement of the entire antennal complex and the occurrence of leg segments were recorded. Antennal mutants were usually able to emerge from the pupal case while insects with damaged compound eyes normally failed and required dissection.

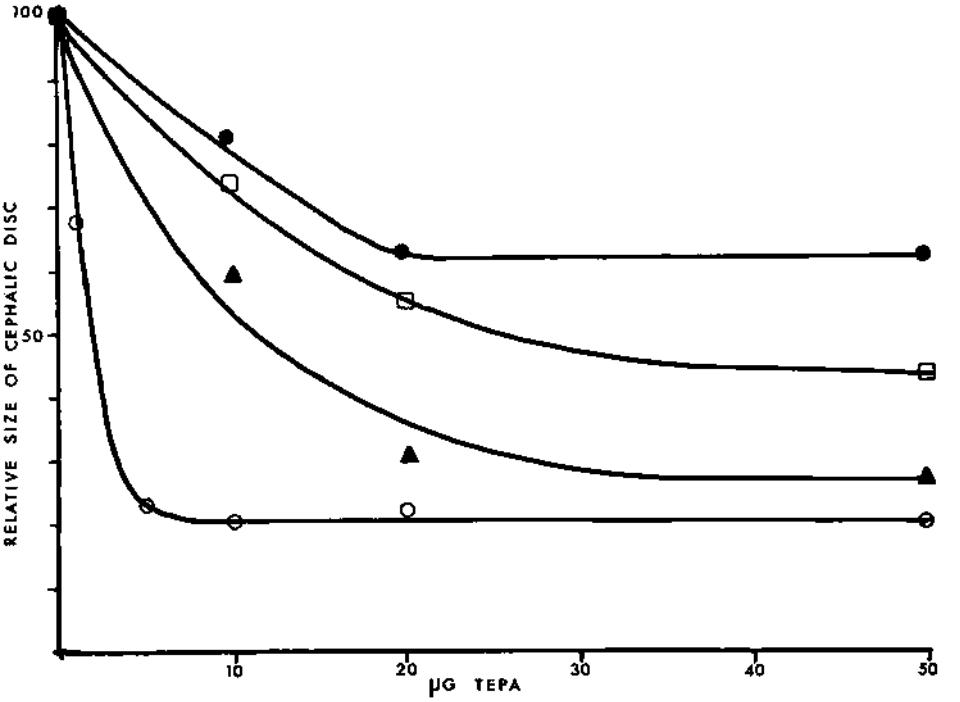


FIG. 5. Effect of tepa applied at different stages during the final instar on the size of the cephalic disc. Each point represents the mean of ten measurements.
○ day 4
▲ day 7
□ day 9
● day 11

Loss of one or both compound eyes entirely reduced the size of the adult head and also displaced the position of the ptilinum above the antennae. As the function of the ptilinum is to thrust off the anterior end of the puparium when the insect is ready to emerge, any interference with this process can easily prevent eclosion. The high pupal mortality induced by tepa may then be explained at least in part by a failure of the insects to emerge following incomplete development of the ptilinum.

ADULTS DERIVED FROM III INSTAR EXPERIMENTS

Adults which emerged from these experiments have so far not included somatic mutants of the types described above. However, the basic structure of the compound eye of this insect was damaged after topical application of 5 - 10 µg tepa during the latter half of the final instar. Treatments did not alter the shape or size of the eye as damage was confined to a vertical band of abnormally constructed facets on the cornea (Fig. 6).

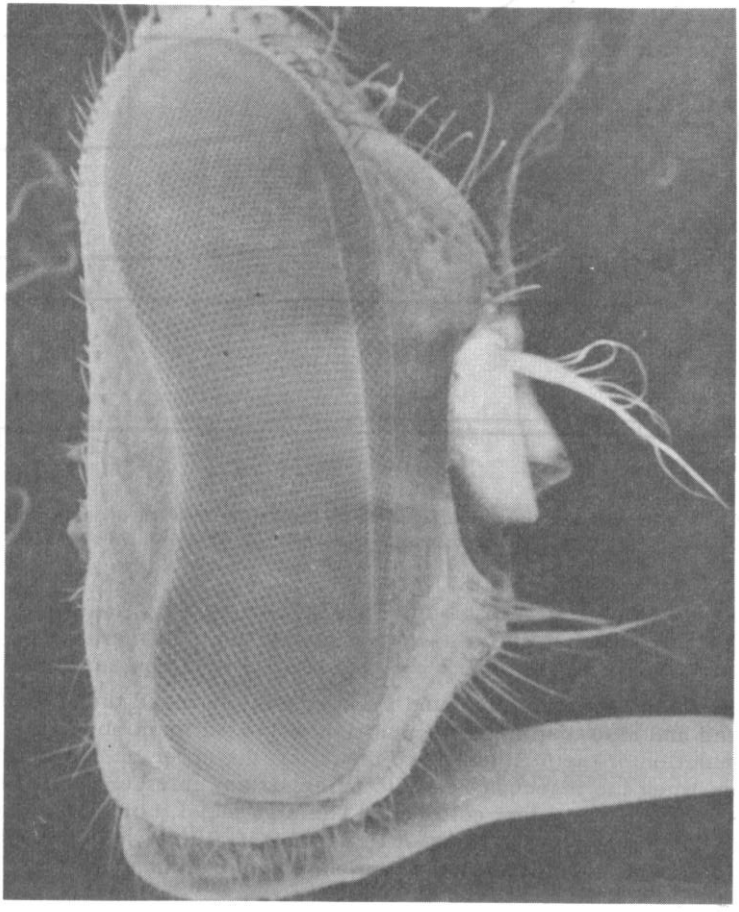
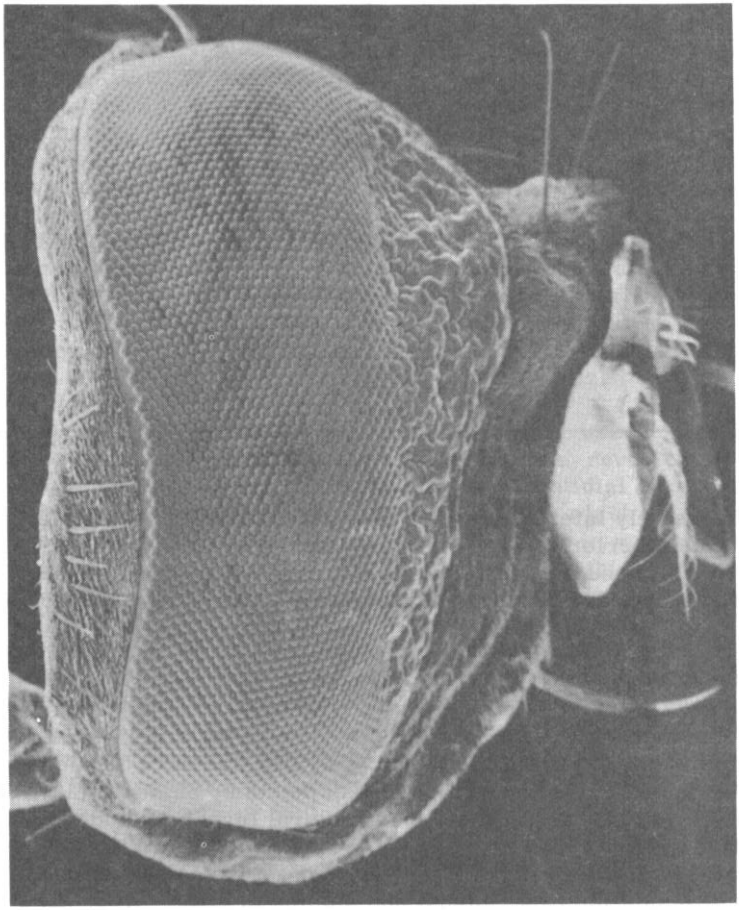


FIG. 6. Lateral views of the compound eye of Stomoxys calcitrans (L.)

(a) Compound eye of an untreated insect showing the regular arrangement of corneal facets × 52



(b) Compound eye of an insect treated with chemosterilant just before pupation showing a vertical band of disorientated facets × 71

TABLE II. FREQUENCY OF SOMATIC ABERRATIONS IN S. calcitrans EXPRESSED AS PERCENTAGES AFTER TREATMENT WITH TEPA IN FIRST AND SECOND INSTARS. (A) EMERGED ADULTS. (B) FORMED ADULTS WHICH FAILED TO EMERGE FROM PUPAE

	A			B			
Dose (ppm)	% Normal	Affected head region		% Normal	Affected head region		Partial differentiation
		Antenna	Compound eye		Antenna	Compound eye	
	First instar			First instar			
Control	100	-	-	100	-	-	-
5	56.4	18.0	43.6	25	-	-	75
10	-	44.4	100	30	10	65.0	35
20	-	-	-	-	-	-	100
	Second instar			Second instar			
Control	100	-	-	100	-	-	-
5	60.7	3.6	42.9	33.3	-	16.6	50
10	18.2	40.9	68.2	-	28.6	64.3	28.6
20	-	-	-	-	62.0	66.0	34.0

Successively later treatments caused the location of this disrupted area to move anteriorly across the eye's surface. In a damaged region uniformly arranged hexagonal facets were replaced first by irregular and then fused facets and finally by complete destruction of the cuticular pattern.

DISCUSSION

The morphological changes which occur during differentiation of the adult Drosophila eye from the larval imaginal disc have been described from the histological and cytological viewpoints by Shatoury [11], Shatoury and Waddington [12] and at the ultrastructural level by Waddington and Perry [13].

In addition Schneider [14] and Gehring [15] have described this process from transplantation experiments and studies in vitro. Recently Fristom and Mitchell [16] have outlined a method for isolating large numbers of imaginal discs from Drosophila larvae, allowing a detailed analysis of the biochemical aspects of differentiation.

The effects of 5-fluorouracil [15], actinomycin D [17], mitomycin C [18], colchicine [19] and nitrogen mustard [10] on development of the compound eye in Drosophila have been described. The authors concluded that compounds known to have a marked effect on cell division disrupt both growth and differentiation of the eye disc. Recently Fristom and Knowles [20] provided fairly conclusive evidence that actinomycin D inhibited protein synthesis in differentiating imaginal discs of third instar Drosophila larvae.

The general similarities between the alkylating mechanisms of aziridines and nitrogen mustards are well documented [21]. The similarity between the major developmental transformation of the arista in tepa-treated Stomoxys and nitrogen-mustard-treated Drosophila also points to a common mode of action. Minor differences in the degree of expression of aristapedia between the two species may be due to the differential responses of the antennal anlagen rather than to differences in the action of the two compounds.

The mosaic nature and regulative capacity of imaginal organs and the cephalic disc in particular have been discussed by Gehring [15]. During the first and second larval instars of the stable fly, cells of the frontal disc become generally determined to form the adult head but local determination into the optic, ptilinal or antennal regions is not yet fully established. Consequently during this period the relative proportions of the prospective eye or antennal regions in the frontal disc can be altered. Imaginal discs which have been dissected from larvae with disproportionately enlarged antennal regions can presumably be correlated with the fully expressed state of aristapedia in the adult.

In the frontal disc of the final instar further differentiation into groups of retinular and cone cells of the ommatidia can begin once the size and position of the compound eye have been determined. The final stages of this differentiation which appear to develop and move in a posterior-anterior direction across the eye's surface can be disorganized by tepa. The results also suggest that the chemosterilant is rapidly metabolized as it exerts its action over a very limited period.

In Aedes aegypti (L.), another dipterous species, use of micro-cauterization techniques instead of chemicals [22] has revealed the passage of a factor capable of inducing cell division and ommatidial differentiation in a posterior-anterior direction. Similar processes which begin in the posterodorsal region and spread across the eye anlage in an anteroventral direction in Ephestia [23] and Bombyx [24] suggest that this pattern of differentiation is a general feature of the eye development in many holometabolous insects.

Two possibilities of chemosterilant action may explain the marked suppression of growth observed in imaginal tissue after treatment. Several independent investigations have shown that apholate and other chemosterilants inhibit the production of DNA in insect eggs, including those of Stomoxys [25], so that it is highly probable that tepa, a closely related aziridine, will act in a similar manner. Thus the suppression of growth at any stage of imaginal disc development could be explained by the interaction of tepa upon DNA or its formation and its indirect effects on subsequent protein synthesis. A possible analogy is the inhibitory action of actinomycin D on protein synthesis of differentiating imaginal cells of Drosophila.

At a different level the amount of growth and differentiation of developing imaginal organs is known to be controlled by a complex balance of hormones which are released by the larval ring gland. Any interference with the normal sequence of synthesis, storage and differential release of these hormones can cause major changes in insect development. The possibility of apholate applied during the larval stage inducing hormonal imbalance in adult A. aegypti has been discussed by Sharma and Rai [5].

They concluded that although damage to the nerve fibres and possibly neurosecretory cells of the brain had occurred, there was either considerable repair of the brain tissue, or neurosecretory cells continued to function

correctly but at suboptimal levels. Presumably if any major functional changes in the hormonal output had occurred the majority would have exerted their effects during the larval stage or at metamorphosis.

Observations of the effects of tepa on the neurosecretory cells of the ring gland during larval development of the stable fly have been made and will be described elsewhere. Impairment of the neurosecretory cells has been recorded and these observations support the hypothesis that tepa may influence the insects' hormonal balance.

ACKNOWLEDGEMENTS

The author wishes to thank Dr. C. T. Lewis for his assistance supervising this work and Dr. A. B. Borhovec, of the U. S. Department of Agriculture, for the supply of chemosterilants.

The project was supported by a research grant from the U. K. Ministry of Overseas Development.

REFERENCES

[1] LaBRECQUE, G. C., et al., J. econ. Ent. 53 (1960) 802.
[2] CHAMBERLAIN, W. F., J. econ. Ent. 55 (1962) 240.
[3] MURRAY, W. S., BICKLEY, W. E., University of Maryland, Agric. Expt. Station Bull. A-134 (1964).
[4] RAI, K. S., Cytologia 29 (1964) 346.
[5] SHARMA, V. P., RAI, K. S., Mosq. News 29 (1969) 9.
[6] PLAPP, F. W., et al., J. econ. Ent. 55 (1962) 607.
[7] HATHAWAY, D. O., et al., J. econ. Ent. 59 (1966) 851.
[8] HARRIS, R. L., J. econ. Ent. 55 (1962) 882.
[9] HOPKINS, B. A., M. Sc. Thesis Univ. London (1966).
[10] BODENSTEIN, D., ABDEL-MALEK, A., J. exp. Zool. III (1949) 95.
[11] SHATOURY, H. H. El, J. Embryol. exp. Morph. 4 (1956) 240.
[12] SHATOURY, H. H. El, WADDINGTON, C. H., J. Embryol. exp. Morphol. 5 (1957) 122.
[13] WADDINGTON, C. H., PERRY, M. M., Proc. R. Soc. Lond. (B) 153 (1960) 155.
[14] SCHNEIDER, I., J. exp. Zool. 156 (1964) 91.
[15] GEHRING, W., J. Embryol. exp. Morphol. 15 (1966) 77.
[16] FRISTOM, J. W., MITCHELL, H. K., J. Cell Biol. 27 (1965) 445.
[17] WADDINGTON, C. H., ROBERTSON, E., Nature, Lond. 221 (1969) 933.
[18] HIROSE, Y., KAJI, S., Experimentia 25 (1969) 199.
[19] VOGT, M., Experimentia 3 (1947) 156.
[20] FRISTOM, J. W., KNOWLES, B. B., Exp. Cell Res. 47 (1967) 97.
[21] ROSS, W. C. J., Biological Alkylating Agents, Butterworths, London (1962).
[22] WHITE, R. H., J. exp. Zool. 148 (1961) 223.
[23] IMBERSKI, R. B., J. exp. Zool. 166 (1967) 151.
[24] WOLSKY, A., Exp. Cell Res., Suppl. 1 (1949) 549.
[25] CHAMBERLAIN, W. F., BARRETT, C. C., Nature, Lond. 218 (1968) 471.

DISCUSSION

E. D. OFFORI: Do you think it is worth pursuing research on the performance of adult Stomoxys calcitrans having deformed compound eyes?

J. S. BADMIN: No, I don't think so. The induction of sterility in the adult stage of this insect is still the most promising area of study.

LA CHIMIOSTERILISATION DES ADULTES DE Dacus oleae (GMELIN) PAR LE TEPA Différences entre les deux sexes

E. FYTIZAS
Institut phytopathologique Benaki,
Kiphissia,
Athènes, Grèce

Abstract — Résumé

CHEMOSTERILIZATION OF ADULT Dacus oleae (GMELIN) WITH TEPA: DIFFERENCE BETWEEN THE SEXES.

The author attempts to compare the data obtained thus far in a summary study of the genital system of Dacus oleae (Gmelin) with those obtained during chemosterilization tests with tepa on this insect. The differences in the effect of tepa on the ovaries and testicles seems to be related to the difference in the genital systems of the two sexes. In his conclusion, the author expresses the view that chemosterilization of Dacus males raises serious problems, whilst that of the Dacus female is less complicated.

LA CHIMIOSTERILISATION DES ADULTES DE Dacus oleae (GMELIN) PAR LE TEPA – DIFFERENCES ENTRE LES DEUX SEXES.

L'auteur tente de rapprocher les données jusqu'ici fournies par une étude sommaire du système génital de Dacus oleae (Gmelin) à celles obtenues au cours des essais de chimiostérilisation de cet insecte par le tepa. Les différences quant à l'effet du tepa sur les ovaires et les testicules semblent être liées aux différences entre le système génital des deux sexes. L'auteur conclut des résultats obtenus que la chimiostérilisation des Dacus mâles pose des problèmes sérieux, tandis que celle des Dacus femelles semble moins compliquée.

INTRODUCTION

Au cours des expériences entreprises dès la fin de 1966 dans notre laboratoire sur les différents effets du tepa sur les adultes de Dacus oleae, nous nous sommes aperçus que les insectes avaient un comportement différent envers le chimiostérilisant, non seulement par rapport à d'autres insectes mais aussi entre eux (selon leur sexe); ainsi les problèmes de la chimiostérilisation ne paraissaient pas être les mêmes pour les adultes mâles et femelles. Nous avons alors émis l'hypothèse que ces différences pourraient être en rapport avec leur cycle génital, jusqu'alors mal connu; c'est pourquoi nous avons entrepris, tout en poursuivant nos essais de chimiostérilisation, un examen sommaire de leur système génital. L'évolution des oocytes dans les ovaires, l'évolution de l'œuf après la ponte (et jusqu'à l'éclosion des œufs) et les caryocinèses dans les testicules ont été étudiées par différentes techniques histologiques.

Dans ce travail, nous avons tenté de rapprocher les données obtenues jusqu'ici concernant l'évolution du système génital des deux sexes de celles obtenues au cours de l'étude des effets du tepa. L'examen du système génital est toujours en cours et la plupart des données que nous rapportons n'ont pas encore été publiées.

RESULTATS

Au cours de l'étude du système génital nous avons obtenu quelques résultats que nous résumons ci-dessous.

Cycle génital des femelles

La différentiation de quelques oogonies s'effectue pendant la sortie des adultes [9] et quelquefois à la fin du stade nymphal.

La croissance des oocytes dure au moins quatre jours pour les insectes issus de l'élevage artificiel, et sept jours pour les insectes «sauvages»; la première moitié de ce temps constitue la période de provitellogénèse et la seconde la période de vitellogénèse et de la formation du chorion [9]; pendant son séjour dans l'ovaire (de la formation de la vésicule germinale jusqu'au passage à travers l'oviducte) l'œuf possède un noyau en repos (membrane nucléaire et nucléole constamment présents).

Après la ponte ont lieu les deux divisions de maturation (5 à 15 min), la fécondation de l'ovule (environ 30 min), les divisions du zygote (45 min à 5 h) et l'organogénèse (23 h à 70 h) (Fytizas, travail non publié).

La différentiation des oogonies et l'évolution des oocytes sont continues et surviennent pendant toute la vie adulte des femelles; dans le même ovaire, l'évolution de chaque ovariole est indépendante de celle des autres ovarioles [9].

Cycle génital des mâles

Baccetti et Bairati [1] décrivent la spermatogénèse, qui s'achève chez Dacus oleae avant la sortie des adultes; ce stock de spermatozoïdes s'épuise avant ou après la seconde spermatogénèse, qui survient 7 à 10 j après la sortie des adultes, et de nouvelles spermatogénèses suivent (une spermatogénèse tous les 6 à 10 j et d'une durée de 3 j) (Fytizas, travail non publié). Après destruction des spermatogonies et des spermatocytes par des rayons gamma [11] ou des substances chimiques [6], les testicules de l'insecte traité sont vidés ou presque au bout de quelques éjaculations successives. D'après le premier des auteurs le stock initial de spermatozoïdes s'épuise entre le 5^e et 11^e accouplement, et d'après le second, dès la 6^e éjaculation on ne trouve dans les spermathèques des femelles fécondées qu'un nombre extrêmement faible de spermatozoïdes, nombre qui d'ailleurs diminue d'un accouplement à l'autre et tombe à zéro pour la plupart des individus entre le 9^e et le 11^e accouplement. Les mêmes auteurs [7,11], quoique travaillant séparément, ont pu constater que la vigueur sexuelle des Dacus mâles est très élevée: les individus mâles peuvent s'accoupler une fois par jour, et pendant au moins un mois.

A l'encontre de ce qui se passe chez les femelles, les spermatogénèses, qui s'effectuent par étapes, sont synchrones pour une population de mâles donnée; en outre, la caryocinèse est aussi synchrone dans toutes les spermatocystes du même ordre et du même testicule (Fytizas, travail non publié).

Relation entre gamétogénèse et éjaculation des spermatozoïdes et la ponte

En bloquant l'un des deux mécanismes, nous avons pu constater que le mécanisme de la ponte est indépendant de celui de l'ovogénèse [5] et que celui de la spermatogénèse est indépendant de celui de l'éjaculation [6].

Le rythme de la formation des œufs et celui de la ponte suivent des courbes quasi parallèles. Chez les mâles, aucune relation n'existe entre le rythme de la spermatogénèse et celui des éjaculations.

Parmi les différences apparentes que nous avons pu jusqu'à présent observer entre les deux sexes, résumons celles que nous considérons comme importantes:

- le degré d'évolution des gamétocytes avant que ceux-ci quittent l'organe génital; chez les femelles ce sont des oocytes de premier ordre immatures, tandis que chez les mâles les spermatozoïdes ont atteint leur forme finale;
- le rythme de l'évolution des gamétocytes: évolution continue et successive des oocytes dans chaque ovariole, évolution par étapes des spermatocytes et caryocinèse synchrone pour les spermatocystes du même ordre et dans le même testicule;
- parallélisme des courbes de la formation des œufs et de la ponte, absence de relation entre le rythme de la spermatogénèse et les éjaculations.

DISCUSSION ET CONCLUSIONS

Les informations que nous avons obtenues jusqu'ici permettraient d'expliquer en partie les différences apparues lors des essais de chimiostérilisation des adultes de Dacus oleae des deux sexes:

– Le tepa, administré pendant les premiers jours qui suivaient la sortie des adultes et à différentes doses, induisait la stérilité totale seulement chez les femelles; les mâles avaient pendant un temps un certain nombre de spermatozoïdes fertiles, leur stérilité complète s'établissant progressivement, au fur et à mesure que les accouplements devenaient plus nombreux. Une explication de cette différence pourrait être donnée par le degré d'évolution différent des gamétocytes des deux sexes au moment de l'administration du chimiostérilisant; chez les femelles les gamétocytes sont soit à l'état d'oogonies, soit à celui d'oocytes de premier ordre, gamètes en évolution n'ayant pas pris leur forme définitive et subissant plus tard des transformations importantes (divisions de maturation); chez les mâles, au contraire, les premiers jours et pendant un certain temps, les testicules possèdent des spermatozoïdes complètement formés, gamètes très ou complètement résistantes.

– Quel que soit le temps de l'intervention, les œufs d'une femelle traitée pondus deux heures après l'administration sont stériles [5]; chez les mâles, l'administration du tepa, même à dose élevée, n'assure pas toujours la stérilité (au moins pour les premiers jours qui suivent le traitement), les résultats dépendant de l'âge de l'insecte. Dans le cas des mâles traités, le fait que la formation des spermatozoïdes est achevée avant le traitement pourrait être la cause de cet échec partiel; le chimiostérilisant ne serait efficace que pour les spermatocytes et les spermatogonies, ceux qui formeraient plus tard les spermatozoïdes stériles.

– Les différentes zones de gamétocytes dans les ovaires et les testicules présentent une différence quant à la sensibilité envers l'action stérilisante du tepa. Cette différence est bien nette chez les ovaires: les œufs déjà formés sont les plus résistants, puis suivent les oogonies et les oocytes n'ayant pas encore achevé la vitellogénèse. La différence de sensibilité entre les oocytes en croissance et les oogonies nous paraît

réelle [5], mais la différence entre oocytes en voie de croissance et œufs formés nous paraît assez obscure: le noyau de l'oocyte ne paraît pas subir des modifications appréciables tant que l'oocyte ne quitte pas l'ovaire. Des changements ont bien sûr lieu surtout en ce qui concerne le volume, les constituants chimiques du protoplasme et les parois de l'oocyte. Peut-être sont-ce les parois et la quantité de chimiostérilisant pouvant les traverser qui pourraient fournir une explication: avant et pendant une bonne partie de la vitellogénèse, les cellules folliculaires faciliteraient peut-être la pénétration du tepa, transporté par l'hémolymphe; à la fin de la vitellogénèse, le rôle de ces cellules disparaîtrait avec elles et le chorion formé créerait une barrière (rôle similaire à celui du puparium empêchant la pénétration de substances comme le tepa) ne laissant au tepa qu'un passage étroit, le micropyle. Si cette supposition se révélait exacte, la différence porterait plutôt sur la quantité pouvant pénétrer dans l'oocyte. En ce qui concerne les mâles, la différence de sensibilité entre les spermatozoïdes et les autres gamétocytes est marquée. Nous avons signalé lors d'une expérience [6] qu'une différence de sensibilité existait entre les spermatocytes et les spermatogonies, ces dernières étant plus sensibles. Pourtant, des essais ultérieurs ont prouvé le contraire: des examens ont montré que dans le premier cas avait lieu une différentiation des spermatogonies, tandis que dans le second cas nous observions des caryocinèses chez les spermatocystes. On peut donc supposer que l'état des noyaux (au repos, en préparation pour la cinèse et en cinèse) plutôt que l'ordre des spermatocytes ou des spermatogonies, jouerait un rôle prépondérant.

Que la sensibilité des différents éléments des testicules change, nous l'avons aussi constaté lorsque le tepa a été administré à des mâles d'âges différents [7], âges correspondant à peu près à un jour avant le commencement de la seconde spermatogénèse et au commencement de celle-ci respectivement. La précision de la méthode utilisée alors laisse à désirer, étant donné que les insectes pouvaient recevoir le tepa, contenu dans leur nourriture, à n'importe quelle heure du jour de l'intervention, alors que chez d'autres insectes on a prouvé que la sensibilité varie selon l'heure du traitement [2].

– Une autre différence encore apparaît, qui concerne la durée de la stérilité induite par le tepa. La stérilité chez les femelles peut être provoquée par une concentration de tepa élevée et par une administration unique; les dégâts que pourrait provoquer cette forte dose priverait la femelle aussi bien d'une descendance viable que des œufs stériles qu'elle aurait pu déposer. Mais pour les Dacus mâles, les doses fortes doivent être évitées, puisque les effets cytotoxiques sur les testicules ne peuvent que nuire à la chimiostérilisation [8] et doivent être classés parmi les effets secondaires. D'ailleurs [7], la durée de la stérilité chez les testicules n'a été satisfaisante que lorsque la dose (administrée en une seule fois) était proche des doses cytotoxiques. Nous avons obtenu les meilleurs résultats en administrant le tepa à doses faibles, soit tous les 8 j et pendant 2 mois, soit tous les 2 ou 3 j et pendant 10 ou 12 j; dans le premier cas, nous avons tenté de faire coïncider les jours du traitement avec ceux où des caryocinèses marquaient la nouvelle spermatogénèse; dans le second cas, les traitements répétés à des intervalles de 2 ou 3 j auraient pour effet, d'après Bacoyannis, une accumulation des effets génétiques.

En conclusion nous pouvons constater que les problèmes de la chimiostérilisation se posent différemment pour les Dacus mâles et femelles.

En premier lieu, le problème du rapport entre les doses et l'efficacité stérilisante ne se pose vraiement que pour les mâles. La stérilité permanente chez les femelles peut être induite par une seule administration de tepa à une concentration affectant les gamétocytes les plus résistants tout en étant inférieure aux doses létales; l'utilisation d'une telle dose n'entraînerait pas de conséquences fâcheuses car le facteur de sécurité est plus que satisfaisant [3]. Mais en ce qui concerne les Dacus mâles, non seulement nous devons éviter les doses qui pourraient provoquer des effets cytotoxiques ou autres effets secondaires (réduction de la vigueur sexuelle, réduction du nombre de spermatozoïdes et inactivation des spermatozoïdes), mais il serait encore souhaitable de déterminer les facteurs de sécurité entre les doses induisant la stérilité et celles induisant un des effets secondaires; la polygamie des mâles exige pour les insectes traités non seulement le maintien de la vigueur sexuelle au niveau des insectes normaux, mais aussi la sauvegarde des spermatogénèses qui suivraient le traitement.

En second lieu, la notion de «moment judicieux» pour l'intervention a un sens différent selon qu'on se réfère à l'un ou l'autre sexe. Quel que soit l'âge des femelles, la présence dans le même ovaire de gamètes à tous les stades possibles d'évolution exclut l'existence d'un temps d'intervention particulièrement favorable. Pourtant, pour la pratique agricole, nous admettons que ce problème peut présenter un certain intérêt: l'administration de tepa avant la formation des premiers œufs aboutirait à une suppression de la fécondité [4] et celle qui suivrait la formation des œufs serait d'autant plus efficace que le nombre des œufs pondus jusqu'à 2 h avant l'administration serait plus faible [5]. Le problème se pose tout autrement dans le cas des mâles: le fait que les spermatogénèses s'effectuent par étapes et les caryocinèses sont synchrones dans les spermatocystes du même ordre nous permettrait une intervention à un moment déterminé et une détermination de zones de sensibilité ainsi que du changement que subit celle-ci. Pourtant, ce problème se complique par le fait que le changement de sensibilité ne concerne pas la totalité des gamétocytes comme c'est le cas chez d'autres insectes, par exemple les femelles de Cochliomyia hominivorax [10]. Le problème dans le cas des Dacus mâles consiste à suivre l'évolution des différents gamétocytes et à déterminer les différentes doses et les différents moments d'intervention convenables afin d'affecter la matière génétique de tous les gamétocytes.

Le cycle génital de l'insecte étudié déterminerait dans un sens la méthode de stérilisation à suivre, et là réside l'importance qu'il acquiert dans le domaine de la chimiostérilisation. Les insectes des deux sexes de la même espèce, comme c'est le cas pour Dacus oleae, n'ont pas obligatoirement le même cycle génital; on peut constater des différences appréciables entre femelles du même ordre, comme par exemple celles de Dacus oleae, de Musca domestica et de Cochliomyia hominivorax; on peut, enfin, trouver quelques ressemblances entre insectes appartenant à une espèce et à un sexe différents, comme c'est le cas pour les mâles de Dacus oleae et les femelles de Musca domestica. Le classement taxonomique des insectes aurait pour l'étude de la stérilisation une importance quelque peu limitée. Mais il serait fort souhaitable de classer les insectes

auxquels pourrait être appliquée la chimiostérilisation d'après leur cycle génital; l'interprétation des expériences de différents chercheurs sur différents insectes serait plus large et la comparaison des données expérimentales plus claire et plus facile.

REFERENCES

[1] BACCETTI, B., BAIRATI, A., Indagini comparative sull'ultrastruttura delle cellule germinali maschili in Dacus oleae Gmel. ed in Drosophila melanogaster Meig. (Ins. Diptera), Redia 49 (1964) 1-31.

[2] CRYSTAL, M.M., in ASCHER, K.R.S., «Approaches to insect control», III[e] Congrès int. des antiparasitaires, Wld. Rev. Pest Control 8 4 (1969) 166.

[3] FYTIZAS, E., Action du Tepa sur les adultes de Dacus oleae Gmel. (Diptera: Tephritidae), en fonction des quantités du chimiostérilisant utilisées, Annls Inst. phytopath. Benaki 8 1 (1967) 32-46.

[4] FYTIZAS, E., Influence de quelques facteurs sur l'action stérilisante du Tepa sur les femelles de Dacus oleae Gmel. (Diptera: Tephritidae), Meded. Rijksfac. LandbWetensch. Gent 32 3 (1967) 717-25.

[5] FYTIZAS, E., BACOYANNIS, A., Action du Tepa sur des femelles de Dacus oleae Gmel., sexuellement mûres, Meded. Rijksfac. LandbWetensch. Gent 33 3 (1968) 917-25.

[6] FYTIZAS, E., Effets cytotoxiques du Tepa sur les mâles de Dacus oleae, Meded. Rijksfac. LandbWetensch. Gent 34 3 (1969) 637-42.

[7] FYTIZAS, E., BACOYANNIS, A., in ASCHER, K.R.S., «Approaches to insect control», III[e] Congrès int. antiparasitaires, Wld. Rev. Pest Control 8 4 (1969) 166.

[8] FYTIZAS, E., BACOYANNIS, A., Effets stérilisants et effets secondaires du Tepa sur les adultes de Dacus oleae Gmel., Paper presented at the 8th FAO Meeting on the Control of the Olive Pests, Athens, 8-12 May 1969 (Preprint).

[9] FYTIZAS, E., BACOYANNIS, A., Evolution des ovaires chez les femelles de Dacus oleae Gmel., Annls Inst. phytopath. Benaki (sous presse).

[10] LACHANCE, L.E., CRYSTAL, M.M., Induction of dominant lethal mutations in insect oocytes and sperm by gamma rays and an alkylating agent: Dose-response and joint action studies, Genetics 51 (1965) 699-708.

[11] TZANAKAKIS, M.E., Experiments with the Olive Fruit Fly at the «Democritus» Nuclear Research Center, Greece, Paper presented at the 8th FAO Meeting on the Control of the Olive Pests, Athens, 8-12 May 1969 (Preprint).

DISCUSSION

M.E. TZANAKAKIS: On the basis of your findings, do you consider tepa to be an ineffective means of sterilizing the olive fruit fly in practice?

E. FYTIZAS: On the contrary, I think that tepa offers considerable scope for the study of chemosterilization in view of the high safety factor of about 50. The effects of various doses can be studied without the risk of causing death.

M.E. TZANAKAKIS: I should like to put another question if I may. Does your statement that the initial sperm stock is exhausted after the first 7-10 days mean that it is exhausted after a certain number of matings by males mating daily?

E. FYTIZAS: I agree with you that exhaustion of the initial stock of spermatozoa occurs after a certain number of matings, whatever the interval between two successive matings; the time at which exhaustion of the initial stock occurs is thus in fact independent of the age of the male.

EFECTOS DEL TEPA EN TRES ESPECIES DE TRIATOMINAE

R.U. CARCAVALLO, C.A. CARABAJAL
Dirección de Epidemiología de la Subsecretaría de Salud Pública de la Provincia de Buenos Aires, Buenos Aires, Argentina

Abstract — Resumen

EFFECTS OF TEPA ON THREE SPECIES OF TRIATOMINAE.

The authors carried out sterilization experiments on three species of Triatominae, using the compound tepa. The species were Triatoma infestans, Triatoma guasayana and Triatoma patagonica. Tepa was applied externally to the specimens (100 of each species) in the fifth nymphal and adult stages by techniques which are described in detail in the paper.

The three species proved sensitive to the treatment, the most highly affected being Triatoma patagonica and the second most highly affected Triatoma guasayana. The best results were obtained by external treatment of the nymphs, which resulted in a male sterility of 88% in the case of Triatoma patagonica, 82% in that of Triatoma guasayana and 78% in that of Triatoma infestans. The treatment of adults was less effective, resulting in corresponding sterility percentages of 70%, 64% and 56% respectively.

No recovery of fertility was observed in these laboratory experiments. In view of the promising results observed, semi-field tests are currently being carried out. The development of an effective attractant is necessary, however, as the species studied do not appear to be attracted by tepa, unlike Rhodnius prolixus.

EFECTOS DEL TEPA EN TRES ESPECIES DE TRIATOMINAE.

Los autores realizaron experiencias de esterilización de tres especies de Triatominae con el compuesto tepa. Las especies fueron Triatoma infestans, Triatoma guasayana y Triatoma patagonica. Los ejemplares (cien de cada especie) fueron topicados en los estadios de ninfa V y adultos, mediante las técnicas que se detallan en el trabajo in extenso.

Las tres especies resultaron sensibles al tratamiento, siendo la más afectada T. patagonica, y en segundo lugar T. guasayana. Los mejores resultados se obtuvieron topicando las ninfas, lográndose porcentajes de esterilidad en machos de 88% en T. patagonica, 82% en T. guasayana y 78% en T. infestans. La topicación en adultos tuvo una eficacia menor, lográndose porcentajes de esterilidad en las especies y en el orden mencionados del 70, 64 y 56% respectivamente.

No se observó recuperación de la fertilidad en estas experiencias de laboratorio. En la actualidad se están realizando pruebas de semicampo, dado que los resultados observados son promisorios. Sin embargo, es necesario desarrollar un atrayente adecuado, dado que las especies estudiadas no demostraron ser atraídas por el tepa, mostrando en este sentido un comportamiento distinto al de Rhodnius prolixus.

Con el propósito de probar las posibilidades del empleo de quimioesterilizantes en el control de los Triatominae, hemos ensayado algunos de ellos con tres especies del género Triatoma Laporte: T. infestans (Klug), T. patagonica Del Ponte y T. guasayana Wygodzinsky y Abalos. La primera es el más importante vector de la enfermedad de Chagas en el sur de Sudamérica y las otras dos son las especies peridomésticas más importantes.

Las sustancias empleadas hasta el momento fueron tepa, metepa, apholate y ticurea, siendo la primera la que hemos estudiado más completamente y de la que exponemos aquí los resultados.

MATERIAL Y METODOS

Cien ejemplares de cada una de las especies fueron separados de sus colonias en el estadio de ninfa IV. La mitad de ellos fueron tratados con el producto a los tres días de la ecdisis de la ninfa V y los restantes, tres

CUADRO I. PROMEDIOS DE HUEVOS POR HEMBRA, PORCENTAJE DE HUEVOS VIABLES Y TIEMPO DE INCUBACIÓN EN TRES ESPECIES DE Triatoma

	N° de huevos por hembra	% de huevos viables	Tiempo de incubación
T. infestans	240	84%	29
T. guasayana	245	84%	28
T. patagonica	169	85%	24

días después de la ecdisis del adulto. Previamente, y fuera de este lote, se habían inyectado veinte ejemplares recién eclosionados de adultos, en la dosis de 0, 05 ml de una solución de tepa de 5 mg/ml para verificar si no perjudicaba la vida de los insectos. También se hicieron topicaciones con una solución del producto al 3% para comprobar efectos esterilizantes.

En la experiencia que describimos se empleó la topicación en el abdomen de una solución de tepa al 0, 4‰, pH 8, 2, lográndose la alcalinidad mediante el agregado de bicarbonato de sodio. La topicación se realizó en ejemplares anestesiados con éter. La cantidad de tepa aplicada en esta forma fué de aproximadamente 0, 1 mg, ya que se empleó 0, 25 ml de solución en la topicación completa, en cara ventral y dorsal.

Quince días después de la aplicación en los adultos, éstos fueron apareados individualmente con ejemplares del otro sexo no tratados. Los ejemplares tratados en estadio de ninfa V fueron apareados 22 días después de producida la ecdisis a adultos.

Cada pareja fué mantenida en las condiciones generales del criadero, habiéndose elegido igual número de especímenes de idéntico origen y edad como grupo testigo. Las observaciones en cada lote se efectuaron diariamente.

CRITERIOS SOBRE ALGUNOS ASPECTOS

Hemos considerado estéril la hembra incapaz de desovar huevos viables después de copular con un macho no tratado. Con igual criterio, el macho que no fecunda a la hembra no tratada fué considerado estéril.

El número de huevos desovados no ha constituido un criterio de evaluación por cuanto es un dato muy variable según los especímenes. Como dato de interés damos el cuadro I.

Un dato que consideramos importante es la posible modificación de la expectativa de vida de los adultos. En estudios anteriores hemos establecido para estas especies que las hembras adultas tienen una longevidad de 138 días T. infestans, 118 T. guasayana y 96 T. patagonica, mientras que los adultos machos, en el mismo orden, tienen una longevidad de 142, 111 y 94 días, respectivamente.

CUADRO II. PORCENTAJES DE ESTERILIDAD EN TRIATOMINAE TRATADOS CON TEPA Y EN TESTIGOS

		T. infestans		T. patagonica		T. guasayana	
		Tratados	Testigos	Tratados	Testigos	Tratados	Testigos
Machos	Adulto	56%	12%	70%	16%	64%	9%
	Ninfa	78%	12%	88%	16%	82%	9%
Hembras	Adulto	62%	16%	67%	8%	64%	11%
	Ninfa	64%	16%	69%	8%	65%	11%

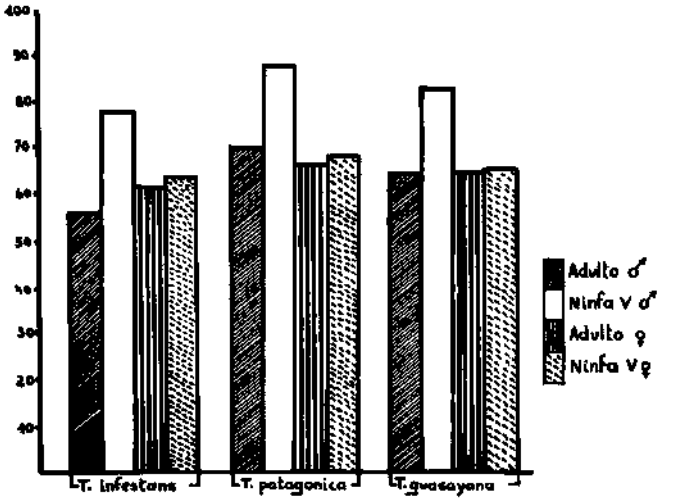


FIG. 1. Porcentajes de esterilidad logrados en tres especies del género Triatoma con aplicaciones de tepa, según sexo y estadio de aplicación.

RESULTADOS

Los resultados se exponen en el cuadro II.

DISCUSION

Si bien los resultados obtenidos demuestran una acción esterilizante del tepa sobre las tres especies estudiadas, no se logró en ningún caso la esterilidad en el 100% de los especímenes, al menos en las dosis empleadas (figura 1).

La acción del tepa parece manifestarse en períodos avanzados de la ovogénesis y espermatogénesis, siendo más sensibles los órganos sexuales masculinos.

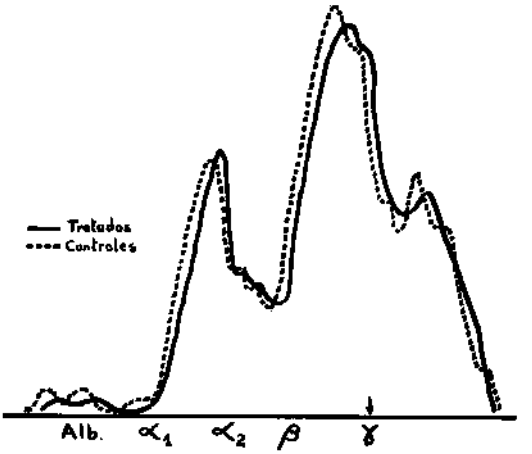


FIG. 2. Leves diferencias en la composición proteica de la linfa, en ejemplares tratados y no tratodos (densitometría de corridas electroforéticas).

El no haberse observado recuperación de la fertilidad es un elemento importante, y aunque no se efectuó estudio histológico sistemático, el daño no parece reversible, o si lo es, tiene una lentitud de recuperación que se aproxima al promedio de expectativa de vida del adulto.

El hecho de no haberse observado diferencias significativas en la longevidad de los adultos permite afirmar que el tepa no resulta perjudicial para otros procesos biológicos del insecto estudiado.

En estudios electroforéticos de la linfa de las tres especies no se observaron diferencias significativas de la calidad y cantidad de las proteínas con respecto a las normales que hemos descrito en un trabajo anterior (figura 2).

CONCLUSIONES

Las tres especies resultaron sensibles al tratamiento con tepa, siendo la más afectada T. patagonica y en segundo lugar T. guasayana. Los mejores resultados se obtuvieron topicando las ninfas, lográndose porcentajes de esterilidad en machos de 88% en T. patagonica, 82% en T. guasayana y 78% en T. infestans. La topicación en adultos tuvo una eficacia menor, lográndose porcentajes de esterilidad en las especies y en el orden mencionado del 70, 64 y 56%, respectivamente.

No se observó recuperación de la fertilidad en estas experiencias de laboratorio. En la actualidad se están realizando experiencias de semicampo. Sin embargo, es necesario desarrollar un atrayente adecuado, dado que las especies estudiadas no son atraídas por el tepa, mostrando en este sentido un comportamiento distinto al de R. prolixus con respecto a otro quimioesterilizante.

BIBLIOGRAFIA

BEROZA, M., BORKOVEK, A.B., The stability of tepa and other aziridine chemosterilants, J. med. Chem. 7 (1964) 44-49.

BORKOVEK, A.B., CHANG, S.C., LIMBURG, A.M., Effect of pH on sterilizing activity of tepa and metepa in male houseflies, J. econ. Ent. 57 (1964) 815-817.

BURDEN, G.S., SMITTLE, B.J., Chemosterilant studies with the German cockroach, Fla. Entom. 46 3 (1963) 229-234.

D'ALESSANDRO, G., BRUNO-SMIRAGLIA, C., LAVAGNINO, A., Saggi di Chemosterilizazione con tepa su A. labranchiae, Stratto dalla Rivista di Malar 45 (1966) 1-8.

HEDING, P.A., WIYGUL, G., VICKERS, D.A., BARTLETT, A.C., MITLIN, N., Sterilizing effect of the chemosterilant tepa on the male boll weevil with respect to dosage, permanency of sterility, gonadal changes and its biological turnover, J. econ. Ent. 60 (1966) 209-214.

WEIDHAAS, D.E., FORD, H.R., GAHAN, J.B., SMITH, C.N., « Preliminary observations on the chemosterilization of mosquitoes », Proc. 48th Ann. Mtng. N.J. Mosq. Ass. (1961) 106-109.

DISCUSSION

A. ECONOMOPOULOS: How do you explain the fact that in your work less sterility was induced when adults were treated?

R. U. CARCAVALLO: I think the reason is that tepa acts on less differentiated cells and also, according to the results of recent studies, on interstitial cells.

D. G. CAMPION: Why did you not use higher doses of tepa to obtain complete sterility?

R. U. CARCAVALLO: We were conducting a comparative study with tepa, metepa, apholate and thiourea, and adopted a standard dose equal to the effective amount of the most active of these four chemosterilants (thiourea).

One facet that I am unable to explain is why some individuals appear to be more sensitive than others to certain chemosterilants. Some remain sterile, while others recover. Even at the dose limit, behaviour has varied widely from one individual to another. I wonder whether anyone here has an explanation for this?

D. S. GROSCH: One suggestion is that insects may differ in their ability to repair lesions induced by chemical agents. In other words, because of differences in genetic make-up, individuals may or may not possess such an ability. A recent thesis by A. C. Hoffman, of my laboratory, demonstrates this for a different agent.

L. E. LaCHANCE: With regard to the variability in sterility from male to male encountered in studies on Triatoma infestans, we found very little variability in thousands of treated male house flies either at moderate or high doses of tepa. I would therefore disagree with the explanation just presented. One should first eliminate variation in the dose to each male and the possible use of a few non-virgin females.

D. S. GROSCH: Mr. LaChance is convinced that biological material is variable, a principle to which I subscribe!

J. C. GÓMEZ-NÚÑEZ: With respect to variations in the response to apparently equal doses of chemosterilants by individual triatomids, it seems advisable to consider the possibility of human error in the dosage before searching for complex biological reasons.

F. M. WIENDL: Mr. Carcavallo, why did you use ether and not carbon dioxide for anaesthesis, in view of the fact that it probably has an influence on longevity?

R. U. CARCAVALLO: Ether is practical and harmless at the doses we use. I don't believe that it affects longevity, and it is used as a matter of routine at our laboratory. The insects are anaesthetized with ether, for example, to prevent their escape when the flasks containing them are cleaned.

AUTOSTERILIZATION OF TSETSE FLIES
A model for use with chemosterilants

E. D. OFFORI
Animal Research Institute,
Council for Scientific and Industrial Research,
Achimota, Ghana

Abstract

AUTOSTERILIZATION OF TSETSE FLIES: A MODEL FOR USE WITH CHEMOSTERILANTS.

The difficulties at present encountered in mass-breeding tsetse flies for use in eradication programs involving the sterile-male technique demand that the problem be tackled from several other angles. One method involves sterilizing flies in the natural population (autosterilization). While this could be effected in various ways, it would appear that with chemical sterilization, an attractant would be required. In the absence of a suitable chemical attractant for use with tsetse flies, it is suggested that the host animals of Glossina (e.g. cattle) or other physical attractant such as a moving vehicle be employed. In the model suggested in this paper a bullock is donned with an 'over-all' impregnated with chemosterilant and driven along well demarcated paths in a tsetse-infested area. Flies that make contact with the 'over-all' thus pick up the chemical and, it is hoped, become sterilized. Some of the pros and cons of this approach are also discussed.

INTRODUCTION

The possibility of applying the sterile-male technique for eradicating tsetse populations has been discussed by several authors [1 - 5]. One of the main initial difficulties that need to be overcome is that of breeding the flies in large numbers. This difficulty arises primarily because of the very low rate of reproduction of tsetse flies. The insect is larviparous; the female produces only one larva every 10 days and a total of 10 to 15 larvae in her life-time. The success of a mass production program is thus limited, among other factors, by this slow rate of reproduction.

In recent years attention has been directed to autosterilization which may be effected by several methods. With chemicals, emerging adults may be exposed to a treated surface or be made to pass through a fine spray of sterilant before the flies are allowed to mix with the natural population. Alternatively pupae may be treated so that emerging flies are sterilized when they come into contact with their own puparia. The model suggested in this paper involves sterilizing flies in the natural population. Such an approach would eliminate the risk involved in releasing large numbers of laboratory-bred flies as well as help to by-pass the difficulty of breeding and maintaining Glossina under laboratory conditions. Dame and Schmidt [5] have pointed out that owing to the mutagenic activity of chemosterilants presently available, it is important to ensure that only the target species is affected. In view of the fact that no chemical attractant is as yet known that could be used effectively with a chemosterilant for tsetse flies, the present paper suggests that the host animals of Glossina or some other physical 'attractant' be employed for the purpose.

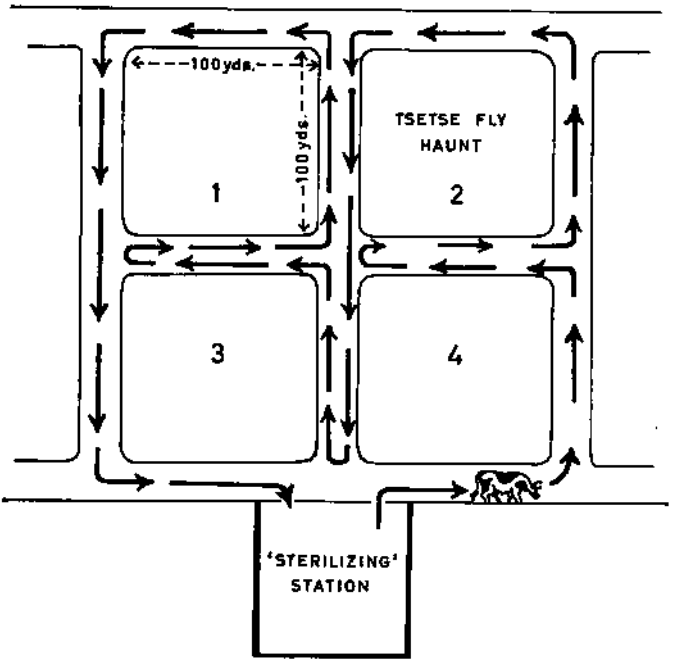


FIG.1. Model 'fly-round' along which a bullock may be driven.

The model is based upon our experience in the field, namely that

(i) tsetse flies are attracted to moving objects — animals, humans, or vehicles, and
(ii) in tsetse survey work, definite paths (fly rounds) are often marked out and followed at regular intervals for the purpose of estimating tsetse population densities.

PROCEDURE

The target area is divided into patches (fly resting haunts) about 100 yards by 100 yards, separated by reasonably wide paths (fly round), as shown in Fig. 1. A tame cow or bullock is procured to serve as both an attractant and chemosterilant applicator. The bullock is donned with an 'over-all' (Fig. 2), preferably of a dull brown or black coloration. The 'over-all' is then smeared or otherwise treated evenly with chemosterilant. To prevent seepage of the chemical through the 'over-all', a non-absorbent material may be used. The bullock thus treated, is driven slowly along the paths and close to the fly haunts (Fig. 1). As the flies alight and begin to probe through the 'over-all', they pick up chemosterilant via the legs and proboscis, as well as other parts of the body that may come into contact with the sterilant.

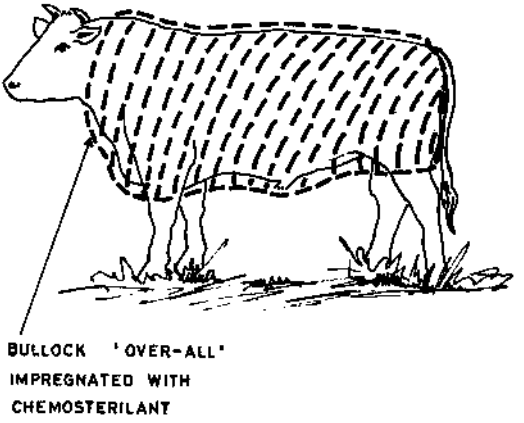


FIG.2. Bullock in 'over-all'. The animal serves as both an attractant for flies and chemosterilant applicator.

DISCUSSION

It is our experience that tsetse flies make several 'landings' on a host animal (or moving vehicle) before settling down to take a blood meal. Thus, the probability of a fly picking up enough sterilant from a treated surface (in this case the bullock's 'over-all') is quite high.

It is possible also that by making several contacts with the treated surface flies would acquire an overdose of sterilant, which could therefore produce high mortality. This may be offset by applying a measured quantity of chemosterilant evenly on the 'over-all'. Dame and Schmidt [5] reported no significant shortening of life span when adult male G. morsitans were exposed for 30-60 min to a glass surface treated with tepa or metepa at the rate of 10mg/ft^2. According to the present model, it is unlikely that a fly would remain in contact with the treated surface continuously for over an hour, especially if it is unable to feed or at least successfully probe through the 'over-all'.

The approach involves also the possibility of sterilizing flies of varying ages with the same dose of chemical. Theoretically this could result in complete sterility of some flies while other become only partially sterilized. Thorough laboratory investigation of the minimum sterilizing doses for flies of different ages is therefore a necessary pre-requisite. In their experiment with tepa and metepa Dame and Ford [6] exposed adult male G. morsitans of varying ages to treated glass surface and obtained complete sterility when the exposure time was 30 or 60 min. Males exposed for 15 min, however, recovered fertility. It would appear therefore that the time of exposure and not the age of the flies might be a limiting factor.

Chemosterilants become degraded upon prolonged exposure to the atmosphere. Thus, although Dame and Schmidt [5] achieved complete sterility in the first 3 months with tepa applied to pupae of G. morsitans, the effectiveness of the chemosterilant declined rapidly thereafter. One of the advantages of the present approach therefore would be that the insects are sterilized without the necessity of exposing the chemical

in the field for too long a time. It would be necessary, however, to repeat the operation at regular and frequent intervals.

It is conceivable that a male fly sterilized by the present method could, in turn, sterilize several females with which it may come into contact during mating. The effectiveness of this (secondary) sterilization by contact would of course depend, among other things, upon the quantity of sterilant picked up by the male at the time it alighted on the treated surface.

The necessity for selecting chemosterilants that would only affect the target species has been pointed out [5]. By using the present method, the host animal of the tsetse is employed as an attractant in order to minimize the possibility of affecting other insects in the environment. Field observations however show that a number of other species especially among the Diptera are attracted to cattle. Most of these, including tabanids, face flies, hornflies and stable flies are either nuisance flies or pests involved in the mechanical transmission of trypanosomiasis in cattle. Thus, even if the present method is not entirely selective against tsetse flies, it is obvious that most of the other insects that may be affected are in fact undesirable species that need to be eradicated.

REFERENCES

[1] SIMPSON, H.R., The effect of sterilized males on a natural tsetse fly population, Biometrics 14 (1958) 159-173.

[2] KNIPLING, E.F., Potential role of the sterility principle for tsetse fly eradication, WHO/Vector Control/27 (1963) 17 pp.

[3] DAME, D.A., FORD, H.R., Multiple mating behaviour of Glossina morsitans and its potential effect on the sterile male technique, Bull. ent. Res. 58 (1968) 213-9.

[4] ITARD, J., "Premiers résultats d' un essai d' irradiation gamma sur des pupes et des mâles adultes de Glossina morsitans morsitans," Control of Livestock Insect Pests by the Sterile-Male Technique (Proc. Panel Vienna, 1967), IAEA, Vienna (1968) 59-63.

[5] DAME, D.A., SCHMIDT, C.H., The sterile-male technique against tsetse flies, Glossina spp., Bull. ent. Soc. Am. 16 (1970) 24-30.

[6] DAME, D.A., FORD, H.R., Effect of the chemosterilant tepa on Glossina morsitans (Westw.), Bull. ent. Res. 56 (1966) 849-58.

DISCUSSION

W.F. BALDWIN: May I ask Mr. Offori if he has considered the distances over which the flies might be expected to respond to a moving object? It seems to me that this would be a very important consideration in developing his model for the autosterilization of tsetse flies.

E.D. OFFORI: Tsetse flies use the olfactory followed by the visual sense to locate their host. If we drive the bullock or the treated vehicle close enough to the vegetation in which the flies were resting, the necessary response will be elicited. This has in fact been demonstrated in the field in Ghana for one or two species by Chapman.

P. MACHILI: Since tsetse flies normally follow a moving animal or any moving object, is it not of some advantage to give the test animal certain types of stimulants or irritants to make it more mobile than usual?

E.D. OFFORI: In point of fact it is better if the animal (or vehicle) moves slowly. We catch more flies when the vehicle is driven slowly (about 5 miles/h) than when it is moving fast.

P. MACHILI: One other important factor would be the effect of the weather. Presumably the test animals would have to be released into the test area during dry weather so that the chemosterilant is not washed off by rain. Unfortunately, dry weather is the time when the animals are less mobile and more likely to be resting in the shade. Have you any comments about this?

E.D. OFFORI: Yes. In this model, I propose to use a tame bullock which I can drive around the area. The bullock will attract flies, which will receive chemosterilant from the animal and return to the wild population. It is immaterial whether the wild host animals are resting in the shade or not.

P. MACHILI: Lastly, have you thought about using wild animals instead of cows, which would have to contend not only with the over-alls, but also with their own survival?

E.D. OFFORI: I don't want to use wild animals. I am sure you appreciate that as chemosterilants are dangerous, we should be able to control the method of application. You would not have this control if you were to apply the chemical to a wild animal. That is why I prefer a tame bullock or, better still, a vehicle.

G.R. SETHI: Your Fig. 2 gives the impression that only the upper portion of the body of the animal is to be covered with material treated with chemosterilant. Would it not be better to cover the whole body to give more treated surface area with which the flies can come into contact?

E.D. OFFORI: The final details of the over-all have not been worked out, but I think your suggestion is a good one.

G.R. SETHI: You propose to try this technique in Africa, where the temperature is rather high in the field. Work on persistent insecticides has shown that the loss of insecticide is considerably greater at high temperatures. Wind velocity has also been found to affect persistence. Would this phenomenon not have an adverse effect on your trials?

E.D. OFFORI: That is quite possible, and I admit that it is one of the many problems we have to contend with. However, according to my 'model', it will not be necessary to expose the chemical in the field for too long a time, so that the danger you refer to will be minimized.

R.C. BUSHLAND: If you wish to sterilize 90% of the flies in a given area using chemosterilant-treated animals, will it not be necessary to have your bait animals outnumber the wild animals at a ratio of 9 to 1?

E.D. OFFORI: I don't think this will be necessary. Using human baits or a moving Landrover vehicle, we were able on many occasions to attract large numbers of flies in the Mole Game Reserve in Northern Ghana, despite the fact that wild host animals were present in the nearby vegetation.

A. MEWS: Am I right in thinking that in a number of tsetse species, the majority of flies attracted to a moving object would be males? Would this have any effect on your theoretical model?

E.D. OFFORI: The answer to your first question is yes. During preliminary studies of Glossina submorsitans in the Mole Game Reserve we observed that the males outnumbered the females in the ratio of 20 to 1. The effect on my 'model' would be advantageous because, after all, it is the males that we wish to sterilize.

EFFECTS OF TRETAMINE ON 4th- AND 5th-INSTAR *Oncopeltus fasciatus* NYMPHS

Development of eggs from tretamine-treated females

A. P. ECONOMOPOULOS*
Department of Entomology,
University of California,
Berkeley, Calif., United States of America

Abstract

EFFECTS OF TRETAMINE ON 4th- AND 5th-INSTAR *Oncopeltus fasciatus* NYMPHS: DEVELOPMENT OF EGGS FROM TRETAMINE-TREATED FEMALES.

One microgram of tretamine, topically applied in acetone solution at the beginning of the 4th- and 5th-nymphal instars of *Oncopeltus fasciatus* (Dallas) (Heteroptera: Lygaeidae), prevented almost completely the next moult; similar application toward the end of the 4th instar permitted the majority of the nymphs to moult into 5ths but a small percentage of them succeeded in turning into abnormal adults. All these treatments, at stages with spermatogonia and spermatocytes present but no sperm produced yet, did not prevent sperm formation. Applications on mid- or late-5th-instar nymphs had no apparent effect on adult moulting and the later-treated male nymphs (more sperm present) showed, as adults, less mating activity and more sterility. The early-treated 5th-instar nymphs had a reduced weight at the time when they should start moulting into adults and many of them survived as abnormal 5ths for an unusually long period; most died in an apparent effort to moult.

One-day-old female bugs were treated topically with 1 μg/insect of tretamine and their ovaries were dissected out after 5 and 25 days. At day 6 most of the ovaries contained no apparent oocytes and very few had ovarioles with small oocytes; at day 26 several ovaries showed various stages of recovery. Females treated on the second day of adult life (oocytes not fully developed yet) and caged with normal males, laid eggs of variable size, the smaller showing higher embryonic death. The striking reduction in egg size suggests injury to the trophocytes.

INTRODUCTION

Tretamine was found highly effective in sterilizing *Oncopeltus fasciatus* males whent treated as adults [1]. Subsequent work showed that female bugs were also sterilized by tretamine. When young females were treated, a low or diminished fecundity was observed; treatment at a later stage, egg laying already started, resulted in a drastic reduction of egg viability for the mature eggs (developed and stored before tretamine application) and an arrest in the production of new eggs [2]. Eggs of small size were always produced in experiments with chemosterilized females; they were detected a few days after treatment and were probably produced by females which suffered minor damage and had started recovering. Lawson and Ball [3] found a similar significant reduction in the number of eggs produced by metepa-treated *O. fasciatus* females.

* Present address: "Democritus" Nuclear Research Center, Aghia Paraskevi Attikis, Athens, Greece.

Sperm formation in O. fasciatus males starts at the beginning of the 5th nymphal instar, and by adult day 1, half of the testis volume is occupied by sperm [2]. Synthetic compounds with juvenile hormone activity (6th-instar nymphs produced) did not alter the sequence of sperm production and the same was true when testes from very young 5th-instar nymphs or late 4ths (sperm not produced yet) were transplanted into adult male or female milkweed bugs.

This work was designed to investigate the effects of tretamine on O. fasciatus nymphs as well as to study the development of small and normal-size eggs from tretamine-treated females.

MATERIALS AND METHODS

The polyfunctional aziridine tretamine (2, 4, 6-tris (1-aziridinyl)-s-triazine) was provided by J. S. Bowman (Agricultural Division, American Cyanamid Co., Princeton, N. J., U. S. A.). Solutions of 1 $\mu g/\mu l$ in acetone were prepared just before topical application. A Hamilton repeating dispenser was used for topical application of one 1-μl droplet on the ventral thorax, between the legs. The insects were anaesthetized with CO_2 before treatment for easier handling. The bugs used were of the CS-18 and CS-19 strains (18 and 19 generations of consecutive rearing on a mixture of cashew nuts and sunflower seeds). Whenever bugs were needed for experimentation, colony cages containing bugs of a stage of development preceding the one needed were transferred into an incubator with a constant temperature of 30°C. At this temperature development is accelerated and the experimental time is shortened. Whenever nymphs were treated they were from both sexes and in a 1:1 sex ratio. The colony bugs were kept at room temperature (20-22°C) in the plastic cages developed in the Department of Entomology, University of California (Gordon and Jao, unpublished data).

For dissections the living females were pinned on their backs to a paraffin-bottom glass dissecting dish covered with an insect saline (6.5g NaCl, 0.25g KCl, 0.25g $CaCl_2$ and 0.25g $NaHCO_3$ in 1 litre of distilled water). The female reproductive systems were studied or photographed soon after dissection in the saline.

Egg development after tretamine treatment was studied in females that were treated on day 2 of adult life. About 40 treated females were mixed 5 days after treatment with an equal number of normal males of similar age. Eggs laid during the 7th and 8th days were discarded and from then on the eggs were harvested daily, from the special egg-laying holes covered with cheesecloth, and held in EP-14 polyethylene cups covered with EP-16 ones (Protective Closures Incorporation, 2207 Elmwood Avenue, Buffalo, New York 14216). The eggs were kept at 30°C for a period of 8 days. The control cage had also 40 bugs from each sex at the beginning.

Only a random sample of 40-80 control eggs were kept daily for examination. Control eggs produced during the first or last days of the fecund period were not used, to avoid the age effect described by Richards and Kolderie [4]. These workers found that very early and very late in the fecund period O. fasciatus females lay fewer eggs that weigh less, take longer to develop and give lower hatching percentages.

TABLE I. PERCENTAGES OF 4th- AND 5th-INSTAR O. fasciatus NYMPHS THAT SUCCEEDED IN MOULTING INTO 5ths AND ADULTS AFTER TOPICAL APPLICATION OF 1 μg OF TRETAMINE

Treated insects	No. of insects	Percent that moulted to 5ths	Percent that ended up as adults
0 - 24-hour-old 4th-instar nymphs[a]	40	7.5[b]	0.0
Late 4th-instar[a] nymphs	50	70.0[b]	30.0[b]
0 - 24-hour-old 5th-instar nymphs	144		0.7[b]
3 - 4-day-old 5th-instar nymphs	55		81.8
6 - 7-day-old 5th-instar nymphs	30		93.3
Control A[c]	26		92.3
Control B[d]	35		94.3

[a] No controls for the early and late 4th-instar treatments were undertaken. However, in other experiments with compounds having juvenile hormone activity, early and late 4th-instar nymphs were treated topically with 1 μl of acetone; more than 80% of the treated nymphs moulted into 5ths and adults respectively.

[b] Mostly weak and abnormal insects.

[c] 0 - 24-hour-old 5th-instar nymphs treated with 1 μl of acetone topically.

[d] Control nymphs not treated with acetone.

TABLE II. AVERAGE BODY WEIGHT IN mg OF 5th-INSTAR O. fasciatus NYMPHS TREATED WITH 1 μg OF TRETAMINE TOPICALLY

The nymphs were treated as 0 - 24-hour-old 5ths and weighed 6 days later (just before moulting to adults)

Insects	Males	Females
Treated	37.3 (33)[a]	53.4 (44)
Control	49.0 (35)	61.2 (22)

[a] In parenthesis the number of insects used.

Eggs recorded as presumably fertilized in Table III remained fully distended for the greater part or all of the observation period of 8 days. Lawson and Ball [3] reported that fertilized O. fasciatus eggs develop a non-cellular structure at the surface; they did not find the above layer in eggs from virgin O. fasciatus females, and these eggs exhibited a shrunken and dull appearance soon after oviposition.

TABLE III. REPRODUCTION VALUES[a] IN NORMAL MILKWEED BUG FEMALES CAGED WITH MALES TREATED AS 4th- AND 5th-INSTAR NYMPHS WITH 1 μg OF TRETAMINE

Seven adult males, surviving from nymphal treatments, were used in each case. The control cage had 25 males. Normal females were added in equal numbers at the beginning of the experiment; at that time both males and females were 5 - 8 days old.

Treated insects	Total eggs produced	TPFE	TN	PFT	PVI	PHCH	MDAYS	FDAYS	MAT	E/FD	PM	PF
Late 4th-instar male nymphs	382	96	0	25.0	0.0	0.0	100	48	0	7.9	0.0	0.0
3 - 4-day-old 5th-instar male nymphs	1154	1063	562	92.0	52.0	48.7	84	61	11	18.9	13.1	18.0
6 - 7-day-old 5th-instar male nymphs	686	397	157	57.9	39.5	22.9	94	79	5	8.7	5.3	6.3
Control	4345	4273	3888	98.3	90.9	89.4	415	350	150	12.4	36.1	42.9

[a] The abbreviations used have the following meaning: TPFE total number of eggs recorded as presumably fertilized, TN total number of nymphs that hatched, PFT percentage of total eggs laid that were recorded as fertile, PVI percentage of eggs that had been recorded as fertile and that subsequently hatched, PHCH percentage of total eggs laid that hatched into nymphs, MDAYS male-days during the experimental period, FDAYS female-days during the experiment, MAT total number of matings observed during the experiment, E/FD total number of eggs divided by the number of female-days, PM percentage of males observed mating, PF percentage of females observed mating.

RESULTS AND DISCUSSION

Nymphal treatments

Table I shows that when very young 4th- and 5th-instar nymphs were treated with 1 μg of tretamine the next moult was prevented in almost all nymphs. The majority of late 4th-instar nymphs treated with tretamine succeeded in moulting into 5ths and a considerable percentage finally moulted into adults. A total of 82% of the nymphs treated as mid-5ths turned into adults and the late-5th-treated moulted in percentages not different from the controls. Fifth-instar nymphs treated at the beginning of the instar had a reduced weight at the end of a normal instar period (Table II). Several nymphs treated as early 4ths or late 4ths remained 4ths and 5ths, respectively, for an unusually long period. The phenomenon appeared more striking (because of lower mortality) in nymphs treated as early 5ths. Thus, out of 144 treated nymphs 30 were found surviving as abnormal 14-day-old 5ths and 20 were alive 20 days after treatment. The 5th-instar usually lasts 6 - 7 days at 30°C. In a control test only 2 out of 112 5th-instar nymphs remained 5ths for up to 15 days; this could be attributed to some hormonic imbalance caused by genetic variability.

Since the same amount of tretamine was applied on all nymphs of Table I, the earlier stages received a much greater dose per unit body weight. The average body weight of a 0 - 24-hour-old 4th-instar nymph was found [2] to be 6.3 mg, of a 0 - 24-hour-old 5th-instar nymph 23.3 mg, and of a 0 - 24-hour-old adult 49.6 mg. Seeing that there is no significant weight difference between a nymph at the end of any instar and the same nymph at the very beginning of the next instar, one can approximate the applied quantities of tretamine to 160, 40, 40 and 20 mg/kg for the early 4th, late 4th, early 5th and late 5th-instar nymphs respectively. Thus one can explain the increased mortality in the early-4th-treated nymphs where only 6 (3 4ths and 3 5ths) out of 40 were surviving 12 days after treatment. In the late-4th-treated nymphs 27 (12 5ths and 15 adults) out of 50 were surviving 12 days after treatment. The fact that the majority of the 4th-instar nymphs treated at the end of the instar succeeded in moulting into 5ths and many of them ended up as adults but only one out of 144 5ths treated at the beginning of the instar moulted into an adult, indicates a special chemosterilant sensitivity at the beginning of the instar. Harwalkar and Nair [5], working on another hemipterous insect, Dysdercus koenigii (F.), found that X-irradiation of an instar during its late phase did not affect its next moult; irradiation during the early stage did affect the next moult. They suggested that treatment at a late stage is not very critical for the approaching moult since the epidermal cells have already divided.

A similar situation could be the case with the chemosterilants that inhibit mitosis. Kuzin et al. [6] showed that pupation in g-irradiated larvae of Ephestia kühniella is not inhibited because of a damage to the DNA of the hypodermis but because of the absence of the moulting hormone, ecdysone. During the 5th instar of O. fasciatus the amount of ecdysone/g of bug is much higher between days 1 to 6 than days 0 and 7, no ecdysone was found on the day of adult ecdysis, and a low level was found in the subsequent days [7]. Therefore, if tretamine damages the neurosecretory cells, as g-irradiation seems to do in Ephestia kühniella, an early treatment in the 5th instar could deprive the insect of the hormone level necessary for

moulting. In all nymphal treatments abnormal forms were observed towards the end of the instar. These forms looked like 'nymphs in an effort to moult' with wing pads abnormally developing and the old cuticle never cast off successfully; all died before moulting. The majority of these abnormalities were found in nymphs treated as young 5ths. Very few were observed in the other treatments, and in nymphs treated as late 4ths the phenomenon appeared towards the end of the next instar. Partial damage to the dividing cells or inadequate quantities of ecdysone could be the cause of these irregularities.

Adult males surviving from the nymphal treatments (Table I) were caged with normal females of similar age. For 15 days the eggs were collected and examined to evaluate the male sterility. The control data represent the 20 1st days from a typical control cage used at that time for the main sterility experiments [2]. In all cases 5 - 8-day-old males were caged with females of similar age. Table III shows that males treated as late 4th-instar nymphs gave zero observed mating activity during the 15-day period that they were caged with normal females. These males were weak insects weighing 36. 6 mg each, on the average, instead of 50 mg which is the average body weight of a normal male. At the end of the 15-day period the surviving males were opened and large quantities of sperm were detected in the testes and the seminal vesicles. Thus, treatment at a stage with no sperm present yet, does not seem to prevent its formation. The substantial number of eggs recorded as fertile, even without any observed mating activity, indicates that the discrimination of eggs into fertile and infertile soon after oviposition is not highly accurate (something already seen in previous work with O. fasciatus [2]).

Males treated in the middle of the 5th instar gave double mating activity compared with those treated at the end of the instar. Fertility, viability and hatchability were also much higher in the case where males were treated as mid-5ths. The much larger number of eggs per female-day in the cage with mid-5th-treated males is probably the result of a more intense mating activity. Gordon and Loher [8] showed that mating activation is the dominant factor controlling egg production in the CS strain. On the other hand, the lower egg production rate in the control cage is probably connected with the larger number of insects in the cage [2].

The observed male mating activity in both mid- and late-5th-instar-treated males was much lower than the control and the phenomenon is especially striking in the late-5th-treated. Seeing that in other experiments, with males treated as young adults with 20 mg/kg of tretamine and with cage density well above the 14 insects, the male mating activity for the 1st 20 experimental days was above 20-25% (percent males mated) [2], one concludes that nymphal treatments are more harmful to the vigour and male mating drive. In both mid- and late-5th-instar treatments the first fertilized eggs appeared 10 - 12 days after treatment and fertility, viability and hatchability were constantly higher in the mid-5th treatment. In both treatments, viability and hatchability are considerably higher than in experiments with males treated as young adults with 20 mg/kg of tretamine [2]. Thus, the data suggest that nymphal treatments caused more damage to the male mating vigour and less sterility. However, the small insect numbers used in the above experiments do not allow any firm conclusion on mating activity and sterility in adults treated as nymphs with tretamine. A preferential major effect of tretamine on mature spermatozoa could explain the lower

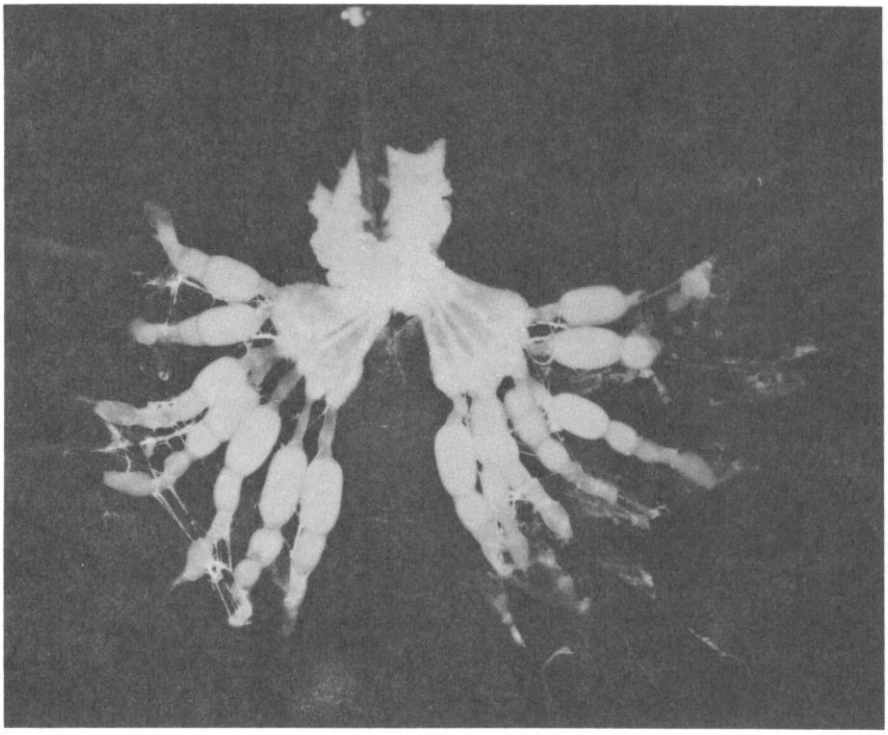


FIG. 1. Ovaries from a 6-day-old O. fasciatus female (control) with many growing oocytes × 9.

sterility induced in nymphal treatments; young adults have more sperm than late-5th-instar male nymphs and the later more than the mid-5ths [2]. Fahmy and Fahmy [9] suggested that the mature spermatozoa are primarily affected by tretamine and Jackson [10] found that the fully formed spermatozoa and spermatids are far more sensitive to tretamine than earlier stages.

Adult treatments

Figures 1 - 3 present the whole reproductive system in control and treated female bugs. As these pictures suggest, tretamine inhibits oocyte development. Upon recovery (Fig. 3), some abnormally small oocytes appeared in some of the ovarioles. On day 26 the control females had the ovarioles full of growing oocytes and the lateral oviducts with many stored ova; ovaries with no signs of recovery on adult day 26 presented a picture similar to Fig. 2. Very few females were found recovering 6 days after treatment. Many chemicals have been used to induce female infecundity, which is usually attributed to severe damage to either the oogonia or nutritive cells or both [11]. Hussein [12] showed that HMPA and metepa,

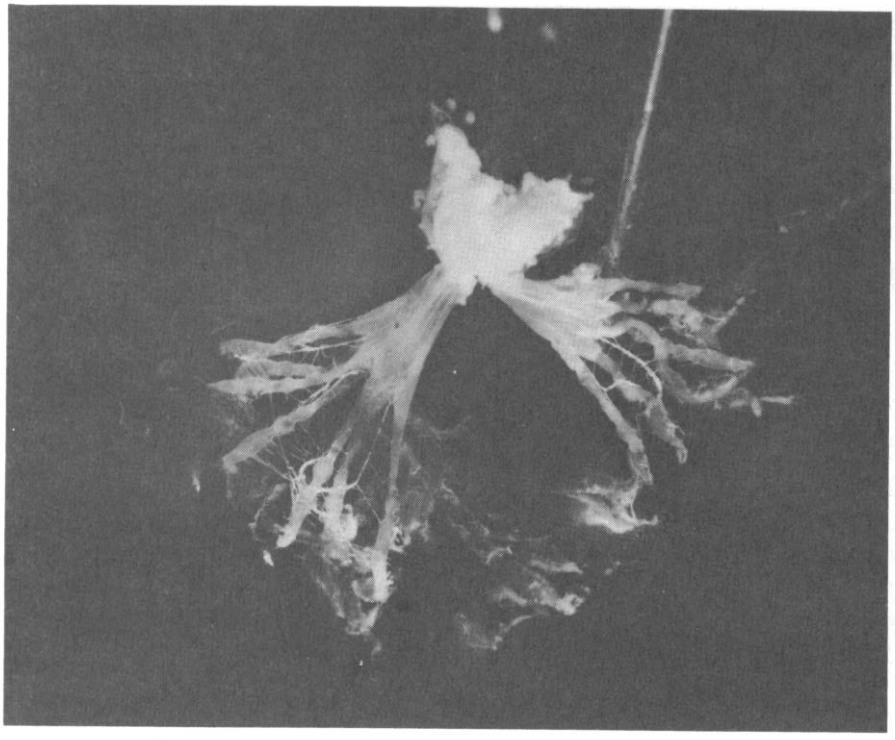


FIG. 2. Ovaries from a 6-day-old O. fasciatus female treated with 20 mg/kg of tretamine on the first day of adult life. Oocyte development is completely arrested × 9.

topically applied, decreased egg production by about 50% when Lygus hesperus females were treated. Morgan [13] found that female house flies maintained on food containing 1% or 2% hempa showed inhibition of egg development followed by vacuolation in the nurse cells and oocytes. Smittle et al. [14] showed that German cockroach females injected with tepa produced few or no oothecae.

In previous work applications of tretamine at 20 mg/kg (1 μg/bug) delayed egg production in milkweed bug females for about 10 days; the subsequent egg production rate was low and many eggs were abnormally small [2]. The above applications were on adult days 1 - 2 when no visible oocytes are seen in the ovarioles. (A minor experiment, on the development of ovaries at 30°C, showed that the first macroscopic signs of oocyte development usually start 3 - 4 days after the adult moulting). Table IV shows that one out of three eggs produced by O. fasciatus females, treated with 20 mg/kg of tretamine on adult day 2, had a significantly reduced size. These small eggs gave an extremely low hatch and almost none of the minute nymphs ended up as adult; most of them died during the 1st instar. Eggs of a normal size, laid by treated females, gave lower hatch and more embryonic death than the controls. The majority of small eggs developed

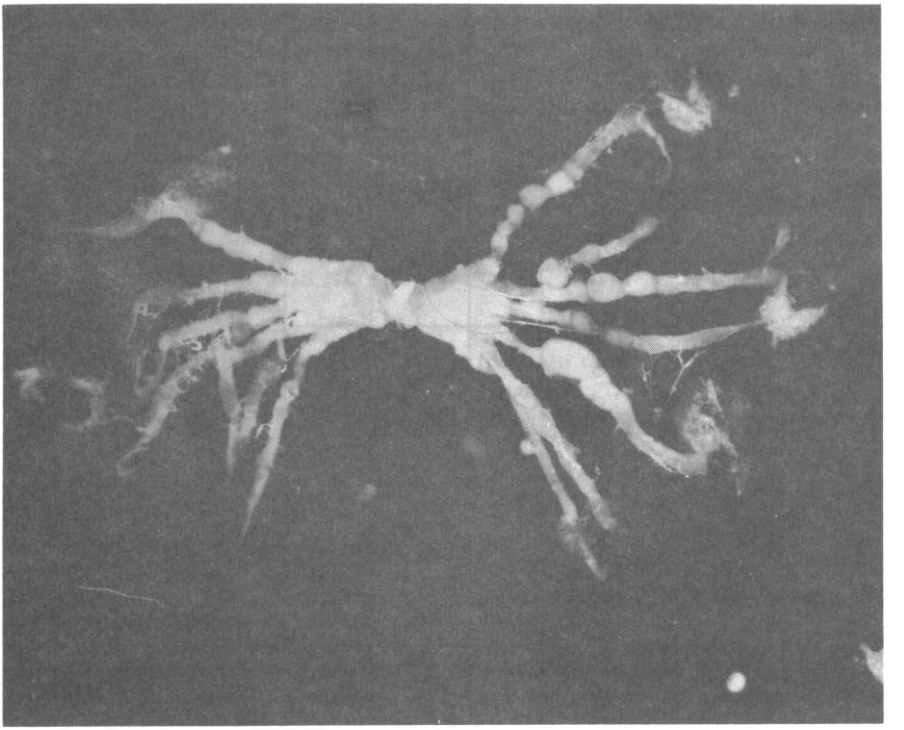


FIG. 3. Ovaries from a 26-day-old O. fasciatus female treated with 20 mg/kg of tretamine on the first day of adult life. Partial recovery of oocyte development can be seen in some ovarioles × 8.

an embryo to some degree but no hatch finally took place (red embryo seen inside). The percentage of adults developed from control eggs (22%) is not considered a representative one since observations in our laboratory usually show a figure of about 30%. The most likely explanation of that low nymphal survival is that for 4 - 5 consecutive days, in the middle of the experimental period, the control eggs were kept in jars which previously housed bugs injected with large quantities of DEF (S, S', S''-tributyl phosphorotrithidate), an aliesterase inhibitor. As the control females started growing older their eggs were often observed to be smaller but not to the extent that one could list them as being $\frac{1}{2}$ - $\frac{1}{3}$ of a normal-size egg. On the other hand the small eggs from treated females were fewer toward the end of the experiment, probably the result of a progressive recovery.

The above effect on fecundity indicates injury to the trophocytes. This is well indicated by the striking reduction in egg size, a result of deficient supply in nutrients. Richards and Kolderie [4] showed that small eggs from normal milkweed bugs have also a reduced hatchability, and Richards [15] pointed out that the weight of eggs is not only correlated with their hatchability but seems to be also related to successful hatching. LaChance and Leverich [16] suggested that g-irradiation hampered oocyte growth in Cochliomyia hominivorax presumably because of damage to the nurse cells.

TABLE IV. REPRODUCTION VALUES IN MILKWEED BUG FEMALES TREATED WITH 1 μg OF TRETAMINE ON ADULT DAY 2

Equal numbers of normal males were added 5 days after treatment. The values are totals for a period of 20 days after mixing the sexes

Insects	Eggs examined		% Nymphs hatched		% Adults produced		% Eggs that had embryo inside but did not hatch	
	Total	% having size 1/2 - 1/3 of normals	From eggs of normal size	From eggs of small size	From eggs of normal size	From eggs of small size	From eggs of normal size	From eggs of small size
Treated	542 [a]	36.5	64.5	4.5	19.8	0.5	29.9	69.2
Control	731 [b]	0.0	73.5		22.0		21.2	

[a] All eggs laid were collected and examined.

[b] Only random samples of 40 - 80 eggs were examined daily.

The same researchers [17], working with an alkylating agent (2, 5-bis(1-aziridinyl)-3, 6-bis(2-methoxyethoxy-p-benzoquinone)) on the same insect suggested that chemical mutagens affect the endomitotic process in nurse cell chromosomes (which leads to the formation of polytene chromosomes) resulting in reduced insect fecundity.

ACKNOWLEDGMENTS

Many thanks are due to Dr. H. T. Gordon of the Department of Entomology, University of California, for help during this work as well as Dr. J.S. Bowman for supplying the chemosterilant. I also wish to thank Mrs. V. Trouposkiadou for assistance in preparing the manuscript.

REFERENCES

[1] ECONOMOPOULOS, A.P., GORDON, H.T., Action of certain chemosterilants on males of the large milkweed bug, J. econ. Ent. 62 6 (1969) 1326.
[2] ECONOMOPOULOS, A.P., "Chemosterilization and reproduction in the large milkweed bug Oncopeltus fasciatus (Dallas), Heteroptera: Lygaeidae", Ph. D. Thesis, Univ. of Calif., Berkeley (1970).
[3] LAWSON, D.E., BALL, H.J., "Effects of metepa on gametogenesis and embryogenesis in the large milkweed bug, Oncopeltus fasciatus (Dallas)", Univ. of Nebraska, College of Agriculture and Home Economics, Res. Bull. 234 (1969).
[4] RICHARDS, A.G., KOLDERIE, M.Q., Variation in weight, developmental rate, and hatchability of Oncopeltus eggs as a function of the mother's age, Ent. News. 68 3 (1957) 57.
[5] HARWALKAR, M.R., NAIR, K.K., Effect of X-irradiation on postembryonic development of the red cotton bug, Dysdercus koenigii, Ann. ent. Soc. Amer. 61 5 (1968) 1107.
[6] KUZIN, A.M., KOLOMIJTSEVA, I.K., YUSIFOV, N.I., Effect of ecdysone on the puparium formation in irradiated Ephestia kühniella Z., Nature, Lond. 217 (1968) 743.
[7] FEIR, D., WINKLER, G., Ecdysone titres in the last larva and adult stages of the milkweed bug, J. Insect Physiol. 15 (1969) 899.
[8] GORDON, H.T., LOHER, W., Egg production and male activation in new laboratory strains of the large milkweed bug, Oncopeltus fasciatus, Ann. ent. Soc. Amer. 61 (1968) 1573.
[9] FAHMY, O.G., FAHMY, M.J., The chemistry and genetics of alkylating chemosterilants, Trans. R. Soc. trop. Med. Hyg. 58 (1964) 318.
[10] JACKSON, H., Effects of alkylating agents on fertility, Brit. med. Bull. 20 (1964) 107.
[11] LaCHANCE, L.E., NORTH, D.T., KLASSEN, W., "Cytogenetic and cellular basis of chemically induced sterility in insects", Ch. 4, Principles of Insect Chemosterilization (LaBRECQUE, G.C., SMITH, C.N., Eds), Appleton-Century-Crofts, New York (1968).
[12] HUSSEIN, E.M.K., "The effects of chemosterilants on Lygus hesperus Knight", Ph.D. Thesis, Univ. of Calif. Davis, Diss. Abstr. 27 3 (1966) 997B.
[13] MORGAN, P.B., Effects of hempa on the ovarian development of the house fly, Musca domestica (Diptera: Muscidae), J. econ. Ent. 60 4 (1967) 812.
[14] SMITTLE, B.J., SCHMITT, J.B., BURDEN, G.S., Effects of tepa on the reproductive organs and embryogeny of the German cockroach, J. econ. Ent. 59 6 (1966) 1419.
[15] RICHARDS, A.G., "Cumulative effects of optimum and suboptimum temperatures on insect development", in. Symp. Soc. Gen. Physiol. (1957).
[16] LaCHANCE, L.E., LEVERICH, A.P., Radiosensitivity of developing reproductive cells in female Cochliomyia hominivorax, Genetics 47 (1962) 721.
[17] LaCHANCE, L.E., LEVERICH, A.P., Cytogenetic studies on the effect of an alkylating agent on insect nurse cell polytene chromosomes as related to ovarian growth and fecundity, Genetics 52 (1965) 453.

DISCUSSION

L. E. LaCHANCE: As tretamine treatment of the 4th instar did not prevent the production of mature sperm, could you tell us whether spermatids were present at the time of treatment.

A. P. ECONOMOPOULOS: No spermatids were found in testes from late 4th O. fasciatus males. The 4th-instar was found to be the period of spermatocyte accumulation, so that at the end of the instar the testis was packed with numerous layers of spermatocytes. At the very beginning of the 5th instar a wave of differentiation starts which proceeds toward the apical region of the testis; by the time the 5th-instar nymph is ready to moult into adulthood, half of the testis is full of sperm. Gordon and I have established this.

BASIC BIOLOGY AND ARTIFICIAL REARING OF INSECTS

(Session V)

Chairman

T. JERMY (Hungary)

Survey paper

RECENT DEVELOPMENTS IN THE BIOCHEMISTRY AND FEEDING BEHAVIOUR OF HAEMATOPHAGOUS ARTHROPODS AS APPLIED TO THEIR MASS REARING

Rachel GALUN
Israel Institute for Biological Research,
Ness-Ziona, Israel

Abstract

RECENT DEVELOPMENTS IN THE BIOCHEMISTRY AND FEEDING BEHAVIOUR OF HAEMATOPHAGOUS ARTHROPODS AS APPLIED TO THEIR MASS REARING.

Due to the large quantities of blood required by parasites in order to complete their development, it is very often not economic to mass-rear them unless a rearing method outside the living host is developed.

Inducing the parasites to feed on blood, even from their natural host, is often complicated. Some insects can be fed blood when it is offered on cotton pads, but most parasites require conditions which simulate more closely their natural feeding manners. In most cases they are fed through membranes which cover the blood meal warmed to body temperature. For mass rearing it is important to have a membrane which is cheap, commercially available and easily prepared for mass feeding. The suitability of a great number of membranes was tested over the years. It was found that blood-sucking arthropods feed better through animal-derived membranes, although in certain cases other membranes were also useful. Animal-derived membranes are relatively expensive since they can be used only a limited number of times before they start to leak. Therefore, the search for proper synthetic membranes should be continued. Once a feeding method has been developed, the feeding stimulants should be identified. Many blood feeders refuse to feed on 'aged blood' or on haemolysed blood, and even more so on dehydrated blood which was dissolved again owing to the deterioration of stimuli. For many blood feeding insects ATP was identified as the feeding stimulant, and addition of ATP to aged blood, serum, or even to saline, induced feeding in mosquitoes, tsetse flies, fleas or Rhodnius. Reduced glutathione was identified as the phagostimulant for soft ticks. Phagostimulants for many other blood feeders have not been investigated.

Using feeding stimulants, natural diet, i.e. blood, could be replaced by artificial diet. So far no blood feeders have been reared from egg to egg on chemically defined media, although rearing was achieved by artificial feeding on blood. Blood from various sources, other than natural host, should be tested since better yields are often obtained on non-natural hosts.

The utilization of the blood of vertebrates as food is a property that evolved independently in a considerable number of arthropods.

Among arthropods with incomplete metamorphosis we find many groups which nourish themselves constantly during their nymphal and adult stages on vertebrate blood and have no other source of nutrition. These include several families of the Acarina and insects - Ixodidae, Argasidae, Gamasidae, Pediculidae, Cimicidae and Triatomidae. Among insects with complete metamorphosis we also find some groups which use blood as a sole nutrient. These are the Glossinae and Pupipara.

On the other hand, many blood-sucking insects with complete metamorphosis are scavengers or omnivorous feeders during their larval stages and use blood only as adults. At their adult stage they may feed on blood alone as do all the Siphonaptera where blood serves as both a source of energy and a source of protein

for reproduction, or they may use blood proteins for egg maturation and carbohydrates for energy consumption. This type of diet we find among Tabanidae, Culicidae, Phlebotominae, Simuliidae, and the blood-sucking species of Ceratopogonidae and Muscidae (other than Glossinae).

Arthropods which require blood for their entire development consume very large amounts until they reach maturity and reproduce, while insects that require blood only for reproduction, take relatively small amounts. However, during the course of their blood imbibing both groups secrete anticoagulants or toxins that act adversely on their hosts.

It seems therefore very important to develop a method for artificial feeding for these insects when their mass-rearing is considered.

Blood feeding away from the host may be a simple problem with some of the Diptera which feed both on blood and nectars. Blood-sucking Muscidae such as Stomoxys, Lyperosya or Haematobia feed readily on blood offered by soaking an absorbent cotton with it [1-3]. If the blood is warmed, the flies are attracted to it immediately, but they also feed readily on blood at room temperature, although they can detect its presence only by contact with their tarsi [4].

Mosquitoes do not possess chemoreceptors which can detect blood on the tarsi, labella or ligula [5] and therefore do not imbibe unwarmed blood. However, sugar receptors are found on these organs [5] and thus supplementing the blood with sugar induces feeding [6]. A careful choice of the most stimulatory sugar for the mosquito -sucrose- can increase the uptake of the blood and thus increase also the egg production [7]. In the Tabanid fly Crysops, addition of glucose increased very markedly ingestion of blood [8].

However, most of the arthropods which feed exclusively on blood, refuse to take blood from a free surface and the food has to be given in a manner simulating more closely the warm-blooded host, i. e. through a membrane which covers the warmed meal.

The membrane-feeding technique has been utilized for studies on transmission of pathogens by their arthropod vectors, already since the beginning of the century [9]. It is a useful tool in nutritional and behavioural studies and has been employed for the collection of oral secretions from mosquitoes and fleas [10,11].

Laboratory colonies of lice, tsetse flies and Pulex fleas were even maintained throughout their life cycle by feeding them blood through a variety of membranes [12-15]. However, the size of the colony was much smaller as compared to a colony feeding on live hosts.

Many of the rapid-feeding, blood-sucking arthropods have been fed artificially as indicated in the review of Tarshis on this subject [16]. The slow-feeding hard ticks seem to possess a greater discrimination towards their site of attachment and no one has, so far, fed them artificially in a satisfactory manner which could be adopted for mass-rearing [17].

From Tarshis's review [16] it seems that the membrane origin and thickness are of great importance for successful feeding. Generally, animal-derived membranes are superior to those of a vegetable or synthetic origin. The animal-derived membranes become soft and pliable and easily penetrable by the arthropods when placed on liquid.

The thickness of the membrane may be a determining factor in the case of an arthropod with short proboscis such as the louse or flea [18,19]. Thin membranes are

advantageous for most blood suckers, with the notable exception of the tsetse fly. Lester and Lloyd [20] claimed that the tsetse flies fed more readily through thicker skins such as those of vultures, fowl and monkeys rather than through thin skins of small birds, rats and lizards. Ox bladder and peritoneal tissue also allowed feeding, although less efficiently than skin [21]. Recent observations [22] also show that mouse skin membrane was much superior to a thin membrane made of ox caecum [22].

In view of these facts it was suggested [23] that a thick membrane is necessary for the haustellum of the fly to be inserted quite deep in order that a series of tactile sense organs be stimulated before the chemical stimulus of contact with blood is received on the sensory plate at the tip of the labellum. Using agar membrane of increasing thickness it was possible to show that it is the thickness of the membrane rather than its nature which is important for successful feeding [23].

Where mass-rearing is concerned, it would be useful to have a cheap, disposable membrane which could easily be prepared under laboratory conditions and, if necessary, sterilized. A cheap, commercially available standard product would definitely be recommended. Some animal-derived membranes are commercially available. Baudruche Capping transparent membranes are prepared on a commercial scale from bovine intestine by Long and Long Co., Beltsville, N. J. The thinnest Baudruche membrane is approximately .0007 in. These membranes may be obtained in sheets of approximately 9 x 36 in. They were found suitable for feeding mosquitoes, fleas and ticks [16, 24, 25], but gave only partial feeding with the tsetse fly [22].

Although commercially available, Baudruche membrane is quite expensive, too fine to be used over large areas, and very often leaks. It would be much preferable to use synthetic or semi-synthetic membranes if the insects could be induced to feed through them.

An extensive survey for the suitability of materials which could be obtained in sheet form was carried out in feeding experiments with lice [26], mosquitoes [16, 27] and fleas [16]. Commercial packaging films, including Saran, Mylar, cellophane and polyethylene, were found unsatisfactory, probably because of their toughness and high tear resistance. Parafilm, which is a mixture of rubber and wax, was reported as suitable for the feeding of body lice [26], soft ticks [28] and is now in routine use for artificial feeding of aphids [29]. Parafilm was found, however, very unsatisfactory for tsetse flies [22, 23] and for the mosquito Aedes aegypti, although Anopheles stephensi fed through it much better. This membrane has many advantages: it is available in any required size, it can be stretched to fit insects which require thin membrane, it may be sterilized easily by U. V., and is very easy to handle. Therefore, the use of this membrane should be tested for each insect to be fed artificially. Lice and Reduviid bugs were successfully fed on various natural rubber products such as gutta percha [26], latex [30] or Durex rubber [31].

The physical and chemical basis for the relative effectiveness of the different membranes is still unknown. We also do not understand why certain membranes are very effective for one insect, and unsatisfactory for another. Much more information on the sensory physiology of feeding is necessary in order to devise an ideal membrane.

In all membrane-feeding experiments the feeding apparatuses were constructed in such a manner that the insects were practically brought in contact with the membrane. In this procedure, environmental factors which influence the alighting of insects on the membrane can be overlooked. However, insects tend to attack, or

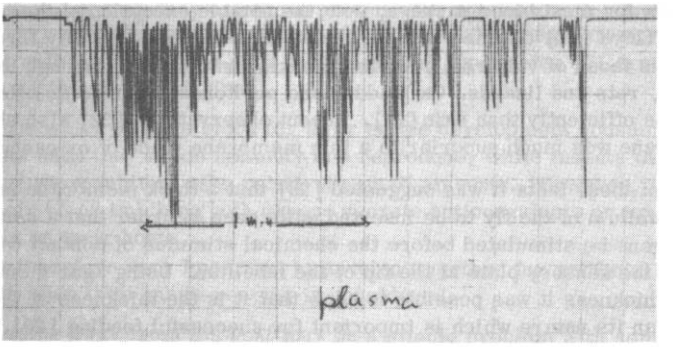


FIG. 1. Probing pattern of Glossina austeni on plasma.

rather probe the membrane, with less alacrity than the skin of a live animal. Recognizing the factors which induce probing may help to increase the efficiency of feeding.

Substrate temperatures, approximating to those of mammalian skin, have been reported to elicit probing in stable flies [4], mosquitoes [32] and tsetse flies [33]. It is, therefore, recommended to heat the blood to 34° - 38° [16]. In addition to temperature, other stimuli may increase probing. Thus Gatehouse [34] found that rapid increase in relative humidity synergizes the temperature effect in Stomoxys. Vapours of butyric and valeric acids were reported to induce probing in the tsetse fly [35], but failed to do so in Stomoxys [34]. In Stomoxys carbon dioxide failed to induce probing when presented alone or simultaneously with increased temperature [34]. In mosquitoes, probing response to a warm surface was enhanced by carbon dioxide [36].

Simuliidae often remain inactive when offered a blood meal. It was found that carbon dioxide induces captive flies to rapidly become active and to swarm to the undersurface of the membrane [37].

Some physical properties of the membrane may also play a role in probing. Thus, colour, reflectance and roughness of the surface may enhance or reduce probing. Duration of probing of Stomoxys was extended on a rough surface of low reflectance [38]. Undulating the membrane surface rather than stretching it into a smooth surface improved the feeding performance of the oriental rat flea [24].

Once probing has been induced, a stimulus of a different type is necessary to stimulate specific "blood chemoreceptors" which supply the sensory output to switch from probing to gorging.

It has been observed by many workers that tsetse flies [29], mosquitoes [39], Rhodnius [40] and fleas [41] gorge very reluctantly on diets lacking erythrocytes.

If offered plasma alone, the tsetse fly will probe into the solution repeatedly for several minutes without gorging, while if red blood cells are present, the first probing will be followed by gorging a full size meal without withdrawal of the fascicles until the meal has been completed. This process is clearly illustrated by electronic recording of the feeding process, using a "bitometer" constructed in a manner similar to that of Kashin and Wakely [42]. In this apparatus, when the fly

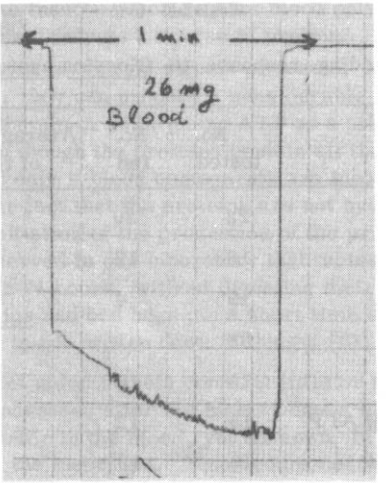


FIG. 2. Feeding pattern of Glossina austeni on beef blood.

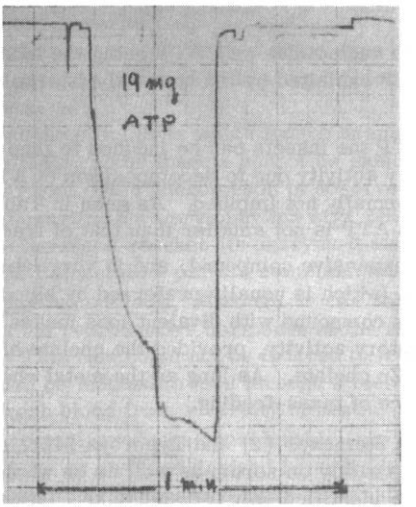


FIG. 3. Feeding pattern of Glossina austeni on 10^{-3} M ATP in 0.85% NaCl solution.

probes through the membrane and comes in contact with the test solution, an electrical circuit is completed and variations in the current flow are recorded on a strip chart recorder. As can be seen in Fig. 1, within 150 seconds the fly inserted and withdrew its fasciles 80 times, while on the blood meal the first probe is switched into active sucking (Fig. 2). Within 48 seconds 26 mg blood were imbibed by the fly [Margalit and Galun - unpublished].

Quite a few works in the last decade identified the feeding stimuli in the blood cells. Mosquitoes [39, 43], Rhodnius [40], fleas [41] and tsetse flies [44] are

TABLE I

Feeding response to Glossina austeni

Solution offered	No. tested	No. fed	Average weight of fly (mg)	Average weight of the meal (mg)
Living rabbit	22	20	14	22
Whole fresh bovine blood	18	17	15	22
Aged whole bovine blood	19	4	13	24
Bovine serum	38	1	15	-
Red blood cells	18	16	13	28
Aged whole blood + 10^{-3} M ATP	15	12	13	23
Bovine serum + 10^{-3} M ATP	19	19	15	30
Bovine albumin + 10^{-3} M ATP in 0.15 M NaCl	26	25	14	24

all stimulated by adenine nucleotides with ATP being the most effective one. The pattern of gorging on ATP exhibited by the tsetse fly is similar to that shown on blood (Fig. 3).

By the addition of ATP the insects can be induced to feed on serum, "aged blood" which lost its stimulatory activity due to decomposition of ATP, and even on synthetic diets which are normally not imbibed. As seen in Table I [taken from 22], the size of the meal with ATP is not smaller than that of fresh blood.

ATP is a relatively expensive compound, and is very labile especially upon heating and at an alkaline pH (which is usually preferred by blood-sucking insects [45, 46]). Chelating this compound with divalent ions makes it more stable, and it does not lose its stimulatory activity, provided the chelate has a stability constant lower than that of ATP-Zn chelate. As long as the metal chelate is not toxic, its addition may lower the price of mass-feeding.

Some insects such as Stomoxys [2] and Lyperoya [47], several species of mites [48] and ticks [49] feed readily on serum as well as on whole blood. Of these species feeding stimuli were identified only for soft ticks. Ticks can be stimulated to feed by either reduced glutathione of the red blood cells, or by several amino acids which are found in the plasma - together with glucose. The most effective amino acids were leucine, isoleucine and proline [49].

Until the phagostimulants of the haematophagous arthropods were recognized it was impossible to evoke optimum amounts of feeding on artificial diets, and in any nutritional study either a small amount of red blood cells was added to the diet [50], or the study was conducted on a host whose diet was controlled in order to render it deficient in certain components [51].

In spite of the fact that a variety of phagostimulants have been identified for several years, a chemically defined diet which supports the development of haematophagous arthropods has not been developed, so far. Studies in composing a defined

diet were limited mostly to insects which require blood only for reproduction, and evidently need the blood meal only as a source of protein.

When mosquitoes are fed enzymatic digests of casein, or a mixture of amino acids in a proper balance, they can produce a considerable number of eggs [6]. When fed bovine serum albumin or milk (using ATP as a stimulant), no ovarian development is induced even though the proteins contain all the essential amino acids. Vitellogenesis is initiated only if blood components are incorporated in the diet [52]. This is probably due to the fact that the proteins are not hydrolyzed into their amino acids due to the lack of initiation of the production of the proteolytic enzymes of the mosquitoes. We also observed in our laboratory that tsetse flies fed on bovine serum albumin died within 24 hours, without digesting their meal (unpublished results). Similarly, Rhodnius and bed bugs die a short time after engorgement of milk. It seems that milk is not broken down in the gut [31, 53].

The digestive system of unfed insects contains little or no proteolytic activity. The proteolytic activity increases after the insect takes a blood meal, and then decreases again [54]. A factor in the blood, yet unknown, is responsible for inducing the proteolytic activity in the insect gut. Blood components such as serum, plasma or washed erythrocytes [54], as well as some commercial proteins, induce proteolytic activity. However, the inducer does not have to be a protein and could easily be some unrelated compound which is normally found in blood but may be missing from many commercially available proteins. Thus, when preparing a synthetic diet, this factor should not be overlooked.

Insects feeding on certain hosts will develop more eggs than on others. Maximal number of eggs may often be obtained by feeding on blood obtained from hosts other than the natural ones.

In spite of the fact that the stimulants which induce haematophagy identified so far are common to all animals, some insects cannot be induced to feed on blood other than of their natural host. This phenomenon is observed especially among some flea spp. The sand-martin flea Cerytophyllus styx takes a small amount of mouse blood, and once the blood reaches its pharynx it stops feeding and does not fill its midgut (M. Rothschild - private communication).

The rabbit flea Spilopsyllus cuniculi, although it can sometimes be found on hare or cat, depends on the reproductive hormones of the rabbit for its normal reproductive cycle [55]. This flea could not be induced to feed through a membrane. However, when insects can be induced to feed through a membrane they will, in most cases, feed on fresh blood from a variety of hosts.

The mosquito Aedes aegypti produces considerably less eggs on human or monkey blood than on bird blood, or on blood derived from frogs or turtles [56, 57]. It was thought that nucleated blood cells were more nourishing. However, the fact that the mosquito produced on guinea pig and rabbit blood as many eggs as on nucleated cells, does not support this hypothesis. The most likely explanation is that the amino acid balance is more suitable in blood of certain hosts than it is in others. It was thus shown that sheep erythrocytes produce hardly any eggs in Aedes, but the addition to the diet of isoleucine increased egg production [50]. Haemoglobin of sheep or of man is very poor in isoleucine, while the haemoglobin of pig or rabbit is richer in isoleucine, and there, the addition of isoleucine does not increase egg production.

The tick Argas arboreus which parasitizes the heron produced many more eggs on heron or pigeon blood than on rabbit blood [58]. The tick Ornithodoros tholozani produced fewer eggs on goose blood, as compared to sheep or camel blood [Galun - unpublished]. Camel blood was found superior to any other blood when artificial

feeding was employed. This is probably due to its resistance to haemolysis. It was often found that when arthropods were fed haemolyzed or partly haemolyzed blood, a high mortality followed [51]. To overcome this possible toxic effect, it would have been much more convenient to feed the arthropods on plasma alone. Plasma can be stored deep frozen for any length of time, and remain unchanged. However, in feeding experiments, plasma is much inferior to whole blood probably because of its low protein content. The tick Argas arboreus laid no eggs on plasma of heron, chicken or rabbit, and only a few eggs on pigeon plasma [58]. Ornithodoros tholozani produced always less eggs on plasma, as compared to whole blood, when the following sources were compared: sheep, beef, goose and camel. However, the number of eggs can be increased by an addition of commercial proteins to the plasma [Galun - unpublished].

Plasma seems also to be insufficient for the developing ticks. When nymphs of Argas arboreus were fed plasma, none molted on heron, chicken or rabbit plasma, and a small percentage molted on pigeon plasma [58]. Ornithodoros tholozani fed on sheep plasma took longer periods to molt and an additional nymphal stage resulted, as compared to the development on whole sheep blood [Galun - unpublished].

From the review presented here, we can see that there is a lot of gaps in our information. Nevertheless, we are well equipped with enough basic knowledge to develop artificial rearing methods which could be adapted for mass rearing.

REFERENCES

[1] McGREGOR, W. S., DREISS, J. M., J. Econ. Entomol. 48, (1955) 327.
[2] KRIJGSMAN, B. J., WINDRED, G. L., Z. Vergl. Physiol. 13 (1930) 61.
[3] SCHMIDT, C. D., HARRIS, R. L., HOFFMAN, R. A., Ann. Entomol. Soc. Am. 60 (1967) 508.
[4] WOODBURY, E. N., "Laboratory procedures in studies of the chemical control of insects". Publ. No. 20, pp. 70-71, Am. Assoc. Adv. Sci., Washington D. C. (1943).
[5] OWEN, W. B., J. Ins. Physiol. 9 (1963) 73.
[6] LEA, A. O., DIMOND, J. B., DeLONG, D. M., Science(1956) 890.
[7] LANG, C. A., WALLIS, R. C., Am. J. Trop. Med. Hyg. 5 (1956) 915.
[8] LALL, S. B., Ent. exp. & appl. 12 (1969) 325.
[9] RODHAIN, J., PONS, C., VANDERBRANDEN, J., BEQUAERT, J., Arch. Schiffs. Trop. Hyg. 16 (1912) 732.
[10] ALLEN, J. R., WEST, A. S., Mosq. News 22 (1962) 157.
[11] BENJAMINI, E., FEINGOLD, B. F., KARTMAN, L., Nature 188 (1960) 959.
[12] HADDON, W., Jr., An. J. Trop. Med. Hyg. 5 (1956) 326.
[13] HUDSON, B. W., Bull. Wld Hlth Org. 19 (1958) 1129.
[14] COCKING, K. L., Rep. E. Afr. Trypan. Res. Org. (1960) 13.
[15] AZEVEDO, J. F., DINHAO, R., TEIA DOS SANTOS, A. M., An. Esc. Nac. Saude pabl. Med. Trop. 2 (1968) 11.
[16] TARSHIS, I. B., Proc. 10th Int. Cong. Entomology (1956) 767.
[17] GREGSON, J. D., Miscell. Pub. Entomol. Soc. Am. 6 (1970) 349.
[18] FULLER, H. S., MURRAY, E. S., SNYDER, J. C., Pub. Hlth Rept. 64 (1949) 1287.
[19] KARTMAN, L., Exp. Parasitol. 3 (1954) 525.
[20] LESTER, H. M. D., LLOYD, L., Bull. Ent. Res. 19 (1928) 39.
[21] YORKE, W., BLACKLOCK, B., Ann. Trop. Med. Parasitol. 9 (1915) 363.

[22] GALUN, R., MARGALIT, J., Proc. 1st Symp. on Tsetse fly breeding under laboratory conditions and its practical application. Lisbon (1969) in press.
[23] LANGLEY, P.A., MALY, H., Nature 221 (1969) 855.
[24] BAR-ZEEV, M., STERNBERG, S., Ent. exp. & appl. 5 (1962) 60.
[25] AVIVI, A., GALUN, R., WARBURG, M., Radiation, radioisotopes and rearing methods in the control of insect pests. (Proc. of a panel, Tel-Aviv 1966) IAEA (1968) 3.
[26] HADDON, W., Jr., Am. J. Trop. Med. Hyg. 5 (1956) 315.
[27] RUTLEDGE, L.C., WARD, R.A., GOULD, D.J., Mosq. News 24 (1964) 407.
[28] GALUN, R., KINDLER, S.H., Science 147 (1965) 166.
[29] MITTLER, T.E., DADD, R.H., Nature 195 (1962) 404.
[30] NICOLLE, P., Bull. Soc. Path. Exot. 34 (1941) 179.
[31] HARINGTON, J.S., Parasit. 50 (1960) 273.
[32] BURGESS, L., Nature 184 (1959) 1968.
[33] DETHIER, V.G., Am. J. Trop. Med. Hyg. 3 (1954) 160.
[34] GATEHOUSE, A.G., J. Ins. Physiol. 16 (1970) 61.
[35] HUGHES, J.C., Bull. Ent. Res. 48 (1957) 561.
[36] RUDOLFS, W., Bull. N.Y. Agr. Exp. St. 367 (1922) 1.
[37] McMAHON, J.P., Bull. Wld Hlth Org. 38 (1968) 957.
[38] GATEHOUSE, A.G., J. Ins. Physiol. 16 (1970) 991.
[39] HOSOI, T., J. Ins. Physiol. 3 (1959) 191.
[40] FRIEND, W.G., Canad. J. Zool. 43 (1965) 125.
[41] GALUN, R., Life Sci. 5 (1966) 1335.
[42] KASHIN, P., WAKELY, H.G., Nature 208 (1965) 462.
[43] GALUN, R., AVI-DOR, Y., BAR-ZEEV, M., Science 142 (1963) 1674.
[44] GALUN, R., MARGALIT, J., Nature 222 (1969) 583.
[45] GALUN, R., Bull. Wld Hlth Org. 36 (1967) 590.
[46] GALUN, R., MARGALIT, J., Trans. Roy. Soc. Trop. Med. Hyg. 64 (1970) 171.
[47] KRIJGSMAN, B.J., Z. Vergl. Physiol. 11 (1930) 702.
[48] CROSS, H.F., WHARTON, G.W., Ann. Entomol. Soc. Amer. 59 (1966) 182.
[49] GALUN, R., KINDLER, S.H., J. Ins. Physiol. 14 (1968) 1409.
[50] GREENBERG, J., J. Nutrit. 43 (1951) 27.
[51] DeMEILLON, B., GOLDBERG, L., J. Exp. Biol. 24 (1947) 41.
[52] CHEN, S.S., The neuroendorine regulation of gut protease activity and ovarian development in mosquitoes of Culex pipiens complex and the induction of imaginal diapause. Ph.D. Thesis, Purdue University (1969).
[53] BELL, W., SCHAEFER, C.W., Ann. Entomol. Soc. Am. 59 (1966) 53.
[54] WIGGLESWORTH, V.B., in "The principles of insect physiology" pp. 427 – 455. Methuen & Co., (1965).
[55] ROTHSCHILD, M., FORD, B., Proc. 12th Int. Cong. Entomol. 12 (1964) 801.
[56] WOKE, P.A., J. Trop. Med. 17 (1937) 720.
[57] BENNETT, G.F., Canad. J. Zool. 48 (1970) 539.
[58] TAWFIK, M.S., GUIRGIS, S.S., J. Med. Ent. 6 (1969) 191.

DISCUSSION

C. T. LEWIS: Is pore size a factor influencing the acceptability of an artificial membrane?

Rachel GALUN: No methodical study using the same membrane composition but changing the pore size was carried out.

C. T. LEWIS: If I may make a second comment, the use of a suitable ATP-metal chelate in an artificial diet would mean that all the flies reared would acquire a marker which could easily be identified by radioactivation analysis. This could be useful in a sterile-male-release program.

A. M. JORDAN: I would like to point out that at Langford we do not apply only 30 Glossina to one rabbit per day. Thirty are applied to one rabbit at one time. It is possible to apply up to 450 Glossina austeni to one rabbit, twice a week, without adversely affecting fly performance. After a period of one year's use the haemoglobin levels of the rabbit blood were slightly lowered, but not sufficiently to affect fly performance. Although a large number of hosts would be required for the mass-rearing of Glossina, it would hardly be necessary to mass-rear the host as well!

A. MEWS: With regard to the use of thin synthetic membranes, both Mr. Langley at Bristol and I myself have found that the conditions under such membranes greatly affect the engorgement response. When the feeding medium is soaked in an absorbent material, and the membrane placed on top, the flies engorge much more readily than when there is no such support under the membrane.

Rachel GALUN: I suppose that the cotton wool prevents settling of the red blood cells, and thus improves contact between the haustellum and the stimulatory fraction.

A. MEWS: Further to this, we have found that ATP is rapidly broken down to ADP and AMP.

The ATP in the saliva is specific and does not break down either GTP or UTP. Neither of these two substances is stimulatory in vivo.

The AMP formed does not appear to be in the cyclic form, nor is cyclic AMP stimulatory in vivo.

Do you think it possible that the saliva of the fly may play at least a mediating role in the process by which ATP stimulates the fly to engorge?

Rachel GALUN: Since it is recorded in the literature that both tsetse flies and mosquitoes can gorge successfully after cutting of their salivary duct, I tend to believe that saliva does not play a role in phagostimulation.

D. W. WALKER: What, in your opinion, are the specific physical properties that are important in selecting membranes for plant sap feeding of Homoptera and Hemiptera?

Rachel GALUN: Aphids were fed very successfully on Parafilm membrane, which apparently simulates the leaves. I assume that this membrane would be suitable for Hemiptera and Homoptera. However, the physical factors which make it a good membrane have never been studied.

P. MACHILI: One of the obvious differences between the artificial feeding system and the natural one is that whereas the artificial system is static, the natural one is dynamic. The presence of pressure in the natural system probably has a considerable effect both on the feeding mechanism of the insect (for example, its rate of feeding and quantity of blood imbibed) and also on the texture of the membrane. The presence of pressure in the fluid would stretch the membrane, thereby making it easier for the proboscis to penetrate it.

I wonder whether consideration has been given to the possibility of installing in the blood-filled membrane containers some sort of mechanism, a micropropeller for example, which would act like a heart and generate the necessary pressure.

Rachel GALUN: Experiments were conducted in which the blood offered was under pressure, but this did not appear to increase the efficiency of feeding.

MAINTENANCE OF *Glossina morsitans* WESTWOOD FED THROUGH AN ARTIFICIAL MEMBRANE ON DEFIBRINATED BLOOD

A.R. MEWS, F. RUHM
International Atomic Energy Agency,
Vienna

Abstract

MAINTENANCE OF *Glossina morsitans* WESTWOOD FED THROUGH AN ARTIFICIAL MEMBRANE ON DEFIBRINATED BLOOD.

The performance of nearly 300 *Glossina morsitans* females, when fed through an agar membrane on defibrinated blood, was determined by means of techniques developed at this laboratory. Ten females were kept per cage and records were kept of mortality and the weights of the pupae produced. A total of 170 females were fed on rabbits' ears to act as a control group. 89.5% of the membrane-fed females were alive after 72 days compared with 64.5% in the rabbit-fed controls. The longevity of the latter group was, however, adversely affected by the poor condition of the rabbits. The productivity (m_x) figures indicated that over 90% of the membrane-fed females produced a pupa in the first three reproductive cycles, but thereafter productivity fell sharply. This was in part due to a lowered mean temperature of the fly-holding room caused by a malfunctioning of a thermostat, and partly by increasing lethargy and reluctance to feed. The mean weight of the F_1 pupae produced by the membrane-fed flies was 22.9 mg or approximately 7 mg less than the rabbit-fed controls. Two groups of 150 F_1 females originating from the membrane-fed group were fed on membranes and rabbits respectively. The mean weight of the F_2 pupae produced by the rabbit-fed group was similar to those from flies that had been fed throughout on rabbits, while the weight of the F_2 pupae from the membrane-fed group was similar to the F_1 pupae from membrane-fed flies. In another experiment using *G. austeni*, in which the flies were caged individually, the membrane-fed flies took about the same weight of blood per meal but fed less often than the rabbit-fed controls. The results indicate that if the productivity of the membrane-fed flies could be sustained, a regime might be established where alternate generations of flies were maintained on membranes and rabbits respectively. This would reduce the number of live animals that needed to be kept as hosts.

INTRODUCTION

The ability to rear tsetse flies (*Glossina* spp.) efficiently without the use of live animals would eliminate many of the problems associated with the mass rearing of the fly for the sterile-insect method of control.

There were a number of early attempts to feed tsetse flies through membranes, including that by Roubaud [1], who fed *G. morsitans* Westwood through a chamois skin. More recently, *G. morsitans* and *G. pallidipes* Westwood were fed through a variety of natural and artificial membranes, on blood from different animals that had either been defibrinated or treated with ethylene diamine tetra acetate salt (EDTA) or heparin [2-4]. Fibrin membranes [5] have also been used by Azevedo et al. [6]. The use of an artificial membrane made from agar-agar was first described by Langley and Maly [7].

The purpose of the main experiment described in this paper was, using techniques described previously [7], to determine the performance of *G. morsitans* when reared on an artificial membrane. To isolate some of

TABLE I. SUMMARY OF DATA FROM EACH CAGE OF G. morsitans REARED ON AGAR MEMBRANES

Cage No.	Age of cage (days)	No. of original flies	No. of flies alive	No. of pupae deposited	Break-even point[a] (days)	Mean weight of pupae (mg) ± 2 × SE
1	83	10	7	46	35	23.4 ± 0.8
2	83	8	8	47	33	25.6 ± 0.8
3	82	10	9	51	38	23.4 ± 0.8
4	82	10	9	55	31	23.2 ± 0.8
5	82	10	9	55	38	22.9 ± 0.7
6	81	10	8	45	37	23.5 ± 1.0
7	81	10	9	59	33	23.0 ± 0.8
8	79	10	8	59	33	22.9 ± 0.8
9	79	10	9	50	32	22.4 ± 0.9
10	79	10	9	62	35	22.6 ± 0.7
11	79	10	9	53	32	21.8 ± 0.8
12	78	10	10	50	34	22.5 ± 0.7
13	78	10	9	41	48	20.7 ± 0.9
14	77	10	8	53	33	22.1 ± 0.9
15	77	10	8	47	34	23.1 ± 0.8
16	76	8	7	32	36	24.7 ± 1.0
17	76	9	5	39	38	23.6 ± 1.2
18	76	10	10	35	41	23.6 ± 1.1
19	75	10	7	42	36	22.3 ± 0.8
20	74	10	8	57	30	23.7 ± 0.9
21	74	10	9	45	35	22.1 ± 0.9
22	74	10	9	43	39	23.2 ± 0.9
23	74	13	11	51	42	22.5 ± 0.9
24	72	10	5	40	34	23.6 ± 0.9
25	72	10	8	40	38	23.4 ± 0.8
26	73	10	7	38	43	22.8 ± 0.9
27	73	10	10	39	43	21.6 ± 0.9
28	72	10	9	41	41	22.8 ± 0.8
29	72	10	10	47	38	23.0 ± 0.8
Total:	-	288	244	1362	-	-
Mean:	77.0	10	8.4	47.0	36.5	22.9 ± 0.2

[a] Break-even point = number of days for each cage to produce a mean of 2 pupae per female.

the factors responsible for the differences in performance of G. austeni Newstead when reared on an agar membrane or on rabbits' ears, a second experiment was run in which these flies were kept individually.

MATERIALS AND METHODS

Group rearing of G. morsitans

Normally, 10 female G. morsitans were kept per cage, although some variations did occur (see Table I). The fly cages were made from polyvinyl chloride (PVC) tubing 12.5 cm in diameter. This was cut into 4.5-cm lengths and then immersed in boiling water and compressed into a 16 × 8 cm oval shape. A 3-cm-diam. hole was cut in one end. The open sides of the cage were covered with black Terylene netting which was secured with PVC glue. The flies were added to, or removed from, the cage via the hole at one end which was closed with a cork. The flies were handled by cooling with a stream of air at 3 - 5° C in a specially designed cold box. The 10 females in each cage were mated 'en masse' at 2 - 4 days old by adding 12 males at least one week old. All but two males were removed from the cage approximately 3 days later. The two males were left to ensure complete mating. The flies were kept at 25 ± 1.5° C and 65 ± 10% relative humidity. The temperature fluctuation may have been greater than 1.5 degC during the last month of the experiment owing to a malfunctioning of a thermostat.

In the membrane-fed group 288 flies were fed on defibrinated bovine blood collected aseptically twice weekly. A pad of cotton wool was placed in the bottom of a sterilized 15 cm × 20 cm porcelain or glass dish and soaked in approximately 40 ml blood. A 2-mm-thick layer of 3% agar-agar in sterile physiological saline, strengthened with coarse black Terylene netting was placed over the blood-saturated cotton wool [7]. The dish was placed in a waterbath at 40 ± 0.25° C. The flies were offered food for 10 min daily except Sundays. The cages were fed in numerical order starting with Cage 1. The weights of the pupae deposited and the post-mortem details of any fly that died were recorded each day.

In the rabbit-fed group, 170 females were held in similar cages which were strapped to the ears of lop-eared rabbits daily, except Sundays [8]. The mean weight of the pupae from the rabbit-fed group was determined by weighing 100 pupae selected at random. This was to ensure that they were of similar weight to those pupae from previous experiments with G. morsitans fed on rabbits' ears (Mews, in preparation).

Individual rearing of G. austeni

In the individual fly experiment, using G. austeni, the flies were kept in small round cages 6 cm in diameter and 3 cm deep made from the same material and in the same way as the larger cages. Each cage was weighed before the fly was offered food on the membrane or rabbit's ear, and if it fed, it was re-weighed 15 min after feeding. This time was chosen to allow the moisture absorbed into the netting from the agar membrane to evaporate. The flies were offered food in exactly the same way as in the other experiment.

TABLE II. MEAN PUPAL WEIGHT OF SUCCESSIVE BATCHES OF 10 PUPAE PRODUCED BY EACH CAGE OF FEMALES SHOWN IN TABLE I (29 CAGES) (G. morsitans)

Pupae deposited	No. pupae considered	Mean weight (mg) (± 2 x SE)
1 - 10	290	23.2 ± 0.4
11 - 20	290	23.1 ± 0.4
21 - 30	290	22.6 ± 0.4
31 - 40	273	22.8 ± 0.4
41 - 50	164	22.9 ± 0.6
51 - 60	53	23.2 ± 0.8
61 - 70	2	22.8 -

TABLE III. MEAN PUPAL WEIGHT OF SUCCESSIVE BATCHES OF 10 PUPAE PRODUCED BY F_1 FLIES FED ON MEMBRANES AND RABBITS (G. morsitans)

Pupae deposited	No. pupae considered	Mean weight (mg) ± 2 x SE
Membrane-fed		
1 - 10	118	22.0 ± 0.5
11 - 20	74	22.4 ± 0.6
21 - 30	14	23.6 ± 1.1
overall	206	22.2 ± 0.4
Rabbit-fed		
1 - 10	144	28.3 ± 0.6
11 - 20	100	30.2 ± 0.6
21 - 30	26	31.9 ± 0.9
overall	270	29.4 ± 0.4

RESULTS

Table I gives a summary of the data recorded from each cage in the membrane-fed G. morsitans test. The mean weight of the F_1 pupae was some 7 - 8 mg less than the mean weight of 30.5 ± 0.7 mg (2 × SE) of the rabbit-fed controls. To determine whether the mean weight of successively deposited pupae tended to increase as is the case of the first 4 - 5 pupae from G. austeni [9] and G. morsitans (Mews, in preparation) females fed on rabbits' ears, the mean weight of successive batches of 10 pupae produced per cage of membrane-fed flies was calculated. Table II shows that there was no significant difference in the weight of pupae deposited early or late in the reproductive period.

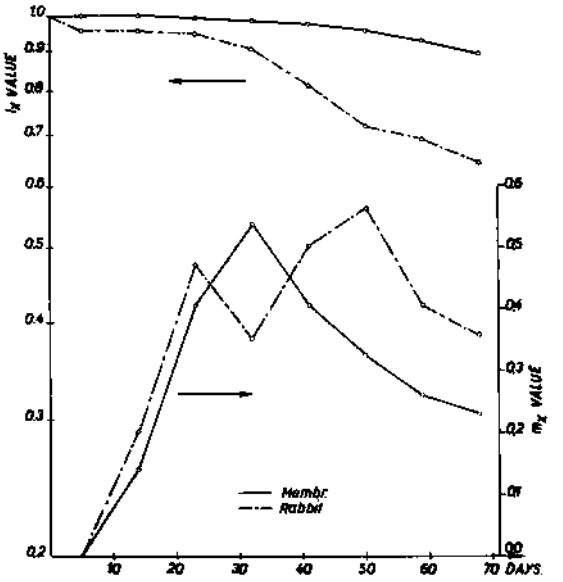


FIG. 1. Graph showing the probability of a female G. morsitans fly during successive 9-day periods of (a) being alive (l_x curves) and (b) depositing a female pupa (assuming a 1 : 1 sex ratio) (m_x curves).

To determine whether the lowering of the mean pupal weight affected the performance of the emerging flies, two groups of 150 F_1 females emerging from pupae deposited by the membrane-fed flies referred to above were fed on membranes and rabbits respectively. The results to date are given in Table III. While the mean weight of the first 206 F_2 pupae deposited by the membrane-fed F_1 group was only slightly lower than that of those deposited by the membrane-fed parental flies, the mean weight of those produced by the rabbit-fed F_1 flies was similar to that of the control group, which was continuously fed on rabbits.

Age-specific fecundity

The number of pupae produced by each cage of G. morsitans is given in Table I. The productivity or age-specific fecundity (m_x) [10] was measured in 9-day age groups and may be defined as the probability that a female fly will deposit a female pupa in any 9-day period. This method assumes an equal sex distribution of the emerging flies. A 9-day period was chosen because, although it is slightly less than the interlarval period for G. morsitans at 25° C, it is convenient for subsequent calculations and comparison with previously published work [11].

The m_x graph for the first eight age groups (72 days) is given in Fig. 1. The results of the first five age groups in both membrane- and rabbit-fed flies indicated that in the first three reproductive cycles the majority of the flies produced a pupa. Thereafter the productivity, particularly of the membrane-fed group, fell off rapidly. The possible causes for this are discussed later.

TABLE IV. RELATIONSHIP BETWEEN WEIGHT OF BLOOD INGESTED BY G. austeni AND WEIGHT OF PUPAE PRODUCED

Flies kept individually, and weight of blood meals (less primary excretion) recorded

Fly No.	No. days to deposit 5 pupae	Total blood uptake to deposition of 5th pupa	No. of blood meals	Mean amount of blood ingested per meal	Sum of pupal weight in mg (5 pupae)
Membrane-fed[a]					
M_1	62	697.8	19	36.7	90.0
M_3	61	747.4	24	31.1	101.4
M_4	63	601.9	30	20.1	88.7
M_5	59	773.8	33	23.9	109.4
Rabbit-fed					
R_1	56	1031.9	30	34.4	130.7
R_2	57	878.9	35	25.1	130.8
R_3	59	873.4	32	27.3	109.3
R_4	57	709.0	39	18.2	108.9
R_5	60	974.1	36	27.1	145.0

[a] Fly no. M_2 died after depositing 2 pupae and the results have not been included.

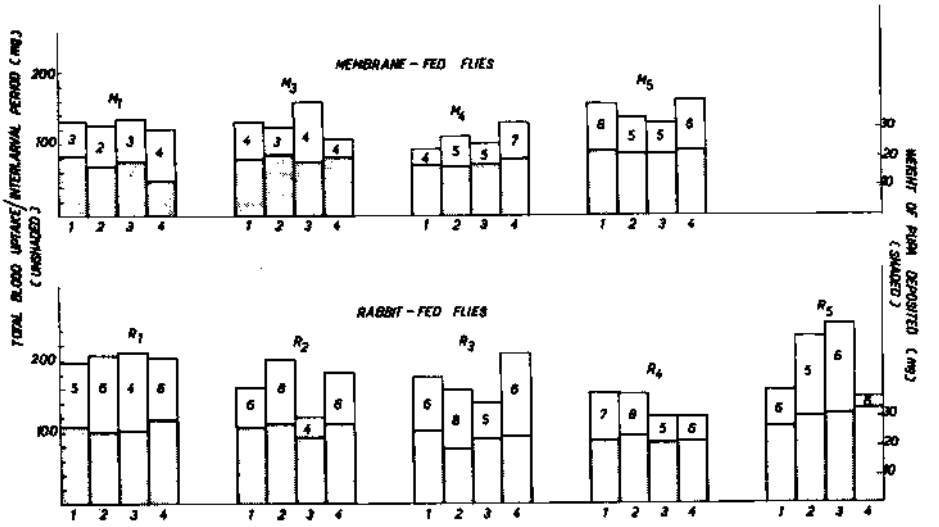


FIG.2. Comparison between membrane- and rabbit-fed G. austeni reared individually. The histograms show the relationship in G. austeni between the weight of blood ingested during each interlarval period and the weight of the pupae produced at the end of that interlarval period. The numbers at the base of each histogram indicate the interlarval period, and those at the top of each histogram the number of blood meals taken during that interlarval period. The total height of each histogram indicates the total amount of blood ingested during the interlarval period; and the shaded portion, weight of the pupa deposited. Because of the long period before the deposition of the 1st pupa, the weight of blood ingested up to the deposition of the 1st pupa, and the weight of the 1st pupa, have not been included. (The membrane-fed fly M_2 died after depositing 2 pupae and the results were not included).

Longevity

The longevity or probability that a female fly will be alive (l_x) was calculated from the number of flies alive at the mid-point of each 9-day age group. The results for both membrane- and rabbit-fed groups is plotted graphically in Fig. 1.

Individual rearing of G. austeni

The results of the individual rearing experiment are given here because, although G. austeni was used, they give a possible explanation for the decreased weight of pupae deposited by membrane-fed G. morsitans in the group-fed experiment. The results for the two groups of five G. austeni flies which were caged individually and fed on membranes and rabbits respectively are given in Table IV and Fig. 2. The membrane-fed group tended to take slightly larger blood meals, but, except for the period up to the deposition of the 1st pupa, this was more than offset by the fact that they fed less often than the rabbit-fed group. For the period up to the deposition of the 1st pupa, the mean amount of blood ingested and the mean weight of the pupa produced at the end of the period was respectively 198.2 mg and 20.8 mg for the membrane-fed and 192.3 mg and 22.5 mg for the rabbit-fed group.

While it may appear from Fig. 2, particularly in the case of the membrane-fed flies, that there was a relationship between the total amount of blood ingested during each interlarval period and the weight of the pupa produced, there was no statistical correlation between the two factors as found by Boyle [12]. This was probably due to the small sample size and the biological variation involved.

DISCUSSION

The longevity of the rabbit-fed G. morsitans over the first 72 days was poor when compared with that of G. austeni reared in a similar manner by others [11], or by Mews (in preparation) in previous experiments. This was probably due to the overuse and poor health of the rabbits. On the other hand, the longevity of the membrane-fed G. morsitans group over the first 72 days was better than that obtained by Jordan and Curtis [11] with G. austeni reared on rabbits. It indicates that the G. morsitans flies were receiving enough nutriment from the defibrinated blood for their basic metabolism to survive well during the period considered.

In other experiments considerable mortality often occurred in a single cage or group of cages and was always associated with a blackened, distended abdomen at post-mortem. The reason for this sudden mortality is being investigated, but it is thought to be due to bacterial contamination of the membrane or blood. The rigorous aseptic precautions that were taken in the present experiment may have prevented the occurrence of this problem, although in one of the cages of F_1 membrane-fed flies considerable mortality from this cause did occur within a period of 48 hours.

There are considerable difficulties in ascertaining the cause of the smaller mean pupal weight of the membrane-fed flies. The results of the individual fly experiment using G. austeni indicated that they fed less often than the rabbit-fed controls. It would seem more likely, therefore, that the lowering of the F_1 pupal weight was due rather to the lowering of the total weight of blood ingested than any basic deficiency in the defibrinated blood. Possible causes of the smaller amount of blood ingested are the relative unattractiveness of the agar membrane (see below) or a lowered rate of digestion of the defibrinated blood in the membrane-fed flies. It was sometimes noted that there appeared to be more undigested blood showing in the abdomen of the membrane-fed flies 24 hours after feeding than those fed on rabbits. However, no differences in the protease activity or rate of digestion could be detected between membrane- and rabbit-fed flies when the techniques described by Langley [13, 14] were used.

The productivity (m_x) graph for both membrane- and rabbit-fed G. morsitans females indicated that over the first three reproductive cycles the majority of the flies produced a pupa, and the figures are comparable with those of G. austeni over a similar period [11]. Over the last month of the experiment the temperature of the fly-holding room sometimes fell at night to as low as 21° C, owing to the malfunctioning of a thermostat. Thus, although part of the fall in productivity in both rabbit- and membrane-fed groups was associated with this cause, the productivity of the membrane-fed group fell faster than that of the flies fed on rabbits, and occurred at the same time as an increasing lethargy and reluctance to feed of the flies. The reasons for this are being investigated.

Attractiveness of the membrane

A variety of stimuli are involved in both the long- and short-range location of a host by the tsetse fly. However, these are greatly modified in the laboratory; for instance, when a live animal was placed in a cage of tsetse flies, few found the animal and fed [15]. Dethier [16] was unable to elicit any probing response of laboratory-reared tsetse flies to human and guinea-pig odours. However, butyric and valeric acid vapours have been shown to attract tsetse flies [17].

Gatehouse [18, 19] has done an elaborate series of tests with another blood-sucking dipteran, Stomoxys calcitrans L. He found that fresh blood, sweat and air drawn over a human subject's arm failed to induce probing, although a rapid increase in relative humidity combined with temperatures approximating to that of human skin did elicit a probing response, particularly when combined with a high concentration of ammonia.

In experiments still in progress, the agar membrane produced a higher probing response than certain inert membranes tested, and this may have been due, at least in part, to the high relative humidity just above the agar membrane. However, the fact that the agar membrane-fed flies did not feed so often as the rabbit-fed ones (Table IV) indicates that this membrane was not as attractive as a rabbit's ear. When a cage of flies that had been offered food on the membrane was then placed on a rabbit's ear, some of those which had refused to attempt to feed on the membrane, fed on the rabbit's ear. The reverse did not hold true, however, and flies that would not feed on the rabbit's ear could not be induced to feed on the membrane.

Stimulus to engorge

Both physical and chemical stimuli are probably involved in stimulating the fly that has probed to engorge. Langley and Maly [7] thought that one of the reasons for the success of the agar membrane was that its thickness made it necessary for the fly to insert the haustellum up to the bulbous base of the theca, a characteristic of the behaviour of flies feeding in nature.

One of the drawbacks of the agar membrane is that it deteriorates after a few hours of use, and while at first the majority of the flies that probed fed rapidly, it was noted that after some time an increasing proportion probed many times before being able to feed. The membrane was, for this reason, not used for more than about 2 - 3 hours.

Once the proboscis has pierced the membrane, the provision of a cotton wool pad to absorb the blood prevents the immediate dispersion of the saliva injected by the fly [7]. This may be the reason for an improvement in the number of flies feeding on an inert membrane when the blood was either soaked in cotton wool or gauze, or the depth of the blood pool was small, as opposed to when a deeper blood pool was provided.

Galun and Margalit [20] found that the presence of ATP was necessary for the engorgement of G. austeni, and it has been suggested that the haemolysis of some of the red blood cells due to contact with the agar membrane even when made with physiological saline, released the ATP from the cells and this helped to stimulate the flies to feed (Langley, personal communication, 1969).

The results described in this paper indicate that there are a number of problems still to be overcome before a self-supporting colony of tsetse flies can be efficiently reared by feeding through artificial membranes on defibrinated blood. However, if the productivity of the older flies could be improved, a regime might be established in which alternate generations of flies were reared with membrane techniques and live animals. This would reduce the number of live animals that would have to be kept as hosts.

Current research

There are a number of problems associated with the use of freshly collected defibrinated blood, and the Seibersdorf Laboratory of the IAEA is investigating the use of freeze-dried blood as a substitute. Preliminary results are encouraging. If this technique can be successfully developed it has a number of distinct advantages over freshly collected defibrinated blood:

(i) Large amounts of blood may be processed at any one time and then stored indefinitely without deterioration;

(ii) A sample may be tested for bacterial contamination and nutritive quality before the batch is released for use;

(iii) The necessity of keeping large numbers of cattle for regular bleeding would be eliminated. The cattle may be brought in as required, bled and then dressed for human consumption. This may be done at a point remote from the mass-rearing laboratory, and the blood shipped in amounts sufficient to last any period of time.

The development and testing of membranes is continuing. One of the main problems associated with any membrane used so far is the lack of attractiveness of the membrane. Studies of the factors involved in the attraction to the membrane and feeding behaviour of G. morsitans are proceeding; in these studies use is made of an electronic recording device based on the technique devised by Kashin and Wakeley [21].

Techniques for mass-rearing and sterilizing the fly for the sterile-insect release method are being investigated.

REFERENCES

[1] ROUBAUD, E., Histoire d'un élevage de Glossina morsitans à l'Institut Pasteur de Paris, Bull. Soc. Path. exot. 10 (1917) 629-40.

[2] COCKINGS, K.L., Laboratory maintenance of Glossina pallidipes, E. Afr. Tryp. Res. Org. Rept. 1959, Govt. Printer, Nairobi (1960) 13-18.

[3] COCKINGS, K.L., Laboratory maintenance of Glossina pallidipes, E. Afr. Tryp. Res. Org. Rept. 1960, Govt. Printer, Nairobi (1961) 32-33.

[4] SOUTHERN, H.A.W., COCKINGS, K.L., Laboratory maintenance of Glossina, E. Afr. Tryp. Res. Org. Rept. 1961, Govt. Printer, Nairobi (1963) 30-33.

[5] KIMBER, C.D., HARLEY, J.M.B., A fibrin membrane for feeding Glossina, E. Afr. Tryp. Res. Org. Rept. 1963-64, Govt. Printer, Nairobi (1964) 45.

[6] AZEVEDO, J.F., PINHÃO, R. da C., dos SANTOS, A.M.T., Artificial breeding of tsetse fly: 1. Factors connected with the feeding membrane and blood meals type, An. Esc. nac. Säude públ. Med. trop. 2 (1968) 11-17.

[7] LANGLEY, P.A., MALY, H., Membrane feeding technique for tsetse flies (Glossina spp.), Nature, Lond. 221 (1969) 855-56.

[8] NASH, T.A.M., JORDAN, A.M., BOYLE, J.A., A promising method for rearing Glossina austeni Newst. on a small scale, based on the use of rabbits' ears for feeding, Trans. R. Soc. trop. Med. Hyg. 60 (1966) 183-88.

[9] JORDAN, A.M., NASH, T.A.M., BOYLE, J.A., Pupal weight in relation to female age in Glossina austeni Newst., Bull. ent. Res. 58 (1969) 549-51.
[10] GLASGOW, J.P., The Distribution and Abundance of Tsetse, Pergamon Press, Oxford (1963).
[11] JORDAN, A.M., CURTIS, C.F., Productivity of Glossina austeni Newst. maintained on lop-eared rabbits, Bull. ent. Res. 50 (1968) 399-410.
[12] BOYLE, J.A., Blood intake in the reproductive cycle of Glossina austeni Newst. in relation to pupal weight, X Seminar on Trypanosomiasis, London, 1969, Trans. R. Soc. trop. Med. Hyg. 64 (1970) 186.
[13] LANGLEY, P.A., The control of digestion in the tsetse fly, Glossina morsitans, Enzyme activity in relation to the size and nature of the meal, J. Insect Physiol. 13 (1966) 439-48.
[14] LANGLEY, P.A., The control of digestion in the tsetse fly Glossina morsitans: A comparison between field flies and flies reared in captivity, J. Insect Physiol. 13 (1967) 477-86.
[15] MELLANBY, H., MELLANBY, K., Rearing tsetse flies in captivity, Proc. R. ent. Soc. Lond. (A) 12 (1937) 1-3.
[16] DETHIER, V.G., Notes on the biting response of tsetse flies, Am. J. trop. Med. Hyg. 3 (1954) 160-71.
[17] HUGHES, J.C., Olfactory stimulation of tsetse flies and blow flies, Bull. ent. Res. 48 (1957) 561-79.
[18] GATEHOUSE, A.G., The probing response of Stomoxys calcitrans to certain physical and olfactory stimuli, J. Insect. Physiol. 16 (1970) 61-74.
[19] GATEHOUSE, A.G., Interaction between stimuli in the induction of probing by Stomoxys calcitrans, J. Insect. Physiol. 16 (1970) 991-1000.
[20] GALUN, R., MARGALIT, J., Adenine nucleotides as feeding stimulants of the tsetse fly Glossina austeni Newst., Nature, Lond. 222 (1969) 583-84.
[21] KASHIN, P., WAKELEY, H.G., An insect bitometer, Nature, Lond. 208 (1965) 462-64.

DISCUSSION

J. ITARD: During artificial feeding of tsetse flies use has to be made of blood to which an anticoagulant has been added or which has been defibrinated. Is there not a danger that the use of blood which cannot coagulate might upset the metabolism of the fly?

A.R. MEWS: The best performance of flies fed on blood through a membrane has been obtained using defibrinated blood. Blood which has been prevented from clotting by the addition of anticoagulants has not proved so successful. Citrated blood severely upsets the metabolism of the fly. It is not known whether the poor performance of flies fed on defibrinated blood through a membrane is due to the fact that the blood cannot clot normally. We have so far been unable to detect any differences in the rate of digestion between membrane-fed and rabbit-fed flies.

J. ITARD: Has Mr. Mews considered the use of an artificial heart for the artificial feeding of the flies?

A.R. MEWS: No, I hadn't considered this interesting suggestion, although, so far as I am aware, anticoagulants are added to the blood to prevent clotting when artificial hearts are used.

Rachel GALUN: I agree with Mr. Itard that membrane feeding is not identical with feeding on the host. Primary excretion in tsetse flies is delayed when they are fed on a membrane. Mosquitoes lay eggs one day later when fed on a membrane than when fed on a host. However, we do not know whether or not these phenomena are due to defibrination of the blood.

INVESTIGATIONS WITH A VIEW TO MASS REARING OF Sparganothis pilleriana SCHIFFERMÜLLER FOR GENETIC CONTROL

K. RUSS
Bundesanstalt für Pflanzenschutz,
Wien, Austria

Abstract

INVESTIGATIONS WITH A VIEW TO MASS REARING OF Sparganothis pilleriana SCHIFFERMÜLLER FOR GENETIC CONTROL.

Sparganothis pilleriana Schiffermüller (Lepidoptera: tortricidae) appears every year in certain localized areas of the wine-growing regions of Lower Austria and Burgenland, causing great damage through larvae feeding on buds and leaves of vine. It develops only one generation per year all over the wine-growing area of Austria. The control of larvae by chemical methods has not been possible because no known pesticides achieve the desired effect. Therefore investigations were started to study using the genetic control technique, since the localized distribution of this grape-moth, its comparatively short flying period of about 3 weeks, and the fact that the females mate only once, greatly favour the sterile-male method of genetic control. It was first necessary to overcome the difficulties of rearing the species under laboratory conditions. A method had to be found to breed larvae on secondary host plants (Vicia sativa, Vicia faba) as well as on artificial diet (Montfavet-recipe). In these feeding tests it was shown that the weight of pupae descending from larvae feeding on this artificial diet is higher than from pupae reared on secondary or natural host plants (vine-leaves). Some differences were found in mating behaviour between moths reared on various diets. Comparing egg production in different breeding methods the highest number of eggs per female was found in females developed on artificial diet. Several investigations were carried out with the aim of advancing from rearing of individual larvae to mass rearing, and it should be possible to develop a promising mass-rearing method in the near future.

INTRODUCTION

In Austria, Sparganothis pilleriana Schiffermüller is one of the most important pests in viticulture. It occurs only, however, in certain parts of the wine-growing areas of Lower Austria and Burgenland [1]. Within these areas it is found only in restricted parts where it favours the low-culture system (vine culture on props). In vineyards with the high-culture system (vine culture on trellises) it is extremely rare [2].

The pest can be found on many other plant species, but never to a great extent; the grape-vine is clearly the favoured host [3].

The larvae cause the damage on the vine mostly by feeding on the tips of the shoots, thus damaging the growing points, but they feed on leaves as well. Feeding damage was rarely observed on inflorescences.

Depending on the weather, the flying time of the moth falls between the beginning and middle of July and lasts hardly longer than 3 weeks. During this period, the females lay eggs in clusters on the leaves in an average number of 150-250. Before starting to feed, the larvae, hatching after one week, move immediately to the parts of the vine close to the ground and produce the cocoons under the bark of the 2-year-old vine branches. In these cocoons they spend the rest of the warm season and the winter up to the middle or end of December in 'eudiapause', followed by quiescence until the start of feeding. At the end of April, they leave the

cocoons and move to the unfolding buds, where they start to feed. At first, two or three larvae feed on one growing tip. Later, they develop a pronounced territorial behaviour which leads to a stage where only one larva is found on each tip [4]. The growing tips become wrapped up with webs, and feeding commences in the centre of the web. As the vine branches grow, the larvae move further up. The webs produced by the larvae outline their feeding territory and are fiercely defended against intra- or interspecific intruders. Investigations proved that this territorial behaviour leads to an even distribution of the larvae in their habitat.

CONTROL

Conventional chemical control

Spraying at budding time. Preparations of the type 'Oleoparathion' used immediately before, or at the time of, budding, are partly successful against the larvae that survive the winter. Complete control, however, is not possible by this method.

Summer treatments. Sprayings with Parathion after budding – about the middle of May – are effective only against the larval stages 1 - 3. The stages 4 - 6 are not controlled effectively enough, and in spite of these efforts damage to the vine can always be observed.

INVESTIGATIONS AIMED AT USING THE AUTOCIDAL METHOD IN AUSTRIA

So far, no tests have been carried out to sterilize males of S. pilleriana. However, important preliminary work has been done.

Investigations into diapause

Mass-rearing the pest under laboratory conditions all the year round is an important prerequisite with the autocidal technique. At first, the 'eudiapause' of S. pilleriana was an obstacle to mass rearing. An effort was made to break up the univoltinity (one generation per year), i.e. to shorten the diapausal development time as far as possible, in order to achieve a more frequent succession of generations. Numerous tests, in which the larvae were exposed to different light treatments, failed to change the univoltinity. But we were able to shorten the diapausal development time considerably by using temperatures between 6 and 8°C, and an optimal succession of generations was obtained by this method.

Figure 1 shows that the optimal reduction of the diapausal development can be obtained at a temperature between 6 and 8°C during a treatment time of 65 to 70 days, counting from the commencement of treating freshly hatched larvae. From this moment on, all young larvae are able to commence post-embryonic development, if temperatures above the developmental minimum of 13°C are used [5]. If the hatched larvae are kept at temperatures below this limiting value, they remain inactive; they are in quiescence, however, and not in diapause any longer. By this technique, large quantities of young larvae can be stored for quite long

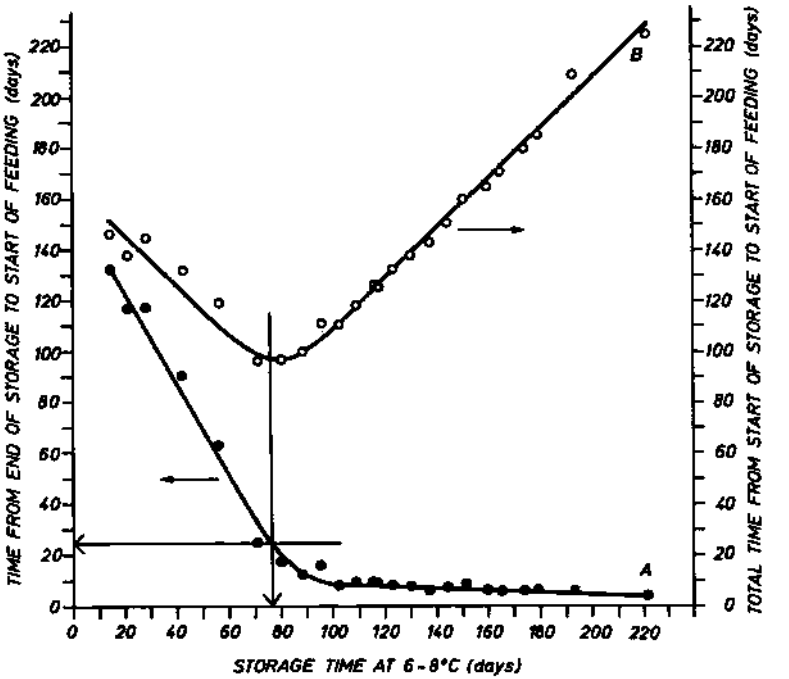


FIG. 1. Relationship between start of feeding and storage time at 6-8°C.
Curve A: Time between end of storage and start of feeding, in relation to storage time.
Curve B: Total storage time plus time between end of storage time and start of feeding, in relation to storage time.

periods. At a given time it is possible to trigger off further development by raising the temperature. Tests showed that this method allows larvae to be stored for up to a year without appreciable mortality. Such stored larvae mostly start developing immediately after a rise in temperature.

Copulation behaviour

In laboratory and field observations it was noted that the mating of S. pilleriana is induced by certain light intensities. In the field, the copulation takes place at the beginning of the astronomical dawn, i.e. when the sun is 18° below the horizon. This observation was tested by simulating this condition in the laboratory [6].

Males mate several times, but females only once. Egg laying takes place in the night following the mating.

INVESTIGATIONS INTO BREEDING IN THE LABORATORY

Laboratory breeding by rearing the larva individually on Vicia sativa

Vicia sativa was selected from the great number of potential hosts because it could be cultivated all the year round. At the appropriate feeding tests, the larvae were kept individually in small polystyrene

TABLE I. CONSTITUTION OF ARTIFICIAL DIET (Montfavet-recipe)

Water	170 ml
Agar	3 - 5 g
Wheat germs	11 g
Maize semolina	11 g
Powdered Vicia faba	11 g
Yeast	11 g
Ascorbic acid	1 g
Maize oil	0.4 ml
Nipagin	0.35 g
Benzoic acid	0.35 g
Aureomycin	5 mg

vessels (125 ml), and the food was changed every second day. As these tests showed, Vicia sativa is well suited for the individual rearing of larvae. Bacterial or fungal diseases occurring from time to time were generally stopped quickly by using 'Dimanin'.

Rearing of larvae on Vicia sativa under greenhouse conditions

Tests carried out to rear the larvae on Vicia sativa under greenhouse conditions had no satisfactory results. The breeding always failed because of the weakness of the host plants. Soon after the plants were inhabited by the larvae they had to be replaced by new plants, because they were so badly covered with webs and damaged. Under these circumstances, a rational rearing of larvae was not possible.

Rearing of larvae on Vicia faba under greenhouse conditions

Plants of Vicia faba, as an alternative to Vicia sativa, were well suited for rearing S. pilleriana in the greenhouse. Vicia faba, which is a more robust plant than Vicia sativa, did not need to be replaced until the development of the larvae.

At the start of the pupal stage, nearly all the pupae could be collected with strips of corrugated cardboard placed between the plants.

We were able to get relatively large numbers of pupae in a very small greenhouse space by using Vicia faba. A total of 20 to 25 larvae per flower pot could be reared on 10 to 15 plants up to the pupal stage. This method yields 400 to 500 pupae/m^2.

The vitality of the larvae reared in this way is as good as that observed in the field; the copulation was normal.

Rearing of larvae on artificial diet

Individual rearing

Tests were carried out to rear S. pilleriana to the imaginal stage on an artificial diet. We employed the diet that had been successfully used on different Lepidoptera by the Montfavet INRA zoological station. Its

composition is given in Table I. The original recipe has been changed only by adding dried and powdered Vicia faba leaves and stems. The larvae reared on this diet in small polystyrene vessels (125 ml) developed without significant losses. The average weight of the pupae found was higher than from pupae reared on Vicia sativa and Vicia faba, and even higher than from pupae developed in vineyards. Tests are being carried out replacing the agar by paper pulp; preliminary tests gave satisfactory results.

Collective rearing

Satisfactory breeding results from individual rearings on artificial diet encouraged us to carry out preparatory tests for mass breeding. As difficulties were to be expected by the pronounced territorial behaviour of larvae, different methods were tested to separate the individuals. Polystyrene dishes (150 mm × 180 mm × 30 mm) and polystyrene buckets (1000 ml) were used as containers.

At first, strips of corrugated cardboard were used to separate the individuals and to provide one tunnel for each. It was noted, however, that the corrugated cardboard cover led to heavy fungus infestation of the artificial diet. This happened even when the cardboard was replaced by corrugated plastic lined with oiled paper. Good results could be obtained by using 'Perlite'. Fungus infections occurred rarely, and the larvae were able to produce proper webs on the granulated substance, where they can live isolated in spite of a great population density.

Tests carried out recently showed that the larvae can also be reared without using different materials for separating the individuals. This seems to make mass breeding possible. Apparently abundance in food supply can suppress the territorial behaviour of the caterpillars.

COMPARISON OF THE PUPAL WEIGHT FROM DIFFERENT REARING TECHNIQUES

Relatively different values of pupal weight from the various rearing methods were noted (Table II). The weight was highest in larvae reared individually on artificial diet. Very favourable results were noted from collective rearing on Vicia faba in the greenhouse. Very similar pupal weights were found on pupae developed in vineyards or in pupae reared individually on Vicia sativa.

Although there was a very high average pupal weight from individual rearings on artificial diet, this led to copulation difficulties and to a changed mating behaviour. Quite often, morphological defects, e.g. deformed wings or twisted or shortened antennae, were observed. Moths from pupae with normal weight, however, showed normal reactions in their adult behaviour and did not display any morphological aberrations. The diet used for rearing the larvae individually is probably too rich, and therefore we are confronted with an effect of 'overfeeding'.

In our collective rearings, this phenomenon has not been observed.

TABLE II. AVERAGE WEIGHT OF MALE AND FEMALE PUPAE FROM DIFFERENT BREEDING TECHNIQUES

Sex	Artificial diet (larvae reared individually)		Vicia sativa (larvae reared individually)		Vicia faba (greenhouse, collective breeding of larvae)		Grape vine (larvae from vineyards)	
	No.	Aver. pupal weight (mg)	No.	Aver. pupal weight (mg)	No.	Aver. pupal weight (mg)	No.	Aver. pupal weight (mg)
♂	32	50.68	48	33.01	80	41.12	36	36.07
♀	33	78.74	32	46.22	80	63.75	54	48.73

TABLE III. AVERAGE NUMBER OF EGGS FROM DIFFERENT REARING TECHNIQUES AND PERCENTAGE OF HATCHED OR UNHATCHED EGGS

Method of rearing	No. of ♀	Aver. number of eggs per female	Aver. number of unhatched eggs	Aver. % of unhatched eggs	Aver. number of hatched eggs	Aver. % of hatched eggs
Artificial diet (larvae reared individually)	52	298.0	107.6	36.1	190.4	63.9
Vicia faba (larvae reared collectively in greenhouse)	34	241.8	29.8	12.3	212.0	87.7

FERTILITY OF FEMALES FROM DIFFERENT REARING TECHNIQUES

There are significant differences in the number of eggs and the percentage of viable larvae hatched from the different rearing techniques (Table III). As can be seen, on average essentially more eggs were laid in individual rearings on artificial diet, but the percentage of hatched viable larvae was remarkably smaller as compared with larvae reared on Vicia faba in the greenhouse. In practice, preference will be given for economic reasons to the rearing on artificial diet, in spite of a probably smaller number of young larvae. The breeding on secondary hosts would have the advantage of supplying imagines of better quality, but intensive mass breeding with this method is not feasible because of the inferior use of space. Certainly it will be possible to improve considerably the collective breeding on artificial diet, since the investigations carried out so far represent preliminary tests only.

DISCUSSION

The genetical control of S. pilleriana would be a great boon to Austrian viticulture. The well-defined isolated habitat of the pest would undoubtedly make for effectiveness in the use of this technique. The relatively low population density caused by the territorial behaviour of the larvae would also help towards success. The fact that there are a large number of potential hosts may cause some problems. That the female mates only once is an advantage, but this is not always a prerequisite for successful application of this method of control. An alternative method to chemical control is needed, and success here with the sterile-release method would encourage its use for controlling other pests.

REFERENCES

[1] RUSS, K., Beitrag zur Biologie und Bekämpfung des Springwurmwicklers (Sparganothis pilleriana Schiffermüller) im niederösterreichischen Weinbaugebiet, Pflanzenschutzberichte XXIII (1960) 129-170.

[2] RUSS, K., Der Einfluss der Erziehungsart des Weinstockes auf den Befall durch den Springwurmwickler (Sparganothis pilleriana Schiffermüller), Der Pflanzenarzt 23 (1970) 2-3.

[3] STELLWAAG, F., Die Weinbauinsekten der Kulturländer, Verlag P. Parey, Berlin (1928).

[4] RUSS, K., Beiträge zum Territorialverhalten der Raupen des Springwurmwicklers, Sparganothis pilleriana Schiffermüller (Lepidoptera: Tortricidae): Vorläufige Mitteilung, Pflanzenschutzberichte XL (1969) 1-10.

[5] RUSS, K., Untersuchungen über den Einfluss des Lichtes auf die Geschwindigkeit der Embryonalentwicklung des Springwurmwicklers, Sparganothis pilleriana Schiffermüller (Lepidoptera: Tortricidae), Die Weinwissenschaft 24 (1969) 248-257.

[6] RUSS, K., Untersuchungen über die Abhängigkeit der Sexualbiologie des Springwurmwicklers (Sparganothis pilleriana Schiffermüller) von diurnalen Licht-Dunkelsituationen, Pflanzenschutzberichte XXXIV (1966) 161-190.

DISCUSSION

M. E. TZANAKAKIS: Is there an explanation for the fact that high-culture vines are more or less free of this pest?

K. RUSS: We have evidence that S. pilleriana infestation is greater by a factor of five in low-culture vines than in high-culture systems. This is also the case if the two culture systems are applied in the same area.

We have also made investigations which showed that egg-laying takes place only at heights not exceeding 0.7 m. So I think that for this insect areas close to the soil provide more favourable conditions. Its behaviour in this respect may enable us to carry out an integrated control program by combining the use of a high-culture system with other control measures.

I. A. KANSU: We have S. pilleriana in Turkey, and have found that it prefers dense cultures of Vitis vinifera, avoiding excessive air and sunlight. Humid weather and shade provide suitable conditions for it. I think this may explain why the insect is rare in high-culture systems.

T. JERMY (Chairman): Did you make estimates of the population on other host plants, Mr. Russ?

K. RUSS: No, but in general S. pilleriana occurs very rarely on other plants.

T. JERMY: I should also like to ask whether any data are available on the migration of this pest.

K. RUSS: No exact data are known. However, observations carried out in vineyards show that the flight range of the moth is limited.

J. G. P. PETTERSON: Have you made any investigations on the possible occurrence of pheromones or sex attractants, for example of the aphrodisiac type? From the description you gave of the behaviour of the insects the existence of something of this nature seems probable.

K. RUSS: We ourselves have not done so, but I know that investigations on this aspect are being carried out at the Weinbauinstitut (Institute of Viticulture) in Freiburg, Federal Republic of Germany, by Dr. Götz and his co-workers.

STERILITY PRINCIPLE AND INSECTS ATTACKING FOOD AND FIBRE CROPS

(Session VI)

Chairman

L. MELLADO (Spain)

Survey paper

GENETIC CONTROL EXPERIMENTS ON THE BEAN WEEVIL, Acanthoscelides obtectus SAY

T. JERMY
Research Institute for Plant Protection,
Budapest, Hungary

Abstract

GENETIC CONTROL EXPERIMENTS ON THE BEAN WEEVIL, Acanthoscelides obtectus SAY.

Papers dealing with experiments on the genetic control of the bean weevil are reviewed, including work on mass rearing, radiation sterilization, population estimation, and competitiveness of sterilized males. Some aspects of the biology of the pest relevant to the use of the genetic control method are also discussed. No field experiments have so far been carried out; however, the data available indicate that the bean weevil is a very good candidate for control by the sterile-release technique in the field, at least in temperate regions.

INTRODUCTION

When comparing the dramatic and continuing increase in published work on the sterile-release technique to the number of agricultural pests which are already controlled in practice by this method, one has to conclude that after the very encouraging initial practical successes achieved by American entomologists in the 1950s and early 1960s, the more recent achievements in practical control bear no satisfactory relation to the increasing efforts made in this work throughout the world. The causes of this slackening in the pace of development are more or less known to those engaged on sterile-release projects, and they have been discussed at several FAO/IAEA meetings, but they are much less known to the agencies and authorities who decide on the distribution of research funds. The situation is made worse by the fact that in many cases the feasibility of the sterile-release technique has been overemphasized by authors of popular scientific works, and sometimes oversold by the scientists themselves.

It can be assumed that most of the scientists working in this challenging but very difficult field have the feeling that there is an urgent need for concentrated efforts, first, to achieve further proofs of the practicability of genetic control, and second, to prevent all further overselling of the results gained so far, because otherwise we could lose face both with the fund-providing authorities and with our farmers.

With these points in mind, the aim of the present review paper is to draw attention to an agricultural pest, the bean weevil, which seems to be one of the species suitable for providing further proof – and within a short period – of the feasibility of genetic control. Confidence in the outcome of such a project is based partly on the relatively ample data available on

the biology of this pest, showing several characteristics favourable to genetic control, and partly on the encouraging experimental work already carried out on this species.

The economic importance of the bean weevil is a further reason for its more intensive study. The pest attacks very valuable crops, causing serious damage both in warm and temperate climates, and since it is a plant quarantine pest, difficulties are encountered in exporting attacked products. Moreover, in many countries chemical control in the field – where the primary infestation occurs – is hindered because the beans may be grown as an intercrop in maize, sunflower, etc.

The following analysis is mainly valid for the temperate regions, since the ecology of the pest has been most intensively studied there.

BIOLOGICAL ASPECTS OF THE BEAN WEEVIL RELEVANT TO GENETIC CONTROL

The bean weevil attacks virtually only the cultivated beans (Phaseolus spp.) and the chick pea (Cicer arietinum L.) [15]. So its population dynamics in a given area depend mainly on the presence and the intensity of cultivation of these plants. No wild plants have been found to be attacked by the bean weevil in Europe. Thus the formation of natural infestation sources is excluded.

The life span of adults is in general less than 100 days [9], but can exceptionally extend to 140 - 180 days [12]. This means that in regions with one bean crop annually, the adults cannot survive in nature from one crop to the next. In such countries overwintering takes place in stored beans where the weevil breeds during the warm season. The adults of these generations developing in granaries, storerooms and the pantries of homes then migrate to the bean fields and lay their eggs in the ripening pods. Thus the population density of the pest can be considerably reduced before genetic control in the field by disinfestation of the stored beans. This can be carried out in granaries of big farms by using fumigants or other methods, but it is very difficult to achieve the disinfestation of beans stored in the pantries of homes which are in most cases the main sources of infestation.

When the adults migrate from the buildings to the bean fields it has been found that they travel no further than a few kilometres [9], so the population density of the pest on bean fields is always higher in the vicinity of settlements.

A critical point regarding the effectiveness of the release of sterile populations to prevent field infestation is the fact that the females can mate before migration. However, Labeyrie [9] reported that females which had had no earlier opportunity of ovipositing were incapable of doing so after several weeks. Thus only adults emerging and mating shortly before the onset of ripening of the bean pods can infest the crop, and it is not necessary to begin the release of sterile populations early in the warm season.

The number of times the insects mate is not yet clear. Szentesi's preliminary observations (unpublished data) indicate the tendency to a behavioural monogamy in females, while the males are considerably polygamous.

It is not known exactly how the adults feed in nature. Feeding of adults on flowers and on bean leaves has been observed [9] but it is unlikely that the adults released in great numbers for genetic control would cause noticeable damage to the bean plants.

MASS REARING

With most agricultural insect pests the practical use of genetic control is impeded by the lack of suitable mass-rearing methods. This obstacle does not exist with the bean weevil, since it can be easily reared on dried beans in the laboratory.

Authors dealing with the mass rearing of the bean weevil have used jars or other containers [13]. We have found, however, that in closed containers the infestation of grains is not homogeneous, thus the utilization of the food is not optimal. In bigger containers metabolic water produced by the larvae in highly infested beans can cause the formation of mould which is detrimental to the insect. Overheating caused by metabolic heat is also dangerous. Therefore Szentesi [14] developed a simple mass-rearing method using shallow trays with only one layer of beans. The eggs are distributed evenly over the beans in suitable numbers for optimal production of adults. The infestation of the beans is quite homogeneous. The trays are piled on racks with 1 cm space between each to allow for ventilation. Even under such conditions overheating of the infested beans has been found to reach 5.0 - 11.1 degC at a surrounding temperature of 28° C.

Before the adults begin hatching the beans are transferred to closed sieves (4 mm mesh) through which the adults are periodically sifted into containers for further handling.

Up to 21 500 adults (average 13 600) could be reared in 1 kg of beans. In the best rearing series the percentage of eggs developing into adults averaged 40.7.

Plastic screen cages were found suitable for producing a large number of eggs of the same age with a minimum of labour. A sieve (1 mm mesh) on the bottom of the cage provided with one layer of beans for stimulating oviposition enabled fast and continuous extraction of the eggs. The number of eggs laid per female varied in these cages on average between 20.0 to 59.9 (overall average 30.0). This is a relatively high value since egg-production under optimal conditions averages about 50 per female, exceptionally above 100 [9, 12].

RADIATION STERILIZATION

Several authors have studied the lethal effect of ionizing radiations as a control method against the bean weevil in stored beans [1-5, 7, 10-12]. In several cases also the sterilizing doses were determined. I shall now discuss the most important results of experiments on gamma-radiation sterilization.

Pesson [12] found that adults treated with 10 to 18 kR were only partially sterilized, and that 20 kR was required for complete sterility.

In his paper reviewing the results of experiments carried out in the USSR Andreev [1] reported that young adults of the bean weevil irradiated

with a dose as low as 1 kR showed substantial decrease in the number of progeny, while 6 kR was enough for complete sterilization. However, in the opinion of Andreev and co-workers [2], based on more extended studies, doses between 12 and 15 kR should be considered as fully sterilizing ones.

Cavalloro and Bonfanti [6] found sexual differences in the susceptibility to gamma-radiation: sterilizing doses for males and females irradiated 4 days before the end of the pupal stage were 4 and 6 kR, respectively, while on the day of emergence 5 and 10 kR were required.

Jermy and Nagy [8] observed that both sexes were sterile when emerging from beans that had been irradiated with 10 kR at the start of adult emergence.

These considerable differences in the sterilizing doses determined by different authors are probably due to the different conditions of irradiation (age of adults, temperature, ventilation, etc.) which are not always mentioned in the publications, but they could be caused by other unknown factors. It is therefore advisable to determine the sterilizing doses for each genetic control project and also to control it from time to time during the release programs.

COMPETITIVENESS OF STERILIZED MALES

Andreev and co-workers [2] studied the competitiveness of males treated with different doses of gamma-radiation in presence and absence of sterilized females on dried beans. There was no significant difference between 10 and 20 kR but above that the competitiveness decreased. The presence of sterilized females considerably lessened the impact of the sterile population on the dynamic of the normal one. The cause of this has not been analysed.

In laboratory experiments carried out with males emerging at 23° C from beans irradiated with 10 kR, Jermy and Nagy [8] found full competitiveness in males emerging on the 1st to 8th day after irradiation. After that time, however, a sudden drop of competitiveness was observed, indicating the noxious effect of the same dose on the earlier ontogenetic stages.

The best way to obtain fully competitive sterile adults for mass release is the irradiation of young adults on the first day after emergence.

ESTIMATION OF POPULATION

The nature of the source of infestation and the small size of the adults as well as the lack of a good trapping method represent great difficulties in estimating populations in the field. The most suitable method known so far is the release and recapture of marked adults. Szentesi (unpublished data) successfully used fluorescent dusts for this purpose.

OUTLOOK ON THE EFFECTIVENESS OF RELEASING STERILE POPULATIONS

It follows from the foregoing that irradiation can be used in principle both to prevent further propagation of the pest in granaries by irradiating the dried beans, and to suppress infestation by the release of sterile

populations. The use of genetic control on beans stored in granaries has not been proposed, and its feasibility is very questionable since the use of fumigants is simpler, and requires less sophisticated organization of control. So the following remarks will only concern the genetic control of field populations.

No field experiments on this pest have so far been carried out. Andreev and co-workers [2] proposed using mobile gamma-sources for irradiation of harvested beans and releasing the sterilized adults emerging from the irradiated product against the adults remaining in the field. The effectiveness of this procedure regarding the release of sterile adults is, however, limited in many areas by the following: (a) since the optimal sterilizing doses (not affecting competitiveness) vary considerably during ontogenesis, and since the weevil individuals of the population present in the harvested beans are in different developmental stages at the time of irradiation, invariably only a part of the population will be suitable for release; (b) in regions where there is only one bean crop a year the field infestation of the next year's crop is mainly due to the adults of later generations developing in stored beans, and the adults sterilized at harvest time would die before the adults infesting the next crop would emerge and appear in the bean field.

Thus in most cases mass rearing of the pest for release programs will be indispensable. Since mass rearing is very simple, large-scale field experiments could be started at any time, enabling us finally to settle the question of the feasibility of the technique for this species. I am confident that the results would be successful, and if other countries could help in the project, this further evidence for the effectiveness of the technique would be obtained more quickly. A new success, I feel, is urgently needed.

REFERENCES

[1] ANDREEV, S.V., "Irradiation effect on six species of agricultural pests", Advances in Insect Population Control by the Sterile-Male Technique (Report Panel Vienna, 1964), Technical Reports Series No.44, IAEA, Vienna (1965) 42.

[2] ANDREEV, S.V., MARTENS, B.K., MOLCHANOVA, V.A., "Development of research in sterilization techniques for agricultural insect pests in the USSR", Sterile-Male Technique for Eradication or Control of Harmful Insects (Proc. Panel Vienna, 1968), IAEA, Vienna (1969) 57.

[3] ANDREEV, S.V., MARTENS, B.K., MOLCHANOVA, V.A., SALDAN, L.N., Luchevaya sterilizaciya v bor'be s vrednymi nasekomymi, Vestn. s. h. Nauki, Moskva 12 1 (1967) 48.

[4] ANDREEV, S.V., MARTENS, B.K., MOLCHANOVA, V.A., SAMOILOVA, Z.I., "Ispol'zovanie radioisotopov i radiacii v bor'be s nasekomymi-vreditelyami rastenii i zhivotnyh", Radiation and Radioisotopes Applied to Insects of Agricultural Importance (Proc. Symp. Athens, 1963), IAEA, Vienna (1963) 115.

[5] BAKER, V.H., TABOADA, O., WIANT, D.E., Lethal effects of electrons on insects which infest wheat, flour, and beans, Part II, Agric. Engng 35 (1954) 407.

[6] CAVALLORO, R., BONFANTI, G., Possibilità dell'impiego di radiazioni ionizzanti contro Acanthoscelides obsoletus Say (Coleoptera, Bruchidae), a difesa dei legumi conservati, Boll. Zool. agr. Bachic., Ser.II, 8 (1966/67) 115.

[7] DE PROOST, M., L'effort belge en matière d'irradiation des aliments, Meded. LandbHogesch. OpzoekStns Gent 30 (1965) 667.

[8] JERMY, T., NAGY, B., "Sterile-male technique studies in Hungary", Sterile-Male Technique for Eradication or Control of Harmful Insects (Proc. Panel Vienna, 1968), IAEA, Vienna (1969) 91.

[9] LABEYRIE, V., "Les Acanthoscelides", Entomologie Appliquée à l'Agriculture (BALACHOWSKY, A.S., Ed.), Masson, Paris (1962) 469.

[10] PEREDELSKY, A.A., "Dejstvie ioniziruyushchih izluchenij na nasekomyh", Itogi Nauki, Biologicheskie Nauki, I, Radiobiologiya (KUZIN, A.M., Ed.), Izdatel. Akad. Nauk SSSR, Moskva (1957) 313.
[11] PESSON, P., "Travaux de recherches utilisant les isotopes et les rayonnements nucléaires en entomologie appliquée en France et dans les pays associés", Radioisotopes and Radiation in Entomology (Proc. Symp. Bombay, 1960), IAEA, Vienna (1962) 297.
[12] PESSON, P., Some experimental data on cobalt-60 radiation doses capable of arresting insect infestation of cereals and flour, Food Irrad. 3 4 (1963) A18.
[13] STRONG, R.G., PARTIDA, G.J., WARNER, D.N., Rearing stored-product insects for laboratory studies: bean and cowpea weevils, J. econ. Ent. 61 (1968) 747.
[14] SZENTESI, A., Studies on the mass rearing of Acanthoscelides obtectus Say (in preparation).
[15] ZACHER, F., "Bruchidae", Handbuch der Pflanzenkrankheiten VI/2 (BLUNCK, H., Ed.), Paray, Berlin (1954) 380.

DISCUSSION

L.E. LaCHANCE: Did you find any decrease in the longevity of weevils irradiated with the fully sterilizing dose? I ask this because in several weevil species radiation sterilization drastically shortens the life span.

T. JERMY: We did not find any significant decrease in the longevity of bean weevils irradiated with the fully sterilizing dose. The differences observed in the radiation sensitivity of different weevils might be due, inter alia, to the fact that some belong to the family of Curculionidae and others to the family of Bruchidae, the latter apparently being less sensitive. Mr. Wiendl's paper (IAEA-SM-138/57) gives some interesting data on the effects of gamma radiation on Zabrotes subfasciatus (Boh.).

WEAK-LINKS IN THE POPULATION DYNAMICS AND DIAPAUSE OF Heliothis zea (BODDIE) WHICH MIGHT BE EXPLOITED BY THE STERILE-INSECT RELEASE TECHNIQUE

P.L. ADKISSON
Department of Entomology,
Texas A&M University,
College Station, Texas,
United States of America

Abstract

WEAK-LINKS IN THE POPULATION DYNAMICS AND DIAPAUSE OF Heliothis zea (BODDIE) WHICH MIGHT BE EXPLOITED BY THE STERILE-INSECT RELEASE TECHNIQUE.

Knipling in 1964 stated that the sterile-insect release method of insect population control 'will not be feasible for those [insects] that have a wide host range'. This limitation would appear to prohibit the use of the sterile-male technique against such important pests as H. zea, which is known to attack more than 70 plant species. However, the present author and co-workers have made an intensive study of the population dynamics of H. zea which reveal certain weak-links in the seasonal cycle of the pest which might be exploited by the sterile-insect technique. In the present report, the author reviews the effects of season on the population dynamics and diapause of H. zea and presents an argument that, with adequate knowledge, multi-host insects may be as suitable – if not more suitable – candidates for control by the sterile-insect release technique as many single-host insects.

INTRODUCTION

It has been suggested that the sterile-insect release technique for insect population suppression may not be feasible for insects that have a wide host range [1]. However, after studying the population dynamics of the bollworm, H. zea, an insect which has many hosts, I have come to the opposite conclusion. Multi-host insects may be very vulnerable to attack by the sterile-insect release technique. The vulnerability of multi-host insects lies in the violent fluctuations in their numbers, from very high to very low, that occur several times each season. In contrast to many single-host species, the increase in numbers by multi-host insects from generation to generation may not be at all constant. One generation may increase to great numbers while the next may almost disappear. The fluctuations in numbers occur in direct response to seasonal change. Therefore, with adequate knowledge of the effects of seasonal change on the population dynamics of the species it may prove relatively easy, at various times of the season, to greatly overflood the natural population of a multi-host pest with releases of sterile insects.

The zea bollworm has a wide range of hosts involving more than 70 plant species [2]. This insect ranks among the most serious pests of crops in the western hemisphere and is a major pest on cotton, corn, soybeans, tomatoes, grain sorghum, alfalfa and many other legumes. Even though the zea bollworm has hosts available at all times of the year, the species maintains a very precarious balance with nature and numbers fluctuate violently during the season.

The survival of the zea bollworm as a species is based on two important adaptations. The first is the ability of the larvae to feed on many hosts. Thus, when a major host disappears during the season, or is no longer favorable for feeding by the larvae, the population switches to a minor host. Here it survives in small numbers until a major host again becomes available.

The second adaptation is the great inherent capacity of the species for increase. Female zea moths may lay great numbers of eggs. A maximum oviposition of 3,000 eggs by a female has been reported [2] with the average oviposition being approximately 1,850 eggs per female [3]. This great capacity for oviposition insures survival of at least a few individuals when on unfavorable hosts or during unfavorable seasons. It also allows for the rapid build-up of great numbers on favorable hosts in favorable times of the season. These adaptations have provided the mechanisms for success by zea as a species; but, here also may be the weak-links which might be exploited by man to bring about their control.

In the present report, I will examine the population dynamics of the zea bollworm and will suggest that this insect might be controlled by the sterile insect release method. Data used to advance this argument were collected at Texas A&M University on population dynamics by Henry and Adkisson [4] and on diapause by Wellso and Adkisson [5] and Roach and Adkisson [6].

RELATION OF HOST SEQUENCE TO SEASONAL ABUNDANCE

Weekly surveys were made on the Texas A&M University Plantation in 1963 to estimate the number of zea bollworms attacking the various crops produced [4]. The plantation is located in a fertile river valley and has 3,182 acres planted to various crops (Table 1).

The zea bollworm was found to have two hosts, alfalfa and corn, available in relatively great acreages during spring emergence and development of the first larval brood in April and May. The species made good use of these hosts for increasing the size of the population. Great numbers of zea bollworm larvae developed on these crops during the early spring (Figure 1). During one period in April, there were more than 200,000 larvae estimated to be infesting the 146 acres of alfalfa. This was equivalent to slightly more than 1,400 larvae per acre (Table 2). Populations of comparable size also were present in corn in early May. On a per acre basis, the larval population in corn was estimated at 2,000 per acre. At times during this two-month period, the combined total zea bollworm population on these two crops of the plantation was estimated at almost 400,000 individuals.

The zea bollworm population attained maximum numbers for the season in June. The June larvae were found mainly on corn; however, small numbers were on cotton. The tremendous size of the population during this period is shown by the number found in corn, which at maximum numbers was estimated at almost 2,000,000 individuals. This amounted to almost 14,000 larvae per acre for the 130 acres of corn. Nearly every corn plant was infested with at least one zea bollworm. These larvae apparently were destined to become the moths which would later infest cotton.

TABLE 1. ACREAGES OF VARIOUS CROPS PRODUCED ON THE TEXAS A&M UNIVERSITY PLANTATION, 1963

Crops	Acres produced in 1963	Percent total acreage
Alfalfa	146	4.6
Corn	130	4.1
Cotton	726	22.8
Grain sorghum	566	17.8
Pasture	760	23.9
Silage	111	3.5
Sudan	280	8.8
Oats	222	7.0
Miscellaneous experimental crops	241	7.5
Total acreage	3,182	100.0

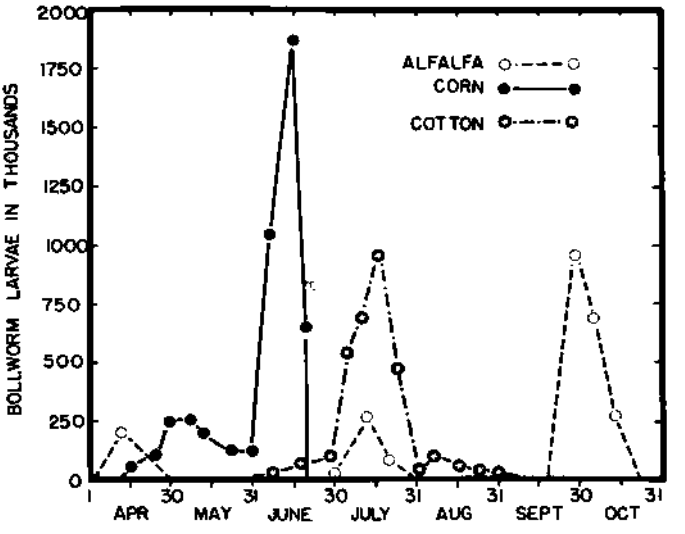


FIG. 1. Estimated numbers of zea bollworm larvae found on the total acreage of alfalfa, corn and cotton of the Texas A&M University Plantation, 1963.

TABLE 2. ESTIMATED NUMBERS OF ZEA BOLLWORM LARVAE PER ACRE ON VARIOUS CROPS OF THE UNIVERSITY PLANTATION, 1963 [4].

Date		Alfalfa	Corn	Cotton	
				Dryland	Irrigated
		Numbers of larvae			
April	2	0	0	0	0
	9	1452	284	0	0
	22	0	726	0	0
	29	0	2134	0	0
May	6	0	2222	0	0
	11	0	1826	0	0
	25	0	968	0	0
June	1	0	880	0	0
	6	0	8404	27	0
	13	0	14220	43	45
	18	0	5412	117	0
	27	0	0	137	39
July	5	54	0	896	348
	9	2292	0	1037	927
	16	313	0	592	2881
	24	0	0	259	1532
Aug.	2	0	0	0	126
	8	0	0	37	479
	16	0	0	0	252
	22	0	0	0	0
	28	0	0	0	0
Sept.	10	0	0	0	0
	22	0	0	0	0
	28	6562	0	0	0
Oct.	5	5000	0	0	0
	12	2560	0	0	0
	19	0	0	0	0

Corn was harvested in late June; cotton then became the most severely infested crop. As indicated in Figure 1, the earlier larval population of the plantation before this time had achieved great numbers. The resulting moths apparently centered their activities on cotton.

The cotton acreage never was infested with as many larvae as corn. There were approximately 5.6 times more acres of cotton than corn on the plantation, but the peak zea bollworm population in cotton was only about half that of corn (Table 2). Apparently there are at least two reasons for this: (1) The cotton was being treated at regular intervals with insecticides primarily for controlling the zea bollworm and (2) cotton may be a less satisfactory host for the pest than corn. Peak larval numbers during July on irrigated cotton were estimated at 2,881 larvae per acre. On the combined irrigated and dryland cotton acreage, the zea bollworm population at maximum numbers in mid-July was estimated at approximately 1,000,000 individuals. In late July and August, zea bollworm populations in irrigated cotton were considerably greater than

in dryland cotton. No doubt, this resulted because the dryland cotton was drouth-stricken and had matured earlier, while the irrigated cotton was still growing lush vegetation that was attractive to the insect.

During August the total number of zea bollworms on the plantation was drastically decreased. This apparently occurred because all host plants had become unsatisfactory for supporting great populations. At this time all corn had been harvested, alfalfa was drouth-stricken and the cotton had matured. The population on the total acreage of all crops of the plantation decreased to less than 100,000 larvae during August and most of September. The greatest number of zea bollworms at this time was in cotton; however, the peak population on this crop during August was small and was estimated at less than 500 larvae per acre (Table 2).

September rains caused the alfalfa to resume growth, and the zea bollworm population transferred its attack to this crop. The total bollworm population then steadily increased to nearly 1,000,000 individuals. Practically all of these were on alfalfa. This amounted to approximately 6,500 larvae per acre and represented the greatest number found on alfalfa throughout the season. On basis of numbers per acre, this was about half the size of the maximum population recorded earlier on corn. Also, this late-fall population on the 146 acres of alfalfa was approximately the same size as the peak population recorded on 726 acres of cotton in mid-July.

Alfalfa may well be the major host utilized in the fall by zea larvae destined to enter diapause. Great percentages of the zea larvae on the crop during the late fall become diapausing pupae [5].

Grain sorghum is a known host of the zea bollworm in this area; however, the numbers of larvae found on this crop during the 1963 season were so small that they were considered to be insignificant.

SEASONAL CONTROL OF DIAPAUSE

The ability of insects in temperate zones to enter diapause is an adaptive mechanism which allows a species to survive the harsh environment of an adverse season such as winter. The zea bollworm is induced to diapause in the fall months in response to climatic factors, namely photoperiod and temperature. Diapause occurs even though there may be ample hosts available for larval feeding and development.

In Central Texas, the zea bollworm is forced into diapause by the seasonal conditions which prevail from late September through early November (Figure 2) [5]. The induction of diapause is controlled by a decrease in the day-length to days having less than 13 hours of light per day and cooler temperatures (Table 3) [6]. Since the primary stimulus for the induction of diapause is day-length, the seasonal occurrence of this event may be predicted with great precision.

The onset of diapause checks further population increases by the zea bollworm. This happens because the development of the diapausing individuals is arrested in the pupal stages. These individuals do not become reproductive until the subsequent spring when they transform to moths. The makeup of the zea population then gradually changes

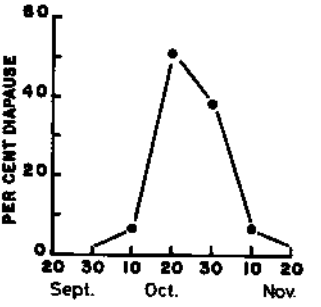


FIG. 2. Seasonal incidence of diapause in zea bollworms reared in an insectary at College Station, Texas. Figure drawn from data reported by Wellso and Adkisson [5].

TABLE 3. EFFECT OF DECREASING PHOTOPERIODS FROM L13:D11 TO L11:D13 AT CONSTANT 21 AND 26.6°C, AND DECREASING TEMPERATURE 3°C PER WEEK FROM 26.6° TO 18.5°C UPON DIAPAUSE INDUCTION IN THE ZEA BOLLWORM

Decreasing Daylengths	Temperature, °C	Percentage Diapause
From To		
13 hrs - 13 hrs	26.6°	0.0
13 hrs - 13 hrs	21°	0.5
13 hrs - 11 hrs	26.6°	0.3
13 hrs - 11 hrs	21°	76.5
13 hrs - 11 hrs	26.6-18.5°	94.3

during the fall months from one composed of eggs, larvae, pupae and adults to one composed entirely of diapausing pupae. Thus, the reproductive capacity of the species declines concomitantly with the increased incidence of diapause.

We know from research with the boll weevil, Anthonomis grandis Boheman, that a large surviving overwintering population is necessary to the survival of the species. When diapausing populations of the boll weevil have been greatly reduced over large geographical regions by insecticidal treatments [7], the insect has been almost eradicated from the treated areas. The insecticidal induced mortality in the fall months combined with the natural mortality of the winter leaves very few weevils to infest crops in the subsequent spring.

The zea bollworm also may be particularly vulnerable to population suppression measures aimed at reducing the size of the diapausing population. It is apparent from the data reported in Figure 2 and

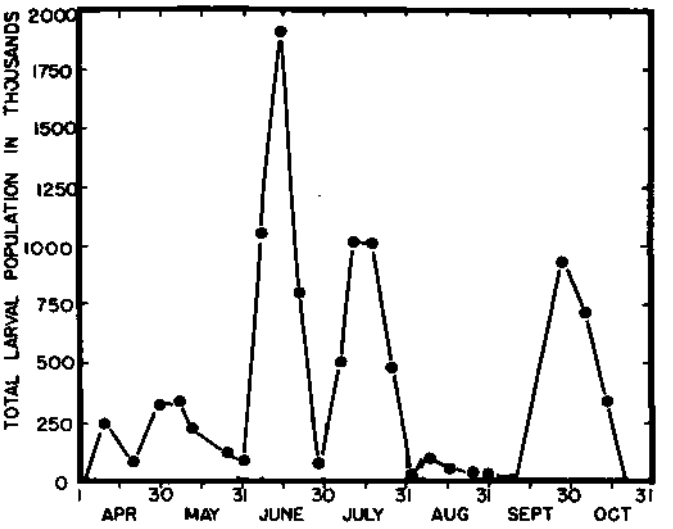


FIG. 3. Estimated numbers of zea bollworm larvae on all crops of the Texas A & M University Plantation, 1963 [4].

Table 3 that diapause occurs at a predictable time of the season. The larvae which develop into diapausing pupae also appear to be confined to a restricted range of hosts which may be growing on a relatively small area of land (Table 2 and Figure 3). Treatment of this relatively small area of land with insecticides, insect pathogens or cultural practices might be the first step in a population suppression program utilizing sterile-insect releases. Great reductions in the size of the overwintering population would mean that there would be few survivors to emerge in the spring. The early spring months, before large acreages of suitable host crops are available for great population increases, would be the most opportune time to overflood the natural population with sterile moths. It also would be the period that would require the fewest numbers of sterile insects in order to achieve the optimum ratio of sterile to fertile moths. This strategy would direct the attack on the zea bollworm at the weakest links in the seasonal cycle of the species.

DISCUSSION

The abundance of a phytophagous insect within its range of distribution is dependent on several factors. If the population is to attain tremendous size, the species first must have a great inherent capacity for increase. The environment then must be such that the species can attain its maximum rate of increase. If a species is to build a population of great size in one season, several other conditions also must be satisfied. The insect must overwinter in great numbers and the early generations must have ample host plants to support large numbers of their brood.

The zea bollworm may build a population of tremendous size within one generation. The species is able to accomplish this because of its great inherent capacity for increase. However, great populations occur only when there are considerable acreages of the most favored hosts available and when natural control from predators, parasites and pathogens is at a low level.

The data reported herein show, however, that at certain times of the year zea bollworm populations may decline to extremely low levels. This occurs in early spring, during periods of drouth in summer, and again in the late fall. These declines may be associated with the disappearance of favored hosts over large acreages. In addition, larvae on hosts untreated with insecticides suffer high mortality from parasites and predators.

The zea bollworm may be particularly vulnerable to population suppression measures during the fall months when the overwintering pupae must develop. In areas of intensive cultivation, the insect often is confined to host plants that are found in relatively small acreages. If the insect could be destroyed during these months, (late September through mid-November) by the destruction of the hosts, or by other means, populations could be drastically reduced. Then, sterile zea moths might be released during the subsequent spring when the species exists in extremely small numbers and is having difficulty in sustaining itself without any interference from man. Later in the season when favorable hosts, such as cotton and corn, become unavailable, or unsuitable, for larval feeding, the zea population again suffers a great natural decline in numbers. During these periods, it should be relatively simple to greatly overflood the natural population with sterile moths. This combined with cultural control of weed hosts, the regulation of growing season of cotton, corn, soybeans, grain sorghum and tomatoes, and the fall clipping of alfalfa and leguminous pastures might prove to be extremely deadly to the zea bollworm. Small acreages of alfalfa and pasture might also be treated with Bacillus thuringensis, Heliothis virus, or insecticides to further reduce the population size.

Thus, I submit the hypothesis that a multi-host insect, the zea bollworm, might be controlled by the sterile insect release technique. For this to be practical, we must have greater knowledge of the population dynamics and population-size of the species on all hosts in the total area to be controlled. Once this information is gathered, it should be possible to devise systems of farming and of controlling host plant sequences that would cause great declines in the population at predictable times in the season. Thus, a combined program of host plant management and the judicious use of insecticides, or insect pathogens, might be used to bring zea numbers to extremely low levels. During these periods, total population suppression then might be easily managed by the release of sterile moths.

REFERENCES

[1] Knipling, E. F., U. S. Dept. Agr. ARS-33-98(1964).

[2] Quaintance, A. L., Brues, C. T., U. S. Dept. Agr. Bur. Entomol. Bul. No. 50(1905).

[3] Isely, D., Arkansas Agr. Exp. Stat. Bull. 320(1935).

[4] Henry, P., Adkisson, P. L., Texas Agr. Exp. Sta. Misc. Publ. 767 (1965).

[5] Wellso, S. G., Adkisson, P. L., J. Insect Physiol. 12 (1966) 1455.

[6] Roach, S. H., Adkisson, P. L., J. Insect Physiol. (1970) In press.

[7] Adkisson, P. L., Rummel, D. R., Sterling, W. L., Owen, W. L., Texas Agr. Exp. Sta. Bull. 1054 (1966).

DISCUSSION

L.E. LaCHANCE: As we all know, the sterility principle for insect control has its detractors as well as its supporters. It is unfortunate that the former were not present to hear a distinguished entomologist, Mr. Adkisson, present this paper. I, for one, am grateful to have these data at my disposal in order to demonstrate to the 'doubters' that even an insect species affecting multiple hosts spread over large areas, and one which is highly radio-resistant, still appears to be a promising candidate for control or eradication by this method.

Marta T. MORALES: I should like to ask whether the population fluctuations of the insect are the same for all the hosts mentioned.

P.L. ADKISSON: As far as we have been able to determine, they are the same.

Marta T. MORALES: In the Philippines the mango trees bear fruit from December to May and there are crops of papayas, bananas and other fruits all the year round. If we release when the insect population in the mango plantation is at its lowest, we shall have the problem of migration from nearby papaya and banana plantations. What do you suggest is the best course of action?

P.L. ADKISSON: I think you will have to make surveys throughout the year on all available hosts and estimate the size of the total population over the entire area. This is the only way in which you will be able to determine when the number of flies is at its lowest during the season.

I.A. KANSU: As an entomologist working on Lepidoptera, and especially as a collector, I would like to mention that moon competition with light traps is the same for almost all noctuid species. When you can clearly see the silhouettes of hills, you collect only few specimens by means of a light trap. That is why the light trap method is not always a good one for surveying noctuids.

I would also point out that changing of the host is a very well-known phenomenon, especially in the case of some desert Lepidoptera. Some species change their host plant several times in a season.

K.L. CHAN: Mr. Philip Corbet of Canada has shown that a number of insects which show lunar periodicity also show synchronization of adult emergence. Did you find this in the case of your moth?

P.L. ADKISSON: We have not yet studied this aspect, but it may well be so.

K.L. CHAN: In such tropical countries as Singapore, where there are no distinct seasons, populations of mosquito species fluctuate only very little. Under such conditions, how and when would you recommend the release of sterile males? It would appear that there are no truly favourable periods, when the populations are low enough.

Although Mr. Pal thinks otherwise, he is speaking only of such tropical countries as Thailand, where distinct seasons do exist. I would agree that in those countries the mosquito populations indeed vary considerably with the seasons.

P.L. ADKISSON: Conditions in the tropics, and especially where there are no distinct rainy and dry seasons, are much more stable than in the higher latitudes. Thus, you may not have the extreme fluctuations in the insect populations that are common outside the tropics. I can only suggest

that you sample your population carefully and determine what its size is during the various months of the year.

R. PAL: May I first attempt to correct what may be a slight misunderstanding on Mr. Chan's part: I consider that even in mosquito populations there are seasonal fluctuations in most instances, and there is a period of lowest and highest density.

I now have a comment on the two papers presented so far this afternoon. The session has been very valuable as some penetrating soul-searching has taken place. Both Mr. Jermy and Mr. Adkisson have raised pertinent questions with respect to whether we are overselling genetic control and whether we collect sufficient information on ecology and population dynamics before attempting to manipulate populations.

D.W. WALKER: What assurance do you have, Mr. Adkisson, that the sterile males will mate during the full moon? If the males in nature do not mate during this period, it would appear to me that the sterile males will not mate either.

P.L. ADKISSON: I did not say moths do not mate during periods of full moon. There are some moths active on most nights. The moon apparently causes a lunar periodicity in the moth generation cycle, so phasing it that greatest numbers appear during each dark moon period (a cycle of about 28 - 30 days). Thus, peak numbers of moths for each generation appear during the dark moon at an interval corresponding to the lunar cycle. Releases should be initiated at the end of the full moon to prevent great numbers of eggs from being laid. They should then be continued throughout the dark-moon period.

D.W. WALKER: What are the possibilities of attracting males from nature to an autosterilization device? It could incorporate pheromone, flickering light, etc. and could obviate the need for mass rearing, but the trap method would have to be very efficient.

P.L. ADKISSON: At the present time I don't think this would work too well. Perhaps in the future the pheromone trap may be very useful when moth numbers in an area are extremely low.

D.G. CAMPION: Do light trap catches merely permit measurement of the relative activity of moths, and not of absolute numbers? In other words, would release estimates based on such data be valid?

P.L. ADKISSON: Light traps provide only some measure of the relative abundance of H. zea and for light-trap data to have much meaning information must be collected over a period of several years. Moth catches provide information on seasonal fluctuations in population numbers, i.e. the peak numbers and low numbers for each generation. Thus, light-trap data should be useful for timing sterile-insect release so that it coincides with the low numbers in each generation.

E.A. TAYLOR: What consideration is being given to the long-distance movement of H. zea?

P.L. ADKISSON: We and our co-workers in the United States Department of Agriculture are devoting much attention to this very important problem, and research is being planned for the immediate future. Unfortunately, the mechanics of the research pose considerable difficulties and the work consequently entails great expense.

FEASIBILITY OF CONTROLLING STORED GRAIN PESTS BY THE STERILE-MALE TECHNIQUE

S. PRADHAN, S.M. CHATTERJI, G.R. SETHI,
M.W. BHAMBURKAR, H. PRASAD
Division of Entomology,
Indian Agricultural Research Institute,
New Delhi, India

Abstract

FEASIBILITY OF CONTROLLING STORED GRAIN PESTS BY THE STERILE-MALE TECHNIQUE.

Apart from one publication, by Crook and colleagues in 1960, no attempt appears to have been made to evaluate the potentialities of the sterile-male technique against pests of stored grains. The paper discusses this question and reports the findings of some laboratory experiments conducted at the Division of Entomology, Indian Agricultural Research Institute, New Delhi.

INTRODUCTION

One of the most tempting lines of pest-control research consequent upon the success in the screw-worm eradication project has been the exploration of other areas of pest problems in which the sterile-male technique can be successfully used. Several scientists, including Knipling, the original formulator of this principle, have listed the essential requirements for success in eradicating a pest by this technique. The following three conditions are to be ensured even for a preliminary selection of a pest species for such a study:

(i) The population of the pest should be either inherently very low or should come down to a very low level as a result of seasonal changes or should be capable of being brought down to that level by other conventional methods of pest control. It should be noted in this connection that the population of the screw worm with which this technique succeeded so well was in the region of a few hundred per square mile whereas the agricultural pests of any significance are generally in thousands per acre. This is the most essential requirement because the number of sterile males released per unit area has to be several times higher than the population of the normal males present in that area. Hence the higher the population level of the pest the larger the number of sterile males to be released and also the higher the cost of the operation; thus beyond a particular level of the pest population this technique ceases to be feasible.

(ii) The area from which the pest is to be eradicated should be so situated that reinfestation can be kept to a negligible level or completely excluded. In the case of the screw worm the strictest possible quarantine measures are being enforced.

(iii) There should be a reasonable chance of evolving a fairly economical method of mass rearing such large numbers of adults as will be needed for release.

Once a prima facie case has been made out in favour of a pest species on the basis of the above three criteria, one can rationally proceed with a serious study of such biological characteristics as:

(a) detailed quantitative life history,
(b) the irradiation dose needed for sterilization without adversely affecting any behavioural characteristics or competitive capabilities,
(c) the ratio of sterile males to normal males for effecting the desired degree of reduction in the population,
(d) the method of mass release so as to ensure uniform distribution in the area,
(e) the feasibility of separating the two sexes on a mass scale at some suitable stage of the life cycle,
(f) the assessment of the harmful effect or nuisance value of the insects released,
(g) the evolution and/or perfection of mass-rearing techniques, and equipment,
(h) the economics of mass rearing and release operations, and the expected economic gain of the whole project.

CHOICE OF STORAGE PESTS

On the basis of the above, we considered that the most promising field for the success of the sterile-male technique was likely to be in the control of storage pests. This theoretical decision was arrived at in spite of the rather disappointing conclusion of Crook et al.[1] after working on the flour-mill moth, Anagasta (Ephestia) kuehniella Zeller. We envisage using the sterile-male technique neither in a normal godown nor in a general way as was attempted by the above-mentioned authors, but in the following planned manner, in which both the storage structure or storage godown and also the general storage practices will have to be modified in view of the requirements of the technique.

The series of steps in which the release of the sterile males has to be fitted in are considered to be as follows:

(a) Earmark certain storage godowns or storage structures for long period of storage;

(b) Make the storage godown or storage structure practically insect-proof by means of any kind of barriers;

(c) Disinfest the storage structure by spraying or fumigation;

(d) Store fresh grain, or fumigated grain which is practically free from insect infestation;

(e) Before closing the storage structure or the godown for long-range storage, introduce suitable populations of sterile insects of the few species of storage pests which cause serious concern in the area so that these sterile insects will make it practically impossible for any small pest population, which might have entered the godown or the storage structure despite the precautions mentioned above, to increase.

---

[1] CROOK, L.J., BULL, J.O., CORNWELL, P.B. Some biological and ecological studies on the flour mill moth Anagasta (Ephestia) kuehniella Zeller, for an appraisal of sterile-male release technique, AERE Report R. 3297, Atomic Energy Research Establishment, Harwell, Berks, United Kingdom.

This last operation is envisaged as a substitute for repeated fumigation and/or for treating the grain with any insecticidal chemical involving health hazards.

Another point which has to be faced during this stage is that we have to write off the small amount of foodstuff which the sterile, non-multiplying, adults of the beetle pests are likely to eat during their own lifetime. This amount of damage is thought to be quite negligible in view of the assumption that only a small number of sterile adults will be needed. Even this minor disadvantage will not exist in the case of several pests, particularly moths, which do not eat in the adult stage.

With the above considerations in mind, we have started studies on one of the several species of storage pests, namely the rust red flour beetle, Tribolium castaneum (Herbst).

DETERMINATION OF GAMMA-RAY STERILITY DOSE

The sterility dose was determined in three replicates by exposing freshly emerged adults of T. castaneum in batches of 50 enclosed in cellophane bags to various doses of gamma radiation and then keeping each batch in separate containers with sterilized wheat flour for repeated observations on the development of the progeny, if any, up to a period of 40 days. No effort was made to segregate sexes in these experiments. The various doses tried were 1000, 2500, 5000, 6000, 7500, 10 000, 15 000 and 30 000 R. The lowest dose at which the development of progeny was almost completely arrested was 7500 R, but a dose of 10 000 R also did not produce any other visible disability in the insects exposed.

It was decided to carry out further investigations with doses of 10 000 R so as to doubly ensure complete sterility in all irradiated batches. A description of these investigations follows.

EFFECT OF DIFFERENT RATIOS OF STERILE TO NORMAL ADULTS ON DEVELOPMENT OF PROGENY

To examine the effect of different ratios of sterile to normal adults on fertility, a dozen different ratios were tried. The general plan and results of this experiment are shown in Table I. As described above, the insects were irradiated in cellophane bags so as to ensure that all of them received a dose of 10 000 R. Thereafter single pairs of normal adults (one male and one female) were separately enclosed with different numbers of pairs of irradiated adults in glass jars (15 cm × 10 cm) covered with muslin and maintained at room temperature and humidity. There were three replications of each ratio between normal and sterilized adults. Also for each of these ratios there were three different types of controls, each of which was also replicated thrice. One type of control contained only normal adults, the number of which was equal to the total of normal and sterile adults (column 6), the second control consisted of only one pair of normal adults (column 8), and the third type of control contained only sterile adults (column 10) the number

TABLE I. Tribolium castaneum (HERBST): EFFECT OF DIFFERENT RATIOS OF STERILE TO NORMAL ADULTS ON DEVELOPMENT OF PROGENY
Glass rearing jars measuring 15 cm × 10 cm and containing 90 g of 'atta' (sterilized wheat flour) and 5% yeast were used.

	Temperature and relative humidity	Treatment			Controls						Percentage reduction in population	
					I		II		III			
		No. of normal and no. of irrad' t adults	Ratio of normal to irrad' t adults	Net increase in population	No. of normal adults	Net increase in population	No. of normal adults	Net increase in population	No. of irradiated adults	Net increase in population	(a)	(b)
1	2	3	4	5	6	7	8	9	10	11	12	13
i	R.H. 51%, temp. 28°C-33°C	2/2	1:1	111	4	435	-	-	2	Nil	74.48	71.68
ii		2/4	1:2	7	6	479	2	392	4	"	98.53	98.21
iii		2/10	1:5	59	12	405	-	-	10	"	87.83	84.94
iv		2/20	1:10	85	22	593	-	-	20	"	85.66	98.31
v		2/40	1:20	19	42	1665	-	-	40	"	98.85	93.60
vi		2/60	1:30	17	62	1995	-	-	60	"	99.14	94.28
vii	R.H. 88%, temp. 28.3°C-33°C	2/80	1:40	17	82	1883	2	297	80	"	99.07	94.28
viii		2/100	1:50	21	102	1513	-	-	100	"	98.61	92.92
ix		2/150	1:75	8	152	1151	-	-	150	"	99.30	93.70
x		2/175	1:87.5	3	177	560	-	-	175	"	94.46	98.99
xi		2/200	1:100	14	202	287	-	-	200	"	95.12	95.28
xii		2/400	1:200	Nil	402	243	-	-	400	"	100	100

(a) Percentage reduction in population in treatment as compared to Control I (column 7).

(b) Percentage reduction in population in treatment as compared to Control II (column 9).

of which was equal to that released in the corresponding treatment. To all these sets sterilized wheat flour was supplied as food at the rate of 90 g per jar. Observations on the progeny were recorded after 40 days from the start of the experiments. This ensured that only one generation was allowed to be completed. Only adults, both dead and alive, were counted.

Table I shows that with every ratio the population in the control jars (column 7) was much more than in those containing both normal and sterile adults (column 5). It is also clear from column 5 that the population in the treatment jars goes on decreasing (with a few exceptions) with the increase in ratio of sterile adults till it becomes nil in the highest ratio of 1:200. As regards the percentage reduction due to the release of sterile adults, it is seen that there is a large percentage of reduction right from the lowest ratio of 1:1 although the percentage reaches 100 only at the ratio of 1:200; this trend is generally the same irrespective of whether the percentage reduction is calculated with reference to the net increase in control No.I or control No.II. Thus the percentage reduction given in column 12 refers to the population shown in column 7, which is the progeny of the number shown in column 6; and the number in column 6, i.e. number of normal adults, is equal to the total of both normal and sterile adults in the corresponding treatments. This had to be done to keep the population pressure of the parent generation equal both in treatment and control. From the results recorded in column 7 it is clear that the number in column 6 has obviously not been the only factor influencing the net increase in progeny in column 7. The limiting factor seems to have been the size of the container, the prevailing climatic conditions, and the number of adults kept in each container. Thus there is not much difference in the net increase in the progeny (column 7) from rows i to vi which represent the results of the experiments carried out during April and May. The case is similar with rows vii to xii, for which the experiments were carried out during September and October. From row x onwards it appears that the high population of the adults (column 6) has begun to show its depressing effect. Rows x to xii most probably reflect the depressing effect of both the autumn season and the high population kept in the jar. Further, when the percentage reduction is worked out with reference to the number in column 9, which represents the net increase in the progeny of only one normal pair when there has been one normal pair in each of the treatment jars, the net increase in the progeny is as given in column 5. The percentage reduction as given in column 13 shows the same trend and leads practically to the same conclusions, that the technique of releasing sterile insects into a normal population can be expected to check the multiplication of normal insects to quite a considerable extent. The net increase in the population (column 14) of irradiated adults was nil, thus confirming complete sterility in all cases. A prima facie case is therefore established for following up this line of work to its logical conclusions.

ACKNOWLEDGEMENTS

The authors are grateful to Dr. M.S. Swaminathan, Director, Indian Agricultural Research Institute, New Delhi, for providing the necessary facilities for this work.

DISCUSSION

F. M. WIENDL: On the subject of using the sterile-male technique for stored-grain insects, I have had experience with applying it to Zabrotes subfasciatus (Boh.). The biological characteristics of this pest offer various advantages for the application of the technique: for example, an average of 30 eggs per female, an adult longevity of barely 6 days and maximum oviposition on the second day; moreover, it is very easy to separate the sexes. A disadvantage is presented by the nature of oviposition, as the eggs are stuck fast to the pea. Sterility is induced with barely 10 krad, and on the basis of a formula developed by Berryman (Canada) there should be approximately 2000 sterilized insects for each 100 fertile ones in the case of aspermia and a few less if there is only immobilization of the sperm, with an average of three matings in both cases.

However, the real problem is the presence in the grain of a large number of dead insects and of eggs, albeit inviable ones. This in fact makes it impossible to apply the technique to this insect.

G. HOOPER: Firstly, Mr. Sethi, it worries me that the data for each ratio treatment are based on only three females. Secondly, for a 1:1 ratio of sterile and untreated insects, and on the assumption that the sterile males are competitive, one would expect a 50% control. Your Table I shows a 74% control for this ratio. How do you explain this?

G. R. SETHI: The data are indeed based on three replications per treatment. Regarding your second observation, I can only say that the data are based on actual observation. They indicate that the sterile males were quite competitive.

M. FRIED: I fear that after starting with a review of some of the requirements for the success of the sterile-male technique you have ended with one of the most difficult situations, both from a technical and economic standpoint and from a food quality point of view. People do not normally want even dead insects in their food nor do they desire the presence of insect excrement. Moreover, in order to achieve success you will have to release sterile insects of many species, each of which will require thorough previous research. You may also have to store the grain in the treatment area for an appreciable length of time to allow the breeding of multiple generations. I really feel that you may have "bitten off more than you can chew", at least at this stage of our knowledge, and that greater attention should be devoted to potentially more successful areas.

G. R. SETHI: As I stated earlier, it is not the intention that this technique should be used wholesale for eradicating stored-grain pests, but in a limited way to check the multiplication of a few individuals which might have entered disinfested storage structures. It would not therefore be necessary to release such large numbers of sterile individuals of the pest species as to pose a serious problem from the consumer-acceptability point of view. I am convinced that these studies give us grounds for believing that the technique could be usefully tried out.

K. K. NAIR: I also am concerned about the application of the sterile-male technique for the control of stored-product insects. Although you can control the insect infestation by this technique, the amount of insect fragments left in the grain will undoubtedly be far beyond the maximum permissible level.

Did you take into account the mortality of adults as a result of cannibalism, which is common in this species?

In my studies I have noticed that a sterilized T. castaneum male is not a competitive individual.

G. R. SETHI: The question of insect remains has already been raised by Mr. Fried, and I would like to state again that it is intended to use this technique only to a limited extent.

As regards your second point, cannibalism was not taken into account as a distinct factor during mortality counts.

Lastly, the data presented indicate that the sterilized males were quite competitive, as we achieve a significant reduction in population even at a 1:1 ratio.

ORGANIZING A PROGRAM FOR ERADICATION OF Culex pipiens fatigans IN RECIFE, BRAZIL*

C. BORGHI
Nuclear Energy Centre
and
A. de MORAIS REGO, Maria L. de OLIVEIRA,
Z. MOTA de AZEVEDO
Nuclear Energy Centre and Bioscience Institute,
Federal University of Pernambuco,
Recife, Brazil

Abstract

ORGANIZING A PROGRAM FOR ERADICATION OF Culex pipiens fatigans IN RECIFE, BRAZIL.

The paper discusses the planning of a program started in Recife to control Culex pipiens fatigans, vector of filariasis, by conventional insecticides and the sterile-male technique. Two alternative projects are discussed. In the first project, the spraying, to be carried out over an area of 10 km², twice per month for a period of 2-3 months, will cost about US$2000 per month; then sterile males will be released at a rate of 100 000 per week. In the alternative project, involving a smaller area, the sterile-male technique alone will be used; a mathematical treatment of the problem is included as an appendix.

INTRODUCTION

The sterile-male technique has been studied in the Centro de Energia Nuclear of Recife (CEN), with a view to controlling Culex pipiens fatigans in the area of Recife, Brazil. Some of our work is mentioned in the Bibliography. The pest is the principal vector of filariasis, a disease that is widespread in the city of Recife. According to Rachu et al. (1956) the infestation index of Culex in Recife reaches 7.3%. Our group reported 2 infected persons among 252 examined cases in 1969 in a suburban part of Recife, and 9 infected among 330 cases in another part.

DESCRIPTION OF THE RESEARCH

The entomological work has involved the collecting of insects in the field by means of conventional nets and traps, and classifying them. Microscopic investigation of anaesthetized insects has revealed a number of mutations of C.p.f., especially white-eyed and green, which have been classified.

A dosimetric investigation has been done to determine the optimum gamma dose for sterilizing male C.p.f. Gamma rays have been used from

* This work has been supported by the Brazilian National Commission for Atomic Energy.

a ^{60}Co source of about 900 Ci. The doses have been measured by the Ficke method (ferrous-ferric transition). The following points have been noted:

(1) The eggs of C. p. f. do not hatch after a 10 000-R dose.
(2) The average life of the larvae from irradiated eggs diminishes rapidly with increasing doses.
(3) With about 4000 R on the eggs, the lifetime of the larvae is almost unaltered, but they do not pupate.
(4) The sterilization dose of adult males is 8000 - 11 000 R.
(5) The same dose appears to be sufficient, when pupae are irradiated, for getting sterile adult males.

A study of the mating competitiveness as between normal and sterile males, with normal females, has shown no disadvantage for the irradiated ones, since the batches of sterile eggs are as frequent as the normal batches, with equal numbers of sterile and normal males.

Chemical sterilization by means of apholate dissolved in the water in which the larvae live, has been studied. With a concentration of 25 mg/litre, sterilization of the males born from the larvae was complete.

A radioactive tracer method, using ^{32}P, has been adopted to determine the dispersion of C. p. f. The concentrations of ^{32}P used were 20 μCi/litre and 25 μCi/litre. The adult insects, anaesthetized, were enclosed in small Al boxes with a thin wall (20 mg/cm^2). Their activity was measured by means of a gas-flow counter. The field part of this research has not yet been done.

THE TWO PROJECTS

We have studied two alternative projects: a two-phase project in which a chemical (insecticide) phase is followed by the use of the sterile-male technique; and an alternative, one-stage project, in which only the latter is used.

THE TWO-PHASE PROJECT

Pesticide phase

As a basis for a cost forecast, let us consider a plane 1 km^2 in area. The insecticide is sprayed along parallel tracks, 100 m apart. Thus 10 tracks cover the whole area, with a distance of 10 + 1 = 11 km for the spraying machine. Let us say 15 km, to include possible detours. These 15 km at a speed of 8 km/h would take 2 hours of work to spray. The cost per hour of the spraying operation, according to information provided by the city of Rio de Janeiro (SURSAN), is about Cr 500 = US$100 for one spraying per km^2. Thus for an area of 10 km^2, and for two sprayings per month, the cost is approximately US$2000 per month.

Sterile-male phase

During the treatment with conventional insecticide, the density of insects in the treated area will be periodically checked by the usual capture methods. When the density drops to 20% of its initial value, the sterile-male technique will be used. The foreseen number of sterile males to be released in the 10-km^2 pilot area is 5000 per day. The production of such a number of insects is already possible in the tanks available at the CEN. The separation of the males is made by means of percolators with holes 2 mm in diameter. The sterilization will be done partly by gamma rays and partly by chemosterilization.

ALTERNATIVE, ONE-PHASE PROJECT

A natural disaster (flood) in Recife upset the plans for the two-phase project. As a result we started planning an alternative, one-phase project consisting of using the sterile-male technique alone in a limited isolated area (Curado-Várzea). The mathematical calculations for our plans are given in the Appendix. We must release at least 5000 males per week per million insects of the starting population n_0. Since our resources appear to give us the possibility of releasing more than 100 000 sterile males per week, a first effort in this direction will be made at the end of the rainy season, probably October 1970. It is possible that this kind of project is useful where scanty sanitation facilities make other methods almost useless.

CONCLUSIONS

From the results so far obtained the work appears to have some chance of success, but the following points are important.

(1) The continuous interest of the Public Authority on whom the financial responsibility lies is essential.

(2) The duration of the project, according to Dr. K.S. Rai (IAEA Expert advising the project) cannot be less than 2 years, and should possibly be 5 years.

(3) The gravity of the filariasis problem in the region makes it possible that the disease could easily spread to other larger areas. Therefore a national as well as an international aid program is required for the elimination of the pest.

APPENDIX

MATHEMATICAL TREATMENT OF THE STERILE-MALE TECHNIQUE FOR CONTROLLING THE INSECT POPULATION

Let us assume the following notations:

n = total number of insects in a given area;
mn = total number of (normal and sterile) males = $\left(m + \frac{J}{n}\right) n$;

J = "artificial" population of sterile males in the area = $\frac{i}{\lambda_1} < mn$;
i = number of sterile males released for unit time;
$(1-m)n$ = total number of females;
f = (number of matings per sterile male)/(number of matings per normal male);
$1/\lambda_1$ = mean life of sterile males;
$1/\lambda$ = mean life of the male population;
B = breeding coefficient (dimensions T^{-1});
v = migration velocity of the insect population n;
D = migration length;
$vD\nabla^2 n$ = net migrating population (inward + outward) per unit time ($\approx$ zero for an isolated area).

The equation for the population variation is

$$\frac{dn}{dt} = -\lambda n + B\left(m - \frac{Jf}{n}\right)(1 - m)\, n^2 + vD\nabla^2 n \tag{1}$$

The first term on the right-hand side is the natural decay; the second is proportional to the product of the number of females $(1 - m)n$ and the number of non-sterile males $(m - Jf/n)n$, taking into account the efficiency coefficient f. For an area which can be considered as almost isolated, the diffusion term $vD\nabla^2 n$ can be taken to vanish. Thus, in this case, Eq.(1) can be written:

$$\frac{dn}{dt} = -an + bn^2 \tag{2}$$

where

$$a = \lambda + B(1 - m)\, Jf$$

$$b = B(1 - m)\, m$$

Integrating, we can write

$$n(t) = \frac{a}{b + \left(\frac{a}{n_0 + J} - b\right) e^{at}} \tag{3}$$

This satisfies the condition $n(0) = n_0 + J$ (according to the assumptions), n_0 being the initial "normal" population. Substituting the values of a and b we get

$$n(t) = (n_0+J)\, \frac{\lambda + B(1 - m)\, Jf}{B(1-m)m(n_0+J) + \left[\lambda + B(1-m)Jf - B(1-m)m(n_0+J)\right] e^{at}} \tag{4}$$

For $J = 0$, Eq.(4) must be $n(t) = n_0 = \text{const}$. This carries the condition

$$\lambda - B\,(1 - m)\, m\, n_0 = 0 \tag{5}$$

which defines the breeding coefficient B, namely

$$B = \frac{\lambda}{m(1 - m)\, n_0} \tag{6}$$

Substituting Eq. (6) into Eq. (4) we have

$$n(t) = (n_0 + J)\, \frac{1 + \frac{Jf}{mn_0}}{1 + \frac{J}{n_0} + \frac{J}{n_0}\left(\frac{f}{m} - 1\right) e^{(\lambda + \frac{Jf}{mn_0})\, t}} \tag{7}$$

Since $f/m > 1$, Eq. (7) steadily decreases for increasing t.

As an example for Culex pipiens fatigans, with

$J = 0.01\ n_0$; $f = 1.5$; $m = 1/3$; $\lambda \approx \frac{1}{15}$ day; $t = 6$ months;

$\lambda t = 12$; $e^{\lambda t} = 1.63 \times 10^5$

one obtains

$$n(6 \text{ months}) = (1.01) n_0 \frac{1.045}{1.01 + 0.035 + 1.63 \times 10^5} = n_0 \times \frac{1}{5400} \tag{8}$$

which shows an important reduction of n_0.

Now the rate of releasing insects, i, is

$$i = J\lambda_1 \tag{9}$$

Thus, in the case shown above, this rate must be, with $1/\lambda \simeq 15$ days:

$$i = \frac{0.01}{15}\, n_0 = \frac{n_0}{1500} \text{ per day} \tag{10}$$

This means, in fact, a release of about 5000 sterile males per week per million insects of the original population, during a period sufficient for the creation of the artificial population J, e.g. 4 months. Hence the decrease of n(t) calculated here requires an initial release of $5000 \times 4 \times 6 \approx 100\,000$ sterile males per million insects. Perhaps this result is somewhat optimistic, but it can be useful as an order-of-magnitude calculation of i.

This suggests the following method:

(a) Release a massive initial number of sterile males, e.g. 0.01 of the initial population.

(b) Release at periods of one week $7i \approx \frac{0.07}{15}$ n sterile males, the number n being the actual population.

(c) Possibly a mixed program with use of insecticide for isolating the chosen area can be utilized.

BIBLIOGRAPHY

DAS, M., Sterilization of Culex pipiens fatigans Wiedemann by apholate, Bull. Wld Hlth Org. 36 6 (1967) 949.

KRISHNAMURTIS, B.S., RAY, S.N., JOSHI, G.C., A note on preliminary field studies of the area of irradiated males for reduction of Culex fatigans populations, Indian J. Malar. 16 (Sept. 1962) 11.

LINDQUIST, A.W., IKESHOJI, T., GRAB, B., MEILLON, B. de, KHAN, Z.H., Dispersion studies of Culex pipiens fatigans tagged with ^{32}P in the Kemmendine area of Rangoon, Burma, Bull. Wld Hlth Org. 36 (1967) 21.

RACHU, R.G., et al., A filariose bancroftiana em Recife etc., Revta bras. Malar. Doenç. trop. 8 2 (1956) 359.

RAI, K.S., The application of the sterile male technique for the control of Culex fatigans, World Health Organization V.B. C/CGGC/15-67.

REGO, A. de M., OLIVEIRA, M.Li., AZEVEDO, Z., Co^{60} gamma radiation effect on the fertility of Culex pipiens fatigans Wiedemann, Recife, UFPe, Comunicações do Centro de Energia Nuclear, No. 10.

REGO, A. de M., OLIVEIRA, M.Li., AZEVEDO, Z., Effects of gamma rays on eggs of Culex pipiens fatigans Wiedemann, Bull. Calcutta Sch. trop. Med. Hyg. XVII 1 (Jan. 1969).

SEBASTIAN, A., MEILLON, B. de, Experiments on the mating of Culex pipiens fatigans in the laboratory, Bull. Wld Hlth Org. 36 1 (1967) 41.

DISCUSSION

R. PAL: Mr. Borghi has stressed the fact that genetic control of insects is a highly sophisticated technique, requiring careful study of such factors as ecology and population dynamics. Consideration must be given to the question whether the services of a short-term consultant allow sufficient preparation for a campaign.

GENETIC MECHANISMS OF INSECT CONTROL

(Session VII)

Chairman

A.M. JORDAN (United Kingdom)

Survey paper

GENETICS OF FACTORS AFFECTING FERTILITY AND OF SEX-RATIO DISTORTIONS IN THE HOUSE FLY

R. MILANI
Istituto di Zoologia,
Università di Pavia,
Pavia, Italy

Abstract

GENETICS OF FACTORS AFFECTING FERTILITY AND OF SEX-RATIO DISTORTIONS IN THE HOUSE FLY.

In amphygonic species fertility properly refers to matings rather than to individuals; the individual contribution to fertility can however be estimated when multiple matings are possible. Fertility of normal house-fly (Musca domestica L.) strains and of mutant stocks differs greatly but the genetic bases of the difference remain undetected. Close inbreeding proverbially reduces fertility; the number of adult flies obtained by single brother-sister pairs may be halved at each generation. Zygotic losses seem to play an important role in this decrease in fertility and possibly provide tools for genetic manipulations. Heterozygous chromosomal translocations obtained by X-ray treatments cause semisterile conditions, which may prove of great value for devising means of genetic control. Sterility factors affecting gametes or fertilization and cytoplasmic sterility have not so far been discussed for the house fly. Sex determination and sex ratios are controlled by genetic mechanisms which differ between populations. The heterogamety is either chromosomal or genic; in the first case the heterogametic sex is the male, which carries a male-determining Y chromosome, while in the case of genic heterogamety, either sex can be heterogametic, according to the factors involved. Male autosomal determining factors inherited as genes have been recognized and assigned to different linkage groups, but strong evidence exists also for the existence of female-determining factors. By variously recombining the known sex factors, new sexual formulae have been obtained. Some crossing combinations between flies of strains differing in their sex formulae allow the production of F_1 hybrid populations composed of males only. The mass production of male populations would facilitate in various ways control based on the sterility principle.

The fertility of an organism is measured by its production of viable progeny. In amphygonic species fertility properly refers to 'pairs' or 'matings' rather than to individuals. However, mean and variance values expressing the fertility of single individuals of either sex can be obtained when multiple matings are possible. These statistics have proved of great value both for the selection of fertility levels with organisms of economic importance and for measuring the effectiveness of sterilizing agents [1].

The fertility of normal house-fly (Musca domestica L.) strains of various origin and of mutant stocks differs greatly. The genetic basis of these differences, however, have hardly been explored; when mutant genes are present, it is possible to ascertain to which extent side (pleyotropic) effects of the mutants contribute to fertility; however, these side effects are generally very sensitive to the action of modifying genes and/or the residual genotype, much more so than the 'major' effects of the same genes. This leads to conspicuous differences of results between crosses, between similar mutant stocks of different origin and between the segregants recovered after outcrosses and their original stock.

TABLE I. MEAN FERTILITY REFERRED TO SINGLE BATCHES OF EGGS OF SINGLE PAIR MATINGS FROM RECIPROCAL CROSSES BETWEEN MUTANT AND NORMAL FLIES OF THE WHO/IN/*Musca domestica*/1 STRAIN
(Franco, M.G., personal communication)

Mutant strain	Mutant females	Mutant males
dl	65.58	58.84
bwb; ctn; conv	47.20	(93)
apt	17	24.60
rp, ar	62.75	79.25
conv, ye	59.45	46.66
ocra, apt	19.55	40.33
apodous	0	49.62
bwb, ge; spi	23.55	59.68
Average	51.72	57.88

TABLE II. FERTILITY OF FIELD-COLLECTED FEMALES AND OF THEIR F_1 AND F_2 PROGENY [3]
(From Milani, 1955 and 1967; by kind permission of the journal "Rivista di parassitologia" and of Elsevier Publ. Co.)

Generation	No. of eggs per batch	No. of batches per female	No. of flies per batch	No. of flies per female	Relative fertility
P	84.21	1.65	44.00	72.60	1
F_1	87.56	1.46	27.05	39.49	0.54
F_2	93.54	0.86	19.64	16.89	0.23

TABLE III. EGG HATCHABILITY OF A FIELD-COLLECTED SAMPLE OF HOUSE FLIES AND OF TWO SUBSEQUENT INBRED GENERATIONS [3]
(From Milani, 1955; by kind permission of the journal "Rivista di parassitologia")

Generation	Batches of eggs	No. of eggs laid	No. of eggs unhatched	% unhatched
P	28	2421	106	4.35
F_1	62	5405	459	8.49
F_2	21	1958	407	20.79

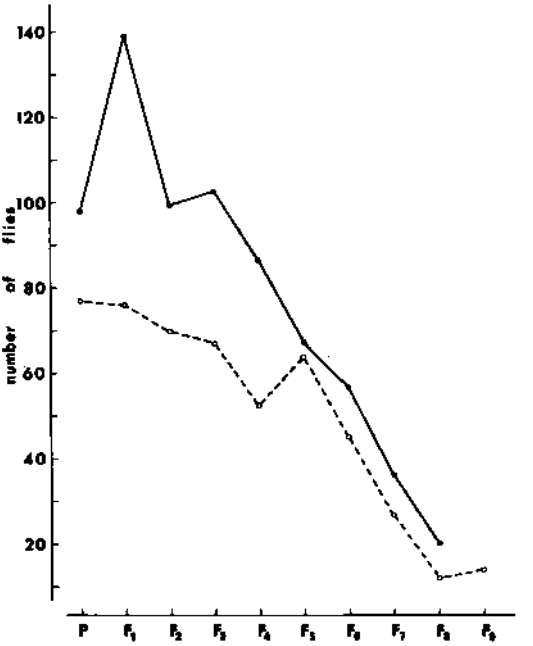


FIG.1. Reduction of fertility in a line inbred for nine generations with selection for high fertility. The mean number of flies obtained from each batch of eggs is the measure of fertility adopted here. The dotted line refers to all the families of the group of those selected for reproduction, represented by the straight line. (Milani, 1967 [4]; by kind permission of Elsevier Publ. Comp., Amsterdam).

The bearing of the type of mating on fertility for the house fly is exemplified in Table I, which shows the mean numbers of flies per batch of eggs obtained from pairs involving a standard fly and a mutant partner; even when the females are standard, the differences between crosses are conspicuous.

With an organism laying eggs in batches, like the house fly, total female fecundity (and fertility) depends on the number of eggs in each batch and on the number of batches which are laid. Under favourable experimental conditions, females differ in the size of their batches of eggs, but each female tends to lay batches of similar size; consequently the contribution to the total variance is greater 'between' than 'within' females of the same or of different strains [2].

Comparing field-collected flies and their F_1 and F_2 progenies, the present author did not observe any decrease in the number of eggs per batch in the three generations, while the number of batches per female decreased steadily (Table II). The differences of fecundity between generations would have remained unnoticed if the chosen parameter for its measurement had been merely the number of eggs per batch. In addition to the loss of fertility consequent upon the lowering of fecundity, a further conspicuous loss was due to increasing mortality in undetermined developmental stages, and possibly to failures of fertilization [2] (Table III).

TABLE IV. AVERAGE HATCH, SEX RATIO OF PROGENY, AND TRANSLOCATION SEGREGATION IN THE PROGENY OF 121 AUTOSOMAL-AUTOSOMAL AND 72 Y-AUTOSOMAL TRANSLOCATIONS (DOUBLE, TRIPLE, AND QUADRUPLE TRANSLOCATIONS TREATED INDEPENDENTLY IN EACH GROUP)
(Wagoner et al., 1969 [8]; by kind permission of the authors and the publisher)

Type of translocation	No.	Average hatch %	No. of eggs	Average % ♂♂	No. of flies	Average % translocations	No. of flies
Autosomal-autosomal	121						
Double	107	44.6	330 525	51.5	139 535	51.0	126 860
Triple	10	34.1	40 346	50.0	12 622	47.0	11 658
Quadruple	4	21.0	25 603	54.0	7860	39.7	7854
Y-Autosomal	72						
Double	64	54.5	253 048	56.5	109 213	54.5	102 640
Triple	6	42.3	38 253	57.8	12 788	57.1	9915
Quadruple	2	25.2	9943	45.4	3935	43.2	3278

Prolonged close inbreeding greatly reduces fertility [3, 4]; the example of this reduction given in Fig. 1 refers to the number of flies obtained from single batches of eggs; the overall reduction would have been much more striking if the number of batches per female and the proportion of infertile pairs had been considered.

Efforts in obtaining highly inbred lines have, however, been successful [4-6].

The genetic basis of the loss of fertility caused by inbreeding remains obscure. Factors operating at different levels are likely to be involved; some cause a loss of zygotes or failures of fertilization, as is shown by the decrease of adult flies obtained from similar numbers of eggs. The (meagre) available evidence indicates that under a close brother-sister mating system the number of adult flies may be halved at each new generation (Table II, last column). Recessive lethals and/or synthetic [7] lethals are a source of zygotic losses which alone may cause such a decrease in fertility. In this case it should be possible to isolate and recognize some of the genetic components responsible for this type of partial sterility and possibly to use it as a tool for genetic manipulations.

Inherited sterility factors, in the strict sense of factors affecting either the gametes or the process of fertilization, have not so far been described for the house fly.

Attention has been given, however, to factors reducing fertility through the production of unviable offspring.

Wagoner et al. [8] examined 193 stocks of house fly containing heterozygous chromosomal translocations induced by X-ray treatment. The translocations examined were both autosomal-autosomal and Y-autosomal. With double reciprocal autosomal translocations, the average hatch of eggs (44.6%) was very close to expectation (50%) on the basis of 50% alternate segregation when double reciprocal translocations are in the heterozygous form. Triple and quadruple translocations reduced egg hatch more drastically (34.1 and 21.0% respectively). From the data published by Wagoner et al., shown here in Table IV, it is clear that post-embryonic mortality was fairly low for double and triple autosomal-autosomal translocations, but was much greater when quadruple translocations were involved.

The Y-autosome heterozygous translocations provided similar results, but the average hatch was somewhat higher than with autosome-autosome translocations.

Most of the autosome-autosome translocation stocks had an approximately equal number of progeny bearing and not bearing the translocation, as expected. Some translocation, however, showed a greater transmission than expected, i.e. in the progeny the proportion of translocation-bearing flies was greater than that of normal progeny.

The loss of half of the zygotes due to unbalanced chromosomal complements causes the state commonly defined as semisterile. Wagoner has pointed out that: "... a study of the regular transmission to progeny of a semisterile state of the type that occurs in heterozygous translocations may give a useful estimate of the feasibility of using a semisterile condition for insect control in general. More specifically, it may prove useful as an approach to a control measure that would be directly applicable to the house fly".

All translocations so far described and/or used have been obtained by X-ray treatment. No case has been described of chromosomal

rearrangements in untreated flies. This is a favourable condition for following the behaviour of experimentally produced translocations after their introduction into experimental or natural populations.

Cytoplasmic sterility, in the sense of sterility which can be traced either to phenomena of an immunological nature or to the action of virus-like particles, has never been mentioned for the house fly, at least to the knowledge of the present author.

At present, the available information about genetic factors affecting fertility is confined to the X-ray-induced chromosomal translocations, which appear as a potential tool for genetic manipulations aiming at the control of house-fly populations by the genetic reduction of fertility.

Much more information is available with regard to sex-determination. The house fly is a very heterodox species in this regard, as it shows a variety of genetic formulae providing efficient systems which ensure heterogamety of one sex and a normal sex ratio in the populations.

Most field populations and laboratory stocks have a standard XX, XY sex-determining mechanism and the males are the heterogametic sex [9]. Sex chromosomes are entirely heterochromatic, and sex-linked traits have not been observed in this species, as was early anticipated by Perje [10].

The integrity of entirely heterochromatic chromosomes is not essential for ensuring a balanced genotype, so variations of size and number can be expected with these chromosomes. At least three types of X and three types of Y chromosomes differing in the length of their arms have been described [11], some of which are shown in Fig. 2; aneuploid chromosomal complements with regard to the number of X or Y chromosomes have been observed in flies from field populations [12] and from laboratory strains [13-16]; by selection, the number of heterosomes has been increased, the supernumerary elements being either X chromosomes (Fig. 3) [17, 18] or Y chromosomes (Rubini, personal information) in different lines; the failure of repeated attempts to synthetize flies without heterosomes (Rubini, personal communication) indicates that the presence of at least one heterosome, either X or Y, is essential for zygote survival.

Observations on aneuploid complements and on autosome-Y translocations indicate that, when the sex alternative is controlled by an XX, XY mechanism, the X chromosomes are 'silent' (their number may change from zero to six without visible effects on the fly) while the Y chromosome acts as the male-determining factor. In fact, all males and only males carry at least one Y chromosome [18]; furthermore the autosomes to which the Y chromosome becomes attached by translocation take a strictly holandric type of inheritance [8, 19]. According to this hypothesis, the female sex appears as the developmental fate of vital house-fly zygotes having at least one X and no Y heterochromosomes, while the Y, or parts of it, actively canalizes embryonic development toward maleness.

This is, however, an over-simplification. Through genetic manipulations (to be discussed later) it has been shown that each type of Y chromosome and at least two of the three types of X chromosomes (the third not having been tested) can be introduced in genotypes where sex differentiation is controlled by autosomal factors; in the new genetic background the Y chromosomes may become 'silent' like the X chromosomes always are, and may pass to the female; they do, however, resume their previous close association with the male sex as soon as the autosomal sex-determining factors are removed. This is a condition which, to the present author's knowledge, does not have any parallel in other organisms [9, 11].

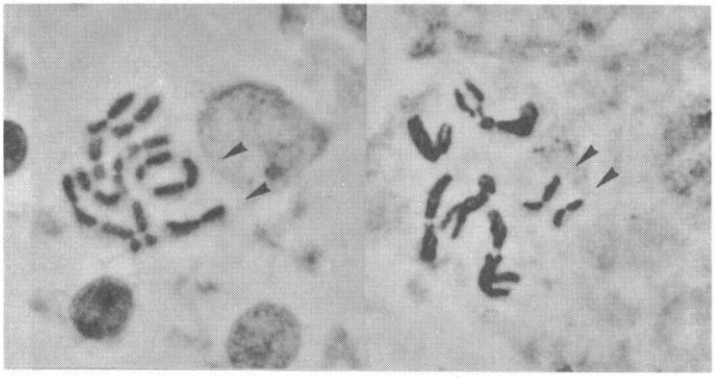


FIG.2. Size differences between heterochromosomes are easily observed in complements of heterozygous flies.
Left: Mitotic metaphase from a female with different X chromosomes (arrows), the one on the periphery of the plate being heterobrachial.
Right: Mitotic metaphase of a male showing two Y chromosomes of different size; the smaller being the standard one.
(Milani et al., 1967 [9] and Rubini, 1967 [11]; by kind permission of the authors and of the journal "Genetica agraria".

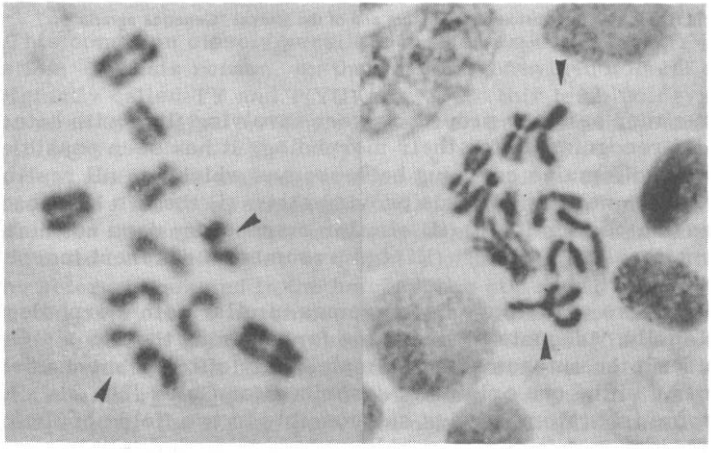


FIG.3. Diploid cells of gonads of male (left) and female (right) flies with 6 heterochromosomes. The heterochromosomes are generally grouped together and differ from autosomes in staining properties, absence of intimate somatic pairing and more delayed division in mitosis. Arrows indicate the groups of heterochromosomes.
(Rubini and Franco, 1966; by kind permission of the authors and of Academia, Prague, and Elsevier Publ. Comp.).

Some field and laboratory house-fly populations differ from the standard condition because both sexes have generally two heterosomes of similar size, indistinguishable from the X of the standard strains; aneuploids (XO, XXX) are more frequent than in standard populations, and they may account for 5-6% of the total population [13]; occasionally an X chromosome with a shortened arm has been observed [11, 17]. The two types of X may

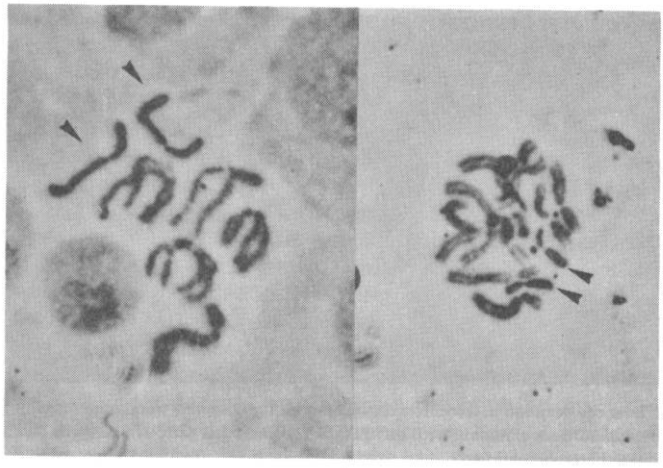


FIG.4. The non-standard heterochromosomes can reach the homozygous state without causing impairment to the flies.
Left: Mitotic metaphase from female house fly homozygous for a heterobrachial X chromosome.
Right: Mitotic metaphase from a male house fly homozygous for the large heterobrachial Y chromosome, shown already in the heterozygous state in Fig.2 right.
(Rubini, 1967[11], by kind permission of the author and of the journal "Genetica agraria").

coexist in either sex; by proper crosses involving flies with heterosomes individually recognizable for their morphology it has been possible to obtain both males and females carrying heterosomes which are all replicas of a single original one (Fig.4), thus proving that: (i) the two heterosomes of both sexes can be identical; (ii) similar morphology does not mask any sort of functional difference; (iii) heterosomes of different morphology behave similarly [11].

When the two sexes have heterosomes similar both morphologically and functionally, adequate mechanisms for ensuring the sex alternative must reside in the autosomes. A simple, fully efficient mechanism essentially similar to the one originally described for Culex [20, 21], has been fixed in some laboratory strains and possibly in two field populations [15, 22-24]. A dominant factor, symbolized M, is carried in the heterozygous condition by all males and only by males. If m is its homologue, females are mm and males Mm; genes in coupling with M follow holandric inheritance (they are transmitted only by males and are received only by (all) the male progeny) while genes in coupling with m can be transmitted by either sex; they can reach the homozygous condition only in females. With recessive characters it is easy to obtain true breeding lines with heterozygous males of normal phenotype and homozygous mutant females [9]. Even if the mutant character is shown by all females and only by them, these lines should not be defined as 'hologynic' because the males receive and transmit the chromosome carrying the recessive gene (Fig.5).

Within these lines, M is strictly limited to the male sex; in house-fly males, crossing over is either unknown or extremely rare and so the association of M with the set of genes of its chromosome is practically ab-

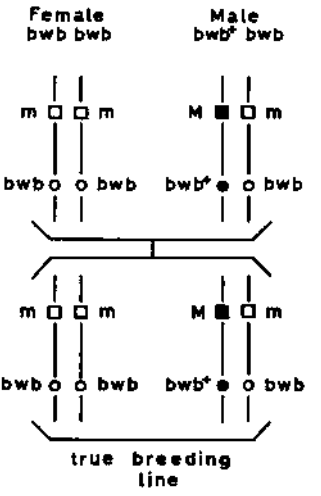


FIG. 5. Linkage between the male-determining factor M and the locus bwb. M and bwb^+ (wild type allele) are in the coupling state, so true breeding lines can easily be established, in which females are of mutant type and males of wild type.

solute. This condition closely parallels the one expected for a Y-autosome translocation; for this reason, on the evidence of hybridological data only, it was originally called TY and T(YII) [15, 25]; this terminology has been abandoned when cytological evidence has shown that no translocation was involved [11].

Abnormal sex ratios and segregation data obtained from crosses between normal flies and flies from strains carrying the M factor could only be explained on the assumption that some atypical males were homozygous for M, that M could be carried also by females of the original atypical strain both in the heterozygous and in the homozygous state, and that the hybrid females could carry the Y chromosome. The evidence for this hypothesis has been discussed in detail elsewhere [9, 23]. It is sufficient to mention here that the evidence that both sexes of the atypical strain and their hybrid derivatives can carry M, even in a double dose, and that the last-named can both have either the XX or the XY combination implies that in these flies sex must be determined by mechanisms other than the XX, XY or mm, Mm alternatives so far discussed and that the factors present in the Y chromosomes and M may lose their function as male-determining factors. Cytological evidence obtained from hybrid females showed their XY or XX structure, in close agreement with the expectation derived from hybridological data [23].

The assumption that a factor or a group of factors limited to the female sex behaves as a suppressor of M and of Y has provided a working hypothesis which accounts for the observed facts and has allowed one to plan and synthetize new sexual formulae [26]. This factor (or factors) has been called F [27], a symbol not italicized[1] because it does not correspond to a gene, even if F can usually be handled in crosses as a single factor.

[1] Italics are represented by underlining in the present text.

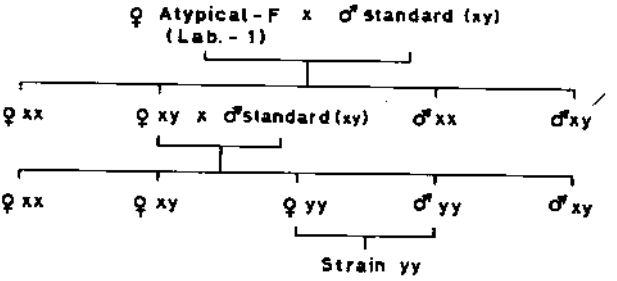


FIG. 6. Genealogy of the first YY strain of house fly synthetized. The sex formula of the parental female Atypical-F was XX MM Ff; by using a genetically marked chromosome 3, the factor M has been excluded from the strain YY, in which females are the heterogametic sex, being Ff. All pairs contributing to the line have been checked cytologically after collection of the second batch of eggs.
(Rubini, P. G., and Franco, M. G., unpublished).

The hypothesis that the F-carrying zygotes develop into females even in the presence of M and/or of the Y chromosome, implies that a proportion of their progeny should be YY or MM. These two conditions have been obtained and proved fully fertile in both sexes (Fig. 6). Moreover, the possibility of introducing M into females allows one to measure its linkage relations with markers of the same chromosome [28].

The cross-over rates obtained in test crosses of females carrying M in opposition with the markers bwb, ge, pcv have been as follows: bwb-M > 50%; ge-M = 41.9%; pcv-M = 8.01%. The order is bwb, ge, pcv, M. The cross-over rates have been practically coincident in families obtained from XY and from XX females.

It has been hinted before that F does not comply with the requisites of a gene. In fact, repeated attempts to assess its linkage relations have failed; furthermore, evidence exists that it can split up into parts of lower valence, giving rise both to unbalanced phenotypes (intersexes) and to new sexual formulae.

Crosses of flies of either sex from the bwb YY strain provided, in the early generations of the strain, progenies with sex ratios in agreement with expectation on the hypothesis that both sexes carried two Y chromosomes and that a normal sex ratio was ensured to the strain by a dominant factor carried only by females in the heterozygous state (female heterogamety for dominant autosomal factors). Males of this strain crossed with standard females gave regularly only male progeny, as expected. However, by the 27th generation, some bwb YY males gave unexpected female progeny when crossed with standard females; each male gave similar progenies with different females, and the observed F_1 sex ratios ranged from males only (as expected), to normal sex ratio, to excess of females, and to females only (Tables V and VI).

It was assumed that genetic recombination had altered the distribution of the factors symbolized by F, leading to various loads of female-determining factors in the population, and that some males could carry parts of F, insufficient to overcome the action of their two Y chromosomes [29]. Support for this hypothesis has been provided by the establishment of a strain (YY M86) in which both sexes still had two Y chromosomes, but according to

TABLE V. PROGENIES OBTAINED FROM MALES OF THE 27th AND 28TH GENERATIONS OF THE bwb YY STRAIN CROSSED WITH STANDARD FEMALES
The appearance of females is unexpected and shows a change in the strain (Rubini, P.G., unpublished data)

♀♀ standard × ♂♂ bwb YY

Generation of parental ♂♂	Type of mating	Progeny			% ♂♂
		♀♀	♂♂	♂♂ ++	
F_{27}	mass culture	2229	3864	103	62.36
F_{28}	single pairs (55 ♀♀; 30 ♂♂)	1036	2218	9	67.97

TABLE VI. RESULTS OBTAINED FROM MULTIPLE MATINGS OF MALES OF THE bwb YY STRAIN, F_{28}, WITH STANDARD FEMALES
The composition of the F_1 families ranged from males only, as expected, to females only; each male has given consistent results with different females
(Rubini, P.G., unpublished data)

♀♀ standard × ♂♂ bwb YY F_{28}

Composition of F_1 families	No. of parents		F_1 progeny			
	♂♂	♀♀	♂♂	♀♀	♂♂ ++	% ♂♂
♂♂ only	10	16	860	-	-	100
Excess of ♂♂	7	17	608	115	1	83.97
Regular sex ratio	10	16	709	682	6	50.78
Excess of ♀♀	2	5	41	215	3	15.83
♀♀ only	1	1	-	24	-	0.00
Total	30	55	2218	1036	9	67.97

all evidence the sex alternative was provided by a fraction of F, called F', carried in a double dose by females and in a single dose by males. In the YY M86 strain, male heterogamety had been re-established and sex was controlled by a balance between sex chromosomes of the Y type and autosomal factor(s). The balance however was not fully effective and stable, and, by the tenth generation, some intersexes appeared; their frequency increased in the following generations, while the sex ratio shifted progressively

in favour of the females, an increasing proportion of which provided progenies which did not conform to expectation. The strain eventually ended at the 20th generation, when only females and a few intersexes appeared (Rubini, personal communication) (Fig. 7).

The decline of this strain has certainly been due to genetic heterogeneity and to greater fitness of some new genotypes, which spread into the strain and reduced the probability of the appearance or of the survival of male zygotes.

Summing up, we now have consistent evidence for the existence in the house fly of at least five sex formulae as follows:

		♀♀	♂♂	heterogamety
1	Standard	: XX mm ff	XY mm ff	male
2	Atypical-M III	: XX mm ff	XX Mm ff	male
*3	Atypical-F	: XX MM Ff	XX MM ff	female
	"	: XX Mm Ff	XX Mm ff	female
	"	: XX mm Ff	XX Mm ff	female
4	bwb YY	: YY mm Fm	YY mm ff	female
5	YY M86	: YY mm F'F'	YY mm F'f	male

The valence of the known factors can be graded as follows: Y > XXX (or more X); M > XXX; F > MM; F > YYY; F > MY; Y < F'; YY > F'; YY < F'F'.

Some of these formulae, namely Nos. 1, 2 and 3, are quite stable, while those experimentally obtained in establishing YY strains are incompletely balanced and the populations carrying them tend to reach new genetic equilibria.

Autosomal sex factors are especially exposed to selective pressures acting on linked genes and so complex adaptations involving the selected trait and sex factors are expected. Examples of correlated adaptations are provided by a series of three atypical strains in which the development of DDT-resistance has been concurrent — to all appearance — with a shift from male to female autosomal heterogamety [30] and by a case of complete replacement of XY males by the atypical XX type in an Australian strain submitted to DDT-pressure [16]; according to Hiroyoshi two Japanese field populations [15] provide evidence of linkage between a viability factor and a male determinant linked to the bwb locus.

This picture is complicated enough, but it does not give the full story for the house fly. In fact, Wagoner [31] in a strain from Australia found evidence for autosomal male-determining factors on chromosomes II, III and V, each factor being adequate to ensure full male development; and Kerr [16] found evidence for a M factor on the II chromosome of another Australian strain. Dr. Vanossi (personal communication) has good evidence of consistent and conspicuous shifts of the sex ratios in 'sister' cultures obtained from subsequent batches of eggs laid by females of a special line.

The standard and the two atypical strains (numbers 1, 2, 3 in the list given above) do not show any impairment of fertility and their sex formulae have remained unaltered for years. On the other hand the YY strains are to some extent biologically impaired and genetically not balanced, at least at the level of the sex formulae.

* The three formulae may coexist in the same population.

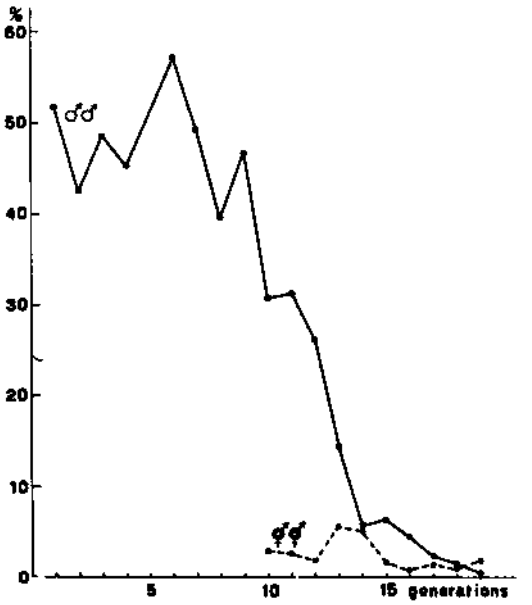


FIG.7. Directional changes of sex ratio in a YY strain of house fly. The proportion of males in the bwb YY M86 strain of house fly has ranged around normality for six generations; subsequently a progressive shift of sex ratio in favour of females has brought the strain to extinction. From the tenth generation onward some intersexes have been observed.
(Rubini and Franco, 1968 [27]; graph unpublished).

All strains discussed above, with the exception of strain YY M86, have normal sex ratios, ensured by one of three types of heterogamety in one sex. When crosses are made between these strains, in most cases the F_1 hybrid progenies have normal or peculiarly distorted sex ratios (Fig.8); but four out of sixteen crossing combinations, however, produce only male progeny, as shown in Table VII.

Males with two Y chromosomes and males MM (homozygous) obviously produce only one kind of sperm, carrying either the Y chromosome or the M factor respectively (allowance being made for non-disjunction), which, on fertilization of eggs produced by females from standard or atypical M strains, will produce only male zygotes without impairment of fertility. These males are perfectly normal, and, being F_1 hybrids between relatively inbred strains, may show some degree of heterosis.

If the sex formulae of natural populations are known, control operations based on the sterility principle may make use of a release of males from strains giving with the local females only male progeny.

Sexually competitive males giving only male progeny released under favourable conditions might in one generation reduce to a minimum the number of females available for ensuring the next generation. If these males should be used in control operations based on induced sterility, the danger of partially sterilizing doses would be cancelled out by the fact that any 'leakage' of fertility would produce only males.

♀ XX Ff MM x ♂ XY ff mm

♂ \ ♀	X F M	X f M
X f m	XX Ff Mm	XX ff Mm
Y f m	XY Ff Mm	XY ff Mm

S. R. 1♀ : 1♂

♀ XX Ff Mm x ♂ XY ff mm

♂ \ ♀	X F M	X F m	X f M	X f m
X f m	XX Ff Mm	XX Ff mm	XX ff Mm	XX ff mm
Y f m	XY Ff Mm	XY Ff mm	XY ff Mm	XY ff mm

S. R. 5♀ : 3♂

♀♀ ♂♂

FIG. 8. Most females of strain Atypical-F are homozygous for the male factor M; some are heterozygous. Crosses with standard males produce F_1 families with sex ratios respectively 1♀:1♂ or 5♀:3♂. The zygotic combinations leading to these ratios are shown in the bodies of the chess-boards below. (Milani et al., 1967 [9], modified).

TABLE VII. SEX RATIOS OBTAINED IN CROSSES BETWEEN STRAINS WITH DIFFERENT SEXUAL FORMULAE

Ratios between brackets are expected when flies from strain atypical-F are heterozygous Mm instead of being homozygous MM

(Milani et al. [9], modified)

	♂ Standard	♂ Atyp.-F	♂ M III	♂ bwb YY
♀ Standard	1:1	♂♂ only (1:1)	1:1	♂♂ only
♀ Atyp.-F	1:1 (5♀:3♂)	1:1	1:1 (5♀:3♂)	1:1
♀ M III	1:1	♂♂ only (1:1)	1:1	♂♂ only
♀ bwb YY	1:1	1:1	1:1	1:1

But the most direct and reliable use of the practical potentialities provided by the various sex formulae of the house fly seems to reside in the use of F_1 hybrid populations composed of males only for sterilization and release; this would reduce the cost of operations at all stages, would provide a more standardized material, and in addition would eliminate the disadvantages of releasing both sexes.

Planning, with the available knowledge and material, the genetic contamination of natural house-fly populations, leading to their self-destruction, still requires much caution. Even if the genetic equilibria of natural populations could be temporarily altered by the introduction of incompatible genetic factors, new equilibria are likely to be selected. Furthermore, we have evidence of already existing fly populations affected by genetic imbalance at the level of sex determinations. A high frequency of intersexes and/or of gynandromorphs has been described in field populations [32-34]; large distortions of sex ratio, usually in favour of males, have been recurrently recorded both for field and laboratory strains, without obvious signs of impairment.

ACKNOWLEDGEMENTS

The author is indebted to Drs. P.G. Rubini and M.G. Franco for providing unpublished material, for giving permission to reproduce several tables, photographs and graphs from their papers, and for continuous help and discussion during the preparation of the manuscript; to Dr. Dale E. Wagoner for permission to reproduce Table IV; to Dr. S. Vanossi for unpublished information; to the publishers of the journals "Rivista di parassitologia", "Genetica agraria" and "Journal of Heredity", and to Academia (Prague) and Elsevier Publ. Co. (Amsterdam) for permission to reproduce certain material, as indicated in the text.

REFERENCES

[1] CURTIS, C.F., The production of partially sterile mutants in Glossina austeni, Genet.Res., Camb. 13 (1969) 289.

[2] MILANI, R., Considerazioni genetiche su alcuni aspetti della biologia della mosca domestica e osservazioni sulla fecondità e sulla fertilità, Riv.Parassit. 16 4 (1955) 257.

[3] MILANI, R., FRANCO, M.G., Modalità di ricombinazione del mutante ac di Musca domestica L. con tre marcatori e osservazioni sulle segregazioni mendeliane, Symp.genet.et biol.Ital. 7 (1960) 59.

[4] MILANI, R., "The genetics of Musca domestica and of other muscoid flies", Ch.9, Genetics of Insect Vectors of Disease (WRIGHT, J.W., PAL, R., Eds.), Elsevier, Amsterdam (1967) 315.

[5] LICHTWARDT, E.T., BRUCE, W.N., DECKER, G.C., Notes on the inbreeding of house flies, J.econ. Ent. 48 3 (1955) 301.

[6] LICHTWARDT, E.T., Genetics of DDT-resistance of an inbred line of houseflies, J.Hered. 47 1 (1956) 11.

[7] DE MAGALHAES, L.E., DE TOLEDO, J.S., BRITO DA CUNHA, A., The nature of lethals in Drosophila willistoni, Genetics 52 3 (1965) 599.

[8] WAGONER, D.E., NICKEL, C.A., JOHNSON, O.A., Chromosomal translocation heterozygotes in the house fly. Egg hatch, sex ratios, and transmission to progeny of 193 translocations, J.Hered. 60 5 (1969) 301.

[9] MILANI, R., RUBINI, P.G., FRANCO, M.G., Sex-determination in the housefly, Genet.agr. 21 (1967) 385.

[10] PERJE, A.M., Studies on the spermatogenesis in Musca domestica, Hereditas 34 (1948) 209.

[11] RUBINI, P.G., Ulteriori osservazioni sui determinanti sessuali di Musca domestica L., Genet.agr. 21 (1967) 363.
[12] BOYES, J.W., COREY, M.J., PATERSON, H.E., Somatic chromosomes of higher Diptera.IX. Karyotypes of some muscoid species, Can.J.Zool. 42 (1964) 1025.
[13] RUBINI, P.G., Polimorfismo cromosomico in Musca domestica L., Boll.Zool. 31 2 (1964) 679.
[14] RUBINI, P.G., Osservazioni citologiche preliminari su Musca domestica L., Symp.genet.et biol.Ital. 13 (1966) 332.
[15] HIROYOSHI, T., Sex-limited inheritance and abnormal sex ratio in strains of the housefly, Genetics 50 3 (1964) 373.
[16] KERR, R.W., Inheritance of DDT resistance in a laboratory colony of the housefly, Musca domestica, Aust.J.biol.Sci. 23 (1970) 377.
[17] RUBINI, P.G., FRANCO, M.G., Polymorphism and aneuploidy of the heterochromosomes in Musca domestica L., Symposium on the Mutational Process, Prague (1965) 89.
[18] RUBINI, P.G., PALENZONA, D., Response to selection for high number of heterochromosomes in Musca domestica L., Genet.agr. 21 (1967) 101.
[19] WAGONER, D.E., Linkage group-karyotype correlation in the house fly, Musca domestica L., confirmed by cytological analysis of X-ray induced Y-autosomal translocations, Genetics 62 (1969) 115.
[20] LAVEN, H., "Formal genetics of Culex pipiens", Ch.2, Genetics of Insect Vectors of Disease (WRIGHT, J.W., PAL, R., Eds.), Elsevier, Amsterdam (1967) 17.
[21] GILCHRIST, B.M., HALDANE, J.B.S., Sex linkage and sex determination in a mosquito, Culex molestus, Hereditas 33 (1947) 175.
[22] FRANCO, M.G., LANNA, T.M., MILANI, R., Eredità legata al sesso nella Musca domestica L., Atti Ass.Genet.Ital. 7 (1962) 198.
[23] RUBINI, P.G., FRANCO, M.G., Osservazioni sugli eterocromosomi e considerazioni sulla determinazione del sesso in Musca domestica L. (Nota preliminare), Boll.Zool. 32 2 (1965) 823.
[24] MILANI, R., FRANCO, M.G., Fertilità, rapporto-sessi e segregazione del mutante bwb in incroci tra ceppi geograficamente separati di Musca domestica L., Symp.genet.et biol.Ital. 6 (1959) 249.
[25] SULLIVAN, R.L., Linkage and sex limitation of several loci in the housefly, J.Hered. 52 6 (1961) 282.
[26] RUBINI, P.G., FRANCO, M.G., Formazione ed analisi genetica preliminare di un ceppo YY di Musca domestica L., Boll.Zool. 33 1 (1966) 141.
[27] RUBINI, P.G., FRANCO, M.G., Additional information on sex-determination in Musca domestica L., Atti Ass.Genet.Ital. 13 (1968) 114.
[28] RUBINI, P.G., FRANCO, M.G., Localizzazione genetica del fattore autosomico di mascolinità M in Musca domestica L., Boll.Zool. 36 4 (1969) 414.
[29] RUBINI, P.G., FRANCO, M.G., Osservazioni sui determinanti sessuali presenti in un ceppo YY di Musca domestica L., Boll.Zool. 35 (1968) 437.
[30] MILANI, R., Le variazioni delle formule sessuali di Musca domestica L. come possibile esempio di coadattamenti genetici, Boll.Zool. 36 4 (1969) 372.
[31] WAGONER, D.E., Presence of male determining factors found on three autosomes in the house fly, Musca domestica, Nature, Lond. 223 5202 (1969) 187.
[32] MILANI, R., The genetics of the house-fly. Preliminary note, Caryologia, (Vol.suppl.) (1954) 791.
[33] MILANI, R., RIVOSECCHI, L., Osservazioni sugli scleriti dei segmenti terminali di individui sessualmente anormali di M. domestica L., Boll.Zool. 22 2 (1955) 373.
[34] SACCA, G., Reperto di ginandromorfi in Musca domestica L. (Diptera, Muscidae), R.C.Ist.sup.Sanità 18 (1955) 380.

DISCUSSION

H. LAVEN: You have compared the sex determination of house flies with that of Culex pipiens, for which it is generally assumed that sex is determined by a single gene with two alleles, M = maleness, m = femaleness. May I remind you that in Culex pipiens we know of one further gene involved in sex determination in another chromosome. Sex determination and sex ratio determination are such fundamental functions in an organism that we can assume a priori that they are determined by polygenic systems. It is dangerous to assume that such systems could be used for control. Sex ratio distortion in Aedes aegypti can serve as an example.

R. MILANI: It seems to me that the analogy with Culex is still valid, at least as far as the M factor is concerned. Regarding the other comments, I thank Professor Laven for having given to the audience roughly the same conclusions as are to be found in the text of my paper, and which I omitted to read owing to lack of time. I think that the potentialities of knowledge of sex determination for the genetic control of the house fly are to be found not so much in their direct use in control operations as in the integration of conventional sterilization methods. A release could be made either of F_1 populations composed of sterilized males only or of males which would produce with local females a progeny of males only. These points are discussed in the paper.

L.E. LaCHANCE: I am afraid I must disagree mildly with the pessimistic point of view that it would be impossible to use sex ratio factors in insect control programs. It might be dangerous to rely solely on this new technique, but I think it might be integrated very nicely into a control program. I would not be pessimistic merely because polyfactorial inheritance is concerned; after all, if animal and plant breeders can make use of such factors, so can entomologists.

USE OF CHROMOSOME REARRANGEMENTS FOR MOSQUITO CONTROL

M.J. WHITTEN
CSIRO, Division of Entomology,
Canberra, A.C.T., Australia

Abstract

USE OF CHROMOSOME REARRANGEMENTS FOR MOSQUITO CONTROL.

The release of males, partially sterilized by translocation heterozygosity, has been suggested as a means of controlling Musca domestica and certain mosquito species in which irradiation-sterilized males are non-competitive. This model of genetic control is examined and several arguments are advanced favouring the rejection of this approach as a practical means of pest control. They include the following: (1) Problems arising from mass rearing partially sterile translocation heterozygotes and culling out the fertile segregants, as well as from the necessity to separate the sexes in some cases, e.g. autosome-autosome translocations. (2) A more serious problem relates to the plateaued response following the release of partially sterile insects such that no advantages result comparable with the swamping effect that normally develops after successive releases of fully sterile insects. (3) Genetic equilibrium conditions lead to rapid removal of translocation heterozygotes.

An alternative use for translocations, involving the release of multiple translocation homozygotes which are viable and fertile, is considered. Particular reference is made to mosquito species and their small number of chromosomes. Theoretical arguments, together with computer simulation studies, suggest that it may be possible to introduce a permanent genetic mortality of the order of 50% into natural populations. A more significant benefit relates to the possibility of manipulating the genetic composition of natural populations to remove insecticide resistance genes or introduce conditional lethals in 10 generations or less.

Semisterility, induced by chromosome translocation heterozygosity, provides a basis for several models of genetic control of insect pests. The release of semisterile males has been suggested as an alternative to radiation-induced sterile insects for mosquito control where radiation reduces competitiveness of males [1, 15]. This use of translocations is significantly different from the approach of using viable homozygous translocations introduced by Serebrovksii [2] and Curtis [3] and discussed by Curtis and Hill [4] for tsetse fly control and by Whitten [5] for control of some other higher Diptera. It is also significantly different from the conventional sterile-male approach. One objective of this report is to demonstrate that it is misleading to group all the various translocation systems under a single heading. The genetic principles involved are often quite different and the goals of the programs are also often quite different. In some instances a program aims at the reduction in numbers of the pest, in others it relies on the replacement of a pest strain by a less objectionable form. It is not my intention to suggest that some approaches will work while others will not. Rather I will attempt to rank the different approaches on some scale of efficiency and show that current programs emphasize systems of control which rank low on this scale. It follows that if it is demonstrated, in the meanwhile, that one of these less efficient genetic systems can lead to population control then we are in a very favourable position indeed.

Let us now look at the systems which involve the release of males rendered semisterile because they are heterozygous for one or more

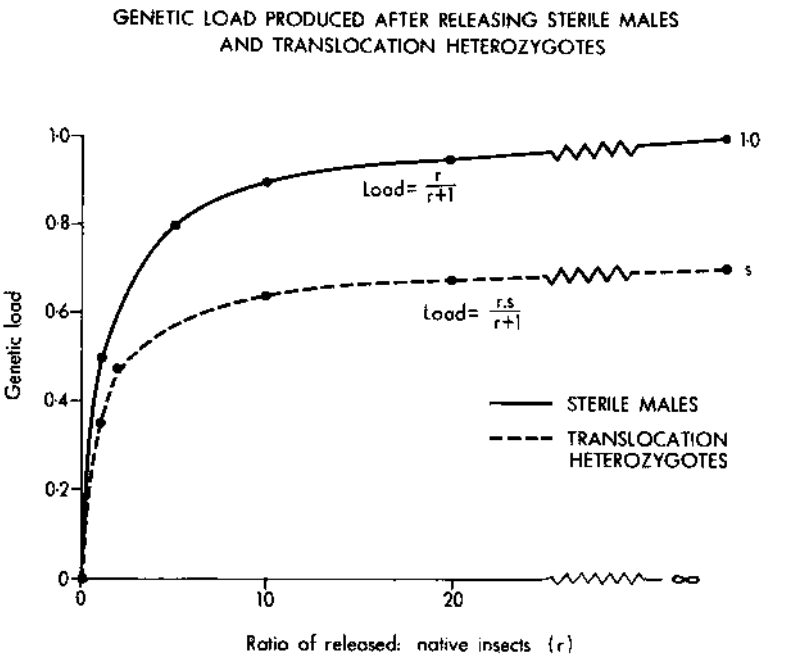


FIG. 1. Genetic load produced after releasing sterile males and translocation heterozygotes.

translocations and see how they compare with the conventional sterile-male technique.

If sterile males are released in the ratio r:1 into a natural population the zygotic mortality or genetic load is equal to $r/(r+1)$. If the released males have a semisterility, s, i.e., (1 - s) of their progeny survive, then the load induced is $r.s/(r+1)$. The curves relating load to release ratio are similar for fully sterile and semisterile males (Fig. 1) but there is a notable biological difference between them. When $s=1$, i.e. the males are fully sterile, the load increases with r. With successive releases the natural population declines causing r to increase. In time the load $r/(r+1) \rightarrow 1$. The releases become more effective until eventually the population is eliminated. Thus emerges the well known fact that the sterile-male method lends itself to programs of eradication rather than simple control.

Where we are releasing translocation heterozygotes s cannot be near 1, otherwise we could not maintain the strain, let alone provide sufficient insects for release. This restriction imposes probable values on s in the range 0.3 - 0.7. Even if we assume it is feasible to supply large numbers of males for release when s = 0.7, the continued release of these males cannot increase the load above 0.7 since $r.s/(r+1) \rightarrow s$ as $r \rightarrow \infty$. Thus, unlike the release of sterile males, there is no incentive to increase r by releasing large numbers of semisterile males or continuing to release them. Inspection of Fig. 1 shows that for s = 0.7 an increase in r from 10 to 10 000 does not effectively increase the load (0.64 - 0.7). When

s = 1 the same change in r increases the load from 0.9 to 0.9999 which is a highly significant increase. This latter change in r would lead to the extinction of many populations.

If it were possible to displace completely normal males in the population with translocation males, then the continued presence of the inherited semisterility and the possible depression in population size that it may generate could, in part, offset the disadvantage I have described. However, when normal males and Y-autosome semisterile males are mixed together in a population the only equilibrium is the trivial one of fixation of normal males. It seems most improbable that we could find realistic conditions where a Y-autosome translocation could become fixed in this manner. Laven [1] has argued that the greater efficiency of the Y-autosome males he used had only a slight effect on the reduction in the population. However, in the absence of their artificially induced efficiency, the Y-autosome males would be quickly eliminated by natural selection. If their sterility is s these translocation males would require a virility in excess of 1/s times normal for them to be favoured by natural selection.

Further problems may arise in preventing the fertile females that are cultured along with the semisterile males from being released with the males. A serious culling problem presents itself if the semisterility stems from autosome-autosome translocations as in some housefly projects [18] since here half the males will not carry any translocation and will be fully fertile. These males could be readily removed with conditional lethals (e.g. white eyes) but it still leaves us with the problem of producing sufficient males from a colony where only one quarter of the insects are of the appropriate genotype and the culture is fairly sterile anyway (s being about 0.7). The prospects of replacing normal males in a population with males carrying autosomal translocations only are zero because half the male offspring from the latter are normal. Furthermore, the loss of translocation males through natural selection will be extremely rapid. If their frequency is p in one generation it will be $p.s/2.(1 - p(1 - s))$ in the following generation. Consequently their impact must be very short-lived.

The pertinent question to ask is: How does the translocation heterozygote system compare with the conventional sterile-male system? Since we cannot release translocation heterozygote males which are fully sterile we should compare their competitiveness with males sterilized to the same degree with irradiation and not, as is common practice, with fully sterile males. It may be the case that fully sterile males are not competitive (though this is by no means a closed case), but are males not competitive who are sterilized with irradiation to a level of 50%, 75%, 95% or even 99%? This study has not been carried out and it must be before a proper comparison can be performed. For obvious reasons, if a male which is sterilized by irradiation in excess of 70% is competitive with normal males, then a control program making use of him would be more efficient than one using a translocation heterozygote.

So far we have considered the use of translocations either where homozygotes are not generated (i.e. y-autosome), or where, if homozygotes are generated (i.e. autosome - autosome), they are inviable. A number of the problems which I have indicated to arise in these cases can be avoided if we use translocations that are fully viable and fertile as homozygotes. I would like to consider four examples in this category.

Example 1

	Male	Female	
Genotypes	$\frac{+}{+}$ $\frac{+}{T(X,2)}$	$\frac{+}{+}$ $\frac{+}{T(X,2)}$	$\frac{T(X,2)}{T(X,2)}$
Fitness	w_1 w_2	w_{11} w_{12}	w_{22}
(Sample values)	1.0 0.5	1.0 0.5	1.0

In this instance, involving a single exchange between the X chromosome and an autosome, an unstable equilibrium exists [7, 8]. That is to say, if we have a mixed population consisting of normal individuals and translocation homozygotes the two types cannot coexist. One type will replace the other. Which type will succeed depends on the initial frequencies. Using Wright's terminology [8] we have

$$\hat{q}_{egg} = \frac{w_{22}w_2 - 0.5\,w_{12}(w_1 + w_2)}{w_{11}w_1 - w_{12}(w_1 + w_2) + w_{22}w_2} \text{ and } \hat{q}_{sperm} = \frac{w_1\hat{q}_{egg}}{w_1\hat{q}_{egg} + w_2(1 - \hat{q}_{egg})}$$

where $\hat{q}$ is the unstable equilibrium value. For the fitness values assigned, $\hat{q}_{egg} = 1/6$ and $\hat{q}_{sperm} = 2/7$. A single release of the X-autosome translocation strain, both sexes included, in excess of the equilibrium value ($r > 5$) should lead to a frequency dependent displacement of normal males by the translocation heterozygote males with an associated load around 0.5. If this permanent load is sufficient to depress population size then the approach may offer theoretical advantages over the release of fully sterile males. However, natural selection would tend to oppose the system by reducing the level of sterility of the translocation heterozygote but there is no a priori means of determining if it could successfully achieve this. It is relevant to note that if Y-autosome translocation males can lead to a population decline then X-autosome males can cause a similar decline with the expenditure of less effort since fewer insects need be released. Both these models assume that the translocation is inseparably attached to the sex locus and this may be very difficult to achieve.

Example 2

The second example I would like to consider combines an autosome-autosome translocation, i.e. T(2,3), with an X-autosome translocation giving the genotypes and suggested relative genetic fitnesses as follows:

	Males				Females		
Genotypes	$\frac{+}{+}$	$\frac{T(X,2,3)}{T(2,3)}$	$\frac{+}{T(2,3)}$	$\frac{T(X,2,3)}{+}$	$\frac{+}{+}$	$\frac{T(X,2,3)}{+}$	$\frac{T(X,2,3)}{T(X,2,3)}$
Relative fitness	1.0	0.5	0.5	0.25	1.0	0.25	1.0

Once again an unstable equilibrium exists at the same point as in Example 1 (i.e. when r = 5). Table 1 shows changes in genotypic frequencies in successive generations after a computer simulated release in excess of the unstable equilibrium point. The liberation of fertile insects leads initially to a population increase, hence the negative genetic load, followed by a permanent load around 0.5. This load results from the fixation of males heterozygous for a T(X,2) translocation.

So far we have achieved a similar result to that provided by Example 1. Another significant change has also occurred. We have, in effect, replaced the native genotype with a translocation type for all three chromosomes in 8 generations. By the appropriate use of translocation breakpoints and inversions we have available a means of rapidly introducing genes into the native population as first suggested by Curtis [6]. We could remove insecticide resistance, introduce conditional lethals, e.g. temperature-sensitive lethals [5], or could change frequency of genes for the inability to transmit disease. In other words, in addition to any control achieved directly from the load of 0.5 we have available another useful tool in the form of genetic manipulation.

Suppose successful control relied on genetic displacement of some undesirable genotype (e.g. vectorial ability) from some designated region. A serious problem may arise if that region represents part of a larger and more or less continuous population rather than an isolated or nearly isolated population. For the displacement to be stable and hence the control system durable, the density of the released form would need to be some 5 times greater than the native strain's density, at least along the border where the 2 forms make contact; otherwise the boundaries will collapse leading to the elimination of the released strain. Partial isolation would be a sufficient condition for stability provided that migration was less than 17% (i.e. $q < 1/6$). The released strain would carry a genetic load of 0.5 which would act towards reducing its density, causing it to fall further short of the equilibrium value. This fact argues against the introduction of a permanent genetic load through sex-linked translocations except for isolated or semi-isolated populations where the whole native populations can be displaced by the initial introduction.

Example 3

I would now like to consider the use of multiple translocations, not involving sex-linkage, to show how we might be able to introduce high genetic loads which persist for 5 or 6 generations which at the same time would permit genetic manipulation. In this instance it should be possible to displace insects from segments of a much larger population without the danger of rapid collapse of the boundaries as predicted for the previous two examples. Here the unstable equilibrium is around 0.5. This implies that the synthetic strain be equally as dense as the surrounding native population for boundary stability. This condition is likely to be satisfied if control is achieved by the removal of some property such as vectorial ability. It would not be satisfied if control operated through some genetic load or a reduction in population size following insecticide treatment over the displaced area, a move made possible by the removal of genes for insecticide resistance. This latter situation was considered elsewhere [5] and it was suggested that either a buffer zone be established along the

TABLE I. CHANGES IN RELATIVE FREQUENCIES OF GENOTYPES AFTER THE RELEASE OF A T(X,2,3) STRAIN ABOVE THE UNSTABLE EQUILIBRIUM VALUE

	Males				Females			
Genotype	$\frac{+}{+}$	$\frac{T(X,2,3)}{T(2,3)}$	$\frac{+}{T(2,3)}$	$\frac{+}{T(X,2,3)}$	$\frac{+}{+}$	$\frac{+}{T(X,2,3)}$	$\frac{T(X,2,3)}{T(X,2,3)}$	
Fitness	1.0	0.5	0.5	0.25	1.0	0.25	1.0	
Generation								Genetic load[b]
0	1000	9000	0	0	1000	0	9000	-
1[a]	10	384	35	92	11	166	404	-4.51
2	1	286	24	56	3	54	298	-0.99
3	1	363	6	43	1	40	354	0.20
4	0	394	13	24	0	17	390	0.58
5	0	445	9	12	0	13	460	0.53
6	0	472	2	5	0	13	492	0.51
7	0	482	0	2	0	2	497	0.51
8	0	487	0	0	0	0	487	0.52

[a] The total number of genotypes in each generation is fixed at 2000 × (1-load).

[b] The genetic load is given by $\left(1 - \frac{\text{actual number of zygotes in population}}{\text{number predicted in the absence of a release}}\right)$.

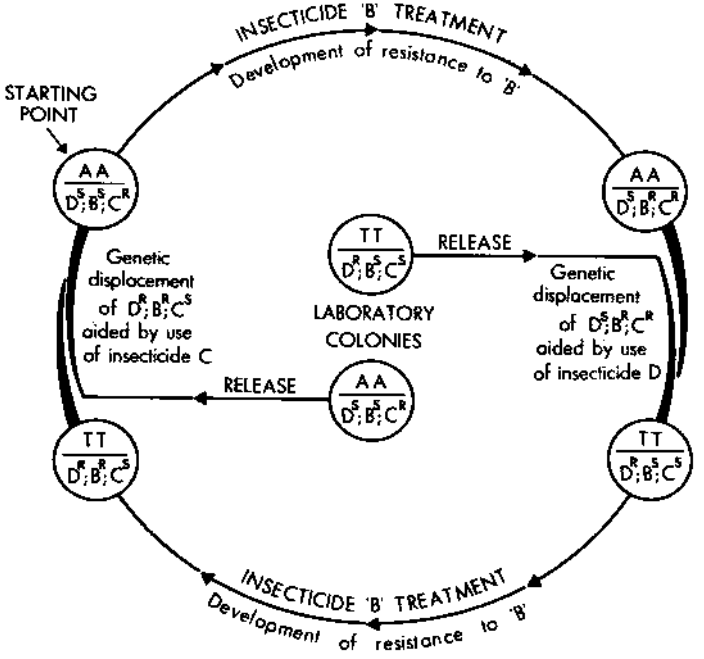


FIG. 2. Scheme for removal of genes for insecticide resistance from natural populations.

boundary of the release area where the insecticide is not used or that the synthetic strain be released repeatedly along these boundaries to maintain stability.

Curtis has considered the use of single translocations in tsetse fly both for direct population control through the presence of partially sterile insects [3,4] and for indirect control by removal of desirable genes [6]. For most species of insect the genetic load from the release of a strain carrying a single translocation would be too low to reduce population size appreciably while the rate of gene displacement in these circumstances (over 30 generations to introduce a desirable gene) is probably too slow to recommend its usage. If these approaches are to become available for many pest species it will be necessary to consider the development and release of multiple translocation strains. Their advantages and problems in their development have been discussed in more detail elsewhere [5,13]. It was argued that the release of (n-1) strains each being homozygous for as few as 2 translocations might lead to genetic losses of the order $(n^2 - 1)/n^2$ persisting for up to 6 generations. This follows from the fact that most individuals, (n-1)/n, would be heterozygous for 4 or more translocations and therefore virtually sterile. As n, the total number of strains, increases, the proportion of hybrids (n-1)/n increases. If the average sterility of the hybrids is s then the load is of the order $s(2-s)-2(1-s)/n$, which tends to s(2-s) for large n (i.e., n = 10 or more). On the other hand the release of a single strain, TT, homozygous for a sufficient number of translocations to render hybrids sterile, could allow rapid introduction of desirable genes (5 - 6 generations).

Since the displacement process just described requires a release above the equilibrium value it would suggest that releases need exceed the native population in size to take advantage of the frequency-dependent selection. This feature mitigates against the model and it contrasts with meiotic drive models where it has been assumed release of large numbers is not essential but a mere seeding of the native population is sufficient. There is a possible solution to this problem if we combine a model described by Wehrhahn and Klassen [9] with one described by Whitten [5,13]. One such composite scheme is outlined in Fig. 2. Three insecticides D, B and C are employed in the program, TT being resistant for D but susceptible for B and C while the field strain is susceptible for D only. TT is released in low numbers while insecticide D is being used. It is easy to show that T will increase in frequency despite its frequency-dependent disadvantage following the small release so long as its resistance to insecticide D provides it with an advantage greater than the reciprocal of its frequency. For example, suppose the release ratio, r, of TT to the native form AA is 0.1 then the insecticide resistance of TT would need to provide it with a more than 10-fold selective advantage, to allow it to exceed the unstable equilibrium. An advantage of this order seems quite likely for the conditions described. After several generations of treatment with D its own frequency advantage should allow the released strain to proceed to fixation without further application of D. Insecticide B could then be used until resistance to it develops. When resistance prevents further control with B, the original AA strain whose resistance type is D^S; B^S; C^R is released and insecticide C is used to permit rapid displacement of TT. Thus insecticide B becomes available once again (Fig. 2). And so the cycle could continue without any need to develop new strains or insecticides. It is quite possible that 1 or 2 generations' usage of insecticides C and D would be adequate to boost the frequency of the liberated strain above the critical equilibrium values. Insecticide B would be used for as many generations as the field insects remained susceptible and in essence would constitute the major control measure in this instance. Where 3 insecticides were available C and D would be the least acceptable of the group while B would be the insecticide whose contribution to environmental contamination is minimal. Since the scheme envisages permanent life for insecticide B, hopefully we could develop a highly specific biological compound similar in action to that described by Slama et al. [17] with little fear of its obsolescence through the development of resistance.

An alternative scheme involves the release of 3 multiple translocation strains T_{11}, T_{22} and T_{33} and two insecticides combined in the following pattern:

AA and T_{11} being D^S; C^R

T_{22} and T_{33} being D^R; C^S where AA represents the native form

T_{22} is released and established using insecticide D in the way described above until it is the common form. T_{11} is then established with insecticide C which also acts to reduce the frequency of T_{22} while at the same time restoring the frequency of AA. On the final swing of the pendulum T_{33} is introduced with insecticide D. By this stage all 4 strains are present

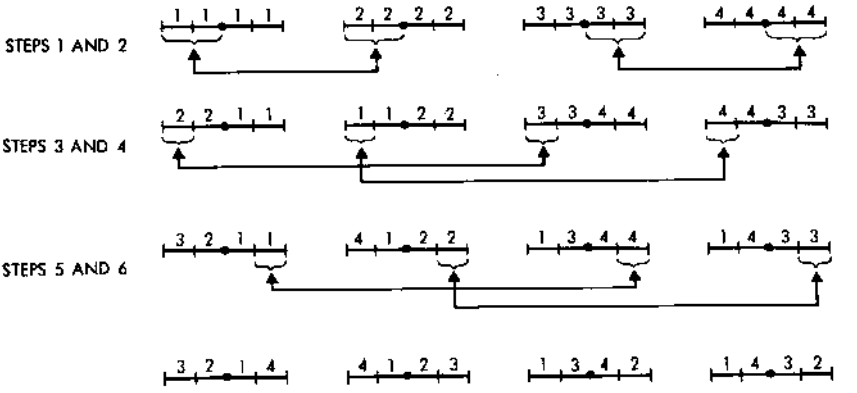


FIG. 3. Multiple translocation strain involving 4 chromosomes and 6 rearrangements.

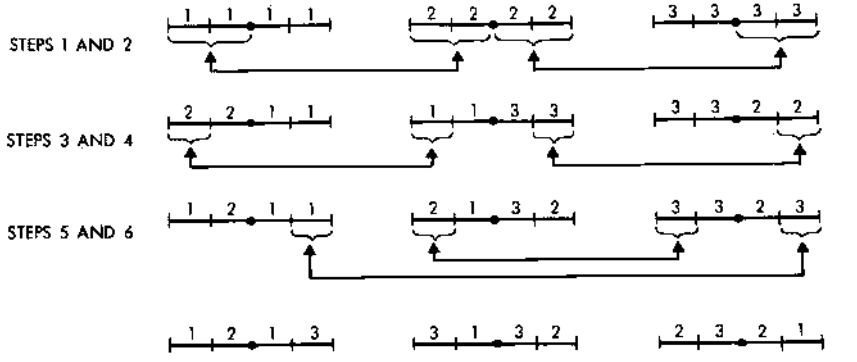


FIG. 4. Multiple translocation strain involving 3 chromosomes and 6 rearrangements.

producing a load of around 90%. The occasional use of insecticides D and C should maintain each strain in the population so that the genetic load imposed on the population by the presence of 4 incompatible strains is around 94%. This latter scheme relies predominantly on the genetic load rather than insecticides for control and on this count would appear preferable.

The important question we must now ask is: Do mosquitoes have sufficient chromosomes to allow adequate chromosomal rearrangement for the heterozygotes to be sterile? Our current understanding of chromosome mechanisms does not permit a reliable answer. Davidson et al. [10] suggest that the sterility in (species A × species B) hybrids of the Anopheles gambiae complex is in part chromosomal, i.e. due directly to mechanical effects of rearrangements. Presumably the greater part of the sterility is derived from gene-determined asynapsis. I am not aware of an example in mosquitoes where a sufficient number of rearrangements have been accumulated to provide total sterility. However, the scarcity of rearrangements in mosquito species is not too surprising because mosquito popula-

tions do not appear to have properties that readily allow fixation of translocations [5,16]. Where these properties do exist, i.e. frequent population bottlenecks and low vagility, translocations arise and become fixed frequently [11]. Four translocation homozygotes in Drosophila virilis that I have examined do not show any obvious reduction in fitness (Whitten, unpublished). Burnham and Stout [12] have demonstrated hybrid sterility in maize following incorporation of artificially induced translocations into viable homozygous strains. From these and other lines of evidence we can reasonably infer that translocations need not involve adverse side effects and their scarcity in a particular group of insects probably reflects demographic features of the group rather than some intrinsic feature of their karyotype.

Where the karyotype contains few chromosomes as in mosquito species it may prove difficult to find the right pattern of rearrangements to induce hybrid sterility. Two hypothetical examples are given for $2n = 8$ and $2n = 6$, both involving 6 rearrangements to indicate how we might approach the problem (Figs. 3 and 4). Both arrangements appear sufficiently complicated to cause high sterility. Their unpredictable behaviour may force one to generate many translocations and select those with the desired features. However, there is really very little profitable theorizing we can engage in until these studies have been attempted.

It is not essential for the hybrid to be completely sterile for rapid displacement, though the question of leakage of the gene being removed through the hybrid to the displacing strain is very important. This problem suggests that optimal strategies lie in the field of introducing conditional lethals such as temperature sensitivity rather than insecticide resistance. A low leakage of the former would be insignificant while it could mean the difference between success and failure in the latter.

Once we have developed a single strain with sufficient rearrangements we then have a very powerful tool for genetic manipulation, either giving permanent life to acceptable insecticides or providing indirect control by rapid introduction of conditional lethals. These advantages are so far-reaching that I feel the onus should be on us to demonstrate for each insect pest why it is not possible to synthesize multiple translocation strains or look for alternative means of providing sterility. The effort in developing the strains may be considerable but in comparison to developing a new insecticide or other control approach it may prove well worthwhile.

Example 4

Each of the preceding three examples is deficient in some manner when we relate them to the special conditions of mosquito species. I would now like to deal with a special type of translocation which avoids many of the problems described above and is particularly relevant to mosquito species as their cytology suggests that they are admirably preadapted to this approach.

E. Lewis at the California Institute of Technology has synthesized a class of chromosome rearrangements in Drosophila melanogaster known as compound chromosomes. In these strains both left arms of a particular autosome pair are attached to one centromere while both right arms are attached to the other centromere. The method of synthesis need not concern

us here other than to say it should be possible to synthesize compound chromosomes for either pair of autosomes in mosquitoes. Compound autosome strains breed true and are fully viable though their fertility is about 25% of the wild type. Such strains are completely genetically isolated from the original type because hybrids between them and the original structural types die in the embryonic stages. Kozhevnekov was so impressed by this fact that when he synthesized a similar strain from D. melanogaster he called it D. artificialis [14].

If we can place any credence on the predictions of population genetics, the outcome of mixing a normal strain with a compound strain will be a frequency-dependent phenomenon. If the initial frequency of a compound strain exceeds 75% it should proceed to replace the original type. A lower frequency will lead to its own replacement. The replacement should be essentially completed within 6 generations. Cage experiments with D. melanogaster are now in progress to confirm these predictions. A summary of this work, together with details of synthesis of compound strains, will be published elsewhere. Suffice it to say that the cytogenetics of mosquitoes, few chromosomes with centromeres centrally located, appear to provide us with a tailor-made candidate for the synthesis of compound chromosomes.

Before we proceed to envisage control systems involving compound chromosomes it is necessary to describe a particularly important advantage of compound strains. It is possible, and has been done often in Drosophila, to replace the whole genome of the compound strain by the genome of the structurally wild-type strain in virtually a single step. In other words, once we have developed a single compound strain for each pest we can replace its whole genome with that of individuals from the area where we would like to make our releases. This opportunity is not available for Example 3 where a strain isolated from the original by a series of rearrangements is rather useless if it does not already contain the appropriate genotype before isolation was commenced. A similar obstacle prevails in the cases of naturally occurring cytoplasmic incompatibility. What we should be able to do, then, is construct a synthetic incompatibility strain with a genotype identical to the one we wish to displace. A similar genotype should entail a similar ecotype without any mating barrier once the strain is released. It is improbable, for very good reasons, that cytoplasmic incompatibility can realize this objective. A single release of the compound strain in excess of 4-fold of the natural population should lead to the latter's replacement in a small number of generations. Providing an artificial fitness to the compound strain with insecticide resistance as described for Example 3 may allow a significant reduction in the numbers of the compound strain needed for release.

The mere displacement of one population by a similar one does not constitute control. However, if we can do this we are in a position to introduce conditional lethals (insecticide susceptibility or temperature sensitivity as is discussed by R.H. Smith in Paper IAEA-SM-138/24 of these Proceedings), or preferably it allows us to replace the vector form by a non-vector form. This latter may sometimes involve merely increasing the frequency of a genotype which already exists in the population we propose to displace.

Finally, if the compound chromosome behaves in mosquitoes in the way we observe it to behave in Drosophila we should be able to replace a

natural population of mosquitoes with one that possesses a zygotic mortality of 75%. The boundary stability of this population would be similar to that described for Examples 1 and 2; that is, it is stable if the population is isolated or if migrants do not exceed 20% of the population each generation. A possible though unlikely outcome is that the 75% genetic load will depress the non-vectorial populations to the point of extinction and we might be faced with the task of further releases, at least on a circumference, to protect the vacated niche. Such a task would be a pleasant one to attack.

ACKNOWLEDGEMENTS

I wish to thank Dr. R. Frankham for contributing his ideas on the use of insecticides to enable a reduction in the numbers of insects required for release and for ensuring the continued presence of high genetic loads.

REFERENCES

[1] LAVEN, H., Eradicating mosquitoes using translocations, Nature, Lond. 221 (1969) 958-59.
[2] SEREBROVSKII, A.S., On the possibility of a new method for the control of insect pests, Zool. Zh. 19 4 (1940) 618-30.
[3] CURTIS, C., A possible genetic method for the control of insect pests, with special reference to tsetse flies (Glossina spp), Bull. ent. Res. 57 (1968) 509-23.
[4] CURTIS, C., HILL, W.G., "Theoretical and practical studies on a possible genetic method for tsetse fly control", Isotopes and Radiation in Entomology (Proc. Symp. Vienna, 1967) IAEA, Vienna, (1968) 233-45.
[5] WHITTEN, M.J., "Genetics of pests in their management", in: Concepts in Pest Management (GUTHRIE, F., RABB, R., Eds.) (1970).
[6] CURTIS, C., Possible use of translocations to fix desirable genes in insect pest populations, Nature, Lond. 218 (1968) 368-69.
[7] HALDANE, J.B.S., JAYAKAR, S.D., Equilibrium under natural selection at a sex-linked locus, J. Genet. 58 (1964) 318-23.
[8] WRIGHT, S., Evolution and the genetics of populations, Vol. 2. The theory of gene frequencies, Chicago University Press (1969).
[9] WEHRHAHN, C.F., KLASSEN, W., Genetic insect control methods based upon the release of relatively few laboratory-reared insects, Ann. ent. Soc. Am. (in press).
[10] DAVIDSON, G., PATERSON, H.E., COLUZZI, M., MASON, C.F., MICKS, D.W., "The Anopheles gambiae complex", in: The Genetics of Insect Vectors of Disease (WRIGHT, J., PAL, R., Eds), Elsevier, London (1967).
[11] WHITE, M.J.D., BLACKITH, R.E., BLACKITH, R.M., CHENEY, J., Cytogenetics of the viatica group of morabine grasshoppers, 1. The "coastal" species, Aust. J. Zool. 15 (1967) 263-302.
[12] BURNHAM, C., STOUT, J.T., Multiple translocations in maize, Maize Genetics Co-operation Newsletter 43 (1969).
[13] WHITTEN, M.J., An approach to insect control by genetic manipulation of natural populations, Science (in press).
[14] DOBZHANSKY, Th., Genetics and the origin of Species (2nd Ed.), Columbia University Press (1941).
[15] McDONALD, P.T., RAI, K.S., Aedes aegypti: origin of a new chromosome from a double translocation heterozygote, Science 168 (1970) 1229-30.
[16] WHITE, M.J.D., Cytogenetics of speciation, J. Aust. ent. Soc. 9 (1970) 1-6.
[17] MASNER, P., SLAMA, K., ZDAREK, J., LANDA, V., Natural and synthetic materials with Insect Hormone activity X. A method of sexually spread insect sterility, J. econ. Ent. 63 3 (1970) 706-10.
[18] WAGONER, D.E., Suppression of insect populations by the introduction of heterozygous reciprocal chromosome translocations, Bull. ent. Soc. Am. 15 (1969) 220.

DISCUSSION

K. KRIMBAS: I would like to make the following points with reference to the problem of using translocations for insect control.

I think it is quite clear that we have to consider two important factors in model building, namely the carrying capacity of the environment and the fertility of the wild strains. For example, if we have a natural population of 10 000 animals, but each leaves 100 individuals as progeny, then 99% of the animals potentially produced die in nature by natural selection and accidental death. By reducing the fertility by 50% we do not necessarily reduce the natural population of 10 000, which is the carrying capacity of the environment. This is well described by the ideas of Bruce Wallace on 'soft' and 'hard' selection. There is also a limit to the reduction in fertility by translocations equal to $(\frac{1}{2})^n$, where n is the number of linkage groups. It should not be forgotten that Oenothera species survive quite well in nature in spite of their translocations. I do not therefore quite see how we could eradicate a natural population by means of translocations. As regards 'infecting' a natural population with undesirable genes transferred by the translocation, we should not forget that natural selection will act at the same time against them and probably prevent a translocation from establishing itself.

M.J. WHITTEN: At no stage have I made any statement concerning the level of zygotic mortality and its effect on population size. I have merely assumed that if a certain level of mortality will cause a population decline, then a higher level of mortality should accelerate the decline. My purpose was to compare the effort involved in applying the different translocation systems to produce a given level of mortality. A system involving less effort for the same level of mortality is assumed to be superior. On the basis of this criterion, Y-autosome systems do not fare well.

As regards your second point, you have misunderstood the genetic principle of unstable equilibria. The only requirement is that the heterozygote must be less fit than both homozygotes. When compound strains are released, the fitness of the hybrid is zero. It follows that an unstable equilibrium exists, its value depending on the relative fitness of the native form and the released compound strain. Natural selection is not the villain of the piece. In fact, we require its operation for the successful implementation of the program.

A.M. JORDAN (Chairman): Has the viability of the females from abnormally coloured pupae been tested? In a colony of flies carrying this factor, would the females be suitable for use in the breeding stock?

M.J. WHITTEN: The black pupa does occur in natural populations, though at a low frequency. This, together with the results of viability and fecundity studies we have carried out in the laboratory, indicates that these females are fully viable and quite fecund. The stock carrying the sex-linked pupal colour is true-breeding and consequently can be mass-reared without any handling. If it were required, the females could be returned to the breeding colonies to boost the production of males for sterilization. However it should be noted that the male is a translocation heterozygote, and his fertility is at a level of about 50%, which would offset the advantage of having the females available as further breeding material.

A.M. JORDAN: If compound chromosome stocks crossed with normal stocks produce no progeny, how can you introduce genes from the normal stock into the compound stock?

M.J. WHITTEN: Firstly, let me say that a complete genome from the normal stock has been introduced into the compound strain of Drosophila on a number of occasions. This operation is effected virtually in one step by irradiating males of the normal stock and crossing these with females from the compound stock. The only progeny produced are those resulting from the fertilization of an egg by a sperm carrying a compounded left or right arm which has been produced by irradiation. This simple screening procedure provides us with a new strain carrying a genome from the normal stock. I hope to be able to describe, in a paper to appear shortly, the advantages and disadvantages of compound strains, together with the results of cage experiments which demonstrate the important properties of these strains.

L.E. LaCHANCE: I was happy to hear Mr. Whitten support the use of fully sterile males in mosquito control programs. Early in the 1960s there were several field tests which failed for one reason or another, and since then the use of radiation sterilization for mosquito control has more or less been abandoned. I feel that we have learned a great deal about the radiation sterilization of insects, ways of improving competitiveness and many other developments in radiation biology. It is a pity that so few people are interested nowadays in investigating the use of fully sterile males for mosquito control. As Mr. Whitten has so neatly shown, it does have advantages.

H. LAVEN: In my view, the release of heterozygous translocation males would not be a practicable means of control.

It is very likely that we can inject a male translocation up to the saturation point, that is, we can replace all normal males in a population by translocation males. When I left France last week we were well on the way to that goal.

Further, I am not so pessimistic about the mass rearing of a strain with males heterozygous for a translocation. A strain with 90% sterility can be built up to a production of 160 million males and females per day in about six months, assuming an average egg production of 100 per female.

Moreover, male translocation systems present no problem with respect to the culling of the sex with the translocation (male) by means of a mechanical sex separator. In no other system has this difficult problem yet been solved.

I am at present very hesitant about using viable homozygous translocations because of the extreme difficulty of measuring the population size during a control program. In the case of male translocations, tests also have to be made in advance of application in the field to make sure that the translocation population cannot become homozygous through crossing-over and give rise to a viable homozygous population.

M.J. WHITTEN: Your field experiments are very important and I think we should await their outcome before we weigh further the pros and cons of the different points of view.

R. PAL: In mosquitoes the synthesis of a genome with all the required attributes would take up considerable effort and time. Furthermore, in practice, repeated release systems have their limitations, so that a self-propagating system would have a considerable advantage.

With respect to the point raised by Mr. LaChance, I would just mention that the competitiveness of sterilized male mosquitoes is under active study at the WHO unit in Delhi.

M.J. WHITTEN: I feel you are suggesting that we need to include all the mechanisms I have described into the one stock for release. I was simply outlining a number of potential applications. We do not anticipate the need to combine several systems in one strain. In other words, it should not be necessary to include insecticide susceptibility, temperature sensitivity and inability to transmit disease into the one stock. Let us suppose that our strategy is to replace a natural population which is polymorphic with respect to its ability to transmit disease by a compound strain without this ability. Our procedure would be to isolate individuals with the right genotype from the field population and introduce their genotype into the compound strain in the manner described in the reply to Mr. Jordan's question. In several generations I hope we should be able to select the appropriate strain with less effort than Mr. Pal suggests.

I have tried to indicate that the establishment of compound chromosome strains need not entail multiple releases, in contrast to techniques involving Y-autosome releases or releases of sterile males. Once the vectorial form has been displaced, we have a self-propagating system.

D.W. WALKER: The displacement of a pest species by a non-noxious species in approximately the same ecologic niche appears to be only part of the potential of this method. Yamamoto, Thorsteinson, Soo Hoo and others have been working on methods of conditioning pest species to develop on and to prefer pest species of plants rather than their natural hosts. Since the former group of plants is undesirable from the point of view of man, and the latter desirable, the advantages appear rather obvious. I believe that this procedure should find application for Coleoptera, Diptera and indeed most of the pest species of insects with monokinetic chromosomes.

M.J. WHITTEN: I think we have to be cautious in our assessment of how many species we could handle with the genetic systems I have outlined. Some schemes require relatively sophisticated techniques, and we would be unrealistically optimistic if we believed that these would become available for too many species. The house fly, the sheep blowfly (Lucilia cuprina), possibly some fruit flies and, we hope, most mosquito species may be suitable candidates. To include all species with monocentric chromosomes would be quite unrealistic, at least at this stage of our knowledge of population genetics.

M. FRIED: I would just like to point out that a population which has been reduced to a lower level will not necessarily remain at that level or be eradicated. It depends entirely on what factors are limiting population in that particular ecology.

M.J. WHITTEN: I agree with Mr. Fried that a reduction in the fertility of individuals in a population may not cause a decline in population size, and if that were our objective, the program could be useless. I was simply suggesting easier ways of producing the same useless result!

SEMISTERILITY FOR INSECT CONTROL

H. LAVEN, E. JOST, H. MEYER, R. SELINGER
Institut für Genetik,
Johannes Gutenberg-Universität,
Mainz, Federal Republic of Germany

Abstract

SEMISTERILITY FOR INSECT CONTROL.

After the eradication of the screwworm fly from the southeastern United States by the use of radiation-sterilized males, there was and still is great interest and enthusiasm for the sterility approach. Unfortunately, the mutagenic treatment often reduces mating competitiveness. Many field trials have failed and others have only led to satisfactory results by the release of sterilized males in ratios up to 100 : 1 between sterilized males and normal males. Recent research on the induction of chromosomal translocations has shown that partial sterilization not only has the advantage that the treated insects suffer a less drastic lack of competitiveness, but also that the surviving offspring of the treated insects carry a high percentage of translocations. Translocations in heterozygous condition lead to semisterility of the carriers and are inherited from generation to generation. Data are presented on the correlation between the degree of induced sterility and the percentage of inherited semisterility, on the nature of chromosomal translocations and their mode of inheritance. Several possible ways of using translocations for the control of insect populations are discussed. Further developments of the semisterility mechanisms are indicated.

Various genetical mechanisms have been suggested as potential means for genetical control of noxious insects, especially for mosquitoes [1, 2]. Most promising are mechanisms which interfere with the normal events during fertilization or embryonic development, because they are as such hardly subjected to natural selection. One such mechanism has been found as naturally occurring between populations of some mosquito species groups, i.e. cytoplasmic incompatibility [3]. It leads in interpopulation crosses to the prevention of syngamy after fertilization. It has already been proven that this mechanism can totally suppress the reproduction of a natural population [4].

Sterility induced by irradiation or chemicals should theoretically have the same result, but unfortunately such mutagenic treatment often reduces the mating competitiveness. All attempts to use the so-called sterile-male technique against mosquitoes have so far failed or have given doubtful results. Also the most recent reports on outdoor cage and field trials with chemosterilized male mosquitoes [5 - 8] are not very promising. The extremely high number of sterilized males employed in these experiments (up to a ratio of 100 sterilized against 1 natural male) and the comparatively low degree of suppression achieved show clearly the lack of normal competitiveness of the treated males. Unfortunately in the last experiments reported, the authors have obscured the evaluation of the efficiency of the sterilized males by destroying every day half of the eggs laid in the population. So they have used two different methods for control simultaneously, but they ascribe the population reduction solely to the influence of the sterile males.

During the last two years we have concentrated our research efforts on other mechanisms which interfere with normal fertilization, namely the production and evaluation of chromosomal translocations in

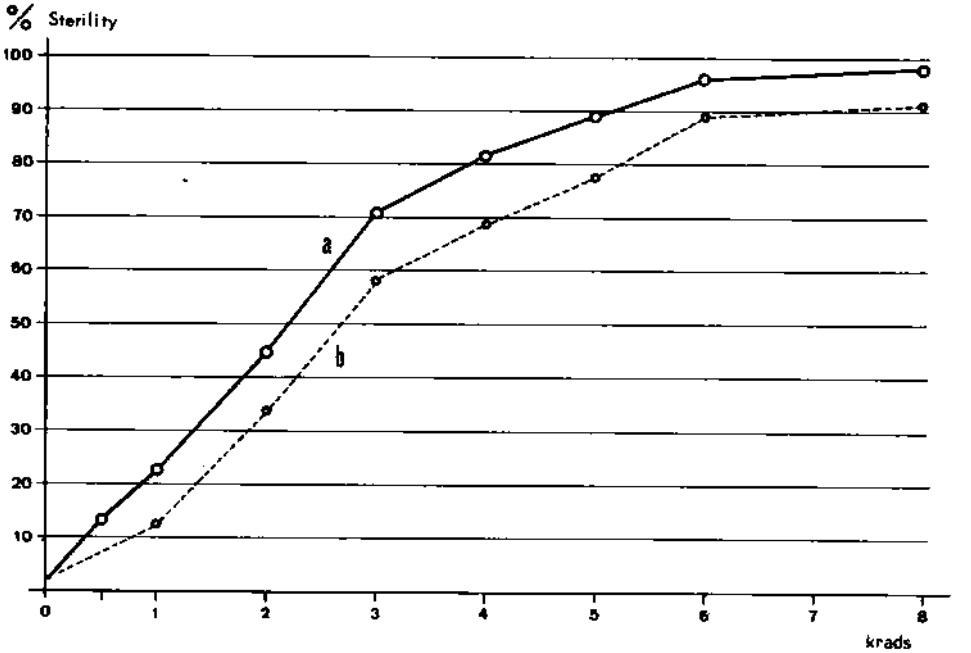


FIG. 1. Effect of different X-ray dosages on dominant lethals induced in mature sperm of Culex pipiens: a = percentage of non-hatching eggs, total sterility; b = percentage of non-embryonated eggs.

mosquitoes as possible means for their control. It is a well known fact that animals which are heterozygous for a translocation have reduced offspring. The phenomenon has been termed semisterility.

As long as 30 years ago Serebrovsky [9] suggested chromosomal translocations as prospective means for pest insect control. The same idea has been expressed recently and independently by several authors [1, 10-12]. Theoretical considerations [1, 13] seem to indicate that the semisterility of translocation heterozygotes could suppress natural populations under certain conditions. Experiments with laboratory populations have already demonstrated that eradication is possible [14, 15] and first field experiments are now being undertaken or considered for the near future.

In this paper data will be presented on the production of translocations in different mosquito species, their isolation and their nature. The relative ease and high yield of production of these chromosomal aberrations open the way for several different methods of their application in control. These methods will be indicated briefly. Furthermore translocations can be used for the development of still more effective genetical control systems. Some of these will be mentioned.

We have produced chromosomal aberrations in our experiment by X-ray irradiation of 2- or 3-day-old males. One series of irradiations has also been conducted with fast neutrons, but as the results are very similar, they will not be presented here but in a subsequent publication.

At the time of irradiation the males have already mature sperm. If these males are immediately mated with normal females, all changes in the chromosomes which become visible in the first or second generation must have occurred in the mature sperm.

The immediate effect of the irradiation is the production of dominant lethals in the chromosomes, either lethal mutations in single genes or gross chromosomal aberrations like deletions or deficiencies. These aberrations are expressed as partial or total sterility of the first generation offspring. The percentage of lethal embryos is correlated with radiation dosage. Figure 1 shows the degree of sterility in the mosquito Culex pipiens for various X-ray dosages (Fig.1, curve a). This dose-response curve is very similar to those for other mosquitoes (Anopheles pharoensis [16]) and dipteran insects in general [17]. It is interesting to note that most of the lethal eggs show no sign of embryonic development, indicating that most of the lethals act predominantly on the first nuclear divisions (Fig.1, curve b).

Most of the non-lethal eggs develop normally and the resulting F_1 adults show normal vitality, at least under laboratory conditions. If these remaining individuals are outcrossed to normal partner, a new type of sterility becomes visible, now in the second generation after irradiation. A certain number of the F_1 adults produces a reduced F_2 offspring. This is due either to a chromosomal translocation or to a pericentric inversion which originated in the sperm of the irradiated males and became heterozygous in the F_1 individuals. In both cases heterozygous individuals are semisterile but from our investigations we can conclude that translocations predominate.

We were in the first place interested to know how many translocations can be produced with a certain radiation dosage. So all available F_1 individuals were tested for semisterility by outcrossing to normal animals. Figure 2 shows the percentage of semisterile individuals of Culex pipiens for different dosages up to 5 000 R. With dosages between 500 and 3 000 R only 10 - 20% of the surviving F_1 animals are semisterile, but with dosages between 3 000 and 5 000 R the percentage increases more rapidly to 50%. This increase of translocations can be explained with the increase of two or more simultaneous breaks with higher dosages.

Our figures of 30 - 50% translocations after application of 3 500 to 5 000 R X-rays seem to be exceptionally high. But they are characteristic for mosquitoes as our figures for translocation production in two other mosquito species, Culex tritaeniorhynchus and Aedes albopictus, are of the same order of magnitude. Unfortunately, data on total translocation production in other insects are non-existent or hardly comparable. In Drosophila melanogaster only 14.78% translocations between all chromosomes have been observed after application of 4452 R X-rays [18]. In more recent experiments, however, 14.39% translocations have been observed only between the second and third chromosome with 4 000 R γ-rays [19]. Whether these almost identical figures on the one hand for total translocation production and on the other hand for translocations between chromosomes two and three only are due to the quality of the radiation applied (X-rays and γ-rays) or due to technical differences in the screening method is an open question. In any case our results are rather outstanding and it remains to be seen whether mosquitoes are exceptional as regards the induction of translocations.

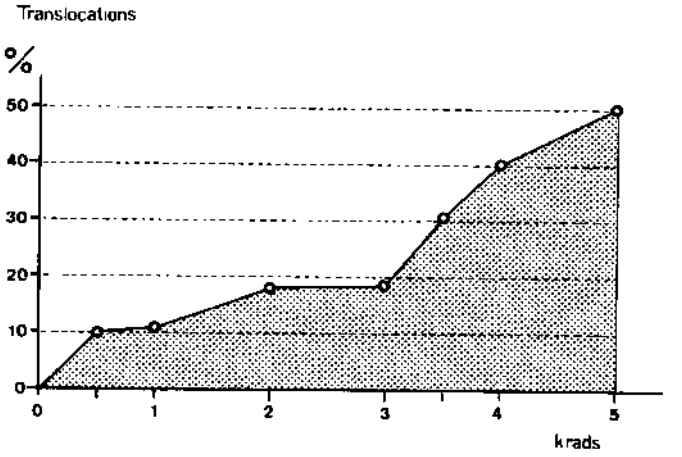


FIG. 2. Percentage of Culex pipiens F_1 individuals carrying a translocation after various X-ray dosages.

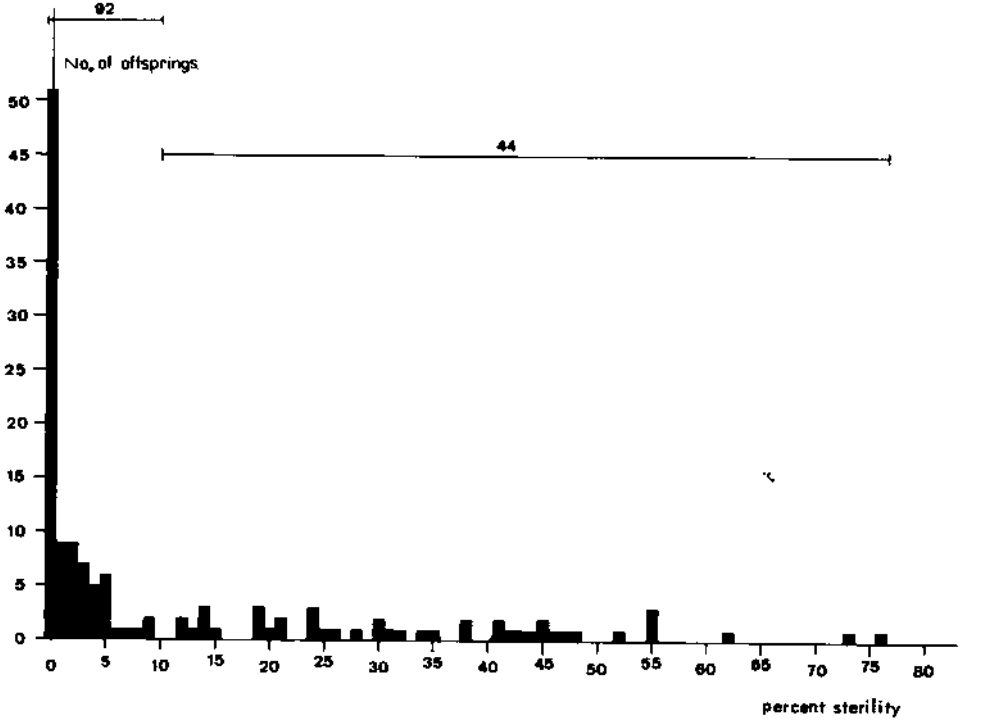


FIG. 3. Degree of sterility in 186 F_2 offsprings of Culex pipiens after 3500 R irradiation of the P generation.

After we had collected these basic data on total translocation production we conducted several experiments with a standard dosage of 3 500 R of X-rays with the aim of characterizing the translocations as regards chromosomes involved and degree of sterility. In one experiment 136 irradiated sperms were tested and 44 different translocations could be isolated. The sterility obtained for these 44 translocations in the F_2 generation varied between 12 and 76% (Fig.3). There is no obvious accumulation of the sterility percentage either around the theoretical value of 66% or the most probable value of 50%.

All 44 translocation lines were maintained for several generations. The average degree of sterility in each line was constant from generation to generation with a variability of plus or minus 10 - 15% between the offspring of different parents of the same line. In two cases a slight increase of 4 - 5% of the sterility has been observed after outcrossing of certain translocations to an unrelated strain. Experiments to raise the degree of sterility in certain lines by selecting the offsprings with the highest sterility have so far given no clear results.

By an adequate screening technique, i.e. outcrossing up to the third and fourth generation and following the mode of inheritance of the semisterility, for 31 out of the 44 lines the type of translocation involved has been established. Culex pipiens has only three almost metacentric chromosome pairs. One of these pairs must be the one which carries the sex-determining factors M for maleness, m for femaleness. Males are heterozygous M/m, females homozygous m/m. According to this chromosome complement three different kinds of translocations are possible, (1) a translocation between the M-chromosome and an autosome, (2) a translocation between the m-chromosome and an autosome and (3) a translocation between the two autosomes. Out of the 31 translocations analysed 7 were of the first kind, T^M-translocations, 3 of the second, T^m, and 21 of the third kind, T^a (Fig.4). The first type of translocations is inherited only through the males, because these are all the time heterozygous for the sex chromosomes, the translocation remaining permanently in the heterozygous condition. So all males in these lines are constantly semisterile from generation to generation. T^m-translocations are inherited through males and females. Males cannot, but females could, become homozygous for such translocations. The third type of translocation is inherited equally through both sexes and it could become homozygous in both sexes.

Our attempts to make the autosomal translocations homozygous have so far met with only a limited success. Only two out of the 21 have become homozygous. In this way the semisterility disappears and the line continues with full fertility. However, outcrossing with any normal line, again translocation heterozygotes are produced and lethality turns up again in the following generation.

It seems to be appropriate to mention at this point that we have verified the nature of the translocations by cytological investigations for about 10 of all the lines we have. With the aid of salivary chromosome preparations the position of the break points have been exactly defined. Investigations are at present being undertaken to find out whether the size of the translocated pieces and the degree of sterility are correlated or not. One translocation line with slightly more than 80% sterility has turned out to contain two translocations, one of the T^M

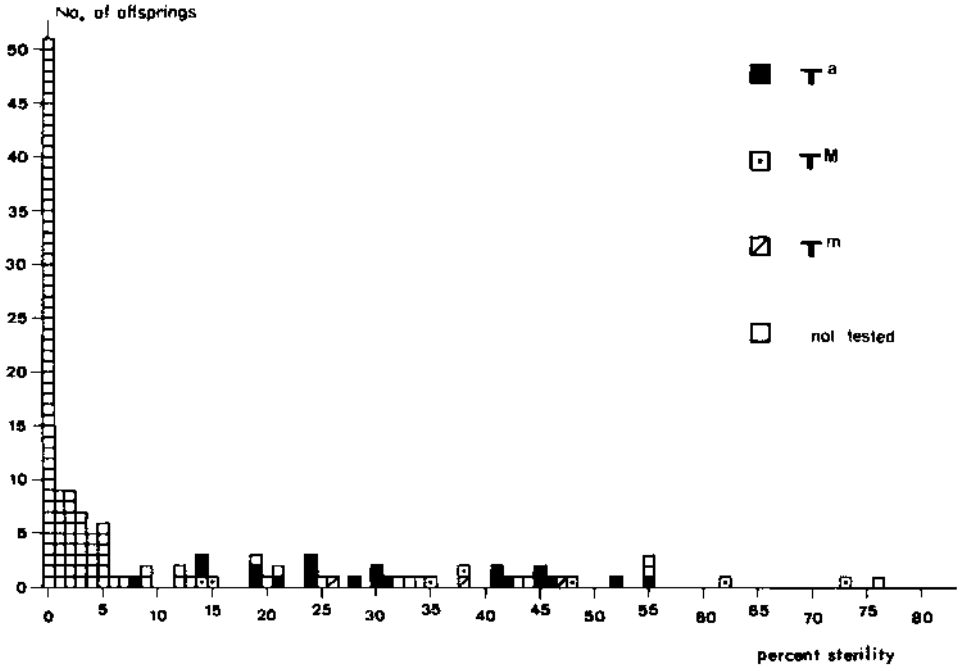


FIG. 4. Classification of translocations in Culex pipiens with respect to the chromosomes involved.

type and the other of the T^a type. Only in one case with inherited semisterility has a pericentric inversion been discovered by cytological investigations.

With so many translocations of the three different types and various degrees of sterility at hand it is, of course, tempting to speculate on the influence which semisterile animals would have on reproduction, if they were released into a natural population. Models and calculations have already been published by several authors [1, 9, 13], so only a few general remarks will suffice here in order to compare or better to contrast the release of semisterile animals with the common method of totally sterilized males.

First, semisterile insects are by no means inferior to normal ones. On the contrary, with certain breeding and selection procedures they can be made more competitive than natural insects.

Second, semisterile males have not only an immediate influence on a natural population owing to their semisterility, but they also transmit at the same time the translocation to half of their offspring. In this way the translocation is injected into the natural population and will constantly increase by repeated releases of translocation heterozygotes. After a certain saturation point for the translocation has been reached, no further releases are necessary. The population would automatically be exterminated.

Third, translocation systems can be produced in all animals. The deleterious effect of translocation heterozygosity is well known

from several insects, but also from mice and man. As far as we know, translocations for control measures have been produced during the last two years in five different mosquito species (Culex pipiens, C. tritaeniorhynchus and Aedes albopictus in our laboratory; Aedes aegypti: Rai [20]; Anopheles gambiae: Davidson and Krafsur, personal communication, 1970), in house flies [12], in tsetse flies [21] and in onion flies (Wijnands, in correspondence, 1970). If it can be proven in field experiments that translocation heterozygotes can suppress a natural population, undoubtedly there will start more research on these lines. It will be extremely interesting to compare species with different chromosome numbers and chromosomes of different sizes regarding percentage of translocations produced by standard irradiation dosages and degree of sterility obtained.

In the application of semisterile translocation animals for control the same technique is used as in the sterile-male technique, i.e. mass rearing and release of males in adequate numbers. The release of translocation homozygotes seems to be the most effective way. But it needs a very careful study of the population size and an adjustment of the numbers of released males from generation to generation, otherwise the translocation homozygotes could replace the natural population and the temporal decline of the population would be reversed. Therefore the application of translocations which cannot become homozygous appears to be much easier and safer. In mosquitoes the T^M translocations would be the best. For obvious reasons only males can be released, and in the T^M lines all males carry a translocation. From theoretical considerations the successive release of different translocations with increasing degree of sterility would lead to the fastest possible decline of a population, as has already been shown in cage experiments [14, 15].

If we adopt the opinion of at least a proportion of the agricultural entomologists, that total eradication of a species is not necessary but only depression of the population to such a level that the economic losses become bearable, and if we adopt the view of the medical entomologists that for breaking the chain of disease transmission it needs only population reduction to a certain level, then we are already in a position to do this with translocations with several mosquito species. Mass production of males for release would not be necessary; eggs of the translocation strain with the necessary characteristics can be seeded in the breeding places. I believe that no method could be cheaper and that countries lacking funds for costly measures would have a chance of overcoming their pest problems in this way. The Culex fatigans problem in Recife in Brazil, mentioned by Borghi et al. in these Proceedings (IAEA-SM-138/63) could be solved in this way.

In species which cannot be mass-produced, it seems, nevertheless, to be possible to use the translocation system. If wild insects can be caught in sufficient numbers and if they are irradiated with such a dosage that only partial sterility is obtained, there is a good possibility that translocations are injected into the population after the release of the treated insects. It is known from experiments with Drosophila that the same number of translocations are produced by irradiation of sperm in males as well as in fertilized females.

Having produced translocations of all sorts and with various degrees of sterility we can now aim at more advanced and more efficient control systems. The next step would be to develop lines with two or more translocations. The way is open for this development as soon as one or the other translocation can be made homozygous. Males of such a line could be irradiated again and the offspring screened for newly arisen translocations. With time and funds available it could be possible to accumulate in one line not only two, but three, four and more translocations. There is reason to assume that such multiple translocation lines are totally or almost totally sterile when outcrossed to a normal population. In other words, a new species has been created.

That such genetic engineering is not merely wishful thinking was already demonstrated some 35 years ago by Koshevnikov [22] in Drosophila. Through the combination of two different translocations by crossing over he got a strain that was totally sterile in outcrosses to normal animals.

Translocations can also be useful tools for other developments. If for example a certain type of translocation is combined with a balanced lethal system a strain can be constructed which produces only males. It can be maintained by crossing with normal females of any other strain. The advantages of such a strain are obvious. Mass production would not be necessary; the eggs could already be dispersed into natural breeding places. The males hatching from these eggs are semisterile and produce again only males, which are in turn again semisterile and male-producing.

No doubt translocations are very useful systems for control, but in the hands of a skilled genetical engineer they turn out as highly efficient tools for the creation of ideal control systems. It seems therefore to be appropriate to stress the need for more research in this field.

REFERENCES

[1] LAVEN, H., Genetische Methoden der Schädlingsbekämpfung, Anz. Schädlingsk. 41 (1968) 1-7.

[2] CRAIG, G.B., Jr., Genetic control of mosquitoes: progress and prospects, Accademia Nazionale dei Lincei, Quedemo N. 128 (1969) 145-154.

[3] LAVEN, H., "Speciation and evolution in Culex pipiens", Ch. 7, Genetics of Insect Vectors of Disease (WRIGHT, J.W., PAL, R., Eds), Elsevier, Amsterdam (1967) 251-275.

[4] LAVEN, H., Eradication of Culex pipiens fatigans through cytoplasmic incompatibility, Nature, Lond. 216 (1967) 383-384.

[5] PATTERSON, R.S., LOFGREN, C.S., BOSTON, M.D., Sterile males for mosquito control: a field cage study with Culex pipiens quinquefasciatus, Mosq. News 28 (1968) 540-544.

[6] PATTERSON, R.S., LOFGREN, C.S., BOSTON, M.D., The sterile-male technique for control of mosquitoes: a field cage study with Anopheles quadrimaculatus, Florida Ent. 51 (1968) 77-82.

[7] PATTERSON, R.S., WEIDHAAS, D.E., FORD, H.R., LOFGREN, C.S., Suppression and elimination of an island population of Culex pipiens fatigans with sterile males, WHO/VBC/70.180. (1970).

[8] PATTERSON, R.S., FORD, H.R., LOFGREN, C.S., WEIDHAAS, D.E., Sterile males: their effect on an isolated population of mosquitoes, Mosq. News 30 (1970) 23-27.

[9] SEREBROVSKY, A.S., On the possibility of a new method for the control of insect pests, Zool. Zh. 19 (1940) 618-630.

[10] CURTIS, C.F., A possible genetic method for the control of insect pests, with special reference to tsetse flies (Glossina spp.), Bull. ent. Res. 57 (1968) 509-523.

[11] CURTIS, C.F., Radiation sterilization and the effect of multiple mating of females in Glossina austeni, J. Insect Physiol. 14 (1968) 1365-1380.

[12] WAGONER, D.E., NICKEL, C.A., JOHNSON, O.A., Chromosomal translocation heterozygotes in the house fly, J. Hered. 60 (1969) 301-304.
[13] CURTIS, C.F., HILL, W.G., "Theoretical and practical studies on a possible genetic method for tsetse fly control", Isotopes and Radiation in Entomology (Proc. Symp. Vienna, 1967) IAEA, Vienna (1968) 233-247.
[14] LAVEN, H., Genetische Schädlingsbekämpfung: Produktion von Semisterilität bei der Stechmücke Culex pipiens und Aussichten für die Bekämpfung mit diesem System, Anz. Schädlingsk. 42 (1969) 17-19.
[15] LAVEN, H., Eradicating mosquitoes using translocations, Nature, Lond. 221 (1969) 958-959.
[16] TANTAWY, A.O., ABDEL-MALEK, A.A., WAKID, A.W., Studies on eradication of Anopheles pharoensis by the sterile-male technique using cobalt-60. II. Induced dominant lethals in the immature stages, J. econ. Ent. 59 (1966) 1392-1394.
[17] LaCHANCE, L.E., "The induction of dominant lethal mutations in insects by ionizing radiation and chemicals - as related to the sterile-male technique of insect control", Ch. 21, Genetics of Insect Vectors of Disease (WRIGHT, J.W., PAL, R., Eds), Elsevier, Amsterdam (1967) 617-650.
[18] PATTERSON, J.T., STONE, W.S., BEDICHEK, S., SUCHE, M., The production of translocations in Drosophila, Amer. Nat. 68 (1934) 359-369.
[19] OSTER, I.I., "The mutational spectrum with special reference to the induction of mosaics", Repair from Genetic Radiation Damage and Differential Radiosensitivity in Germ Cells (SOBELS, F.H., Ed.), Pergamon, Oxford (1963) 51-62.
[20] RAI, K.S., "Techniques for studying the effects of radiation on meiosis and related processes in mosquitoes with particular reference to Aedes aegypti", Effects of Radiation on Meiotic Systems (Proc. Panel Vienna, 1967) IAEA, Vienna (1968) 185-200.
[21] CURTIS, C.F., The production of partially sterile mutants in Glossina austeni, Genet. Res. 13 (1969) 289-301.
[22] KOSHEVNIKOV, B.Th., Experimentally produced karyotypical isolation, Biol. Zh. 5 (1936) 741-752.

DISCUSSION

J.W. WRIGHT: The value of today's discussion is that it brings into perspective the potentialities that lie in the future. We must be realistic about them, however. A technique must be developed that can be applied by developing countries in the tropics and subtropics with limited manpower and material resources. Any sophisticated procedure that might be developed without this being taken into account may never be used. It seems essential that if a technique is to be practical, it should be self-propagating, involving a minimum of releases and a minimum of insects in each release.

H. LAVEN: The genetic system that I am suggesting for the control of Culex pipiens is an integrated translocation system which needs only multiplication in the sense of mass production. What I have in mind is not the production and release of adult males, but only the production of fertilized egg rafts. The eggs can be seeded in the natural breeding places or, better still, in tanks or ponds prepared for this purpose. I cannot imagine a simpler and cheaper control measure. It requires no sophisticated work, expensive equipment or hazardous chemicals.

K. KOJIMA: Complete eradication is impossible. The only thing one can do is to keep the population small. There are two main reasons for this situation. First of all, eradication represents an unstable point. Secondly, when the insect concerned has a large reproductive capacity, a single mutation (affecting chemical or mating systems) can lead to a tremendous number in a few years.

Even to restrict an insect population to a low level by biological means is extremely costly. The best method is probably to bombard

the pest insect population initially, and then use the sterile-male release method on a smaller scale.

H. LAVEN: A translocation system cannot in itself lead to eradication. What we can achieve with a translocation having 90% sterility is a population depression to 10% of the original size. In this situation environmental factors could reduce the population still further or perhaps even to zero.

L.E. LaCHANCE: I am sorry that I must disagree completely with Mr. Kojima. Every day we hear that certain species are on the verge of extinction owing to purely random factors, and it seems strange that man cannot achieve the same result, eradication, by means of concerted well-conducted pest control programs. I would remind you that in Africa, for example, the tsetse fly can be eliminated from certain regions merely by clearing the bush and settling the area. Furthermore, in Cyprus and Sardinia there were very good mosquito eradication programs based on the use of insecticides shortly after the Second World War. I think many other examples could be cited. Lastly, I should like to recall that self-sustaining populations of the screw-worm fly have not been found in the south-east of the United States since 1959. If this does not constitute eradication, then it is very good control.

K. KOJIMA: Pest insects flourish wherever their foods are available. As I indicated, they can be controlled at a lower level than the environment's carrying capacity if they do not have a very high rate of reproduction. However, this is an extremely costly operation when a fecund female lays vast numbers of fertile eggs. Regarding 'random effects', to which Mr. LaChance has referred, I shall talk about these in my paper IAEA-SM-138/22 in Session VIII. As for insecticides, it was my understanding that we were not dealing with them here.

K.S. RAI: I think you indicated, Mr. Laven, that the egg papers from the translocation stocks could be seeded in natural habitats. I am wondering how this would be possible, particularly in the case of the type of translocations (Y-autosomal) that you are using. Would you not also be releasing normal females along with your translocation males?

H. LAVEN: In the system I have in mind, use is made of both incompatibility and translocations. It would take too much time to describe it in detail.

C. BORGHI: I am grateful to Mr. Laven for his suggestion that Recife's filariasis problem could be solved by means of the translocation method. I should also like to ask his opinion on the cost of that method in relation to the cost of complete sterilization.

H. LAVEN: The cost of such a program depends on many factors, such as the size of the area, the population density, the degree of isolation, the availability of material and the level of salaries. But the control system itself is available and involves no sophisticated work or equipment.

EXPERIMENTS ON BREEDING TRANSLOCATION HOMOZYGOTES IN TSETSE FLIES

C.F. CURTIS
Tsetse Research Laboratory,
University of Bristol School of Veterinary Science,
Langford, Bristol, United Kingdom

Abstract

EXPERIMENTS ON BREEDING TRANSLOCATION HOMOZYGOTES IN TSETSE FLIES.

The introduction of chromosome translocations into tsetse populations is expected to cause a serious depression of fertility because of the semisterility of the heterozygotes. It would only be practicable to mass-rear the translocations in the form of fertile homozygotes. Mutations with the properties of translocation heterozygotes were obtained in Glossina austeni by irradiation and selection for semisterility. The descendents of such mutant individuals were inbred to try to produce homozygotes. A suitable translocation would show full fertility when the homozygote was mated to wild type and all the products of such matings would be semisterile heterozygotes. Two generation tests for these properties were used to diagnose translocation homozygotes among the products of inbreeding. About 300 individuals belonging to 7 different mutant stocks were tested and in three of the stocks translocation homozygote individuals were fairly certainly identified. In one of the mutant lines it was possible to mate individuals identified as translocation homozygotes to close relatives and, as expected, this produced several translocation homozygotes and heterozygotes and one unexplained wild type. Further inbreeding in these families has yielded some fully fertile matings which are expected to be the founders of pure translocation homozygote families. There are preliminary indications of reduced viability in these families but it is not yet clear whether this is due to recessive effects of the translocation itself, or to the effects of the intense inbreeding to which they have been submitted. Two of the mutant lines originally showed transmission of semisterility to a proportion of both sexes, but, after several generations of inbreeding and selection, semisterility is now transmitted to all males and no females. It may be that in these lines the effect of selection at each generation for semisterile males has been to transfer the sex-determining function from the X and Y chromosomes to a locus at or linked to the translocation break points.

INTRODUCTION

Most schemes for autocidal control depend on the mass rearing of genetically normal insects and treatment of them just before release to induce a large number of assorted dominant lethals and/or translocations. An alternative approach is to induce translocations in a laboratory population, to select out one with desirable properties and then to replicate it in a mass-rearing plant. It is essential that the translocation is not selectively eliminated during the mass-rearing process and one way of ensuring this is to use a translocation involving the Y chromosome or linked tightly to the male-determining gene. A colony founded from males carrying a translocation of this type and normal females will maintain this composition. Laven [1] demonstrated the possibility of eradicating an artificial population of Culex pipiens by introducing males carrying translocations linked to the male-determining gene. The present author has produced Y chromosome translocations in the tsetse fly Glossina austeni [2] but does not think that they could be economically used for population control. Even with the best possible rearing methods

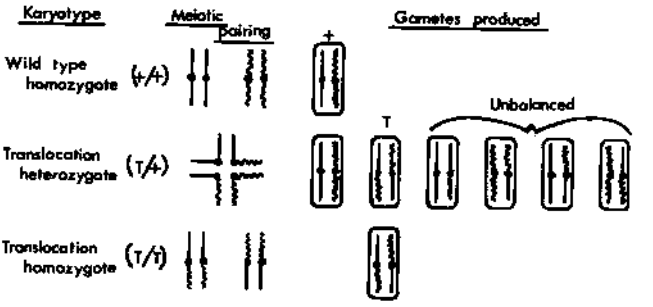


FIG. 1. Diagram of chromosome pairing and gamete production by wild-type homozygotes, translocation heterozygotes and translocation homozygotes.

the weekly output of males from a colony of fully fertile G. austeni would be only about one-third of the number of breeding flies that have to be continuously maintained [3,4]. All matings in a colony in which the males had Y chromosome translocations would be partially sterile, so the weekly output of males would be reduced to somewhat less than one-sixth of the number of breeding flies and the present author does not think that the operation would be economically competitive with other possible control methods.

Some autosomal translocations yield fully fertile homozygotes but the heterozygotes are partially sterile. Fig.1 illustrates chromosome pairing in the wild type (symbolized +/+), translocation heterozygote (T/+) and translocation homozygote (T/T). Because of the abnormal pairing in the T/+ a proportion of genetically unbalanced gametes are produced (which give inviable zygotes) as well as some balanced T and + gametes. In the T/T, however, pairing is normal and only balanced, T, gametes are produced. It should be possible to mass-rear a selected autosomal translocation as a pure T/T fully fertile population and it would only begin to cause a reduction in fertility when it was released into the wild, where partially sterile heterozygotes would be produced [5, 6]. The prolonged depression of population fertility which could be achieved by the release of a carefully controlled number of T/T individuals might provide an efficient means of control for slow breeding insects such as the tsetse fly [7, 8]. There is the alternative possibility of using an autosomal translocation to increase the frequency of a desirable gene, such as one for inability to act as a disease vector [9].

Using gamma radiation the present author produced several mutations in Glossina austeni which each have characteristic degrees of partial sterility and which are inherited like dominant autosomal factors [2]. He concluded that these were almost certainly translocations between autosomes, in the heterozygous state. This has recently been confirmed for two of the stocks by Dr. D. Southern (personal communication) who observed characteristic translocation heterozygote pairing configurations in male meiosis.

Seven of the most suitable translocations were selected for inbreeding in an attempt to produce T/Ts.

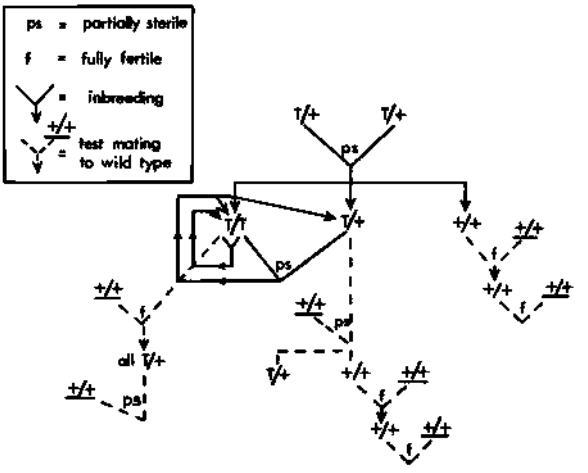


FIG.2. Diagram of the method of production of translocation homozygotes by inbreeding and the method for identifying them by test matings to wild type.

METHOD FOR THE PRODUCTION AND SELECTION OF TRANSLOCATION HOMOZYGOTES

There are no marker genes available in tsetses. Cytological methods for detecting translocations at male meiosis are only just becoming available and they require the killing of the individual in the early pupal stage, which is the only time at which male meiosis occurs [10]. The author has therefore used measurements of fertility as the sole means of identifying T/+ and T/T individuals; this procedure has the advantage that one automatically selects for those translocations which have the properties required for an autocidal project, i.e. constant full fertility of the T/T and constant partial sterility of the T/+.

An individual's fertility is tested by mating it to wild type drawn from our unirradiated laboratory colony of G. austeni. One mating is used for testing females and four for testing males [2]. The mated females are maintained by the methods described by Nash, Jordan and Boyle [11, 12] and records are kept of the number of pupae produced over a test period. The female ovulates at approximately nine-day intervals and in control (+/+ × +/+) matings these eggs develop to the pupal stage in either 90% or 100% of cases (depending on the maintenance method used). Most of the translocations that were studied as heterozygotes caused about 50% of early embryonic deaths and the consequent reduced rate of pupal production enabled matings involving a T/+ to be distinguished with reasonable clarity from those involving only homozygotes, though there is a margin of sampling error [2].

To produce T/Ts, matings must be made between descendents of the same translocation-carrying sperm. The males for these matings can be previously shown to be T/+ by their partial sterility in test matings, but the females have to be taken at random as virgins from the progeny of T/+ males, because once a female is inseminated it uses the sperm

received throughout its life. Only half of the inbred matings are therefore of the desired T/+ × T/+ type. Fig.2 illustrates the procedure from the occurrence of such a mating. Three karyotypes are produced and these are distinguished by the test matings shown. The T/+ gives partially sterile test matings and the T/T and +/+ both give fully fertile matings. However, the +/+ gives all +/+ progeny from the test matings which are fully fertile when they, in turn, are test-mated. The T/T gives all T/+ progeny which show partial sterility in their subsequent test matings. When an individual is suspected of being T/T two or more of its progeny are separately test-mated to check that each one is partially sterile, i.e. that the supposed homozygote is conforming with expectation in not producing a segregation among its progeny. A partial control on this test for T/Ts follows from the fact that they should only arise from inbreeding; when a T/+ is outcrossed to +/+ all the fully fertile progeny should score as +/+ and not T/T, as illustrated in the lower part of the middle column of Fig.2. This has so far been checked in ten instances and all agreed with expectation.

When a male T/T has been identified it is used for matings to its close relations to try to obtain a T/T × T/T mating. As already explained, the females cannot be test-mated before use and so daughters of suitable matings have to be used at random and only subsequently identified by the progeny that they produce. It is very unlikely that a T/T × T/T mating will be made in the first round of inbreeding, but more likely that a T/T male × T/+ female mating will occur and that subsequent rounds of inbreeding will yield the desired pure T/T family. It may be that such repeated inbreeding will lead to partial sterility, not due to the action of translocations but to inbreeding depression [13]. Inbreeding is known to affect fertility in G. austeni [14]. However, this could not invalidate the identification of males as T/T in test matings because they are only required to contribute a haploid set of chromosomes to these and the state of their diploid set would not affect the issue.

RESULTS OF TESTS FOR TRANSLOCATION HOMOZYGOTES

Over 300 individuals from inbreeding in 7 translocation stocks have been tested by the methods described and T/Ts have been identified in three of the stocks, but in two stocks only single T/T individuals have been obtained. The author will discuss this low yield of T/Ts in six of the stocks later and meanwhile will describe the one stock (number 68 in Ref.[2]) which has yielded several individuals scored as T/T and in which further inbreeding of these has been possible.

Figure 3 shows the data for the branch of this stock which has been most completely analysed so far. Where space allows, the fertility scores of the matings are indicated in the diagram to give an idea of the kind of evidence on which the identification of each individual is based. The scores are given in terms of pupae produced and number of ovulation cycles completed by the mated females in the test period. As shown the male A, derived from an inbred mating, was fully fertile in a test mating to +/+ females and the sons of these, B and C, were partially sterile, i.e. A scored as T/T. A was mated to its sister D,

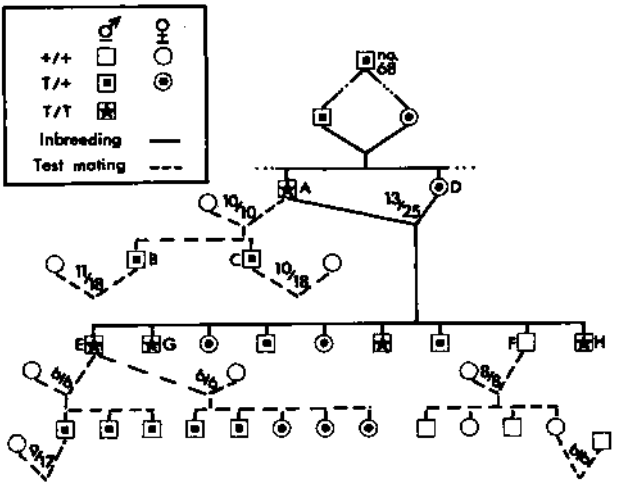


FIG.3. Part of the pedigree of stock 68 to demonstrate production of a T/T and its progeny when inbred. Where space permits, the fertility of the matings is indicated in terms of the pupae produced per ovulation cycles completed.

which was deduced to be T/+ because the mating was partially sterile. Their son, E, was fully fertile in two separate test matings and the eight male and female progeny tested were all partially sterile, which is very good evidence that E was T/T. Three other sons of A × D were similarly classified and two sons and two daughters scored as T/+. These results are consistent with the expected 1 : 1 ratio of T/+ and T/T from a T/T × T/+ mating. However, F scored unequivocally as a +/+. This type is quite unexpected and the author can see no obvious explanation for it.

Males E and G have been mated to other inbred members of stock 68. These matings were partially sterile but backcrossing the progeny to G and H has yielded two fully fertile matings with fertile progeny. These matings would appear, therefore, to be T/T × T/T, but this requires confirmation by test matings on the progeny. If these tests are positive there should be no difficulty in founding a small colony of T/Ts by matings within the family.

VIABILITY OF THE TRANSLOCATION HOMOZYGOTES

Two of the 7 stocks that were inbred now show holandric inheritance of the translocation, so it is not surprising that no T/Ts have been produced. Four of the stocks have shown conventional autosomal inheritance and yet only two individuals scoring as T/T have been obtained out of about 140 tested. In matings of T/+ males to females, half of which are T/+ and half +/+, one would expect about one-thirteenth of chromosomally balanced zygotes to be T/T, and it therefore seems likely that these four translocations are all recessive lethals or semi-lethals so that the T/Ts usually die before maturity. This result is not

TABLE I. DATA ON THE INBRED MALE PROGENY OF TRANSLOCATION HOMOZYGOTES IN STOCK No.68

	+/+ control	Progeny of T/Ts	
Upcurved wings	Rare or absent	4/33	
Survival to day 50	19/20 ← P* = 0.01	→ 19/31	
		Partially sterile (T/+)	Fully fertile†
Survival from days 10-50	9/10	9/10 ← P* = 0.113 →	9/15

* P calculated from exact test for 2 x 2 contingency table.

† Includes eight individuals shown to be T/T and tests on the progeny of the remainder have not so far been completed.

at all surprising in view of the large proportion of recessive lethal translocations in Drosophila [15]. The other possible explanation is that the T/Ts of these four translocations are not fully fertile and hence are not picked up by the test procedure. Whichever explanation is correct, these translocations would not be suitable for an autocidal control project.

In stock 68 several T/Ts have already been shown to survive up to an age at which mating tests can be made but it is important to know whether there is any degree of reduction in viability compared to wild type, since even quite small reductions would seriously reduce the efficiency of a control project [7, 8]. A proper test of this point must wait until there is a good yield of progeny from a pure T/T family but some data can already be assembled for the male progeny of matings between T/Ts of stock 68 and their close relatives. The maternal karyotypes are in many cases not yet established. The data are shown in Table I, together with the available control data for outbred male wild types maintained in the same way (i.e. in individual cages). As shown a visible abnormality (upcurved wing tips) occurred in a few of the male progeny of T/Ts (and some of the females too); this condition is rare or absent in controls. The condition is probably a symptom of generalized weakness because a similar abnormality was found after irradiation of G. morsitans with a sub-lethal dose in the middle of the pupal period (Langley and Curtis, unpublished data). The proportion surviving from emergence to day 50 is significantly less in the progeny of the T/Ts than in controls. This and the upcurved wing condition might be interpreted either as phenotypic effects of the translocation with low penetrance or as effects of the inbreeding to which the stocks had been submitted. Adequate comparative tests on inbred families free from translocations have not been made. The progeny of the T/T males were not all themselves T/T because many of the mothers were T/+. Five died before the age at which the first test mating is made (day 10) and these cannot be identified as one or the other karyotype. However, among those that did survive to day 10 a comparison can be made of the relative chances of survival from day 10 to day 50 of males shown subsequently to be

either partially sterile (i.e. T/+) or fully fertile. The latter are all expected to be T/T but progeny tests to establish this have not yet been completed in all cases. As shown in Table I, the survival appeared to be lower among the homozygous males (9/15 against 9/10), but this difference is not statistically significant. Inbreeding depression would be expected to affect the T/+ to approximately (though not exactly) the same extent as their T/T brothers, so these results give some support to the view that reduced survival is due to the translocation itself, but more data are required. The exact cause of the reduced survival is of practical importance, as any effects of inbreeding could be avoided by crossing two separately bred lines homozygous for the same translocation, whereas effects of the translocation itself would cause an inescapable reduction in the efficiency with which this particular translocation could be mass-reared and used against a wild population.

It seems likely that if one tested sufficient different translocations, the necessary one fully viable example could be found, but it is impossible to predict how much effort this would entail.

STOCKS HAVING A SWITCH-OVER TO HOLANDRIC INHERITANCE

Among the 18 translocations that the author produced in Glossina austeni he was able to distinguish clearly between the majority, which showed transmission of partial sterility to a proportion of both sexes, and two stocks, which showed holandric inheritance, i.e. all the males and none of the females inherited partial sterility [2]. The former group were interpreted as autosomal translocations and the latter as translocations involving the Y. The wild-type tsetse male has been shown by cytological means to have differentiated X and Y chromosomes (A. Haring, personal communication). One of the Y translocation stocks is still being maintained and continues to show holandric inheritance after 9 generations of breeding.

Two of the stocks which were originally scored as autosomal translocations (stocks 626 and 73 in Ref. [2]) have since shown a switch-over in pattern of inheritance to the holandric type. Fig.4 presents the data for one of the stocks diagrammatically. The data for the other stock are similar. Successive generations were reared from males shown to be partially sterile in test matings. In this stock (no.626) the switch-over in behaviour can be traced to one individual, A, which was ancestor to all the later members of the stock. The right side of the figure shows the score of normal and partially sterile males and females among those tested (this was a small minority of the total number hatching). In the first 6 generations there was segregation in both sexes; the apparent excess of partially sterile males above a 1 : 1 ratio is not significant. In the last 4 generations inheritance appears to be purely holandric and the heterogeneity between the first six and the last four generations is highly significant. The figure gives data for the mates of the partially sterile males on the mean rate of production of pupae and of abortions; the abortions are apparently due to a delay in the lethality, due to chromosome imbalance, until quite late in development of the larva in the uterus [2]. As shown, the pupal and abortion rates were very similar before and after the switch-over to holandric inheritance.

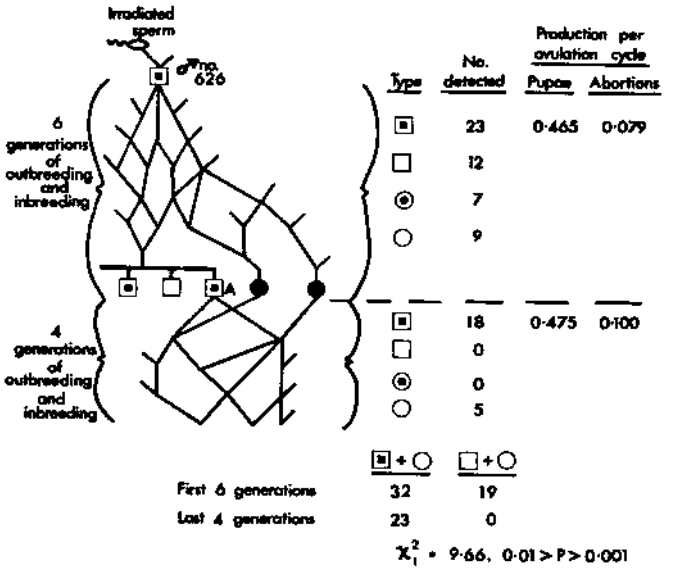


FIG. 4. Diagram of the pedigree and summary of data for stock 626 to show the switch-over from autosomal to holandric inheritance of partial sterility. Symbols as in Fig. 3; black circles = unclassified females.

It is unlikely that the explanation of this switch-over is the occurrence of a new translocation (involving the Y) in the sperm from which A was derived, because different translocations in G. austeni cause characteristic pupal production and abortion rates [2] and it is unlikely that a new translocation would be so similar to the old one in both these respects. The same argument counts against the suggestion that at the time of the switch-over the Y chromosome became attached to a pre-existing autosomal translocation. The author would expect this to affect the segregation of the heterozygote and hence the rate of pupal production.

Another possibility is that the translocation has always involved the Y chromosome but in the first 6 generations non-disjunction frequently occurred giving X0 (non-translocation) males and XXY (translocation) females. One might postulate that some adjustment occurred in individual A which prevented further non-disjunction. There is evidence [16, 17] for non-disjunction in house-fly translocations involving the Y, but in this species it is the presence of the Y (regardless of the number of X chromosomes) which determines maleness. If the same is true in tsetse flies a Y translocation could never show other than holandric inheritance.

Another suggestion is that the translocation in stock 626 is autosomal but in later generations the determination of sex has been transferred from the X and Y chromosomes to a site at or close to one of the translocation break points. This transference might have occurred by the evolution of modifying genes under the pressure of the selection the author applied by propagating the stock from males which were partially sterile. This hypothesis implies that both sexes now carry two X chromosomes. There is a precedent in the work of Winge on Lebistes [18]

for a change in the mechanism of sex determination under the pressure of artificial selection, but he found extreme distortion of the sex ratio in the early stages of this change. The present author did not find this in stock 626.

Which, if any, of these ideas is correct can only be established by cytological studies and these are now being carried out by Dr. Southern. The results may well elucidate the sex determination mechanism of Glossina, for example they may be able to distinguish a mechanism dependent on the number of X chromosomes (as in Drosophila) from one dependent on the Y (as in house flies). Information about the sex determination mechanism is of importance in view of the suggestions that have been made for the control of insect pests by the use of sex ratio distorting factors.

REFERENCES

[1] LAVEN, H., Eradicating mosquitoes using translocations, Nature, Lond. 221 (1969) 958.

[2] CURTIS, C.F., The production of partially sterile mutants in Glossina austeni, Genet. Res. 13 (1969) 289.

[3] JORDAN, A.M., CURTIS, C.F., Productivity of Glossina austeni Newst. maintained on lop-eared rabbits, Bull. ent. Res. 58 (1968) 399.

[4] CURTIS, C.F., JORDAN, A.M., Calculations on the productivity of Glossina austeni Newst. maintained on goats and lop-eared rabbits, Bull. ent. Res. 59 (1970) 651.

[5] SEREBROVSKII, A.S., On the possibility of a new method for the control of insect pests, Zool. Zh. 19 (1940) 618.

[6] CURTIS, C.F., A possible genetic method for the control of insect pests, with special reference to tsetse flies (Glossina spp.), Bull. ent. Res. 57 (1968) 509.

[7] CURTIS, C.F., HILL, W.G., "Theoretical and practical studies of a possible genetic method for tsetse fly control", Isotopes and Radiation in Entomology (Proc. Symp. Vienna, 1967), IAEA, Vienna (1968) 233.

[8] CURTIS, C.F., HILL, W.G., Theoretical studies on the use of translocations for the control of tsetse flies and other disease vectors, Theoret. Pop. Biol. (in press).

[9] CURTIS, C.F., Possible use of translocations to fix desirable genes in insect pest populations, Nature, Lond. 218 (1968) 368.

[10] HARING, A., FRASER, D.M., Spermatogenesis of Glossina austeni, Trans. R. Soc. trop. Med. Hyg. 62 (1968) 125.

[11] JORDAN, A.M., NASH, T.A.M., BOYLE, J.A., The rearing of Glossina austeni Newst. with lop-eared rabbits as hosts. I. The efficacy of the method, Ann. trop. Med. Parasit. 61 (1967) 182.

[12] NASH, T.A.M., JORDAN, A.M., BOYLE, J.A., A method of maintaining Glossina austeni Newst. singly, and a study of the feeding habits of the female in relation to larviposition and pupal weight, Bull. ent. Res. 57 (1967) 327.

[13] CURTIS, C.F., "An attempt to produce stocks homozygous for chromosome translocations in Glossina austeni", Proc. Symp. Tsetse Fly Breeding under Laboratory Conditions and its Practical Application, Lisbon, 1969 (in press).

[14] JORDAN, A.M., "Inbreeding of Glossina austeni", Proc. Symp. Tsetse Fly Breeding under Laboratory Conditions and its Practical Application, Lisbon, 1969 (in press).

[15] BURNHAM, C.R., Discussion in Cytogenetics, Burgess, Minneapolis (1962).

[16] HIROYOSHI, T., Sex limited inheritance and abnormal sex ratio in strains of the house fly, Genetics 50 (1964) 373.

[17] WAGONER, D.E., Linkage group karyotype correlation in the house fly, Musca domestica L., confirmed by cytological analysis of X-ray induced Y-autosomal translocations, Genetics 62 (1969) 115.

[18] WINGE, Ö., The experimental alteration of sex chromosomes into autosomes and vice versa as illustrated by Lebistes, C.R. Trav. Lab. Carlsberg ser. Physiol. 21 (1934) 1.

DISCUSSION

M.J. WHITTEN: I think Mr. Curtis is to be congratulated on his successful production of a translocation homozygous strain of tsetse fly. In my estimation, tsetse probably represents a most difficult subject on which to perform this type of operation, which it should therefore be possible to repeat for better suited insects.

I should like to make one other comment about translocation homozygotes. It is commonly believed that they must invariably be associated with position-effects which reduce their fitness. It must be stressed that translocations arise often in nature and, against enormous odds, become fixed in natural populations. M.J.D. White has documented this point in some detail in his studies on Australian grasshoppers. He has argued that the abundance of translocations in some groups (e.g. grasshoppers) and their near absence in others (e.g. mosquitoes) is an aspect of the different population dynamics in the two groups. His grasshoppers pass through more bottle-necks, allowing stochastic events to increase their chances of fixation. In any case, the important thing from the point of view of evolutionary studies is that translocations need not involve a reduction in fitness.

C.F. CURTIS: Certainly, some translocations allow full fitness, but it is impossible to estimate how many deleterious translocations occurred and were eliminated in the evolution of the grasshoppers to which you referred. Hence one cannot be sure that the necessary one or two translocations compatible with full fitness could be obtained with a reasonable expenditure of effort.

R.C. BUSHLAND: I congratulate Mr. Curtis not only on his fine accomplishment, but also on his moderation in not claiming more vigour for his translocation stocks than would be shown by insects irradiated to achieve 100% dominant lethality. I think that the claim of greater fitness has been oversold by earlier speakers in this session. The failure of sterilized Anopheles quadrimaculatus several years ago in Florida was because the laboratory-adapted strain was unfit for nature. House flies on the island of Grand Turk preferred to mate with each other rather than with the laboratory strain of Florida flies. According to Muller, by irradiation you induce four recessive detrimentals for every dominant lethal mutation. This suggests that at the 90% dominant lethality dose rates used to induce translocations almost every viable sperm should bear detrimentals. These may not be selected out under cage conditions. I believe in the translocation approach, and I support Mr. Wagoner's research at our Fargo laboratory, but I do not think it is more certain of success than radiation sterilization. Prime attention must be given to insect ecology and population dynamics if any technique is to succeed.

C.F. CURTIS: I agree that the question of the possible loss of genetic adaptation of laboratory populations to the environment is important and that it would be desirable to induce translocations in a population recently isolated from the wild flies. As previously mentioned by Mr. Jordan, there is now more evidence that the population of Glossina morsitans at Langford has not lost its genetic adaptation to the environment after two years of laboratory breeding.

No doubt much other genetic damage was done by the radiation that I used to induce translocations. However, before starting inbreeding,

I repeatedly backcrossed the translocation heterozygotes with unirradiated stock. I think that this would have removed almost all radiation damage, which I do not believe can explain the reduced fitness of the homozygotes. These are now separated by about twelve generations from the irradiated flies.

M.J. WHITTEN: I concur with what Mr. Curtis has said concerning Mr. Bushland's comments. I would even go further and say that Muller's data are quite irrelevant to the present discussion, or at least they should be. It is the rearrangement itself that we make use of, not the genes it contains. Deleterious genes induced by the irradiation treatment should be removed along with other naturally occurring lethals and semi-lethals by an appropriate backcrossing program before a homozygous translocation strain is synthesized and its fitness estimated. An adequate understanding of the genetic principles involved should preclude an unreasonable comparison of this type.

L.E. LaCHANCE: Mr. Curtis, do you foresee any advantage in maintaining 2 T/T stocks in the laboratory and crossing them before release as compared with maintaining and releasing a 'double T/+ stock'?

C.F. CURTIS: I do see advantages, both because the captive populations of T/Ts would be fully fertile and because they would automatically maintain their genetic purity. Full fertility is important in a tsetse stock if it is to be economically mass-reared, but may be less important in oviparous species. If a double autosomal heterozygote stock were reared, there would be segregation at each generation and selective elimination of the translocation, unless the wild-type segregants were culled. I do not think that this culling process would be practical during mass rearing and believe that a stock which automatically remains genetically pure would be essential.

CHROMOSOMAL TRANSLOCATIONS AND GENETIC CONTROL OF *Aedes aegypti**

K.S. RAI, P.T. McDONALD
Department of Biology,
University of Notre Dame,
Notre Dame, Ind., United States of America

Abstract

CHROMOSOMAL TRANSLOCATIONS AND GENETIC CONTROL OF *Aedes aegypti*.

Genetic control mechanisms for insect pests are currently being developed for a number of important species. In view of the environmental contamination that ensues from the use of insecticidal chemicals and the fact that insect populations rapidly become resistant to most insecticides, it is imperative that alternative control methods be developed.

In their laboratory, the authors are interested in evaluating the use of chromosomal translocations for genetic control of *Aedes aegypti*, which transmits diseases such as yellow fever, dengue and haemorrhagic fever. This species is thus one of the most important disease vectors. The authors have recently analysed two radiation-induced sex-linked reciprocal translocations in this species. One, RT(1 : 2), involves linkage groups I and II with the original break points 0.3 cross-over unit from the gene for male-determining allele (M) on group I and 1.6 units from the wild-type allele of spot abdomen (s^+) on group II. The other translocation, RT(1 : 3), involves linkage groups I and III with the original break points 0.4 cross-over unit from the wild-type allele of red-eyed gene (re^+) on group I and 0.6 unit from the normal allele of black tarsus (blt^+) on group III.

Though originally both these translocations involved the male-determining chromosomes, female translocation heterozygotes have been established by appropriate crosses. Furthermore, two types of males heterozygous for each translocation have been constructed. In one type (T_M) the male-determining chromosome is translocated and in the other (T_m) the female-determining chromosome is translocated. In addition, by intercrossing both these translocations, double translocation heterozygous males have been established. Whereas each translocation heterozygote is associated with semisterility, the fertility of double translocation heterozygous males is much lower (7 - 12% against a multiple marker RED stock).

Population dynamics of males heterozygous for each translocation have been tested in sequential generations to evaluate their usefulness for genetic control purposes. Results obtained have demonstrated their potential usefulness. Detailed data from genetic and population studies and computer simulations where different release strategies have been evaluated are presented. In addition, prospects for field testing of the translocation method against an isolated population of *Aedes aegypti* are discussed.

INTRODUCTION

Notable progress has been made, at least in theory, in recent years in the field of insect pest control through genetic manipulation of populations. As a result, genetic control mechanisms are currently being developed for a number of important pest species. In view of the environmental contamination that ensues from the use of insecticidal chemicals and the fact that insect populations rapidly become resistant to most insecticides, it is imperative that such control methods be developed and field-tested.

One possible genetic control method that has attracted much attention during recent years is the use of inherited semisterility associated with chromosomal translocations. Although the potential of this method was

* This work received support from U.S. Atomic Energy Commission Contract AT(11-1)-38 with the Radiation Laboratory, University of Notre Dame. This is AEC Document No. C00-38-748.

originally proposed three decades ago [1], it was not until recently that its use for a number of insect species, e.g. mosquitoes [2-4], tsetse flies [5] and houseflies [6] has been contemplated and progress made.

The use of translocations for population control may provide some advantages over the sterile-male technique. Sterile males do not transmit sterility to members of the next generation and, therefore, continued reintroductions are essential to maintain control. On the contrary, Curtis [5] has calculated that a single release of translocation homozygotes of both sexes will reduce an idealized tsetse-fly population to zero in about 12 generations; whereas releases of sterile males of equal numbers cannot do that. Furthermore, an additional advantage for the translocation system may accrue from the facts that no physical treatment is given the released insects in the generation in which they are released and that the translocation systems are usually associated with an essentially unaffected genotype and phenotype. As a result, the competitive ability of translocated insects may not be hampered. Such reduced competitive ability often becomes a limiting factor in mutagen-induced dominant lethality [7].

In our laboratory, we have been interested in inducing reciprocal translocations in the yellow-fever mosquito, Aedes aegypti, and evaluating the same for population control purposes. This species is well suited for studies of this sort. It has a low chromosome number: three pairs which are individually recognizable [8]; it is easily maintained and manipulated in the laboratory, has a rich store of genetic variability and its three linkage groups are well established [9]; and mechanization for its mass rearing for release purposes has been adequately developed [10]. Furthermore, the economic importance of this species is well known. It transmits diseases such as yellow fever, haemorrhagic fever and dengue and is thus one of the most important disease vectors. As a result of all these attributes, this species is regarded as a promising subject for the application of genetic methodology for population control purposes [11].

We have induced with radiation five sex-linked and two autosomal translocations in this species. Of the five sex-linked, one is a double translocation involving all three pairs. However, this paper includes data on two sex-linked translocations only, the work on which has been completed. One of the translocations has been designated RT(1 : 2) and the other RT(1 : 3). The former involves linkage groups one and two and the latter one and three. It may be mentioned that sex in this species is determined by a single gene, M vs. m with Mm genotype determining maleness and mm femaleness [12].

MATERIAL AND METHODS

The usual procedure that we employ to induce and isolate reciprocal translocations in this species is to irradiate young males of a wild type, ROCK strain, to sub-sterilizing doses of radiation, mate these males with a multiple marker (RED) stock and backcross the F_1 males from cultures showing 50% or lower fertility with the RED stock. The RED stock is homozygous for the adult recessive markers, red-eye (re), spot abdomen (s) and black tarsi (blt) on linkage groups one, two and three respectively. The phenotypes of the backcross progenies are scored and the translocations are originally picked up through 'pseudolinkage' between markers known to

be present on different linkage groups. The presence of the translocations is then confirmed by cytological examination of meiotic and/or mitotic tissues.

RT(1 : 2) was isolated following 1200 R egg irradiation for four successive generations. A 10-kCi cobalt-60 source giving a dose rate of approximately 123 R/min at a fixed spot was used for irradiation [13]. RT(1 : 3) was isolated following X-irradiation of 0 - 26-hour-old adult ROCK males. The source of radiation was a 230 KVP Picker Console Therapy unit operated at 15 MA and 1.0 cm of Al filter. The total dose to which the young adult males were irradiated was 5000 R at a rate of approximately 200 R/min.

Using semisterility and the cytogenetic evidence as criteria it was obvious that both these translocations were male-linked, i.e. they were transmitted from father to sons and were therefore maintained as such originally.

The eggs were hatched in de-oxygenated water and the larvae were reared in tap-water to which adequate amounts of beef-liver powder were added from time to time as food. The rearing was done in an insectary maintained at 80 ± 5°F and 80 ± 10% relative humidity. For oviposition, the females were fed on anaesthetized mice. Sugar cubes or apples were used as food for the males. Techniques for making crosses in this species have been described by Craig and VandeHey [14]. Experimental procedures used to study various competitive parameters of RT(1 : 2) and the population dynamics of the two translocations are included in the results section.

RESULTS

1. Genetics

1.1. Mapping of break points and recovery of female translocation heterozygotes

Preliminary genetic results from back crosses indicated that RT(1 : 2) and RT(1 : 3) involved linkage groups one and two and one and three respectively. Both were linked with the male-determining (M) locus. Genetic mapping of the radiation-induced breaks on the individual linkage groups in the two translocations was undertaken as follows.

1.1.1. RT(1 : 2)

In the case of RT(1 : 2), crosses between RED stock females carrying the gene spot abdomen (s) in the homozygous state and wild-type males heterozygous for this gene and the translocation $\left(\frac{M\ s^{+}}{m\ s}\right)$ were set up and the following progeny were obtained:

824	wild-type males
1001	spot females
22	wild-type females
14	spot males
1861	total progeny

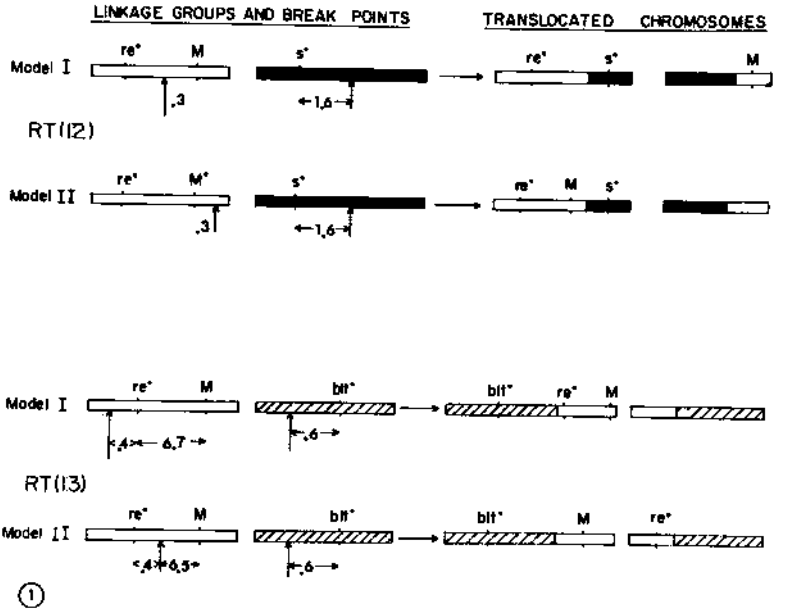


FIG. 1. Genetic mapping of radiation-induced break points on linkage groups one and two in RT(1 : 2) and on linkage groups one and three in RT(1 : 3). Vertical arrows indicate the position of breaks. For each translocation, two possible models are indicated.

This gives a cross-over distance of 1.9 units (36/1861) between the genes for sex (M) on one side and wild-type allele of spot abdomen (s^+) on the other side of the break.

The wild-type females recovered from the previous crosses were tested to determine whether or not they were translocated. These females were crossed with RED stock males. In all crosses approximately half the progeny (both males and females) were s and half s^+. The wild-type F_1 male progeny were then back-crossed with RED stock females and the progeny scored for assortment of s^+ and female-determining gene (m). Those showing linkage between m and s^+ must have been translocated females. Of the 22 recovered s^+ females, 14 died without leaving progeny and 2 produced progeny that were infecund or sterile. Of the remaining six, five were non-translocated and one was translocated.

Since the non-translocated female must have been produced as a result of an exchange between the s^+ locus and the break point, and the translocated females because of an exchange between the sex locus and the break point, the latter distance must be considerably less (1/6 of 1.9 or approximately 0.3 cross-over unit) than the former distance (5/6 of 1.9 or 1.6 cross-over units). These break points on linkage groups one and two and the linkage distances are included in Fig. 1.

1.1.2. RT(1 : 3)

Genetic mapping in the case of RT(1 : 3) was accomplished by making crosses between RED stock females and translocated males that were carrying wild-type alleles for red-eye and black tarsi on the translocated

chromosomes and the recessive alleles on the normal chromosomes. From these crosses, progeny with the following phenotypes were obtained:

1048 re^+ blt^+ (wild) males
883 re blt (red, black tarsi) females
73 re^+ blt^+ (wild) females
60 re blt (red, black tarsi) males
7 re^+ blt (black tarsi) males
8 re blt^+ (red) females
5 re^+ blt (black tarsi) females
1 re blt^+ (red) males
2085 total progeny

These data indicated that the breakage on linkage groups one occurred 0.4 cross-over unit from the wild-type allele of red-eyed gene (re^+) and 0.6 unit from blt^+ on linkage group three. However, the above data fit with two models depending upon whether the breakage on linkage group one occurred to the left or right of the wild-type allele of the red-eye gene (re^+). As a result, the distance between the breakage point and the sex locus may be 6.5 or 7.1 cross-over units (Fig. 1).

Seventy-three wild-type females recovered from the previous experiment must have resulted from an exchange between the genes for sex and red-eye (Model I) or between sex and the translocation breakage point (Model II). Consequently they were expected to be translocation heterozygotes. Eight of these females were tested by crossing these with RED males. The progeny from these crosses showed that all the females tested were translocation heterozygotes.

1.2. Synthesis and genetics of double translocation heterozygous males

The original male-linked translocations and the recovery of the translocated female chromosome from these, has made possible the synthesis of the following two types of male translocation heterozygotes for RT(1 : 2) and RT(1 : 3):

(a) males in which the male-determining chromosome is translocated (T_M)
(b) males in which the female-determining chromosome is translocated (T_m).

Furthermore, in an effort to determine the effectiveness of individuals carrying more than one translocation in reducing population sizes, a program was undertaken to produce individuals that would be heterozygous for both RT(1 :2) and RT(1 : 3). Two interesting, albeit unusual, results have emerged from this work [15]. First, a new karyotype, with new linkage relationships, has been created. As a result of a cross-over between the sex and the red-eye genes in the double heterozygous individuals, a 'new' chromosome containing parts of linkage groups one, two and three has been created. This chromosome, together with two of the translocated chromosomes forms a haploid set (new karyotype RT(1 : 2 : 3)) that is capable of functioning, in the presence of a normal haploid set, in a way that allows normal development to the adult stage.

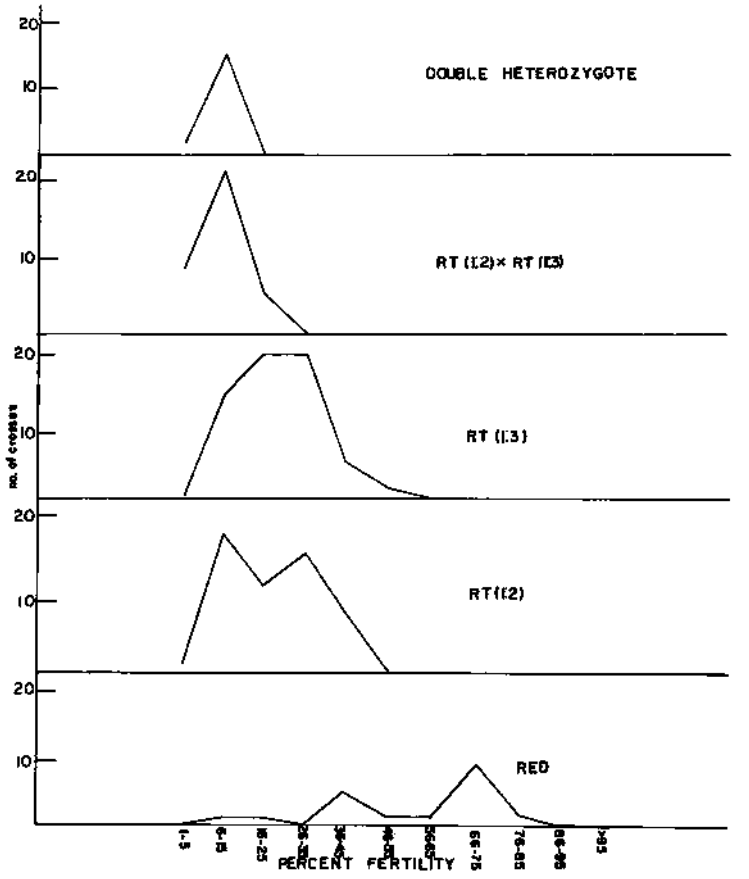


FIG. 2. Frequency distribution of fertilities of RED, RT(1 : 2), RT(1 : 3), RT(1 : 2) × RT(1 : 3) and the double heterozygotes among the two translocations. Data obtained from egg papers hatched within eight days after collection and containing over 100 eggs.

The second interesting aspect of the double translocation heterozygote system is greatly enhanced crossing-over that occurs in the regions between genes for sex and red-eye as well as between those for sex and spot. This has the effect of producing a higher than expected frequency of new karyotype individuals from double heterozygote individuals and conversely, a higher than expected frequency of double heterozygote individuals from new karyotype individuals.

2. Fertility

In general, both translocation heterozygotes are characterized by semisterility. However, a great degree of variability in fertility was observed in the different crosses including those of the non-translocated controls (Fig. 2).

The control crosses involving the RED stock (in which the translocations were maintained) has a mean fertility of 55% with a range of 15% to 81%. The fertility of translocation heterozygotes for RT(1 : 2) was determined for males with the translocation associated with the M locus (15%) and with the m locus (24%) and also in females (25%). Against the RED stock, the overall mean fertility for RT(1 : 2) was 20% with a range of 4% to 48%. The fertility of translocation heterozygotes for RT(1 : 3) was similarly determined for males with the translocation associated with the M locus (24%) and with the m locus (27%) and also in females (24%). The overall mean for RT(1 : 3) was 25% with a range of 5% to 48%. Crosses involving females heterozygous for RT(1 : 2) and males heterozygous for RT(1 : 3) or vice versa showed a mean fertility of 9% with a range of 1% to 21%. The double heterozygotes obtained by crossing these two translocations had a mean fertility of 8% with a range of 3% to 11%.

3. Mating competitiveness, fecundity and longevity

In order to evaluate whether translocation heterozygosity per se was associated with any undesirable biological attributes and whether translocated males could be used for possible genetic control purposes, the following parameters of males heterozygous for RT(1 : 2) were compared with those of the normal males.

3.1. Mating competitiveness

One hundred crosses, each involving one RED stock female, one ROCK male and one RT(1 : 2) male homozygous for black tarsi, were set up. Since multiple inseminations do not take place as a rule in Aedes aegypti, a female inseminated by a ROCK male would be expected to produce progeny with normal tarsi whereas females inseminated by translocated males would produce progeny homozygous for black tarsi (blt).

Of the 100 females tested, 46 were fertilized by ROCK males and 47 by RT(1 : 2) males. Three females were fertilized by both types of males and four died without leaving any progeny. Thus males heterozygous for this translocation are equally competitive with normal males for matings with females.

3.2. Male fecundity

To test their overall fecundity, one RT(1 : 2) or one ROCK male were placed with successive batches of three virgin ROCK females at three-day intervals. Twenty such matings (ten for RT(1 : 2) males and 10 for ROCK males) were set up. After the females had been with the male for three days, spermathecae of the females were dissected and scored for the presence of sperm. The ten RT(1 : 2) males filled 271 or a mean of 27.1 spermathecae per male. The ROCK males filled 274 or 27.4 spermathecae per male. Thus, the two types of males were similar in their overall reproductive potential. It may be pointed out that, in this species, normally only two of the three spermathecae receive sperm following insemination. Neither type of male inseminated any additional females beyond the sixth batch although most males still contained sperm in their testes and seminal vesicles.

TABLE I. DATA FROM POPULATION CAGES CONTAINING NORMAL (RED) FEMALES AND DIFFERENT PROPORTIONS OF NORMAL (RED) AND RT(1:2) MALES IN SEQUENTIAL GENERATIONS

Gen. No.	No. used at start			% Hatch	Phenotype of the progeny						Total progeny	s^+, RT(1:2)					
					$\sigma^*\sigma^*$			♀♀				$\sigma^*\sigma^*$ progeny		Total progeny		% RT(1:2)	Efficiency index[a] RT(1:2)
	Red ♀	Red σ^*	RT(1:2) σ^*		s	s^+	Total	s	s^+	Total		obs.	exp.	obs.	exp.		
I (Cont) normal	50	50	-	6	127	-	127	91	-	91	218						
I (Cont) RT(1:2)	50	-	50	20	5	102	107	98	2	100	207						
I-A	50	10	40	23	3	81	84	50	2	52	136	81	56	83	45	61	Outcompet.[b] or (1.25)
I-B	50	10	40	38	32	50	82	63	1	64	146	50	55	51	49	35	1.02
II-A	30	10	20+40	20	4	70	74	72	6	78	152	70	55	76	57	50	Outcompet.[b] or (1.16)
II-B	30	10	20+40	21	-	47	47	66	1	67	114	47	35	48	43	42	Outcompet.[b] or (1.08)
II-C	30	10	20	30	77	21	98	99	-	99	197	21	49	21	49	11	0.58
II-D	30	10	20	22	24	13	37	20	-	20	57	13	18	13	14	23	0.97
III-A	17	4	13+40	15	-	53	53	43	3	46	99	53	46	56	43	56	Outcompet.[b] or (1.07)
III-C	20	10	10	24	67	44	111	93	4	97	208	44	37	46	34	22	1.24

[a] Assuming that the efficiency index of normal males (RED stock) is 1.

[b] If analysis is based on male progeny alone, 3 s males in I-A, 4 in II-A, and 1 in III-A could have originated through crossing over in RT male parents. Thus, no RED fathers may have produced any of the male progeny and, therefore, RT males could have completely outcompeted the RED males. Figures in parenthesis are based on the total progeny.

3.3. Longevity

The adult longevity of the two types of males was compared by placing 100 males of each type and of similar age (± 8 hours) in separate gallon cages. These were maintained under similar conditions and their longevity was measured by counting the total days elapsed until 1/2 (i.e. 50 adults) were dead. The figure was 93 days for RT(1 : 2) males and 82 days for normal males.

4. Population studies to determine the feasibility of the use of RT(1 : 2) and RT(1 : 3) for genetic control

One of the major objectives of our studies has been to evaluate the potential of the translocated males for possible population control purposes. Laboratory population cages containing normal females and a variable proportion of normal and either RT(1 : 2) or RT(1 : 3) males were set up for this purpose. To ascertain which progeny was fathered by RT males and which by non-translocated, control males, the females and the two types of males were genetically marked.

4.1. RT(1 : 2)

In the case of RT(1 : 2), the normal males and females used were from the RED stock and therefore homozygous for spot abdomen (s) and the RT(1 : 2) males used were heterozygous for this gene with the s^+ allele linked with the male-determining allele. An experimental cage was set up with the following proportion of adults:

RED stock (normal) females = 50
RED stock (normal) males = 10
RT(1 : 2) males = 40

In all the population experiments, the ages of the two types of males were similar. The experiment was run in duplicate and the eggs were collected at weekly intervals. All the eggs recovered were hatched and the progeny were scored for sex and spot abdomen.

If the parental RT(1 : 2) and RED males were equally competitive and if semisterility for RT males is assumed (though in practice the fertility of RT males in this experiment was never more than 20%, while that of the RED stock was approximately 60%), two-thirds of the male progeny and one-third of the total progeny would be expected to be wild-type (s^+) or RT(1 : 2) heterozygotes. It is obvious from the results entered in Table I that of the two replicates, one (I-B) produced expected numbers of wild type and mutant progeny. Thus, the parental RT and RED males that produced this progeny must have been equally competitive.

However, the second cage, I-A, produced 83 wild-type or RT(1 : 2) individuals and 53 mutant or non-translocated progeny. These data, particularly the male progeny, indicate that of the 50 parental females used to start this cage, none may have been inseminated by a non-translocated (s) male. That is, the 40 RT males introduced into the cage appear to have completely outcompeted the RED males. (These results are comparable with those of the RT, control cage).

TABLE II. DATA FROM POPULATION CAGES CONTAINING NORMAL (RED) FEMALES AND DIFFERENT PROPORTION OF NORMAL (RED) AND RT(1:3) MALES IN TWO GENERATIONS

Gen. No.	No. used at start			% Hatch	Phenotype next generation progeny						Total progeny	re+, RT(1:3)			
					♂♂			♀♀				Total progeny		% RT(1:3)	Efficiency index[a]
	Red ♀	Red ♂	RT(1:3) ♂		re	re+	Total	re	re+	Total		observ.	expect.		
I-A[b]	30	12	48	30	86	214	300	201	9	210	510	223	170	44	1.18
I-A[c]				34	18	41	59	42	1	43	102	42	34	41	1.10
I-B[b]	30	12	48	52	105	90	195	182	16	198	393	106	131	27	0.88
I-B[c]				35	106	140	246	160	29	189	435	169	145	39	1.10
II-A	18	6	12+24	33	77	116	193	149	5	154	347	121	130	35	0.95
II-B	18	5	30	27	7	94	101	80	7	87	188	101	71	53	1.16
II-C	18	6	12	41	38	36	74	63	0	63	137	36	34	28	1.07
II-D	18	6	12	51	110	70	180	134	7	141	321	77	80	24	0.99

[a] Assuming that the efficiency index of the normal males (RED stock) is 1.
[b] Progeny obtained from first egg hatch.
[c] Progeny obtained from second egg hatch.

If the population is assumed to be stable under normal conditions, the second generation may be calculated as follows. Of the 50 females, one-fifth that are expected to be fertilized by normal males will produce 10 normal females and 10 normal males. The remaining 40 females inseminated by RT(1 : 2) males will produce 20 normal females and 20 RT males (assuming semisterility for the RT males). Thus, the next generation will be expected to consist of 30 s (normal) females, 10 s (normal) males and 20 s^+ (RT) males. This is how population II-C and II-D were set up. To two replicates (II-A and II-B), 40 s^+ or RT(1 : 2) males were added. Eggs were harvested from all these second generation population cages, the hatchability ascertained and the progeny phenotypes scored. The results from these cages mostly substantiated the conclusions drawn from the first generation populations. Except population II-C, the RT males were either equally competitive with normal males (II-D) or outcompeted them (II-A, II-B).

Similar results have been obtained from the third generation experimental populations which, using the assumptions mentioned above, may be expected to consist of 17 normal females, 4 normal males and 13 RT males. Again 40 additional RT males were added to one such cage (III-A). Populations II-C and II-D were expected to give rise to 20 normal females, 10 normal males and 10 RT males (III-B).

Beyond the fourth generation, it may be unnecessary to introduce any additional RT males into the declining populations, for the fourth generation will be expected to consist of nine females, one normal male and 8 RT males. Fifth generation will be down to 4.5 females and 4.5 RT males. Such a population would be eliminated by the eighth generation if the two basic assumptions made (stable population, a pair replacing only a pair) are valid.

4.2. RT(1 : 3)

To test the population dynamics of RT(1 : 3), population cage experiments were initiated using RT(1 : 3) males and RED stock males. The design was similar to that employed for RT(1 : 2) males. Translocation heterozygous males carrying re^+ and blt^+ on the translocated chromosome and re and blt on the normal chromosomes were introduced in cages in the following proportion:

RED stock (normal) females = 30
RED stock (normal) males = 12
RT(1 : 3) males = 48

The experiment was run in duplicate and the eggs collected at weekly intervals. The results from two generations are included in Table II. These results have confirmed those obtained with RT(1 : 2) males. If the efficiency index of normal (RED) males is assumed to be 1, those of RT(1 : 3) males was slightly higher, except in two cases where it was 0.88 and 0.95.

5. Computer simulations

Computer programs based on releases of the following types of translocated males in idealized populations have been simulated in collaboration with Max Whitten of the University of Chicago:

a: Males in which the male-determining chromosome is translocated (T_M).
b: Males in which the female-determining chromosome is translocated (T_m).
c: Alternating releases of T_m and T_M males.
d: Double translocation heterozygous males.
e: New karyotype, RT(1:2:3) males.

These programs were simulated under different proportions of translocated to normal mosquitoes (2 : 1, 4 : 1, 6 : 1). If it is assumed that the idealized population is stable and that density-dependent factors do not operate, the computer simulations indicated that:

(1) The optimal proportion of RT to normal males was four.

(2) Assuming an initial population of 2000 and in some cases 20 000, it takes eleven generations to 'eradicate' a population if T_M males are released, eight generations if alternating releases of T_m and T_M males are made, six generations if T_m males are released and five generations if either double heterozygotes or new karyotype males are released. In all cases these results were obtained after the introduction of translocated males for five to six generations. When the population growth rate is up to five-fold per generation, the double heterozygote is capable of effecting 'eradication'.

DISCUSSION

The successful eradication of the screw-worm fly from the island of Curaçao and the southeastern United States through the release of males sterilized by gamma radiation has stimulated great interest in the possibility of extending this and other genetic techniques for the control of several other noxious species of insects. With mosquitoes, three trials with the sterile-male technique have been unsuccessful. However, in spite of these unsuccessful attempts, theoretical considerations indicate that this technique should be applicable for mosquito control [7]. Nevertheless, considerable effort, in several laboratories around the world, is underway to develop alternative genetic methods for controlling mosquito populations.

One method that is currently being evaluated for application to the yellow-fever mosquito, Aedes aegypti, is the use of chromosomal translocations. We have recently isolated seven translocations in this species and completed work on two. The radiation-induced break points have been genetically mapped in both translocations. This localization is important, particularly in the case of sex-linked translocations, for the distance between the male locus (M) and the break point will determine what frequency of normal (non-translocated) sons will appear among the progeny of translocated fathers.

Though originally both these translocations involved the male-determining chromosomes, female translocation heterozygotes have been established by appropriate crosses. Furthermore, two types of males heterozygous for each translocation have been constructed. In one type (T_M), the male-determining chromosome is translocated, and in the other (T_m), the female-determining chromosome is translocated. In addition, by intercrossing both these translocations, double translocation heterozygous males have been established.

Whereas each translocation heterozygote is associated with semisterility, the fertility of double translocation heterozygous males is approximately 25% of RED stock, often much lower. Fertility of RT(1:2) heterozygotes was slightly lower than that of RT(1:3). This may be due to the additional presence of a pericentric inversion in one of the translocated chromosomes. Confirmatory evidence for this comes from the cytological analysis and from a reduced rate of recombination in the area across the centromere in the translocated stock.

Results included in this paper have demonstrated that the mating competitiveness, fecundity and longevity of RT(1:2) males were comparable to those of the wild-type ROCK males. These studies have been further extended to laboratory population cages. When RT(1:2) males and RT(1:3) males (in which the male-determining chromosome, T_M, is translocated) were tested against RED males in proportions of 1:1, 2:1, 4:1, 6:1 or 13:1 for matings with RED females, the translocated males were either equally competitive or often outcompeted normal males and thus in subsequent generations produced expected or more than expected translocated progeny respectively. In most cases, the only genetic difference between the two types of males consisted of a single gene: spot versus wild-type abdomen in RT(1:2) experiments and red versus wild-type eyes in RT(1:3) experiments. It should be mentioned that Laven [4] also observed an efficiency index of male-linked translocations in Culex pipiens ranging between 1.7 and 2.1 compared with the efficiency index of 1 for normal males.

Another observation of interest from the population cage experiments was a preponderance of males in several populations. This does not appear to be a consequence of translocation heterozygosity but may be due to the sex distorter gene [16] which is very tightly linked with the M locus. A combination of the translocation with the 'male producing' gene could provide an additional advantage for population control.

A likely objection that may be cited against the population cage experiments is that the results were predicated on the premise that a natural population will be stable to the point that in the absence of the introduced translocation-related sterility, it does not undergo any increase or decrease from generation to generation. Admittedly, this assumption is artificial. It is well known that mosquito populations do undergo substantial increases as well as decreases. Nevertheless, according to Laven [4] "releases of translocation males into a population which is continuously decreasing would enhance the downward trend and, theoretically, lead to total eradication".

On theoretical grounds, it is expected that the rate of population suppression following releases of males in which the female chromosome is translocated (T_m) should be more rapid than comparable releases of males in which the male-determining chromosome is translocated (T_M). This happens because in the former case female translocation heterozygotes will be produced among the progeny which will themselves be semisterile.

Furthermore, when such females mate with males heterozygous for the same translocation, translocation homozygotes will be produced and if these homozygotes are inviable, the overall population will be proportionately reduced. The most rapid rate of population decline is expected to ensue from releases of males which are heterozygous for both RT(1 : 2) and RT(1 : 3). Not only is the fertility of such males approximately one-half of each translocation heterozygote, but they also generate into a breeding population RT(1 : 2), RT(1 : 3), a new translocated karyotype, RT(1 : 2 : 3), and the normal individuals in approximately equal proportions [15]. These expectations have been borne out by the computer simulation programs.

Rai [17] has developed a technique for the induction and isolation of sex-linked recessive lethals in A. aegypti. If a recessive lethal mutation is induced on the female-determining chromosome in the translocation heterozygote males, daughters of matings involving such males and normal females will be heterozygous for the lethal in the F_1 generation. Half of the females formed in the subsequent generations arising from matings involving males and females heterozygous for this lethal will die. It will be most desirable to have the lethal very tightly linked with the female-determining locus or associated with an inversion so that it will not be lost through genetic recombination. Such a combination of the translocation system and a recessive lethal factor should bring about a more rapid population decline than will be possible through the use of either one of the two systems.

Although actual large-scale field releases using the translocation system have not yet been undertaken with any insect species, Wagoner [18] has reported that an experimental release of both sexes of Musca domestica carrying a heterozygous translocation between chromosomes II, III and V at a 9 : 1 ratio to wild type reduced the population to approximately 0.25% of the control level in two generations.

In addition to their population suppression potential, Curtis [19] has proposed a possible application of the use of translocation homozygotes to fix desirable genes in insect populations. If such homozygotes (TT), carrying genes for refractoriness to diseases or genes conferring insecticide susceptibility, are released into populations in numbers "so that the T frequency in the wild population exceeded the equilibrium point, the population would gradually approach fixation both for the T chromosomes and the gene for non-infectibility". Thus the genetic structure of a vector population could be changed such that a population becomes unable to transmit a disease.

Although it has not yet been possible to obtain translocation homozygotes for RT(1 : 2) and RT(1 : 3), the failure of getting translocation homozygotes may be due to the presence of similar recessive lethals in each of the translocated chromosomes, as they were originally derived from the same individual. A program of outcrossing translocation heterozygotes is currently underway. It is expected that this may rid the translocated chromosomes of recessive lethal genes through crossing over. Crossing the female and male translocation heterozygotes following the above procedure may produce viable homozygotes. If such homozygotes can be obtained, a considerably improved potential for population control purposes would ensue.

Population cages containing normal females and males and either T_m males or double translocation heterozygote males will soon be set up.

The results will be compared with those obtained with RT(1 : 2) and RT(1 : 3). It may also be mentioned that the potential of the use of the translocated males will be tested in large field cages and finally, if these results warrant it, against an isolated small-scale natural population in collaboration with the USDA laboratory on "Insects Affecting Man" in Gainesville, Florida and/or with the World Health Organization field unit on "Genetic Control of Culicine Mosquitoes" in India.

ACKNOWLEDGEMENT

The technical assistance of Miss Nancy Choitz is gratefully acknowledged.

REFERENCES

[1] SEREBROVSKII, A.A., On the possibility of a new method for the control of insect pests, Zool. Zh. 19 (1940) 618.
[2] RAI, K.S., Manipulation of cytogenetic mechanisms for genetic control of vectors, Wld. Hlth. Org. Scientific Group on the Cytogenetics of Vectors of Disease of Man, SC/VG. 67.34, (1967) 1.
[3] RAI, K.S., ASMAN, Sr. M., Possible application of a reciprocal translocation for genetic control of the mosquito, Aedes aegypti, Proc. XII Int. Congr. Genetics, Tokyo, Japan, 1 (1968) 164.
[4] LAVEN, H., Eradicating mosquitoes using translocations, Nature, Lond. 221 (1969) 958.
[5] CURTIS, C.F., A possible genetic method for the control of insect pests, with special reference to tsetse flies (Glossina spp.), Bull. ent. Res. 57 (1968) 509.
[6] WAGONER, D.E., NICKEL, C.A., JOHNSON, O.A., Chromosomal translocation heterozygotes in the house fly, J. Hered. 60 (1969) 301.
[7] RAI, K.S., "Status of the sterile-male technique for mosquito control", Sterile-Male Technique for Eradication or Control of Harmful Insects (Proc. Panel Vienna, 1968), IAEA, Vienna (1969) 107.
[8] RAI, K.S., A comparative study of mosquito karyotypes, Ann. ent. Soc. Am. 56 (1963) 160.
[9] CRAIG, G.B., Jr., HICKEY, W.A., "Genetics of Aedes aegypti", Ch.3, Genetics of Insect Vectors of Disease (WRIGHT, J.W., PAL, R., Eds.), Elsevier, Amsterdam (1967).
[10] SMITH, C.A., "Mass breeding procedures", Ch.22, Genetics of Insect Vectors of Disease (WRIGHT, J.W., PAL, R., Eds.), Elsevier, Amsterdam (1967).
[11] KNIPLING, E.F., LAVEN, H., CRAIG G.B., Jr., PAL, R., KITZMILLER, J.B., SMITH, C.N., BROWN, A.W.A., Genetic control of insects of public health importance, Bull. Wld. Hlth. Org. 38 (1968) 421.
[12] McCLELLAND, G.A.H., Sex-linkage in Aedes aegypti, Trans. R. Soc. trop. Med. Hyg. 56 (1962) 4.
[13] ASMAN, Sr.M., Cytogenetic and Developmental Effects of Gamma Radiation on Aedes aegypti (L.), Ph.D. Thesis, Univ. Notre Dame (1966).
[14] CRAIG, G.B., Jr., VANDEHEY, R.C., Genetic variability in Aedes aegypti, I. Mutations affecting color pattern, Ann. ent. Soc. Am. 55 (1962) 47.
[15] McDONALD, P.T., RAI, K.S., Aedes aegypti: origin of a "new" chromosome from a double translocation heterozygote, Science 168 (1970) 1229.
[16] HICKEY, W.A., CRAIG, G.B., Jr., Genetic distortion of sex ratio in a mosquito, Aedes aegypti, Genetics 53 (1966) 1177.
[17] RAI, K.S., "Techniques for the study of genetics and cytogenetics of vectors", Ch.23, Genetics of Insect Vectors of Disease (WRIGHT, J.W., PAL, R., Eds.), Elsevier, Amsterdam (1967).
[18] WAGONER, D.E., Suppression of insect populations by the introduction of heterozygous reciprocal chromosome translocations, Bull. ent. Soc. Am. 15 (1969) 220.
[19] CURTIS, C.F., Possible use of translocations to fix desirable genes in insect pest populations, Nature, Lond. 218 (1968) 368.

DISCUSSION

M. J. WHITTEN: I wonder whether Mr. Rai has undertaken computer simulations involving conventional sterile males and would be able to discuss the relative merits of the X-autosome translocations that he has described and of the conventional sterile-male technique. I notice that the maximum zygotic mortality provided for in the application of his scheme is 80 - 90%. What is the efficiency of these releases compared with the release of males irradiation-sterilized to the same level?

K. S. RAI: We have not conducted any computer simulations involving so-called 'conventional' sterile males. Nevertheless, if I recall correctly, Mr. Curtis discussed the results of just such a study in a paper in 1968 and showed that a single release of translocation homozygotes could theoretically eradicate a tsetse-fly population in something like twelve generations, while a single release of sterile males in equal numbers could not result in eradication. Thus, in terms of the cumulative load, the homozygote system was more effective than the sterile-male technique. Perhaps Mr. Curtis can comment on this further.

Of course, it will be most worthwhile to compare the effect of our heterozygous translocations of various types with that of sterile males, and we intend to carry out such simulations shortly.

C. F. CURTIS: It is true that the release of a given number of autosomal translocation homozygotes can be expected to cause a greater accumulated genetic load on a population than the release of the same number of equally competitive sterile males, but the load at any one generation would inevitably be limited. In this connection, I think it worth re-emphasizing that the effectiveness of limited loads in causing a population reduction depends on the extent to which the population size is buffered by density-dependent factors.

INDUCED CONDITIONAL LETHAL MUTATIONS FOR THE CONTROL OF INSECT POPULATIONS*

Roger H. SMITH
Biology Division,
Oak Ridge National Laboratory,
Oak Ridge, Tenn., United States of America

Abstract

INDUCED CONDITIONAL LETHAL MUTATIONS FOR THE CONTROL OF INSECT POPULATIONS.

Conditional lethal mutations have been induced in microorganisms, insects and mammalian tissue cultures. The molecular basis of these types of mutations is now well understood and is being used for a variety of genetic analyses; to map the genome and study macromolecular synthesis in microorganisms, and to study gene action during development in Habrobracon and Drosophila.

Temperature-sensitive lethal mutations, a class of conditional mutations, can be induced by radiation and a variety of chemical mutagens. For example, ethyl methanesulfonate is a mutagen that induces temperature-sensitive mutations at a relatively high frequency in insects. These mutations are expressed as wild type at one temperature (the permissive temperature) but as a lethal phenotype at another temperature (the restrictive temperature). As with most induced mutations the majority of these mutations are recessive, but as a matter of prime importance for insect control it should be noted that dominant conditional mutations have been induced in microorganisms and insects.

There are many possibilities for types of induced conditional dominant lethal mutations which undoubtedly would be useful for the control of insect populations. Mutations could be selected on the basis of temperature-sensitivity, either 'hot' or 'cold' sensitive lethal mutations, which could be used under different climatic restrictions. Also, mutants could be selected that would not feed on their natural food source but only on an artificial diet. These particular variants may have defective mouthparts or be some form of chemical-sensitive mutants. Along the same line, a mutant might be induced which could change the feeding habits of the population to use some economically unimportant food source instead of the primary source.

A conditional lethal mutation allows survival and growth of a cell or organism under one set of environmental conditions (permissive) but not under a different set (restrictive). Nutritional mutations found in microorganisms are conditional lethals in this sense. The restrictive condition is the absence of a particular nutrient in the growth medium. If the nutrient is added, conditions are permissive. However, there are many mutations, in higher organisms and microorganisms, the effects of which cannot be overcome by the addition of nutrients to a growth medium.

Temperature-Sensitive Lethal Mutations

Some mutations result in a single amino acid substitution in the protein coded by the particular gene. This altered gene product may function as well as the wild-type protein. Usually it does not. Sometimes it may function at one temperature but not at another. In the latter case we call the mutant "temperature-sensitive." If the gene is indispensable, the mutant is a temperature-sensitive lethal. Under the permissive temperature the organism expresses wild-type phenotype (survival and growth) while under restrictive temperatures it expresses the mutant phenotype (death).

* Research sponsored by the U.S. Atomic Energy Commission under contract with the Union Carbide Corporation.

Temperature-sensitive lethal mutations have been induced and used in genetic, developmental, and biochemical studies in *Neurospora* [1], bacteriophages [2], bacteria [3], and yeast [4].

P. W. Whiting [5] was probably first to recognize a temperature-sensitive mutation in an insect. The mutant found in *Habrobracon juglandis* had kidney-shaped eyes at low temperature but was expressed as a lethal at a high temperature. Now that the molecular nature of temperature-sensitive mutations are understood, they have been induced and used in genetic analyses in *Drosophila* [6, 7, 8] and *Habrobracon* [9, 10]. These mutations are heritable and map throughout the genome. Most are recessive, but dominant temperature-sensitive mutants have been found [4, 8].

Conditional mutations of almost all genes can be induced with radiation and chemical mutagens. In insects, the mono-alkylating compounds such as ethyl methanesulfonate (EMS), are the most effective in inducing point mutations, especially those of the temperature-sensitive type [6].

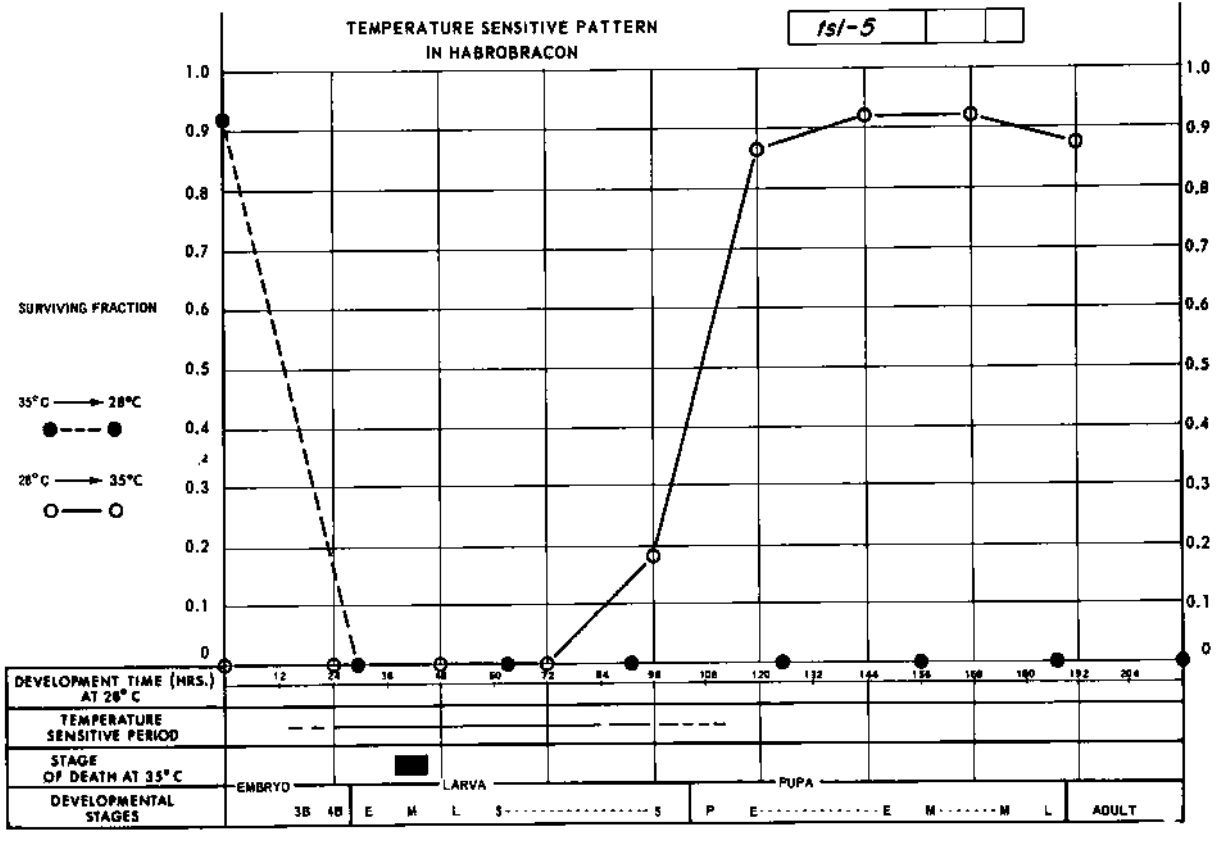
Ten to 20% of all recessive lethal mutations induced by EMS in sperm of *Habrobracon serinopae* are temperature-sensitive. Mutations are easily detected in this wasp because the diploid virgin females produce haploid male progeny parthenogenetically. Thus the entire genome can be screened for mutations in the second generation after a treatment with a mutagen. Many temperature-sensitive mutations have been made homozygous and maintained in the laboratory under permissive conditions.

As in microorganisms, the very nature of these conditional mutations has permitted the study of gene action during development. The phenotype of each mutant is death at a particular stage of development at the restrictive temperature (35°C) but survival to adulthood, or wild-type phenotype, at the permissive temperature (28°C). By shifting the developing wasp from 35°C to 28°C at different time intervals, the time and stage can be determined at which the gene product becomes necessary for survival to adulthood. By shifting the wasps from 28°C to 35°C at different time intervals, the time and stage can be determined at which the gene product is no longer necessary. From these experiments a pattern of gene action can be obtained for each temperature-sensitive mutation in a developmental gene [11].

Figure 1a shows the pattern of temperature-sensitivity for a particular mutant, *tsl-5*. Each point on the graph represents the frequency of survival to adulthood of individuals transferred from one temperature to the other at the specified stages of development. When eggs from a female homozygous for *tsl-5* are placed at 35°C, the progeny die soon after hatching as indicated in the figure. However, as shown by the pattern of temperature-sensitivity, the gene product remains necessary until the pupal stage for survival to adulthood.

In contrast to *tsl-5* development of *tsl-6* is arrested in the pupal stage at the restrictive temperature, and the gene product is necessary for only a short time before the lethal phase (Figure 1b). Some individuals carrying this mutation show different degrees of differentiation between the left and right sides, or between their head and thorax when development is arrested. One side or the head may be in the early pupal stage (no body pigment) while the other side or thorax is in the mid-pupal stage (with adult body pigment). These animals are haploid, and each is carrying the mutant gene; i.e., these are not genetic mosaics. The phenotype seems to be the result of differential gene action which is at present unexplained.

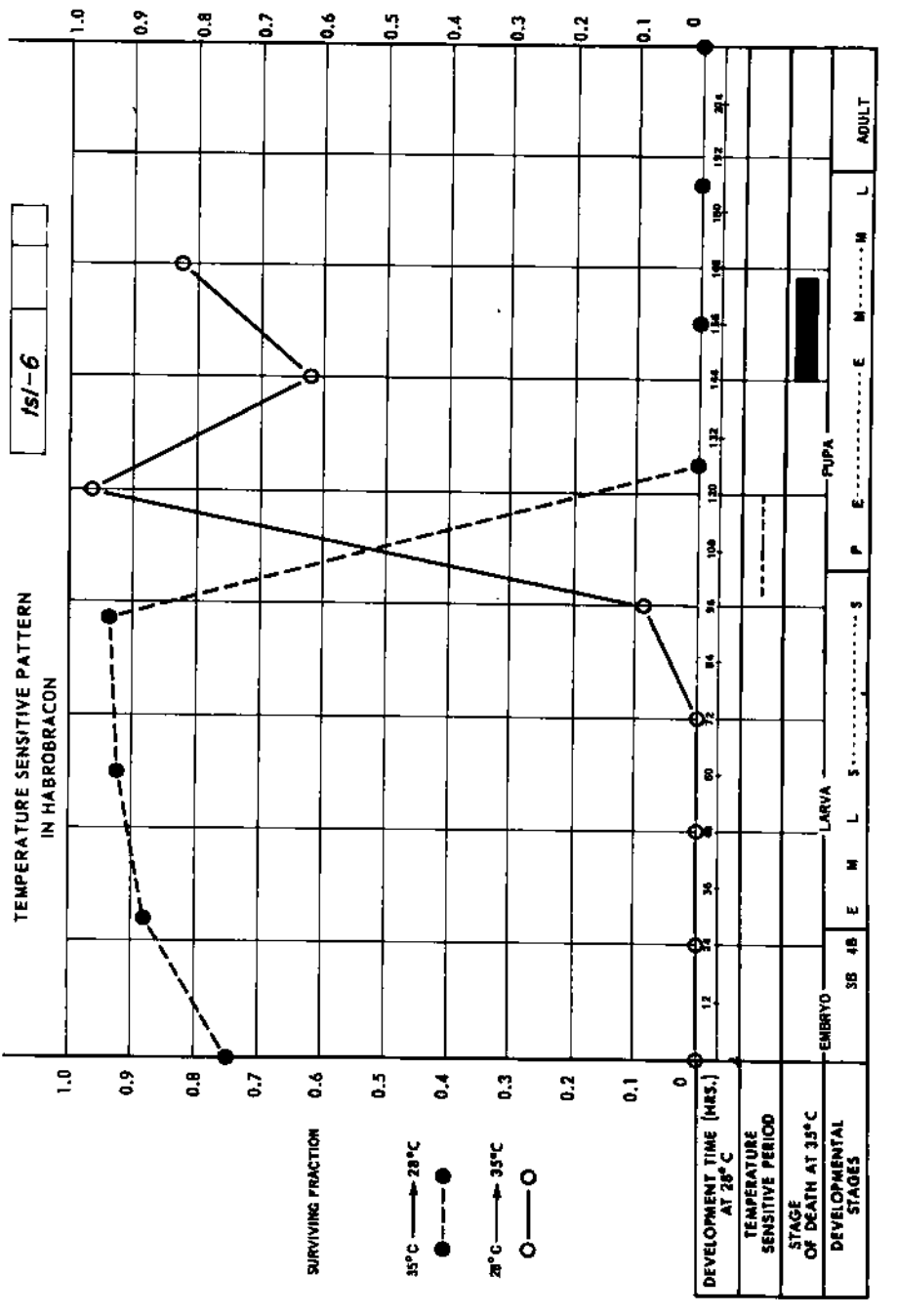
Figure 1c shows the pattern for *tsl-1* where the stages of death are spread over a long period of time, and the time of gene action coincides with the same period. The gene product is available over a long period but is not absolutely necessary for survival in the later stages of development.



(a)

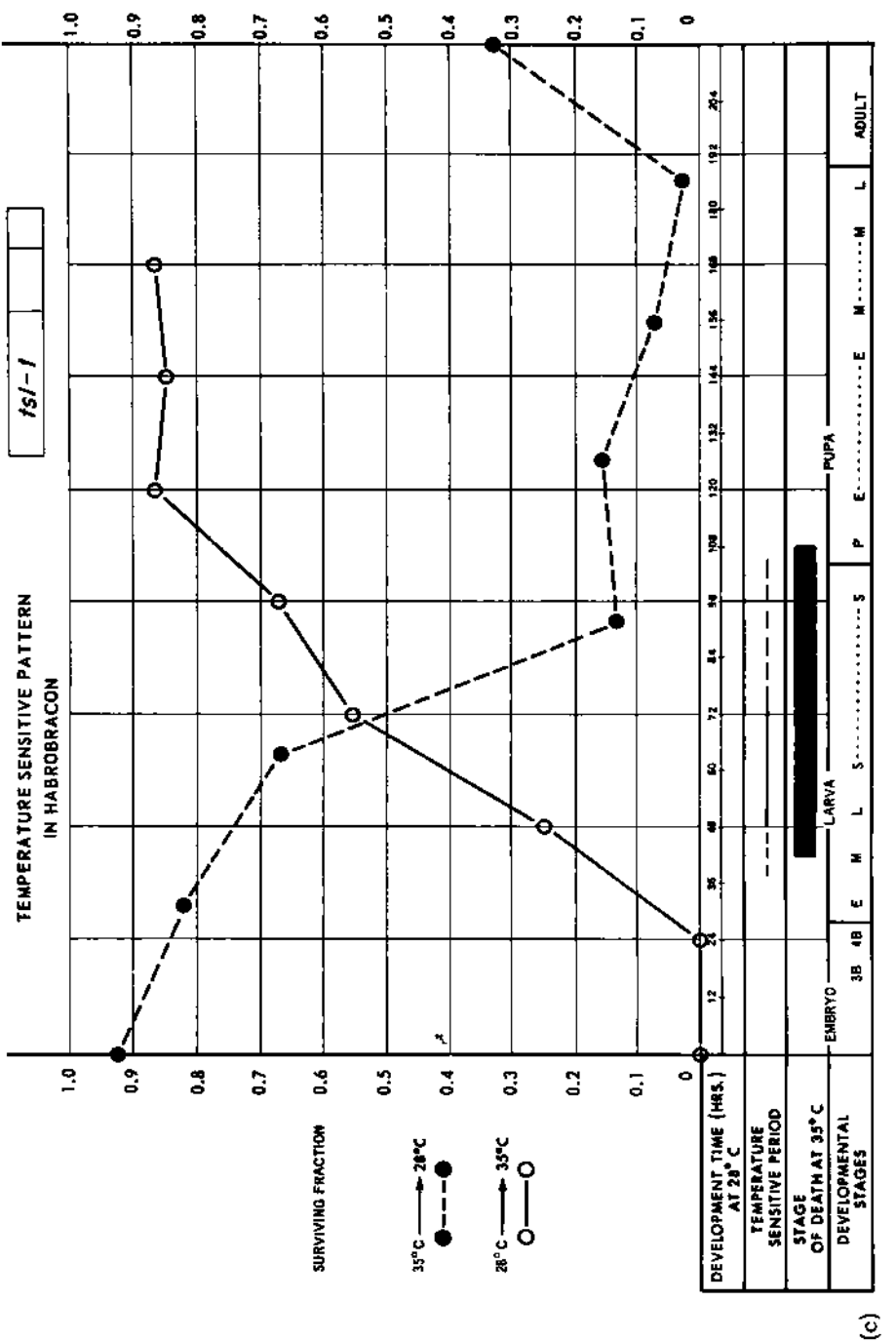
FIGS 1a, b, & c. The pattern of temperature sensitivity for three temperature-sensitive lethal mutants in Habrobracon serinopae (redrawn from Ref. [11]). Each point represents the adult survival after the developing wasp was transferred from one temperature to the other. The pattern of temperature sensitivity represents the time the gene product is necessary for survival to adulthood.

IAEA-SM-138/24


TEMPERATURE SENSITIVE PATTERN
IN HABROBRACON
ts/-6
SURVIVING FRACTION
35°C → 28°C
28°C → 35°C
DEVELOPMENT TIME (HRS.) AT 28° C
TEMPERATURE SENSITIVE PERIOD
STAGE OF DEATH AT 35°C
DEVELOPMENTAL STAGES
EMBRYO
LARVA
PUPA
ADULT
SB
48
E
M
L
S
P
E
M
L

(b)

FIG. 1. (cont.)


TEMPERATURE SENSITIVE PATTERN
IN HABROBRACON
tsf-1
SURVIVING FRACTION
35°C → 28°C
28°C → 35°C
DEVELOPMENT TIME (HRS.) AT 28° C
TEMPERATURE SENSITIVE PERIOD
STAGE OF DEATH AT 35° C
DEVELOPMENTAL STAGES
EMBRYO
LARVA
PUPA
ADULT

(c)

FIG. 1. (cont.)

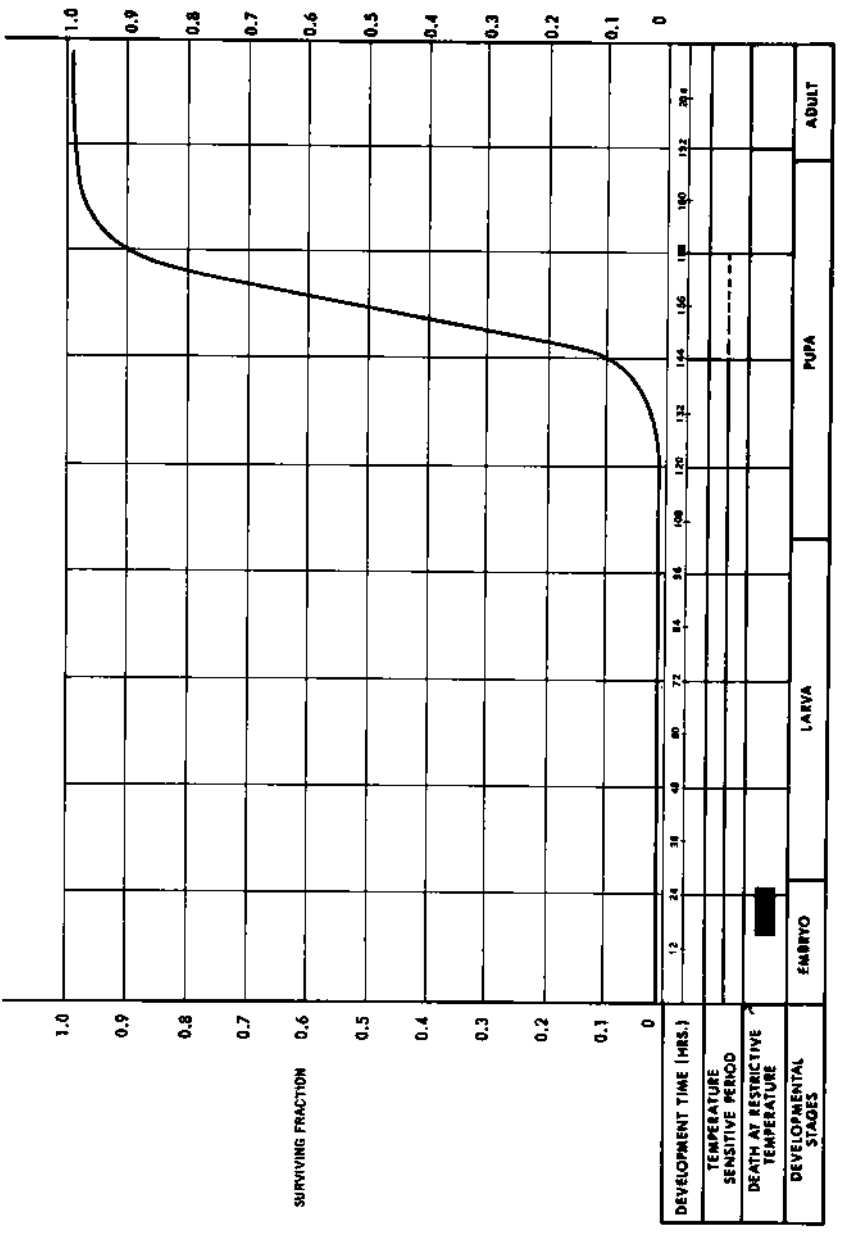


FIG. 2. Pattern of temperature sensitivity of a hypothetical tsl dominant mutant with a function which is indispensable from the time of oviposition until mid-pupal stage. The pattern of temperature sensitivity is determined as in Fig. 1.

These are just a few examples of several temperature-sensitive mutations found in *Habrobracon serinopae*. The pattern of gene action and the time of death have been unique for each mutant so far examined. Many show pleiotropic effects, such as male or female sterility and morphological variations (body color, wing structure). At least one shows evidence of dosage compensation, in that the haploid is completely lethal at the restrictive temperature, but a low frequency of diploids survive to adulthood, and some die at later stages of development than the haploid individuals. Some of the mutants can be maintained heterozygous but are lethal at both the restrictive and permissive temperatures when the female is homozygous for the mutant gene.

Noticeably, I have avoided stating what the products of the genes are in which we have isolated *ts* mutants. The reason is that I do not know. I have many ideas, but I cannot say which is the right one. It is our goal to understand better the developmental processes in a higher organism by the use of conditional lethal mutations, or what might be called the "genetic dissection of development." The important point for our discussion here is that it is possible to isolate and characterize mutants with no inkling of the underlying physiology. Among the many kinds of mutants which one finds in this way, some will hopefully be useful for purposes of insect control. Let us examine some possibilities.

A dominant "hot-sensitive" mutant where the gene functions throughout the life cycle, except as an adult, could be very effective in insect control. The mutant would be raised in the laboratory under permissive conditions and then released into nature to interbreed with the natural pests. When the eggs were laid from such crosses, the eggs, larvae, and pupae would die when exposed to the natural restrictive temperatures. Of course, the restrictive temperatures as tested in the laboratory should be determined by the minimum average day-time temperature of that region during the critical season. An ideal theoretical temperature-sensitivity pattern for such a mutation is shown in Figure 2.

A dominant "cold-sensitive" mutation would work in the same manner, except that it would be effective in regions where temperatures drop to low levels at night in the spring and, possibly, early summer. The idea would be to kill the eggs and small larvae before they got a chance to feed. Also, it would be very effective for control of insects which have to overwinter as larvae or pupae. The critical phase for a mutant which had to overwinter would be just before, or after, diapause. In fact, a cold-sensitive mutant would be an example of a non-diapausing mutation.

A third type of temperature-dependent mutant would be a hot- or cold-sensitive sterility mutant. Raised in the laboratory at the permissive temperature this mutant would be fertile. However, if the adult were placed at a restrictive higher temperature in the laboratory or released when its gonial cells were starting to develop and/or differentiate, it would be rendered sterile. Matings with animals in nature would yield no offspring.

Other Lethal Mutations of Potential Use

Temperature-sensitive lethal mutations are a class of "generalized" conditional lethal mutations, in that such mutations can be induced in most indispensable genes. There exists at least one other class of generalized conditional lethals, such as the genetically suppressible mutants found mainly in microorganisms. However, these have not been studied in insects in great enough detail for us to predict their usefulness in insect control. The concept of conditional lethality, however, includes more specialized types of mutations. In these cases, the specific functions of the

genes involved are important. Each specific type of mutant sought will require a special isolation and selection technique. With no pretense of being exhaustive, I shall discuss here a few types which come to mind.

Non-diapausing mutants. – Klassen *et al.* [12] have discussed the use of non-diapausing mutations in insects. Essentially, the idea is to use strains of insects, such as the boll weevil, from regions where they do not undergo diapause, and to allow those strains to interbreed with natural populations that must diapause to overwinter. Of course it is necessary that diapausing be recessive.

Non-feeding mutants. – Mutations which affect the feeding habits of insects in nature would be very useful in insect control. Mutants with defective mouthparts that would feed on artificial diets but are unable to feed on their natural food source would be one type. Another type would be behavioral mutants which would not or could not seek out and feed on their natural food source.

A mutant type which would change its diet to an economically unimportant energy source, or even feed on another pest, is another possibility. Recessive genes could be used to change the population to a new food source by combining this technique with the sterile-male method or one of the conditional dominant lethal genes. The dominant lethal genes would eliminate the insects' attacking the economically important crop while their feeding on the alternate food source would be less affected. Repeated treatments would shift the recessive gene frequency drastically towards feeding entirely on the alternate food source until finally only the new population (ecospecies) existed.

Behavioral traits are inherited in all animals, and mutations have been induced which alter behavior in insects [see 13, 14]. It should be possible to induce and use mutations of the type mentioned above for control of many, if not most, species of insect pests.

Cocoonless mutants. – In our laboratory a mutation was induced with EMS in the parasitic wasp, *Habrobracon serinopae*, which was unable to spin a functional cocoon [11]. The silk was made by the larva but it then pupated on the surface of the randomly spun mat of silk. Only under special conditions of temperature (28°C) and relative humidity (50–60%) did the individuals survive to adulthood.

What would happen to an insect in nature if it could not construct a functional cocoon or produce the necessary silk? Its exposure to extreme temperature and humidity fluctuations, soaking by rain or snow, and subjection to easy predation by natural enemies would drastically decrease its chances of survival to adulthood.

Multiple conditional recessive lethal mutations. – Genomes could be constructed containing several conditional recessive lethal mutations which would be expressed at different times during development. These mutations could be used in conjunction with genetic methods, such as meiotic drive [15] and translocations [16] to increase the frequency of the conditional mutations before their critical phases were expressed, so that the population would be drastically reduced several generations after the initial introduction of the genes.

Schemes for Selecting Useful Mutations

Much research has gone into the biology (life cycle, physiology, behavior, genetics, ecology) of many insect pests. We shall take as our example the codling moth, *Carpocapsa pomonella*, for a concrete possibility of selecting induced conditional lethals. This insect is an internationally

important pest to pomes [17, 18] and artificial diets are available which do not require its natural food source as an ingredient [19, 20]. Undoubtedly many other pests such as the sugarcane borer, *Diatraea saccharalis* and the olive fly, *Dacus oleae* fulfill these criteria as well.

Mutation and selection of non-feeding mutants. – Either one or both parents of the codling moth are treated with a chemical mutagen or by radiation. EMS, or some other potent mutagen, is fed or administered as an aerosol [21] or an aerosol under reduced pressure [22]. In *Drosophila* [6] and *Habrobracon* [23], feeding of EMS yields a high frequency of point mutations in the F_1 generation with low frequencies of dominant lethality in sperm. A dose-action curve for dominant lethality in sperm and eggs (F_1) should be determined in the codling moth and then compared to second generation mutation frequency (F_2) for temperature-sensitive dominant and recessive lethal mutations. Based on the experiments in which EMS was administered to *Drosophila* and *Habrobracon*, one would expect a high yield of second generation point mutations without a high yield of dominant lethal mutations (chromosomal aberrations).

When the dose of a mutagen is determined for the effective induction of point mutations, the eggs of the treated parents are placed on the artificial diet. In, or close to, the artificial diet an apple or some other suitable pome is placed which contains a lethal dose of insecticide, or preferably, a more lethal poison which does not permit insecticide-resistant mutants to arise. Only those individuals which *do not* seek out and eat the pome will survive. In this way, several thousands or even millions of eggs can be screened for "non-feeding" mutations. Larvae of the codling moth are known to prefer the natural food source and will seek it out when given a choice between the artificial diet and a pome fruit [24].

The survivors can then be progeny-tested and selected again under the same conditions to determine the "true" mutants from the "lucky" escapees.

With a slight modification, this technique can be used to give the newly hatched larvae a choice between the natural food source containing the insecticide and an economically unimportant food source. Again the individuals selecting the alternative food source are progeny-tested.

When a mutation is found, it must be made homozygous and compared to the wild type for competitiveness, and experimental cage studies must be made. Successfully tested mutant lines can be maintained in small cultures in the laboratory; large populations need be built up only when they are to be used in the field. The kinetics of population depression should be similar to those described by Klassen *et al.* [12]. Of course, male and female codling moths will not have to be separated, since both sexes will be homozygous for the trait. The appeal of selection of these mutants is that they will in no way endanger the crop when released in vast numbers.

Selection of temperature-sensitive mutants. – Dominant temperature-sensitive mutations will require progeny-testing of each zygote from the treated parents. If genetic variants are known for an insect, the screening procedure for temperature-sensitive lethal mutations is simple. If genetic variants are not available for an insect pest such as *Carpocapsa* then visible mutations (eye or body color, isozymes, etc.) can be induced or obtained from nature as a first step to screening for the mutations which can be used in insect control. Of course, mutations which are sex-linked and sex-limited will be the easiest to obtain for those insects where one sex is hemizygous for sex chromosomes. Once these mutations are isolated on the sex chromosome, then the temperature-sensitive mutations can be detected.

Figure 3 illustrates a method by which sex-linked dominant or recessive temperature-sensitive lethal mutations can be obtained. This method would require one, and preferably two, sex-linked visible marker genes. For example, assume that two sex-linked markers, *r* and *s* are known for

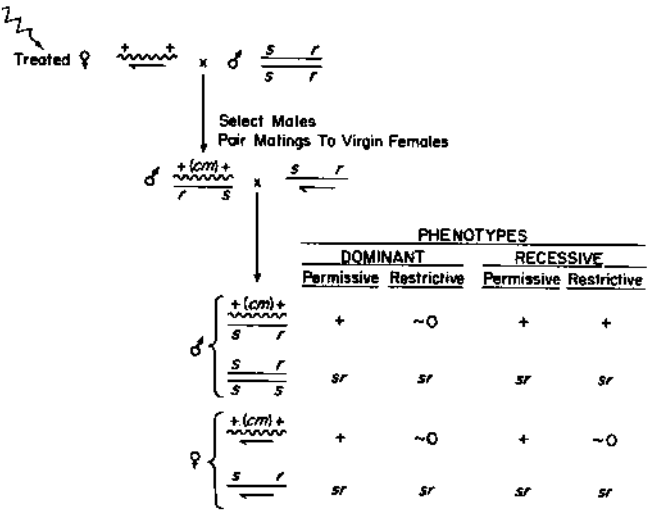


FIG. 3. The induction of sex-linked conditional lethal mutations. The permissive condition is the optimal temperature or a particular set of conditions within the laboratory. The restrictive conditions are high or low temperatures or some set of conditions expected in nature. For simplification this example assumes no genetic crossing over between the sex-linked markers, r and s. The objective would be to look for pair matings which produce very few or no wild-type progeny under restrictive conditions. These progeny would be carrying the conditional mutation (cm) induced by a mutagen.

Carpocapsa. The hemizygous, wild-type female parent is treated with a chemical mutagen and then mated to an untreated male homozygous for the two sex-linked markers. The resulting male progeny are pair-mated to virgin females hemizygous for *r* and *s*. The progeny of this cross are then exposed to high and low temperatures. If there is an absence of wild-type males and females at the high but *not* at the low temperature, then a dominant conditional lethal mutation has been induced in the sex chromosome. An absence of only wild-type females indicates that a conditional recessive lethal mutation has occurred on the sex chromosome. By back-crossing the wild-type animals and progeny-testing again at both high and low temperatures, the mutant type can be made homozygous.

Female progeny from the same cross can be mated to wild-type males and tested in a similar manner to detect sex-limited conditional lethal mutations (Figure 4). These types might be of limited value, however, because only females would be killed, while males would be unaffected. On the other hand, it would be possible to rear the female progeny at high and low temperatures to determine whether or not they carried a mutation for sterility. If they did, they could be exposed to high temperature in the laboratory before they were released into the field. The high temperature would render the females sterile, so mating with wild-type males in nature would produce no progeny.

Temperature-sensitive lethals which are found independent of the sex ratio are autosomal in origin and are dominant. Of course, without markers these would be hard to detect. Tedious F_2 adult/egg viability studies would have to be made from each cross, following the treatment of either or both parents. Therefore, a genome which contained more genetic variants would be more versatile in screening for temperature-sensitive mutations.

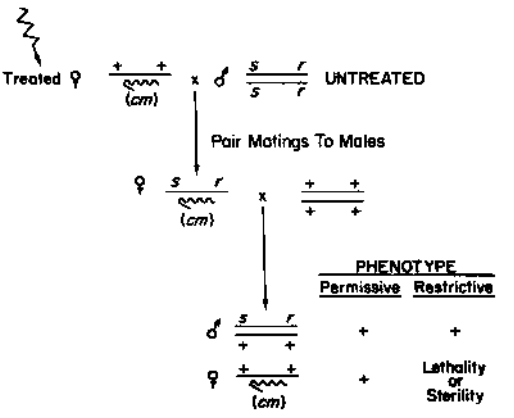


FIG.4. The induction of sex-limited conditional mutations in females. See Fig.3 for the explanation of the symbols.

In all cases, the time and stage of death and the time of gene action should be determined for each temperature-sensitive mutation. Female sterility, death during embryogenesis, or death shortly after hatching would be ideal. The time of gene action, then, would be early in development. For a margin of safety, the gene preferably should remain nonfunctional throughout the larval and pupal state, in case some individuals escaped the critical temperature during an earlier stage (Figure 2).

In the above discussion, the term "temperature-sensitive" has been used in its conventional sense meaning "hot-sensitive." The same procedures would apply for selecting "cold-sensitive" mutants.

General Discussion

Knipling *et al.* [25] and Klassen *et al.* [12] recognized the potential of conditional dominant lethal mutations in the control of insect populations when they proposed the use of non-diapausing mutant types introduced into local populations where diapause is necessary for overwintering. The potential of conditional mutations for the control of any insect population may be realized by the induction of mutations that cause death under natural conditions but allow development to adulthood in the laboratory. The ideal conditional lethal should be dominant, and should be expressed at some stage before the insect feeds.

The introduction of a geographically isolated strain into a new locality could have some undesirable effects. Mating between the two strains might not be random. If the populations had been isolated for a period of time long enough to have evolved a different mechanism for diapause, some degree of mating behavior might also have changed. The two strains would probably contribute a great amount of genetic variability into the local population. A few escapees with some resistance to the non-diapausing traits could evolve into resistant forms. In other words, by introducing new genetic variability, the rate of natural evolution of this *new* local strain might be increased. The escapees could be heterotic in many respects, or they might be naturally selected

for several traits such as fecundity, mobility, and mating proficiency. On the other hand, conditional mutations induced by mutagens and used in the local populations would contribute only a limited amount of new genetic variability.

In time, as with insecticide resistance, the natural populations would evolve resistance (modifying genes) to the conditional mutations. However, with a continuing program of inducing and isolating new conditional lethal mutations, a maintenance of several useful mutant strains should allow the program to keep up with, or ahead of, natural selection. As a natural population developed resistance to one conditional dominant lethal mutant, another mutant of a different allele or gene would be used in the next generation. Further, introduction of insects from the wild population on a continual basis into the breeding population in the laboratory would obviate the evolution of a new species that would not interbreed with the wild (natural) species.

Summary

Temperature-sensitive mutations have been induced and studied in *Habrobracon serinopae*. The mutants show a wide spectrum of variation in times of death and gene action. The same is true for *Drosophila*. There is no reason to believe that a similarly rich spectrum of mutations could not be isolated in any insect. I would like to recommend that the use of such mutations be seriously considered for the control of insect populations. In addition, many other types of conditional lethal mutations should be considered; I have tried to suggest only a few possibilities for a particular species. Modern techniques of producing point mutations with potent chemical mutagens, advances in artificial media for rearing insects, and relatively straightforward genetic analyses make such a program feasible.

ACKNOWLEDGMENTS

The author would like to thank Mrs. Margaret Yetté and Mrs. Julie Tindall for their excellent technical assistance in the laboratory, Dr. B. A. Butt of Entomology Research Division, USDA, Yakima, Washington, for the abundant information on the codling moth, and Drs. C. M. Steinberg and R. C. von Borstel for their helpful discussions and critical review of this manuscript.

REFERENCES

[1] HOROWITZ, N. H., Advan. Genet. *3* (1950) 33.
[2] EPSTEIN, R. H., BOLLE, A., STEINBERG, C. M., KELLENBERGER, E., BOY DE LA TOUR, E., CHEVALLEY, R., EDGAR, R. S., SUSMAN, N., DENHARDT, G. H., and LIELAUSIS, A. Cold Spring Harbor Symp. Quant. Biol. *28* (1963) 375.
[3] NISHINARA, M. and ROMIG, W. R. J. Bacteriol. *88* (1966) 1220.
[4] HARTWELL, L. H. J. Bacteriol. *93* (1967) 1662.
[5] WHITING, P. W. Genetics *17* (1932) 1.
[6] SUZUKI, D. T., PITERNICK, L. K., HATASHI, S., TARASOFF, M., BAILLIE, D., and ERASMUS, U. Proc. Natl. Acad. Sci., USA *57* (1967) 907.
[7] BAILLIE, D., SUZUKI, D. T., and TARASOFF, M. Can. J. Genet. Cytol. *10* (1968) 412.
[8] SUZUKI, D. T., and PROCUNIER, D. Proc. Natl. Acad. Sci., USA *62* (1969) 369.
[9] SMITH, R. H. Genetics *60* (1968) 227.
[10] SMITH, R. H. Mutation Res. *7* (1969) 231.

[11] SMITH, R. H. (in press) 1970.
[12] KLASSEN, W., KNIPLING, E. F., and MCGUIRE, JR., J. U. Ann. Entomol. Soc. Amer. *62* (1970) 238.
[13] WHITING, A. R. Adv. Genet. *10* (1961) 295.
[14] LINDSLEY, D. L., and GRELL, E. H. Genetic Variations of *Drosophila melanogaster*. Carnegie Institute of Washington Publication No. 627 (1967).
[15] VON BORSTEL, R. C., and BUZZATI-TRAVERSO, *in* Proc. Colloquium on Radioisotopes and Radiations in Entomology, Vienna (1962) 273.
[16] WHITTEN, M. J. (in press) 1970.
[17] TADIC, M. The Biology of the Codling Moth. Published for the Department of Agriculture and the National Science Foundation, Washington, D.C. by the NOLIT Publishing House, Terazije 27/II, Belgrade, Yugoslavia (1963).
[18] PUTMAN, W. L. Proc. Entomol. Soc. Ontario *93* (1963) 22.
[19] BRINTON, F. E., PROVERBS, M. D., and CARTY, B. E. Canad. Entomol. *101* (1969) 577.
[20] PROVERBS, M. D., and LOGAN, D. M. Canad. Entomol. *102* (1970) 42.
[21] LÖBBECKE, E.-A., and VON BORSTEL, R. C. Genetics *47* (1962) 853.
[22] SEGA, G. A., and LEE, W. R. Drosophila Inf. Serv. *45* (1970) 179.
[23] SMITH, R. H., unpublished.
[24] BUTT, B. A. (personal communication).
[25] KNIPLING, E. F., LAVEN, W., CRAIG, G. B., PAL, R., KITZMILLER, J. B., SMITH, C. N., and BROWN, A. W. A. Bull. Wld. Hlth. Org. *38* (1968) 421.

INSECT RADIOBIOLOGY

(Session VIII)

Chairman

J. ITARD (France)

USE OF NITROGEN DURING IRRADIATION TO IMPROVE COMPETITIVENESS IN STERILE MALES OF Rhodnius prolixus

W.F. BALDWIN, G.D. CHANT
Chalk River Nuclear Laboratories,
Atomic Energy of Canada Limited,
Chalk River, Ont., Canada

Abstract

USE OF NITROGEN DURING IRRADIATION TO IMPROVE COMPETITIVENESS IN STERILE MALES OF Rhodnius prolixus.

The control of insects by the sterile-male method requires a high level of sterility in males; also, these males must be able to compete successfully with untreated individuals. Radiation exposures sufficient to produce sterility tend to reduce mating frequency and life-span in irradiated males. A more favourable balance between these two effects has been obtained by irradiating males of Rhodnius prolixus in nitrogen. In groups treated at three exposure levels (5, 10 and 15 kR) in air, the two higher exposures killed the insects or interfered with mating within the first 7 weeks. Conversely, the insects exposed in nitrogen at these doses survived more than 15 weeks, and demonstrated continued mating activity over this period. Tests of competitiveness between irradiated and unirradiated groups, in which different ratios of sterile/normal males were tested with females, indicated that anoxia during irradiation produced a substantial improvement in competitiveness for a given degree of sterility. The results showed that the sterile insects could successfully lower egg hatch to less than 20% over two months at 9 : 1 ratios and to less than 50% over the same period at 7 : 3 ratios of sterile to fertile males.

INTRODUCTION

In the application of the "sterile male" method of insect control, sterilizing exposures of ionizing radiation usually result in a reduction in survival and mating activity of treated males. Thus the effectiveness of irradiated males in controlling the growth of populations soon disappears, and the proportion of viable eggs producing young returns to normal. In Lucilia, Donnelly [1964] has stated that irradiated sterile males could mate only once or twice, whereas normal males average at least six and sometimes twelve copulations. In studies with Rhodnius, Baldwin and Shaver [1962] have shown that adult males exposed to 17,500 R died by the end of 9 weeks, and that mating activities declined during the third and fourth weeks, ceasing altogether in the second month. Also, Gomez et al. [1964] found that longevity and mating in Rhodnius were seriously affected by sterilizing exposures of γ and X-ray radiation.

The results presented in this paper deal with protection afforded by anoxia during irradiation of males, i. e. in terms of survival and mating ability. Data on the sterilizing effect of radiation at three different dose levels in both air and nitrogen are presented as well as the results of population tests in which different ratios of males irradiated in nitrogen and normal individuals were tested with a constant number of normal female insects.

METHODS

Adult Rhodnius males from a large laboratory culture were irradiated in air and in nitrogen. The insects were placed in a small plastic cylinder through which nitrogen gas or air could be circulated and rotated near a cobalt source. The nitrogen-irradiated males were held in the gas (3 liters/min.) for 10 minutes before and during the radiation exposure. In the nitrogen atmosphere, the insects immediately became unconscious and immobile on the floor of the cylinder. They recovered, however, in a very short time after the chambers were opened to the air. The irradiations were done with a 5000 Ci source at an exposure rate of 628R/min. Following this treatment the males were offered a blood meal [Baldwin and Shaver, 1962] and were mated to females. The feeding and mating to new females were repeated at intervals (see Table 1), and the mating frequency, the number of eggs deposited and the number of eggs hatching were recorded. The frequency of mating data was obtained by direct observation of single pairs. In these tests, the same males were used throughout; new females were mated to these males at the time intervals explained above. Mating usually occurred within half an hour after the females were placed in vials with the test males; after mating, the females were held separately in oviposition jars.

The effect of sterile males on small laboratory populations was tested by placing different ratios of irradiated/fertile males (total of 10) with 10 normal females in large glass jars with blotting paper for egg deposition. The hatch of eggs deposited during successive months for a 4-month period was recorded (see Fig. 1). Females were replaced at the end of each month. The radiation exposure employed in the population studies was 15 kR.

All experimental material was held in a rearing room at 23°C (70% R. H.). Full details of the rearing and maintenance of Rhodnius have been presented in a prior publication [Baldwin and Shaver, 1962].

RESULTS

Irradiated adult males

The first experiment was designed to test the protective effects of anoxia during sterilizing exposures of radiation. Different groups of 5 males each were irradiated at 5, 10 and 15 kR in air and nitrogen; unirradiated males were mated to females as controls (Table 1). Records of eclosion of eggs from different groups of females paired at intervals with the irradiated males showed that at the lower exposure (5 kR), the irradiation did not interfere with mating in either air or nitrogen (Table 2), but did produce a reduction in the egg hatch with both treatments (Table 1). Although egg hatch was depressed in each case to less than 50 percent, there was no obvious difference in the results. Higher doses in air which produced sterility did interfere with mating, a situation which was not found with nitrogen treatment during irradiation (Table 1). For instance, at 10 kR, complete sterility occurred within 7 weeks in males irradiated in

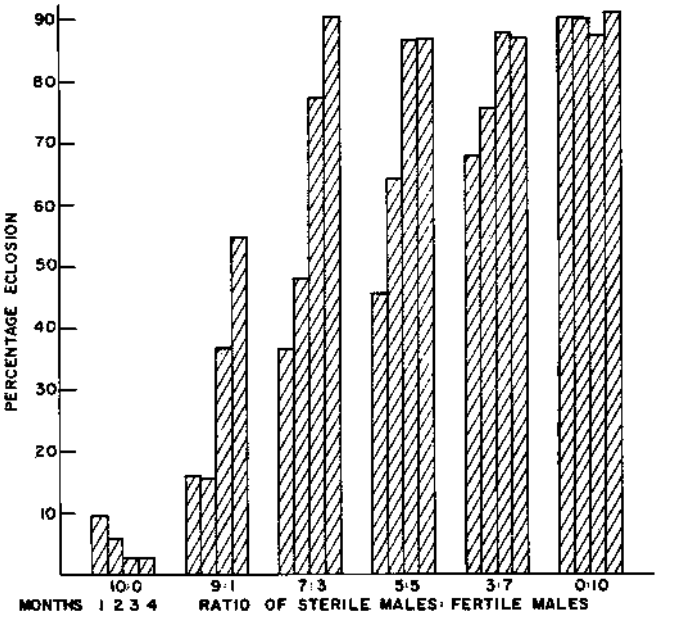


FIG. 1. Monthly hatch of eggs from groups of 10 females confined with different ratios of sterile : fertile males.

TABLE 1

Eclosion of eggs from females mated under observation at intervals to males irradiated in air and nitrogen at three different exposure levels.

Matings*	5,000 R				10,000 R				15,000 R				Controls	
	Air		N_2		Air		N_2		Air		N_2			
	Eggs	% Hatch	Eggs	% Hatch	Eggs	% Hatch	Eggs	% Hatch	Eggs	% Hatch	Eggs	% Hatch	Eggs	% Hatch
0 weeks	124	17	132	31	103	3	83	16	65	0	116	0.8	169	59
2 weeks	99	30	123	48	131	18	118	28	43	0	64	0	124	70
7 weeks	170	42	98	24	44	0	126	17	-		58	0	176	65
10 weeks	111	40	64	31	**		109	6	-		21	0	136	46
15 weeks	88	28	87	14	-		70	9	-		46	0	18	50

* Although five normal females were placed with treated males at each different date, matings did not occur in some cases. (See Table 2).

** Dash indicates that mating did not take place.

air; however, mating activity among the males ceased at 7 weeks following this treatment. In nitrogen, the 10 kR exposure reduced hatching to less than 10 percent after 7 weeks, while mating activity continued over the whole period. At the highest exposure (15 kR), complete sterility in males irradiated in air was found at the first mating, but here again, mating was seriously affected, and none of these males attempted copulation after the second week. Nitrogen treatment at this dose produced males which were actively mating over the whole 15 week period. These individuals were almost completely sterile, only the 0 week group producing a hatch of 0.8 percent.

TABLE 2

Frequency of observed matings among 5 males irradiated at 3 different exposures in air and nitrogen.

Mating	Control	5,000 R		10,000 R		15,000 R	
		Air	N_2	Air	N_2	Air	N_2
0 weeks	5	5	5	5	5	5	5
2 weeks	5	5	5	5	5	3	5
7 weeks	5	5	4	3	4	None (1 dead)	4
10 weeks	5	5	3	None	4	None (3 dead)	3
15 weeks	5	5	4	None	4	None (5 dead)	3

In Table 2, the records of the number of matings among males from the above experiment confirms that nitrogen during exposure not only extends the period during which the males will mate, but shows, at the highest dose, that high mortality among insects irradiated in air did not occur with N_2, at least during the experimental period. As shown in Table 2, a few individuals irradiated in nitrogen at each dose failed to mate after seven weeks; this might be a combined effect of advancing age and radiation effect.

Population Study

In previous tests of the effects of sterile males on laboratory populations, fertility levels returned to normal after a month, presumably because of inhibition of mating activity prior to early death in the irradiated males [Baldwin and Shaver, 1962]. These insects were irradiated in air (at 17.5 kR) and did not survive beyond 9 weeks. In the present experiment, groups composed of different ratios of males irradiated in nitrogen (at 15 kR) and untreated males (10:0, 9:1, 7:3, 5:5, 3:7 and 0:10) were held with a constant number (10) of normal females in glass containers for 4 months. The insects were fed on rabbit blood and the females in each jar were replaced each month. The percentage hatch of eggs from each group was recorded at monthly intervals (Fig. 1).

With the first group (ratio - 10:0) in which only nitrogen irradiated males were present, egg hatch was reduced from 9 percent in the first month to 3 percent in the 3rd and 4th months. Although the presence of one untreated male in the 9:1 ratio group produced a considerable rise in hatching rate, egg eclosion did not return to normal levels over all 4 months, indicating that the treated males were actively mating over this whole period. Again, at the ratio of 7:3, the effects of the nitrogen irradiated males were evident over a three-month period, although the activity of the 3 normal individuals caused fertility to rise rapidly. The effect of sterility was visible for two months at the 5:5 and 3:7 groups; however, the normal males in these latter groups soon obliterated the effects of the sterile insects.

DISCUSSION

These studies on radiation-induced sterility in Rhodnius show that irradiation in nitrogen will produce sterility while affording considerable protection from the deleterious effects of gamma exposures on mating activity and survival. The protection afforded by anoxia is well illustrated in the tests of sterilizing exposures, where the two highest exposures (10 and 15 kR) in nitrogen produced males which mated over the whole period of 15 weeks, while in air, mating ceased at an early date and at 15 kR, the males were dying at 7 weeks. At 5 kR, it was interesting that both air and nitrogen irradiation at this comparatively low exposure depressed fertility to comparable levels in both cases, with no indication of impairment of survival or mating ability.

In the present work, it has been shown in laboratory populations that sterility among males will inhibit egg eclosion when high ratios of sterile: fertile males are placed with normal female insects. The males, irradiated in nitrogen, proved to be effective in some cases for long periods of time, and at ratios of 9:1 and 7:3 of irradiated vs untreated males, egg hatch was depressed for 4 and 3 months respectively. This means that the irradiated were competing with untreated males, showing that irradiated individuals continued mating over extended periods of time. In prior studies, the effects of males irradiated in air disappeared after a month [Baldwin and Shaver, 1962] and although a slightly higher radiation exposure was employed (17.5 kR), it is probable that nitrogen treatment during irradiation would have extended the period when these males would compete with normal males.

In Rhodnius, females mate repeatedly, and because of this factor even a single fertile male in a group can eventually mate with most of the females and successfully compete with a large group of irradiated insects. Thus we have not been able to predict, through laboratory studies, that the "sterile male" method of control could have practical application in the control of this insect. The method of irradiating the males under anoxic conditions substantially improved the survival and mating ability of sterilized individuals, results which will undoubtedly have application in the "sterile male" method of control in other species of insects.

ACKNOWLEDGEMENTS

The authors wish to acknowledge the technical assistance of Mr. A. Knight.

REFERENCES

BALDWIN, W.F., SHAVER, E.L., 1962. Radiation-induced sterility in the insect Rhodnius prolixus. Can. J. Zool. 41, pp.637-648.

DONNELLY, J., 1964. Possible causes of failure in a field test of the "sterile males" method of control, Proc. 12th Int. Congr. Entomology, London, July 1964, pp.253-254.

GOMEZ-NUÑEZ, J.C., GROSS, A., MUCKADO, C. 1964. Las radiaciones gamma y el comportamiento reproductivo del Rhodnius prolixus macho, Acta cient. venez. 15 (3), pp.97-104.

DISCUSSION

J. ITARD (Chairman): Could Mr. Baldwin tell us the age at which Rhodnius prolixus was irradiated in his experiments?

W.F. BALDWIN: We irradiated the adults soon after they had changed from the fifth to the sixth nymphal instar (within 2-4 days). The irradiation was immediately followed by the first blood meal.

R.U. CARCAVALLO: I should like to know how long, in your experience, the insects could be kept in nitrogen without risk of killing them.

W.F. BALDWIN: We found that 10 minutes was sufficient time to give a maximum of protection. I have kept Rhodnius in nitrogen for up to half an hour, however, without any apparent deleterious effects. I have also kept Dahlbominus, a small wasp, in nitrogen for up to 2 hours.

R.C. BUSHLAND: In our experience the anoxia does not allow us to achieve sterility while avoiding radiation damage. At Fargo several years ago, Flint and Riemann attempted to prevent gut damage to the boll weevil by irradiating it in an atmosphere of nitrogen. The gut was protected, but so were the gonads, so that what had been a sterilizing dose in air was ineffective in nitrogen. Raising of the dose to the sterilizing level in nitrogen also damaged the midgut. North and Holt of our laboratory made similar observations in the case of Heliothis virescens.

W.F. BALDWIN: These experiments of mine certainly show that we obtained sterility with decreased somatic damage by irradiation under anoxic conditions. Also, in work in Canada on the codling moth, Proverbs has reported that he irradiates adults at 25 or 30 krad in nitrogen, with good results.

J. THEUNISSEN: Did you observe any differences in the radiation effects on the gonads of insects treated in air and in nitrogen?

W.F. BALDWIN: As I remember, insects irradiated at a dose of 15 krad in air did show damage, especially females 3 - 4 weeks of age. In nitrogen this damage was not evident.

G. HOOPER: With respect to the interpretation of your Fig.1, is the first mating or the last mating of major importance in the case Rhodnius in determining the egg hatch, or is there sperm mixing? In

other words, what is the effect on the egg hatch of mating females alternately with irradiated and untreated males?

W.F. BALDWIN: In tests with Rhodnius entailing alternate mating of females with sterile and fertile males, we found that even after five or six matings with sterile individuals a mating with a fertile male raised the percentage of eclosion substantially. Mr. Gómez-Núñez can probably tell you more about this aspect than I, however.

J.C. GÓMEZ-NÚÑEZ: We have noticed that when the sequence is irradiated-normal-irradiated male, eclosion increases and continues to increase. When the sequence is normal-irradiated-normal male, eclosion decreases and continues to decrease. These effects vary according to the dose applied and the time between matings. The mechanisms involved are being studied at present.

C.T. LEWIS: Is the mechanism by which nitrogen, or oxygen deficiency, provides protection from radiation damage fully understood yet?

W.F. BALDWIN: It is well understood that anoxic conditions do protect organisms from radiation damage; the mechanism is in some way connected with the low metabolic rate in the absence of oxygen.

C.T. LEWIS: I believe you exposed insects to nitrogen for 10 minutes before irradiating them. Have you any information on the minimum period of nitrogen pretreatment which is required before insects exhibit resistance to radiation damage? That might provide some indication of the rate at which nitrogen displaces oxygen from cells.

W.F. BALDWIN: In studies of somatic damage after irradiation of epidermal cells of Rhodnius prolixus, I found that maximum protection was afforded by an exposure to nitrogen of 10 minutes. In fact, we adopted the period of 10 minutes because it gave a good effect.

M.E. TZANAKAKIS: Was your criterion of effective mating insemination or just the act of mating?

W.F. BALDWIN: We took as our criterion of successful mating the period during which the male and female remained coupled. Previous experience had shown that after 15 minutes the female would certainly be inseminated; for these studies we judged matings to be successful after half an hour.

STOCHASTIC MODELS FOR EFFICIENT CONTROL OF INSECT POPULATIONS BY STERILE-INSECT RELEASE METHODS*

Ken-ichi KOJIMA
Department of Zoology,
University of Texas,
Austin, Texas, United States of America

Abstract

STOCHASTIC MODELS FOR EFFICIENT CONTROL OF INSECT POPULATIONS BY STERILE-MALE RELEASE METHODS.

Because of air and water pollution due to indiscriminant use of insecticides and herbicides, various alternative methods for controlling undesirable insects and plants have been proposed. The dangers of such chemical usage were amplified by Rachel Carson's famous book "Silent Spring" (1962).

The present paper aims to make efficiency comparisons of the sterile-insect release method among various modes of insect-mating systems on a theoretical model basis. This is one of a variety of biological control methods, such as an introduction of predators. However, essentially no quantitative evaluation was made for its effectiveness when pest insects have different mating characteristics such as monogamous vs. polygamous and the degree of released male competitiveness and fertility and that of female fecundity, etc.

The author, a population geneticist, considers that various facets have to be examined before adopting a particular sterile-male release system. The paper mentions four different mating possibilities: (1) male and female are both monogamous, (2) male is polygamous and female is monogamous, (3) male is monogamous and female is polygamous (rare case), and (4) male and female are both polygamous. In each model discussed, various fitness components are included to lower pest insect population size to a reasonable level. Since there exists a chance of invasion of a new population of a similar insect, a complete eradication of pest insects is not possible. In other words, a complete eradication is a point of unstable equilibrium in mathematical terminology.

Some cases analysed in this paper are fairly simple, and their analytical solutions can be obtained. However, most cases are complex, and numerical solutions are obtainable in table and graph forms by computing the values of complicated equations and simulating the systems on a computer (CDC 6600). Such tables and figures are included.

Introduction

The use of insecticides is one of the most effective methods to control pest insects. However, there is a serious drawback, namely environmental pollution by chemicals used. How such a pollution initiates a chain of imbalance among plant and animal species is well illustrated in Rachel Carson's famous book, Silent Spring (1962) [1]. Since then, various alternative means to control insect population have become a very important subject in various scientific communities. The active and capable investigators such as Berryman (1967) [2], Knipling (1968) [3], Proverbs (1969) [4], and Varley and Gradwell (1970) [5], have intensified their research activities in theories and experiments. There are a number of genetic and non-genetic approaches to control the size of insect populations.

* The financial support of AT-(40-1)-3681 and USPHS GM-15769 is acknowledged.

The objectives of this paper are to investigate the effectiveness of controlling population size by releasing sterile male, or sterile male-female insects with a few different modes of mating, by the method of stochastic computer simulations, and to observe their effectiveness and the change in genetic makeup when such control methods are used.

The Outline of Simulation Scheme and Parameters

A number of variable parameters and constant parameters must be defined to accommodate the simulation run without errors. The specification of these parameters has to be precise and definitive.

The environment is specified by the carrying-capacity, IK, and its variance, $(VK)^2$. The IK is a normal random variable with given mean IK and standard deviation VK. The IK used is always 400,000 individuals and the value of VK is 40,000, although IK and VK can be changed to any number.

The number of released sterile insects is a constant for a given set of simulations runs, and designated by NS. One-half of NS are males and the other half are females. However, NS stands for the total number of sterile males, when only males are released. Another biological parameter is the mating propensity of released males, C, which is a constant for a given set of runs, although it can be modified for different runs. The case of C = 1.0 represents the situation where sterile males released compete equally well in successful mating. However, the value of C is 0.9 in all cases in this paper. The parameters, NF and NM, stand for the numbers of wild females and males. At the initial generation, they are constant inputs, but they become stochastic numbers. The parameter, P, is "population expansion factor" after the size of a population in a particular generation is reduced by genetic and environmental pressures. The P is a normal variable with a given mean and variance, $(VP)^2$. The total number of insects before cutting the population by IK is designated by ISUM.

The genetic parameters are survival rate and fecundity-fertility combinations. The population for a given survival rate, S, is generated by a binomial distribution with eleven (11) classes, 0.0, 0.1, 0.2 --- 1.0. The population for a given fecundity-fertility is also generated by a binomial distribution with eleven (11) classes. All possible combinations of these genetic classifications will form an 11 x 11 checker board or matrix M (Figure 1). The all-wild females are put into these pigeonholes at the initial generation. Then, the individuals from each cell for the next generation are randomly generated according to the normal variate with mean = cell value and standard deviation = 0.10 x mean, where cell values are the product of survival rate and fecundity-fertility corresponding to row and column in M. An example of the genetic death, genetic fecundity-fertility and the use of P may be helpful at this point: suppose that 1,000 individuals are put in the cell with survival rate of 0.5 and fecundity-fertility value of 0.8 and P = 10. Then, the expected number of individuals contributed to the next generation is:

$$1{,}000 \times 0.5 \times 0.8 \times 10.0 = 4{,}000$$

The actual number will be distributed around this mean value. If the value of P generated happens to be 2.0 (recall P is a random variable) and the cell value (another random variable) deviated to + 0.05 from its mean 0.5 x 0.8, then the actual number of contributions becomes only 900 (1,000 x 0.45 x 2.0) from this cell. However, mass killing by M

```
Begin Experiment Number 5
     0     2    11    31    54    65    54    31    11     2     0
     2    25   116   310   543   651   543   310   116    25     2
    11   116   523  1396  2443  2392  2443  1396   523   116    11
    31   310  1396  3724  6517  7820  6517  3724  1396   310    31
    54   543  2443  6517 11405 13686 11405  6517  2443   543    54
    65   651  2932  7820 13686 16423 13686  7820  2932   651    65
    54   543  2443  6517 11405 13686 11405  6517  2443   543    54
    31   310  1396  3724  6517  7820  6517  3724  1396   310    31
    11   116   523  1396  2443  2932  2443  1396   523   116    11
     2    25   116   310   543   651   543   310   116    25     2
     0     2    11    31    54    65    54    31    11     2     0
Begin Generation Number 1   Population Expansion Factor  462.812   Maximum Population 396020
     Population at Capacity
        .004
     Total Population          135920
     0     0     0     0     0     0     0     0     0     0     0
     0     0     3    15    36    53    49    35    12     2     0
     0     3    32   136   327   417   486   378   143    34     2
     0    13   137   524  1394  2107  2151  1188   682   191    19
     0    38   243  1353  3047  3820  4875  3802  1659   438    69
     0    49   433  1530  4558  8106  7114  6406  2533   842   122
     0    56   530  2393  4642  6989  7806  5688  3232   879   177
     0    31   381  1369  3819  5917  5946  4290  2371   769   150
     0    13   148   665  1690  2524  2900  2555  1257   472    89
     0     3    33   165   509   816   905   730   504   167    37
     0     0     2    16    63   142   173   160    96    40     7
Row Sums
     0   205  1958  8406 19342 31693 32392 25043 12313  3869   699
Column Sums
     0   206  1942  8166 20085 30891 32403 25232 12489  3834   672
Begin Generation Number  2   Population Expansion Factor   454.426  Maximum Population      373833
```

FIG. 1. An example of matrix M. (Survival fecundity-fertility for wild females).

does not directly improve the genetic makeup of populations. On the contrary, the genetic makeup of fitness for pest insects may decline under this kind of selection.

Another facet of genetics built in the computer program is a genetic improvement of the pest populations from natural selection under the insect control system used. This part of the program has an option to improve the survival rate and fecundity-fertility by a random percentage, PE, (say 10%), which has a given mean. This maneuvering is done by picking out the specified random portion of individuals, PE, from each cell, and moving the corresponding number of individuals from one cell to another cell diagonally in the checker board (or matrix) mentioned earlier. When the survival rate ≈ 1.0 or fecundity = 1.0, the transfer is not in the diagonal direction, but only to the next higher survival cell or the next higher cell in fecundity, respectively. The main purpose of this operation is to improve the pest population by natural selection under the control mechanisms used.

All other parameters are control parameters; the number of generations to be run, IGEN, the number of replicates to run, IREP, and so forth. The computer used is a CDC 6600, which is a very fast computer. However, the simulation of one set of data (40 generations and 20 replications) took considerable central computer time, 3 sec. to 123 sec., per set of parameters, depending upon population size and complexity of mating scheme. Since there were more than 96 sets of parameter combinations, this paper will deal with: (1) male and female are both monogamous and only sterile males are released, (2) the same as (1) but sterile males and females were released. A very simplified flow chart is presented in Figure 2. Since the program is a long one, each set of parameters was put in without another "Do-loop" to include "Read New Parameters".

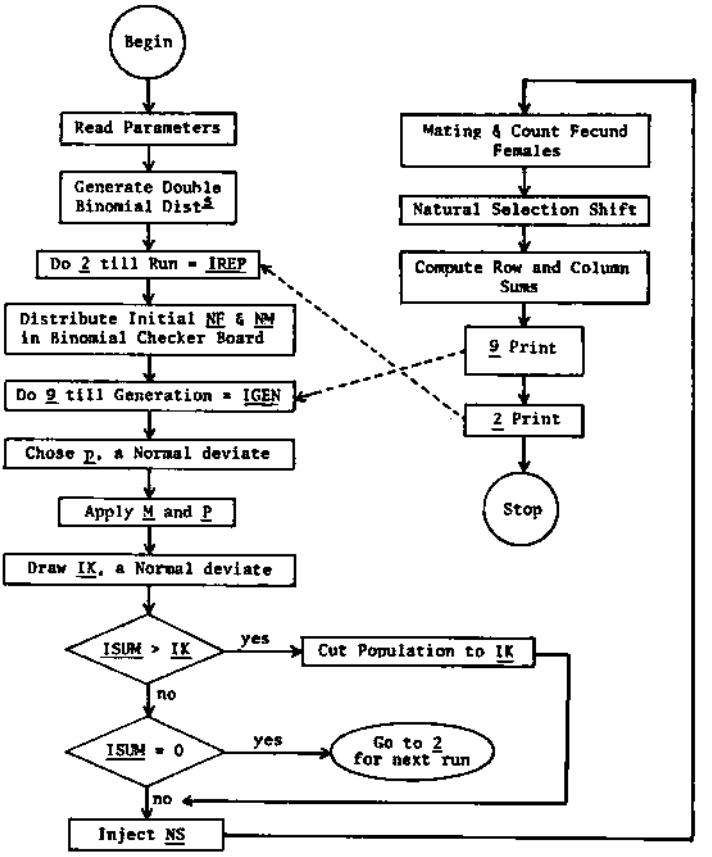


FIG. 2. Flow chart of the computer program.

In nature, there are many insects which have male-polygamy and female-monogamy. However, the results of this condition do not change very much from those of insects which are monogamous in both sexes. The reason is that there are many more males than females and there is no chance for the second mating for females. Consequently, the case of male-polygamy and female-monogamy, which is widely spread in nature, was not specially considered. This point was brought up by Proverbs (1969). There are a few other cases which are rather rare in insects. For example, the praying mantis, the males of which are eaten by copulating females during mating, in this case are "male monogamy and female polygamy". There are a number of insect species which are polygamous in both sexes. This last case is difficult to simulate by a computer program, because the program must trace individual females in their mating order with wild and sterilized males. Furthermore, the sexually active longevity must be incorporated in such a program.

Results and Discussions

Figure 3 represents the case of 200,000 sterile males released every generation up to 40 generations. This number of released males is one-half of the carrying capacity. The eight cases, each with 20

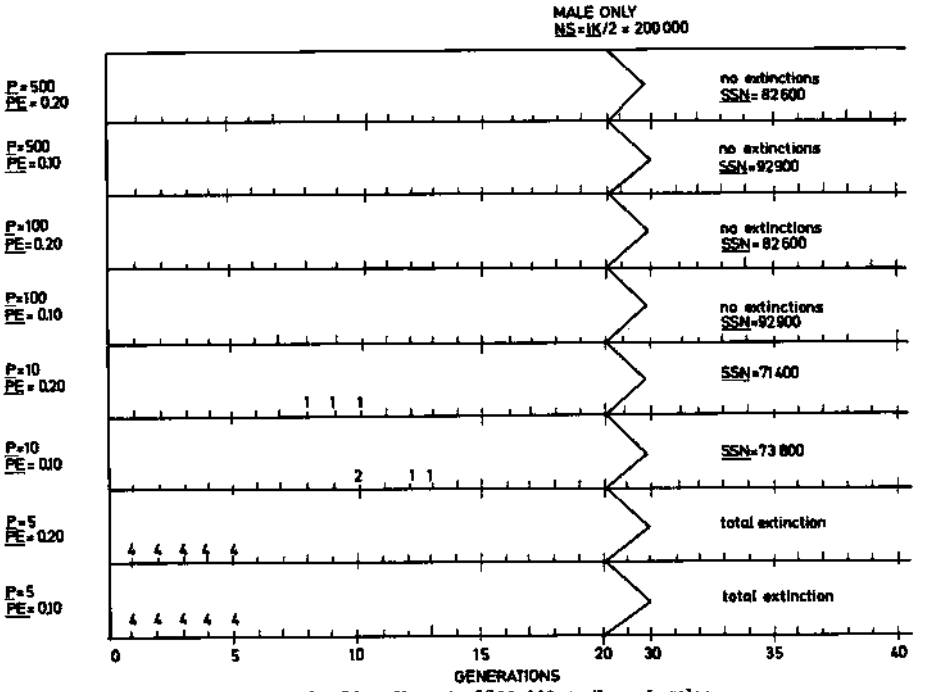


FIG. 3. Results (20 replicates) of 200, 000 sterile male release.

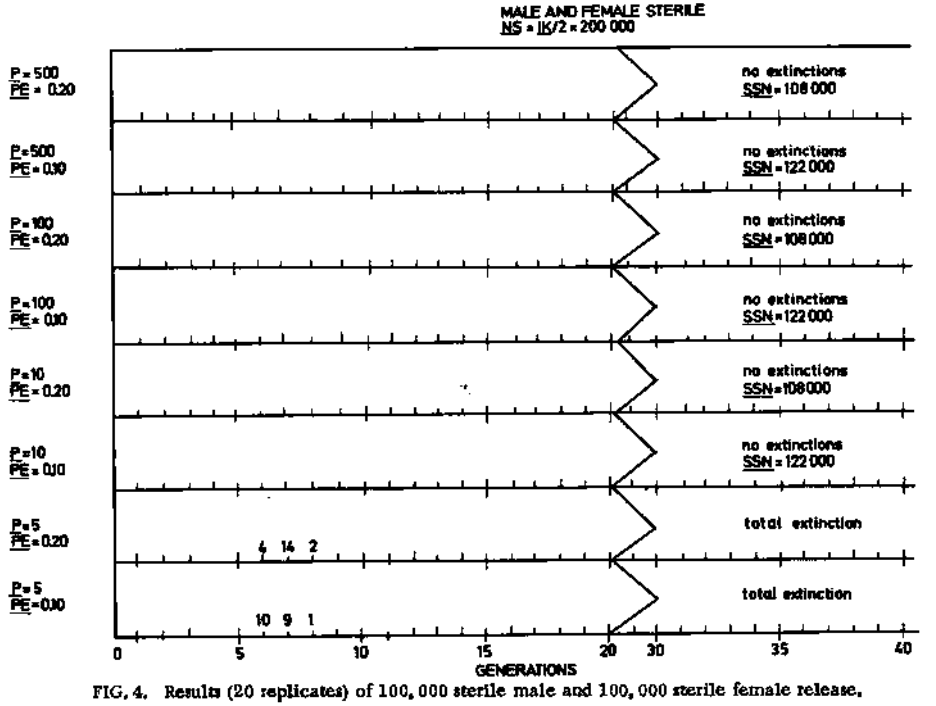


FIG. 4. Results (20 replicates) of 100, 000 sterile male and 100, 000 sterile female release.

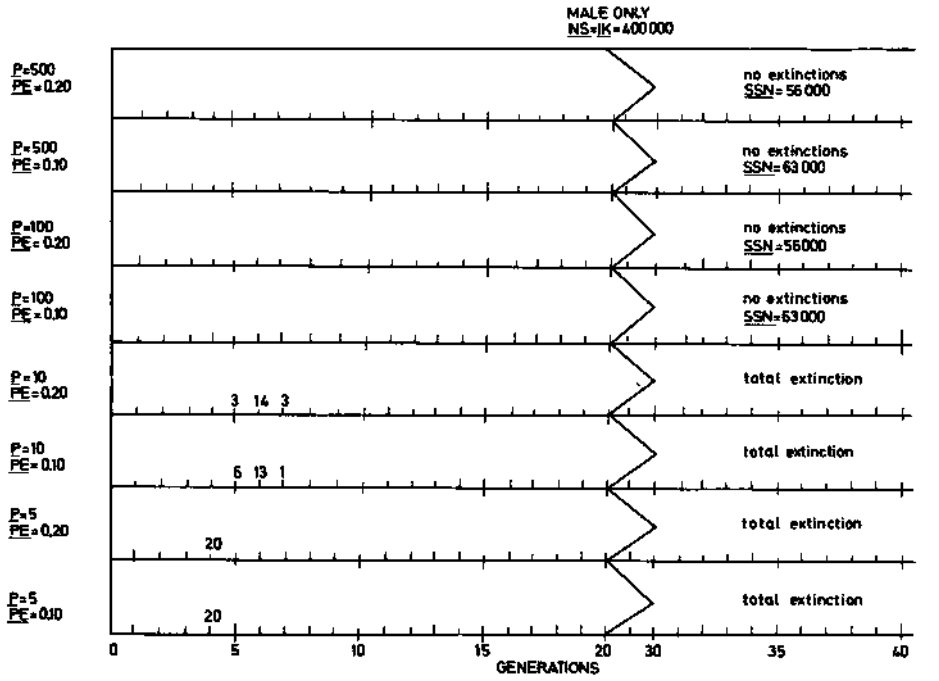


FIG. 5. Results (20 replicates) of 400,000 sterile male release.

replications, are presented according to the various combinations of P and PE. As stated before, P is a random variable and IK also a random variable after initial generation. The initial IK is 400,000 individuals. As one can see, with the mean P equal to 5, all populations become extinct by generation 5, regardless of the value of PE (Figure 3).

When the mean values of P = 10 and PE = .10, there are 4 populations out of 20 which become extinct by generation 13 and the rest (16) of the populations are maintained at generation 40. At this point, the average number of wild male mated with wild female (SSN) is about 70,000 to 100,000. If one relaxes or stops the method of sterile male release, the pest population is likely to reach the IK (capacity) value in one or two generations.

Almost the same case applies to the case of P = 10, and PE = .20. Here 3 populations become extinct by the 10th generation, but the remainders do not go to extinction by generation 40. The value of SSN is about 70,000 to 80,000. When mean P becomes 100 or over, there is no population going to extinction. However, the values of SSN are kept under 100,000, if the sterile male release is continued.

Almost the same situation prevails in the case of 100,000 sterile males and 100,000 sterile females released (Figure 4). However, the values of SSE at generation 40 are considerably higher than the case of 200,000 male release.

Figure 5 represents the case of dumping sterile males of 400,000 individuals, which is the mean of IK, every generation. Now the four cases out of eight parameter combinations go to total extinction (namely P = 5

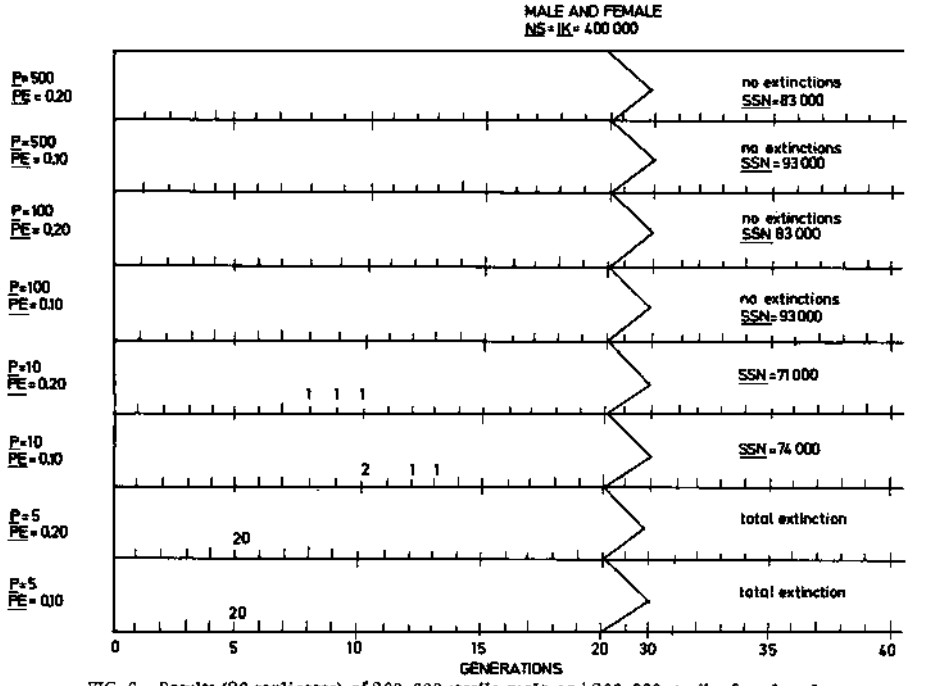


FIG. 6. Results (20 replicates) of 200, 000 sterile male and 200, 000 sterile female release.

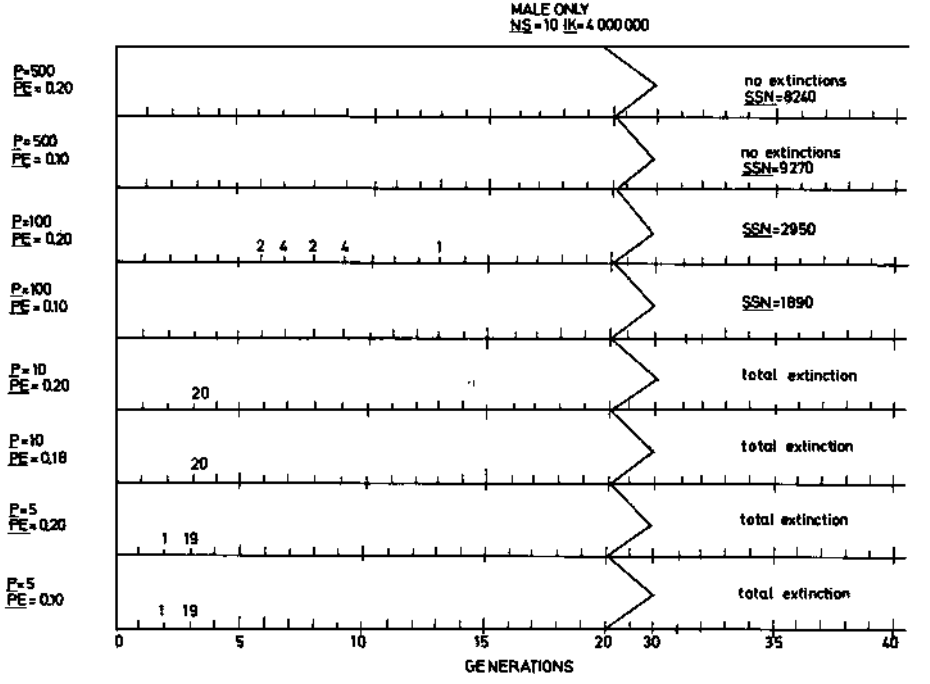


FIG. 7. Results (20 replicates) of 4, 000, 000 sterile male release.

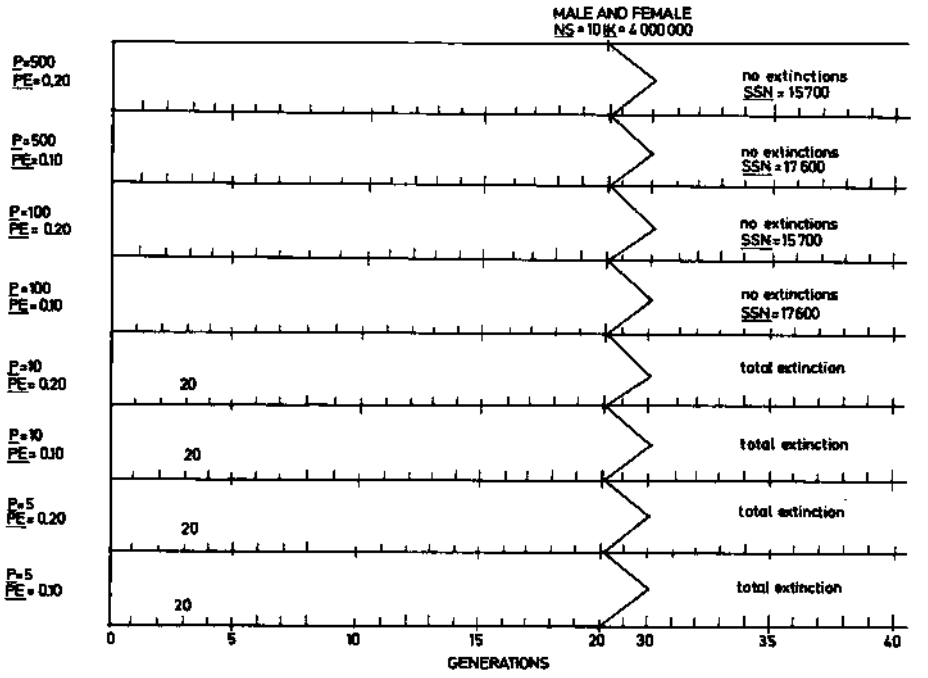


FIG. 8. Results (20 replicates) of 2,000,000 sterile male and 2,000,000 sterile female release.

and $\overline{P}$ = 10). Furthermore, the values of SSE are significantly smaller than the previous cases. However, it is necessary to keep releasing sterile males every generation to maintain low SSE values. With the parameter combination of $\overline{P}$ = 10, $\overline{PE}$ = .10 or .20, there are some extinctions (4 for $\overline{PE}$ = .10 and 3 for .20). Moreover, the values of SSN are about 60,000 at generation 40. The main difference between Figure 5 and Figure 6 data is that the values of SSN are larger in the case of Figure 6 than that of Figure 5, but the chance of total extinction occurs with $\overline{P}$ = 10 in Figure 5.

In order to see the effectiveness of sterile male and/or female, the case of 4,000,000 male release and another case of 2,000,000 male plus 2,000,000 female release were examined (Figure 7 and Figure 8). Figure 7 shows that total extinction happens for the case of $\overline{P}$ = 10 and $\overline{P}$ = 5; however, only 16 populations become extinct by generation 9 with $\overline{P}$ = 100, and $\overline{PE}$ = .10, and 13 populations become extinct with the same $\overline{P}$ value, but $\overline{PE}$ = .20. The average number, SSE, is extremely small. (In all cases SSE is less than 10,000). Figure 8 is a surprise case. Up to mean P equal to 10, all 20 replicated populations become extinct by generation 3; but no extinction occurred for the cases of P = 100 or more. The average SSE is not particularly low.

During 40 generations of rather strong selection, the genetic makeup of insect fitness has become very much improved in survival rate and fecundity-fertility value. The computer output of M showed that 90% of individuals in a given population are in the right bottom corner of M.

This will make the eradication nearly impossible. The only thing which one can do is to keep the pest population low by releasing sterile males every generation.

Another point of interest is the sterile male release is more effective than the sterile male and female release. This observation assumes that NS is all male for the former case and one-half NS males and one-half NS females for the latter case. Thus, if separation or identification of male and female is easy, one can recommend the sterile male release method over the sterile male and sterile female release method. However, if the separation of males and females is difficult, one can take a huge number of sterile males and sterile females, and release them at the appropriate time.

Eradication or extinction did occur in the Monte Carlo simulation runs (Figure 3 to Figure 8). But this will be an unstable condition, since immigrants probably move in fairly rapidly.

Another avenue available, is to find a line of stronger fitness or to artificially select a line with stronger fitness and a higher mating propensity. This author is treating the subject in a naive manner. However, he would like to point out that predictions based on deterministic and stochastic (more real than deterministic) models are widely different.

If eradication is a goal, a suggestion is the release of an adequate number of sterile insects for several generations, and then the release of a 10-fold or 100-fold number of sterile insects in one of the generations.

Finally, the use of the particular M may need some discussion. It is probably better to use a Poisson distribution for the survival rate scale and binomial distribution for fecundity-fertility scale. But this author considers the results would be probably similar to those of this study.

Summary

A stochastic process for pest insect populations was studied by means of a Monte Carlo computer program. Several constant factors in other authors' parameters were changed to stochastic or random variables. The prediction based upon 20 replications of 48 parameter combinations was surprisingly different from the prediction based upon deterministic or constant parameter sets. This author acknowledges the help of Mr. Gordon Beavers in running the computer program.

References

[1] CARSON, Rachel. Silent Spring, Houghton, Mifflin Company, Boston, (1962) 368.

[2] BERRYMAN, Alan A., "Mathematical description of the sterile male principle", The Canadian Entomologist, 99, (1967) 858-865.

[3] KNIPLING, E. F., "The potential role of sterility for pest control", Principles of Insect Chemosterilization, LaBrecque, G. C. Smith, (1968) 7-40.

[4] PROVERBS, M. D., "Induced sterilization and control of insects", Annual Review of Entomology, 14, (1969) 81-95.

[5] VARLEY, G. C., GRADWELL, G. R., "Recent advances in insect population dynamics", Annual Review of Entomology, 15, (1970) 1 -19.

Appendix Table 1

In order to examine more details, the cases of P = 10 with coefficient of variation of 10%, were studied, and the results are presented in the following Table. All cases were IK = 400,000 with a coefficient variation of 10%.

IGEN = 50 and IREP = 100

NS	input sex	PE	# extinction	SSN at IGN = 50*
2×10^5	M	10%	0	8.47×10^4
2×10^5	M	20%	2	8.42×10^4
2×10^5	M & F	10%	0	1.21×10^5
2×10^5	M & F	20%	0	1.10×10^5
4×10^5	M	10%	100 (5.82)**	0
4×10^5	M	20%	100 (6.02)**	0
4×10^5	M & F	10%	23	7.06×10^4
4×10^5	M & F	20%	8	7.60×10^4
4×10^6	M	10%	100 (3.00)**	0
4×10^6	M	20%	100 (3.00)**	0
4×10^6	M & F	10%	100 (3.00)**	0
4×10^6	M & F	20%	100 (3.00)**	0

*For only surviving populations.

** Average duration of pest populations before extinction.

Appendix Table 2

Some extra runs, which were not included in the text, are listed in this table. Note IK is large.

IK = 10^6 with a coefficient of variation of 10%.

P = 10^2 with a coefficient of variation of 50%.

IGEN = 40 and IREP = 100.

NS	input sex	PE	# extinction	SSN at IGN = 40*
10^6	M	10%	0	1.66×10^5
10^6	M	20%	0	1.50×10^5
10^6	M & F	10%	0	2.40×10^5
10^6	M & F	20%	0	2.13×10^5
2×10^6	M	10%	3	9.25×10^4
2×10^6	M	20%	3	8.60×10^4
2×10^6	M & F	10%	1	1.60×10^5
2×10^6	M & F	20%	2	1.40×10^5
10^7	M	10%	66	7.46×10^3
10^7	M	20%	63	6.30×10^3
10^7	M & F	10%	36	2.81×10^4
10^7	M & F	20%	35	2.43×10^4

*Only for surviving populations.

RECHERCHES SUR LA STERILISATION DE Oryctes rhinoceros L.*

B. HURPIN
I. N. R. A. - Station de recherches de lutte biologique et de biocoenotique,
La Minière, Versailles, France

Abstract — Résumé

RESEARCH ON THE STERILIZATION OF Oryctes rhinoceros L.

Under a contract from the joint United Nations Special Fund/South Pacific Commission Project on means of controlling Oryctes rhinoceros L. (Coleopt. Scarabaeidae), the study of ways of applying autocidal control procedures to this palm tree pest has been continued. Improved rearing procedures ensure the production of approximately 3000 insects per year, with a yield of the order of 70 fullgrown adults from 100 larvae hatched. These insects were used for research on problems of the biology of reproduction: e.g. rate of ovogenesis, influence of the age of males and females on the time of first mating and on the number of copulations for the same female, action of the number of males and females respectively on the fecundity of the female and on the fertility of the eggs. It was noticed, in particular, that the majority of females were already mated at the time of their emergence from the larval form and showed developing ovocytes. Sterilization of the males is obtained by irradiation of the imagos, the age of the latter being of no great significance. On the other hand, the dose rate of the gamma rays is a factor which must not be ignored, even for doses of as high as 10 000 rad. At that dose a low-rate irradiating facility (50 rad/min) produces only 60% sterilization, while with a dose rate of 500 rad/min sterility is total. The absence of delayed irradiation effects was demonstrated on the basis of larvae exposed to weak doses (2000 rad) and of adults hatching from larvae resulting from the mating of normal females with males irradiated with small doses. The competitiveness of the irradiated males was studied in reared insects and in Oryctes just emerged from larval forms. The ratio of males irradiated at 10 000 rad to normal males must be greater than 10 to have any marked effect on egg fertility. The prospects for applying the procedures to control Oryctes rhinoceros are discussed.

RECHERCHES SUR LA STERILISATION DE Oryctes rhinoceros L.

Sous contrat du Projet conjoint du Fonds Special des Nations Unies et de la Commission du Pacifique-Sud sur les moyens de lutte contre l' Oryctes rhinoceros L. (Coleopt. Scarabaeidae) l' étude des possibilités d' application à ce ravageur des palmiers des procédés de lutte autocide a été poursuivie. La mise au point de l' élevage assure la production d' environ 3000 insectes par an, avec un rendement de l' ordre de 70 adultes formés à partir de 100 larves à l' éclosion. Ces insectes ont été utilisés pour des recherches sur la biologie de la reproduction: rythme de l' ovogenèse, influence de l' âge des mâles et des femelles sur la date du premier accouplement et sur le nombre de colts pour une même femelle, action du nombre respectif de mâles et de femelles sur la fécondité des femelles et la fertilité des œufs. Il a été constaté, notamment, que la plupart des femelles étaient déjà accouplées au moment de leur sortie du gîte larvaire et présentaient des ovocytes en développement. La stérilisation des mâles est obtenue par irradiation des imagos, sans influence notable de l' âge de ceux-ci. Par contre, le débit des rayons γ joue un rôle qui ne doit pas être négligé, même pour une dose aussi élevée que 10 000 rad. A cette dose un irradiateur de faible débit (50 rad/min) ne provoque que 60% de stérilisation alors qu' avec un débit de 500 rad/min la stérilité est totale. L' absence d' effets différés de l' irradiation a été montrée, soit à partir de larves exposées à des doses faibles (2000 rad), soit à partir d' adultes issus de larves provenant de l' accouplement de femelles normales avec des mâles irradiés à faibles doses. La compétitivité des mâles irradiés a été étudiée avec des insectes d' élevage et avec des Oryctes venant de sortir des gîtes larvaires. Le rapport entre les mâles irradiés à 10 000 rad et les mâles normaux doit être supérieur à 10 pour qu' il y ait un effet sensible sur la fertilité des œufs. Les perspectives d' application à la lutte contre O. rhinoceros sont discutées.

* Recherches effectuées sous contrat du Projet PNUD (FS)/CPS de recherche sur les moyens de lutte contre l' Oryctes rhinoceros.

La stérilisation des mâles est une des méthodes prise en considération par le Projet du Fonds spécial des Nations Unies et de la Commission du Pacifique Sud de recherche sur les moyens de lutte contre l'Oryctes rhinoceros. Ce projet, dont le siège est à Apia (Samoa Occidentales), a pour but, depuis 1964, de définir des procédés efficaces pour limiter les ravages que les adultes de ce Coléoptère occasionnent aux cocotiers, en particulier dans les îles du Pacifique où son introduction est plus ou moins récente (1909 à 1963) [1]. Sous contrat de ce Projet qui envisage les différentes possibilités offertes par les connaissances modernes en défense des cultures, c'est-à-dire par les méthodes chimiques, biologiques, agrotechniques, physiologiques, nous avons entrepris l'étude des modalités d'application aux Oryctes des principes de la lutte autocide. Dans ce but il était nécessaire de disposer d'un élevage permanent de ces insectes qui ne soit pas soumis aux hécatombes généralement constatées du fait de la mycose à Metarrhizium anisopliae Metsch., il était utile de préciser les processus et les conditions de la reproduction et il fallait déterminer la sensibilité aux rayons ionisants des insectes selon leur état physiologique et évaluer les effets de l'irradiation sur le comportement des individus traités ainsi que sur leur descendance.

Les résultats des premiers essais réalisés dans cette voie ont été présentés [2] lors du précédent Colloque sur l'utilisation des isotopes et des radiations en entomologie (Vienne, 1967). Dans la présente note nous nous proposons de résumer l'ensemble des données acquises à ce sujet.

1. Méthode d'élevage des Oryctes

Elle est fondée sur l'utilisation, pour le développement des larves, d'un mélange de deux tiers d'un substrat végétal en décomposition (terreau, sciure de bois, bois pourri) et de un tiers de fèces d'animaux domestiques (bouse de vache généralement), dans un local à 28°/30°C. Après 6 générations successives obtenues à La Minière, il apparait que le rendement moyen est de 70 imagos pour 100 larves du premier stade. Les chiffres présentés antérieurement [3] sont confirmés par les élevages conduits ces dix derniers mois selon la méthode permettant d'utiliser au mieux le matériel dont nous disposons : mise en élevage en serres à 30°C de groupes de 120 larves âgées de quelques jours dans des coffres en bois renfermant environ 600 litres d'un mélange de sciure de bois et de bouse de vache desséchée ou non selon son état d'humidité afin que l'humidité relative du mélange soit voisine de 70 %. Les insectes ne sont introduits qu'après une semaine au cours de laquelle la fermentation porte la température de la masse à 60°C. Ils restent dans ce milieu pendant 3 mois puis sont transférés à l'état de larve du troisième stade bien développée dans des bacs en polyéthylène contenant 30 ou 45 litres, selon les modèles, du mélange sciure + bouse ou bois décomposé + bouse, à raison de 20 ou 30 L_3 par bac. 24 séries de ce type ont permis l'élevage de 2850 L_1 qui ont donné naissance à 1988 imagos, soit un rendement de 69 % avec seulement 45 cas de Metarrhizium, représentant 1,5 % de la population initiale.

Les adultes sont placés, par lots de 10 couples généralement, dans des bacs de 50 litres en polyéthylène, tronconiques, munis d'un couvercle percé de quelques trous pour éviter les condensations. Ces récipients maintenus en serres à 28°C sont remplis au tiers de leur capacité de terreau de couche criblé, à 70 % H.R. Des rondelles de banane renouvelées deux fois par semaine assurent l'alimentation. Les pontes, déposées le plus souvent au fond des récipients dans une couche triturée et ameublie par les femelles, sont recherchées chaque semaine par tri du terreau.

Les premiers dépôts d'oeufs ont lieu après 2 à 4 semaines suivant l'âge des femelles lors de la constitution des couples et se prolongent en général jusqu'à 4 mois après cette date. La fécondité moyenne est de 55 oeufs par femelle avec une fertilité de 82 % pour 440 femelles. Des élevages par couples ont montré une grande variabilité individuelle de la ponte qui peut aller de 0 à 310 oeufs par femelle.

2. Etude de la reproduction

Les recherches en ce domaine ont porté principalement sur le rythme de l'ovogenèse et des accouplements, le pouvoir fécondant des mâles et l'influence du mâle sur la fécondité des femelles.

2.1. Ovogenèse et accouplement

L'évolution des ovaires et les délais d'accouplement ont été examinés par dissection, tous les 3 ou 4 jours, de 4 femelles prélevées dans les élevages de 10 couples en bac, en fonction de l'âge des deux sexes au moment de la mise en élevage. 7 séries ont été comparées pendant 2 mois en associant à des femelles de 3 jours, 1 semaine, ou 3 semaines à jeun à 20°C, des mâles de ces trois âges selon les combinaisons suivantes :

mâles 3 jours	× femelles 3 jours
mâles 3 jours	× femelles 1 semaine
mâles 3 jours	× femelles 3 semaines
mâles 1 semaine	× femelles 1 semaine
mâles 3 semaines	× femelles 3 jours
mâles 3 semaines	× femelles 1 semaine
mâles 3 semaines	× femelles 3 semaines

En outre, l'ovogenèse de femelles vierges âgées de 2 à 3 jours a été suivie dans les mêmes conditions.

A la dissection étaient notés : l'état du tissu adipeux, le nombre d'ovocytes dans les ovarioles, la longueur et le diamètre des ovocytes de 3 ovarioles, la présence de corps jaunes, l'aspect de la poche copulatrice (nombre et couleur des spermatophores).

Le tableau I schématise les données obtenues. Il montre que les accouplements et la ponte ont lieu d'autant plus tôt que les insectes sont plus âgés et que, généralement, les deux phénomènes se produisent à la même époque : lors du premier accouplement les ovaires terminent leur premier cycle ovogénétique. La série mâles de 3 jours x femelles de 3 semaines fait exception pour des raisons qui restent à préciser : des processus de dégénérescence du plus gros ovocyte ont été observés, d'autre part les accouplements ont concerné seulement certaines femelles pendant les trois premières semaines. Le parallélisme entre l'accouplement et la maturation des ovocytes, quelque soit l'âge initial des partenaires, suggère l'intervention d'interactions entre les deux sexes : l'accouplement des mâles plus jeunes est avancé par la présence de femelles âgées dont l'ovogenèse est plus rapide, et réciproquement la ponte de femelles jeunes est accélérée par la présence de mâles plus âgés.

A en juger par le nombre de spermatophores décelables dans la poche copulatrice, les accouplements successifs d'une même femelle se limitent à deux ou trois, dans nos élevages tout au moins.

TABLEAU I. EVOLUTION DES ORGANES GENITAUX FEMELLES SELON L'AGE DU MALE ET DE LA FEMELLE

Age des insectes	Délai en jours pour observer			Deuxième accouplement
	premier accouplement	oeufs dans ovarioles	corps jaunes	
mâles 3 jours x femelles 3 jours	24	24	28	32
mâles 3 jours x femelles 1 semaine	16	16	20	24
mâles 3 jours x femelles 3 semaines	8	16	20	-
mâles 1 semaine x femelles 1 semaine	16	16	24	32
mâles 3 semaines x femelles 3 jours	12	14	14	14
mâles 3 semaines x femelle 1 semaine	14	16	24	28
mâles 3 semaines x femelles 3 semaines	6	8	15	8

2.2. Rythmes de ponte

Pour déterminer le rythme des pontes des élevages ont été conduits par couples dans des pots cylindriques en polyéthylène de 25 cm de diamètre et 30 cm de hauteur munis d'un couvercle solidement fixé par des crochets pour éviter les fuites et renfermant un tiers de leur capacité, soit 6 litres de terreau criblé. Deux séries de 30 couples ont été comparées : l'une constituée avec des insectes âgés de 3 jours, l'autre avec des mâles et des femelles de 3 semaines.

Dans les deux cas une variabilité individuelle très importante a été mise en évidence aussi bien quant à la fécondité que pour les rythmes de dépôt des oeufs ainsi qu'en témoignent les quelques exemples du tableau II.

Certaines femelles déposent leurs oeufs régulièrement et sans interruption pendant toute la période de ponte, qui peut dépasser 3 mois (femelles 1 et 44) , mais se limiter à quelques semaines (femelle 24); d'autres présentent un rythme de ponte plus ou moins marqué (femelles 26 et 60) qui montre l'existence de 4 cycles ovogénétiques successifs à intervalles de 2 à 5 semaines ; d'autres (femelles 4, 18, 38) ne font preuve d'aucune régularité. La même variabilité s'enregistre pour le début de la ponte qui peut intervenir dès la 2ème semaine ou être reportée à 3 mois sans qu'il y ait de ce fait de conséquences marquées sur la fécondité totale.

Les causes de ces différences individuelles n'ont pas pu être déterminées jusqu'à présent.

2.3. Pouvoir fécondant des spermatozoïdes

La longévité des mâles est inférieure à celle des femelles. Ainsi, dans les deux expériences précédentes, en couples, la longévité moyenne fut de 13 semaines pour les mâles (max.: 26 semaines, min.: 8 se-

maines) et de 18 semaines pour les femelles (max. : 34 semaines, min. : 7 semaines) dans le cas des insectes âgés au départ de quelques jours, tandis que pour les Oryctes ayant déjà passé 3 semaines à 20°C, les durées de vie dans les dispositifs de ponte furent de 19 semaines en moyenne pour les mâles contre 24 semaines chez les femelles avec des maxima respectifs de 30 et 32 semaines. De ce fait bien souvent des femelles subsistent sans mâle pendant un délai plus ou moins long. Aucune différence sensible dans la fertilité des oeufs émis après la mort du mâle n'est cependant constatée, même si la ponte se prolonge pendant de nombreuses semaines, voire 3 ou 4 mois, comme nous l'avons noté à plusieurs reprises. Les spermatozoïdes peuvent donc dans la spermathèque conserver leur vitalité pendant au moins 3 mois.

Le pouvoir fécondant du mâle a été examiné d'une part en proposant successivement à un même mâle 3 séries de 4 ou 6 femelles à intervalle de 2 puis de 6 semaines, d'autre part en plaçant en bacs en serres des mâles et femelles selon différents sex ratio. La première expérience a confirmé que le premier accouplement n'intervenait pas avant 2 semaines au minimum et montré qu'un seul mâle était capable d'assurer une fertilité normale à 13 femelles.

La deuxième expérience réalisée en faisant varier le rapport entre le nombre de mâles et celui des femelles dans la même enceinte de 3/1 à 1/24 avec 9 intermédiaires : 3/2, 1/1, 1/2, 1/3, 1/4, 1/6, 1/8, 1/12, 1/18, indique que la fertilité reste analogue dans tous les cas. Il apparaît donc qu'un mâle d'O. rhinoceros est susceptible de féconder au moins une vingtaine de femelles.

2.4. Etat physiologique des femelles à la sortie des gîtes larvaires

Nous avons cherché à déterminer, au moins dans les conditions de nos élevages, à quelle période de la vie imaginale se situait le premier coït et s'il intervenait avant ou après la sortie des adultes des gîtes larvaires.

Nous avons eu recours à un dispositif de capture posé en surface du milieu d'élevage de larves dans les coffres en serre à 30°C de façon à récolter les Oryctes lors de leur première manifestation d'activité épigée et par dissection, à examiner l'état de l'appareil génital femelle. Ce dispositif est constitué par une plaque de polyester de 1 m^2 environ, formant couvercle jointif appliqué en surface du substrat. Cette plaque comporte 16 trous de 5 cm de diamètre prolongés par un tube de même diamètre et de 5 cm de haut dans lequel pivote une plaquette permettant la sortie de l'insecte mais non son retour. Un signal électrique transmis à un enregistreur permet d'inscrire les passages en fonction du temps. Ainsi que nous l'avons exposé dans une note antérieure [4], ce système utilisé à 5 reprises a prouvé que la plupart des femelles sont accouplées et sont prêtes à pondre lorsqu'elles apparaissent pour la première fois en dehors du milieu où se sont déroulées la vie larvaire et la métamorphose.

Ce phénomène, dont l'importance pour la mise en oeuvre de la lutte autocide n'est pas à souligner, doit être vérifié dans les conditions naturelles car les dispositifs expérimentaux peuvent provoquer des modifications de comportement. Toutefois, chez O. monoceros tout au moins, d'après D. MARIAU (communication orale), en Côte d'Ivoire, les femelles récoltées dans les cocotiers ont en général leurs ovaires développés et une poche copulatrice pleine.

TABLEAU II. NOMBRE D'ŒUFS DEPOSES CHAQUE SEMAINE PAR UNE FEMELLE DE O. rhinoceros EN ELEVAGE PAR COUPLES A 28°C

Femelles de 3 jours N°	Semaines 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	Fécondité
1										5	12	3	7	12	9	13	12	14	19	18	9	22	39	19									215
4		4	14		32							8	8																				66
18											9		8		7		14							21			8	7	2	3	6	6	91
24		24	60	49	5																												138
26		8	4						4	20	13	9			8	7	13	16						19	33	39							203
Femelles de 3 semaines N°																																	
38		5	26	8		17	19	8				8	13																				104
44												4	9	10	13	10	8	4	11	17	24	35	13										158
52					11	27	16				16	24	27	13	14	18	8	3	13	8	16	36	60										310
60			21			17			2	40			9	6				6	7	6	5					7		4					130

TABLEAU III. MODALITES DE STERILISATION DES MALES DE O. rhinoceros AUX RAYONS γ

Dose (en rad)	Débit (en rad/min)	Nombre de mâles	TL 50 (semaines)	Oeufs/ femelle	% L_1 [a]
3000	1500	56	9	35	59
	1100	10	9	76	95
	500	66	11	39	50
	40	60	14	71	83
	Témoin	20	12	85	82
4000	1100	42	7	43	64
	Témoin	21	8	75	72
4500	1100	15	8	26	6
5000	1500	39	8	23	9
	1100	40	8	50	12
	500	32	9	16	2
	40	110	10	52	63
	Témoin	40	8	43	81
6000	1100	10	8	27	2
	500	32	7	40	1,5
	40	16	6	67	29
7000	1500	38	11	45	0
	500	51	8	32	1
	40	46	9	34	50
	Témoin	30	7	41	78
10000	1500	40	7	35	0
	500	40	5	38	0
	40	48	11	53	36
	Témoin	40	7	61	81
12000	500	40	5	45	0
	Témoin	20	8	60	81

[a] L_1 = larves du premier stade

3. Détermination des conditions de stérilisation des mâles

3.1. Doses stérilisantes

Quatre irradiateurs au ^{60}Co et un générateur de rayons X ont été utilisés pour nos essais, les débits de dose des appareils à rayons γ étant d'environ 1500, 1100, 500 et 40 rad par minute, tandis que l'appareil à rayons X avait un débit de dose de l'ordre de 170 rad/min sous une tension de 200 kV, à 12,5 m A, en utilisant un filtre en cuivre de 0,5 mm d'épaisseur. A chaque opération le débit était déterminé.

En général les insectes étaient traités par groupe de 5 ou 10 dans une boîte cylindrique en polystyrène de 250 ou 500 ml remplie de tourbe pour satisfaire leurs exigences thigmotactiques et éviter qu'ils ne s'agitent sans arrêt. La cellule de l'irradiateur de 1500 rad étant trop exiguë, dans ce cas les Oryctes étaient irradiés isolément dans une cage en plexiglass d'un volume légèrement supérieur à celui de l'animal .

TABLEAU IV. MODALITES DE STERILISATION DES MALES DE O. rhinoceros PAR LES RAYONS X

Dose (en rad)	Débit (en rad/min)	Nombre de mâles	TL 50 (semaines)	Oeufs/ femelle	% L_1 [a]
3000	170	10	5	31	50
5000	"	50	8	38	51
7000	"	60	9	34	19
10000	"	60	7	48	4
12000	"	40	6	55	0

[a] L_1 = larves du premier stade

Après l'irradiation les mâles étaient placés en bacs de ponte avec des femelles du même âge, non traitées, par groupes de 10 couples. Des Oryctes de même origine, non soumis aux rayons, étaient mis en élevage dans les mêmes conditions pour constituer les témoins. Des contrôles hebdomadaires avaient pour but de noter la mortalité, de récolter les oeufs et, après mise en incubation de ceux-ci dans de la tourbe + terreau, d'estimer leur fertilité par dénombrement des jeunes larves. Le tableau III présente les résultats des irradiations de 1000 mâles par rayons γ, le tableau IV ceux relatifs aux rayons X appliqués à 220 mâles. Ils font ressortir l'influence conjointe du débit de dose et de la dose. En effet, pour une même dose, 5000 rad par exemple, au moins 90 % des mâles sont stériles lorsque le débit des rayons γ est de 500 rad/min ou plus, alors qu'il n'y en a que 37 % pour un débit de 40 rad, et 50 % par traitement aux rayons X à 170 rad/min.

Au point de vue pratique, il est donc préférable de recourir à une source au ^{60}Co assez puissante dont le débit de dose soit au moins de 500 rad/min. La stérilisation quasi totale des mâles de O. rhinoceros est alors assurée avec une dose de 6000 ou 7000 rad. Dans le cas de sources à faible débit (rayons γ ou rayons X) il est nécessaire d'employer des doses deux fois plus importantes, de l'ordre de 12000rad, pour aboutir à une stérilisation analogue.

Du point de vue théorique ces résultats posent le problème de l'influence du débit dans les effets biologiques de l'irradiation. Il n'est pas dans notre intention de discuter ici de cette question qui a fait l'objet de publications contradictoires selon les auteurs et les animaux considérés. Nous pensons, comme BYDSHKOVSKAJA et al.[5] que le facteur "durée de l'irradiation" intervient et peut expliquer certaines différences d'activité des radiations ionisantes en permettant ou non aux processus de défense, voire de régénération, cellulaire ou tissulaire de se manifester. Selon la puissance de l'irradiateur, en effet, la durée du traitement est très variable : près de 3 heures pour 40 rad/min, contre 40 minutes pour les rayons X à 170 rad/min et respectivement 14 minutes et 4 min 40 sec pour 500 et 1500 rad/min, dans le cas d'une dose de 7000 rad.

Comme il a déjà été mentionné par plusieurs auteurs, il n'y a pas de répercussions notables de l'irradiation sur la longévité des

TABLEAU V. FERTILITE DES OEUFS SELON L'AGE DU MALE LORS DE L'IRRADIATION

Dose (en rad)	Débit (en rad/min)	Age des mâles			
		Moins d'une semaine		3 semaines	
		Nb. mâles	% L_1 [a]	Nb. mâles	% L_1 [a]
3000	1500	26	60	30	58
4000	1100	20	62	22	66
5000	1500	20	17	20	6
	170	40	47	10	69
	40	10	49	10	63
7000	500	36	0	15	1,5
	170	40	24	20	13
	40	38	30	8	73

[a] L_1 = larves du premier stade

mâles, appréciée par le temps nécessaire pour avoir 50 % de mortalité (TL 50). On note même un certain accroissement de la longévité aux faibles doses et aux faibles débits. Quant à la fécondité moyenne des femelles elle ne parait pas affectée par le traitement subi par leurs partenaires.

3.2. Influence de l'âge des imagos

L'action stérilisante des rayons Y a été comparée chez trois types de mâles : des insectes issus de nymphes et maintenus vierges et à jeun à 20°C soit pendant quelques jours, soit pendant 3 semaines, d'autre part des Oryctes pris à la sortie du substrat d'élevage larvaire et par conséquent présumés ayant déjà copulé.

D'après le tableau V la fertilité de la descendance est un peu moindre chez les mâles plus âgés seulement lorsque l'irradiation est effectuée à faible débit; ce qui suggère que les phénomènes de régénération seraient plus intenses chez ces insectes.

Le tableau VI schématise les résultats de 3 séries d'essais effectués avec des O. rhinoceros d'origine différente. Il indique que les mâles recueillis en surface du substrat où ont évolué les larves, c'est-à-dire les mâles dits de "gîtes larvaires", déterminent, après exposition aux rayons Y, une stérilité des oeufs encore satisfaisante (au moins 50 %) qu'ils soient mis en présence de femelles nées de nymphes isolées en élevage ou de femelles provenant également de gîtes larvaires. Par contre, 66 % des oeufs restent fertiles lorsque des femelles issues de gîtes larvaires, et par conséquent vraisemblablement déjà accouplées, sont associées à des mâles irradiés formés à partir de nymphes depuis quelques jours seulement ; alors que ce pourcentage n'est plus, en moyenne, que de 27 % si des femelles de même origine sont accouplées avec des mâles traités issus également de gîtes larvaires. Ceci confirme le délai nécessaire pour le début d'activité génitale des mâles après leur sortie de la dépouille nymphale et atteste des possibilités de stérilisation par irradiation d'insectes ayant déjà copulé.

TABLEAU VI. FERTILITE DES ŒUFS SUIVANT L'ORIGINE DU MALE: NYMPHOSE EN ELEVAGE OU GITE LARVAIRE (Irradiation: 10 000 rad à 500 rad/min)

Femelle	Mâle		
	Elevage Irradié	Irradié	Gîte non traité
Elevage	0 - 0	0 - 50 - 0	81
Gîte	68 - 64	4 - 50 - 26	81 - 77

3.3. Compétition entre les mâles irradiés et mâles normaux

Cet aspect très important pour les applications sur le terrain a été étudié de deux façons: d'une part, par remplacement dans l'enceinte d'élevage (bacs de 50 litres avec 10 couples) de mâles normaux par des mâles traités et vice versa ; d'autre part, par emploi simultané de ces deux catégories de mâles pour la fécondation de lots de femelles.

Dans le premier cas notre expérimentation a prouvé qu'en remplaçant après 2, 4 ou 6 semaines des mâles soumis à 10 000 rad par des mâles non irradiés, les oeufs déposés par les femelles recouvraient leur fertilité dans la proportion de 60 à 90 %. Mais, réciproquement, la substitution après 1 ou 2 mois de mâles traités à 10 000 rad à des mâles normaux en bacs de ponte avec les mêmes femelles permet de rendre celles-ci stériles.

Il semble donc que le premier accouplement n'ait pas de rôle prédominant et que ce soit les spermatozoïdes le plus récemment déposés dans la bourse copulatrice qui déterminent la fertilité des oeufs.

La compétitivité simultanée entre mâles normaux et mâles stérilisés a été examinée en plaçant dans le même bac de ponte des mâles exposés à différentes doses de rayons γ, des mâles de même âge non irradiés et des femelles normales dans des rapports variables, de façon à déterminer le nombre minimum de mâles traités à adjoindre à 1 mâle normal pour obtenir un effet significatif sur la fertilité des oeufs.

Dans quelques cas, des femelles irradiées ont également été mises en compétition.

Le tableau VII résume les multiples essais réalisés dans ce domaine en irradiant soit des Oryctes formés au laboratoire à partir de nymphes, soit des imagos prélevés en surface du milieu d'élevage larvaire. Dans les deux cas il faut plus de 10 mâles traités par mâle normal pour que l'effet sur la descendance soit suffisant. Cette proportion est en accord avec les chiffres donnés pour d'autres ravageurs d'intérêt agricole: Carpocapse [6], Anthonomus grandis [7], Dacus cucurbitae [8], par exemple, pour lesquels il faut respectivement au moins 15, 20, 9 mâles stériles par mâle normal. Toutefois dans le cas des Oryctes, nuisibles à l'état imaginal, le nombre élevé de mâles à stériliser pour espérer une incidence sensible sur l'évolution des populations constitue un handicap sérieux pour l'application pratique de la méthode.

TABLEAU VII. COMPETITIVITE DES MALES IRRADIES PAR RAPPORT AUX MALES NORMAUX EN ELEVAGE A 30°C

Doses en rad pour les mâles traités	Rapport mâles irradiés / mâles normaux/femelles normales	% fertilité des oeufs
A – Mâles issus d'élevage		
5000	6 : 3 : 9	5 – 80 – 73 – 82
	8 : 2 : 10	82 – 81 – 85
6000	6 : 3 : 9	84 – 64 – 78 – 77
	8 : 2 : 10	82
7000	6 : 1 : 6	84 – 85
	12 : 1 : 6	50 – 72
10000	6 : 1 : 6	0 – 0
	6 : 1 : 12	64 – 81
	12 : 1 : 6	0 – 88
B – Mâles sortant de gîtes larvaires		
7000	18 : 2 : 10	44 – 68
10000	15 : 1 : 10	43 – 57
	18 : 2 : 10	66

4. Effets différés de l'irradiation

Ils ont fait l'objet de deux types d'expérimentations : d'une part, l'irradiation de larves ou de nymphes de divers âges avec étude du pouvoir reproducteur des adultes formés dans les essais à faibles doses, d'autre part, l'élevage des larves puis des imagos issus d'oeufs déposés par des femelles accouplées avec des mâles irradiés à des doses insuffisantes pour assurer la stérilité totale.

Trois séries d'expériences ont été réalisées pour examiner l'influence des rayons γ (2000, 4000 et 6000 rad) sur les larves et les nymphes de O. rhinoceros des deux sexes. D'une façon générale chez les larves des deux derniers stades comme chez les nymphes plus ou moins âgées, la dose de 6000 rad détermine une mortalité totale du stade traité, 4000 rad permettent la formation de nymphes et d'adultes dans une proportion d'autant plus élevée que les larves ont été irradiées plus jeunes, mais tous les imagos présentent des malformations, en particulier au niveau des ailes et des élytres ; ces malformations se retrouvent chez les adultes issus de nymphes soumises à cette dose.

A 2000 rad, pour les larves ainsi que pour les nymphes, le développement se déroule normalement et donne des insectes parfaits apparemment normaux. Ces Oryctes ont été mis en élevage pour comparer leur fécondité et leur fertilité à celles des insectes provenant de larves non irradiées. Aucune différence n'a été relevée dans le pourcentage d'éclosion des oeufs ni dans l'importance des pontes.

Des élevages en boites individuelles avec le mélange bois + bouse ont été conduits à partir de 11 issues de pontes déposées par des femelles accouplées avec des mâles ayant subi une irradiation de 3000, 4000 ou 5000 rad. 11 lots de telles larves, comptant au total 1050 insectes, ont été suivis sans faire apparaître de différences dans l'évolution de ces Oryctes par rapport aux larves venant de mâles non traités, aussi bien d'après le pourcentage d'imagos formés que pour le poids de ces animaux, ou les cas de Metarrhizium ainsi que pour la durée de développement. Une soixantaine de ces adultes ont été mis en ponte pour vérifier leur longévité et leur fécondité : elles se sont montrées analogues à celles des O. rhinoceros normaux.

Conclusion

Parmi les résultats acquis au cours de l'expérimentation que nous venons de résumer, les principaux points méritant d'être soulignés sont, à notre avis, les suivants :

- La mise au point d'une méthode d'élevage éprouvée maintenant par 3 années de pratique, soit 6 générations successives, qui assure un rendement moyen de 70 imagos pour 100 larves à l'éclosion.

- La détermination du rythme de l'ovogenèse et de la synchronisation entre le premier accouplement et la fin du premier cycle ovogénétique.

- La caractérisation, au moins dans nos conditions d'élevage, de l'état des femelles lorsqu'elles sortent pour la première fois du milieu où se sont effectués le développement larvaire puis la nymphose.

- L'établissement de la dose de rayons X ou γ, en fonction du débit de la source utilisée, provoquant une stérilité suffisante chez les mâles, sans altération marquée de leur longévité.

- La mise en évidence des possibilités de stérilisation, plus ou moins prononcée, des insectes issus de gîtes larvaires et par suite déjà accouplés.

- La nécessité de recourir à plus de 10 mâles irradiés pour un mâle normal.

- L'absence d'effets différés notables dans la descendance des insectes irradiés.

Toutes ces données obtenues en laboratoire constituent les principaux éléments physiologiques permettant d'apprécier dans quelles conditions l'application de la lutte autocide aux Oryctes est possible. Il reste toutefois à préciser des aspects écologiques notamment en matière de dynamique des populations et dans le domaine des attractifs.

Du fait de la biologie de ces insectes interdisant le lâcher de mâles stériles produits en élevage et de la compétitivité relativement faible des insectes irradiés, l'emploi de chimiostérilisants associés à un attractif suffisamment efficace parait être une formule mieux adaptée à la lutte contre les Oryctes. Aussi, dans le cadre du Projet avons nous entrepris l'étude de quelques chimiostérilisants qui sera exposée dans une publication ultérieure.

REFERENCES

[1] CATLEY, A., The Coconut Rhinoceros beetle Oryctes rhinoceros (L.) (Coleoptera Scarabaeidae: Dynastinae), PANS 15 1 (1969) 18-30.

[2] HURPIN, B., «Application aux Oryctes (Coleoptères Scarabaeidae) de la méthode du lâcher de mâles stériles», Isotopes and Radiation in Entomology (C.r. Coll. Vienne, 1967), AIEA, Vienne (1968) 301.

[3] HURPIN, B., FRESNEAU, M., Etude en laboratoire du développement larvaire de Oryctes monoceros et de O. rhinoceros (Col. Scarabaeidae), Annls. Soc. ent. Fr. (N.S.) 6 1 (1970) 193-214.

[4] HURPIN, B., Sur la place du premier accouplement dans les différentes séquences de l' activité imaginale de Oryctes rhinoceros (Coleopt. Scarabaeidae), C.r. hebd. Séanc. Acad. Sci., Paris D 268 (1969) 2701.

[5] BYDSHKOVSKAJA, I.B., OTSHINSKAJA, G.K., VOLYNOVA, T.P., Importance du facteur temps lors de l' irradiation d' imago d' insecte, Radiobiologija 8 2 (1968) (en russe).

[6] PROVERBS, M.D., NEWTON, J.R., LOGAN, D.M., Autocidal control of the codling-moth by release of males and females sterilized as adults by gamma irradiation, J. econ. Ent. 60 (1967) 1302-1306.

[7] DAVICH, T.B., "Sterile male technique for control or eradication of the boll weevil, Anthonomus grandis Boh.", Sterile-male Technique for Eradication or Control of Harmful Insects (C.r. Groupe d' étude, Vienne), AIEA, Vienne (1969) 65.

[8] QURESHI, Z.A., "The ability of sterilized irradiated males of Dacus cucurbitae Coq and Dacus zonatus (Saunders) to compete with normal males", Insect Ecology and the Sterile-male Technique (C.r. Groupe d' étude, Vienne, 1967), AIEA, Vienne (1969) 43.

DISCUSSION

E.A. TAYLOR: Has this insect been observed mating in its natural environment?

B. HURPIN: It has rarely been observed mating as the adult is crepuscular in its habits. It leaves the resting site after sunset and burrows into the base of palm leaves. During larval population surveys Oryctes rhinoceros has occasionally been found copulating in rotting coconut trees or compost.

ALTERACIONES EMBRIOLOGICAS EN HUEVOS IRRADIADOS DE Triatoma infestans Y SU POSTERIOR EVOLUCION

R.U. CARCAVALLO, E. REWALD, C.A. CARABAJAL
Dirección de Epidemiología de la Subsecretaría de Salud Pública de la Provincia de Buenos Aires, Buenos Aires, Argentina

Abstract — Resumen

EMBRYOLOGICAL CHANGES IN IRRADIATED EGGS OF Triatoma infestans AND SUBSEQUENT DEVELOPMENT.

The development of a technique for separating the contents of the egg of Triatomina made possible a complete microscopic study of the embryonic structures in the various stages of development, from the earliest until hatching of the first nymphal stage.

Using a mixture of chloroform, methyl alcohol and acetic acid in equal parts (a percentual modification of Carnoy's fluid), we obtained three simultaneous effects: spontaneous opening of the operculum, projection of the embryo outside the egg and instantaneous fixation of the cellular structures. A complete study of the structures can be made by subsequent staining with selective dyes and impregnation with silver.

A large number of eggs were divided up into four batches, three of which were exposed to different doses of radiation, the fourth serving as a control.

In the results obtained using X-rays, the higher the radiation dose and the earlier applied, the greater were the changes. In embryos less than two days old the mean and high doses shown in detail in the tables are lethal; cytological studies revealed profound changes in the nucleus: pyknosis, karyorrhexis and protoplasmic vacuolation.

The low doses to young embryos produce less profound changes, but give rise to a physiological weakening of the first-stage nymphs which hatch from them and do not survive to the second stage. The mean doses applied to 8- to 9-day-old eggs cause a delay in the hatching of the first nymph and some slight malformations (semi-atrophy of the tibia and tarsus, malformations of the tergites, etc.), the majority being non-viable as from the second stage. High doses for similar times cause 100% lethality in second-stage nymphs owing to an inability to feed from the first alteration.

ALTERACIONES EMBRIOLOGICAS EN HUEVOS IRRADIADOS DE Triatoma infestans Y SU POSTERIOR EVOLUCION.

El desarrollo de una técnica que permite la separación del contenido del huevo de los triatominos, permitió un completo estudio microscópico de las estructuras embrionarias en las distintas etapas del desarrollo, desde las más precoces hasta la eclosión de la ninfa I.

Empleando una mezcla de cloroformo, alcohol metílico y ácido acético en partes iguales (modificación porcentual del líquido de Carnoy) se logran tres efectos simultáneos: apertura espontánea del opérculo, proyección del embrión fuera del huevo e instantánea fijación de las estructuras celulares. La posterior coloración con tinciones selectivas e impregnaciones argénticas, permite un estudio completo de las estructuras.

Tomando un elevado número de huevos, se procedió a la confección de cuatro lotes: tres en los que se aplicaron diferentes dosis de radiaciones y un cuarto que sirvió de testigo.

Los resultados obtenidos con el empleo de rayos X demuestran alteraciones tanto más profundas cuanto mayor es la dosis de radiación aplicada y cuando más precozmente se aplica. En los embriones menores de dos días, las dosis medias y altas que se tabulan en el trabajo in extenso resultan letales, mostrando los estudios citológicos alteraciones nucleares profundas, picnosis, cariorexis y vacuolización protoplasmática.

Las dosis pequeñas en embriones jóvenes producen alteraciones menos profundas, pero que debilitan fisiológicamente las ninfas I eclosionadas de los mismos, las que a su vez no sobreviven al segundo estadio. Las dosis medias aplicadas a huevos de seis a nueve días provocan un retardo en la eclosión de

la ninfa I y algunas malformaciones leves (semiatrofia de tibias y tarsos, malformaciones en los tergitos, etc.), siendo la mayoría incompatibles con la vida a partir del segundo estadio. Las dosis altas a iguales tiempos provocan un cien por cien de letalidad en las ninfas II, por incapacidad de alimentación después de la primera muda.

PROPOSITOS Y OBJETIVOS

Los Triatominae, como vectores de la tripanosomiasis americana, son insectos que están siendo estudiados en distintos aspectos de su biología. Uno de los menos conocidos, por las dificultades técnicas de su estudio, es su embriología. La principal dificultad estriba en la extracción del embrión del huevo, dado que la consistencia del mismo, por su dureza y friabilidad después de la acción de los líquidos fijadores, hacen muy difícil el tratamiento que permita el estudio microscópico.

En 1968 desarrollamos una técnica que permite la fácil extracción y conservación del embrión entero, libre de la cubierta del huevo. Esto, una vez aplicadas técnicas clásicas de corte y tinción, nos ha permitido completar un estudio embriológico que se halla actualmente en prensa.

El empleo de radiaciones, cada vez más usado en adultos y ninfas, lo ha sido pocas veces en huevos, con miras a conocer la influencia que puede tener en el desarrollo del embrión y posteriores estadios.

En este trabajo nos propusimos:

1) Conocer si las radiaciones tenían influencia en el desarrollo embrionario del Triatoma infestans Klug.
2) Determinar las dosis de radiaciones letales para el embrión.
3) Establecer las sensibilidades embrionarias a las radiaciones en función de la edad del huevo.
4) Detectar posibles anomalías o mutaciones inducidas.
5) Comparar la fisiología de las ninfas nacidas de huevos irradiados con controles normales.

MATERIAL Y METODOS

De los frascos donde se crían Triatoma infestans en el laboratorio se procedió a separar diariamente los desoves de las anteriores 24 h. La operación se practicó siempre a la misma hora, con el objeto de tener una periodicidad de menos de un día en la edad de los huevos.

Una tercera parte de los huevos fueron separados como controles en tubos rotulados con la fecha y la marca 000 que nos indicaban la condición de testigos. Los huevos restantes, destinados a ser irradiados, se separaron en seis tubos diferentes con la dosis de radiación marcada. Hemos empleado 2000, 5000 y 10 000 R, usando radiaciones gamma y rayos X.

Se eligió el día 12° para la irradiación de todos los tubos, con lo que obtuvimos similar número de huevos de edades que oscilaron entre menos de 24 h del desove hasta 12 días de evolución post-desove, con una periodicidad de un día de evolución entre uno y otro.

Cada tubo conteniendo huevos de igual edad e idéntica dosis irradiada sirvió para separar tres lotes: uno fué destinado para fijarse a las 48 h de irradiado; otro para fijarse a los cinco días y el último para esperar la eclosión de la ninfa I y/o comprobar la no viabilidad del embrión. En el caso de nacer ninfas, estas fueron criadas y alimentadas en idéntica forma que los controles. También los huevos separados como testigos fueron procesados en iguales períodos de tiempo que los irradiados.

La experiencia fué realizada a temperatura constante de 27°C y las ninfas fueron alimentadas con conejillos de la India.

El líquido empleado para la fijación de los embriones fué el que hemos descrito en otro trabajo, consistente en una mezcla por partes iguales de alcohol metílico, ácido acético y cloroformo. Este reactivo tiene la particularidad de separar el opérculo del huevo, aumenta el volumen del contenido y fija las estructuras celulares, especialmente del núcleo. Los huevos sumergidos en este reactivo comienzan a abrir su opérculo a los 30 min. A partir de ese momento comienza a proyectarse el contenido hacia afuera.

Cuatro horas más tarde casi la mitad del contenido del huevo se encuentra afuera, lo que permite que con la ayuda de una pinza fina se extraiga el embrión cómodamente, el que ya está fijado y en condiciones de ser recogido el alcohol etílico puro. Posteriormente se hidrata para su coloración en bloque o se completa la deshidratación para su inclusión para cortes histológicos.

Los métodos de coloración empleados fueron la hematoxilina de Dalafield, la hematoxilina férrica, el método tricrómico según Van Gieson, el panóptico según Mallory y la impregnación argéntica según Bielchowsky.

RESULTADOS

No se observaron diferencia entre los huevos tratados con radiación gamma y los irradiados con rayos X, en cuanto a los resultados.

La totalidad de los huevos irradiados a 10 000 R interrumpieron el desarrollo embrionario, siendo la dosis letal para el embrión.

Las alteraciones citológicas observadas son picnosis, cariorexis, y cariolisis, mientras el citoplasma muestra vacuolización (figura 1). En cortes observados a pequeños aumentos se muestra alteración general de las estructuras (figura 2).

En los huevos irradiados a 5000 R se observó la muerte embrionaria en casi todos los más jóvenes (entre uno y cinco días de evolución de la oviposición), mientras que los más evolucionados presentaron retardo del nacimiento de la ninfa I, porcentaje alto y variable de letalidad y disminución fisiológica manifiesta de las ninfas que determinó la muerte de las mismas dentro de los tres primeros días de nacidas. En algunas se observaron alteraciones morfológicas consistentes en acortamiento disgenésico de las patas y alguna anomalía de los tergitos, esquematizada en la figura 3.

La disminución fisiológica más importante observada fué la incapacidad para alimentarse. Mientras en el lote testigo alrededor del 65% de las ninfas se alimentan dentro de los tres primeros días de

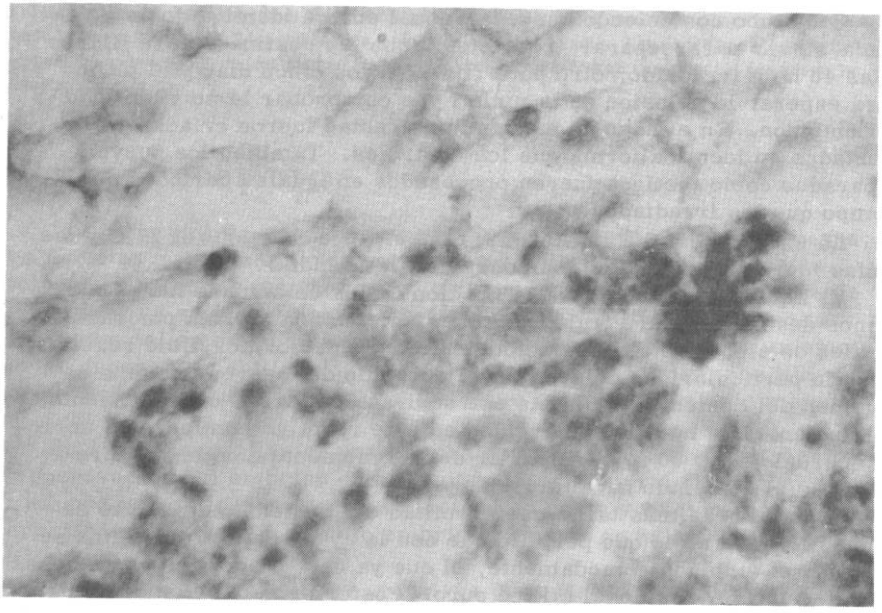


FIG.1. A mayor aumento se observan las alteraciones nucleares y citoplasmáticas.

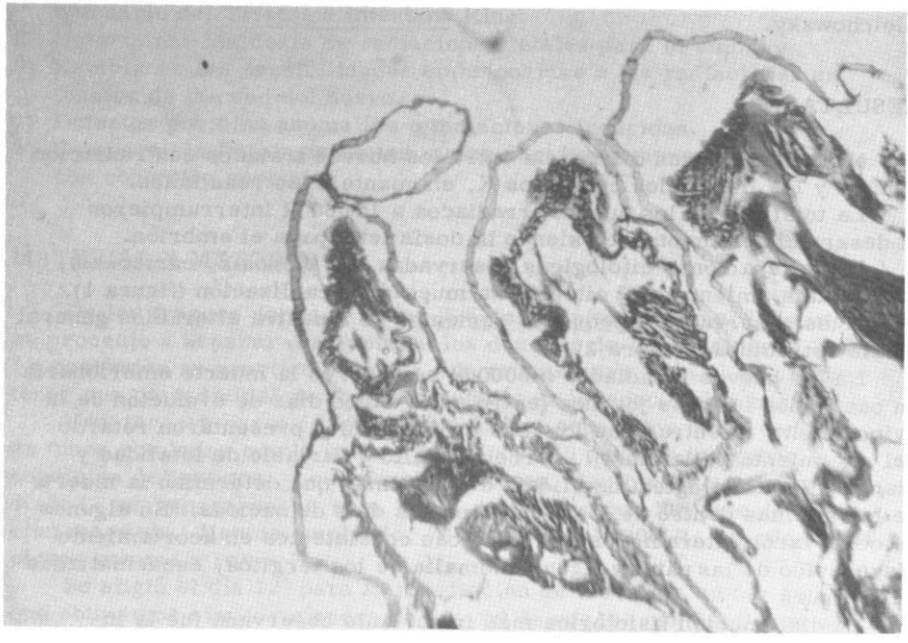


FIG.2. Fenómenos degenerativos en embrión de 14 días tratados con 10 000 R.

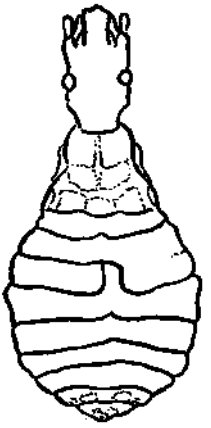


FIG.3. Malformación en tergitos de una ninfa nacida de un huevo irradiado a 5000 R (esquemático).

TABLA I. LETALIDAD EN EMBRIONES IRRADIADOS CON 5000R, EN RELACION AL TIEMPO DE EVOLUCION EMBRIONARIA EN EL MOMENTO DE SER TRATADOS

Edad del huevo en días	N° de huevos irradiados	N° ninfas nacidas	Embriones muertos	Letalidad (%)
1	80	0	80	100
2	80	0	80	100
3	75	0	75	100
4	60	0	60	100
5	100	4	96	96
6	100	9	91	91
7	50	5	45	90
8	50	6	49	88
9	80	16	64	80
10	100	31	69	69
11	50	15	35	70
12	100	39	61	61
Totales	925	125	800	85,4

TABLA II. LETALIDAD EN EMBRIONES IRRADIADOS CON 2000 R EN RELACION AL TIEMPO DE EVOLUCION EMBRIONARIA EN EL MOMENTO DE SER TRATADOS

Edad del huevo en días	N° de huevos irradiados	N° ninfas nacidas	Embriones muertos	Letalidad (%)
1	50	0	50	100
2	60	0	60	100
3	50	3	47	94
4	50	3	47	94
5	100	7	93	93
6	50	9	41	82
7	100	29	71	71
8	50	15	35	70
9	50	20	30	60
10	50	24	26	52
11	60	40	20	33
12	60	39	21	35
Totales	730	189	541	74,1

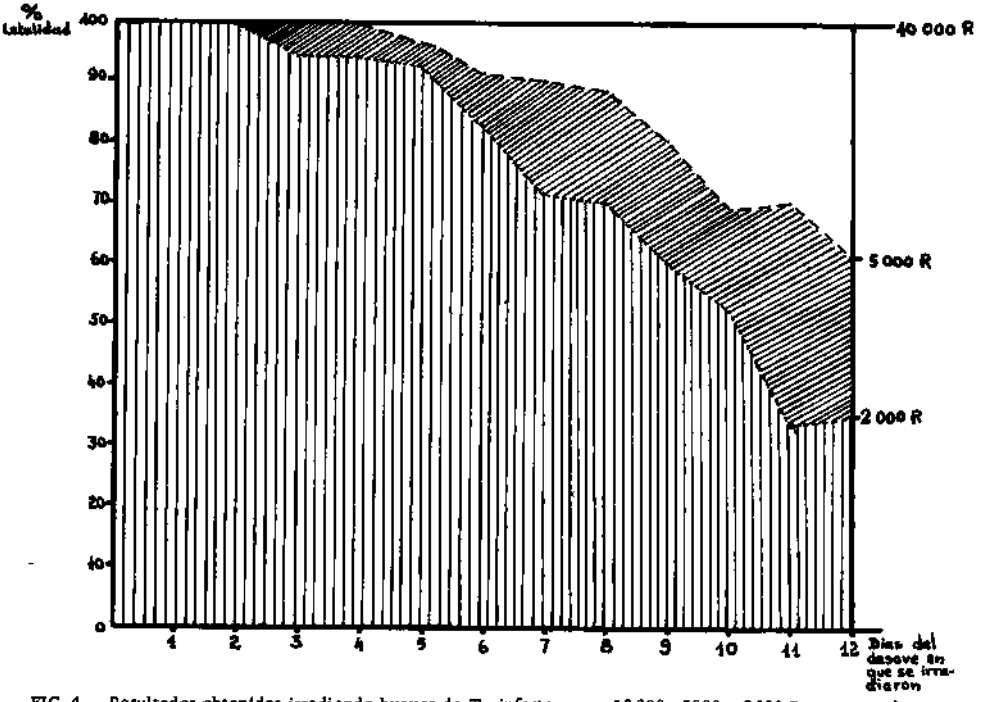


FIG. 4. Resultados obtenidos irradiando huevos de T. infestans con 10000, 5000 y 2000 R, comparados con testigos no irradiados. Letalidad embrionaria en relación a la edad del embrión cuando fue irradiado.

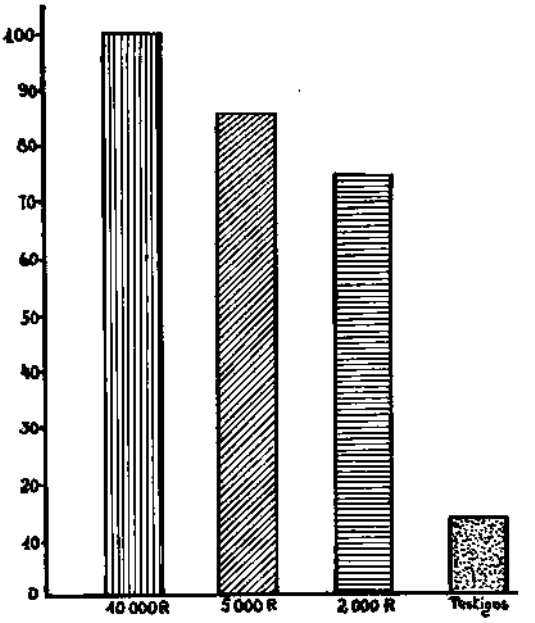


FIG. 5. Letalidad embrionaria general en irradiados a 10 000, 5000 y 2000, comparada con el grupo testigo.

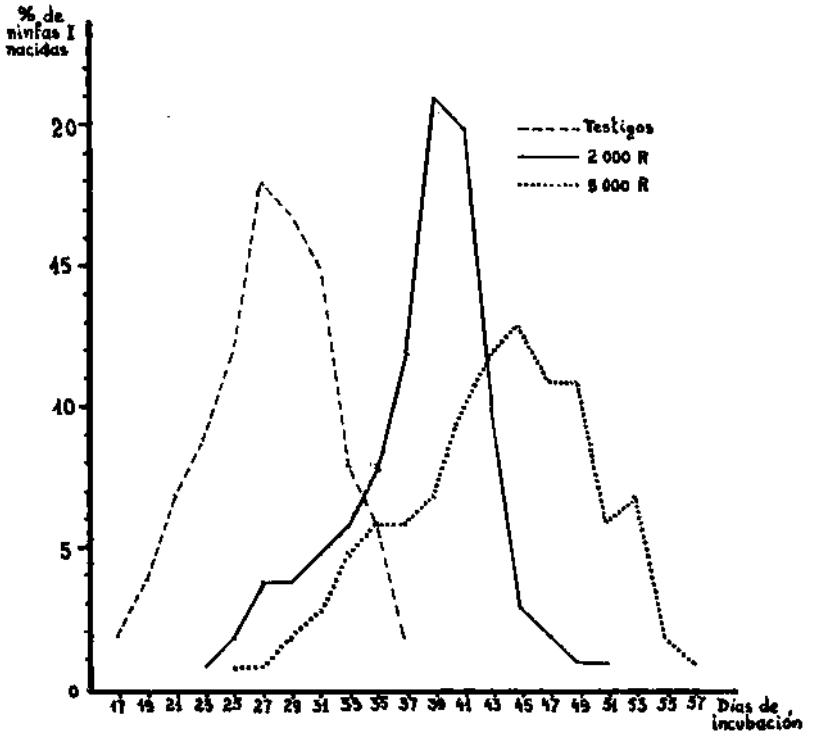


FIG. 6. Nacimiento de ninfas I a partir del día de desove en huevos viables, irradiados con 2000 y 5000 R y en no irradiados.

nacidas, en las irradiadas a 5000 R no se comprobó alimentación en ninguna de ellas.

La aplicación de dosis de 2000 R provoca similares consecuencias, aunque algo atenuadas: el porcentaje de viabilidad embrionaria es mayor, las ninfas I eclosionadas presentaron en pocos casos malformaciones congénitas y el 32% llegó a ninfa II. En este estadio se produce un fenómeno de disminución fisiológica que provoca la muerte, por imposibilidad de alimentarse. Los huevos más jóvenes irradiados a esta dosis son en su mayoría los que determinan el porcentaje de letalidad embrionaria antes expresada, ya que la eclosión de las ninfas por parte de huevos irradiados después de 10 días del desove difiere poco de lo observado en los especímenes del grupo testigo. En las tablas I y II se analizan los datos obtenidos en huevos irradiados a 5000 y 2000 R respectivamente. En las figuras 4, 5 y 6 se muestran los resultados obtenidos.

DISCUSION

La aplicación de radiaciones en dosis que varían entre 2000 y 10 000 R provocan alteraciones en el embrión que varían en función de la edad del huevo y de la dosis aplicada. Estas alteraciones van desde la muerte del embrión en forma más o menos inmediata, hasta una sobrevida que puede extenderse con apariencias de normalidad hasta las ninfas II. La incidencia de fenómenos mutagénicos está dentro de lo que podría esperarse en la hipótesis de trabajo, al igual que la letalidad observada.

Un fenómeno no muy claro es la incapacidad de alimentarse que se observó en algunas ninfas irradiadas en estado embrionario a 2000 R, hecho que se observó recién después de la primera ecdisis. La buena disposición a alimentarse de las ninfas I contrastó evidentemente con lo sucedido posteriormente. El estudio anatómico de las mismas no demonstró ningún tipo de alteración. La declinación fisiológica después de la ecdisis puede estar relacionada con indeterminados mecanismos hormonales, pero es significativo el hallazgo de una alteración de las proteínas linfáticas en las ninfas irradiadas, cuando se estudian mediante electroforesis. Intrigados por la explicación del fenómeno, decidimos realizar tiradas electroforéticas, aplicando la técnica utilizada para un trabajo anterior. Comprobamos que las ninfas II provenientes de huevos irradiados presentan una disminución global de las proteínas, pero especialmente muy manifiesta en el sector de velocidad de corrida correspondiente a las globulinas beta y gamma del suero humano que empleamos como patrón.

Esto podría explicar la alteración fisiológica de consecuencias letales, que si bien no impiden la ecdisis, altera la capacidad alimentaria de la ninfa II, pudiendo incluso considerarse la hipótesis de una alteración con la relación simbiótica.

La aplicación de dosis inferiores a 1000 R, que estamos realizando actualmente, se basa en la hipótesis de encontrar un mecanismo de regulación fisiológica lo suficientemente alterado para evitar la propagación de la especie, pero lo suficientemente conservado como para permitir el completo desarrollo de los especímenes. Gómez Núñez,

estudiando el Rhodnius proxilus, encuentra que la irradiación de huevos y ninfas provoca esterilidad completa pero acompañada de graves alteraciones morfológicas en los adultos que surgieron de ellos.

Es interesante también el retardo en la eclosión de las ninfas que están directamente en relación con las dosis empleadas, como se puede ver en la figura 6. Puede apreciarse que mientras en los testigos eclosionan el 94% de las ninfas entre los días 21 y 39 de la oviposición, en los irradiados con 2000 R sólo lo hacen el 63% en igual período, y en los de 5000 R sólo el 31%, tomando en los tres casos los porcentajes sobre el total de ninfas nacidas y descartando los huevos no viables.

CONCLUSIONES

1) Las radiaciones gamma y los rayos X afectan el desarrollo embrionario del Triatoma infestans.
2) Las alteraciones del desarrollo son tanto más profundas cuanto mayor es la dosis irradiada, siendo letales en el 100% de los embriones cuando se aplican 10 000 R, en un alto porcentaje cuando la dosis es de 5000 R y menor cuando es de 2000 R.
3) Las alteraciones del desarrollo son tanto más profundas cuanto más jóvenes son los embriones irradiados.
4) Es dable observar algunos fenómenos mutagénicos, con aparición de anomalías en las extremidades y en los tergitos de las ninfas I.
5) La muerte celular presenta microscópicamente figura de picnosis, cariorexis y cariolisis, acompañadas de vacuolización citoplasmática.
6) Las dosis experimentadas demuestran la sensibilidad embrionaria a las radiaciones, pero no parecen útiles para ningún tipo de control de estos vectores. Las actuales experimentaciones con dosis menores de 1000 R son promisorias, aunque se encuentran todavía en la primera etapa.
7) Existe un fenómeno de disminución fisiológica que se observa en las ninfas I de huevos irradiados a 5000 R y en las ninfas II de los irradiados a 2000 R, cuya consecuencia es letal.
8) En relación a lo anterior, se observa una disminución marcada de las proteínas linfáticas de estas ninfas, especialmente en el sector que corresponde en velocidad de corrida a las globulinas beta y gamma.

BIBLIOGRAFIA

BALDWIN, W.F., SHAVER, E.L., Radiation induced sterility in the insect Rhodinus prolixus, Can. J. Zool. 41 (1963) 637-48.

CARCAVALLO, R.U., A method to study Triatoma eggs and embryos (1970) (en prensa).

CARCAVALLO, R.U., CARABAJAL, C.A., Embryos of Triatoma infestans (1970) (en prensa).

CARCAVALLO, R.U., "Epidemiología de la enfermedad de Chagas y las posibilidades del control biológico", Srivastava Commemoration Volume, Izatnager, India (1970) 881-90.

D'ASCOLI, A., GOMEZ-NUÑEZ, J.C., Red-eye mutation in R. prolixus (Hemipt.-Reduv.), Am. Midland Naturalist 77 (1967) 222.

GOMEZ-NUÑEZ, J.C., GALLIMORE, J.C., FERNANDEZ, M.J., GROSS, A., El efecto de las radiaciones ionizantes sobre la biología y la ecología del R. prolixus, Acta cient. venez. 2 (1962) 46-52.

GOMEZ-NUÑEZ, J.C., GROSS, A., MACHADO, C., Radiaciones gamma y el comportamiento reproductivo del R. prolixus macho, Acta cient. venez. 15 (1964) 97-104.

DISCUSSION

F.M. WIENDL: Could you tell me why there is a delay in the eclosion of the nymphs? Your Fig.6 shows a great difference in the eclosion percentage from day 23 to day 31.

R.U. CARCAVALLO: I can only hazard a guess. The most feasible interpretation is that embryonic cells with endocrine functions are affected, with a consequent delay in development.

W.F. BALDWIN: You spoke about mutagenic damage from radiation, and I am wondering whether this damage occurred in irradiated individuals or in progeny of these individuals.

R.U. CARCAVALLO: The malformations occur in the nymphs from irradiated eggs.

W.F. BALDWIN: I had an idea that the damage might be malformations caused by the irradiation of cells which were later involved in the formation of appendages.

R.U. CARCAVALLO: I agree with you. It might be preferable to use the term 'teratological' instead of 'mutagenic'.

J.C. GOMEZ-NUÑEZ: It would be interesting to follow up this work by a study of the effect of radiation on symbiotic organisms, as the results suggest nutritional deficiencies in nymphs from irradiated eggs.

EFFECT OF GAMMA IRRADIATION ON IMMATURE SUGARCANE BORERS

D.W. WALKER, V. QUINTANA-MUÑOZ, F. PADOVANI
Puerto Rico Nuclear Center,
University of Puerto Rico,
Mayaguez, Puerto Rico

Abstract

EFFECT OF GAMMA IRRADIATION ON IMMATURE SUGARCANE BORERS.

The effect of gamma radiation was assayed in respect to induced sterility and mortality in the embryonic stage, the five larval stages and the pupal stage of the sugarcane borer Diatraea saccharalis (Fab.) (Lepidoptera: Crambidae). Radiosensitivity was greatest in embryos, followed by the larval stages. Each stage was progressively more radioresistant. Pupal radioresistance was greater, and resistance increased with age within the pupal stage. It is impractical to irradiate any immature stage for producing sterile adults because somatic damage is so severe that only a small proportion of individuals survive to the adult stage. Those few that survived were incapable of mating effectively.

INTRODUCTION

The objective of this experiment was to determine whether the sugarcane borer, Diatraea saccharalis (Fab.) would be sterilized with gamma radiation in the immature stages. Walker [1] reported that adults could be sterilized at relatively high exposures, i.e. 20 to 30 krad. His preliminary work showed that even exposure to low doses caused high mortality in larval stages. When irradiated larvae or pupae survived irradiation and completed development to adulthood they had low mating frequency and low fertility rates. The work reported here was undertaken to test these earlier observations in a more refined and systematic manner.

Gamma-induced sterility has been studied in many Lepidoptera. LaChance et al. [2] summarized much of this information. Judging from the publications available one would conclude that it is only practical to sterilize adults by treating the late pupal stages or the adults themselves. Although we know from personal knowledge that many workers have experimented with gamma-induced sterility of immature Lepidoptera, very few of these data have been published. Proverbs and Newton [3] report the effects of gamma exposure on immature life stages of the codling moth on the basis of developmental units. This is a measure of developmental rate that is time-, temperature-, and light-dependent. Unfortunately it is not an expression of stage sensitivity directly. Husseiny and Madsen [4] present data for the radiation effect on early life stages of the navel orangeworm and El Sayed and Graves [5] with Heliothis virescens. The general conclusions that can be made from these experiments are that the immature stages are relatively much more radiosensitive, and that post-treatment survival is low when larvae are irradiated.

We have attempted to evaluate this in a systematic manner by treating individuals of each immature life stage.

Vigorous, long-lived, productive, sexually-active adults with high genetic load are desired in a sterile-release program. Ideally these adults should out-live normal adults in nature, and they would be superior in

mating capability. Post-treatment survival, sexual performance, and detrimental genetic factors are the three important aspects to be considered when immature insects are to be sterilized. The genetic factors are the cause of the dominant lethality, but if survival is low, or if the sterile adults produced are ineffectual mating competitors, their useful contribution in an overflooding program is limited.

The various contributing factors to male sterility have been examined in detail by several workers. Cheng [6] has made an elegant analysis of these factors in the gray sugarcane borer (Eucosma schistaceans) while Flint and Kressin [7] have made an equally competent study with Heliothis virescens. Both works have emphasized the need to be aware of the precision of our objective: fertilization with death in the embryo. Females that have received spermatophores do not produce zygotes if there has been sperm immobility, faulty sperm transfer, or aspermia. Our approach to this problem has been considerably less profound, but more direct. We are fortunate that egg development is easy to observe, and the blastula stage can be identified in fertile eggs within 24 hours after oviposition. Thus our determination of sperm effectiveness is based upon fertilization rather than the suitability of the sperm itself. This is described in detail by Walker and Quintana [8].

MATERIALS AND PROCEDURE

The laboratory strain tested originated from material that had been collected as fifth instars and pupae in cane and corn from western Puerto Rico. The insects irradiated were grown on PRNC diet, or on corn stalk. See Walker [1] for a discussion of rearing methods.

The source used is a water-shielded 2 000 Ci cobalt-60 installation that has the exposure field in the centre. The dose rate was 2 000 R/min for all exposures. Since most of the exposure times were short the insects were not aerated during exposure.

Four adults were irradiated simultaneously in the one-ounce polyethylene exposure chamber. Up to 20 larvae, or 30 egg clusters were irradiated at the same time. The chamber was positioned inside a celluloid column which was in turn held in position by a polyethylene collar inside a weighted one-gallon polyethylene bottle. Dose was measured by the Fricke method. The position effect within the exposure chamber was measured using silver phosphor dosimeters and were read by a Turner Fluorometer. Variation within the chamber was less than $\pm 3\%$.

Individuals to be irradiated were selected and then isolated before placing them in the exposure chamber. They were removed immediately after exposure and returned to artificial diet if larvae, to the normal handling procedure if embryos or pupae, and were mated when they became adults. Mating containers were four-ounce polyethylene cups.

Five hundred embryos were irradiated in the black head (head capsule) stage. They were 72 hours old when treated. The larvae that emerged were collected twice daily as they hatched. Individuals to be treated as larvae were removed from food and the developmental stage was determined by measuring the width of the head capsule. Fifty larvae of each stage were treated. Larvae were removed from the food for irradiation and immediately returned to food after irradiation. Larvae were examined

TABLE I. STERILITY AND MORTALITY IN IRRADIATED EMBRYOS[a]

Dose (kR)	% Mortality corrected	Stages of death[b]	Comments
0	100	None	30 days dev't. time
0.5	0	E, adult, pupae, 5, 4	45-60 days to death
1	0	E, adult, pupae, 5 prepupal	50-70 days
2	0	E, adult, pupae, 5 prepupal	None mated
4	0	E, 5, pupae, prepupal	None mated
6	0	E, 5, 4	None pupated
8	0	E, 3, 2, 1	None pupated Lived 10 days
10	0	E, 2, 1	None pupated Lived 8 days
12	0	E, 1, 2	None pupated Lived 5 days

[a] 72 hours old, 500 individuals at each dose

[b] Larval stages: E = embryonic, 1 = first, 2 = second, 3 = third, 4 = fourth, 5 = fifth, prepupal

every fifth day after irradiation, and the dead larvae were removed from the food. Larvae were considered to be dead when they failed to respond to the tactile stimulation of an artist's brush. Pupae were examined daily after irradiation until the fourteenth day. By the end of this period all the living insects had emerged as adults.

Matings were made as individual pairs in four-ounce plastic cups. The treated individual was paired with a normal mate within 24 hours of emergence. Each pair was held in the dark for 48 hours to ensure high mating frequency. All females were dissected after death to determine the number of times that each had mated; but fertility of eggs was only calculated on the basis of egg development as described previously [8].

In our method of rearing it was impossible to select larvae of uniform age within the particular developmental stage. This does not present difficulties except in the fifth stage. Young fifth instars are more radioresistant than those which have already begun prepupation tissue changes. Fifth instars were selected by stage on the basis of head capsule width and overall size, and within the stage they were selected on the basis of their activity: inactive or sluggish individuals were not selected for treatment.

RESULTS

Embryos (Table I)

Approximately 80% of the 500 normal embryos completed development to the adult stage, and of these nearly 50% of the females mated and laid fertile eggs. Development time to the adult stage was 30 days or less. Among the irradiated embryos a large number died in the embryonic stage,

TABLE II. MORTALITY OF IRRADIATED LARVAE

Dose (kR)	Stage irradiated[a]									
	First		Second		Third		Fourth		Fifth	
	% Survival[b]	Stage of death[c]	% Survival	Stage of death	% Survival	Stage of death	% Survival	Stage of death	% Survival	Stage of death
0	100	A, P	100	A, 5	100	A, 5	100	A, 5	100	A, 5
0.8	12.5	A, 5, P	96.5	P	100	A, P	100	A, P	100	A, P
3.2	2.5	P, 5	89.4	P	96.5	P, A	100	A, P	84.2	A, P
6.4	2.5	5, 1	23.5	P, A	58.8	P, A, 5	94.1	A, P	52.6	A, P, 5
8.0	17.5	3, 1	16.5	5, P	35.3	P, A, 5	82.4	A, P, 5	42.1	P, A, 5
8.8	0	3, 2, 1	0	5, 4, 3	29.4	P, 5, A	82.4	A, P, 5	42.1	P, A, 5
9.6	0	2, 3, 1	0	5, 4, 2	0	5, P, 4	58.8	P, 5, 4	31.6	5, P, A
10.4	0	2, 1	0	4, 3, 2	0	4, 5, P	47.1	5, P, 4	31.6	5, P, A
12.8	0	2, 1	0	3, 2	0	3, 4, 5	35.3	5, 4, P	21.1	5, P
16.0	0	2, 1	0	2, 3	0	3, 4	23.5	4, 5, P	0	5, P
25.0	0	1	0	2	0	3, 4	0	4, 5, P	0	5, P

[a] Explanation of symbols:
1 = first larval stage
2 = second larval stage
3 = third larval stage
4 = fourth larval stage
5 = fifth larval stage, prepupal stage
P = Pupa
A = Adult

[b] 50 larvae treated at each dose; mortality corrected (Abbot)

[c] Stage at which the death occurred in descending order

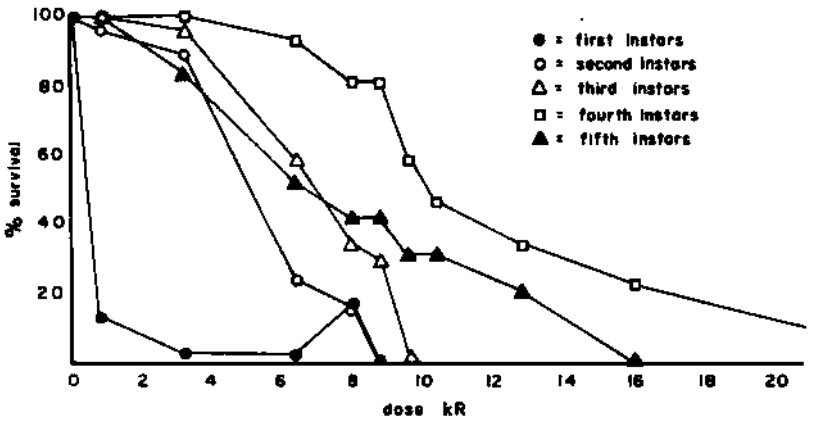


FIG.1. Larval survival at different doses.

i.e. they failed to hatch, and development time was longer than the unirradiated embryos. There was high mortality in the embryonic stage at all doses. None of the adults produced eggs that hatched when mated to normals, although some of the eggs that were laid were fertile. At 4.0 kR and above none survived to the adult stage. Because of early death life-span was shortened at 4.0 kR and above, but at 1.0 and 2.0 kR development time was prolonged to as long as 70 days.

Larval stages (Table II, Fig.1)

Survival was reduced by radiation at all doses, and development time between larval stages was doubled or tripled. Adults were harvested from only the four lowest doses (8.0 kR and below), but they failed to mate. Above 8.8 kR none survived beyond the third larval stage even though some of these lived as long as 40 days. At 25 kR all irradiated larvae died before reaching the second larval stage.

Non-irradiated larvae

Survival of non-irradiated larvae to the adult stage was high regardless of stage. Of the 50 larvae of each stage 40 first instars survived to become adults, 32 second instars, 32 third instars, 32 fourth instars, and 44 fifth instars. Even among the normal individuals there is a range in time spent in each developmental stage. The averages for each stage in normals was first 3 days; second 4 to 5 days; third 4 to 6 days; fourth 5 to 8 days; fifth 5 to 8 days; and pupal stage 5 to 6 days. Thus the minimum time that could be expected from first instar to adult is 26 days, from second to adult is 23 days, third to adult is 19 days, fourth to adult is 15 days, fifth to adult 10 days. Averages for developmental rate of non-irradiated individuals were actually about one-fourth greater than these minimum time periods.

TABLE III. MORTALITY AND STERILITY OF IRRADIATED PUPAE

Sex	Dose kR	Number tested	Number survived	% Survival to adult [a]	% Survivors mated [b]	Fertile eggs per female	% of fertile eggs hatched
Pupae of 0-3 days age when irradiated							
M	0	28	25	100.0	90.3	276.0	96.0
M	2	25	10	44.3	40.0	120.0	66.5
M	4	23	8	38.5	34.8	73.5	0
M	6	22	4	20.2	18.2	63.0	0
M	9	32	0	0	0		
M	12	40	0	0	0		
M	16	42	0	0	0		
M	25	21	0	0	0		
F	0	21	17	100.0	81.0	295.0	92.1
F	2	16	10	77.2	62.5	214.0	1.4
F	4	21	13	75.8	61.4	156.8	0
F	6	29	9	37.0	30.0	63.0	0
F	9	26	3	14.2	11.5	18.0	0
F	12	22	0	0	0		
F	16	28	0	0	0		
F	25	20	0	0	0		
Pupae of 3-4 days age when irradiated							
M	0	21	19	100.0	90.5	304.8	95.1
M	2	15	11	81.0	73.3	71.3	43.9
M	4	14	4	30.5	27.6	31.3	0.9
M	6	12	2	18.5	16.7	24.5	0
M	9	18	6	36.8	33.3	13	0
M	12	17	0	0	0		
M	16	20	0	0	0		
M	25	15	0	0	0		
F	0	19	16	100.0	84.2	296.3	91.1
F	2	11	6	64.8	54.6	106.0	6.3
F	4	10	6	71.3	60.0	83.0	0
F	6	14	5	84.8	71.4	29.0	0
F	9	13		0	0		
F	12	15		0	0		
F	16	17		0	0		
F	25	16		0	0		

TABLE III (cont.)

Sex	Dose kR	Number tested	Number survived	% Survival to adult[a]	% Survivors mated[b]	Fertile eggs per female	% of fertile eggs hatched
Pupae 4-5 days age when irradiated							
M	0	23	20	100.0	87.0	300.5	86.4
M	2	21	18	98.5	85.7	101.0	54.4
M	4	20	13	74.7	65.0	123.0	29.5
M	6	28	24	98.5	85.7	68.2	0
M	9	29	23	91.1	79.3		
M	12	27	20	85.1	74.0		
M	16	30		0	0		
M	25	32		0	0		
F	0	30	28	100.0	93.3	298.9	96.8
F	2	16	8	53.6	50.0	268.3	45.0
F	4	15	12	85.7	80.0	93.0	25.8
F	6	18	9	53.6	50.0	13.0	15.4
F	9	27	18	71.5	66.7	14.5	0
F	12	29	22	81.4	75.9		
F	16	31		0	0		
F	25	20		0	0		

[a] Corrected (Abbot)
[b] Percentage of survivors that mated

Irradiated larvae

The two effects that can be attributed to irradiation are mortality before becoming adults and prolongation of development time.

None of the irradiated larvae produced offspring upon reaching the adult stage, and mating frequency of these adults was very low.

First instars: At 8.8 kR and above none of the irradiated first instars survived beyond the third larval stage. At 0.8, 3.2, 6.4, and 8.0 kR 5, 1, 1, and 14 larvae survived to become adults. None of these adults mated. Duration of development time in each stage was twice or three times as long as the unirradiated larvae.

Second instars: Larvae survived to become adults at 0.8 to 8.0 kR, and none of these adults mated. At 8.8 kR and above no larvae survived beyond the fifth stage. Larval development times were considerably longer in the dose range 0.8 to 8.0 kR.

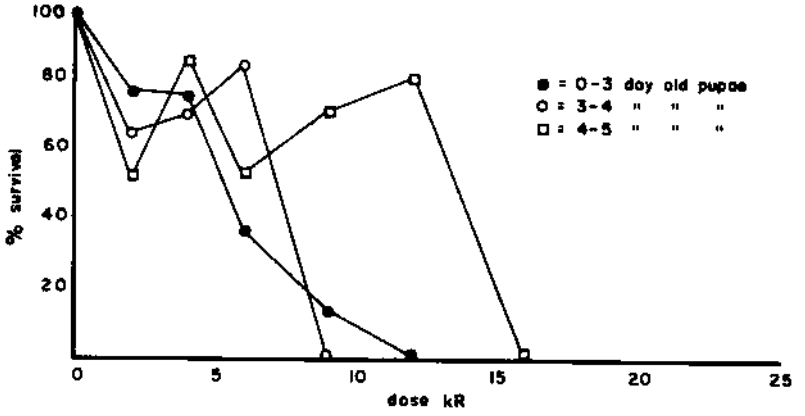


FIG. 2. Percent survival of irradiated female pupae.

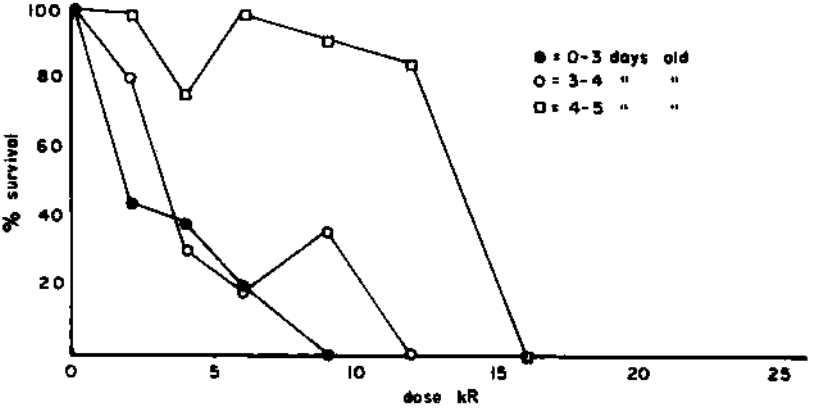


FIG. 3. Percent survival of irradiated male pupae.

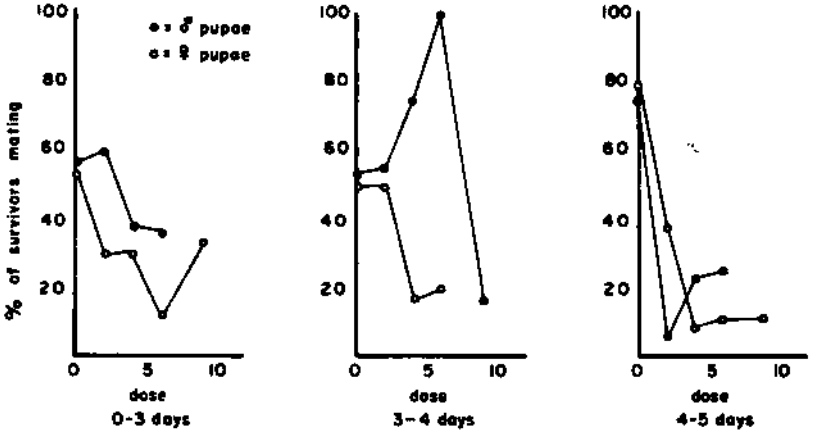


FIG. 4. Percent mating of irradiated survivors of irradiated pupae.

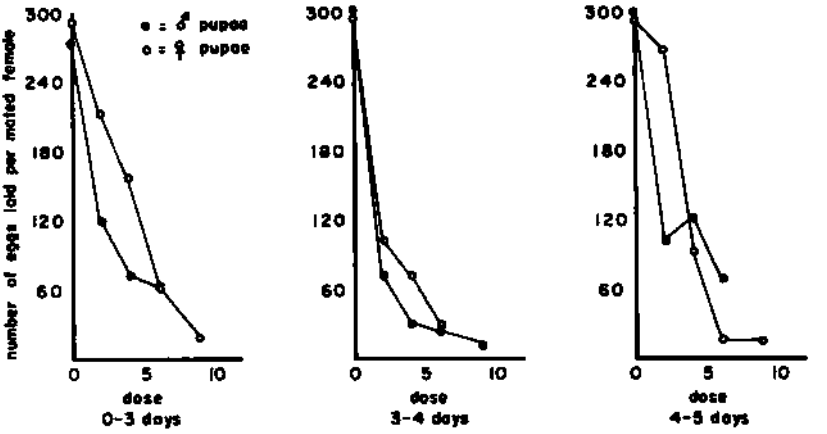


FIG. 5. Number of eggs laid per female that mated from irradiated pupae.

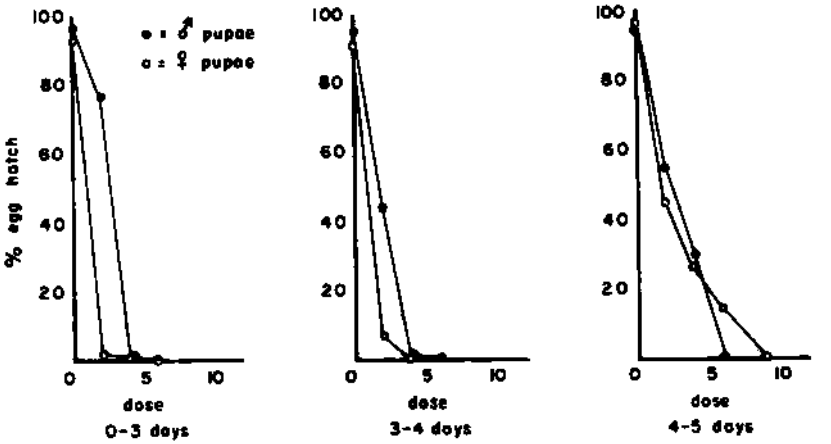


FIG. 6. Percent of fertile eggs that hatched from irradiated pupae.

Third instars: Adults were produced from larvae irradiated at 0.8, 3.2, 6.4 and 8.8 kR as follows: 42, 41, 25, and 12. None of these adults mated. Development time in each stage was nearly three times as long in all individuals that survived the radiation at doses above 3.2 kR. A small proportion of the larvae treated at 8.8 and 9.6 kR reached the fifth stage, but at 10.4 kR and above no larvae lived beyond the fourth stage.

Fourth instars: Larvae survived to the adult stage after exposure to 16.0 kR or less, but none of these adults mated. Progressively more survived than third instars at the same doses in the 0.8 to 8.8 kR range, and there were more survivors to the adult stage at all the dosages in comparison to third instars when compared on the basis of survival.

Development times were 1½ to twice as long as non-irradiated larvae. In relation to previous stages fourth instars have greater survival at equivalent doses.

Fifth instars: Larvae developed to the adult stage from the 12.8 kR treatment and from lower doses. Relative survival at equivalent dose was about one-half that of fourth instars. Development time is more difficult to evaluate in this group, and death was common in the stage irradiated.

Pupae (Table III, Figs 2-6)

Male pupae of 0 to 3 days age survived to become adults at treatments of 6 kR or less, but mating frequency was low in these survivors and the number of fertile eggs produced by the normal females to which these treated males were mated was low.

Female pupae of 0 to 3 days age were relatively more radioresistant than males of the same age. Females survived 9 kR or less to become adults, but the number of fertile eggs produced was greatly reduced by treatment of 6 to 9 kR.

Male pupae of 3 to 4 days age had higher survival rates and higher mating frequency than comparable males of the younger age group with lower rates of production of fertile eggs at equal doses.

Female pupae of 3 to 4 days age did not lay as many fertile eggs as comparable females at the same dosage in the 0 to 3 day group. None of the females exposed to 9 kR mated. Females of this age group were less radioresistant than the younger females.

Male pupae of 4 to 5 days age survived, mated and the normal females to which they mated produced fertile eggs at dosages of 6 kR and less. At 9 kR and 12 kR males mated, but no fertile eggs were produced.

Female pupae of the 4 to 5 days age group survived, mated, and produced fertile eggs after exposure to 9 kR or less. At the 12 kR exposure they survived, but none mated.

Pupal development time was at least twice as long as the development time in unirradiated pupae.

DISCUSSION

Embryos

Very low doses of radiation, as low as 0.5 kR, cause severe damage as evidenced by the failure of the survivors to produce offspring. The developmental period is greatly extended, probably owing to inhibition of cell division. And this is for an insect that requires over 20 kR to produce complete sterility in adults [1]. The damage caused by these low doses seems incomprehensible. However, we must assume that embryonic cells are rather unspecialized and that damage to very few cells could alter several tissues arising from the damaged embryonic cells. This hypothesis is further strengthened by the fact that doses of 6 kR and above prevented all surviving individuals from pupating.

Two factors have to be considered in the irradiation of embryos: (1) duration of larval life, and (2) survival to adulthood. At very low doses (up to 4 kR), larval life is lengthened (for the reason already mentioned) and some individuals reach adulthood. These adults were useless since they had difficulties mating and those that mated produced no embryonated eggs. The higher doses (above 4 kR) are very effective in reducing larval lifespan. If we assume that originally the larval lifespan was increased as a result of mitotic inhibition we must conclude that the shortening of lifespan is caused by the same phenomenon. Very short larval lifespan is probably caused by total inhibition of cellular division accompanied by severe cytological damage at the higher doses.

Larvae

The most radiosensitive larval stages are the first and the second, even though the second is considerably more resistant than the first (Table II). As with embryos, very low doses (0.8 kR) are damaging enough to reduce survival to adulthood by at least 90%. However, there is a steady increase in radioresistance with age up to, but not including, the 5th instars. Originally, the fact that 5th instars were more radiosensitive than 4th instars was surprising. After examining the survival data on our control colony we found that only under the best conditions were 5th instars capable of surviving to the pupal stage. In fact mortality in our laboratory colony is more common in the 5th stage. This suggests that individuals in this particular stage are intrinsically weak and probably the weakest link in normal development. If this is the case, it should not be surprising that 5th instars are more radiosensitive than 4th and probably 3rd instars.

Survival to adulthood as a measure of radioresistance could be misleading if the survivors were to be used in a sterile-release program. High adult survival rates were obtained when fourth instars (the most radioresistant) were irradiated at or below 6.4 kR. These adults were incapable of mating, which is a very undesirable characteristic. A more meaningful basis of comparison would be the yields of sexually competent but sterile adults. On this basis all larval stages must be considered as very radiosensitive since even the lowest treatments greatly impaired the mating capacity in all larval stages.

First, second, and third instars have a narrow range of tolerance to radiation damage, but some fourth and fifth instars were able to reach adulthood after treatments of 12 kR or higher. This can be explained in terms of chronological age within stage. The fourth and fifth stages are probably the longest stages in the life cycle. Since our experiment is based on larval stage rather than age, more variation in age within the stage would be expected in the older groups, the younger individuals within a stage being more radiosensitive.

Pupae

Using several criteria for radioresistance, the pupal stage appears to be the least sensitive of all the stages studied. The trend of increasing radioresistance with age holds true here also since pupae are more radioresistant in terms of survival to adulthood than any of the other stages tested (Figs 2 and 3). On this same basis radioresistance increases with age

within the stage. It has been demonstrated that the adult is the most radioresistant of all stages [1]. Since the dose required to reduce survival considerably in 4 – 5-day-old pupae is quite high one could suspect that pharate adults were irradiated. However, Walker [1] has observed that the mating frequency increases when adults are irradiated at low doses and this did not happen when 4 – 5-day-old pupae were irradiated. In fact, a marked decrease in adult mating frequency was observed when pupae of one age were irradiated.

A successful sterilization program must provide high yields of individuals capable of laying high numbers of embryonated eggs with enough dominant lethals to cause early death. This cannot be accomplished by irradiating pupae since egg production is sharply reduced even by low radiation treatments (Fig. 5). In irradiated female pupae egg damage prevents high production of eggs while in males irradiation causes either aspermia or immotile sperm. The number of embryonated eggs and the total number of eggs produced by normal females mated to the surviving irradiated males demonstrate that immotile sperm or aspermia is the main cause.

CONCLUSION

It is immediately evident from our discussion that irradiation of immature stages of D. saccharalis fails to meet all the requirements for an effective sterility program. Irradiation of larvae not only produces low yields of adults, but also lengthens the life cycle tremendously. Moreover, the few adults produced are sexually incompetent. Moderate yield of fairly competent adults can be obtained by irradiating pupae, but the yield is sharply reduced at the sterilizing dose, and sperm are defective. We must therefore conclude that it is impractical to irradiate immature stages with the purpose of using them in a sterile-release program.

REFERENCES

[1] WALKER, D.W., "Potential for the control of the sugarcane borer through radio-induced sterility", Radiation, Radioisotopes and Rearing Methods in the Control of Insect Pests (Proc. Panel Tel Aviv, 1966), IAEA, Vienna (1968) 131-40.

[2] LaCHANCE, L.E., SCHMIDT, C.H., BUSHLAND, R.C., "Radiation induced sterilization", Pest Control (KILGORE, W.F., BOUTT, R.L., eds.), Academic Press, New York (1967) 148-96.

[3] PROVERBS, M.D., NEWTON, J.R., Influence of gamma radiation on the development and fertility of the codling moth, Carpicapsa pomonella (L.) (Lepidoptera: Olethreutidae), Can. J. Ent. 40 (1962) 401-20.

[4] HUSSEINY, M.M., MADSEN, H.F., Sterilization of the navel orangeworm, Paramyelois transitella (Walker) (Lepidoptera: Phycitidae), by gamma radiation, Hilgardia 36 (3) (1964) 113-37.

[5] EL SAYED, E.I., GRAVES, J.B., Effects of gamma radiation on the tobacco budworm. III. Irradiation of eggs and larvae, J. econ. Ent. 62 (1969) 296-8.

[6] CHENG, Wen-yi, Components of the sterility induced in late-stage male pupae of the sugarcane gray borer, Eucosma schistaceana (Lepidoptera: Olethreutidae), with gamma radiation, Ann. ent. Soc. Amer. 62 (5) (1969) 1106-13.

[7] FLINT, H.M., KRESSIN, E.L., Transfer of sperm by irradiated Heliothis virescens (Lepidoptera: Noctuidae) and relationship to fecundity, Can. Ent. 101 (1969) 500-07.

[8] WALKER, D.W., QUINTANA-M.V., Mortality stageing of dominant lethals induced in the F. generation of the sugarcane borer, Diatraea saccharalis (F.), Radiat. Res. 36 (1) (1968) 138-43.

SOME GAMMA-IRRADIATION EFFECTS ON SURVIVAL, LONGEVITY AND REPRODUCTION OF Zabrotes subfasciatus (BOH.)

F.M. WIENDL
Nuclear Energy Center for Agriculture,
College of Agriculture "Luiz de Queiroz",
Piracicaba, São Paulo, Brazil

Abstract

SOME GAMMA-IRRADIATION EFFECTS ON SURVIVAL, LONGEVITY AND REPRODUCTION OF Zabrotes subfasciatus (BOH.).

Gamma-irradiation effects (from a ^{60}Co source) on Zabrotes subfasciatus (Boh.) (Coleoptera: Bruchidae) were studied to determine efficient doses for controlling the worst pest insect attacking stored beans (Phaseolus vulgaris L.), the largest source of proteins in Brazil.

Five dosages were used for the irradiation of 0 - 24-hour eggs: 0.5, 1, 2, 5 and 10 krad; there was no emergence at the 2-krad dosage.

Three-day-old larvae (8 days after oviposition) and 2 - 3-day-old pupae (19-20 days after oviposition) were irradiated with doses of 1, 2, 5, 10 and 20 krad. The larvae irradiated with 5 krad and the pupae irradiated with 20 krad did not emerge into adults. With the low irradiation doses the time elapsing before emergence into adults was greater than that for the control, and adults developing from pupae irradiated at 5 krad were infertile.

1 to 2-day-old adults irradiated with doses of 1, 2, 5, 10 and 20 krad became non-fertile from 10 krad upwards. Irradiated with 14 cumulative doses of 25 krad, there was an increase of 6 days in the longevity with the 25-krad dose, which decreased gradually with the increase of the doses, the adults irradiated with 350 krad living only 24 hours.

In order that the results could be compared with confidence the same dose rate was used throughout, namely 19.5 krad/h, with the exception that for the determination of the effect of radiation on the longevity of adults a dose rate of 105 krad/h was used.

From the results obtained it was concluded that irradiation of this bruchid is highly effective with relatively low doses, which do not cause side effects on beans, as proved by field and laboratory sprouting, survival, growth and production tests.

INTRODUCTION

In Brazil Toledo [1] estimated the losses caused by weevils in stored beans to be 20%. Rossetto and Carvalho [2] stated also that Zabrotes subfasciatus (Boh.) is the species which does most damage in warehouses and silos.

For many years the effectiveness of irradiation at sterilizing doses for controlling insect pests in grains and stored products has been known. However, most of the work on this subject has been carried out in technologically advanced countries, which do not have this bean weevil species.

In fact, in the advanced countries beans are very rarely used for human consumption; the 'per capita' consumption varies from 3 to 16 g/day, while in Brazil in the areas with relatively well fed population the consumption is 68 g/day [3]. Perhaps this is the reason why kwashiorkor, or protein hunger, is relatively rare in the country. The disease exists, however, in the slums and in the zone of the Brazilian northeastern states with abundant vegetation, between the coast and the interior infertile areas [4, 5]. To halt the destruction of beans in this zone — a food which provides proteins

with lower value than the animal proteins but which is the only protein source for a large part of the population – work was undertaken to determine such factors as the sterilizing doses and the lethal effect.

MATERIAL AND METHODS

Irradiation source and dose rate

A cobalt-60 source with approximately 1000 Ci was used for the irradiation. Because the dose rate is of great importance in this kind of work, it was kept at 19.5 krad/h throughout the experiments [6], except in a test to determine the lethal dose, in which 105 krad/h was used. The insects were collected from a stock of many generations reared in the laboratory.

All the tests, including irradiation and post-irradiation rearing, were run in vials 37 mm high and 44 mm diameter, covered with paper with a number of holes in it. During irradiation these covers were replaced by polyethylene plastic ones and the unused space was filled with cotton. This care was taken to avoid injuries by the moving of the grains and, in the case of irradiation of imagoes, to avoid changes in the way the beans were positioned.

Post-irradiation rearing tests were run at 30°C and 70-72% r.h.

Irradiation of eggs

Eggs aged 24 to 48 hours were used. The doses were of 0, 0.5, 1, 2, 5 and 10 krad. Each dose was replicated 4 times, with 100 to 300 eggs for each replication. The sterilizing dose was determined by post-irradiation count of the eggs, which either turned white, indicating origination of a larva, or dried out, indicating sterility.

Also determined were the time for emergence of the first adult, and the number and oviposition of all the adults emerging from irradiated eggs.

Irradiation of larvae

Eight days after oviposition the larvae were irradiated, i.e. 3-day-old larvae. The doses were of 0, 1, 2, 5, 10 and 20 krad, the number of larvae varying from 25 to 60 for each replication, with 4 replications at each dosage.

The lethal dose for larvae was determined by a post-irradiation count of the emerged adults. Also determined were the number of days required for emergence of the first adult, as well as its oviposition and fertility.

Irradiation of pupae

The pupae were irradiated 19 - 20 days after oviposition. The doses used were of 0, 2, 5, 10 and 20 krad. There were 25 pupae for each replication and 4 replications for each dosage. The number of adults emerging and their oviposition were also determined.

TABLE I. RADIOSENSITIVITY OF EGGS

Dose (krad)	No. of eggs irradiated	% of eggs hatched	% of emerged adults	No. of fertile eggs laid by adults	No. of days for emergence of 1st adult
0	562	84.16	72.77	11240	23
0.5	774	63.95	51.55	466	29
1	567	9.88	0.53	0	32
2	980	0	0	0	-
5	799	0	0	0	-
10	648	0	0	0	-

TABLE II. RADIOSENSITIVITY OF LARVAE

Dose (krad)	No. of larvae irradiated	% of emerged adults	No. of fertile eggs laid by adults	No. of days for emergence of 1st adult
0	151	86.09	2513	19
2	163	7.36	72	23
5	133	0	0	-
10	152	0	0	-
20	152	0	0	-

TABLE III. RADIOSENSITIVITY OF PUPAE

Dose (krad)	% of emerged adults	No. of fertile eggs laid by adults
0	100	2613
2	91	87
5	8	0
10	5	0
20	0	0

Irradiation of adults

Two tests were carried out to determine the sterilizing dose for adults, one for males and one for females. All the insects were taken out of the beans, with a needle, to make sure that they were unmated. The doses were 4, 6, 8 and 10 krad, with 15 males for each dose, in 3 replications.

To determine the sterilizing dose for the males, they were paired, after irradiation, with 24-hour-old unmated normal females, and from their oviposition the fertility was determined. The same process was repeated, but vice versa, i.e. irradiated females with unmated normal males, always with a count of the fertile and sterile eggs.

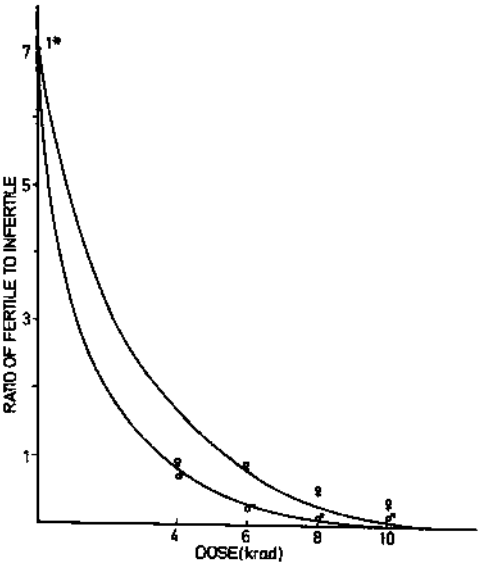


FIG. 1. Ratio between fertile and infertile eggs laid by irradiated females mated with normal males (♀♀ curve) and by normal females mated with irradiated males (♂♂ curve).

To determine the lethal dose for imagoes two tests were made, with mortality counts taken daily. The first test consisted of irradiation at 0, 2, 5, 10 and 20 krad; the second one of irradiation at cumulative doses from 25 to 350 krad, with a dose rate of 105 krad/h. Daily counts were made until all the individuals were dead.

RESULTS AND DISCUSSION

Table I shows the data obtained from the irradiation of the eggs.

Table II gives the results obtained from the irradiation of the larvae, which, compared to the eggs, were found to be slightly more resistant.

Table III is a summary of the results obtained from irradiating the pupae, showing a slight increase in the resistance to gamma radiation.

Figure 1 shows the ratio of fertile to infertile eggs laid by irradiated females mated with normal males (♀♀ curve) and by normal females mated with irradiated males (♂♂ curve). From this it can be seen that the males are more radiosensitive than the females. There was complete sterilization of the males at 10.5 krad and of the females at 11.8 krad. Point 1* in the curve was obtained by calculating the results obtained from the controls for the other tests. The relatively low doses causing sterilizing and lethal effects on eggs, larvae and pupae were observed with due consideration to the work of several different authors on species of the same family [7-10].

The longevity of irradiated adult insects undergoes an increase of up to 6 days at a dose of 10 krad as compared with the control, and to judge from the longevity test this insect is highly resistant as compared to other species of the same family. A continuous decrease in longevity, down to only 24 hours at 350 krad, is observed (Fig. 2).

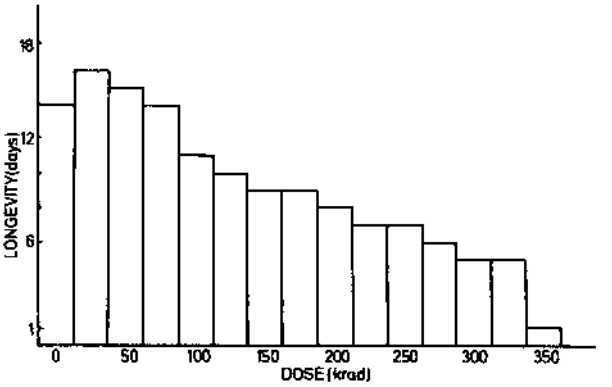


FIG. 2. Longevity of adults as a function of dose.

CONCLUSIONS

For Zabrotes subfasciatus (Boh.), irradiation is a highly efficient method and, as proved by some sprouting, survival, growth and production tests of the beans, these doses do not bring about morphological changes in the plant development, mainly because they are relatively low.

It is clear that there is still much to be done in the commercial application of this method in Brazil but there is no doubt as to its efficacy as a means of solving the problem of stored beans, without causing any problems with the accumulation of toxic insecticide residues. The only inconvenience, already long known, is the easy reinfestation, and to avoid this, the beans must be stored in suitably protected places.

ACKNOWLEDGEMENTS

The author wishes to express his sincere thanks to Prof. Admar Cervellini, of CENA – Centro de Energia Nuclear na Agricultura (Center for Nuclear Energy in Agriculture) – for the facilities provided during the work, to Prof. Domingos Gallo for his suggestions, and to Dr. Moshe Calderon for the initial ideas which originated the study.

REFERENCES

[1] TOLEDO, A. A., Chacaras Quint. 74 2 (1946) 184.
[2] ROSSETTO, C. J., CARVALHO, R. P. L., Bras. Ent. 13 (1968) 105-17.
[3] AYKROYD, W. R., DOUGHTY, J., Las Leguminosas en la Nutricion Humana, FAO, Rome (1964) 156 pp.
[4] ANDRADE, E., BETSUEM, J., Realidade, São Paulo 29 (1968) 146-60.
[5] CASTRO, J. de., Geopolítica da Fome, Brasiliense (1961) 186 pp.
[6] VEREECKE, A., PELERENTS, C., Meded. LandbHoogesch. OpzoekStns Gent XXXII 3/4 (1967) 890-903.
[7] NEHARIN, A., CALDERON, M., YACOBI, S. O., Israel Atomic Energy Commission IA-1010 (1965) 11 pp.

[8] QURAISHI, M.S., METIN, S.M., in: Radiation and Radioisotopes Applied to Insects of Agricultural Importance (Proc. Symp. Athens, 1963), IAEA, Vienna (1963) 479-84.
[9] HUQUE, H., KAHN, M.A., Fd Irrad. 4 3 (1964) A2-A7.
[10] HOWE, R.W., CURRIE, J.E., Bull. ent. Res. 55 3 (1964) 437-77.

DISCUSSION

C. BORGHI: The inhibition dose of 5000 rad seems considerably lower than the 8000 rad encountered in other cases. Is this due to a weakness of the species, or is there perhaps some doubt as to accuracy of measurement of the doses?

F. M. WIENDL: This weevil is particularly sensitive to gamma radiation. I am quite sure that the dose was measured correctly; we have a very good dosimeter.

CHAIRMEN OF SESSIONS

Session I	M.E. TZANAKAKIS	Greece
Session II	E.A. TAYLOR	United States of America
Session III	J.W. WRIGHT	World Health Organization
Session IV	Rachel GALUN	Israel
Session V	T. JERMY	Hungary
Session VI	L. MELLADO	Spain
Session VII	A.M. JORDAN	United Kingdom
Session VIII	J. ITARD	France

SECRETARIAT OF THE SYMPOSIUM

Scientific Secretary	L.E. LaCHANCE	Joint FAO/IAEA Division of Atomic Energy in Food and Agriculture
Administrative Secretary	Caroline de MOL van OTTERLOO	Division of Scientific and Technical Information, IAEA
Editor	L.A. SELF	Division of Publications, IAEA
Records Officer	A. MACRAE	Division of Languages, IAEA
Liaison Officer, Greek Government	P. PAPADIMITROPOULOS	

LIST OF PARTICIPANTS

ALBANIA

Murraj, X.O.	Universiteti Shteteror, Tirana
Zela, F.H.	Universiteti Shteteror, Tirana

ARGENTINA

Carcavallo, R.U.	Dirección de Epidemiología, 49 No. 885, La Plata
Zerbino, M.C.	Comité Interamericano de Protección Agrícola (CIPA), Avenida Pueyrredón 1969, piso 13-"A", Buenos Aires

AUSTRALIA

Whitten, M.J.	CSIRO, Division of Entomology, P.O. Box 109, Canberra City, ACT 2601 (See also under CSIRO)

AUSTRIA

Russ, K.	Bundesanstalt für Pflanzenschutz, Trunnerstrasse 5, 1020 Vienna

BRAZIL

Borghi, C.	Centro de Energia Nuclear, Universidade Federal de Pernambuco, Cidade Universitaria, Caixa Postal 2007, Recife
Wiendl, F.M.	Centro de Energia Nuclear na Agricultura, Caixa Postal 9, Piracicaba, São Paulo

BULGARIA

Berberov, D.N.	Ministère d'Agriculture et Production Alimentaire, Section d'Application de l'Energie Atomique et l'Industrie Alimentaire, Sofia
Lazarov, A.W.	Academy of Agricultural Sciences, 8 Boul. Dragan Tzankov, Sofia

CANADA

Baldwin, W.F. — Atomic Energy of Canada Limited,
Chalk River Nuclear Laboratories,
Chalk River, Ont.

Nair, K.K. — Department of Biological Sciences, Simon Fraser University,
Burnaby-2, B.C.

CHINA, REPUBLIC OF

Liu, H.Y. — Joint Commission on Rural Reconstruction,
37 Nan Hai Road, Taipei, Taiwan 107

CZECHOSLOVAK SOCIALIST REPUBLIC

Landa, V. — Institute of Entomology (Entomologický Ústav Csav),
Czechoslovak Academy of Sciences,
Viničná 7, Prague 2

CYPRUS

Serghiou, C. — Agricultural Research Institute, Nicosia
(Present address: IAEA Laboratory,
Seibersdorf 2444, Austria)
(See also under IAEA)

DENMARK

Mourier, H. — Government Pest Infestation Laboratory,
Skovbrynet 14, 2800 Lyngby

FRANCE

Boiteau, R. — Institut technique français de la betterave industrielle,
43-45 rue de Naples, 75 Paris 8e

Huignard, J. — Laboratoire d'écologie et de biocoenotique expérimentales,
Université de Tours,
Parc Grandmont, 37 Tours

Hurpin, B. — Station de recherche de lutte biologique et de biocoenotique, INRA,
78 La Minière par Versailles

Itard, J. — Institut d'élevage et de médecine véterinaire des pays tropicaux,
10 rue Pierre Curie, 94 Maisons-Alfort

GERMANY, FEDERAL REPUBLIC OF

Haisch, A.	Bayerische Landesanstalt für Bodenkultur, Pflanzenbau und Pflanzenschutz, Menzingerstrasse 54, 8000 Munich 19
Jäger, A.	Schering AG, Berlin und Bergkamen, Müllerstrasse 170-172, 1000 Berlin 65
Laven, H.	Institut für Genetik, Universität, 6500 Mainz (Present address: Entente interdépartementale pour la démoustication, B.P. 6036, 34 Montpellier - St. Clément, France)
Stüben, Mechthild	Biologische Bundesanstalt für Land- und Forstwirtschaft, Königin Luise Strasse 19, 1000 Berlin

GHANA

Offori, E.D.	Council for Scientific and Industrial Research, P.O. Box M 32, Accra

GREECE

Bouchelos, T.	Plant Protection Service, Ministry of Agriculture, Athens
Economopoulos, A.	Greek Atomic Energy Commission, "Democritus" Nuclear Research Centre, Aghia Paraskevi - Attikis, Athens
Pytizas, E.	Benaki Phytopathological Institute, 3 Delta Kifissia, Athens
Giannakakis, Areti	Greek Atomic Energy Commission, "Democritus" Nuclear Research Centre, Aghia Paraskevi - Attikis, Athens
Kalmoukos, P.	Benaki Phytopathological Institute, 3 Delta Kifissia, Athens
Karahalios, Catherine	Greek Atomic Energy Commission, "Democritus" Nuclear Research Centre, Aghia Paraskevi - Attikis, Athens
Krimbas, C.	Agricultural College of Athens, Athens
Manoukas, A.	Greek Atomic Energy Commission, "Democritus" Nuclear Research Centre, Aghia Paraskevi - Attikis, Athens
Orphanidis, P.	Benaki Phytopathological Institute, 3 Delta Kifissia, Athens

Tzanakakis, M.E. — Faculty of Agriculture and Forestry, University of Thessaloniki, Thessaloniki

Vakirtzi-Lemonias, Catherine — Greek Atomic Energy Commission, "Democritus" Nuclear Research Centre, Aghia Paraskevi - Attikis, Athens

HUNGARY

Jermy, T. — Hungarian Research Institute for Plant Protection (Növényvédelmi Kutató Intézet), Herman Ottó Út 15, Budapest II

INDIA

Sethi, G.R. — Agricultural Research Institute, Division of Entomology, New Delhi 12
(Present address: Rothamstead Experimental Station, Harpenden, Herts, United Kingdom)
(See also under IAEA)

INDONESIA

Hatmosoewarno, S. — Jogjakarta Plantation Institute (State Sugar Academy), Djl. Sala 40a, P.O. Box 6, Jogjakarta

IRAN

Bagheri-Zenouz, E. — Faculté d'Agriculture, Université de Téhéran, Karadj

ISRAEL

Galun, Rachel — Israeli Institute for Biological Research, P.O. Box 19, Ness-Ziona

ITALY

Cirio, U. — Laboratorio Applicazioni Agricoltura, CNEN, S. Maria di Galeria, Casaccia, 00060 Rome

Girolami, V. — Istituto di Entomologia Agraria, Università di Padova, Via Grademigo 6, Padova

Milani, R. — Istituto di Zoologia dell'Università, Piazza Botta, I 27100 Pavia

MADAGASCAR

Ravelojaona, G. — Institut de recherches agronomiques de Madagascar, B.P. 1444, Tananarive

NETHERLANDS

Noordink, J.Ph.W.	Institute for Phytopathological Research, Binnenhaven 12, Wageningen
Theunissen, J.	Institute for Phytopathological Research, Binnenhaven 12, Wageningen
Ticheler, J.H.G.	Institute for Phytopathological Research, Binnenhaven 12, Wageningen

PAKISTAN

Huque, Heshamul	Department of Plant Protection, Ministry of Agriculture, Jinnah Avenue, Karachi-27

PARAGUAY

Aranda Centurión, B.R.	Ministerio de Agricultura y Ganadería, Calle Presidente Franco y 14 de Mayo, Asunción

PHILIPPINES

Morales, Marta T.	Philippine Atomic Energy Commission, 727 Herran Street, P.O. Box 932, Manila

ROMANIA

Grosu, Silvia	Comité d'Etat pour l'Energie Nucléaire, Bul. Ilie Pintilie 7, Bucharest

SINGAPORE

Chan, Kai-Lok	Vector Control and Research Branch, Ministry of Health, 151-P Kim Chuan Road, Singapore 19

SOUTH AFRICA

Malan, J.R.	Veterinary Research Institute, Onderstepoort (Present address: c/o Dr. Shaw, CTB, Berkhamstead, United Kingdom)

SPAIN

Mellado, L.	Instituto Nacional de Investigaciones Agronómicas, Avda. Puerta de Hierro, Madrid 3
Rey, J.M.	Instituto Español de Entomología, Pinar 19 dpdo, Madrid 6

SWEDEN

Pettersson, J.G.P.	Department of Plant Pathology and Entomology, 750 07 Uppsala 7

SWITZERLAND

Boller, E.F.	Eidg. Forschungsanstalt für Obst-, Wein- und Gartenbau, 8820 Wädenswil

TURKEY

Kansu, I.A.	Adana Agricultural Faculty (Adana Ziraat Fakültesi), Ankara
Merter, Ülkü	Commissariat à l'énergie atomique, Mehterler Sokak No. 13, Etlik-Ankara

UNITED KINGDOM

Badmin, J.S.	Imperial College Field Station, Silwood Park, Ascot, Berks
Campion, D.G.	Tropical Pesticides Research Headquarters and Information Unit, Ministry of Overseas Development, 56-62 Grays Inn Road, London WC1
Curtis, C.	Tsetse Research Laboratory, University of Bristol School of Veterinary Science, Langford, Bristol BS18 704
Jacob, F.H.	Pest Infestation Control Laboratory, Block "B", Government Buildings, Hook Rise South, Tolworth, Surbiton, Surrey
Jordan, A.M.	Tsetse Research Laboratory, University of Bristol School of Veterinary Science, Langford, Bristol BS18 704
Lewis, C.T.	Department of Zoology and Applied Entomology, Imperial College, London, SW7

UNITED STATES OF AMERICA

Adkisson, P.L.	Department of Entomology, Texas A&M University, College Station, Texas 77843
Bushland, R.C.	Metabolism and Radiation Research Laboratory, US Department of Agriculture, State University Station, Fargo, N.D. 58102
Grosch, D.S.	Genetics Department, North Carolina State University, Raleigh, N.C. 27607

Harris, E.J.	US AID, c/o American Embassy, Tunis, Tunisia
Kojima, Ken-ichi	University of Texas at Austin, Austin, Tex. 78712
Kunz, S.E.	Veterinary Toxicology and Entomology Research Laboratory, USDA, ARS, ENT, P.O. Drawer GE, College Station, Tex. 77801
Rai, K.S.	University of Notre Dame, Department of Biology, Notre Dame, Indiana 46556
Smith, Roger H.	Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tenn. 37830
Taylor, E.A.	Entomology Research Services, USDA, ARS, Plant Industry Station, Beltsville, Md. 20705
Walker, D.W.	Puerto Rico Nuclear Center, University of Puerto Rico, Mayaguez, Puerto Rico 00708

URUGUAY

Boroukhovitch, M.	Ministerio de Ganadería y Agricultura, Millán 4715, Montevideo

VENEZUELA

Gómez-Núñez, J.C.	División de Endemias Rurales, Dirección de Malariología, Ministerio de Sanidad, Av. Bermúdez, Maracay

YUGOSLAVIA

Maksimović, M.	Institute for Plant Protection (Institut za zastitu bilja), Teodora Drajzera 7, Belgrade

ZAMBIA

Machili, P.	National Council for Scientific Research, Tsetse Research Team, P.O. Box 9, Chilanga

ORGANIZATIONS

CCE (COMMISSION DES COMMUNAUTES EUROPEENNES)

Hoeck, F. van	200 rue de la Loi, 1040 Brussels, Belgium

CSIRO (COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANIZATION)

Whitten, M.J.	CSIRO, Division of Entomology, P.O. Box 109, Canberra City, ACT 2601

FAO/UNDP (FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS/ UNITED NATIONS DEVELOPMENT PROGRAMME)

Moericke, V.	36 Amalia Avenue, Athens 119, Greece
Sigwalt, B.A.G.	36 Amalia Avenue, Athens 119, Greece

UNDP (UNITED NATIONS DEVELOPMENT PROGRAMME)

Brédo, H.J.	Consultant to the Administrator, New York, N.Y., USA

WHO (WORLD HEALTH ORGANIZATION)

Pal, R.	Vector Biology and Control, 1211 Geneva 27, Switzerland
Wright, J.W.	Vector Biology and Control, 1211 Geneva 27, Switzerland

IAEA (INTERNATIONAL ATOMIC ENERGY AGENCY)

Hoedaya, M.S. (IAEA fellow)	Djumat Research Centre, P.O. Box 2, Djakarta, Indonesia (Present address: Laboratorio Applicazioni in Agricoltura, CNEN, S.P. Anguillarese, Casaccia, Rome, Italy)
Hooper, G.	Division of Research and Laboratories, P.O. Box 590, 1011 Vienna, Austria
Mews, A.R.	Division of Research and Laboratories, P.O. Box 590, 1011 Vienna, Austria
Serghiou, C. (IAEA fellow)	Agricultural Research Institute, Nicosia, Cyprus (Present address: IAEA Laboratory, Seibersdorf 2444, Austria)
Sethi, G.R. (IAEA fellow)	Agricultural Research Institute, Division of Entomology, New Delhi 12, India (Present address: Rothamstead Experimental Station, Harpenden, Herts, United Kingdom)

AUTHOR INDEX

(including Participants in Discussions)

The numbers underlined indicate the first page of a paper by an author.
Other numbers denote the page numbers of discussion comments.

IAEA SALES AGENTS AND BOOKSELLERS

Orders for Agency publications can be placed with your bookseller or any of the addresses listed below:

ARGENTINA
Comisión Nacional de
Energía Atómica
Avenida del Libertador 8250
Buenos Aires

AUSTRALIA
Hunter Publications
23 McKillop Street
Melbourne, C.1

AUSTRIA
Publishing Section
International Atomic Energy Agency
Kärntner Ring 11
P.O. Box 590
A-1011 Vienna

BELGIUM
Office International
de Librairie
30, Avenue Marnix
Brussels 5

CANADA
Information Canada
Ottawa, Canada

C.S.S.R.
S.N.T.L.
Spolena 51
Nové Mesto
Prague 1

DENMARK
Ejnar Munksgaard Ltd.
6 Norregade
DK-1165 Copenhagen K

FRANCE
Office International de
Documentation et Librairie
48, Rue Gay-Lussac
F-75 Paris 5e

HUNGARY
Kultura
Hungarian Trading Company
for Books and Newspapers
P.O. Box 149
Budapest 62

INDIA
Oxford Book & Stationery Comp.
17, Park Street
Calcutta 16

ISRAEL
Heiliger & Co.
3, Nathan Strauss Str.
Jerusalem

ITALY
Agenzia Editoriale Commissionaria
A.E.I.O.U.
Via Meravigli 16
I-20123 Milan

JAPAN
Maruzen Company, Ltd.
P.O. Box 5050,
100-31 Tokyo International

MEXICO
Librería Internacional, S.A.
Av. Sonora 206
México 11, D.F.

NETHERLANDS
Martinus Nijhoff N.V.
Lange Voorhout 9
P.O. Box 269
The Hague

PAKISTAN
Mirza Book Agency
65, Shahrah Quaid-E-Azam
P.O. Box 729
Lahore - 3

POLAND
Ars Polona
Centrala Handlu Zagranicznego
Krakowskie Przedmiescie 7
Warsaw

ROMANIA
Cartimex
3-5 13 Decembrie Street
P.O. Box 134-135
Bucarest

SPAIN
Librería Bosch
Ronda Universidad 11
Barcelona - 7

SWEDEN
C.E. Fritzes Kungl. Hovbokhandel
Fredsgatan 2
Stockholm 16

SWITZERLAND
Librairie Payot
Rue Grenus 6
CH-1211 Geneva 11

U.S.S.R.
Mezhdunarodnaya Kniga
Smolenskaya-Sennaya 32-34
Moscow G-200

U.K.
Her Majesty's Stationery Office
P.O. Box 569
London S.E.1

U.S.A.
UNIPUB, Inc.
P.O. Box 433
New York, N.Y. 10016

YUGOSLAVIA
Jugoslovenska Knjiga
Terazije 27
Belgrade

IAEA Publications can also be purchased retail at the United Nations Bookshop at United Nations Headquarters, New York, from the news-stand at the Agency's Headquarters, Vienna, and at most conferences, symposia and seminars organized by the Agency.

In order to facilitate the distribution of its publications, the Agency is prepared to accept payment in UNESCO coupons or in local currencies.

Orders and inquiries from countries not listed above may be sent to:

Publishing Section
International Atomic Energy Agency
Kärntner Ring 11
P.O. Box 590
A-1011 Vienna, Austria

Photography: Dale Whitner

NCY

SUBJECT GROUP: I
Life Sciences/Entomology