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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 radio-isotope 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 33 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.

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Chairman

M.E. TZANAKAKIS (Greece)

STERILITY PRINCIPLE FOR INSECTS

The sterile-male technique eradicates the sex-ratio
of the population. Laboratory-scale experiments have shown
that a dose of about 10–15 krads is adequate for this
purpose. A similar dose administered
moistened was observed to be effective in
the field, but it is important to note that
the dosage must be carefully controlled.

SERTILITY PRINCIPLE FOR INSECTS

The sterile-male technique
was initially used in the control of the
sandfly. Laboratory experiments have shown
that a dose of about 10–15 krads is adequate for this
purpose. A similar dose administered
moistened was observed to be effective in
the field, but it is important to note that
the dosage must be carefully controlled.

INTRODUCTION

I was invited to participate in a research project
aimed at developing a sterile-male technique for
insects. The goal is to use this technique to
control the population of a particular species
of insect, which is a pest in the area. The
method involves exposing the male insects to
a specific dose of radiation, which makes them
sterile and unable to reproduce. The sterile
males are then released into the field, where
they mate with the females, resulting in
sterile eggs that do not hatch.

The introduction of sterile males into the
population is expected to reduce the population
size of the pest, ultimately leading to a reduction
in the number of pests and a decrease in their
impact on crops and the environment.

METHODS

The method involves the following steps:

1. Selection of the sterile-male technique
2. Designing radiation equipment
3. Testing the effectiveness of the technique
4. Field trial

RESULTS

The results of the field trial were promising.
The sterile males successfully reduced the
population of the pest, and the area showed
a significant decrease in pest damage.

DISCUSSION

The sterile-male technique is a promising
candidate for controlling pest populations.
It offers a non-chemical solution to pest
management, reducing the risk of resistance
development and environmental impacts.

CONCLUSION

The sterile-male technique has potential
for controlling pest populations. Further
research is needed to refine the technique
and optimize its application in different
settings.
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 screwworm, 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 Diprora 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 Leptopisma requires about 30 krad of gamma radiation to induce dominant lethal mutations in all the sperm. Somatic damage from this large dose reduces vigour and mating competitiveness. Alterations in spermatogenesis trigger commonly result in the sperm of irradiated males not being competitive.

A dose of about 15 krad does not completely sterilize males, but their chromosomes undergo so many modifications, such as reciprocal translocations, that their F2 progeny, though active at mating, are almost all sterile. The irradiated adults, since they are reared with only about one-third the irradiating 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 treated in the laboratory or collected in the field, be reared, 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 these females develop a mating avoidance response as a result of a chemical received in the setting. 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 to nature, the expense of rearing, rearing, 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 infected 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 5,000 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 cooperated with livestock
growers and State agricultural officials in attempting to eradicate screw-
worms by treating infested wounds with a larvicidal and by protecting live-
stock 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 irradiation of the sterilizing effect to do with laboratory
screw-worms. We went back to screw-worm.

By 1946, Knipling Insects Affecting Man Quarantine, and livestock
laboratory at Kerrville compounds had been and were now to be at
and arachnids effect in a concurrent study of techniques of sterilize
the flies that survived screening tests did no.

It was not until 1951 at the Entomology Res attention to the paper
of radiation-induced d.

Outlined the Florida ever be possible to induce could survive and con
felt that the experim.

On preliminary ex
tired gamma radiation it was most efficient in a
dose of 2500 rad six females; and that pups. Then, since it was not
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Beginning in 1952
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A field test on the
actually achieved erad
100 sterile males/mil
males/mile2 per week
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 1948.

By 1948, Knipping 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. Knipping therefore suggested a concurrent study of the mating behavior 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. Knipping 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 32P 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 32P 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/mile2 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/mile2 per week did not succeed, but a rate of 400 sterile males/mile2 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 estimate of the dimensions of the screw-worm populations.

Curacao has an area of only 170 mile², so the experiment had involved the release of only 138,000 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 flies a larger area. We selected a 20,000-mile² section bordering the Atlantic Coast near Cape Kennedy, where screw-worms were even more abundant than on Curacao. Then we released 500 males/mile² per week for 10 weeks. Our purpose 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 1953 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/mile² 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 live-stock screw-worms were eradicated.

Our original concept of isolating the population of the screw-worm of the banana-moths of the Florida program had a good idea, average years at the time of that treated successfully; but western states were not. In Florida, we were able to achieve eradication at the South-west and to use the estimated cost of 3 million dollars in the way it was thought could be appropriate, to get rid of the entire infestation. Within 2 years of that project, the whole of the southeastern United States was eradicated, with the exception of the area in Texas. The project was then turned over to the states, and by the end of 1959, 90% of the population was eradicated, with the remaining 10% occurring in the southern parts of the state. In 1960, the project was terminated.

STERILIZATION OF MIGRANTS

One insect that hatched in the south, the Anthonomus grandis, that is, the beetle of the same name that attacks the Klassen and Ear mix, was used to sterilize the populations of this insect. This was done by mixing the beetles with the steriley granules, except that the female granules were not separated from the male granules, and the males were not separated from the females. The result was the complete elimination of the population in the area where the granules were applied. This was done in order to reduce the population to a level where it would no longer be a threat to the population.
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 miles², 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 mid gut 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 mid gut.

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.
rain of boll weevils, their fertile non-dominant conditional population models in insects might be controlled by 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 Ral [22] described a translocation involving three chromosomes in Anopheles gambiae (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, Wagener [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 103 translocation heterozygotes by irradiating males with 2000-2500 R of X-rays [25]. When these translocations involved two chromosomes, sterility of the heterozygotes averaged 58%; when they involved three chromosomes, it averaged 88%; and when they involved four chromosomes, it averaged 79%. However, the development of vigorous, fertile, translocation homozygotes has been difficult.

Last year, Wagener, lacking a translocation homozygote, released male heterozygotes at a semi-isolated location in Florida in cooperation with G. C. LabBrecq 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.) Wagener (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 detrimental in the shelter of the laboratory. Therefore, it is important to back-cross a laboratory strain to a wild-type strain and 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 rotated at intervals that would indicate how quickly each strain moved from the release point to the traps. Also, avoidance responses 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 lethals. 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 blow fly, and even with the blow fly 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 55% 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 the reaction in female the same response, i.e., breeding of house flies frequently rest inside sprayed with a substance which would be exposed to the effect on a fly in a killing. However, flies which would have no effect may therefore lead to gnomous insect pests, female house flies of species, Phormia to cause mating avoid monogamous Diptera fly, Haematobia irrit.

Also, many mosquito described a substance accessory glands of secretions on the part of a blocking of the vagina.

I think that much a mating avoidance chemical that will in chemical variously a chemosterilant for a model illustrating by a field population the however, involve could develop resistant inactivator, Whitter 95 - 99% monogamous mating, the monogamic STERILIZATION OF

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R3 STERILITY

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laboratory cages, to mark a strain of Caucasus, near the regular two kinds of flies that catches in fly. For example, the quickly each strain was continued. It took 3 years of trials that was not such, markers were labeled.

be similar, but this on the difficult course, of chemosterilants, that affects only sterilize both sexes [28], effective sterilants are those lethal to the weevil, hazards of group, when conditions the boll weevil, resistance studies to date. I cannot foresee any control. However, that is needed is a cause. House flies showed that even response in the testes were water-soluble and was injected into the adults of this laboratory house flies secrete a hormone in the mixture is with tritiated males with radioactive material. Some males at intervals of 2 min after copulation became radioactive. At 30 min, the material could be detected. The male house flies do not develop resistance before they would not become radioactive. This inability to become radioactive would not prevent mating. The effect 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 resting 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 matrona which is produced by the accessory glands of male *A. aegypti*. Matrona 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. Knipping [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 Lu, *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 sterilize of 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 some 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.

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**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 chromosomes. 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 spermatothecae in the proper position for the sperm to reach the spermathecae. Also, sterile males often fail to incorporate sperm in their spermathecae.

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 F1 progeny. They found that males treated with about a 50% sterilizing dose had progeny that were more than 80% sterile. They explained this effect as the result of the many broken chromosomes in the sperm that had recombined with fragments of other chromosomes to form multiple translocations. The F1 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 spermatothecae was more nearly normal as was the incorporation of sperm. The F2 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 F2 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, Oncopelus 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 F2 generation, much less evident in the F3 generation, and had largely disappeared by the F4 generation. The fact that some fertile progeny are produced in the F2 generation and that almost normal fertility is recovered by the F3 generation indicates that this technique has more value for suppressing lepidopterous populations than it does for actual eradication. Knipping [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. Knipping [42] recently extended his calculations and professed his hope for the application of F2 sterility to control the cabbage looper in the eastern United States and the corn earworm, Heliothis z. sterility could be used Laspeyresia pomelia gossypirlla (Saunders of a program; final e: sterile males.

CONCLUSIONS

As you can see, be sterilized with rat of sterility to control
The first practical lethal mutations in 10 and small-scale field development by application to or very lar insects from adjacent released insects must be released at a frequency mates in the native portion at such interval and will outnumber the especially important be made so that eno.

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Sterility in insect new and promising as strains homozygous 1 with wild-type insect so that sterile hetero is underway with hom.

A simpler approach involves F2 sterility in enthusiastic about the genetic control.

[1] LACHANCE, L. E., SCID Pest Control (KILGER, W
[2] KELVIN, R., BUSHLAND
earworm, Heliothis zea (Boddie), in California. He pointed out that F₁ 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 founded that screw-worms could be sterilized by 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 F₁ 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

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MALE PU
Lymantria
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M. MAKSIM
Institute for F
Belgrade, Yu

Abstract

EFFECT OF COBALT-60 (RAD) ON BIOLOGICAL FUNCTIONS OF

The effect of irradiating 30,000 and 40,000 rad. Certain irradiated pupae were found to be The age of pupae when irradiated
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functions in mating. The oviposition

1. INTRODUCTION

The effect of sex has been investigated [2]. These studies have
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In the present two
doses of 30,000 and 40
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2. METHODS

The gypsy moth in greenhouse and then proved to pupae.
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 moth. 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 8 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 45.9% with male pupal irradiation of 30,000 rad. In 86.89% eggs,
although the caterpillar larvae were 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 Vasić [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

<table>
<thead>
<tr>
<th>Age at which pupae were irradiated (days)</th>
<th>Pupae irradiated with 30,000 rad</th>
<th>40,000 rad</th>
<th>Unirradiated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total no. of pupae irradiated</td>
<td>Dead</td>
<td>%</td>
</tr>
<tr>
<td>2-5</td>
<td>13</td>
<td>12</td>
<td>22.3</td>
</tr>
<tr>
<td>8-10</td>
<td>11</td>
<td>2</td>
<td>18.1</td>
</tr>
<tr>
<td>11-15</td>
<td>6</td>
<td>0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

### TABLE II. EFFECT OF IRRADIATION ON FEMALE GYPSY MOTHS

<table>
<thead>
<tr>
<th>Male pupae of var. M. arbustorum with females</th>
<th>Number of male pupae alive after irradiation</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>10</td>
<td>2</td>
</tr>
</tbody>
</table>

3. RESULTS OF IN VIVO TREATMENTS

The mortality of 1st instar larvae, which were irradiated, was significantly higher than in the controls. The number of survivors after irradiation was significantly lower than in the controls.

Male pupae of var. M. arbustorum with females. The mortality of male pupae was significantly higher than in the controls. The number of survivors after irradiation was significantly lower than in the controls.
<table>
<thead>
<tr>
<th>Number of male moths</th>
<th>Body length (mm)</th>
<th>Number of matings</th>
<th>Average number of matings per male moth</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>1</td>
<td>0.33</td>
</tr>
<tr>
<td>5</td>
<td>11 - 12</td>
<td>4</td>
<td>0.80</td>
</tr>
<tr>
<td>9</td>
<td>13 - 17</td>
<td>12</td>
<td>1.33</td>
</tr>
</tbody>
</table>

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 of 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

<table>
<thead>
<tr>
<th>Irradiation dose (Rad)</th>
<th>Temperature (°C)</th>
<th>Duration of copulation (min)</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>30,000</td>
<td>11</td>
<td>10</td>
<td>2.02</td>
<td>4.27</td>
<td>1.20</td>
</tr>
<tr>
<td>40,000</td>
<td>12</td>
<td>22.5</td>
<td>6.5</td>
<td>14.1</td>
<td>1.42</td>
</tr>
<tr>
<td>50,000</td>
<td>13</td>
<td>32</td>
<td>13.1</td>
<td>20.5</td>
<td>4.99</td>
</tr>
<tr>
<td>Unirradiated</td>
<td>14</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table IV. Effect of Irradiation Dose on Life Span of Male Moths

<table>
<thead>
<tr>
<th>Irradiation dose (Rad)</th>
<th>Number of males</th>
<th>Temp. (°C)</th>
<th>Life span of male moths (days)</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>30,000</td>
<td>11</td>
<td>12</td>
<td>1.04</td>
<td>4.75</td>
<td>7.5</td>
<td>6.46</td>
</tr>
<tr>
<td>40,000</td>
<td>10</td>
<td>13</td>
<td>2.10</td>
<td>13.0</td>
<td>11.0</td>
<td>9.07</td>
</tr>
<tr>
<td>50,000</td>
<td>9</td>
<td>14</td>
<td>1.90</td>
<td>2.10</td>
<td>2.07</td>
<td>2.17</td>
</tr>
<tr>
<td>Unirradiated</td>
<td>14</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### 3.3. Effect of Irradiation

Irradiation of the duration of copulation of males irradiated with maximum duration of moths.

The harmful effect is clearly shown by Table VII with 30,000 rad. The average figures are not reliable a number of moths used.

### 3.4. Effect of mating of female moths

Table VIII shows that the results with males are more significant than for females. The cross figure was obtained with 40,000 rad. The lower result obtained for females shows the higher critical dose for female moths.

### 3.5. Effect of sterilizability

Eggs obtained from the irradiated females showed wide differences. The hatched ones were irradiated with sterile males, whereas the irradiated ones were not completely sterile.

### 4. DISCUSSION AND CONCLUSION

Cobalt-60 irradiation was found to have a harmful effect on the pupae when pupae were irradiated with males. However, when pupae were irradiated with females, the effect was not as pronounced. Further studies are needed to determine the optimal dose and method of irradiation for effective control of pests.
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 males.

The harmful effect of irradiation on life span is more marked, as Table IV clearly shows. The maximum durations are shorter for 40,000 rad 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 variations 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 2.51 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 82.8% hatched in the control. However, among eggs resulting from crossing with sterile males there were 56.3% containing larvae that died unhatched, whereas there were 17.2% such eggs in the control. There were 43.2% completely sterile eggs, and only 8.2% 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 82.8% (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 OVIPPOSITION

<table>
<thead>
<tr>
<th>Irradiation dose of male moths (rad)</th>
<th>Number of female moths</th>
<th>Temp. (°C)</th>
<th>Duration of oviposition (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30,000</td>
<td>5</td>
<td>21 - 26</td>
<td>Minimum: 1.88 Max: 4.31 Ave: 2.96</td>
</tr>
<tr>
<td>40,000</td>
<td>6</td>
<td></td>
<td>1.90 Max: 2.68 Ave: 1.65</td>
</tr>
<tr>
<td>Unirradiated</td>
<td>4</td>
<td></td>
<td>3.86 Max: 5.72 Ave: 4.40</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Irradiation dose of male moths (rad)</th>
<th>Number of female moths</th>
<th>Temp. (°C)</th>
<th>Life span of female moths (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30,000</td>
<td>5</td>
<td>21 - 26</td>
<td>Minimum: 1.16 Max: 8.44 Ave: 4.89</td>
</tr>
<tr>
<td>40,000</td>
<td>6</td>
<td></td>
<td>1.70 Max: 5.00 Ave: 4.08</td>
</tr>
<tr>
<td>Unirradiated</td>
<td>4</td>
<td></td>
<td>4.75 Max: 7.75 Ave: 0.39</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Egg-masses from copulation with:</th>
<th>Egg-masses</th>
<th>Total number of eggs</th>
<th>Percentage of eggs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male moth irradiated with 30,000 rad</td>
<td>3</td>
<td>562</td>
<td>38.6% Non-embryonated</td>
</tr>
<tr>
<td>Unirradiated male moth</td>
<td>3</td>
<td>819</td>
<td>17.2% Embryo-nated</td>
</tr>
</tbody>
</table>
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%. Vasić et al. [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. Vasić et al. [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

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 18 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 Développement 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 and 1000 rad and in 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 rad and 1 Mrad; the difference between the control medium and the medium irradiated at 25 rad 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 decreased more significantly in the males than in the females in rearing on irradiated media. This effect is observed at irradiation doses of 25 rad but is greatest for the medium irradiated at 500 rad. 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 rad and 1 Mrad can modify this factor and so to create factors that promote longevity and fertility in adults of *Sitophilus granarius*.

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

L'auteur a étudié l’influence de l’irradiation de grains de blé par le rayonnement gamma de $^{60}$Co sur les conditions de développement et de reproduction de *Sitophilus granarius*, qui vit normalement sur ce produit. Les grains de blé ont été irradiés à 25, 100, 500, 1000 rad et 1 Mrad. Dans une série d’expériences, la fécondité et la longévité des insectes élevés sur des milieux irradiés et ceux 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, les 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 rad et 1 Mrad. La différence n’est plus significative entre le milieu témoins et le milieu irradié à 25 rad. Cette différence se retrouve pour des adultes issus de la deuxième génération élevés sur ces milieux irradiés.

Pour la longévité, on remarque que, dans les élevages sur milieux irradiés, celle-ci est moins significativement que celle des témoins. Ce fait se manifeste pour des doses d’irradiation de 25 rad, mais il est maximal pour le milieu irradié à 500 rad. Les différences de longévité moyenne entre le lot témoins 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 rad à 1 Mrad peut modifier cet aspect de manière 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).
<table>
<thead>
<tr>
<th>Duration of the experiment (d)</th>
<th>Number of females present</th>
<th>Number of females present</th>
<th>Male:female ratio</th>
<th>Number of females present</th>
<th>Number of females present</th>
<th>Male:female ratio</th>
<th>Number of females present</th>
<th>Number of females present</th>
<th>Male:female ratio</th>
<th>Number of females present</th>
<th>Number of females present</th>
<th>Male:female ratio</th>
<th>Number of females present</th>
<th>Number of females present</th>
<th>Male:female ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 12</td>
<td>60</td>
<td>995</td>
<td>16,66</td>
<td>60</td>
<td>995</td>
<td>16,66</td>
<td>60</td>
<td>995</td>
<td>16,66</td>
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<td>995</td>
<td>16,66</td>
<td>60</td>
<td>995</td>
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<tr>
<td>15 - 20</td>
<td>60</td>
<td>995</td>
<td>16,66</td>
<td>50</td>
<td>1218</td>
<td>24,36</td>
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<td>24,36</td>
<td>50</td>
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<td>24,36</td>
<td>50</td>
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<td>16,66</td>
<td>40</td>
<td>1096</td>
<td>27,43</td>
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<td>27,43</td>
<td>40</td>
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<td>20</td>
<td>634</td>
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<tr>
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<td>16,66</td>
<td>10</td>
<td>184</td>
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<td>184</td>
<td>18,40</td>
<td>10</td>
<td>184</td>
<td>18,40</td>
</tr>
</tbody>
</table>

**Moyenne par semaine au bout de 60 d:** 70,70

**Moyenne per week at 60 d:** 70,70
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 issues de ce milieu irradié.

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

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 krad 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 krad 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 (1A1, 1A2, ..., 1A500, 1A100) et ceux de la deuxième génération 2A (2A1, 2A2, ..., 2A500).

Pour évaluer la fécondité nous avons dénombré tous les imagos qui sortaient ultérieurement 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.
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 d'établir la fécondité à 60 jours.

On peut remarquer que la période la plus élevée dans les 3 lots est celle du lot 1A (sur du nombre des descendants) au cours de laquelle le taux de fécondité de l'élevage est le plus élevé.

Au cours de la radiation, les lots 1B et 1C ont sodés réduits, tandis que le lot 1A (témoin) est resté le même que celui des 3 lots suivants.

Les figures 1 et 2 montrent clairement que les lots les plus dégradés sont ceux de la radiation, tandis que le lot 1A (témoin) est resté inchangé.

Les résultats de cette expérience montrent que la radiation a un effet néfaste sur la fécondité des femelles, en particulier dans le groupe des descendants les plus petits.

Ces expériences répétées de 10 coups, nous avons obtenu des résultats comparables.

2. INFLUENCE DE

Nous avons étudié l'influence des milieux sains, permis de noter, à l'élevage, la mortalité.

Nous avons pour l'étude de la mortalité, les résultats pour chaque milieu, les rapports pour chaque milieu.
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 lot témoin. Seul le lot 1A_1 (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_1 (milieu irradié à 25 krad) et une autre dans le lot 1A_2; 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 krad et 500 krad, et deux femelles mortes dans le lot irradié à 1 Mrad, alors que dans le lot témoin et le lot irradié à 100 krad 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 1 (témoin) et le lot 1A_1 n'est pas significative, mais qu'elle l'est entre ce même lot 1A_1 et les lots 1A_2, 1A_3 et 1A_4.

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 bébés (lot témoin et lots irradiés aux mêmes doses que précédemment) dont les graines étaient plus petits.

Pour cette raison sans doute, et pour d'autres encore inexplicées, les lots expérimentaux 2A_6, 2A_7, 2A_8 et le lot 2A_2 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_9 et 2A_10 (tableau II). Seul le lot 2A_9 a donné moins de descendants que le témoin, la différence n'étant toutefois pas significative.

Ces expériences ne portaient d'ailleurs que sur 30 couples (deux répétitions de 10 couples) au lieu de 50 couples pour les lots de la série 1A.

2. INFLUENCE DE LIRRADIATION 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**

<table>
<thead>
<tr>
<th>Décès de l'expédition [J]</th>
<th>$\lambda_A$</th>
<th>$\lambda_M$</th>
<th>$\lambda_M$</th>
<th>$\lambda_M$</th>
<th>$\lambda_M$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nombre de femelles produisant</td>
<td>Nombre total de descendants</td>
<td>Moyenne</td>
<td>Nombre de femelles produisant</td>
<td>Nombre total de descendants</td>
</tr>
<tr>
<td>0 - 15</td>
<td>20</td>
<td>184</td>
<td>9,20</td>
<td>30</td>
<td>247</td>
</tr>
<tr>
<td>15 - 30</td>
<td>20</td>
<td>184</td>
<td>9,10</td>
<td>20</td>
<td>183</td>
</tr>
<tr>
<td>30 - 45</td>
<td>20</td>
<td>188</td>
<td>9,45</td>
<td>20</td>
<td>185</td>
</tr>
<tr>
<td>45 - 60</td>
<td>20</td>
<td>186</td>
<td>9,30</td>
<td>20</td>
<td>180</td>
</tr>
<tr>
<td>Moyenne par femelle au bout de 60 J</td>
<td>36,50</td>
<td>31,90</td>
<td>31,70</td>
<td>42,15</td>
<td>55,50</td>
</tr>
</tbody>
</table>

**TABLEAU III. COMPARAISON DE LA LONGEVITE (en jours) DES ADULTES, MALES ET FEMELLES, DE**
*Sitophilus granarius* **ELVES SUR DU BLE NORMAL ET DU BLE IRRAĐIE DE 25 krad à 1 Mrad**

<table>
<thead>
<tr>
<th></th>
<th>$\lambda_f$</th>
<th>$\lambda_M$</th>
<th>$\lambda_M$</th>
<th>$\lambda_M$</th>
<th>$\lambda_M$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>min. moy. max.</td>
<td>min. moy. max.</td>
<td>min. moy. max.</td>
<td>min. moy. max.</td>
<td>min. moy. max.</td>
</tr>
<tr>
<td>Milé</td>
<td>22,5 124,4 172,5</td>
<td>22,5 124,5 121,5</td>
<td>22,5 124,5 172,5</td>
<td>22,5 124,5 172,5</td>
<td>22,5 124,5 172,5</td>
</tr>
<tr>
<td>Fémelle</td>
<td>67,5 121,8 210</td>
<td>22,5 124,8 204,5</td>
<td>67,5 123,6 172,5</td>
<td>37,5 120,6 202,5</td>
<td>22,5 122,8 177,5</td>
</tr>
</tbody>
</table>
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. À la différence de ce que nous avons observé à propos de la fécondité, les milieux irradiés à une faible dose (25 krad) sont déjà favorables à un accroissement de la longévité; celle-ci est toutefois maximale sur les milieux irradiés à 500 krad.

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 krad 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 krad, mais il est en général maximal pour une dose d’irradiation de 100 krad.

REMERCIEMENTS

Je me dois 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


DISCUSSION

G.H.S. HOOVER: 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. BACHIERI-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 the 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. BACHIERI-ZENOUZ: I have no detailed information on this aspect. I have compared histological sections of the gonad 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 gonad 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. BACHIERI-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 ON THE OF Durat

HEISHALMI I Department of Agriculture, Karachi, Pak

Abstract

EFFECT OF GAMMA RADIATION

The effects of gamma radiation was studied to explore the technique. One aspect of this male and female flies, Is shown in the inset material used was the irradiated fruit fly. The study was made in three times. The main finding is that the insect was reduced, at 5 day to the 15th day with the normal flies. After 15th day of emergence could see up to mid-stage.

INTRODUCTION


MATERIAL AND METHOD

The insect mating (Psidium guajava) fru fruit, collected from
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 effect 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 sex 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 Camay’s fixative for fixation.

The main findings are that the sex of both male and female reproductive organs of irradiated pupae is revealed. At 3 weeks the formation of spermatocytes was completed on the 15th day as against the 15th day of the normal insect. At 8 hr spermatozoids and spermatids were not observed even after the 18th day of emergence. At 16 hr nucleolus started in the apical region of the testes which could be seen up to mid-region when the dose was increased to 18 hr. The testicular sheath also shrunk.

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 eradication of 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. citri (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 infected with larvae of D. zonatus. The infested fruits, 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

<table>
<thead>
<tr>
<th>Dose of gamma radiation applied to pupae (kR)</th>
<th>Pairs of testes examined</th>
<th>Testicles appearing normal when compared to control (%)</th>
<th>Testicles appearing partially normal (%)</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>20</td>
<td>85</td>
<td>15</td>
<td>(1) Spermatocytes in abundance, (2) Spermatzoa few in number.</td>
</tr>
<tr>
<td>8</td>
<td>20</td>
<td>0</td>
<td>4</td>
<td>(1) Testicles reduced in size, (2) Primary cells very few in number and scattered.</td>
</tr>
<tr>
<td>10</td>
<td>20</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>20</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

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 Gammarcell 200 irradiation chamber. The source used for these experiments was cobalt-60 housed in

TABLE II. THE ME. IRRADIATED FEMALES EMERGENCE FROM PUPAE

<table>
<thead>
<tr>
<th>Dose of gamma radiation applied to pupae (kR)</th>
<th>Pair ovary examined</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>9</td>
<td>2</td>
</tr>
<tr>
<td>Untreated</td>
<td>2</td>
</tr>
</tbody>
</table>

a fool-proof cell man of doses varying from emerging from irradiated to 24 days after emergence with haematoxylin and eosin.

RESULTS

Effect of radiation on ovary development

At a dose of 5 kR of sperm was slightly spermatogenesis to form unirradiated males showed normal sperm (Table I). With an 8- and the normal process a few scattered at high magnification. 1 increased to 10 kR. 1 necrosis started in the mid-region with images also shrunk.

Effect of radiation on ovary development

From Table II it is seen that oocytes were taken from the ovaries contained a 0 and the oocytes did not ovary taken from th seen that oocytes were
TABLE II. THE MEAN LENGTH AND WIDTH OF OVARIES OF IRRADIATED FEMALES OF Dacus zonatus 16 DAYS AFTER EMERGENCE FROM PUPAE

<table>
<thead>
<tr>
<th>Dose of gamma radiation applied to pupae (kR)</th>
<th>Pairs of ovaries measured</th>
<th>Length (mm)</th>
<th>Width (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Range</td>
<td>Mean</td>
</tr>
<tr>
<td>3</td>
<td>20</td>
<td>4.8-6.2</td>
<td>4.9</td>
</tr>
<tr>
<td>5</td>
<td>20</td>
<td>3.8-4.0</td>
<td>3.99</td>
</tr>
<tr>
<td>7</td>
<td>15</td>
<td>3.5-3.7</td>
<td>3.68</td>
</tr>
<tr>
<td>9</td>
<td>20</td>
<td>2.0-2.6</td>
<td>2.26</td>
</tr>
<tr>
<td>Untreated</td>
<td>20</td>
<td>7.3-9.5</td>
<td>7.4</td>
</tr>
</tbody>
</table>

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),
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 dif-
ferentiation 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.
post-emergence development of the pupae was adversely affected by various doses applied, especially at 3 kR, and emergence of females from irradiated ovaries was markedly reduced. In some cases, no females emerged and no differentiated eggs were observed.

Development of the post-emergence female pupae was adversely affected by various doses applied, especially at 3 kR, and emergence of females from irradiated ovaries was markedly reduced. In some cases, no females emerged and no differentiated eggs were observed.

Haque 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 formation was 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


DISCUSSION

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

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

J. 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. THURINSSON: 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 6, 8, 12 and 20 krad were applied at a rate of 30,3 krad/h. Use was made of 24 Petri dishes, representing five treatments with five replications. Ten unused 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 fue determinar el efecto que producen cuatro dosis diferentes de radiación gamma sobre adultos de Sitotroga cerealella Olivier. Se utilizaron dosis de 6, 8, 12 y 20 krad, con una «dose-rate» de 30,3 krad/h. Se utilizaron 24 platos Petri correspondientes a cinco tratamientos con cinco repeticiones. Se colocaron 10 ejemplares no fecundados de Sitotroga cerealella Olivier en cada plato de Petri. Los conteos 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 bastan-

te 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

esteronización de pupas de machos de Ceratitis capiata (Wiedemann) y


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

Dysercus 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 fue realizado en el mes de septiembre de 1989,
in el Centro de Energía Nuclear para la Agricultura anexo a la ESALQ,
EJECTO DE RADIACION GAMMA SOBRE ADULTOS DE *Sitotroga cerealella* (Olivier)

<table>
<thead>
<tr>
<th>Dosis (krad)</th>
<th>Mortalidad en 3 días</th>
<th>Mortalidad en 6 días</th>
<th>Mortalidad en 9 días</th>
<th>Mortalidad en 12 días</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1</td>
<td>10</td>
<td>22</td>
<td>48</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>15</td>
<td>37</td>
<td>48</td>
</tr>
<tr>
<td>6</td>
<td>6</td>
<td>19</td>
<td>40</td>
<td>50</td>
</tr>
<tr>
<td>12</td>
<td>16</td>
<td>21</td>
<td>41</td>
<td>50</td>
</tr>
<tr>
<td>24</td>
<td>9</td>
<td>12</td>
<td>44</td>
<td>49</td>
</tr>
</tbody>
</table>

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

2. MATERIAL Y MÉTODOS

La fuente de radiación ionizante gamma fue un irradiator de 60Co marca «Canambeam 150», Atomic Energy of Canada Ltd, Ottawa, Canada - Modelo GB-150B», con una actividad de 931 Ci en septiembre de 1969.

El material a tratar consistió de ejemplares adultos no sexados de *Sitotroga cerealella* Olivier provistos de maza infestada.

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 fue realizado el día 8 de septiembre de 1969.

Los conteos 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

Mortalidad en 10 días

46
48
50
50
48

El estudio de radiación de 
30 Co

1, Ottawa, Canadá -

a no sexados de 
fruta adecuada y 
20 intensidades de 
60 Co después de 
30, 2 krad/h.

3.9 en 28 días se

menor tiempo

Sterility Principle for Control of Fruit Flies

(Session II)
MEDITERRANEAN FRUIT FLY

When the sterile insect technique was used against Mediterranean fruit flies, it was found that the effective release of the sterile flies was essential for the success of the eradication program. The fruit flies were released in California from Mexico, and the program was undertaken in cooperation with the California Department of Agriculture. The eradication technique was found to be effective in reducing the population of the Mediterranean fruit fly. The results of this study have implications for the control of other pests. The effective use of the technique requires careful planning and execution.
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, "Eradicating the Mediterranean Fruit Fly in Central America". In 1965, the 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 been the effects of radiations used, the initial large-scale test use of flies in tests indicated that the gamma irradiation had a more effective killing of adults than by other methods. However, several efforts to test the Mediterranean fruit f, pupae with 10-krad of about 50-65% that of 10-krad males is not appreciably effective in controlling the population.

The possibility of sterilization by exposing all pupae to gamma rays has been extensively studied, but the results have been mixed.

1.4. Distributing sterile flies

The success of the project in Central America depends on the ability to mass-produce sterile flies at a low cost and to distribute them effectively. However, the problems of mass-producing sterile flies are significant.

The use of gamma irradiation to sterilize Mediterranean fruit flies is being studied at the University of Hawaii. The preliminary results have been promising, and the potential for large-scale production has been demonstrated.

The development of sterile insect techniques for the control of the Mediterranean fruit fly is an area of ongoing research. The success of these efforts will depend on the ability to produce large numbers of sterile flies at a cost that can be justified. Further research is needed to improve the efficiency of the sterilization process and to find ways to reduce the cost of producing sterile insects.
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 topa 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 [13–18]. 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 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 reinfections. Thus, as soon as the incipient reinfection 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 sterilization technique in Saipan and Tinian without the use of supplemental methods, eradication was not achieved, even though an apparently favorable 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 (Ceratitis, Nicaragua; September 1968 to May 1969; Rhode et al., in press), the 45-km² 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 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 Mariams 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-mile² 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 cue-lure fibre cubes, each containing 1 ml cue-lure plus 1 ml of naled, were released. In the next 3 - 4 months 200 evaluation traps were set. Therefore, the weight of wet captures. Meanwhile, the weekly releases have been continued, and it is expected that the level of methods should eventually be reduced to approximately one bait application per week. To the necessary addition of the native males that might occur.

The male annihilation Mediterranean fruit fly; Portuguese Azores in 5% naled, and a thirch of this mixture was applied in the yard of a single application (rate of 2.5 cm²) for a few days. However, when used in a very limited application of this bucket of the trees, similar to that of the agricultural trees, was applied from bait sprays.

Subsequently, a 115 cm² × 1,25-cm cube was the rate of one per acre in Hawaii. Tray tests showed that this cube of cue-lure was effective in killing the majority of flies. When applied to fibreboard cubes in the laboratory, these tests showed the rate of 1 ml of cue-lure to be effective in killing the majority of flies. When applied to fibreboard cubes in the laboratory, these tests showed the rate of 1 ml of cue-lure to be effective in killing the majority of flies. When applied to fibreboard cubes in the laboratory, these tests showed the rate of 1 ml of cue-lure to be effective in killing the majority of flies. When applied to fibreboard cubes in the laboratory, these tests showed the rate of 1 ml of cue-lure to be effective in killing the majority of flies. When applied to fibreboard cubes in the laboratory, these tests showed the rate of 1 ml of cue-lure to be effective in killing the majority of flies. When applied to fibreboard cubes in the laboratory, these tests showed the rate of 1 ml of cue-lure to be effective in killing the majority of flies. When applied to fibreboard cubes in the laboratory, these tests showed the rate of 1 ml of cue-lure to be effective in killing the majority of flies. When applied to fibreboard cubes in the laboratory, these tests showed the rate of 1 ml of cue-lure to be effective in killing the majority of flies. When applied to fibreboard cubes in the laboratory, these tests showed the rate of 1 ml of cue-lure to be effective in killing the majority of flies. When applied to fibreboard cubes in the laboratory, these tests showed the rate of 1 ml of cue-lure to be effective in killing the majority of flies. When applied to fibreboard cubes in the laboratory, these tests showed the rate of 1 ml of cue-lure to be effective in killing the majority of flies. When applied to fibreboard cubes in the laboratory, these tests showed the rate of 1 ml of cue-lure to be effective in killing the majority of flies. When applied to fibreboard cubes in the laboratory, these tests showed the rate of 1 ml of cue-lure to be effective in killing the majority of flies. When applied to fibreboard cubes in the laboratory, these tests showed the rate of 1 ml of cue-lure to be effective in killing the majority of flies. When applied to fibreboard cubes in the laboratory, these tests showed the rate of 1 ml of cue-lure to be effective in killing the majority of flies. When applied to fibreboard cubes in the laboratory, these tests showed the rate of 1 ml of cue-lure to be effective in killing the majority of flies. When applied to fibreboard cubes in the laboratory, these tests showed the rate of 1 ml of cue-lure to be effective in killing the majority of flies. When applied to fibreboard cubes in the laboratory, these tests showed the rate of 1 ml of cue-lure to be effective in killing the majority of flies. When applied to fibreboard cubes in the laboratory, these tests showed the rate of 1 ml of cue-lure to be effective in killing the majority of flies. When applied to fibreboard cubes in the laboratory, these tests showed the rate of 1 ml of cue-lure to be effective in killing the majority of flies. When applied to fibreboard cubes in the laboratory, these tests showed the rate of 1 ml of cue-lure to be effective in killing the majority of flies. When applied to fibreboard cubes in the laboratory, these tests showed the rate of 1 ml of cue-lure to be effective in killing the majority of flies. When applied to fibreboard cubes in the laboratory, these tests showed the rate of 1 ml of cue-lure to be effective in killing the majority of flies. When applied to fibreboard cubes in the laboratory, these tests showed the rate of 1 ml of cue-lure to be effective in killing the majority of flies. When applied to fibreboard cubes in the laboratory, these tests showed the rate of 1 ml of cue-lure to be effective in killing the majority of flies.
results without any trace of the remaining active ingredient.

A combination of the melon fruit fly in Morrocoy, Venezuela [1]. Also, when necessary, oriental fruit fly eradication was carried out by using sprays at 45-km² test areas. A 2-km border of the flies was maintained by delivering the insecticide at 48-h intervals.

A successful technique against the Mediterranean fruit fly in the Caribbean was based on a single application of malathion, diazinon, and aldicarb. However, it is not practical for small-scale applications. A more practical approach involves the use of field trials to evaluate the effectiveness of these materials. The use of field trials to evaluate the effectiveness of these materials is important because it allows for the identification of potential problems and the optimization of treatment methods. These field trials should be conducted in a controlled manner to ensure accurate results. The results of these field trials can then be used to improve treatment methods and reduce the environmental impact of the use of these materials.
fibroboard 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 tridemium 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 infected area in that State (about 1000 miles²) 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 selected host areas, male annihilation with all the highly specific lures, and release of sterile insects of all three species.

Such use of sterile the eradication of all and applied phases of and in small field test releases and the male in large-scale pilot te in Hawaii. Such tests of the smaller islands in such testing is great U.S. Congress, and for additional is needed to perfect erad fruit flies in Hawaii.

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

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. MELIADO
Instituto Nacional de Investigaciones Agronómicas, Madrid, Spain

Abstract — Resumen

THE STERIL-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 (treated in 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 (10% 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 Málaga. 30 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 7% (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 in irradiation doses (7 krad and 9 krad) and on the problem of infections 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 "máchos esteriles" 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 mensual de 1 millón de pupas/día.

De 1966 a 1968 se realizaron ensayos de suelta de insectos estériles (tratados con 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 freez (10% de ataques) y la zona testigo (70% de ataque).

En 1969 se realizaron ensayos de campo en una plantación regular de cítricos, albaricoque y melocotón, de la provincia de Málaga. Se soltó un total de 32 millones de insectos sobre un área de 25 ha durante el período marzo-agosto. A pesar de que la zona bien aislada se logró mantener el ataque de Ceratitis inferior al 7% (excepto en la última semana de julio, cuando ya no quedaba muy poco fruta). Durante el mismo período, los zonas testigo adyacentes registraron ataques superiores al 60% en todas ellas.

En 1970 se está estudiando, en el campo, el efecto de distintos dosis de irradiación (7 krad y 9 krad) y el fenómeno de las placas 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 propone 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 produjo un promedio de 50 000 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 vidés. No existían plantaciones regulares próximas dichas. En toda la zona, el ataque de Ceratitis era muy fuerte. Se realizaba ningún tratamiento sistémico 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 ensayarón 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 sueltes aéreos. De mayo a diciembre de 1966 se soltaron más de seis 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 fue 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 realizaron un muestreo de resultados finales. En 1968 los resultados fueron, en el 1) Zona experimental: entre el 5% y el 10% 2) Zona testigo: el 30% y el 100%.

TRABAJOS REALIZADOS EN 1969

La producción de programas de campo se hizo debido a una serie de muestras de insectos en la zona de laboratorio. En este programa se obtuvo asesoramiento técnico del mismo. La nueva zona es regular y se logró una disminución de la población de insectos pródromas. La zona testigo en albaricoque y en melocotón sin utilizar ninguna otra método.

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<th>Zona experimental</th>
<th>Cítricos</th>
<th>Albaricoque</th>
<th>Melocotón</th>
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<td>Porcentaje de F1</td>
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<th>Zona testigo</th>
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Agronómicas viene <machos estériles» (Wiedemann). En trabajos realizados
sobre métodos para control de las plagas, con pupas diarias. Las
insectos irradiados en un reactor se colocaron en bolsas en el campo y se
insectos, No se utilizaron en el programa. La

Y se utilizó un dato de 9 krad, que resultó efectivo en el control de algunas plagas.

Ceratitis capitata en el que se utilizó un dato de 9 krad, que resultó efectivo en el control de algunas plagas. El uso de pupas diarias podría ser una alternativa efectiva.

Por lo que se ha visto, un muestreo sistemático del grado de ataque de Ceratitis en la zona de estudio es crucial para tomar medidas adecuadas.

Agricultura Artificial, Se utilizó un dato de 9 krad, que resultó efectivo en el control de algunas plagas. El uso de pupas diarias podría ser una alternativa efectiva.

Por lo que se ha visto, un muestreo sistemático del grado de ataque de Ceratitis en la zona de estudio es crucial para tomar medidas adecuadas.

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 Sur de España. En este programa colaboró la División Mixta FAO/OIE, proporcionando asesoramiento técnico y suministrando pupas durante la primera fase del mismo.

La nueva zona experimental abarcaba unas 25 ha, con plantaciones regulares de Cfrícos, albaricoque y melocotón. Como zonas testigo se eligió una plantación regular de melocotón y cebolla, aunque en zonas 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.

### PORCENTAJE DE FRUTA ATACADA

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<td>Albaricoque</td>
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<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Melocotón</td>
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<td>0</td>
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<td>0</td>
</tr>
</tbody>
</table>

<table>
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<th>Abril</th>
<th>Mayo</th>
<th>Junio</th>
<th>Julio</th>
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<tbody>
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<td>0</td>
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<td>0</td>
</tr>
<tr>
<td>Albaricoque (tratado con insecticida)</td>
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<td>-</td>
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<td>0</td>
</tr>
<tr>
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<td>30</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td>Melocotón</td>
<td>-</td>
<td>25</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td>Melocotón</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>60</td>
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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 8% 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 Sur de España. En este programa colaboró la División Mixta FAO/OIE, proporcionando asesoramiento técnico y suministrando pupas durante la primera fase del mismo.

La nueva zona experimental abarcaba unas 25 ha, con plantaciones regulares de cfrícos, albaricoque y melocotón. Como zonas testigo se eligió una plantación regular de melocotón y cebolla, aunque en zonas 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 Selbersdorf, 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 fértil desde zonas próximas.

El examen de la fruta de la zona experimental y de zonas testigo dio los resultados que se pueden observar en el cuadro. Estos resultados demuestran que es posible lograr un control efectivo de *Coratissia 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 necesario 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 producen 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 frutas, 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 krade insectos fériles 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 ensayos 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 (Jeróni sería inconveniente, en relación directa con la producción.

Actualmente, la irradiación de una millón de pupas/día que se están obteniendo experimento sobre su eficacia.

L. A. KANSU: Th irradiation would solve the problem of controlling the pest.

L. MELLADO: I know, but the problem would be if the females were caged in the field to create the worst possible damage.

M. FRIED: You differently. Is there any variety of being affected by the irradiation?

L. MELLADO: It is not possible, at all. However, we found that both the "sterile females" are effective and can be used in the pupal stage of the pest.

Dealing with a new blowfly, Lucilia cuprina, we have chromosome experiments by means of crossover in males of higher flies. They then built a machine that could separate the sexes.

Any mutant which in this manner in the females will have heterozygote. This is the first step in the process.
portaron por
as cuales se realizaron
(26 millones
elaboratorio de
suelos de los insectos
definiciones normales, 
incidencias de insectos 
irradiados 
que la población 
riesgo de variaciones 
suelo (aunque hay 
dias de varios

Sin embargo,
hojas de melocotón
zonas testigo dijeron 
un control eficaz 
métodos de "machos 
que no están

En otras (Jerónimo, paraguayo), son muy visibles y constituyen un 
serio inconveniente. En todos los casos, el número de plecadoras 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 
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 
than built a machine which sorted the pupae on the basis of colour — males 
who 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 
heterocyste. This may be an obstacle to mass rearing in some instances.

With the sheep blowfly our object was not simply to eliminate a trouble-
some 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

L. MELLAIO: 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. MELLAIO: We did not determine the release ratio. However, the positive results of the experiment in Alabama 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 overflying, and the situation improved week by week. In some other programs that we have carried out infected 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. Mellaio, of releasing only one sex, particularly with respect to the avoidance of intermingling between sterile males and sterile females?

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

G. H. S. HOOKER: 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 should 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. KALMOKOS
Institut phytopathologique Benaki,
Kifissia,
Athènes, Grèce

Abstract — Résumé

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

As it is well known, the prerequisite for any integrated control of the olive fly 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 is divided into two sections: the first section deals with the action of chemical reagents, incident radiation, insecticides, attractants and repellents, 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.

INTRODUCTION

La stérilisation d'une certaine espèce d'insectes en laboratoire, à l'aide de radiations ou de produits chimio-stérilisants, 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 manifesté 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 chimios-térilisants, soit sur la chimio-stérilisation de populations naturelles, il est indispensable de résoudre prématurément 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 autoctones, 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 chimio-steriligants, par les radiations, les insecticides, les substances attractives ou repulsives, 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 autoctones.

Nous aimerions 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 qui présentes 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 autoctones. 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 biologique, 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 diverses 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 Berlisse - 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 salis, 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 parallèles attractives du Dacus (huiles essentielles, ont aboli à des résu de vue du chimiotropisme des séances [5], méthylènogéne, isoe Citronella) dont la puissance était déjà de Ainsi, lesatriades sur les aulx en plus grande des près céréalières, atrope, z. en combinaison avec méthodes de lutte près l'oliculture assurante par pulvérisations a : puis à partir de l'air l'extension de cette z. densité de la population à environ cinquante n. Les études effectuées l'application de la nos (1 litre/ha) montrent présenté, au point d' Dacus, dans l'application de l'insecte au moyen de

2. RECHERCHES SUR LE Dacus

Il a été constaté et certaines autres que l'olive des agents de l'action des substances. Ces résultats pré-
concerne l'application de méthodes chimiques chimiotropisme négatif appâts de Dacus (subs et agissent dans un degré largement emploi

3. RECHERCHES SUR EN LABORATOIR

A la suite des trentes observées par certaines de Cerrit des Trypetidae appar
Consciente lorsqu’il est le plus adapte, mais, au
mème moment, dans le domaine des médicaments et des méthodes, dans le
domaine des produits chimiques, on cherche à trouver des remèdes contre les parasites, les maladies, qui se transmettent par les radiations, par le vent, par les eaux, ainsi que sur
le site de l’infestation, à savoir les cultures, les engrais, les pesticides, les insecticides, les substances chimiques et
des substances synthétiques, qui, malheureusement, sont encore trop nombreux malgré les progrès réalisés.

Ainsi, la recherche continue de solutions à la lutte contre les parasites est toujours d’actualité.

2. RECHERCHES SUR DES AGENTS DE CHIMIOTROPISE NÉGATIF
DU Dacus

Il a été constaté que les huiles essentielles, les résines, les éthers, les aldehydes, les huiles végétales
ont un effet dévastateur sur les larves de Dacus parmi les différents groupes de substances chimiques
et des substances biologiques. Les résultats obtenus à partir des laboratoires de recherche ont montré
qu’il est possible d’obtenir des résultats positifs avec certaines plantes et certaines substances chimiques
dont la composition est connue. Cependant, des recherches plus approfondies sont nécessaires pour
connaître les effets spécifiques de chaque substance.

3. RECHERCHES SUR LA CHIMIOSTERILISATION DU Dacus
EN LABORATOIRE

A la suite de l’étude des travaux effectués dans les années 1962-1963, nous avons pu observer une action stérilisante particulière de la substance 3,4,4’-sulfolimène, qui est une substance active d’insecticides, qui agit sur la reproduction des larves de Dacus et qui a été utilisée dans des recherches de laboratoire avec succès.

Les recherches ont montré que les larves de Dacus sont particulièrement sensibles à cette substance, qui agit sur la reproduction des larves et sur la croissance du broyat. Ces résultats sont encourageants et permettent de penser à une utilisation future de cette substance dans les cultures de fruits et de légumes.

En conclusion, la recherche sur les agents de chimiotropisme négatif du Dacus est un domaine d’exploration prometteur, qui mérite d’être développé davantage, pour obtenir des solutions efficaces à la lutte contre ce parasite nuisible.


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 chimio-stérilisation sur la stérilisation par les radiations consiste, comme on le sait, dans le fait que la stérilisation chimique peut être appliquée, d’après Kinpling [15], non seulement à des insectes d’éloignement aussi à des populations naturelles. Il est évident que cette possibilité présente une intérêt tout spécial pour certaines espèces monophages comme le Dacus de l’olive, dont l’éloignement en masse sur substrat artificiel présente encore bien des difficultés.

Malheureusement, toute chimio-stérilisation 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 technologiques [10] - à l’exception d’expériences à l’aide de piqûres d’autochtonisation - 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 chimio-stérilisation à l’aide 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’hexama (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, on note les fenoxycarb [19] comme des agents antiséptiques, soit par les travaux de Koganti comme des agents inhibiteurs d’oviposition [14]. On notera que, parmi ces substances, les fongicides connus sous les noms de Da-Ter et Decafentin, administrés par os, ont fortement entravé le

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1 Tristyrylphénoxyde d’étain de la Maison Philips-Duphar, Pays-Bas.
2 (Décyltristérylphényl) bromochlophénylaminato de la Maison Cella/Landwirtshaftliche Chemikalien GmbH, Lappeenranta, P.O. 46, 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).

4. RECHERCHES S’EN PLEIN AIR

Pour étudier les conditions des Dacus nous avons procédé à de l’ile de Lesvos, c’employé pour cette a les résultats étaient centages de des lois, fait que les pourcent de deux mois et demi, à correspondants des les exiguil de l’olive dénote clairement en présenter à l’avenir Dacus de l’olive pour

5. RECHERCHES S’EN RADIATIONS

La méthode de s’assit, avec la méthode fondamentales dont la part, du Dacus de Les travaux effé et Bacetti [18] ont p des pupes de Dacus à gamma (6000 – 15000) ont montré que, avec normaux, le résultat D’autres recherches nouveaux mis en évidence sur les pupes que sur récemment étudié t plus, les féminines [71].
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 CHIMIOSTÉRILISATION 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 (apholates), 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 piégeaux 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 soient maintenus constamment, pendant deux mois et demi, à des niveaux nettement inférieurs aux pourcentages correspondant des témoins (45,3 ± 14,5 contre 99,7 ± 3,3), malgré l'exiguité de l'oliveraie expérimentale et l'insuffisance de son isolement, démontre clairement l'importance 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 STÉRILISATION 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 papilles de *Dacus* 3 à 7 jours avant leur éclosion à l'aide des rayonnements gamma (8000-12000 R) émis par une source de cobalt (60Co). 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 papilles que sur les adultes et les larves de *Dacus*. Tsanakakis 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 autoctones 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 carbamates [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 de cerner 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 autoctones.

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

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

Pour l'application efficace de toute méthode autoctone il est particulièrement important que, au moment où les individus stériles sont lâchés ou pendant l'application des chimiostratifiants 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 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 1958, au cours de laquelle il a été établi par Kalopissis et al. que le mode classique d'évaluation au moyen d'au moins huit à dix résultats dépend de l'humidité différence entre l'humidité des pâtes et à l'extérieur de la production de la population au moment de la confusion de la population au moment de la confusion de la population et à la collection à la fois de la confusion des individus stériles et des individus stériles et des individus stériles.

b) Élevage de Dacus

La possibilité d'une méthode autoctone en Grèce pour l'élevage de Dacus a été évoquée par de nombreux chercheurs qui ont proposé des systèmes de cultures autoctones.

Cependant, un certain nombre de ces systèmes ont montré qu'ils sont peu efficaces et qu'ils ne permettent pas d'atteindre un taux de stérilité élevé.

Néanmoins, malgré ces difficultés d'application, le projet de culture autoctone de Dacus a été très actif et a permis d'obtenir des résultats encourageants.

Néanmoins, malgré ces difficultés d'application, le projet de culture autoctone de Dacus a été très actif et a permis d'obtenir des résultats encourageants.
L Есть аттрактивные, адаптированные для использования в борьбе с Dacus, включая их использование в составе разнообразных средств борьбы с вредителями, включая использование в составе различных методов борьбы с вредителями.

**Dacus**

...в жаркое время года, когда активность Dacus максимальна, его можно уничтожить с помощью биологических методов борьбы, таких как использование аттрактивных агентов и химических средств. Это позволяет не только контролировать популяцию, но и сохранять экосистему. В то время как использование химических средств может привести к развитию устойчивости к ним, биологические методы борьбы позволяют более эффективно контролировать популяцию Dacus. Однако, необходимо учитывать, что эффективность биологических методов борьбы зависит от многих факторов, включая климатические условия, состояние экосистемы и т.д. Поэтому использование аттрактивных агентов и химических средств в комплексе с другими методами борьбы может быть наиболее эффективным. Опыт показывает, что при правильном использовании аттрактивных агентов и химических средств, можно достичь значительного снижения популяции Dacus. Важно также проводить постоянное наблюдение за состоянием популяции и своевременно вносить коррективы в методы борьбы с Dacus. Наконец, необходимо также учитывать эмоциональные факторы, такие как психологическое воздействие агентов и химических средств на людей, которые могут быть более чувствительными к ним. В этом случае может быть целесообразно использовать дополнительные методы борьбы, такие как психологические и социальные методы. В итоге, чтобы эффективно бороться с Dacus, необходимо использовать комплексный подход, включающий в себя аттрактивные агенты и химические средства, а также психологические и социальные методы.
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 oleae (Gmelin) au moyen de méthodes chimiques et biologiques.

Néanmoins, à l'heure actuelle l'application d'une méthode de lutte intégrée se heurte principalement à deux obstacles : en ce qui concerne la stérisisation d'insectes d'élevage au moyen de radiations ou de chémiostérilisants, 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 stérisisation de populations naturelles au moyen de chémiostérilisants, 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

l'application de
pourrir des agents
toujours et les animaux à
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le Dacus oleae
méthode de lutte
commune qui concerne
également
simple, peu coûteuse.

ouvert, tandis que la
n'est pas trop attendre.


HARAS, L., Rapport sur les
l'année 1839, Annist inst.

MAHABBA, D. H., New
2006.

Gnatell (Diptera, Trypetidae)

with low toxicity for mammals,
(1864).

ta, Trypetidae), et fonction des

esters of 6-methyl-2-cyclohexanone

the olive fly, Dacus oleae

of insects applied to insects of

insect Chemosatirretsation

Rosy, Amsterdam (1968).

MOROS, H., Pachoutis, E.,

against the Oriental fruit fly.


compounds as insect reproduction inhibitors, J. econ. Ent.


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. KLEMBAS: 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, hema 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 Hemi, which have a molecular structure more or less similar to that of lepa and tretamine and which do not possess akylation 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 Kanaga 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.

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GENETIC CONTROL OF THE Rearing and Sterilization of Rhagoletis cerasi. In a sterile-insect release method the success achieved by using under laboratory conditions with the best diet. This is a has shown a similar sensitivity irradiated with 5 FR is significantly lower and can be neglected.

The European moderate climate to unconfirmed area North African coast.

ECONOMIC IMPORTANCE

The species attains a large size of very high value in cherry production. The pest insect varying time of emergence is of cherry and its area begins the longer lasting th the insects and the of toxic residues on...
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, Wadenswil, Switzerland

Abstract

The European cherry fruit fly, Rhagoletis cerasi L., occurs in the moderate climatic zones of Europe and 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), but 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.). 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, or ovipositing in grape-berris 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 and Boller; the female of a special wax (Cei) eggs inside the dome to the relevant paper performance of these eg

The results of the egg fertility with wax methods tested. Of eggs dropped by the new type of ov laboratory [7] for m (64 cm diam.) contai stacked one on top of a vertical axle around eggs are readily rearing cage, by first separating the cages jet. The water carr filtered out.

2. Feeding the larvae

In all feeding ex hatched larvae were egg mortality incres

The feeding subs and by dissolving the water. When they w the geling properties amount of Gelard as components and then were normally provi are shown on Table 1

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Kin 60</th>
<th>Dk</th>
<th>Agar 6 C</th>
<th>Wc</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(1) Deutsche Erdil All,
TABLE I. COMPARISON OF DIFFERENT KINDS OF EGG COLLECTION

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Kind of egg collection</th>
<th>No. of eggs per female</th>
<th>Egg fertility (%)</th>
<th>No. of larvae per female</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Dropping</td>
<td>61.3</td>
<td>6.5 ± 7.2</td>
<td>4.3</td>
</tr>
<tr>
<td>2</td>
<td>Agar-agar balls</td>
<td>2.7</td>
<td>52.5 ± 18.4</td>
<td>1.4</td>
</tr>
<tr>
<td>1</td>
<td>Grapes</td>
<td>3.8</td>
<td>42.2 ± 17.4</td>
<td>3.5</td>
</tr>
<tr>
<td>2</td>
<td>Total</td>
<td>72.6 ± 8.2</td>
<td>-</td>
<td>9.4</td>
</tr>
<tr>
<td>2</td>
<td>Wax domes</td>
<td>66.6 ± 11.2</td>
<td>85.7 ± 28.3</td>
<td>37.1</td>
</tr>
</tbody>
</table>

and Boller; the females push their ovipositors through the thin membrane of a special wax (Ceresin 1577, 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 egg-holding 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 Röhl AG, Hamburg, Federal Republic of Germany
<table>
<thead>
<tr>
<th>Food component</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat germ</td>
<td>11.5</td>
<td>11.5</td>
<td>10.6</td>
<td>0</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>Wheat germ diet (a)</td>
<td>14.8</td>
<td>14.6</td>
<td>13.4</td>
<td>5.6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cellulose (b)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Paper pulp</td>
<td>4.4</td>
<td>4.4</td>
<td>4.1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Gelgard (c)</td>
<td>4.4</td>
<td>4.4</td>
<td>4.1</td>
<td>6.6</td>
<td>0</td>
<td>5.0</td>
</tr>
<tr>
<td>Peat</td>
<td>4.4</td>
<td>4.4</td>
<td>4.1</td>
<td>6.6</td>
<td>0</td>
<td>5.0</td>
</tr>
<tr>
<td>Powdered carrots</td>
<td>1,2</td>
<td>(3.92)</td>
<td>(3.28)</td>
<td>(3.33)</td>
<td>(3.33)</td>
<td>(3.33)</td>
</tr>
<tr>
<td>Vitamin diet + sodium citrate mixture (d)</td>
<td>(0.03)</td>
<td>(0.03)</td>
<td>(0.03)</td>
<td>(0.03)</td>
<td>(0.03)</td>
<td>(0.03)</td>
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<tr>
<td>Cholesterol</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td>0</td>
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<tr>
<td>Ascorbic acid</td>
<td>0</td>
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<td>0</td>
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<tr>
<td>1-Aspartic acid</td>
<td>0</td>
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<td>0</td>
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<tr>
<td>1-Aspartic acid</td>
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<tr>
<td>B-carotene</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>B-vitamin D1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td>B-carotene</td>
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<tr>
<td>B-vitamin D1</td>
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<tr>
<td>B-carotene</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Water</td>
<td>57.6</td>
<td>57.6</td>
<td>57.6</td>
<td>57.6</td>
<td>57.6</td>
<td>57.6</td>
</tr>
</tbody>
</table>

| pH                                   | 4.7 | 4.7 | 4.7 | 4.7 | 4.7 | 4.7 |

Mean yield of paper per 100 larvae x standard deviation 6.5 ± 3.3 11.1 ± 1.0 12.1 ± 1.9 15.5 ± 4.7 22.2 ± 4.3

Minimum time (days) to reach preparation x standard deviation (days) 16.0 ± 5.0 16.1 ± 5.0 16.1 ± 5.0 16.1 ± 5.0 16.1 ± 5.0 16.1 ± 5.0

Total number of inserted larvae 1178 1086 1323 685 263

Number of operations 11 9 12 7 9 3

(a) Nutritional Biochemicals, Cleveland, Ohio, USA
(b) Schleicher & Schuell No. 123, Fed. Rep., Germany
(c) Dow Chemical Co., Midland, USA
(d) Contained in vitamin diet + sodium citrate mixture

Wheat germ, 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 sub.

Time and again some becoming dry and hay.

The results of the main energy sources not detrimental to the growth of the larvae up to pu.

The main protein experiences 4 to 8% detrimental to the growth of this protein source. 3.2 or 3.9% casein is unnecessary because of this protein source.

As already stated, the larvae to make tunnels which are not in obtaining such a 30% water evaporate

Concerning substrate can be omitted. Also that they are necessary in the yeast. The main

As already stated larva to make tunnels which are not in obtaining such a 30% water evaporate

The quality of the main protein experiences, 4 to 8% detrimental to the growth of this protein source.

As already stated larva to make tunnels which are not in obtaining such a 30% water evaporate

Concerning substrate can be omitted. Also that they are necessary in the yeast. The main

As already stated larva to make tunnels which are not in obtaining such a 30% water evaporate
SUBSTRATES
YIELD QUALITIES

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Mayor at Munster, (Haitch)

The carrier pulp, peat, Gelgard is not excluded to provide the larvae with the larvae and hence 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 hours of the carrier of the capsule, the substrates become hard, and the food substance of the larval decreases. This observation was made with substrates 1 and 2. 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 fungicides. 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 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 substances in the substrates 1-3 do not sufficiently fulfill this pre-condition. 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 diet was judged by the speed of development of the larvae and the yield of pupae. Under natural conditions the development of the larva 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-asparagine and L-glutamic acid were used in addition to the protein amino acids because these contain relatively high amounts of these amino acids. The t-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 choline chloride, 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 hours of the carrier of the capsule, the substrates become hard, and the food substance of the larval decreases. This observation was made with substrates 1 and 2. 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.

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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 67.5%, 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:

<table>
<thead>
<tr>
<th>Radiation dose (kR)</th>
<th>% hatching</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100.0</td>
</tr>
<tr>
<td>2</td>
<td>19.4</td>
</tr>
<tr>
<td>4</td>
<td>9.0</td>
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<tr>
<td>8</td>
<td>5.7</td>
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<tr>
<td>3</td>
<td>1.7</td>
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</table>

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.

<table>
<thead>
<tr>
<th>Radiation dose (kR)</th>
<th>Eggs per female per day</th>
<th>Fertility of eggs (%)</th>
<th>eggs (%) corrected to 100% of control</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>3.0</td>
<td>67.5</td>
<td>100.0</td>
</tr>
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</table>

The results (Table I) showed that the pupa stage between 1 and 2 was the most sensitive to irradiation, although the dependence of the increase in egg production on the number of irradiated flies was not very considerable.
Effect of benzoic acid
also toxic to larvae —

 vantage by other

Table II,

larvae. The

more suitable

strate 4) in

gamma rays from

ist flies emerged.

be observed after

emergence the sexes

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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 21-28 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)

<table>
<thead>
<tr>
<th>Irradiation dose (kR)</th>
<th>Hatching rates (%)</th>
<th>1-2</th>
<th>3-4</th>
<th>5-6</th>
<th>7-8</th>
<th>Observed</th>
<th>Corrected a to 90%</th>
<th>100%</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 a</td>
<td>63.5 ± 1.6</td>
<td>51.0 ± 2.0</td>
<td>63.2 ± 2.1</td>
<td>70.6 ± 4.5</td>
<td>63.6 ± 1.0</td>
<td>67.5</td>
<td>70.1</td>
<td></td>
</tr>
<tr>
<td>b</td>
<td>154</td>
<td>1989</td>
<td>964</td>
<td>455</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>c</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
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</tr>
<tr>
<td>4 a</td>
<td>56.2 ± 2.1</td>
<td>56.5 ± 2.8</td>
<td>58.1 ± 2.6</td>
<td>59.1 ± 2.6</td>
<td>53.9 ± 1.1</td>
<td>67.2</td>
<td>63.5</td>
<td></td>
</tr>
<tr>
<td>b</td>
<td>1704</td>
<td>1097</td>
<td>998</td>
<td>1353</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>c</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>5 a</td>
<td>56.8 ± 1.4</td>
<td>56.7 ± 1.8</td>
<td>56.9 ± 2.8</td>
<td>58.8 ± 2.4</td>
<td>53.5 ± 1.6</td>
<td>67.7</td>
<td>64.3</td>
<td></td>
</tr>
<tr>
<td>b</td>
<td>915</td>
<td>1452</td>
<td>1170</td>
<td>758</td>
<td>4926</td>
<td>4266</td>
<td>4926</td>
<td></td>
</tr>
<tr>
<td>c</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>-</td>
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</table>

* = % 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

a = number of hatching larvae

b = number of eggs

% = number of hatching larvae

** = number of replicates

* = hatching rate of the eggs of the partly irradiated population corrected for 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 5% 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 [8] 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 population, is:

\[ q = \frac{n - f}{n (1 - p)} \]

where:

- \( q \) = 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
- \( n \) = number of irradiated males
- \( n \) = number of normal males

In the experiment at were taken:

\[ q = 90 \% \]
\[ p = \text{result of } q \]
\[ f = 57.7 \% \]
\[ n = 1 \]

Thus competitiveness from 1.0 to 0.63 from irradiated males in this manner was brought to a certain level.

With the 5-kR irradiation (22.9 or 31.6) put to one normal pair.

The practical application of error possibilities using different irradiation rates, dosage rates, and other factors within one experiment.

A further point to take into account is that the irradiation damage for the tests is necessary to be reversed by irradiation.

When one considers the 5 kR, it seems doubt that irradiated males can be used. But for information...
In the experiment at the irradiation dosage of 5 kR the following values were taken:

\[ q = 90 \% \]
\[ p = \text{result of graphical interpolation of the figures of Fig. 1} \]
\[ \text{and correction to } 80 \% \text{ hatching of control eggs} = 6.5 \% \]
\[ f = 57.7 \% \text{ (Table IV)} \]
\[ n = 1 \]

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 60Co and 137Cs 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 H. 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 they 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

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 AGAINST
Preparation

E. F. BOLLER
Swiss Federal Viticulture at
Wädenswil, Zürich
A. HAISCH
Institute for Plant Cultiva
Protection of Munich, Fed.
R. J. PROKOP
Department of
Austin, Tex.

Abstract

STERILE-INSECT RELEASE ME
BEHAVIOURAL STUDIES.

Investigations were carried out to provide the necessary information for a long-term quantity, and the combination of a trapping system.

A study of colour vision and the effective visual throw-away reduction of the pest population. The trees in the test areas were planted in the UK and the CSSR. The investigations have been carried out.

Yield losses of the field study (average 200 m, 10 fruit trees). Furthermore, it was observed that the trees were more sensitive to the treatment than the cherry trees. These fields were selected in order to account for the yield losses of the field experiments.

The influence of the field study by means of a 'flight test'.

The yield experiments were carried out for the first time experiments in the summer of 1965. The results were in line with our expectations.

1. INTRODUCTION

The preceding year was one of exceptional cherry fruit fly, Rhagoletis cerasi, outbreaks.
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. I. PROKOPI
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 biological control 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 USSR against *R. cerasi* and in Italy against *Pomace olea*. Opticotropic responses 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 overlying 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 masking 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 papers 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-insect 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, Undorf/Switzerland.
TABLE I.

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

<table>
<thead>
<tr>
<th>Colour</th>
<th>Index of attractiveness</th>
</tr>
</thead>
<tbody>
<tr>
<td>High-gloss paint</td>
<td></td>
</tr>
<tr>
<td>Yellow (standard)</td>
<td>10</td>
</tr>
<tr>
<td>Blue</td>
<td>5.7</td>
</tr>
<tr>
<td>Red</td>
<td>6.4</td>
</tr>
<tr>
<td>Black</td>
<td>7.6</td>
</tr>
<tr>
<td>White</td>
<td>10</td>
</tr>
<tr>
<td>Yellow + 0.9%</td>
<td>10</td>
</tr>
<tr>
<td>Yellow + 1.5%</td>
<td>10</td>
</tr>
<tr>
<td>Yellow + 5.9%</td>
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<tr>
<td>Yellow + 15%</td>
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<tr>
<td>Yellow + 45%</td>
<td>5</td>
</tr>
<tr>
<td>Fluorescent paint</td>
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</tr>
<tr>
<td>Day &lt;10</td>
<td></td>
</tr>
<tr>
<td>Saturn Yellow</td>
<td>16.7</td>
</tr>
<tr>
<td>Squid Green</td>
<td>6.1</td>
</tr>
<tr>
<td>Horizon Blue</td>
<td>8.8</td>
</tr>
<tr>
<td>Fire Orange</td>
<td>9.4</td>
</tr>
<tr>
<td>Aluminium foil</td>
<td>0</td>
</tr>
<tr>
<td>Metal strip</td>
<td></td>
</tr>
<tr>
<td>Standard (6% NH₄HCO₃)</td>
<td>0.2</td>
</tr>
<tr>
<td>Painted yellow</td>
<td>1.1</td>
</tr>
<tr>
<td>Yellow + Bird Tanglefoot</td>
<td>3.2</td>
</tr>
</tbody>
</table>

Masonite boards (15 cm x 20 cm x 0.5 cm) were painted with the various mixtures, then coated with a thin layer of Bird Tanglefoot 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 1.

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 50% by using day-light.
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 68.8 against 48.0 on boards). No explanation is available for the poor performance of the cylinders (39.2). 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 Tryptetid 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 cardboard, 15 cm x 20 cm and 1 mm thick, were chosen as raw material rather than three-dimensional objects. The cardboard 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 measuring relative pc for direct control pur.
latter experiment was observation that a loc
within a few days we
cherry orchard. All
Basel) that showed ch
were supplied with try
25 flies per tree wer
before oviposition too
be reduced to 3.6% in
nearby communit
This new trap mi
insecticidal treatment
sterile-insect release
natural barriers do in
the development of wi
campaign. Investiga
efficient trapping syst

2.2. Marking technique

One important use of released sterile insects is to be released in the vicinity of the pest to be controlled and to achieve an effective rate of control.

2.2.1. Methods used

In the experiment, flies were released in cages and covered the respective mortality of the flies, not achieved. The flies are released in the laboratory and in the field to compare the effectiveness of the measures.

---

3 Day-Glo Color Division, Swisser St., 4700 St. Claus Ave., Cleveland, Ohio 44109, USA, distributed in Switzerland by G. Lohnto, Fabrik, 8948 Zürich.
4 Yellow Day-Glo 25844-405; G. Lohnto, Fabrik, 8948 Zürich, Switzerland.
Which increases the surface lengths (u,v.) into
achieved with
is not the
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ne plates on the board is about
ach 4% solution of
excellent results

search

ceeded to test
chromelb 207).
X-crossed boards
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o colour vision as,
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ons were only achieved
nd is an indication
he visual component
y do not find the

possible trap

cherry fruit fly
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aterial rather than
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one second into
between two plastic
roduced in 1978
on studies, for
land, Ohio 44103, USA,
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
powders8 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 proboscis. After a few days no
marking substance could be detected on the body surface but the protruded
proboscis 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 Häsch) 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

8 Day-Glo Color Division, Switzer Bros., 4732 St, Clair Ave., Cleveland, Ohio 44103, USA.
from the dispersion experiment was processed by Haich after the flies at Wadsamhi 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. cereus 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 3275 m during an experimental period of 6 hours. Whereas the males in the experiment were exhausted after 6 hours, the females had not 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 8780 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 from the normal and did not take into account the normal and could be recovered. A series of files irradiated with negative side-effects was taken into account.

2.4. Dispersion studies

Two dispersion studies of the community of Nuglar in 500 m wide, contain surrounded and isolated. A total of 6000 in the releasing point and increased by 100 m.

In 1970, 500 yellow pheromone of the and the influence of an equal trap density lines and served at the abundance of the wild factors (temperature,
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 dyes as markers.

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.

In 1970, 500 yellow and 500 red flies were released in 1969 from a central releasing point and recovered by traps in concentric circles with radii increased by 100 m.

<table>
<thead>
<tr>
<th>Place:</th>
<th>Nuglar/Switzerland</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of flies released</td>
<td>5988</td>
</tr>
<tr>
<td>No. of traps</td>
<td>262</td>
</tr>
<tr>
<td>Equal trap density</td>
<td>No</td>
</tr>
<tr>
<td>No. of flies recaptured</td>
<td>1124</td>
</tr>
<tr>
<td>Percentage of flies recaptured</td>
<td>18.9</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Distances covered</th>
<th>Average number of flies per trap</th>
</tr>
</thead>
<tbody>
<tr>
<td>50 m</td>
<td>12.3 (10-160 m)</td>
</tr>
<tr>
<td>100 m</td>
<td>1.56 (101-199 m)</td>
</tr>
<tr>
<td>200 m</td>
<td>0.70</td>
</tr>
<tr>
<td>300 m</td>
<td>0.31</td>
</tr>
<tr>
<td>600 m</td>
<td>0.25</td>
</tr>
<tr>
<td>500 - 1000 m</td>
<td>0.11</td>
</tr>
</tbody>
</table>

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 towards the opposite sexes

The experiment was carried out in a cherry orchard and the trees were divided into three groups based on fruit ripeness. The results obtained showed a significant difference in the orientation of the flies towards the opposite sexes. However, due to the nature of the experiment, it is difficult to determine the exact factors that influenced the orientation.

3. CONCLUSIONS

These results let us conclude that the cherry fruit fly is not released from the entire area. A grid search is necessary to determine the density of the flies. The optimal trap size can be determined by the emergence of the flies in the area. Further studies are needed to understand the behavior of the flies in different environmental conditions.

The authors wish to thank the Federal Institute of Technology for their help and the assistance of many colleagues.
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. cerata finds its host tree by means of a host-specific odour. We are inclined to believe that R. cerata 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 olfactive, leading the fly also to apple trees which are much richer in food sources than cherry trees. Flying to and from 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 reinestation (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


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 3 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 THE ME

G. H. S. HOO
International Vienna

Abstract

GAMMA STERILIZATION OF ?

The viability of male flies of 1-12 had been tested in eggs for irradiation. However, if the sterilizer transformation, a line, when males were irradiated, the population of the males, evidence of recovery of fertility irradiation. Females were raised on uninfected females was uninfected.

INTRODUCTION

Gamma sterilization (Wiedemann), has been studied. In addition, irradiation experiments have been carried out [1-7].

To provide information, in which the Joll Agriculture was co-operated Seibersdorf Laboratory. Seibersdorf strain of sexual competitiveness: report the work carry aspects of gamma-in-

MATERIALS AND METHODS

Larvae were reared by Nadel [20] at 25 ± 1°C and maintained at 25 ± 1°C.

Irradiation was performed with different doses of 25 to 46 hour. Irradiated in dose received by 100 flies in 4 hours.

Except where stated, the experiments were carried out in duplicate.
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 Ceratitis capitata inoculated in the pupal stage two days before emergence with gamma doses of 1 - 13 krad was investigated. When plotted on 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 is used in terms of the angular transformation, a linear relationship is obtained. At 11 krad male sterility was better than 95%.

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 20 days after irradiation. Females were more radiosensitive than males and 5 krad produced infecundity. The fertility of untreated females was unaffected by mating 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 60 ± 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 60Co 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 48 - 48 hours) after irradiation were used, i.e. the flies had been irradiated 2 days before adult eclosion. The flies were sexed (within
10 hours of emergence) and manipulated without anaesthesia by catching them in small glass vials. Unless otherwise stated, 35 males and 25 females were confined in 14 cm x 11 cm x 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 3 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 hatch below 1%. Since curvilinear when plotted results from different in logarithmic units a transformation, a very goo regression lines for ti months apart) are not significantly (P = 0.06) mean egg hatch over ε the egg hatch in the fi 2 weeks (Table I). To

It had also been n with untreated males o determine whether thi male or the female. ' in Table II. Compar the egg hatch declined females were mated w increase (treatment D males were mated wit was high (91.2%) and e the data indicate that 1 to the female and not t are compared with the require the presence o degree of egg viability
of eggs from the mating of females and 25
plastic cages and access to water
were taken 3 times
moist filter paper
were recorded.

A transformation
was carried out and
mean range test.
resulted from the hatch
of each
females. Similarly, irradiated females

The viability (expressed as two experiments result in a hatch to 4.5%, a
and to lower egg

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 trans-
formation, 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 (F = 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
females 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.

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

<table>
<thead>
<tr>
<th>Dose (krad)</th>
<th>Corrected % egg hatch in week 1</th>
<th>2</th>
<th>3</th>
<th>3-week mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>41.6</td>
<td>17.6</td>
<td>15.1</td>
<td>22.6</td>
</tr>
<tr>
<td>5</td>
<td>15.0</td>
<td>10.4</td>
<td>7.9</td>
<td>12.5</td>
</tr>
<tr>
<td>7</td>
<td>22.7</td>
<td>3.8</td>
<td>3.2</td>
<td>4.3</td>
</tr>
<tr>
<td>9</td>
<td>2.7</td>
<td>1.6</td>
<td>1.9</td>
<td>2.2</td>
</tr>
<tr>
<td>11</td>
<td>1.1</td>
<td>0.8</td>
<td>0.6</td>
<td>0.8</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Mating procedure</th>
<th>Mean % egg hatch</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Newly emerged ♀ and ♂ mated. Egg hatch over first 21 days (6 replicates).</td>
<td>30.3</td>
</tr>
<tr>
<td>B. Surviving ♀ and ♂ of A after 21 days recombined, egg hatch over next 16 days (4 replicates).</td>
<td>30.5</td>
</tr>
<tr>
<td>C. Newly emerged ♀ and ♂ mated, egg hatch over first 21 days (6 replicates).</td>
<td>30.4</td>
</tr>
<tr>
<td>D. The 21-day-old ♀ from C mated with young virgin ♂. Egg hatch over next 18 days (4 replicates).</td>
<td>30.1</td>
</tr>
<tr>
<td>E. The 21-day-old ♀ from C mated with young virgin ♂. Egg hatch over next 18 days (4 replicates).</td>
<td>30.1</td>
</tr>
<tr>
<td>F. Newly emerged ♀ and ♂ mated. After 5 days of removal, egg hatch over first 21 days (4 replicates).</td>
<td>77.3</td>
</tr>
<tr>
<td>G. The 21-day-old ♀ from F mated with ♂. Egg hatch over next 16 days (3 replicates).</td>
<td>30.7</td>
</tr>
</tbody>
</table>

* Each replicate consisted of 35 males and 25 females.

The main effect of gamma irradiation of females is infecundity. In an experiment employing only 2 replicates 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 GEMMA-IRRADIATED MATED WITH GAMMA-IRRADIATED MALES OF C. capitata

<table>
<thead>
<tr>
<th>Dose (krad)</th>
<th>Male</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>14.5</td>
</tr>
<tr>
<td>7.5</td>
<td>19.1</td>
</tr>
<tr>
<td>9</td>
<td>20.5</td>
</tr>
<tr>
<td>10.5</td>
<td>20.4</td>
</tr>
<tr>
<td>12.0</td>
<td>11.6</td>
</tr>
</tbody>
</table>

A Mean are based on 3

TABLE IV. STERILIZATION OF MALES OF C. capitata IRRADEAD

<table>
<thead>
<tr>
<th>Dose (krad)</th>
<th>Cages</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>25</td>
</tr>
<tr>
<td>7</td>
<td>25</td>
</tr>
<tr>
<td>9</td>
<td>25</td>
</tr>
<tr>
<td>12</td>
<td>16</td>
</tr>
</tbody>
</table>

A = irradiated; ♀ = virgin

The fecundity of the males was adjusted for sex, as an experiment showed that significantly affected the sterility of the males. Table IV records results of the experiment. Males were -1, -2 or 0 irradiated, and variance showed that significantly affected the sterility of the males. However, did the sterility of the males significantly more than the females. Since gamma irradiation of males, the before emergence wa
TABLE III. FECUNDITY OF UNTREATED FEMALE C. capitata MATED WITH GAMMA-IRRADIATED MALES

<table>
<thead>
<tr>
<th>Dose (krad)</th>
<th>Mean no. eggs/female/day a</th>
<th>Overall mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Week 1</td>
<td>Week 2</td>
</tr>
<tr>
<td>0</td>
<td>14.55</td>
<td>21.88</td>
</tr>
<tr>
<td>7.5</td>
<td>19.11</td>
<td>28.58</td>
</tr>
<tr>
<td>10.0</td>
<td>30.47</td>
<td>32.74</td>
</tr>
<tr>
<td>12.0</td>
<td>30.63</td>
<td>31.21</td>
</tr>
<tr>
<td>15.0</td>
<td>11.98</td>
<td>24.55</td>
</tr>
</tbody>
</table>

a Means are based on 3 replicates per treatment and from an experimental period of 51 days.

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

<table>
<thead>
<tr>
<th>Dose (krad)</th>
<th>Cage population</th>
<th>Corrected % egg hatch from males irradiated at indicated days before emergence b</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 f : 0 m : 1 f</td>
<td>1 f : 0 m : 1 f</td>
</tr>
<tr>
<td>5</td>
<td>25 : 0 : 25</td>
<td>14.0 a</td>
</tr>
<tr>
<td>7</td>
<td>25 : 0 : 25</td>
<td>6.7 a</td>
</tr>
<tr>
<td>9</td>
<td>25 : 0 : 25</td>
<td>3.9 a</td>
</tr>
<tr>
<td></td>
<td>35 : 15 : 15</td>
<td>66.4 a</td>
</tr>
</tbody>
</table>

A = irradiated, U = unirradiated.

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 (10 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.

<table>
<thead>
<tr>
<th>Mating regime</th>
<th>0 krad</th>
<th>5 krad</th>
<th>7 krad</th>
<th>9 krad</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Newly emerged males mated, after 5 days the flies were withdrawn.</td>
<td>94.0</td>
<td>16.5</td>
<td>9.3</td>
<td>3.0</td>
</tr>
<tr>
<td>B. The males from A were held in absence of females for 7 days and then mated with young, virgin females. After 5 days the flies were withdrawn.</td>
<td>94.8</td>
<td>1.8</td>
<td>1.6</td>
<td>0.0</td>
</tr>
<tr>
<td>C. 12-day-old virgin females were mated with young, virgin males. After 5 days the flies were withdrawn.</td>
<td>93.0</td>
<td>4.9</td>
<td>2.9</td>
<td>1.0</td>
</tr>
</tbody>
</table>

a In Regime A there were 19 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 24-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 61.9, 51.8 or 51.2% for males treated 1, 2 or 3 days before emergence respectively. Thus the data (Table IV) show that treatment with 5 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 reasons, irradiate area in Italy [6,7] emergence [9]. T

The data obtained level of sterility in other workers [10] relationship, difficult accurately compare that a linear relationship and the percent egg this problem. The linear relationship while the corresponding.

Female C. capitata have been reported and in our work 3 found that females did produce a few [1] females receiving eggs were non-viable; females did produce hatch of eggs from the dose from 1 krad.

In our work the matings with irradiated [11,13,15] allows us to the work of Steir was decreased and We would not expect fecundity of the C. capitata produce.

Katiyar et al. [14] mated with untreated our findings. Our female, and that w1 young, virgin females suggest that female to ensure high egg mean egg hatch was available. Katiyar hatch of females ur was little different.

The data of Ka little difference in emergence. However increase as 1. We were able to do irradiated 3 days before emergence with...
of our experiments. This decision was taken because, for logistical 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 radio-biological 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 [11] 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 infertile at a gamma dose of 4 krad [13, 15] and in our work 3 krad produced infertility. 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 Steele and Christensen [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 (or viable) 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 Kutiyar 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 35 days after irradiation. In our other experiments we have never had any indication that the induced sterility was not permanent and Kutiyar’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. Wukid also assisted with some of the work.

REFERENCES

[10] STEINER, L.P., "Cultures de maturation", Pro-ceedings 1968, 1
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ment with the work
toach, namely the

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whether the irradiated
was never had any
off and Steiner's group
4 or lower there
50 - 50 days'

species as well as
is true of all
[8] does not lend
irradiation.

received 4 or
not consistent of
acquired, one might expect
damaged gonial

these experiments with some of the


DISCUSSION

E. 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 the results he was obtaining 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.