

PROCEEDINGS OF A PANEL
VIENNA, 1-5 JUNE 1970

ORGANIZED BY THE
JOINT FAO/IAEA DIVISION
OF ATOMIC ENERGY IN FOOD AND AGRICULTURE



APPLICATION OF INDUCED STERILITY FOR CONTROL OF LEPIDOPTEROUS POPULATIONS



INTERNATIONAL ATOMIC ENERGY AGENCY, VIENNA, 1971

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PANEL PROCEEDINGS SERIES

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FOREWORD

The order Lepidoptera includes some of the most damaging pests of agriculture and forestry. Daily they compete with man for his food and fibre and present a continuing threat to our efforts to increase agricultural production throughout the world. Since indefinite reliance on pesticides to control these species is costly and deemed undesirable, alternative control methods are urgently needed. Although the sterile-insect release method is theoretically applicable to all sexually reproducing species of insects its successful large-scale application to the control of lepidopterous pests awaits implementation. Progress has been slow, mainly because of a dearth of investigators able to devote their energies to the problem, and a lack of information on the ecology and population dynamics of many species. Difficulties in mass rearing, in radiation sterilization without loss of competitiveness, and in area-wide distribution also contribute to the problem.

The present panel, which brought together a group of experts in Vienna on 1 - 5 June 1970, dealt chiefly with recent advances in laboratory and field trials. Although at times the problems seemed difficult to tackle, the general outlook was encouraging and the group ended their meeting with reasons for optimism and with a sense of resolve.

Shortly before the meeting, on 18 - 21 May 1970, a panel and research co-ordination meeting had been held in Bogotá, Colombia, on Ecology and Behaviour of the Heliothis Complex as Related to the Sterile-Male Technique. A report of the Bogotá meeting was given to the present panel, and the recommendations from that meeting are included at the end of the present volume.

It is hoped that the publication of these papers and recommendations will serve to guide other researchers in the application of the sterility principle to the control of lepidopterous pests and will also stimulate the additional research that is essential before we can claim preparedness to tip the scale in our favour in the struggle with these insects.

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EXPLORATORY STUDIES ON THE POSSIBILITY OF INTEGRATED CONTROL OF THE FIG MOTH, Ephestia cautella Walk.

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Abstract

EXPLORATORY STUDIES ON THE POSSIBILITY OF INTEGRATED CONTROL OF THE FIG MOTH,
Ephestia cautella Walk.

Results of some preliminary studies on the fig moth, Ephestia cautella Walk., and its parasitic wasp, Habrobracon hebetor Say, are reported. Sterilizing and sub-sterilizing doses of the fig moth were used. It is contemplated that Ephestia population density could be reduced by rearing and releasing the parasitic wasps at certain intervals and by applying the sterile-insect methods for the control or eradication of the pest. Before embarking on such a program, a thorough knowledge of the biology and ecology of both host and parasite is essential. In addition, the sterility technique for Ephestia should be carefully investigated using different sterilizing and sub-sterilizing doses.

1. INTRODUCTION

Ephestia cautella Walk. is a serious pest of stored dry dates in Iraq. It also attacks ripe fruits still in the field causing extensive damage [1]. Preliminary studies of the biology of the fig moth (Ephestia cautella Walk.) and its parasite Habrobracon hebetor Say were initiated at this laboratory in 1967 [2]. The biological studies of the host and its parasite are essential for the evaluation of insect control or eradication by the sterile-male techniques. It is established that if parasites are released in small numbers at the beginning of the breeding season of the host insect, the pest population may be held at a low level [3]. It is also well known that the technique of insect control through the release of sterile insects gathers increasing effectiveness as the natural population declines. If the normal population is reduced by 95%, it might then be feasible to dominate the natural population with sterile insects in order to complete the elimination of the whole population [4]. It is considered advisable to combine sterile insects and parasites for eradication purposes rather than sterile insects and insecticides, since the latter usually produce harmful residual effects and may cause more problems [5].

In this work different sterilizing and sub-sterilizing doses of gamma radiation were used to determine the rates of induced sterility in both males and females of the fig moth. The possibility of inherited sterility in F_1 individuals was also studied in a similar way to that described in Refs [6-8]. The present paper also deals with the possibility of reducing the fig moth population by the parasitic wasp (Habrobracon hebetor Say), which is an efficient parasite on Ephestia larva [1, 2, 9].

TABLE I. EFFECT OF DIFFERENT DOSES OF GAMMA RADIATION ON EGG PRODUCTION AND HATCHABILITY OF THE FIG MOTH (Ephestia cautella Walk.)

Dose (krad)	Individual pair matings ^a female x male	No. of replicates (pairs)	Total No. of eggs laid	Average No. of eggs per female	Hatchability (%)	Temperature (°C)
Control	N x N	69	10225	148.2	62.1	13.5 - 39
Control	N x N	15	2781	185.4	75.4	24.5
54-64	R x R	6	784	136.7	0.0	26 - 29
32	R x R	20	2097	104.8	0.05	13.5 - 30
20	N x R	6	1783	297.2	36.5	24.5

^a R and N designate irradiated and unirradiated moths, respectively.

2. MATERIALS AND METHODS

Fig moth (E. cautella Walk.) adults and their parasitic wasps (H. hebetor Say), which were collected from dry-date stores in Baghdad, were reared in beakers of different sizes half-filled with dry dates. A continuous rearing of the fig moth was carried out in the laboratory. Adults of Ephestia were let to mate (single-paired matings) and oviposit on dry dates contained in small vials (25 × 75 mm). Daily broods were obtained by transferring the adults from the old vial to a new one. Thus, the eggs could be counted and transferred to a piece of date (or to a wet dark thick cloth) spread in a petri-dish.

The stocks as well as the insects used for the tests were kept in the laboratory at temperatures ranging from 13.5 to 39°C. Some tests were carried out inside incubators at 24.5°C.

For sterilization studies, virgin moths of uniform age were obtained by collecting pupae and placing them separately in small vials for emergence. The age of the newly-emerged moths used in the control and irradiation tests varied from 4 to 20 h. Different doses were used (from 20 to 64 krad) of gamma radiation from a ⁶⁰Co source (Gammacell-220) at a dose rate of approximately 16 krad/min. Males, females or both were treated. The progeny of two irradiated male parents were tested for inherited sterility [7, 8].

Oviposition of the parasite females (H. hebetor) was initiated by giving them access to larvae of E. cautella in small vials [10]. Fresh Ephestia larvae were supplied daily in new tubes with pieces of date and the wasps were gently transferred into them. Sex ratio, lifespan and the number of hosts parasitized were studied.

3. RESULTS AND DISCUSSION

3.1. Laboratory studies at temperatures of 13.5-39°C

(a) The fig moth (Ephestia cautella)

The average number of eggs per female was 147 ± 21 . The maximum number of eggs laid by one female was 350. Egg hatchability varied greatly from one female to the other in the range from 0% to 95% and averaged 62% (see Table I).

A total of 3295 adult fig moths were collected from laboratory stocks and sexed, including 1641 females and 1654 males. Therefore, it is concluded that the sex ratio in E. cautella is 1:1 (female/male) ratio ($\chi^2 = 0.05$, $P > 0.80$).

Adult females of Ephestia cautella usually live longer than males. The average age of females and males was 5.5 ± 0.4 and 4.1 ± 0.25 days respectively. The value of t for the longevity of females and males was 3 for 124 degrees of freedom (64 females + 62 males). This value of t corresponds to a probability of less than 0.01. Therefore, the mean ages of both sexes are significantly different.

TABLE II. ADULT EMERGENCE, HATCHABILITY AND LONGEVITY OF THE FIG MOTH AND ITS PARASITIC WASP FEMALES (14° - 39°C)

Species	No. of females examined	Average age of adult female in days \pm S. E.	Average No. of eggs per female \pm S. E.	Average No. of pupae per female \pm S. E.	% of adults emerged	Duration of all life-cycle stages in days		
						From	to	average
<u>Ephestia cautella</u>	64	5.5 \pm 0.42	147 \pm 21	-	22	31	49	40
<u>Bracon hebetor</u>	8	13.0 \pm 2.5	-	97 \pm 34	94	10	35	22.5

(b) The parasite (Habrobracon hebetor Say)

The sex-ratio of the parasite appeared to be 1:1. The maximum number of Ephestia larvae paralyzed (killed) by one Habrobracon female was 124. The average number of larvae paralyzed was 41.4. This parasitic wasp proved to be an efficient natural enemy of Ephestia larvae. In Table II the percentage of adult emergence of Habrobracon is higher than that of E. cautella. The duration of all life-cycle stages of Habrobracon is shorter. So the biotic potential [11,12] of the parasite is higher than that of the fig moth. Therefore, it may be possible to disrupt the existing natural parasite-host balance by increasing the population density of these different parasitic wasps.

3.2. Rearing the fig moth at 24.5°C

The average number of eggs laid by one female increased to 185.4. Egg hatchability also increased to 75.4 (see Table I). One female laid the extraordinarily large number of 820 eggs. The maximum number of eggs laid by this female in a night was 221. The temperature of 24.5°C appears to be more satisfactory for rearing the fig moth.

3.3. Irradiation

Preliminary work aimed at sterilizing the fig moth is carried out using different doses of gamma radiation. Complete sterility for both sexes was achieved by crossing irradiated male and female moths with a dose of more than 50 krad (see Table I). When males were irradiated with a sub-sterilizing dose of 20 krad and crossed individually with unirradiated virgin females an increase in egg laying was noticed (297 eggs per female), though this difference is not considered statistically significant (see Table I). The maximum number of eggs laid by one female crossed with an irradiated male (20 krad) was 400.

Table III presents the results of an attempt to study the inherited sterility in the F₁ generation after irradiating parent males with 20 krad of ⁶⁰Co gamma radiation.

The level of sterility in F₁ male progeny was higher than that of the irradiated male parent, as is already known [6-8]. The cross between

TABLE III. INHERITED STERILITY IN F_1 GENERATION OF IRRADIATED MALE FIG MOTHS (24.5°C)

Dose (krad)	Individual pair matings female \times male	No. of replicates (pairs)	Average No. of eggs per female	Hatchability (%)
Control	N \times N	15	185.4	75.4
20	N \times R	2	233.5	55.4
0	N \times F_1R^a	4	204.2	48.8
0	$F_1R^a \times F_1R^a$	2	157.0	30.0

^a F_1R : F_1 individual whose male parent moths were treated with 20 krad of radiation and crossed with untreated virgin females (see the second row of the Table).

F_1 female and F_1 male (both coming from a male fig moth that received a sub-sterilizing dose and crossed with an unirradiated female) resulted in even higher level of sterility (70%) as shown in Table III. However, information on the biology of the fig moth and sterilizing and sub-sterilizing doses are being accumulated since *E. cautella*, as a lepidopterous insect, seems to be an excellent organism for self-extermination by inducing chromosomal rearrangements, mainly reciprocal translocations. If a high number of possible independent translocations can be induced, a 99% embryo mortality will result [6]. It is also interesting to select a strain with viable homozygous reciprocal translocation by inbreeding the progeny with apparent inherited sterility. If such a viable stock can be "synthesized", a large number of such translocation homozygotes could be reared and released into a wild population where matings with normal moths would produce translocation heterozygotes, and hence cause a reduction in the fertility of the population [13].

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POTENTIALITIES FOR APPLICATION OF THE STERILE-MALE TECHNIQUE TO THE CONTROL OF THE COCOA MOTH, Cadra cautella Walk.

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Abstract

POTENTIALITIES FOR APPLICATION OF THE STERILE-MALE TECHNIQUE TO THE CONTROL OF THE COCOA MOTH, Cadra cautella Walk.

Cadra (Ephestia) cautella Walk. is one of the major pests of prepared cocoa beans during storage in Ghana. Damage is caused by the larva which spends almost the whole of its life inside the bean. The damage, though often quantitatively small, may seriously affect the quality of the cocoa as determined by grading.

The insect is currently controlled by the daily application of pyrethrum fog into the storage premises. The frequency of this treatment has raised questions of cost and pesticide residues. As an alternative control measure the possibility of applying the sterile-male technique is being considered. Various aspects of the biology of the insect and the storage practice of cocoa suggest that this may successfully be done at a comparatively low cost.

INTRODUCTION

Cadra (Ephestia) cautella Walk. is one of the major pests of prepared cocoa beans during storage in Ghana. The damage is caused by the larva which bores into the bean and spends almost the whole of its life within it, feeding on its contents. The actual damage caused, when estimated in terms of the quantity of food destroyed or in terms of weight loss, is usually small, but the depreciation in quality that results from it and the consequent financial loss are often considerable. This is because cocoa is a highly commercialized crop that is subject to rigid quality control measures. According to the latest recommendations of an FAO committee on cocoa grading [1], Grade I cocoa should not contain more than a total maximum of 3% by count of insect-damaged, germinated and flat beans, and Grade II cocoa not more than a total maximum of 6%. Anything above this is to be rejected as sub-standard cocoa that can be marketed only under special contract. As an illustration of this grading system, if in a random sample of 100 beans two flat ones and two germinated ones are found, the presence also of more than two insect-damaged beans is enough for the consignment to be rejected.

This is the basis of the great importance that is attached to insect infestation of prepared cocoa beans in Ghana.

ORIGIN OF INFESTATION

Cocoa is prepared for the market by drying the fermented beans until the moisture content is reduced to about 7%. During this drying stage the initial infestation takes place. The female moth deposits her eggs

among the drying beans and the young larvae, on hatching out, bore their way into the beans. The infestation builds up in the finished crop during storage and the first signs become apparent after 4 to 6 weeks of storage. The storage period may vary from one to nine months and studies [2] have shown that there is a close correlation between the degree of infestation and the length of storage period.

BIOLOGY OF THE INSECT

The biology of C. cautella has been studied by Rawnsley and his colleagues of the Insect Control Unit of the Ghana Cocoa Marketing Board [2-9]. The findings show that under laboratory conditions the life cycle of the moth takes about 50 days on cocoa beans. The incubation period of the eggs is 4 days, the larval period 38 days, and the pupal stage 8 days. The female moth lays an average total of 100 eggs in her average life time of four days. Most of the eggs are laid within the first 48 hours after emergence. The eggs are laid singly and loosely among the beans. Adult moths emerge from the pupae mainly in the afternoon. The adults show a diurnal rhythm of flight activity with two peaks: the first, a minor one, occurring between 5 a.m. and 8 a.m. and the second, the major one, between 5 p.m. and 7 p.m. Adults become sexually mature within two hours after emergence, but mating occurs mostly after the flight activity in the evening. The female appears to mate only once. The adults do not feed and have a life-span of about four days.

OTHER HOST PLANTS

In addition to cocoa beans, C. cautella has been recorded on maize, groundnuts, palm kernels, copra and cowpeas. All these food crops appear to be more suitable than cocoa for the development of the insect, for its life cycle on them occupies a much shorter period. On maize, for example, the life cycle is completed in about 30 days as compared to 50 days on cocoa beans. On these crops, however, the insect is not regarded as a serious pest. This is firstly because the damage caused is assessed quantitatively in terms of the actual amount of food destroyed rather than qualitatively in terms of its effect on the grading of the commodity; and secondly because these crops are not produced and stored in quantities and under conditions that would favour rapid multiplication of the insect.

CURRENT CONTROL MEASURES

The pest is currently being controlled by daily application of pyrethrum which is injected as a fog into the cocoa sheds by means of a thermal fog generator. The insecticide is applied at the rate of 1 pint per 40 000 ft³. This treatment is directed against the adult stage of the moth and is timed to coincide with the peak of flight activity which occurs in the evening. The treatment has little effect on the immature stages of the insect.

One significant observation that has been made in connection with this treatment is that knocked-down females are still capable of laying viable eggs.

POSSIBLE APPLICATION OF THE STERILE-MALE TECHNIQUE TO
CONTROL Cadra cautella

The chemical treatment using pyrethrum fog has so far proved effective in controlling the moth, but the high frequency of application required to give satisfactory results has raised questions of cost and of the hazards of pesticide residues. Alternative methods of control are, therefore, being considered, and the one that is currently receiving serious attention is the method of induced sterility.

Several aspects of the biology of C. cautella and of the cultural practice followed in cocoa storage suggest that this may be one of the few cases in stored-product entomology where the sterile-male technique may be successfully applied. Among these aspects are the following:

1. Cocoa beans are stored in closed sheds which vary in size from 100 000 ft³ to about 1.8 million ft³. The moth population in any one shed therefore forms, in effect, a small isolated community occupying a well-defined and enclosed space, and can therefore be readily swamped by the release of a relatively small number of sterile males.
2. Because the moth population consists of these small isolated communities confined to a small space the release aspect of any eradication or control program would be relatively easy and would not require any special machinery, such as aircraft, for its operation.
3. The smallness of the area occupied by the moth community also increases the chances of the two sexes meeting.
4. The majority of matings occur at a definite time of the day, that is soon after the flight activity between 5 p.m. and 7 p.m. [7]. The release of sterile males can, therefore, be timed to coincide with the period they are likely to offer maximum competition to normal males.
5. Preliminary observations suggest that the female moth mates only once. Although this may not be a necessary condition for the application of the sterile-male technique [10] it is nevertheless a desirable one because it increases the chances of success.
6. The adults of C. cautella do not feed, therefore the release of large numbers of sterile males will not add to the problem of infestation.
7. The insect can be bred in large numbers with relative ease on such common food products as maize, wheat flour or bran. Under laboratory conditions the life cycle on a mixture consisting of equal proportions of maize flour and bran fortified with yeast takes 28 days. This period may be shortened by adjusting temperature and humidity conditions.
8. Cocoa beans are normally stored bagged in jute sacks arranged into large stacks inside the shed and the adult moth normally lives outside the bags. The risk of contaminating the beans with dead insect bodies through the release of large numbers of sterile males is, therefore, minimal.
9. The pupal stage of the moth is a fairly hardy stage that will lend itself to easy handling and would therefore constitute a suitable stage for irradiation.

The proposed application of the sterile-male technique to C. cautella would aim at control rather than complete eradication because eradication

would not only be expensive but also difficult to achieve. This is because of the existence of alternative host crops such as maize, the storage of which is neither standardized nor centralized so that they would not easily lend themselves to the application of the sterility technique. And although cross infestation from these crops to cocoa is considered negligible under normal storage conditions, reinfestation from this source cannot be ruled out completely.

ADVANTAGES OF THE STERILE-MALE TECHNIQUE FOR CONTROL OF Cadra cautella COMPARED WITH CHEMICAL CONTROL

Apart from the inherent superiority of the sterility method over the chemical method in the control of insect populations [10], in the particular case of C. cautella the sterility method has additional advantages:

1. Fertilized females of C. cautella, which have been knocked down by pyrethrum, are still capable of laying viable eggs before they die, and because the chemical applied as a fog has little effect on the eggs, larvae or pupae, there is always the chance that some stages of the moth will survive treatment. This is not so with the sterility method where no viable eggs are produced.
2. One of the factors that militates against the application of the sterile-male technique is the cost involved in mounting the large-scale operations that are usually required for success. In the proposed limited application of this technique as a control measure against C. cautella the cost will be relatively small because, apart from the cost of the radiation source (which in any case would be shared by other projects), the only major expenditure would be that involved in the large-scale breeding of the insect. On comparing the cost of the products used as food media for breeding the insect (e. g. maize or bran) with that of pesticides used in its chemical control (e. g. pyrethrum) it can be said that the sterile-male technique will be a more economical method to apply. This is particularly so in the case where the chemical treatment has to be applied daily in order to obtain effective control as is required by the pyrethrum fog treatment. With the sterile-male technique daily releases may be necessary only for a short time. It can be envisaged that after some time the moth population will be reduced to a level that will make further releases unnecessary.
3. The sterile-male technique has the additional advantage that it avoids the problem of pesticide residues in food commodities.

RADIATION STUDIES

Because of the lack of radiation facilities, studies on the radiation response of the moth have not yet started. Huque [3] and Papadopoulou [5] have studied the effect of gamma radiation on the various stages of C. cautella but they were concerned with the determination of lethal doses rather than

sterilizing doses. Arrangements for the supply of a ^{60}Co source are now in progress. As soon as this becomes available the radiation aspects of the problem will be studied and if these yield suitable results a pilot control project using a few storage sheds will be started.

CONCLUSION

Cadra cautella as a storage pest of cocoa beans clearly presents a near ideal situation for the small-scale application of the sterility technique. It is hoped that the proposed project will not only solve the infestation problem associated with this moth but will also offer the example of success that is needed to demonstrate the feasibility of this technique under the conditions of the limited resources of developing countries.

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POPULATION FLUCTUATION OF LEPIDOPTEROUS FOREST INSECTS AND THE APPLICATION OF INDUCED STERILITY FOR CONTROL

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Abstract

POPULATION FLUCTUATION OF LEPIDOPTEROUS FOREST INSECTS AND THE APPLICATION OF
INDUCED STERILITY FOR CONTROL.

The fluctuation in insect populations in time and space may be understood as the result of determination (setting of density limits) and limitation (density-dependent and chance regulation around these limits). This dualistic process is interpreted with the findings from the various fluctuation types of the larch bud moth, *Zeiraphera diniana* Gn. The population dynamic of this and other forest Lepidoptera is compared to the fluctuation of such insects which have been successfully controlled by the application of the method of induced sterility.

INTRODUCTION

Knipling listed several criteria that are important when judging whether an insect species is likely to be a good candidate for control by the sterile-male technique. Since many forest insect pests meet these criteria it may be of interest to review some data on the population dynamics of forest pests.

This paper first refers to the general principles of population dynamics, then discusses the population dynamics of the larch bud moth compared with various forest insect pests and the classic species which were successfully controlled by the sterile-male technique.

REMARKS ON PRINCIPLES OF POPULATION DYNAMICS

The reasons for considering an insect as a pest depend not only on the insect itself but equally on human requirements. These requirements determine the economic threshold level, which may depend on insect numbers or on the kind of insect damage. In this context, however, discussion of population dynamics is restricted to insect numbers, or abundance. The functional theory of population dynamics put forward by Wilbert (1962, 1970) and refined by Schwerdtfeger (1968) considers three basic processes:

1. The primary fluctuation, i.e. the variation of the abundance with time caused either by fertility or mortality, or by immigration and emigration.
2. The abundance varies with the limits determined by the environmental capacity. This determination of the upper and lower density limits is achieved by a complex of density-independent

factors which in their turn exhibit a characteristic pattern of variation. Thus, the upper or lower density limits may be either fixed or variable.

3. Finally, these determined density limits are observed by the action of density-dependent processes which rely on the feed-back principle. This process, called regulation, may operate through various factors, the ultimate regulation being the intraspecific competition. This regulator can be influenced from the outside only by way of shifts in environmental condition.

POPULATION DYNAMICS OF THE LARCH BUD MOTH, Zeiraphera diniana Gn. (Tortricidae)

To demonstrate this theory on population dynamics the example of the larch bud moth is used. The fluctuation of larval densities over several generations is discussed with the help of the reproduction curve as described by Takahashi (1968). This method correlates densities of two generations in sequence; as long as the population increases the curve lies above the 45° line, with falling populations the curve is below the 45° line. This curve is characterized by the three important points where the 45° line is crossed. Point E and T indicate a stable density situation, termed equilibrium points, whereas at R, the release point, the population trend is variable as density may either rise or fall. But once point R is overshot, the population increases more or less linearly to point T.

The population fluctuation of the larch bud moth is of a remarkable periodicity in its optimum area, the subalpine region between 1700-2000 m altitude. Defoliation damage to the extensive larch stands has been recorded at regular 9-10 year intervals since 1850, and a quantitative census by Auer (1961) covers the last two cycles from 1949 to 1969. From the graphical representation of the larval densities it is evident that there is hardly any variation at the upper equilibrium point. The density of 100 larvae per 1 kg of larch branches is the lower threshold level for general defoliation in the area. Above this, density overpopulation phenomena (such as an epizootic virus disease, parasites and intraspecific competition for food) induce the population collapse. In addition, a change in proportions of ecotypes, which are differently susceptible to stress, was found to occur after peak densities. This selective process functions permanently, whereas the influence of parasitism or food quality ceases to be of quantitative importance three years after peak density. In contrast to the upper equilibrium point, densities at the lower point vary very much; it appears as if chance factors exert a great influence below a medium density, which might be considered as the region of the release point. Since the larch bud moth occurs also at low altitude on the Swiss plateau at 600 m without causing any defoliation damage to the planted larch stands, we are able to compare the cyclic fluctuation of the optimum area with the latent fluctuation. At medium altitudes of 1200 to 1400 m, defoliation damage appears in an irregular sequence. Thus, moving along the altitudinal gradient the two dualistic processes of determination and regulation change accordingly.

The regression for population increase shows the least standard deviation in the optimum area which indicates a regular, undisturbed

population growth, until at the fixed upper limit ultimate regulation is induced by a feed-back process between the insect and the host plant. Defoliation of the larch tree causes a change in food quality, chemically as well as morphologically, for the two following generations which brings about a change in population quality by selection. These conditions result in the cyclic fluctuation type. At medium altitude determination becomes more variable, the density amplitude is at its maximum. It may reach defoliation densities but also may dwindle to zero. This situation is demonstrated by the intermittent fluctuation type. At low altitude determination is most variable in intensity as well as in time, therefore narrower limits are set to the amplitude. It is established that temperature causes heavy and variable mortality in the egg stage (Baltensweiler, Giese, Auer, in press). Regulation processes are not yet clearly understood here. However, the proportion of stress-resistant ecotypes is higher in this fringe area than in the optimum area.

COMPARISON OF POPULATION DYNAMICS

This complete system of fluctuation-types is summarized in Fig.1 which shows the variation of the determination of upper and lower density limits in relation to space. This graph may serve to compare the population dynamics of various lepidopterous forest insects and those which were successfully controlled by the method of induced sterility.

The well-known German forest pests (Schwerdtfeger, 1957) are taken as representative. They all have large population peaks in common; differences are only in amplitude or frequency. They may be considered as representing the same three fluctuation types as found with the larch bud moth; in opposition to this, those species which were successfully controlled by the sterile-male technique generally have a low density and small amplitudes (LaChance et al., 1967):

<u>Cochliomyia hominivorax</u>	200 individuals/sq. mile
<u>Lucilia sericata</u>	2 000 individuals/sq. mile
<u>Melolontha melolontha</u>	1 500 000 individuals/sq. mile

The fruit fly, which may increase to higher densities, was cited as successfully controlled in fringe areas (Dacus tryoni) or under environmental stress (Dacus dorsalis) when the host trees were destroyed by typhoons. Also, because of their feeding habits, none of these species is able to induce a density-dependent change in population quality by a feed-back mechanism, since the food of the insect (which is fruit or livestock) does not represent an integral element in the production process of the host plant and is removed by management. Therefore, ultimate regulation is never evoked which implies that density-independent determination restricts population increase before the absolute possible density is reached.

This may be interpreted that due to the low mean density and the restricted amplitude, the successfully controlled insects are, in effect, better suited for application of the sterile-male technique than most of the forest insects.

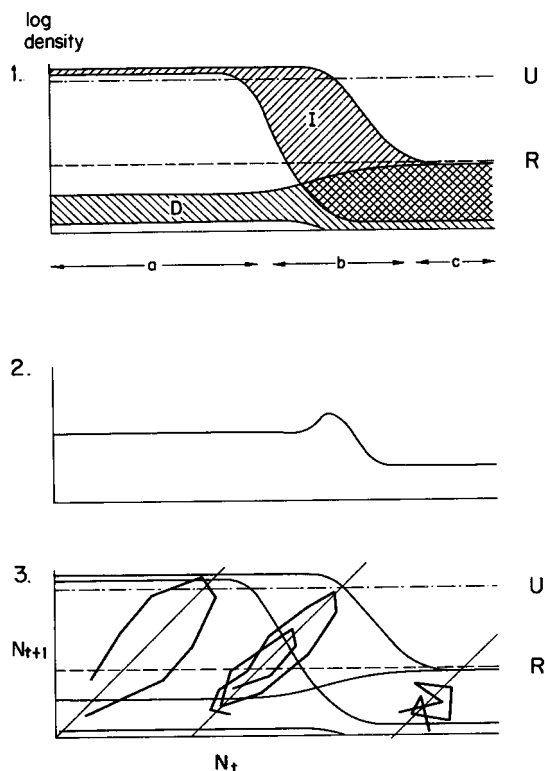


FIG. 1. Fluctuations in insect populations.

1. The range of determination of insect numbers (schematic)
 - I : range for population increase
 - D : range for population decrease
 - U : threshold of ultimate regulation
 - R : threshold of population release
 - a : optimum area, cyclic fluctuation type
 - b : suboptimum area, intermittent fluctuation type
 - c : fringe area, latent fluctuation type
2. Amplitude of density
3. Examples of fluctuation.

However, the well-documented shift of the sex ratio in favour of the males after stress situations emphasizes, in combination with sex-phenomenon attraction, a principle of evolutionary importance. The large number of the generally more motile males with their capacity to inseminate several females ensure lepidopterous populations to survive at very low densities. It seems, therefore, only logical to apply the same strategy by using sterilized males to keep populations low. And, finally, since neither insecticidal, biological nor integrated control methods are efficient at very low densities, further efforts are justified to pursue the development of the sterile-male technique for lepidopterous forest insects.

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COMPUTER SIMULATION OF POPULATION REDUCTION BY RELEASE OF STERILE INSECTS

I. A study of the relative importance of the parameters of a mathematical model

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Presented by M. Fried

Abstract

COMPUTER SIMULATION OF POPULATION REDUCTION BY RELEASE OF STERILE INSECTS.

I. A STUDY OF THE RELATIVE IMPORTANCE OF THE PARAMETERS OF A MATHEMATICAL MODEL.

A computer program was written to calculate the minimum ratio of sterile to fertile males necessary to eradicate an insect population in five generations using Berryman's 1967 model. In this deterministic simulation, only one of the parameters at any one time was varied. The simulation was designed to test the effects of three levels of complexity of the model. The results of the study show that the probability of survival from egg to adult is probably the most crucial parameter affecting the results of the release strategy. Mating and oviposition habits of the insect have an impact on the numbers of sterile insects required for eradication. The studies indicate that sterile males with reduced competitiveness as low as 50% could be successfully used in eradication programs.

INTRODUCTION

The sterile-male principle, which was first expounded by Baumhover et al. (1955), Bushland et al. (1955) and Knipling (1955), is based on release of large quantities of male insects sterilized by gamma irradiation which compete with wild individuals to mate with females. The principle was tested and proven by the eradication of the screw worm fly, Cochliomyia hominivorax (Coquerel) from Curacao in 1954 (Bushland et al. 1955).

Several factors are known to affect the outcome of sterile-male releases. These are competitiveness of sterile males and females, competitiveness of sterile sperm, mating frequency of females, oviposition sequence of females and survival rates in the field. The theoretical aspects of the sterile-male principle were first expounded by Knipling (1955, 1959, 1964). He used a tabular method for predicting the effects of continuous release of sterile males on wild populations. Recently more detailed mathematical analyses of the sterile-male theory have been published. Berryman (1967) described the theoretical effect of sterile-male release on wild populations and took into consideration the consequences of unequal competitiveness of sterile males and sterile sperm, and various mating habits and oviposition

habits of the female. He also developed computer methods for making decisions on release strategies and predicting population decline following release.

The following paper describes a computer study of the relative importance of the parameters suggested by Berryman (1967) in his mathematical description of the sterile-male principle.

METHODS

Berryman's (1967) prediction equation is the following:

$$N_{g+1} = N_g F_p S \sum_{m=1}^M P_m \left\{ \sum_{i=1}^m E_i [1 - (P_s^i + C_s P_s - C_s P_s^i)] \right. \\ \left. + \sum_{i=m+1}^M E_i [(1 - (P_s^m + C_s P_s - C_s P_s^m))] \right\}$$

where N_g is the number of wild adults in the g^{th} generation, F_p is the proportion of females in the population, S the proportion of the generation that would normally survive, M is the maximum possible number of matings per female, P_m the probability of a female mating m times, E_i the number of eggs laid per female after each successive mating, C_s the competitiveness of sterile sperms (the ratio of the probability of a sterile sperm fertilizing the ovum to the probability of a fertile one fertilizing the same ovum), P_s is the probability of mating with a sterile male which can be expressed as:

$$P_s = \frac{N_s C_m}{N_g M_p + N_s C_m}$$

where C_m , the competitiveness of a sterile male, is the ratio of the probability of a sterile male mating with a female to the probability of a fertile male mating with the same female, N_g is the wild population size and N_s is the number of sterile males in the population.

A computer program was written to solve the equation described above and, in particular, to calculate the minimum ratios of sterile to fertile males necessary to eradicate an insect population in a given number of discrete generations (in this study five generations) by varying only one of the parameters at a time and holding all the others constant. The simulation was designed to test the effects of three levels of complexity:

- (a) The most complex situation where females mate several times, interspersed throughout the oviposition cycle, and sperm are utilized independently from mating sequence (i.e. Berryman's (1967) Eq. [8])
- (b) Females mate several times, but mating ceases after oviposition starts and sperm are utilized independently of mating sequence (i.e. Berryman's (1967) Eq. [7]).
- (c) Females mate once only or if multiple

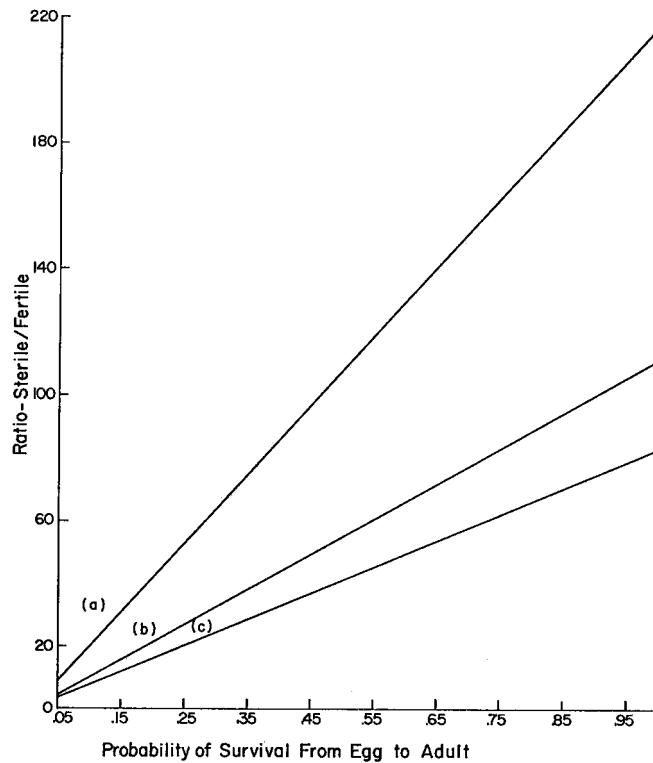


FIG. 1. Effect of the probability of survival from egg to adult on the ratio of sterile/fertile males required for complete extinction in five generations simulated at three levels of complexity: (a), (b) and (c) (see text).

matings occur sperm are utilized from first or last mate only (i.e. Berryman's (1967) Eq. [4]).

Whenever the parameters were held constant they were held at the following levels: size of wild population = 1000, proportion of females = 0.5, mean number of eggs per female = 100, probability of survival from egg to adult = 10%, competitiveness of males = 0.75, sperm competitiveness = 0.75.

The extension of the model to fit species where multiple matings occur introduces many new possibilities and an almost infinite combination of mating distributions with differential sperm utilization. For the sake of simplicity it was decided to simulate the case where females may mate up to five times with the following probabilities: 0.3, 0.3, 0.2, 0.1, 0.1.

In order to keep the study comparative it was assumed that the number of eggs laid was constant, i.e. those which mated more than once laid the same total number of eggs than those which mated only once.

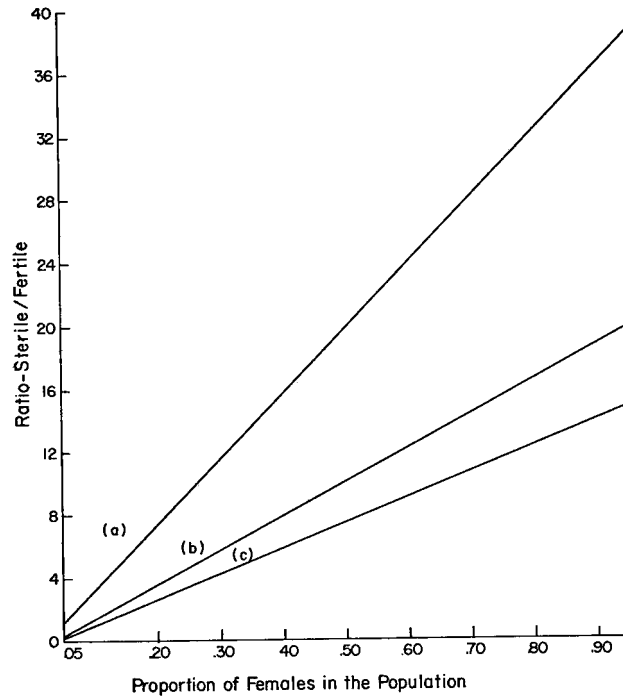


FIG. 2. Effect of the proportion of females in the population on the ratio of sterile/fertile males required for complete extinction in five generations simulated at three levels of complexity (a), (b) and (c) (see text).

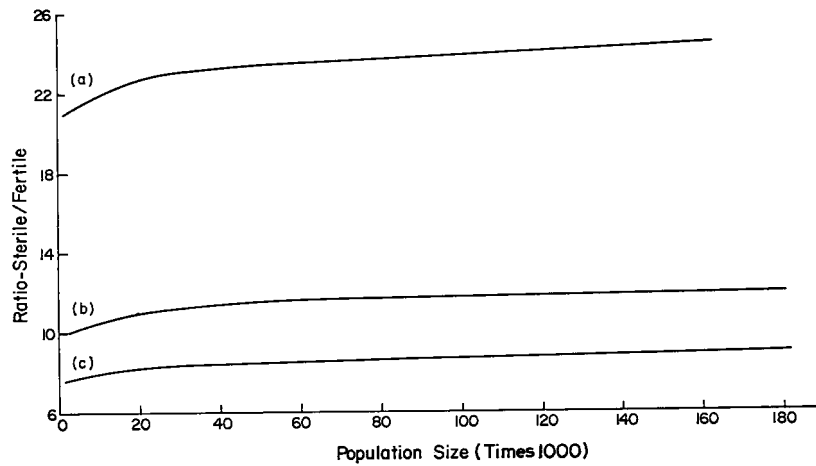


FIG. 3. Effect of population size on the ratio of sterile/fertile males required for complete extinction in five generations simulated at three levels of complexity (a), (b) and (c) (see text).

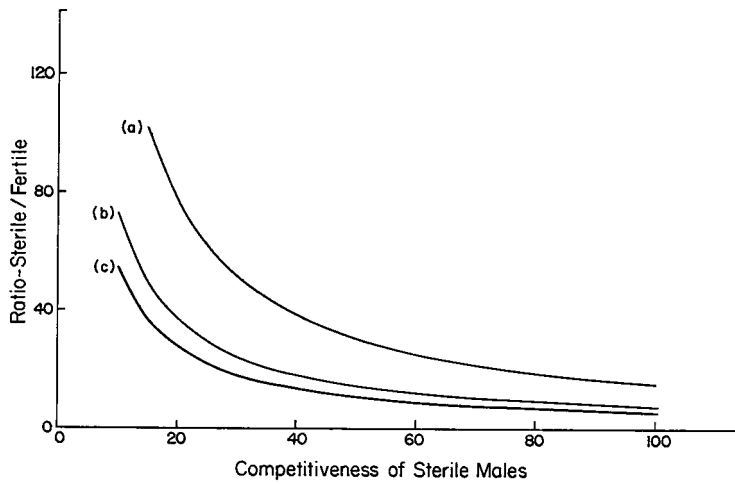


FIG. 4. Effect of competitiveness of sterile males on the ratio of sterile/fertile males required for complete extinction in five generations simulated at three levels of complexity: (a), (b) and (c) (see text).

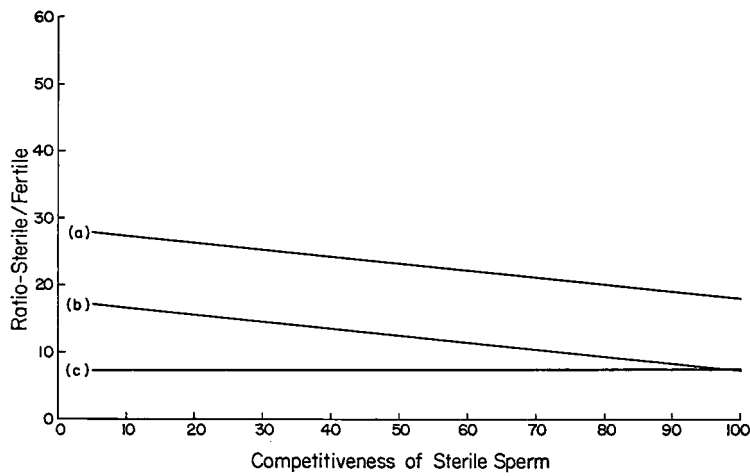


FIG. 5. Effect of competitiveness of sterile sperm on the ratio of sterile/fertile males required for complete extinction in five generations simulated at three levels of complexity: (a), (b) and (c) (see text).

RESULTS

Probability of survival from egg to adult

The effect of this parameter is strictly linear (Fig.1). The ratio of steriles to fertiles increases in direct proportion to survival rate. The effect of increasing complexity of mating and oviposition habits is to increase the slope of the regression line.

Proportion of females

The effect of this parameter is also linear (Fig. 2) although the magnitude of the effect is five-fold less than that due to survival rate. The slope of the regression is also influenced by mating and oviposition habits.

Population size

The effect of this parameter increases logarithmically with increasing population size, but the increase is rather small (Fig. 3). Once again the effect is greatly magnified by the more complex mating and oviposition habits.

Competitiveness of sterile males

Increasing this parameter will have a substantial negative exponential effect on the ratio of sterile males to fertile ones with the greatest effect being induced by the higher levels of complexity (Fig. 4).

Competitiveness of sterile sperms

This parameter has a negative linear effect on the ratio of sterile to normal males and the effect is also magnified by mating and oviposition complexity (Fig. 5).

DISCUSSION

From Berryman's prediction equation, which in its present form is still an oversimplification, it is not easy to determine the relative effects of the various parameters. This study was undertaken to obtain a "feel" for them. In deciding on a reasonable release policy, one should be in possession of good estimates of the true values of these parameters, but very often this is not possible. One would, therefore, like to know how seriously an under- or overestimation of the respective parameter will affect the results of the release. Some of the parameters are easier to estimate than others. The adult population size should be fairly readily estimated from sampling of the larval or pupal stage. According to the results of this study variations in this parameter have relatively little effect, and the larger the population the less the rate of increase of the required ratio of steriles to fertiles. Sex ratio should be fairly constant in most insect populations and easy to estimate. The linear effect of this parameter should enable planners to estimate the effect of small misjudgments accurately. The probability of survival from egg to adult is probably the most difficult and also most crucial parameter affecting the results of the release strategy. Not only is this parameter difficult to estimate, but it will probably always remain somewhat unpredictable because it is affected by so many different variables. Survival probability may change from generation to generation and methods to estimate survival probabilities for a particular season by the utilization of environmental and ecological data should be worked out.

We should like to emphasize the importance of co-operation between biologists and mathematicians in this field. Not only will it be necessary to refine field estimation techniques and methods of ecological observations, to work out new models in which variable survival patterns can be incorporated, but it will be imperative to co-ordinate field work and designing sampling methods to determine the effectiveness of the predictions. Field testing of predictions should be an essential part of the program. The study of the effect of competitiveness of the sterile males indicate that even a 30% decrease in competitiveness will not seriously affect the results. This is particularly important for Lepidopteran species, where the large doses of radiation, that are usually necessary to induce complete sterility, will also affect the competitiveness of the males. These studies seem to indicate that insects with slightly reduced competitiveness could be successfully used in programs designed to eradicate insect pests within a few generations.

This study has also shown that the mating and oviposition habits of the insect has considerable impact on the release strategy. This further emphasizes the need for sound biological data in evaluating the potential of the sterile-male technique for controlling insect pests.

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SUMMER FRUIT TORTRIX MOTH, Adoxophyes orana F. R.

Studies on biology, behaviour and population dynamics in relation to the application of the sterility principle

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Abstract

SUMMER FRUIT TORTRIX MOTH, Adoxophyes orana F. R.: STUDIES ON BIOLOGY, BEHAVIOUR AND POPULATION DYNAMICS IN RELATION TO THE APPLICATION OF THE STERILITY PRINCIPLE.

In the Netherlands, a program to control the summer fruit tortrix moth with the sterile-male technique is conducted by a working group. The program started with studies on the distribution, the host plants and how the insect developed into a pest of economic importance. Since 1968 the activities of several institutes and laboratories have been co-ordinated in a three-year program. Experiments are conducted with cheap methods for massive rearing on artificial media. Preliminary experiments indicated that survival from eggs in August 1968 to moths in June 1969 must have been about 0.06. By sampling of clusters and shoots, the population density of an eight-year-old apple orchard in May has been established at about 1500 to 3600 individuals per ha. However, densities of 10 000 to 20 000 individuals per ha occur also in orchards. Application of insecticides may reduce these numbers to about 500 or 1000 per ha. Studies are made on sex-ratio, mating behaviour and reproduction. The dispersal of moths and young larvae and problems related with reinfections from surrounding fields are also studied. Preliminary experiments indicated that dosages of 25 krad are effective in sterilizing the males and females. It is likely that a sufficient rate of sterility of male moths will be obtained while retaining sufficient competitiveness. Computer models for release programs, conducted with the aid of the formula of Berryman, gave information on some parameters, such as the influence of reinfections by moths from outside the orchard.

INTRODUCTION

Distribution

Adoxophyes orana occurs in Europe and Asia. It is known from south-east Russia [1, 2], central and south-Europe, the west and north-west of the Continent including Scandinavia, except the northern regions. It occurs also in Japan [3, 4] and it is presumably the same species as Adoxophyes fasciata Wlsm. from India and China [5].

In the Netherlands the first moth was recorded in 1939, and in the following 12 years the species spread in the country as a pest of apple and pear [6-8]. In Belgium the first moth was captured in 1942 [9], and it has spread as an orchard pest since 1949 [10]. In 1951 A. orana was observed for the first time in England, first in apple orchards in Kent and later also in Essex [5, 11-14]. It is likely that it was accidentally imported into England from the Continent [15]. In Germany, where A. orana is known to have lived on birch and other plants for a long time,

the occurrence as a pest has been recognized in the Rhine region [16, 17] and in South Bavaria [18, 19] from 1950 onwards. In north Italy the occurrence on apple was reported in 1949 [20].

Host plants

A. orana seems to be very polyphagous. In the Netherlands A. orana is captured in light-traps and sex-traps, in several biotopes, namely: orchards, woodlands, parks and home gardens. Because there are indications that the moths only slightly incline to migrate from the orchards to the surrounding fields [21], it is likely that the specimens captured originate from other host plants than apple and pear. The caterpillars are collected from many plants which are always found in these biotopes.

Development as a pest

Because the reasons for this species reaching a pest status are unknown, some possibilities are discussed. In the Netherlands, and presumably also in other countries, the development into a pest in orchards coincided with changes in orchard management, which led to better plant growth, offering the phytophagous insects an abundance of food from spring till autumn. Moreover, the absence of natural enemies, probably caused by the use of broad spectrum insecticides, has contributed to this pest status [16]. The assumption of Steinhausen [22] that the warm summers in 1945, 1947 and 1949 led to massive multiplications near Berlin, and that the species might decrease during the following years, was not justified [23, 24].

Hering [25] concludes from his observations that the larvae have changed their feed behaviour from 1940; from entirely leaf eaters, they changed into both leaf and fruit eaters.

Such an alteration has taken place simultaneously in many widely separated regions of the world. In contrast, however, it was already in 1932 and 1937 that A. orana was described as a pest on apple fruits in the Caucasus and Crimea [1, 2]. A preference for apple and pear or a deviation in two subspecies is not likely in the Netherlands, according to recent observations [26]. Adaptation to different conditions seems to exist in Japan. There A. orana occurs as a pest on apple in the cooler, northern regions, where the young larvae enter diapause. It occurs as a pest on tea in the warmer, southern regions, where no real diapause occurs [27].

Van der Meer [28] reviewed the literature on the pest status of A. orana in Europe.

Differences between apple varieties

A. orana occurs on all apple, pear and plum varieties. We indicated, however, that the population density of the caterpillars and the rate of fruit damage may vary from one variety to the other. These differences may be caused by several factors, such as the texture of the leaves and the fruits, the length of the fruit stems and the extent to which the fruits are covered by the leaves.

Economic importance

Notwithstanding two to four sprays, an average of 4% of the apples is damaged annually. In 1967, a total area of 33 000 ha under apples in the Netherlands produced 317 million kg valued at 30 million US dollars. This means a loss of 1.2 million US dollars. The costs of insecticides used to control A. orana amount to 0.3 million US dollars, so the total costs for A. orana were that year about 1.5 million US dollars. In spite of the reduction in the total acreage under apples in recent years, we may assess the annual loss by A. orana at more than 1 million US dollars.

Background for this study

Resistance to pesticides is unknown in A. orana, but closely related species, as the red-banded leafroller in the USA, have developed resistance against pesticides. Although in our opinion the chances of resistance development are relatively small, alternative methods for control are highly desirable.

The application of Bacillus thuringiensis and a nuclear polyhedral virus of A. orana [29, 30] did not give satisfactory control. Parasitization by the egg parasite Trichogramma evanescens Westwood [11], and releases of laboratory reared Trichogramma [31-33] never resulted in a satisfactory control of A. orana. Some larval parasites are bred from A. orana. Ankersmit observed in some parts of an apple orchard in the centre of our country that Teleutea striata (Ichneumonidae) parasitized about 20% of the A. orana larvae in September 1969. Generally, parasitization is low and always insufficient. Predatory bugs, such as Atractotomus mali and Anthocoris memorum, sometimes kill young larvae but never important numbers. Since the effect of natural enemies may be reduced by repeated applications of broad-spectrum pesticides, used to control A. orana, alternative methods have to be developed. From the point of view of public health the use of pesticides with a long-lasting effect must be restricted.

Since A. orana has to be controlled by means of broad-spectrum insecticides at various periods in the season and these applications interfere with biological agents of its own and other pest species, it may be regarded as a key pest in fruit growing. Selective control methods will facilitate the development of natural enemies, so that other pests, for example the fruit tree red spider mite Panonychus ulmi, may be kept in check naturally. If A. orana can be controlled by selective methods, the number of sprays may be reduced from five to about two, the remaining ones being applied in acceptable periods, thus resulting in an integrated control system.

Organization

In view of the aforementioned problems it was considered desirable to study alternative control methods. In 1968 the section for genetic control of the Dutch working group, Integrated Control of Pests TNO, formulated a three-year program. In this program, the planning for

induced sterility methods is pointed out and the activities for several institutes and laboratories are co-ordinated.

Results of earlier researches are collected and new studies are conducted in starting a release program in field experiments.

PROBLEMS

Detailed knowledge of the ecology of A. orana is fundamental for a release program. Several aspects will be mentioned, methods will be described and results, so far as available, will be recorded.

Bionomics

In the Netherlands and most other European countries, A. orana develops two generations annually; the flights occur mainly in June and in August-September. The overwintered young larvae damage the buds, flowers and young leaves in spring. The full-grown larvae pupate on the trees. In June-July the young larvae of the new brood make small webs on the leaves and enter terminals, which are spun together. The larger larvae attach one leaf to another or to a fruit, which may be damaged. The larvae of the last brood damage the ripening fruit before hibernating on the branches in September-October [7, 8, 10]. Bovey [34] gives a more detailed description of the biology.

In winter no lethal temperatures occur in the Netherlands. In spring, the reactivation of the overwintered larvae is influenced by temperature. Summer temperatures are not so high that the fecundity of the moth is adversely affected, but in that season the weather is alternately favourable and unfavourable for flying and egg laying. Light-traps are used to collect data on the flight periods and flight activities [35, 36]. Sex-traps especially indicate the beginning of the flight periods [37-40]. Temperatures may advance or delay the flight periods [41, 42].

Under Dutch orchard conditions the moths are typically nocturnal (Minks [38]), the highest numbers being caught by light-traps around midnight; he did not distinguish between females and males. In Japanese conditions, greater numbers of females of the smaller tea tortrix were captured in black-light-traps before midnight, while more males were captured after midnight [36].

Oviposition increases greatly at temperatures above 15°C, while temperatures below 13°C inhibit about all activity. Consequently, oviposition only takes place if distinct temperature limits are passed. A practical rule is that peak-numbers captured in light-traps generally coincide with important oviposition periods [41]. In Dutch orchards it appeared that the temperatures at sunset were at least 13 to 15°C on days with maximum temperatures of at least 20°C or 23°C, and these temperature limits may be used in practice to indicate when nights will be favourable for egg laying [43].

More information on phenological observations and their use in forecasting spraying dates is given in Refs [21, 35, 39, 43, 44].

Rearing *A. orana*

In Wageningen and Wilhelminadorp the larvae are reared continuously on wheat-germ diets [45] modified by Ankersmit [46, 47]. Since cheap methods for massive rearing are necessary, experiments are conducted with cheaper media without agar, as indicated by Roelofs [48] and Karpel and Hagmann [49]. Pupae are difficult to collect, but the moths can be collected by means of their positive phototaxis. Moths, pupae and larvae can be tagged by rearing the larvae on media containing radioactive phosphorus ($0.1 \mu\text{Ci Na}_2\text{H}^{32}\text{PO}_4$ per ml food) [50-53].

Irradiation of *A. orana*

According to Ankersmit, moths can be sterilized by X-rays. Dosages of 25 krad, produced by an electron generator, are effective in sterilizing the males and females. It is likely that a sufficient rate of sterility of male moths will be obtained while retaining sufficient competitiveness [54, 55]. Further work on irradiation dosages and its effect on mating competitiveness are needed.

Host plants

A. orana larvae are found on many trees, shrubs and herbaceous plants. In our country we found them feeding on many Rosaceae such as apple, pear, plum, quince, damson, cherry, gooseberry, black-, red- and white currant, blackberry, raspberry, strawberry, hawthorn and roses [8]. They are also found on several members of other plant families such as maple, ash, birch, willow, alder, poplar, elm, honeysuckle, privet, *Carpinus*, *Ligustrum*, *Laburnum*, *Tilia* and stinging nettle [8, 16]. In the Netherlands and Belgium, *A. orana* is observed as a pest on lilac [56, 57]; it attacks also cotton in Russia [58] and tea in Japan [3].

Dispersal of *A. orana*

For experiments on sterile-male methods, information about the movements of the moths is highly necessary. Both movement in a biotope and dispersal from one biotope to another have to be taken into consideration.

Since 1967 investigations on the dispersal of *A. orana* have been conducted. Radioactively tagged moths (^{32}P) were released and recaptured with light-traps or sex-traps [53, 59]. The pupae were placed in the orchard where the moths emerged. In the first experiments the moths emerged in the centre of an orchard, in subsequent experiments they were released in a group of *Crataegus* shrubs at a distance of 40 m from the orchard and separated from it by an open field [60].

Light-traps with a limited range of attraction (5 m) and with a wide range of attraction were arranged systematically around the centre of release, at intervals of 4 to 250 m [53]. Sex-traps, containing one or two living virgin females or an extract of the abdomens of virgin females, were also used to recapture males and proved to be very effective [53, 61, 62]. Males were recaptured at distances up to more than 200 m from the place

where they were liberated. Females behave more passively and were only recaptured at 4 to 25 m from the centre. We occasionally observed that a disturbed female rose 10 to 15 m into the air and flew a distance of about 100 m [63,64]. The experiments of 1969, conducted by Barèl [60], confirmed that both virgin and mated females only rarely leave their biotope to cross open fields.

It is observed that egg larvae may also contribute to reinfections because they spin silken threads which may be blown away from the leaves by the wind. This will be studied by Barèl in Wageningen.

Sex-ratio and mating behaviour

The sex-ratio in the field is approximately 1 : 1. At the beginning of a flight period males prevail, at the end the females are more numerous. In the laboratory and in the field, mating may begin already in the night following the day of emergence. After mating, a spermatophore is generally left in the bursa of the female which can be recognized by dissecting the female. Field-collected samples of females show differences in age and the number of spermatophores in the bursa, depending on the sampling method used. According to Minks [38], 50 to 60% of the females captured in light-traps bear one spermatophore, 25 to 30% bear two and some three or four spermatophores; nearly 25% of the females had deposited about all eggs, and 10% were unmated. In samples, beaten from trees, only 6 to 8% had deposited all eggs and 4 to 8% were unmated. The importance of a second mating is unknown.

The attractivity of virgin females in sex-traps decreased greatly after approximately 8 days. The longevity of the females is approximately 8 to 14 days, which is a few days longer than that of the males.

Reproduction

The egg production in the laboratory depends on the culture methods and averages often 175 eggs per female, in other cases 200 to 300 per female. The numbers of eggs laid by a female vary sometimes with the weight of the moths [54]. In cages in the open field they produced generally an average of 150 to 200 eggs. However, females, which are carefully reared individually, reached in some cases a total egg production of 600. Most eggs are laid in the first part of life; these have the best viability and larvae are often hatched from more than 90% of these eggs. Under field conditions often 80 to 90% of the eggs were hatched.

Population density

Light-trap captures indicate the population density of the moths and their activity; sex-traps are still more complicated. Visual methods and beating methods give most reliable information on the population density. We estimated the actual population of trees in its entirety by counting the numbers of larvae. Taking a sample of 100 clusters or shoots may facilitate the calculation of the population density. As a result of such investigations we established the population density of an eight-year-old apple orchard in May at about 1500 individuals per ha, and in an identical orchard at 3600 individuals per ha.

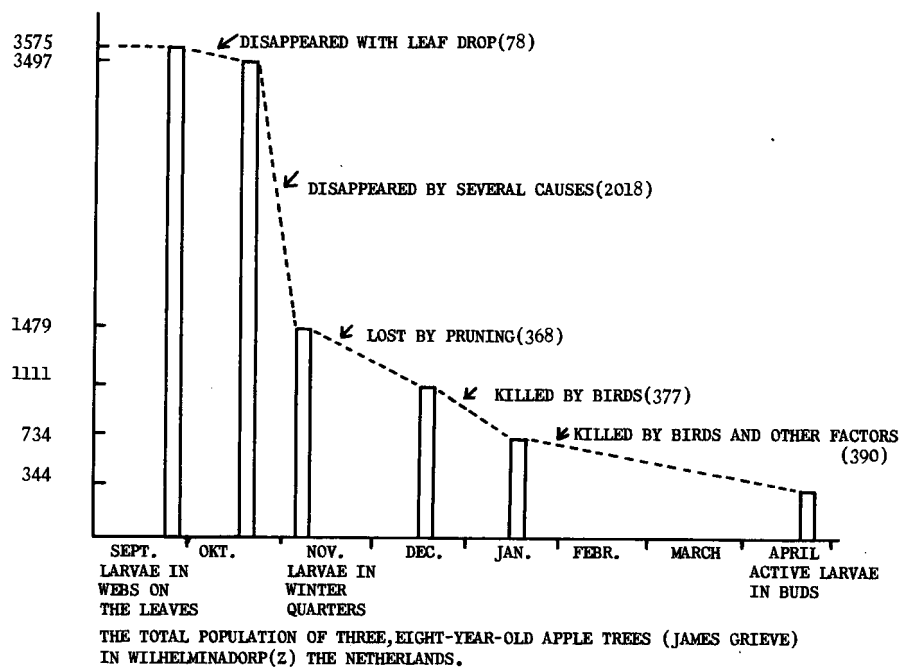
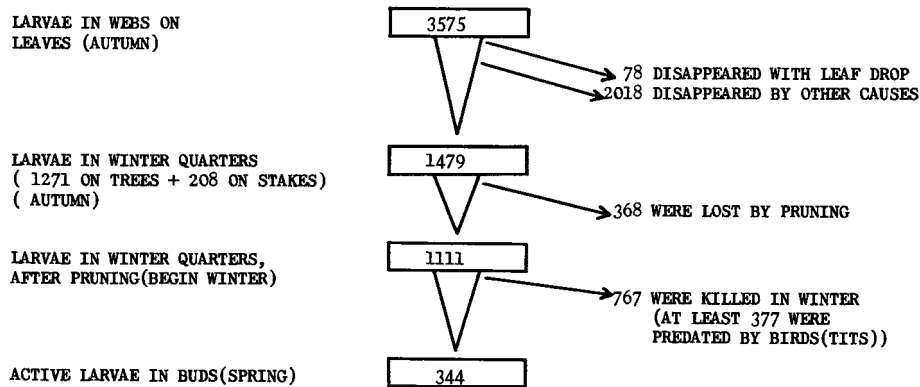


FIG. 1. Relation between the larvae population of *Adoxophyes orana* F.R. in the autumn of 1968 and the spring of 1969.

Populations of 1000 to 5000 individuals, but also of 10 000 to 12 000 individuals of almost full-grown larvae per ha, occur in orchards [39, 54]; by application of insecticides these numbers may be reduced to 500 or 1000 per ha.

Survival

Detailed knowledge of the numerical relations between successive generations is of great importance for integrated control and for sterile-male methods. After some preliminary investigations on winter mortality of hibernating larvae, we conducted a detailed study on the numerical relations between the population in August 1968 and the population in spring the next year [65, 66]. The population densities were counted in the orchard, and the investigations were carried out on several groups of trees because many insects were disturbed or killed by the inspections. Besides information on the influence of natural enemies and weather conditions, the data inform us on the influence of the pruning procedure. The investigations were conducted on seven-year-old apple trees, variety James Grieve. The results are summarized in Fig. 1. The figure shows that many larvae, which were hatched in August-September and made webs against the leaves, disappeared in the autumn. Nearly 2% were lost with leaf drop, and more than 56% disappeared by other causes so that only 41% started to hibernate: almost 6% on the stakes and 36% on the trees. Nearly 10% of the over-wintering larvae were removed by the normal pruning procedure, and another 10% were killed by tits in December-January. Eleven percent of the autumn generation were killed by unknown causes, probably partially also by birds, so that only 10% of the autumn generation (26% of the larvae that started to hibernate) survived winter and became active in spring.

The mortality of the over-wintering larvae on creosote-impregnated stakes was much higher than on the trees. In the winter 1969-1970 only 2% of the over-wintering larvae survived on the stakes, while 86% survived on the trees.

Besides, moths emerged from 92% of the pupae in June 1969, and larvae were hatched from nearly 85% of the eggs laid on the trees in June. In August, larvae were hatched from nearly 80% of the eggs laid during the second flight. Many eggs laid at the end of the second flight in the second half of September were not hatched.

Based on countings in 1968-1969, we estimated that nearly 180 000 caterpillars per ha made webs on leaves during the second flight in 1968; 18 000 survived winter.

The multiplication from the second flight in 1968 to the first flight in 1969 must have been nearly five-fold, while the survival from eggs laid in August-September 1968 to moths in June must have been about 6%, ($S = 0.06$).

Models for release programs

Simulating programs may be conducted with the aid of the formula of Berryman [67]. Two models of simulated programs, which were conducted by Ankersmit [68], are shown in Models I and II. They are based on the

foregoing results and arbitrary parameters. Though they are no real programs, they may give information on some parameters, such as the influence of reinfection from outside the orchard.

Model (I) of a simulated release program without reinfection

N_g = 1000 moths/ha during the first flight in June
 F_p = 0.5
 E = 175
 S_{June} = 0.30
 S_{August} = 0.06

Competitiveness of sterile males = 100%

Goal : 100 moths in the next year

No more moths are to be released than strictly necessary.

Result: Release in June of the first year = 20 230 sterilized males

Release in August of the first year = 10 207 sterilized males

Flight in June of the second year: 100 moths

(this number is not considered dangerous for fruit damage).

To maintain the population on the same level in second and subsequent years the following numbers of sterile males must be released:

In June : 1263

In August: 213

Model (II) of a simulated release program with reinfection

N_g = 1000 moths/ha during the first flight in June
 F_p = 0.5
 E = 175
 S_{June} = 0.80
 S_{August} = 0.06

Competitiveness of sterile males = 80%

Reinfection, both in June and August = 100 moths/ha

Goal : 5 moths of the original population will be maintained and

100 moths by reinfection in the next year.

No more moths are to be released than strictly necessary.

Result: Release in June of the first year = 110 299 sterile males

Release in August of the first year = 50 638 sterile males

Flight in June of the second year: 105 moths

To maintain the population on the same level in second and subsequent years the following numbers of sterile males must be released:

In June : 36 110

In August: 7 170

$$\text{Berryman's formula: } N_{g+1} = N_g F_p E S \left(1 - \frac{N_s}{N_g M_p + N_s} \right).$$

(assuming that mating activity of sterile males equals that of natural males)

N_s = number of sterile males
 N = number of wild adults in a release generation
 F_p^g = proportion of females
 S = proportion of survivors
 M_p = proportion of males
 E = average egg-production per female

$\left(1 - \frac{N_s}{N_g M_p + N_s}\right)$ represents the probability of a fertile mating in a release program.

DISCUSSION

The models show that reinfections of 100 moths per ha per flight period may be very inconvenient for release programs provided that the immigrants are ecologically equivalent to the local population. Dispersal of egg larvae by the wind, which, however, is still unknown, may also interfere unfavourably.

It may be discussed whether eradication has to be the ultimate goal or whether reduction to very low population densities, under the limit for economic damage, is to be maintained by repeated releases of small numbers of sterile males. Attention must be paid to possibilities for the application of substerilized males. The costs of repeated releases of small numbers of sterilized moths, as necessary in the first model, are presumably not higher than those of the insecticides which are annually necessary for a sufficient chemical control of *A. orana*.

The fact that the insect is very polyphagous and does not only occur in orchards, but also in many other biotopes around the orchards, indicates that dispersal and reinfection may be of great importance for the release programs. Recent studies suggest, however, that the female moths only occasionally leave their biotope. Strips of open field around orchards, or orchard complexes which will be treated, may probably act as barriers.

Considering the economic importance of *A. orana* and other interfering complications as discussed before, we are convinced that further research is justified.

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EFFECT OF GAMMA IRRADIATION DOSE
ON THE REPRODUCTIVE PERFORMANCE
OF THE P AND F₁ GENERATIONS
IN THE CODLING MOTH,
Laspeyresia pomonella L.

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Abstract

EFFECT OF GAMMA IRRADIATION DOSE ON THE REPRODUCTIVE PERFORMANCE OF THE P AND F₁ GENERATIONS IN THE CODLING MOTH, Laspeyresia pomonella L.

The paper gives the results obtained in the laboratory on males irradiated at doses from 10 to 100 krad. Their behaviour, sterility after crossing with normal females and the value of the F₁ offspring were studied. The sterility of the males is high from 10 krad and is complete at 40 krad. Longevity and the number of matings diminish as the dose increases. The offspring may die at any stage of development, which considerably reduces the hatching of the F₁ moths. The latter are practically all sterile even if the male parents were irradiated at 10 krad. They show very poor competitiveness in the laboratory.

Since the sterile P males are hardly competitive, consideration can be given to the release of sub-sterilized insects whose vigour has been proved in the laboratory. The scarcity of the F₁ offspring and the poor competitiveness would probably make their role negligible. The use of very low doses might perhaps permit the treatment of the L₅ larvae for the purpose of releasing substerile diapause larvae.

1. INTRODUCTION

The codling moth is the major entomological problem as far as the protection of our orchards is concerned. Control of this pest requires three to five antiparasitic treatments per annum and is thus a serious hindrance to the implementation of integrated control projects. For this reason, since 1967 we have been studying the possibility of applying the sterile-male technique.

In our initial field tests during the 1969 season, we released codling moths sterilized at a dose of 40 000 rad of gamma rays. This dose induces total sterility in males [1, 2, 3]. However, it has been proved that the sperm of sterile males is not competitive [4]. On the other hand, in an experiment in which males were released and recaptured in sex lures, Granges (unpublished paper) has shown that the sterilized codling moth males are less competitive. In seven releases the irradiated, as well as the normal, males were labelled alternately with red and green powders. By this method the effect of labelling on the comparison between irradiated and normal males could be eliminated. The sterilizing dose of gamma rays reduces the recapture rate by 40% (see Fig.1).

The quality of the insects released is one of the important factors for practical success so that it is necessary to test other irradiation techniques. For example, there is the possibility of treating males at substerilizing

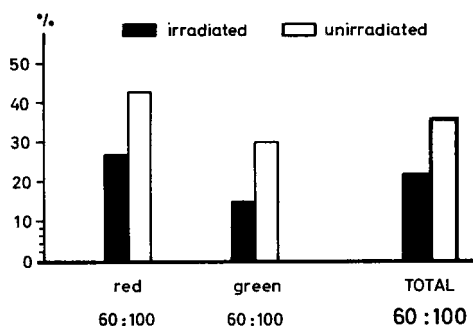


FIG. 1. Recapture of irradiated and normal males released in orchards (in percentage of released insects).

doses and using the sterile F_1 generation, as suggested by North and Holt [5]. Low-dose treatments would not change the competitiveness of the irradiated insects.

In order to ascertain the effectiveness of this technique under our conditions, we need to study the response of our codling moth strain to various gamma irradiation doses and determine the value of the F_1 moths. For this purpose, we carried out a series of laboratory experiments, in which we measured longevity, mating ability and sterility level in males irradiated at doses from 0 to 100 000 rad. We also studied the same parameters for the F_1 males.

2. MATERIAL AND METHOD

The material used was obtained by artificial breeding on apples. A room maintained at $24 \pm 1^\circ\text{C}$ and at a relative humidity of about 50% was used for rearing the larvae. The laying of eggs and embryonic development took place in a room kept at $27 \pm 1^\circ\text{C}$ and at a relative humidity of 70%. In both rearing rooms a 19-h photoperiod was maintained.

Only the male moths were irradiated at different doses with a Gammacell-220 irradiator at an intensity of about 5000 rad/min. A thermal enclosure stabilized at 60°C was used for keeping the moths immobilized during the treatment.

The irradiated males and normal females were mated in polyethylene boxes (volume 1 litre) provided with a water-soaked cotton-wool pad. At each dose we crossed 20 pairs, distributed in four boxes.

In the case of the F_1 moths, we observed their development up to adulthood and studied mortality in relation to penetration of the apples, number of L_5 larvae, chrysalises, normal moths and the sex ratio. The seemingly normal F_1 males were crossed with the reared females. The same observations were made on the moths and eggs as in the parent generation.

In view of the limited number of the F_1 females, it was not possible to cross the F_1 females with the reared males.

TABLE I. IRRADIATED MALES CROSSED WITH NORMAL FEMALES
Effect of gamma irradiation dose on embryonic mortality

Dose (krad)	No. of eggs	Percentage of unhatched eggs		Total
		without embryonic structure	with embryo	
0	5614	4.3	8.5	12.8
10	4942	15.2	24.8	40.0
20	5462	17.5	36.6	54.1
25	4547	35.8	38.7	74.5
30	4491	40.0	45.4	85.4
40	4656	70.9	22.6	93.5
60	3110	87.5	12.3	99.8
80	2575	91.9	8.1	100
100	3322	90.1	9.9	100

TABLE II. IRRADIATED MALES CROSSED WITH NORMAL FEMALES
Effect of gamma irradiation dose on longevity and sexual behaviour

Dose (krad)	Percentage of females mated	Average number of spermatophores		Longevity in days	
		per female mated	per male	female	male
0	100	2.4	2.4	11.4	14.7
10	90	2.6	2.3	13.0	13.6
20	95	2.7	2.6	11.5	16.1
25	90	2.0	1.8	9.4	13.1
30	75**	2.3	1.7	10.9	12.4*
40	75**	2.2	1.6	11.3	12.6*
60	55**	2.1	1.1**	14.4**	12.0**
80	60**	1.7	0.8**	14.3**	9.3**
100	40**	1.7	0.7**	13.5**	8.6**

Significant difference relative to the control, error levels of 5% (*) and 1% (**).

3. RESULTS AND DISCUSSION

The observations on the crossing of irradiated males with normal females are summarized in Tables I and II.

The results confirm that a dose of 40 krad is sufficient to induce almost total sterility. Sterility, as indicated by the number of unhatched eggs, is high even after treatment at 10 krad.

TABLE III. IRRADIATED MALES CROSSED WITH NORMAL FEMALES
Post-embryonic development of the F₁ offspring

Dose (krad)	No. of L ₁ larvae	Percentage of population in different stages of development					Male/female ratio
		Penetration in apples	L ₅ larvae	Chrysalises	Imagoes	Moths without deformation	
0	300	76.0	75.7	60.0	59.3	57.7	1.1 : 1
20	500	44.8	41.4	36.6	21.0	14.0	7.7 : 1
25	500	44.0	40.2	36.0	20.4	12.0	10.3 : 1
30	500	35.8	32.4	30.4	16.6	8.4	10.8 : 1
40	500	37.4	32.2	29.6	11.2	5.4	8.3 : 1

TABLE IV. F_1 MALES CROSSED WITH NORMAL FEMALES
Embryonic mortality

Dose received by P males (krad)	No. of eggs	Percentage of unhatched eggs		Total
		without embryonic structure	with embryo	
0	5464	7.6	10.2	17.8
10	2509	55.4	35.7	91.1
20	2217	94.3	1.3	95.6
25	1719	92.2	7.8	100
30	1507	99.0	1.0	100
40	730	98.8	1.2	100

TABLE V. F_1 MALES CROSSED WITH NORMAL FEMALES
Longevity and sexual behaviour

Dose received by P males (krad)	Percentage of females mated	Average number of spermatophores		Longevity in days	
		per female mated	per male	female	male
0	70	2.9	2.0	9.2	12.7
10	65	1.8*	1.1**	8.4	8.8
20	50**	2.0*	1.0**	9.5	10.5
25	35**	2.3	0.8**	12.0	12.2
30	25**	1.8*	0.4**	10.9	10.9
40	35**	1.6*	0.5**	8.7	8.0

Significant difference from the control, error levels of 5% (*) and 1% (**).

Mortality in the initial embryonic stages increases with dose, reaching 90% of the total mortality. These were dead eggs without any apparent embryonic structure. The rate of premature mortality increases sharply from 40 krad. This may be due to increase of the lethal factors induced in the spermatozoid. These factors might explain the non-competitiveness of sperm irradiated at the sterilizing dose.

The longevity of the irradiated males diminishes significantly from 30 krad. This contributes to the poor competitiveness in the field of the males irradiated at high doses.

The number of matings per male and the number of females mated decrease progressively as the dose increases. However, the average number of spermatophores per female does not vary. If it is assumed

that each mating gives rise to only one spermatophore, it will be seen that the activity of the highly irradiated males is concentrated on an increasingly limited number of females. This factor, together with the increased longevity of the females, seems to explain the lower activity of the males.

The lower activity and longevity at 40 krad shown in the laboratory confirm the observations made in the field. The conclusion that the males irradiated at 40 krad are not competitive seems to be borne out. The preliminary laboratory tests do not, however, show any decrease in the activity of the males irradiated at doses lower than 30 krad.

The transmission of sterility to the F_1 generation is proved in the codling moth [4, 6] and other Lepidoptera [5, 7, 8, 9], and its explanation is known [5, 10, 11]. It was, therefore, possible to develop the theoretical models designed for using the sterility of F_1 and F_2 after a release of substerilized insects in the field [12, 13]. However, the action of F_1 is associated with their quantity and quality. For that reason, we made a laboratory study of the value of F_1 in our strain. The results are given in Tables III, IV and V.

The mortality of F_1 is high, regardless of the dose received by the parent male. Death may occur at any stage of development, showing that certain lethal factors are active in the post-embryonic stages. Mortality is particularly high in the L_1 larval stage. The proportion of hatched moths with deformed wings is as high as 50% in all the cases studied. The females rarely reach the imaginal stage.

Mortality, the proportion of adult males and deformations of the imago increase with the dose received by the parent male. Apart from the hatching of a few eggs after treatment at 10-20 krad, the sterility of the F_1 males is total. Embryonic mortality occurs in the initial stages of development of the egg, thus showing the importance of the lethal factors transmitted by the sperm.

The number of matings per male and the number of mated females are low, but the average number of spermatophores per fertilized female hardly varies.

From the results it can be concluded that the F_1 males behave in the same way, under favourable laboratory conditions, as the moths which have received an overdose of gamma rays.

4. CONCLUSIONS

The results obtained in the laboratory, together with those of release in the field, confirmed the poor competitiveness of the males irradiated at 40 krad. This factor can be compensated for only by raising the ratio of the irradiated to wild males. However, the rearing of Lepidoptera is a delicate operation and the need to release a surplus of insects considerably diminishes the effectiveness of the technique.

The advantage of using substerilized males is that the competitiveness of the insects is not impaired. The vigour that they show in the laboratory should obviously be confirmed in the field. It would appear that consideration should not be given to this technique in view of the use of the F_1 generation. Our tests in the laboratory show the low activity of this generation, which will probably be negligible in the field. This confirms the observations made on Heliothis virescens (F.).

A dose of 25 krad can be chosen for practical applications. Treatment at this dose induces satisfactory sterility in males without apparently changing their competitiveness. It also ensures the sterility of the females, thus making sexing unnecessary. This technique can easily be applied to orchards with a small native population. It would also enable us, even in the first year, to keep the damage below the economically permissible limit [14].

The release of substerile insects already seems to be a feasible technique. The use of subdosages perhaps paves the way for new techniques which deserve to be studied. A very low irradiation would permit, for example, the treatment of the larval L₅ stage. The insects could then be released in the stage of diapause larvae. The release technique would thus consist in distributing the larvae in the autumn. The larvae, distributed in the field, would produce a population whose flight curve and period of sexual activity would be twice that of the natural population [15]. The practical importance of this method is evident and justifies the need for further research on the subject.

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DETERMINATION OF STERILE INSECT COMPETITIVENESS*

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Abstract

DETERMINATION OF STERILE INSECT COMPETITIVENESS.

A method of determining competitiveness for sterilized insects is demonstrated. The method is independent of the ratio of treated to normal insects used and can be determined from one ratio test provided that the egg hatch data are known for normal matings and matings between treated and normal insects.

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RESEARCH ON STERILITY METHODS FOR CONTROL OF THE CABBAGE LOOPER, Trichoplusia ni (Hübner)

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Abstract

RESEARCH ON STERILITY METHODS FOR CONTROL OF THE CABBAGE LOOPER Trichoplusia ni (Hübner).

The potential of sterility as a means of controlling the cabbage looper, Trichoplusia ni (Hübner), appears encouraging. Sterile insects for release can be produced by using irradiation or chemosterilants. However, excessive exposure or overdoses do diminish mating, sperm transfer, response to the female sex pheromone, and longevity, and can cause other adverse behaviour, all of which reduce the effectiveness of the released insects. Males exposed to substerilizing doses of irradiation were found to be more competitive and their progeny also exhibited sterility. This phenomena may provide a new and effective sterility method involving release of partially sterile insects. Preliminary tests with laboratory and field cages indicate that electromagnetic radiation can attract native male cabbage loopers to a source where they can be exposed to a chemosterilant; thereafter returning to the population to compete with unsterilized insects for mating and ecological niches.

INTRODUCTION

The sterile-release method of suppressing or controlling insect populations is one of the outstanding recent contributions to entomology. Its demonstrated potential, the successful control and eradication of the screw-worm, Cochliomyia hominivorax (Coquerel), from Curaçao and from the southeastern United States [1,2], has had a significant impact on the direction and intensity of research by scientists in the United States and in many other countries. Thus, today, the method is being studied to determine its applicability to the control of a wide variety of important insect pests of agricultural commodities and man. These specific programs are in different stages, from preliminary research to pilot studies being made in preparation for actual field releases, and are directed against many different insects, including such important Lepidoptera as the pink bollworm, Pectinophora gossypiella (Saunders); the codling moth, Carpocapsa pomonella (Linnaeus); the tobacco hornworm, Manduca sexta (Johannson); the tobacco budworm, Heliothis virescens (Fabricius); the oriental fruit moth, Grapholitha molesta (Busck); the sugarcane borer, Diatraea saccharalis (Fabricius); the European corn borer, Ostrinia nubilalis (Hübner); the corn earworm, Heliothis zea (Boddie); the gypsy moth, Porthetria dispar (Linnaeus); and the cabbage looper, Trichoplusia ni (Hübner).

The first method of inducing sterility in insects that were to be used for release was exposure to ionizing irradiation. However, the discovery of chemicals that induce insect sterility [3] suggested another new concept,

that an entire native population of insects (or a large percentage of it) might be sterilized in its own environment [4]. If safe, effective chemo-sterilants, or safe methods of using those presently available, can be developed, we may have a second new method of insect control. Moreover, this second method has several important theoretical advantages [5] compared with releases of sterile insects.

To date, no large-scale field tests have been made to determine how feasible the new concept is, but the potential is so great that extensive re-research is warranted.

The present paper is a review of the research that has been done, or is underway, into methods of using sterility to control the cabbage looper.

STERILE-RELEASE METHOD

Rearing cabbage loopers

A continuous, dependable method of mass rearing which produces healthy, vigorous insects is essential to any release system, but as recently as 10 years ago the laboratory rearing of many lepidopterous insects was considered impossible. The great progress that has been made since 1960 must be attributed to the many investigators in the field of insect nutrition which have provided us with the basic information necessary to develop successful techniques for rearing insects under laboratory conditions.

With cabbage loopers, the early attempts at rearing were frustrated because of repeated disease epizootics. McEwen and Hervey [6] then overcame that problem by isolation, careful control of temperature, the rearing of small numbers of insects per unit, proper sanitation, and liberal use of antimicrobial agents. Next, satisfactory artificial media were developed for mass rearing the insect [7-10]. Today, 20 000 adult cabbage loopers per week are reared routinely at the Bean, Berry, and Leafy Vegetable Insects Investigation Laboratory at Riverside, California, and as many as 100 000 per week can be reared when there is a special demand. We, therefore, have an efficient and dependable method of rearing cabbage loopers at a cost of about \$3000 per million insects [11] and, with additional research and attention to automation, should be easily able to produce greater numbers even more cheaply.

Larval and adult diets, techniques of insect handling, and factors affecting rearing were reported by Henneberry and Kishaba [11]. Also, information regarding the biology of the species has increased markedly since laboratory rearing has been possible.

Sterilization by irradiation

The doses of radiation required to sterilize species of Lepidoptera have proved to be relatively high compared with those required to sterilize Diptera, Hymenoptera, Coleoptera, Orthoptera, Hemiptera, and Homoptera. For example, among 16 lepidopterous species listed by LaChance et al. [12], the estimated doses required to induce sterility ranged from 4 to 50 krad, and only two species were sterilized by doses below 10 krad.

TABLE I. EFFECTS OF GAMMA RADIATION ON FERTILITY OF THREE-DAY-OLD ADULT CABBAGE LOOPER MALES (TREATED MALES, UNTREATED FEMALES)^a

Dose (krad)	Eggs/♀ ^b	Hatch (%)
0	466	91
5	349	83
10	313	59
15	182	55
20	44	15
30	195	4
40	52	0

^a Data extracted from North and Holt (1968).

^b Mean 4-22 females.

These high levels of irradiation may cause a number of undesirable effects in insects being prepared for releases — reduced longevity, inability to mate, noncompetitive behavioural responses, reduced vigour, or other biological characteristics that limit the activity of the released insect in nature.

Data extracted from a publication of North and Holt [13] show that 30 krad of gamma irradiation will cause nearly complete sterility in adult male cabbage loopers (Table I). However, longevity was markedly reduced. If the released insects were otherwise completely competitive, this shorter lifespan would mean only that more frequent or larger releases would be necessary to maintain the required ratio of sterile to normal insects. (With many insect species, such a solution would be feasible, but rearing costs for most Lepidoptera are relatively high, so the additional costs might be prohibitive.) However, the irradiated cabbage looper males also sometimes transferred only a spermatophore during mating and failed to supply the female an adequate amount of sperm. In one test, no more than 30% of the males treated with 20 krad transferred enough sperm to supply the females with an adequate amount; a substerilizing dose (15 krad) allowed a higher percentage to transfer sperm. This difficulty was much more serious than reduced longevity. If releases are to be successful, the male cabbage loopers must contribute competitive sperm at each mating.

The radioresistance of Lepidoptera remains a matter of speculation. However, most species of this order are characterized by large numbers of small, holokinetic chromosomes and heterogametic females compared with Diptera which are radiosensitive and have fewer large chromosomes, localized centromeres, and heterogametic males [12]. The diffuse centromeres of Lepidoptera could impart a high degree of resistance to induced sterility since the chromosomal fragments would probably not be lost, and would therefore not lead to dominant lethality [13]. In addition, these diffuse centromeres of Lepidoptera may be the basis for the partial sterility in the progeny of irradiated male parents [13, 14] reported by

TABLE II. INHERITED STERILITY OF PROGENY OF MALE CABBAGE LOOPERS THAT RECEIVED SUB-STERILIZING DOSES OF GAMMA RADIATION^a

Dose to P ₁ male (krad)	P ₁		F ₁ ♂♂ × N ♀♀ ^b	
	Eggs ^c	% Hatch	Eggs ^d	% Hatch
0	951	84	1025	72
5	828	68	901	50
10	810	54	605	6
15	755	32	526	3

^a Data from Debolt (unpublished).

^b Untreated virgin females.

^c Means of 30 moth pairs.

^d Means of 10 moth pairs.

TABLE III. COMPARISON OF THEORETICAL EFFECT OF RELEASING COMPLETELY STERILE AND PARTIALLY STERILE MALES INTO INSECT POPULATIONS IN PARENT GENERATION ONLY AT 9:1 RATIO OF STERILE TO NATIVE MALES^a

Generation	Native insect population (No.)	If 100% sterile ♂♂ released (No.)	If partially sterile ♂♂ released (No.)
Parent	1 000 (500 ♂♂ + 500 ♀♀)	4 500	4 500
F ₁	500	None	2 300
F ₂	2 500	None	540
F ₃	12 500	None	2 700
F ₄	62 500	None	13 500

^a Assumes: (1) 5-fold increase per generation in untreated population; (2) Treated P₁ males × native females produce 60% fewer progeny than native parent mating; and (3) F₁ treated male × untreated female progeny are completely sterile.

various workers in many species [15]. Reciprocal translocations may not involve the loss of chromosomal material in progeny; instead, rearrangements may occur that contain duplicate or deficient amounts of genetic material which, during meiosis in the progeny, could result in sperm lethal to the embryo. The mechanisms by which irradiation or chemosterilants induce sterility in Lepidoptera should therefore be studied in detail.

The importance of the effect of irradiation on cabbage loopers through the sterility factor introduced into the progeny by substerilizing doses (Table II) was not recognized until Knipling (1967, unpublished data)

considered the possibility of keeping physiological and somatic cell damage at a low level in released insects and used the sterility in progeny to introduce a new concept into the method of sterile releases. He then encouraged researchers to investigate the practical application of the idea in programs designed to suppress populations.

Initially, male cabbage loopers treated with 15 krad were found to be as competitive in small cage tests as unirradiated males based on the reduction in egg hatch [15]. Then the results of large field cage tests at the Riverside, California laboratory corroborated these findings (Toba and North, personal communication). Partially sterile (15 krad) and completely sterile (30 krad) males released into untreated cabbage loopers at ratios of 9:1, reduced developing populations by 90 and 80%, respectively. This reduction was slightly less than calculated but still very encouraging as a successful demonstration of a new method of controlling cabbage loopers.

Knipling's theoretical appraisal of the possible effect of releasing partially sterile insects into a native population of the same species indicated that fewer of these more competitive insects would need to be released to achieve greater suppression because of the inherited sterility. From the model in Table III (taken from Knipling [16]), nearly four times as many completely sterile males as partially sterile males would have to be released in the parent generation to produce comparable effects on the reproductive potential of an insect population over four generations. Moreover, the impact of releases of partially sterile males in both the parent and F_1 generations would be greater than that of completely sterile males.

The results of releases of partially sterile insects have still to be demonstrated in the field. However, losses caused by lepidopterous insect pests are so great in spite of conventional methods of control that additional investigations should be undertaken to evaluate the practicality of the method.

Sterilization with chemicals

A number of investigators have studied chemicals as sterilants of lepidopterous insects that might be used for sterile releases. Several chemicals are available that may be sprayed, fed, applied topically, or used as dry residues to contact the insects. Tepa appeared to be the most satisfactory in terms of reliability and reproducibility of results [17], and gave complete sterility in males and females at doses of 85 and 125 μg , respectively. Substerilizing doses (SD) were as follows [18]:

Sterility (%)	Males		Females	
	SD ($\mu\text{g}/\text{moth}$)	95% confidence limits	SD ($\mu\text{g}/\text{moth}$)	95% confidence limits
30	7.37	7.00 - 7.76	15.92	14.50 - 17.48
50	9.50	9.02 - 9.92	24.99	22.76 - 27.44
70	12.13	11.52 - 12.78	39.27	34.59 - 44.58
90	17.41	16.14 - 18.77	75.44	61.79 - 92.11

TABLE IV. EFFECTS OF TEPA SPRAYS ON THE FECUNDITY, VIABILITY OF EGGS, MORTALITY OF MALES AT END OF TEST PERIOD, AND MATING OF CABBAGE LOOPER MOTHS, WHEN MALES WERE TREATED WITH DIFFERENT CONCENTRATIONS OF THE CHEMOSTERILANT
Means of 12 pairs (3 pairs, 4 replications)

% of sterilant in spray	Eggs		Percentage of		
	Laid	Hatch (%) ^a	♂♂ that died	♀♀ that mated	Mating aberrations
♂♂ treated when less than 1-day old					
0.25	725	67	0	100	0
0.5	556	5	8	100	0
1.0	482	2	41	92	0
2.0	497	t	17	100	0
Check	553	82	0	100	0
♂♂ treated when 1-day old					
0.25	467	51	25	100	0
0.5	442	t	50	100	8
1.0	394	0	58	100	0
2.0	136	0	58	100	42
Check	529	85	25	92	0
♂♂ treated when 2-days old					
0.25	636	59	25	100	0
0.5	698	1	58	92	8
1.0	393	0	83	100	17
2.0	285	0	75	100	8
Check	496	83	58	92	0

^a t = less than 1%.

Cabbage looper males fed tepa died sooner than untreated males [17]. Also, male cabbage loopers fed 1% tepa in 10% sugar solution for 48 h and then confined with virgin females for 8 days were not competitive. When the females were dissected to determine the presence of spermatophores, the results were as follows [17]:

Treatment	Mean spermatophores
	(20 ♀♀)
Untreated males	1.3
Males fed 1% tepa	0.2

TABLE V. EFFECT ON CABBAGE LOOPER REPRODUCTION WHEN MALES WERE EXPOSED TO DIFFERENT RESIDUES OF TEPA ON GLASS SURFACES COMPARED WITH THAT IN THE SAME NUMBER OF MOTHS USED AS A CONTROL

Material and strength (mg) ^a	No. moth pairs in test	Eggs			
		Laid		Viable (%)	
		Treated	Check	Treated	Check
Tepa					
240	20	405	1122	0.0	69
120	24	2162	3969	0.0	71
60	8	1548	1720	0.0	76
30	18	4736	4075	26.0	79
15	10	679	1127	17.0	67

^a per 500-ml Erlenmeyer flask.

Competitiveness of moths fed 0.1 or 1% tepa in 10% sucrose solution was also tested by degree of response to the female sex pheromone [19]. They proved to require at least 40-fold as much pheromone to elicit a response from 50% of the males.

Doses of chemosterilants applied in food proved difficult to control because of wide variations in feeding habits. Chemosterilant sprays, in contrast, induced sterility without causing immediately apparent adverse effects. The results obtained when aqueous sprays containing four concentrations of tepa (ranging from 0.25 to 2%) were applied to male or female moths [17] are shown in Table IV. Females mating with males treated with all but the lowest dose produced about the same number of eggs as females mating with untreated males, but at least 95% of the eggs were not viable. Also, males sprayed with 0.5 or 1% tepa were as responsive to the sex pheromone as untreated males [19]. However, females mating with males treated with 2% tepa laid fewer eggs in two of the three tests [17]. The treated males may have been incapable of adequate transfer of sperm in the same way as irradiated cabbage looper males, or the ability to mate may have been reduced by the treatment, the latter appears rather likely. The number of abnormal copulations was high (Table IV). It appeared to be related to the concentration and the age of the males at the time of treatment, but 12% of 240 matings between untreated females and treated males were abnormal; no abnormal copulations occurred in 60 matings of untreated pairs. The reason for these abnormalities and the relationship to sterilization is obscure at the present time.

Table V shows the results obtained when cabbage looper moths were exposed to contact with dry residues of five concentrations of tepa [20] in 500-ml Erlenmeyer flasks. The untreated females mating with males exposed to the three higher doses for 2 h produced no hatchable eggs, but the two lower doses were ineffective though males exposed for as little as 15 min to 120 mg per flask were completely sterilized (Table VI). When males exposed to sterilizing doses of tepa as residues for 2 h were

TABLE VI. EFFECT ON REPRODUCTION OF CABBAGE LOOPERS WHEN MALES WERE EXPOSED FOR DIFFERENT TIMES TO RESIDUES OF 120 mg OF TEPA PER 500-ml ERLLENMEYER FLASK

Exposure time (Min)	Eggs ^a			
	Laid		Viable (%)	
	Treated	Check	Treated	Check
5 ^b	1603	-	85	-
15	3243	2820	0.0	92
30	1288	2535	0.0	85

^a Mean 10 pairs.

^b Mean 5 pairs.

TABLE VII. EFFECT ON REPRODUCTION OF CABBAGE LOOPERS WHEN TEPA-STERILIZED MALE MOTHS WERE RELEASED INTO LARGE FIELD CAGES CONTAINING CABBAGE PLANTS ^a

No. moths released into cage ^b				
$\sigma\sigma$			No. F ₁ larvae	Reduction (%)
S	U	♀♀, U		
100	5	5	7	97
75	5	5	61	76
50	5	5	90	65
25	5	5	205	21
0	5	5	259	0

^a 150 plants in each test.

^b S = sterilized, U = untreated.

released in cages with untreated pairs, the numbers of developing larvae decreased as the ratio of sterile to normal males and females increased. At the highest ratio, 20:1:1, the larval population was reduced 97% (Table VII). Methods of determining the effects of chemosterilants on insect vigour proved difficult to develop. Therefore, since flight and flight characteristics appeared to be criteria for vigour, and since cages of any size are limiting, flight mills were developed by a number of investigators. The technique involves tethering the insects on a rotating arm or other device that permits it to fly in a small space [21].

When the device was used to test male and female cabbage loopers treated with tepa spray [21], their flight characteristics proved not to be affected (Table VIII). However, insects forced to fly immediately after

TABLE VIII. EFFECTS OF TEPA ON FLIGHT CHARACTERISTICS OF 3-DAY-OLD MALE AND FEMALE CABBAGE LOOPER MOTHS

μg tepa/ moth	Distance (miles)	Hours in flight when suspended for 24 h	Velocity (miles/h)	No. of stops
Males				
0 ^a	14.67	6.83	2.25	32
7.6 μg	18.32	9.31	2.04	36
9.6 μg	16.41	8.85	2.04	29
12.1 μg	15.35	8.87	1.87	41
85.0 μg	14.27	7.79	1.82	50
Untreated	13.58	6.83	1.98	40
Females				
0 ^a	12.26	5.78	2.19	18
15 μg	15.21	6.71	2.11	33
23 μg	12.21	6.22	1.99	37
36 μg	17.23	6.73	2.64	47
125 μg	12.09	5.56	2.26	32
Untreated	12.05	6.39	1.82	28

^a Treated with 1 μlitre water + 0.3% Triton X-100.

treatment with the sterilant showed less effect of the chemical. The SD_{50} was increased about 1.4 and 1.6 times for males and females, respectively, as follows:

Moths	Males		Females	
	SD_{50} (μg)	95% confidence limits	SD_{50} (μg)	95% confidence limits
Flown	18.83	16.37 - 21.63	58.88	47.94 - 72.30
Not flown	13.37	11.85 - 15.10	39.81	33.73 - 46.98

Although the fate of tepa in the cabbage looper has not been investigated, the effects of this chemosterilant on the physiological and metabolic systems of insects should be studied. A reduced sterilizing effect in active moths could affect the success of a sterile-release program.

Sterilizing native moth populations

The advantages that might accrue from sterilizing a large percentage of a native insect population in the field were discussed by Knippling [5].

TABLE IX. EFFECT ON REPRODUCTION OF CABBAGE LOOPERS WHEN MOTHS WERE EXPOSED IN FIELD CAGES EACH CONTAINING A BLACK-LIGHT ENCLOSED BY A TEPA-COATED CELLULOSE NITRATE CYLINDER

Pairs of moths/ cage	No. of plants examined/ cage	No. of larvae present in:		Reduction (%)
		Cage with black- light	Check cage	
30	30	1	107	99
60	50	459	1309	65
30	30	21	118	82

Such a method would, of course, eliminate the need to rear, sterilize, and release insects. However, for such a procedure, we need ways to induce the insects to the source of the sterilant. Insect baits and attractants appear promising, and electromagnetic irradiation has also been reported as an attractant for many species of Lepidoptera. In addition, sex pheromones have been demonstrated in many species [22].

Investigations to determine whether it is possible to combine an attractant and a chemosterilant to achieve sterilization of a native population of lepidopterous insects have been limited. However, the results of preliminary cage studies at the Riverside laboratory were encouraging [20]. In these tests, fluorescent black-light lamps (15 watt) were enclosed in cellulose nitrate cylinders about 8 inches in diameter by 20 inches long, and the outer surfaces of the cylinders were coated with an 8% tepa solution. Then one cylinder was installed in each field cage over cabbage plants and turned on from 7 p.m. to 7 a.m. for 14 consecutive nights. The first night, 30 or 60 pairs of untreated virgin moths were released into the cages containing treated cylinders and like numbers into cages containing untreated cylinders. The test was replicated three times. Eighteen days after the releases, the number of larvae on 30 to 50 plants chosen at random in each cage were counted. Table IX shows that larval populations in the three cages containing coated cylinders were reduced 99, 65, and 82%, respectively, compared with the populations in the check cages.

When male cabbage looper moths are exposed to dry residues of tepa, they must remain on the treated surface for 15 min or more before sterilization is complete [20]. Therefore, methods of incorporating tepa into mixtures of lanolin and *n*-hexanol were tested to determine whether these materials could be used to produce sterility by a briefer contact [23]. In this test, two concentrations of tepa, 16 and 4%, were incorporated into 75% lanolin, the weight of the mixture was adjusted with *n*-hexanol, and the product was used to coat glass microscope slides. Then male moths and female moths (2 days old) from the laboratory colony were held by the wings so the tarsi touched for about 2 sec one of the two tepa

mixtures (15 of each sex exposed to each mixture). After treatment, three males were paired with three untreated virgin females, or three untreated males were paired with three treated virgin females (five replications, a total of 15 pairs) and placed in 1-pint oviposition cages lined with blotting paper. The number of eggs laid and the hatch was recorded 7 days later.

The male moths were completely sterilized by the exposure to the mixture containing 16% tepa; the female moths treated at the same dose laid fewer eggs than untreated females, and only a small percentage hatched as shown:

Per cent tepa ^a	Males treated		Females treated	
	No. eggs laid	% eggs viable	No. eggs laid	% eggs viable
16	387	0	154	3
4	499	23	266	29
0	462	78	528	32

^a Per cent tepa after incorporation in 75% lanolin with the weight of the mixture adjusted with n-hexanol.

As a result of the encouraging preliminary work, additional studies were made in 12 cages (10 ft wide, 24 ft long, and 6 ft high) covered with nylon screen, each set over four rows of cabbage plants. A mixture of 75% lanolin, 9% n-hexanol, and 16% tepa was painted on 4 × 6 × 24-inch Plexiglas containers made to cover 15-watt fluorescent black-light lamps; other similar containers were not painted. The lamps were then installed, one per cage, in each of the 12 cages (three contained untreated containers), and the lights were turned on from 7 p.m. to 7 a.m. after 25 untreated male cabbage looper moths were released in each cage. The lamps in the three check cages were left on for 3 nights; the lamps in the other nine cages were left on for 1, 2, or 3 nights (three cages for each period). After each treatment was complete, 25 virgin females were introduced into each cage and left for 14 days. Larval populations on cabbage plants in cages containing the treated containers were reduced 90% (estimated by counting the number of larvae on 30 plants in each cage) and compared with the number on plants in the check cages. The results were as follows:

Black-light lamps on number of nights indicated	Number of larvae per 30 plants	% reduction
1	2	99
2	5	98
3	18	92
3 (check)	209	-

No significant difference in percentage sterility was apparent whether the lamps were turned on for 1, 2, or 3 nights. Thus, most male moths were sterilized the first night.

DISCUSSION

The cabbage looper has been consistently rated as one of the most damaging insect pests in various parts of the United States. The results of research to date indicate that it may be possible to use sterility to control this insect. However, critical information regarding the population dynamics of the species in the field is lacking. The insect is a strong flier [24, 25] and capable of migrating long distances. Since it apparently does not diapause, a critical determination of the northernmost limits of the overwintering should be made. If the area delineated proves to be sufficiently restricted, it may be possible to suppress these insect populations. For example, Knipling [16] hypothetically assumed an area of winter survival of 100 000 square miles in the southeastern United States with an average density of 500 moths per square mile. Against this level of population, partially sterile males released against the parent and F_2 generations could conceivably keep the number of progeny at a low level for 5 generations. Control of the cabbage looper by such a system of suppression is an exciting and promising concept, and may be applicable to a number of other important damaging lepidopterous insect pests.

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GENETIC CONTROL STUDIES OF Carpocapsa pomonella (Linnaeus) IN HUNGARY

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Abstract

GENETIC CONTROL STUDIES OF Carpocapsa pomonella (Linnaeus) IN HUNGARY.

The codling moth is the most important pest of commercial apple orchards in Hungary. However, its population density is low due to extensive insecticide treatments.

Studies on the genetic control of the codling moth included mass rearing of the Yakima strain on small apples. The Hungarian strains showed less adaptability to laboratory rearing (low egg production). A new type of rearing tray made of wood, a wire screen, plastic foil and linen was used. In 1969 the productivity of rearing varied between about one adult per apple in May and June, and 0.22 adults per apple in October. This reduction was due mainly to granulosis virus infection. At 17 hours photophase the diapause rate was less than 0.3%, and it did not exceed 3.2% when the larvae were reared in total darkness with one hour daily illumination. By using a combined adult emergence and oviposition device the handling of adults was simplified and a homogeneous distribution of the eggs on wax paper strips was achieved.

In preliminary migration experiments, 40-krad irradiated males were recaptured within 150 m. Males released outside the orchard migrated up to 650 m.

Possible pitfalls in the genetic control of the codling moth in Hungary are: (1) whether the method would be economical; (2) programing of mass rearing and release so that the population density of adults in nature, showing two more or less sharp peaks, can be followed; and (3) how to control other insect pests which at present are controlled by insecticide treatments applied for codling moth control.

INTRODUCTION

The codling moth has been chosen as the main species to be studied concerning the possibilities of using the sterile-male technique in Hungary because: (1) this species is one of the most dangerous pests of apple production in the country; (2) the new commercial apple orchards planted during the last two decades represent large continuous areas with restricted secondary infestation sources (scattered trees); (3) as a result of intensive chemical control the natural population density in the commercial orchards is low; and (4) experience gained abroad has already shown the competitiveness of irradiated males and the possibility of developing mass-rearing methods [1, 2].

This paper discusses the main results of the studies that have been carried out so far in Hungary (see also Ref. [3]).

EXPERIENCE IN MASS REARING

Several authors have described methods for mass rearing the codling moth. The American results have been summarized by Hamilton and Hathaway in Smith [4]. Petrushowa et al. [5] reported on work carried out in the Soviet Union. The rearing techniques used abroad were modified in several points to develop a method more suitable for our conditions and to simplify the whole procedure.

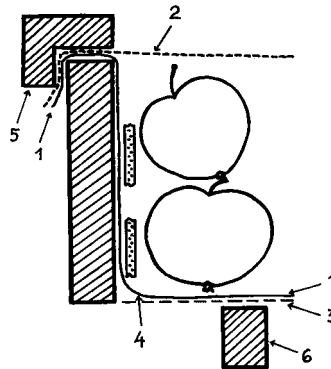


FIG. 1. Partial cross-section of a "double" rearing tray. 1. plastic foil; 2. linen cover; 3. wire screen bottom; 4. corrugated paper strip; 5. wooden frame; 6. counter-lath.

Substrate

Mass rearing was only carried out on small apples between 3 and 4 cm in diameter. Seven apple varieties were tried for cold storage. Only Jonathan and Nemes Sóvári were suitable for this purpose. Wooden boxes containing about 30 kg of apples were used for storage. Since the apples were stored together with other commercial goods, the temperature of the storage room sometimes became raised above the ideal $+3$ to $+5^{\circ}\text{C}$. It is likely that this caused the decay of apples which reached in some cases 20 to 30% by the end of April.

The apples were washed in a 0.5% sodium hypochlorite solution for 5 to 10 min, rinsed in tapwater for 5 to 10 min and dried on a wire grid.

Trays

Since, in Hungary, disposable cardboard trays would be more expensive in the long run than trays for repeated use, the following types of trays were developed. Our "simple" tray has a wooden frame ($40 \times 60 \times 6$ cm) with a 1-2 cm mesh wire-netting bottom. The tray is lined with a plastic sheet reaching over the rim and is covered with linen which is held on the tray by a wooden frame (Figs 1-3). Two 2-cm counter-laths on the under side of the tray enable the trays to be illuminated and ventilated when they are stacked. The wooden parts of the tray are soaked with a lacquer to facilitate cleaning and to prevent the mature larvae from boring into the wood. The linen cover is coated with oil-paint along the border to reduce the boring out of the larvae. The apples are placed in the tray in a single layer. Corrugated paper strips on the sides of the tray serve as co-cooning places.

The "double" tray has the same structure and size but is higher (8 cm) so that there is enough space for two layers of apples (Fig. 1). It was found that reduction of light intensity due to the double layer of apples did not change the diapause rate.

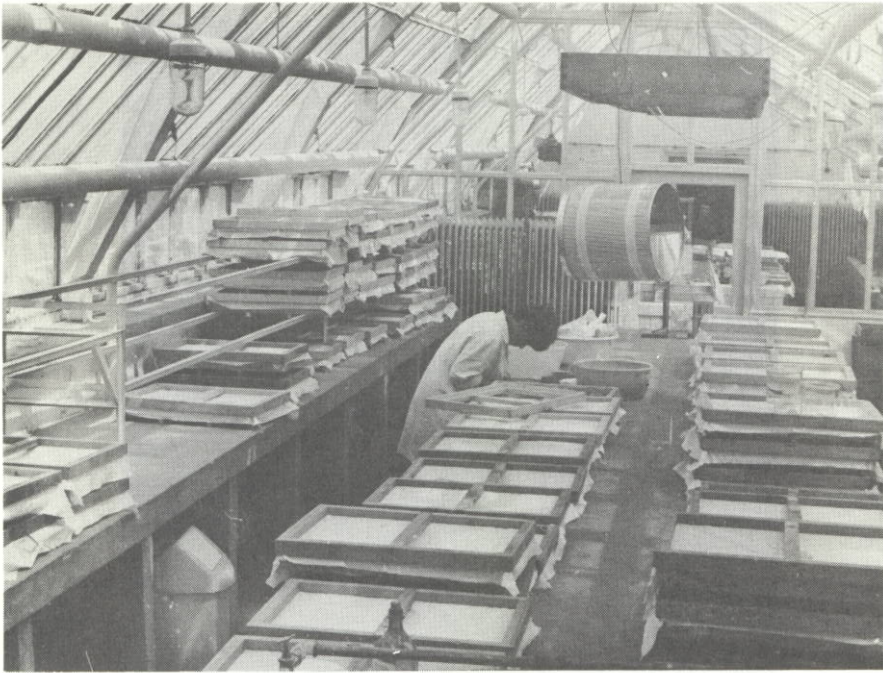


FIG. 2. Mass rearing in the glasshouse at Keszthely.



FIG. 3. Examination of a tray at the end of larval development.

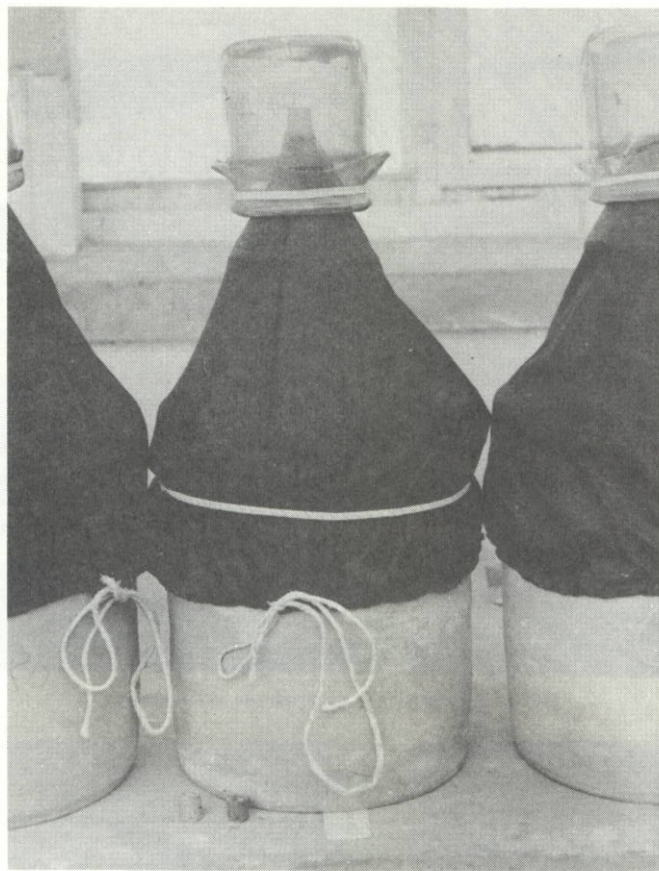


FIG. 4. Adult emergence pots with collecting jars.

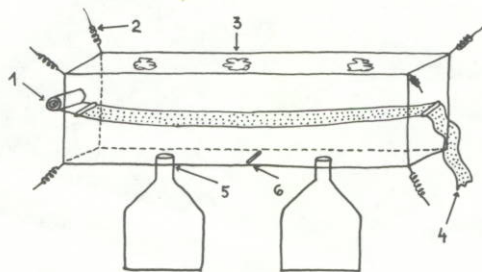


FIG. 5. Combined emergence-oviposition device. 1. wax paper strip rolled up; 2. suspension of the plastic screen cage; 3. wet cotton-wool wad; 4. wax paper strip; 5. connection between emergence pot and oviposition cage; 6. opening with zipper for removal of dead adults.

Eggs in the black-head stage were placed on the apples. The paper strips containing the eggs were cut into pieces 1 to 4 cm² or strips 0.5 to 1 cm wide, and were distributed evenly on the apples. About 400 eggs were put into the "simple" trays and 800 into the "double" ones. The eggs were, in general, not disinfected.

The larvae were reared in a glass house (Fig. 2) at temperatures varying between 23 and 30°C. The air humidity was not regulated. Illumination with fluorescent tubes in the morning and evening completed the photophase of 17 h.

The corrugated paper strips were collected from the trays (Fig. 3) 2 to 3 weeks after the apples were infested, and replaced by a second series of strips which were collected after a further 7 to 10 days. To save work the strips were often collected once only, namely at the emergence of the first adults in the trays.

Emergence pots

For emergence of adults, 10-litre cylindrical earthen vessels were used. Their orifice was covered by a cone-shaped black linen top ending at the tip in a plastic funnel. One-litre glass jars with an inverted conical wire-screen cover were placed on the plastic funnels to collect the adults (Fig. 4).

Combined emergence-oviposition device

The second author developed a combined device for the emergence of adults and oviposition (Fig. 5). It consists of a plastic screen cage 100 × 20 × 20 cm with a 1 to 2 mm mesh. The emergence pots containing the pupae are connected with the bottom of the cage. The pots can be replaced by other ones when emergence has ended. The eggs are laid on a 12-cm wide wax paper or cellophane strip which enters through a slit at one end of the cage and is pulled horizontally across it through another slit at the other end. Dead adults must be removed through an opening, which is closed by a zipper, on the bottom because the eggs can be laid on it. The number of living adults varied between 500 to 1000. The devices were placed in a dark room at 20 to 26°C and lit from above by red incandescent lights to ensure even distribution of egg-laying adults in the cage. Water was given the adults by placing cotton-wool wads soaked with tapwater on the top of the cage.

Advantages of this simple device are: (1) no additional handling is necessary between emergence and oviposition which means a considerable reduction in work and in damaging the moths; (2) distribution of the eggs on the strips is quite homogeneous; and (3) the homogeneity of the age of the eggs can be controlled by the periods of changing the strip.

Codling moth strains reared

The mass rearing was established by using the Yakima laboratory strain (Y-strain) which has been kindly placed at our disposal by Dr. B. Butt. This strain seems to be well adapted to laboratory conditions while, until now, all attempts to establish mass rearing of strains collected in different parts of Hungary failed because the females of the latter strains laid, on the average, much less eggs than the Y-females.

TABLE I. RESULTS OF CODLING MOTH MASS-REARING ON SMALL APPLES IN HUNGARY, 1969

Month	No. of trays examined	Average No. of				Dead larvae in cocoons	Dead pupae	Diapausing larvae	Average No. of		
		eggs	apples	cocoons	adults per tray				cocoons	adults	
per tray				in % of all cocoons			per apple				
May	83 S	419.7	123.3	122.0	0.32	3.23	-	117.6	0.99	0.95	
Jun.	71 S	359.1	168.3	128.5	0.66	3.79	0.10	122.4	0.76	0.73	
	11 D	800	301.3	344.6	0.85	6.03	0.08	319.8	1.14	1.06	
Jul.	26 S	400	167.0	163.9	4.56	8.10	0.06	141.3	0.98	0.85	
	27 D	800	332.3	266.7	2.62	8.43	0.22	237.8	0.80	0.72	
Aug.	9 S	355.6	104.0	70.1	4.20	4.31	-	63.9	0.67	0.61	
Sep.	15 S	344.7	163.3	88.7	1.70	3.24	-	84.5	0.54	0.52	
Oct.	42 S	392.0	177.3	51.6	17.12	1.78	-	39.6	0.29	0.22	

Note: S = "simple" trays (see text).
D = "double" trays (see text).

Results of mass-rearing experiments

Table I summarizes the main results of mass rearing from May to October 1969. It can be seen that productivity of the rearing diminished gradually from about 1 adult per apple in May and June to 0.22 adults per apple in October. This was mainly due to the granulosis virus causing increased mortality probably in all larval stages. It is assumed that the decrease in mean temperature in the glasshouse and other unknown factors could also have played a role.

The diapause rate was very low. In separate experiments Sáringer [6] has shown that only 3.2% of larvae entered in diapause when reared at $28 \pm 0.9^\circ\text{C}$ in total darkness with one hour daily illumination only. Thus, long-day illumination can be replaced by one-hour photophase which means economy in energy requirements of mass-rearing plants. However, the influence of one-hour photophase on the daily activity rhythm of adults has to be examined.

DISPERSAL EXPERIMENTS

In a quite well-isolated 6-ha apple orchard in a district near Budapest that is surrounded by woody hills, preliminary migration experiments were carried out with 40-krad irradiated males of the Y-strain. The moths were labelled with fluorescent powders of different colours for each release point. The latter were chosen in a line according to the main wind direction 500 and 2000 m from the centre of the orchard in both upwind and downwind directions. One release point was in the centre of the orchard. Sex-traps with five virgin females in each served for recapture. Altogether 2051 males were released. Of the males released in the orchard 7.8% were recaptured, most of them within 40 to 60 m, and only a few specimens up to 150 m. Only 1.05% of the males released on the 500-m points came to the traps, the greatest flight distance being about 650 m. No males from 2000 m were recaptured.

The above preliminary results show that there was no significant migration within the orchard. Further studies are necessary to determine the migration range in orchards of other areas.

SOME ECOLOGICAL DATA RELEVANT TO THE GENETIC CONTROL OF THE CODLING MOTH

The codling moth has two generations a year in Hungary. On the average, emergence of the over-wintering generation adults begins in the first half of May and lasts until the middle of July with a peak at the end of May. The first full-grown larvae are to be found in the second half of June. Diapause rate is less than 20% until the middle of July and reaches 100% during the first days of August [7, 8]. The summer generation adults emerge from the first half of July, their flight curve shows a flattened peak at the end of July and lasts until the end of August. There is no sharp limit between

the two generations. However, the number of adults in nature varies considerably in time because the peak of emergence is quite sharp, particularly that of the over-wintering generation. The time and the shape of the flight peaks depend on the weather conditions and are, therefore, not exactly forecastable.

OUTLOOK ON THE FEASIBILITY OF THE GENETIC CONTROL METHOD AGAINST THE CODLING MOTH

Research on the sterile-male technique against the codling moth is only beginning in Hungary. Nevertheless, several questions have arisen which require thorough considerations to prevent unfounded optimism on the one hand, and paralyzing scepticism on the other.

Since thinning of apples is not carried out in the commercial orchards in our country, mass rearing on small apples would not be economical in practical applications of the method. Therefore, feasibility of the genetic control depends at first on the possibility of developing an inexpensive mass-rearing method based on a synthetic diet. So far only a few laboratory experiments on this have been carried out in Hungary so the costs of industrial-scale rearings cannot be calculated. The expense of mass-rearing plants, and of irradiation and release procedures cannot be estimated either. Therefore, no well-founded calculations on the economy of the method can be made at present.

A serious difficulty which the practical application of the genetic method has to face is represented by the above-mentioned sharp peaks of adult emergence which means sudden changes in population densities in nature. Therefore, the mass rearing should be programmed so that the number of adults to be released at a given time could follow these sudden changes.

The insect pests which are controlled now by insecticide treatments, together with the codling moth, represent a further problem. These pests include the San José scale and several tortricid moths, the most important species being the following [9]: Adoxophyes reticulana Hübner; Pandemis ribeana Hübner; Spilonota ocellana F.; and Simaethis pariana Cl. Despite the fact that the biology of these pests is quite well known, predictions cannot be made with certainty on the number of insecticide treatments per year that will be necessary against them if the codling moth is controlled by the sterile-male technique. Only large-scale field experiments with the latter technique can give a final answer to this question. However, some observations seem to show that the population density of the above-mentioned moth species is not as constantly high as that of the codling moth so some insecticide treatments could probably be spared. Parasites seem also to be more effective against them than against the codling moth, and their activity would probably increase when the number of insecticide treatments could be reduced. On the other hand, the San José scale occurs very often only in small parts of the commercial orchards, so that the treatments against it could be limited to those foci.

Anyway, the problem of coexisting insect pests may be a serious pitfall in introducing the sterile-male method, and it seems to be more serious in the temperate zone of Europe than, for example, under the semiarid conditions of American apple-growing areas. In trying to surmount these

coming difficulties we included into our codling moth project research on the possibilities of controlling the San José scale and the main tortricid species by selective methods.

A C K N O W L E D G E M E N T S

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APPLICATION OF THE STERILE-MALE TECHNIQUE TO THE GYPSY MOTH, Lymantria dispar L.

A field trial

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Abstract

APPLICATION OF THE STERILE-MALE TECHNIQUE TO THE GYPSY MOTH, Lymantria dispar L.:
A FIELD TRIAL.

Gypsy moth males irradiated in the pupal stage with ^{60}Co at a dose of 30 krad were liberated into an isolated area in Jelsa Park on the island of Hvar, Yugoslavia. A release ratio of 0.5 sterile to 1 normal males was obtained. Despite the low release ratio, 22% of the egg-masses that were obtained resulted in a 0-28.7% egg hatch.

INTRODUCTION

During the last quarter of this century several different methods have been applied in Yugoslavia to control the gypsy moth which is the most destructive pest of forests and orchards. The method most applied was chemical control. Nevertheless, it was found that repeated attacks could not be prevented nor could the areas of attack be limited. This resulted in more intensive research on biological control measures, one of which was the application of the sterile-male technique in an isolated area situated in Jelsa Park on the island of Hvar. Results of the first application are reported here.

The work was supported by the International Atomic Energy Agency, Vienna, the Republic Fund for Scientific Research of SR, Serbia and the Institute for Plant Protection, Belgrade.

METHODS

Work was carried out in 1968 and 1969 and continued in 1970. The first sterile-male releases were in 1969.

In Jelsa Park, which has an area of about 1.3 hectares, the gypsy moth has appeared regularly although there have been no apparent attacks in the surrounding areas. So it can be considered as an isolated area. Five test plots were established in an area of 4.85 hectares.

During the gypsy moth season a detailed survey of the test plots was made each 7 - 10 days. Tree trunks were examined to the height of 2 metres and, on each tree, 2-4 of the lowest branches were examined to the length of about 70 cm. The number and development stage of the gypsy moth was recorded.

TABLE I. DENSITY OF GYPSY MOTH EGG-MASSSES IN JELSA PARK AND VRBA PARK

Year	Survey date	Jelsa Park		Vrba Park	
		No. of egg-masses		No. of egg-masses	
		on the test plots	in the whole park	on the test plots	in the whole park
1967	25 April 1968	1	No data	0	No data
1968	28 Feb. 1969	17	73	11	54
1969	26 Nov. 1969	157	542	99	389

All the egg-masses were taken off on careful survey of the whole park in order to decrease the number of wild moths. A further decrease in the gypsy moth population was achieved during the period when the adult caterpillars descended from the tree trunks to rest or to form cocoons.

Vrba Park, Stari Grad, about 10 miles from Jelsa, served as a check plot. The gypsy moth occurrence has also been observed here, but its host plants are different, which is the subject of another paper (Maksimović and Politeo [1]). In this park, which measures about 1 hectare, three test plots were established on the whole area of 3.64 hectares. A survey was carried out in the same way and at the same time as the survey in Jelsa, with the one difference that the abundance of the gypsy-moth egg-masses were recorded but not removed.

An analysis of the number of eggs in the egg-masses, the parasitism and the sterility of the eggs was made in the Biological Control Laboratory.

Male pupae between 9 and 12 days old were irradiated with ^{60}Co at a dose of 30 krad at the Institute for Nuclear Energy, "Boris Kidrič", Belgrade.

Irradiated pupae were transported to the island of Hvar within one day of treatment in four shipments at intervals of 21 days. Close to Jelsa Park pupae were held in the open in cages and protected against the sun. On emergence, the moths were released from the cages twice a day.

RESULTS OF RESEARCH

(a) Density of the gypsy moth population

In both parks the gypsy moth population has fluctuated between 1967 and 1970. From Table I it is evident that there was a low number of gypsy moth egg-masses in Vrba Park in 1967. In the next two years, the number of egg-masses increased. Similar year to year increases in egg-masses

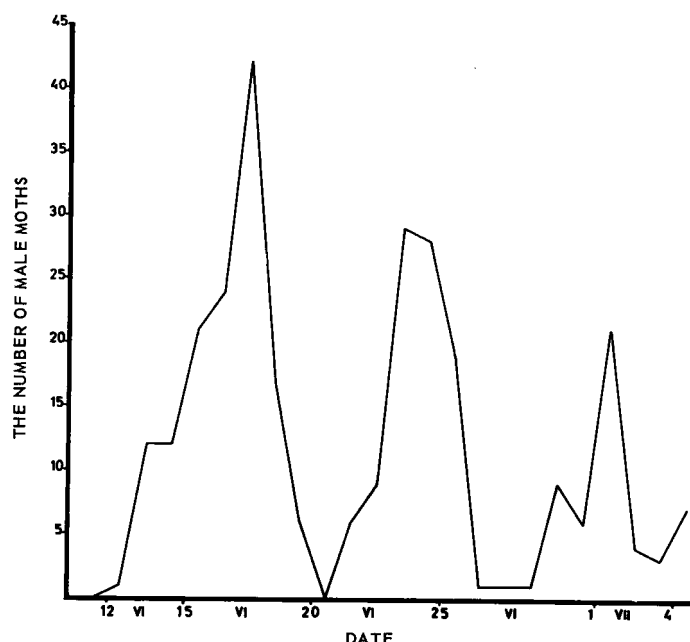


FIG. 1. Emergence rate of irradiated gypsy male moths in Jelsa Park, 1969.

occurred in Jelsa Park. However, since in Jelsa Park 73 egg-masses were removed in 1968, the population was probably increasing more rapidly. The data indicate that the numbers of moths were increasing in both parks.

To bring about a further decrease in the gypsy moth population of the 1969 generation, adult caterpillars, pronymphae and pupae were collected in Jelsa Park in May and June. A total of 1024 were destroyed.

(b) Laboratory rearing of the gypsy moth for irradiation

The gypsy moths were reared for irradiation in a greenhouse. The rearing was synchronized with the moth development in Jelsa Park. When caterpillars reached the 3rd instar they were put in entomological cages and further reared in an insectarium to the end of their development. 3620 caterpillars were put in cages and 970 male pupae were produced from them. The occurrence of polyhedrosis among the caterpillars resulted in a high mortality.

The first pupae in the cages were found on May 28. In Jelsa Park 6 pupae were found on June 1, indicating full synchronization of the rearing with the development in natural conditions of the Jelsa Park.

As soon as the cocoons were spun, male pupae were isolated according to age. The 8-13 day old pupae were exposed to ^{60}Co irradiation at a dose of 30 krad. The pupae were irradiated in four groups: 312 pupae on June 12, 234 pupae on June 21, 354 pupae on June 28 and 70 pupae on July 3. On completed irradiation the pupae were immediately transported by air to Jelsa. A total of 966 pupae were dispatched.

(c) Sterile-male release

Male pupae were reared in a cage on their arrival in Jelsa and kept on the terrace of a house, about 80 m from the poplar site, till the emergence of moths. As soon as males started to emerge from pupae, they were released twice a day. Figure 1 shows the emergence rate of male moths from irradiated pupae. The first moth emerged on about June 12 and observations showed that emergence at Jelsa Park was similar. Therefore we obtained full synchronization in emergence of treated and wild moths.

The total number of male moths that emerged was 289 of 966 pupae. The low percentage of emergence (29.9%) was due to the following deleterious effects:

Pupae with dead moth	8.8 %
Pupae destroyed by polyhedrosis	46.4 %
Exhuvia of pupae from which moths emerged	33.8 %
Emerged moths with wings not developed	8.9 %
Moths with developed wings, but small	2.1 %

The data presented show that polyhedrosis was the main cause of death.

(d) Laboratory tests with sterile males

The effect of the 30-krad dose on the fecundity is presented in Table II. The data show that when irradiated males were crossed with untreated females, no deposited eggs hatched even though 20.4% of them were embryonated. When normal males were crossed with normal females, 95.8% and 59.9% of the eggs were embryonated and hatched, respectively.

(e) Effect of sterile males in Jelsa Park

Egg-masses were collected in Jelsa Park in November 1969 and March 1970 to determine the degree of control obtained due to release of sterile males. Table III shows that of 100 egg-masses sampled, eggs in 2 did not hatch and that in 9 only 8.5% hatched. In addition, with 11 egg-masses, 28.7% of the egg-masses hatched. The above represents a total of 22% of the egg-masses in which the percentage of egg hatch was 28.7% or less.

(f) Determination of ratio of sterile to wild males

To determine the ratio of released sterile males to those of the natural population in the park, the whole park was examined for egg-masses. A total of 542 were found. By assuming that only 95% of the masses were found, we calculated that there were 570 egg-masses in the park. From these figures we estimated that with a release of 289 irradiated males, the ratio of sterile to wild males was 0.5 : 1 (S : N).

TABLE II. EFFECT OF FERTILITY OF UNTREATED (N) FEMALES WHEN CROSSED WITH MALES STERILIZED WITH 30 krad OF GAMMA RADIATION (S)

Mating	No. of egg-masses	Eggs		
		average No. per egg-mass	per cent embryonated	per cent hatched
N♂ × N♀	17	379.5	95.8	59.9
S♂ × N♀	8	406.8	20.4	0.0

TABLE III. PER CENT HATCH OF GYPSY MOTH EGGS COLLECTED FROM JELSA PARK WITH RELEASE OF STERILE MALES IN 1969

No. of egg-masses sampled	Percentage hatched eggs
2	0.0
9	8.5
11	28.7
5	46.0
73	71.2

DISCUSSION

The first trial with the sterile-male technique in Yugoslavia was partially successful in Jelsa Park on the island of Hvar in 1969. Only 289 irradiated males were available for release because of the high mortality due to the polyhedrosis virus. Consequently, we estimated that the ratio of sterile to normal males was 0.5 : 1. Despite this low ratio, some effects of the sterile release were recorded. Of the egg-masses sampled in the release area, 22% of them resulted in an egg hatch of 0 to 28.7%. This low egg hatch was probably due to the sterile males.

The influence of low ratios of sterile males to untreated (normal) ones has been shown by different authors. In laboratory experiments on the pine processionary moth, *Thaumetopoea processionea*, at a sterile (40 krad) to normal ratio of 2 : 1, Baccetti and Zocchi [2] reported a strong reduction in fecundity. With Lepidoptera it is evident that one can demonstrate competitiveness of sterile males with normal ones for females with low ratios.

In laboratory experiments, when males irradiated with 30 krad were crossed with untreated females, embryogenesis was detected in 20.4% of the deposited eggs (Table II) but they failed to hatch. However,

conclusions should be made with care since the observation was based on only 8 egg-masses.

The hatch of gypsy moth eggs from females mated with males from irradiated (20 krad) pupae older than 7 days was investigated by Godwin et al. [3]. They observed embryogenesis in 92.8% of the eggs. Similar results were obtained with the unirradiated check. They also found that 2.4% of the eggs hatched but the larvae failed to complete development. Vasiljević [4] found that 14.8% and 14.4% of the eggs hatched from untreated females mated to males from pupae irradiated with 20 and 30 krad, respectively. Proverbs [5] found that the reproductive potential, based on the numbers of mature larval progeny, was reduced 85% at the ratio of 10 : 1 and 98% at the ratio of 20 : 1 for the codling moth, Carpocapsa pomonella.

The results of these investigators are encouraging and we are optimistic that we will be able to obtain economic control when we can overwhelm the natural population with sterile males.

CONCLUSION

Despite a male ratio of only 0.5 : 1 (S : N), a measurable effect was obtained by release of sterile males from pupae irradiated with 30 krad into a natural population in Jelsa Park, Hvar Island, Yugoslavia.

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REARING OF THE COTTON WORM, Spodoptera littoralis (Boisduval), ON A CALCIUM ALGINATE MEDIUM

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Abstract

REARING OF THE COTTON WORM, Spodoptera littoralis (Boisduval), ON A CALCIUM ALGINATE MEDIUM.

The rearing of Spodoptera littoralis (Boisduval) on an artificial medium based on a calcium alginate gel instead of agar is described. With this medium three serial generations were bred individually and 10 collectively. Various biological parameters are given.

INTRODUCTION

Reasonable cost of insect production is considered the most important basic requirement for the practical development of control by the sterile-insect release technique [1]. One of the most costly items in the artificial rearing of many insects is agar which, according to Knipling [1], may represent on its own 75% of the cost of the nutritional medium.

Recently, Spodoptera littoralis (Boisduval) – the most injurious pest of cotton in Israel and one of the insects on which a previous Panel [2] recommended further research – was successfully reared on an artificial medium containing sodium alginate instead of agar [3]. S. littoralis is affected by a nuclear polyhedrosis and during the latter part of its larval development it requires increasing amounts of ascorbic acid and is cannibalistic. These biological characteristics pose special problems in rearing this insect for integrated control purposes.

The present paper describes further developments in the technique of rearing and the nutrition of the cotton worm on a calcium alginate medium. The methods are presented in some detail since they could be adapted to the development of the mass rearing of other Lepidoptera with similar characteristics prior to their control by the sterile-male technique.

MATERIALS AND METHODS

The composition of the artificial medium used is given in Table I.

The implements used are a laboratory stirrer and a 15-litre capacity restaurant mixer equipped with a manual rotary scraper¹.

The steps in preparing the medium are as follows. The casein is dispersed by stirring with a solution of triethanolamine in 4800 ml water until the pH shifts to about 7. To this, Calgon dissolved in 200 ml water is added, followed by the sodium alginate. To complete dissolution, these

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TABLE I. COMPOSITION OF ARTIFICIAL MEDIUM

1. Triethanolamine	28.0 g
2. Casein (vitamin-free)	200.0 g
3. Full-fat soy meal	450.0 g
4. Torula yeast	350.0 g
5. Methyl phydroxy benzoate (Nipagin)	13.0 g
6. Calcium carbonate	6.0 g
7. Sodium hexametaphosphate mixture (Calgon)	7.0 g
8. Sodium alginate ^a	110.0 g
9. Ascorbic acid	33.5 g
10. Glacial acetic acid	67.0 ml
11. Distilled water	5500.0 ml

^a "Protanal S.F." - Protan a/s Drammen - Norway.

ingredients are re-transferred to the bowl of the mixer and the soy, nipagin and calcium carbonate are mixed in (this salt is added last to delay gelling as much as possible). The ascorbic and acetic acids in 500 ml water are then very rapidly mixed in (about 15 sec) using the motor as well as the scraper. The mixture is allowed to set for 15 min. With the mixer used, a 6 - 7 kg load seems optimum.

The medium is stored at 10°C on a grid to allow draining of any syneretic fluid.

Breeding methods

The larvae are kept in plastic boxes (25 × 33 × 7.5 cm) with flat rims. These boxes are stored at 22 - 27°C and 60% R.H. Each breeding unit consists of two such boxes, the bottom one containing the larvae, the upper one serving as a lid. The top of the lid is cut out and replaced by cloth glued around the perimeter. The bottom box contains wood sawdust on which rests a cardboard partitioning unit followed by a plastic grid and again a partitioning unit dividing the box into 40 rearing cells. The rim of the bottom box is lined with a strip of plastic foam to prevent larvae from escaping. Each rearing cell contains about 5 larvae together with a 1 - 2 g block of medium (Fig. 1).

To check the adequacy of the medium without interference from cannibalism, larvae were also bred individually for three successive generations. This was done in small plastic boxes lined with absorbant paper.

Food is renewed every 3 days; except for larvae kept individually, a population steadily supplying 100 pupae per day requires about 8 kg medium per week.

Pupae are kept in paper bags (46 × 26 cm) at the rate of 10 couples per bag; the upper end of the bag closes around a drinking device for the

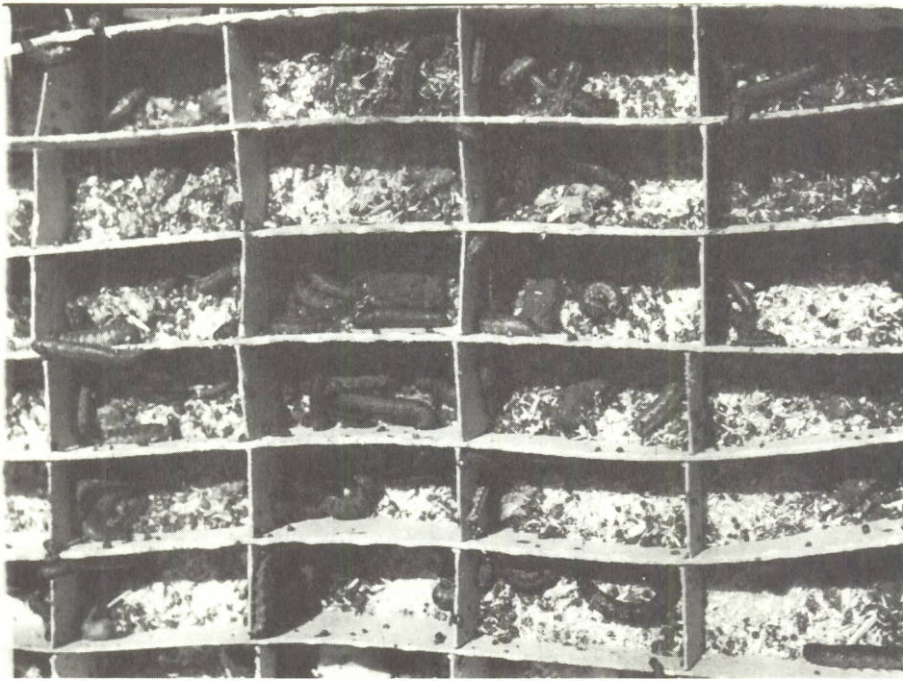


FIG.1. Larval rearing units for *Spodoptera littoralis*.

adults. This device is an inverted vial closed with cloth and containing an aqueous solution of 10% sucrose, 0.5% ascorbic acid and 0.1% yeast extract. Every 2 days, the egg masses are cut out and the moths transferred to new bags.

Sanitation measures

When a virus epizootic is suspected, fresh egg masses are dipped for 1 min in an aqueous sodium hypochlorite solution containing 1.5% active chlorine and a few drops of detergent ("7 X") followed immediately by rinsing in running water. Incubation then takes place in a water-saturated atmosphere.

The sawdust and the cardboard partitions are discarded after each generation completes its development. The rearing boxes are washed in a solution of trisodium phosphate, soap and water (12:6:100 parts respectively).

Larvae observed to be affected by virosis or any other pathogen(s) are discarded immediately.

RESULTS AND DISCUSSION

Table II sums up the biological parameters recorded when larvae were bred individually.

TABLE II. BIOLOGICAL PARAMETERS FOR SECOND AND THIRD GENERATION INDIVIDUALLY BRED

Generation	No. of larvae	Mean larval development period (days)	Mean pupal weight (g)		Mean adult weight (g)		Sex ratio (male/female)	Mean fecundity	Adult return (%)
			Female	Male	Female	Male			
II	30	29.7	398	353	204	179	1.0/0.5	1564.1	60.0
III	32	31.3	417	358	261	213	1.0/1.3	1294.6	67.5

Statistical tests did not reveal any significant difference between corresponding parameters in Table II. This shows there is no deterioration from the second to the third serial generation and that the diet is nutritionally adequate. On the other hand, the sex ratio appears to be given to wider fluctuations than the other parameters.

On another medium also based on calcium alginate [3], the mean pupal weight was 283 mg, i. e. considerably less than the values shown in Table II. This difference in favour of the present medium probably is due to both a nutritional and a physical factor. The two media are similar except that the present one contains twice more casein and four times more triethanolamine. The effect of the increase in casein seems obvious while the increase in triethanolamine results in a better solution of the casein, thereby making it more available. This is particularly important in the case of neonate larvae which in nature scrape the host-leaf surface and drink the oozing liquid.

The high adult return recorded in Table II, can be ascribed not only to adequate nutrition, but also to individual breeding which prevented cannibalism and contamination by pathogens (nuclear polyhedrosis mainly; see also below).

At the time of writing, 10 successive generations were obtained in the collective rearings. The adult return was about 50% which is enough for a colony to expand rapidly.

The partitions used in the rearing boxes and on which the larvae climb, spread out the population on a larger surface somewhat like the host plant in nature. This fulfils an important hygienic requirement: without these partitions and under conditions of high density rearing, virus epizootics are very frequent and drastically reduce the population. In addition, the aerating effect of the partitions allows the maintenance of a relatively low humidity in the boxes – also an important factor in curtailing disease. In particular, these measures counteract regurgitation, a habit frequently observed among older larvae and facilitating cross contamination.

The use of acetic acid in the present medium successfully prevents spoilage by micro-organisms and allows renewing the larval food every 3 days and even at longer intervals. The limiting factor here is the lability of ascorbic acid which, at the level used, requires the supply of fresh food every 3 days. Without this precaution, morphological damage to the pupae resulting from insufficient vitamin is observed [4].

The system for obtaining a calcium alginate gel in the present medium differs from that previously described [3]: acetic acid is used instead of gluconodeltalactone, calcium carbonate replaces dibasic calcium phosphate and Calgon has been added. This system has been adopted because it is considerably cheaper than the previous one, but here the preparation of the gel requires some dexterity. This is so because instead of the slow release of acidity obtainable with gluconodeltalactone, acetic acid acts immediately; therefore Calgon, a retarder of calcium ions, was included. Also, calcium carbonate replaced dibasic calcium phosphate because it is much cheaper and even less soluble in water. With this system, although a slow setting starts almost immediately after adding the acids, there is still time enough for a thorough mixing of the ingredients without breaking the gel. Finally, replacing agar by sodium alginate reduces the cost of the nutritional medium by a factor of five.

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RADIATION STUDIES OF SPERM TRANSFER IN RELATION TO COMPETITIVENESS AND OVIPOSITION IN THE CABBAGE LOOPER AND CORN EARWORM*

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Abstract

RADIATION STUDIES OF SPERM TRANSFER IN RELATION TO COMPETITIVENESS AND OVIPOSITION IN THE CABBAGE LOOPER AND CORN EARWORM.

Male cabbage loopers, *Trichoplusia ni* (Hübner), sterilized with 30 krad of gamma radiation failed to transfer sperm to the spermathecae. Thus, sterile matings did not elicit a normal oviposition response. Irradiated (25 krad) adult corn earworms, *Heliothis zea* (Boddie), showed no adverse effects on ability to mate or properly transfer sperm. Male cabbage looper pupae sterilized 5 days before emergence with fast neutrons subsequently mated and transferred sperm more successfully than pupae of the same age sterilized with gamma radiation. Evidence is presented which indicates that the male accessory secretion of the cabbage looper is responsible for eliciting normal ovipositional response rather than either the quantity or type of sperm transferred. The importance of sperm transfer, both in terms of type and quantity, in producing a competitive male in polygamous lepidopterous species is presented.

1. INTRODUCTION

The largest single drawback in the use of radiation-sterilized male moths to control natural populations of lepidopterous insects has been the inability of the irradiated males to compete successfully with natural males. North and Holt (1968) pointed out that the reason for the lack of competitiveness of radiosterilized cabbage loopers, *Trichoplusia ni* (Hübner), was the inability of irradiated males to transfer sperm successfully. Irradiated male Lepidoptera mated to unirradiated females often fail to elicit a normal ovipositional response. This has been reported by many workers (Godwin et al. 1964, Ouye et al. 1964, Raun et al. 1967, North and Holt 1968, and Flint and Kressin 1969). However, the exact relationships involving sperm transfer to the female and the elicitation of the oviposition response are not presently clear. The inability of the irradiated male cabbage looper to transfer sperm that will reach the spermatheca is dependent on the dose of radiation received (North and Holt 1968).

The routine experimental procedure to determine the mating ability of treated moths has involved dissecting the female bursa copulatrix and counting the spermatophores. Taylor (1967) pointed out the fallacy of assuming a direct correlation between the presence of a spermatophore and

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the transfer of sperm; he showed that male Atteva punctella (Cramer) may transfer spermatophores without transferring sperm. This was also pointed out for the cabbage looper by North and Holt (1968) and for the tobacco budworm, Heliothis virescens (F.) by Flint and Kressin (1969); and George and Howard (1968) indicated that after continued mating, males of the oriental fruit moth, Grapholitha molesta (Busck), are capable of transferring sperm successfully without transferring a spermatophore. Therefore, it is evident that assaying for a spermatophore is not a sound technique to determine sperm transfer. The procedure that has evolved is the dissection of the bursa copulatrix (to determine the presence of a spermatophore) and the dissection of the spermatheca (to determine the presence of sperm). This is usually done after the completion of oviposition by the female and is often misleading in terms of the type of sperm present. The successful mating of the cabbage looper (and presumably other Lepidoptera) requires the incorporation of both eupyrene and apyrene sperm in the spermatophore bulb, the positioning of the stalk at the opening of the seminal duct, and the movement of the sperm from the spermatophore bulb to the spermatheca via the seminal duct and common oviduct (Holt and North 1970). An unsuccessful mating occurs when any one of the criteria is not met.

Taylor (1967) noted that the occasional failure of Atteva punctella (Cramer) to transfer sperm in mating is rather common, even when the male is not irradiated. North and Holt (1968) observed this to be true with unirradiated pair matings of the cabbage looper. Therefore, the condition that results in unsuccessful sperm transmission by irradiated male moths also exists, but to a lesser degree, in normal untreated insects.

The reproductive systems of Lepidoptera are similar, but variation does occur in size, shape, and placement of the female accessory organs. In almost every species there is a differently shaped bursa copulatrix, and there is a distinctive variation in the size and shape of the spermatophores.

Callahan and Chapin (1960), in their study of the comparative morphology of three noctuids, noted that there is some evidence that certain specimens are more likely to successfully complete the mechanics of copulation than others. The number of unsuccessful matings appeared to be directly correlated with the complexity of spermatophore inversion. Callahan reported 2.8% aberrations in matings of untreated corn earworms, Heliothis zea (Boddie), 0% in the variegated cutworm, Peridroma saucia (Hübner), and 15.5% in the armyworm, Pseudaletia unipuncta (Haworth). A number of aberrations in matings for this last species were attributed to the complexity of the spermatophore insertion as opposed to 0.0% in the variegated cutworm. North and Holt (1968) reported 22% aberrations for singly mated untreated male and female cabbage loopers; Flint and Kressin (1969) reported 15% aberrations of sperm transmission in the untreated tobacco budworms. Studies of the reproductive tracts of the cabbage looper, tobacco budworm, and the corn earworm and a comparison of the diagrammatic illustrations of the three species mentioned in the literature cited above reveal considerable variation in the endophallus and cuticular lower simplex between males; they also reveal considerable difference in bursa copulatrix among females. These variations in males of various lepidopterous species offer some explanation of the variation in sperm transfer under normal conditions and after irradiation.

As shown by Holt and North (1970), the irradiated male cabbage looper does not really fail to transfer sperm but, because of a disruption in the

timing of the mating process, the sperm of the irradiated male are ejaculated directly into the bursa copulatrix rather than incorporated into the bulb of the spermatophore. They pointed out that though only a small percentage of the males actually have a normal complement of sperm incorporated in the spermatophore bulb, when the total sperm count of the ejaculate is considered (whether it fills the spermatophore or is placed into the bursa copulatrix) the number of sperm ejaculated for both control and irradiated males is the same. This means that when irradiated males transfer sperm, the placement of the sperm becomes the all important factor.

Holt and North (1970) also pointed out that the eupyrene sperm of the cabbage looper, which are nucleate and capable of fertilization in contrast with the anucleate apyrene sperm (Riemann 1970), do not become motile until they have reached the spermathecae of the female. Therefore, any eupyrene sperm placed directly into the bursa copulatrix cannot find their way up the seminal duct. On the other hand, since apyrene sperm possess motility when incorporated into the ejaculate, they can find their way to the spermatheca by random chance. This explains why irradiated males often appear to transfer only apyrene sperm.

If radiosterilized males (at least in some species of Lepidoptera) are to be used in a release program for population control, a way must be found in a large percentage of cases for irradiated males to successfully transfer a normal complement of eupyrene and apyrene sperm to the female. This becomes important in polygamous species because irradiated males in mating with previously mated females must be capable of displacing the original sperm in the spermatheca. If this does not occur, the irradiated male mating would be totally ineffective. Competition in Lepidoptera may well become a problem of sperm competition and, possibly even more important, a problem involving the quantity of sperm as it relates to matings by unirradiated and irradiated males.

This paper deals with the two basic aspects of sperm competition: (1) whether a particular stage and type of radiation is more advantageous in obtaining better sperm transfer or sperm utilization; and (2) the effect that sperm transfer per se or related accessory fluids have on the ovi-positional response elicited in the female.

2. MATERIALS AND METHODS

The insects used in these experiments were reared and handled under the same conditions as described by Holt and North (1970). Any variations are mentioned as they relate to a particular experiment. Both the cabbage looper and the corn earworm were reared on a larval diet formulated by Ignoffo (1963). The adults of both species were fed a 10% sucrose solution.

The normal eupyrene to apyrene ratio is considered to be 2 eupyrene: 1 apyrene. Any ratio below 1:1 was considered abnormal in sperm found in the spermathecae of the females.

All dissections were made in either Belar's saline solution (6 g of NaCl, 0.02 g KCl, 0.29 g CaCl₂, 0.29 g NaCO₃ in water to make 1 litre) or in a saline solution described by Twarog and Roeder (1957). No estimates of sperm motility were made because sperm in any of these saline solutions failed to live beyond a 20-30 min period.

TABLE I. RELATIONSHIP OF DOSE OF GAMMA RADIATION TO THE ABILITY OF 3-DAY-OLD ADULT CABBAGE LOOPERS TO TRANSFER SPERM (males allowed to mate for 3 nights)

Dose of gamma radiation given to $\sigma\sigma$ (krad)	No. of individuals tested	% $\sigma\sigma$ mated	% of tested $\sigma\sigma$ transferring sperm	% of $\sigma\sigma$ tested that transfer normal ratio of eupyrene:apyrene sperm
0	20	100.0	90.0	70.0
5	19	100.0	94.7	70.3
15	20	90.0	70.0	43.5
30 ^a	19	94.8	63.2	10.6

^a This is considered to be the sterilizing dose.

3. SPERM TRANSFER BY ADULTS

The sperm transferred in the cabbage looper and the corn earworm during mating are those stored in the duplex regions of the male reproductive tract; cabbage looper males are not ready for mating until they are 2-3 days old, whereas corn earworm males are ready when 1 day old. Only 3-day-old males of both species of insects were used in these tests. The results of single matings of both unirradiated and irradiated male cabbage loopers are given in Table I. These data show the problems relating to sperm transfer of irradiated males. In the controls, 90% of the males tested transferred sperm at the first mating but of these only 70% transferred a normal complement of eupyrene and apyrene sperm. The percentage of males transferring sperm decreased as the dose of radiation increased, but, even more important, the percentage decreased that transferred a normal ratio of eupyrene to apyrene sperm. At the sterilizing dose (30 krad), 10.6% of the tested males transferred a complement of sperm having a normal ratio of eupyrene to apyrene. The relation of this to competitiveness at the sperm level is apparent. The difference between those males receiving 15 and 30 krad is the number of individuals that transferred a normal ratio of eupyrene to apyrene sperm. The reasons for this were discussed by Holt and North (1970). When sperm are deposited in the bursa copulatrix by an irradiated male, the eupyrene are immobile and cannot get to the spermatheca, but the apyrene sperm apparently can. Examination shows the females have sperm, but when the males received high doses of radiation, very few females received sperm capable of fertilization.

The type of sperm transferred is important. Apyrene sperm are anucleate and therefore incapable of fertilization. They are smaller but more motile than eupyrene sperm (Iriki 1941). Eupyrene sperm are nucleate and undergo (at least in the cabbage looper and probably in other Lepidoptera) gross morphological changes during spermiogenesis from the time they leave the testes until they reach the spermatheca (Riemann 1970).

TABLE II. EFFECT OF GAMMA RADIATION ON THE ABILITY OF 3-DAY-OLD ADULT MALE MOTHS OF *Trichoplusia ni* AND *Heliothis zea* TO SUCCESSFULLY TRANSFER SPERM ($\sigma\sigma$ allowed to mate 3 nights)

Dose of gamma radiation to 3-day-old $\sigma\sigma$ (krad)	% males mated		% mated males transferring sperm		% of males transferring normal ratios of eupyrene:apyrene sperm	
	<u>T. ni</u>	<u>H. zea</u>	<u>T. ni</u>	<u>H. zea</u>	<u>T. ni</u>	<u>H. zea</u>
0	70.7	80.0	88.0	75.0	78.0	100.0
5	66.1	85.7	87.4	80.0	63.5	100.0
10	-	71.4	-	100.0	-	100.0
15	68.5	85.7	80.6	100.0	42.7	100.0
20	-	80.0	-	75.0	-	100.0
25	-	85.0	-	100.0	-	100.0
30	66.9	-	64.7	-	13.7	-

The irradiated corn earworm is an example of a Lepidopteran with no apparent problem in sperm transfer. A comparison was made (Table II) of the ability of unirradiated and irradiated 3-day-old male cabbage loopers and corn earworms to transfer successfully sperm when allowed to mate for 3 nights. The greatest difference was in the percentage of males able to transfer a normal ratio of eupyrene:apyrene sperm. The cabbage looper male, even though allowed to mate for 3 nights, failed to transfer sperm successfully as the radiation dose increased; 100% of the corn earworm males that mated transferred a normal ratio of eupyrene:apyrene sperm (even at 25 krad). Apparently the irradiated corn earworm should be competitive, both from a standpoint of mating and ability to transfer sperm, when placed with unirradiated males. We therefore conclude that the release of sterile corn earworm males is more promising than the release of sterile cabbage looper males for population suppression.

The success of using delayed sterility in population suppression depends on a large percentage of the progeny coming from irradiated males (North and Holt 1969). It appears that the corn earworm is an ideal candidate for this type of release program. The little effect irradiation has on the mating ability and sperm transfer in the corn earworm suggests that fully sterile moths are capable of population suppression. A program where both partially sterile and fully sterile moths are released at different times could increase the efficiency of population suppression.

To test the effect the number of matings had on sperm transfer and irradiated male cabbage loopers, experiments were conducted where 3-day-old males were mated consecutively for 8 nights (Table III). A large percentage of the control males failed to transfer a normal ratio of eupyrene:apyrene sperm the first night; but after the first night they maintained a

TABLE III. RELATIONSHIP OF 8 CONSECUTIVE MATINGS AND THE ABILITY TO TRANSFER SPERM BY UNIRRADIATED AND IRRADIATED ADULT MALE CABBAGE LOOPERS
(♂♂ were 3 days old at beginning of test)

Number of consecutive matings	Number of individuals	% mating	% ♂♂ mated transferring sperm	% ♂♂ that transferred normal ratio of eupyrene:apyrene sperm
<u>Unirradiated males</u>				
1	56	85.7	64.6	32.3
2	58	77.6	97.8	84.1
3	53	69.8	94.6	71.4
4	38	73.7	100.0	75.0
5	43	72.1	71.0	77.3
6	43	48.8	100.0	80.9
7	39	71.8	96.4	77.8
8	35	57.1	95.0	100.0
<u>Males that received 5 krad</u>				
1	58	79.3	67.4	71.0
2	57	64.9	91.9	76.5
3	56	71.4	97.5	71.8
4	38	78.9	100.0	73.3
5	56	64.3	77.8	35.7
6	51	41.2	90.5	-
7	44	68.2	93.3	50.0
8	35	60.0	90.5	89.5
<u>Males that received 15 krad</u>				
1	60	78.3	61.7	17.2
2	60	58.3	77.1	44.4
3	58	70.7	95.1	43.6
4	36	72.2	100.0	-
5	54	59.3	71.9	60.9
6	52	36.5	100.0	63.2
7	40	42.5	70.6	25.0
8	28	35.7	80.0	50.0
<u>Males that received 30 krad^a</u>				
1	60	81.7	34.7	35.3
2	58	79.3	69.6	25.0
3	56	69.6	89.7	8.6
4	38	63.2	83.3	5.0
5	44	70.5	74.2	13.0
6	41	61.0	56.0	0.0
7	40	50.0	55.0	9.1
8	35	42.9	60.0	0.0

^a 30 krad is the sterilizing dose to adult males.

TABLE IV. RELATIONSHIP OF GAMMA RADIATION TO THE ABILITY OF 3-DAY-OLD ADULTS TO MATE AND TRANSFER SPERM OVER A 3-DAY PERIOD WHEN IRRADIATED AS PUPAE 5 DAYS BEFORE EMERGENCE

(♂ allowed to mate for 3 nights)

Dose (krad)	% mated	% ♂ tested transferring sperm	% ♂ tested transferring normal ratio of eupyrene:apyrene sperm
0	75.0	75.0	58.3
5	69.2	69.2	61.5
10	42.9	7.1	7.1
15	22.2	22.2	11.1
20 ^a	8.3	0.0	0.0
25	14.3	0.0	0.0

^a This is considered a sterilizing dose.

normal percentage. Males that received 5 krad of radiation transferred sperm as well as the controls. However, males that received 15 krad showed a definite reduction in the percentage of individuals that transferred a normal ratio of eupyrene: apyrene sperm. These males reached their peak between the 4th and 6th matings. This is probably the reason that in competitive tests reported by North and Holt (1969) males given 15 krad were competitive. Males receiving a sterilizing dose of 30 krad showed a greater ability to transfer a normal ratio of eupyrene: apyrene sperm in the first two matings than they did thereafter. It is generally true in other tests with the cabbage looper that a male that fails to transfer a normal complement of sperm in the first several matings rarely recovers this ability. This means that in multiple matings a sterile male would not be able to compete effectively with unirradiated males. It is expected that during the first night of mating a sterile male would compete with unirradiated males simply because the latter often fail to transfer sperm the first mating.

4. ABILITY OF THE CABBAGE LOOPER MALES IRRADIATED AS PUPAE TO TRANSFER SPERM SUCCESSFULLY

There are many economic advantages in irradiating pupae rather than adults, but pupal irradiation has not proved successful in producing competitive males (Proverbs and Newton 1962, Flint and Kressin 1967, Cheng 1969). Male pupae were irradiated with various doses of gamma radiation 5 days before emergence. The ability of the resulting adult male moth to mate and transfer sperm is shown in Table IV. These data show (1) that the percentage of males that mated decreased rapidly as the radiation dose increased, and (2) at the sterilizing dose (20 krad) only 8.3% of the males tested mated and none of these mated males transferred sperm. This

TABLE V. RELATIONSHIP OF DOSE OF 0.43-MeV FAST NEUTRONS ON THE RESULTING 3-DAY-OLD ADULT MALE CABBAGE LOOPERS WHEN IRRADIATED AS PUPAE 5 DAYS BEFORE EMERGENCE ($\sigma\sigma$ allowed to mate for 3 nights)

Dose of gamma radiation (krad)	No. of individuals tested	% $\sigma\sigma$ mated	% $\sigma\sigma$ tested transferring sperm	% $\sigma\sigma$ tested transferring normal ratio of eupyrene:apyrene sperm
0	22	86.4	81.8	81.8
1	18	94.0	88.9	77.8
3	21	95.0	95.2	95.2
5	20	75.0	55.0	50.0
10	25	56.0	52.0	52.0
15 ^a	25	60.0	44.0	36.0
20	28	28.6	17.9	10.7
25	13	7.7	0.0	0.0
30	23	21.7	6.7	0.0

^a The average per cent fertility of these males is 7.0% and can be considered the sterilizing dose.

clearly shows that the percentage of sterility resulting from radiation of pupae at this stage with gamma radiation is the same as that of aspermia. Even at relatively low doses (10 krad), only 7.1% of the males transferred a normal ratio of eupyrene: apyrene sperm. Irradiation of cabbage looper pupae therefore does not produce very competitive sterile insects.

Studies were done to determine whether the type of radiation used on pupae had any bearing on the production of competitive males. Pupae were irradiated 5 days before emergence with various doses of 0.43-MeV fast neutrons, and the ability of the resulting adult males to transfer sperm was studied (Table V).

The percentage of the males that mated decreased with the increase in dose as was found when gamma radiation was used. But when compared with the pupae irradiated with gamma radiation, the decrease was not as severe. Also, the percentage of males transferring a normal complement of sperm was high. Thirty-six per cent of the males irradiated with 15 krad as pupae transferred a normal ratio of eupyrene: apyrene sperm as compared with none of the males irradiated with a sterilizing dose of gamma radiation. When pupae received a sterilizing dose of fast neutrons, only 60% of the resultant males mated but there was still a sufficient number that transferred a normal complement of sperm; this was not true when adult males were given a sterilizing dose of gamma irradiation (Table I). The advantages of using fast neutrons in insect sterilization have not yet been fully explored. Since these data appear encouraging the authors plan more research on the use of neutron irradiation of Lepidoptera.

TABLE VI. EFFECT OF TYPE AND QUANTITY OF SPERM TRANSFERRED ON OVIPOSITION RESPONSE OF FEMALE CABBAGE LOOPERS

Type of sperm transferred	No. of individuals	Average eggs per oviposition
Normal eupyrene:apyrene	118	164.7
Largely apyrene	21	154.5
No sperm	50	57.6
Virgin females	28	31.4

5. TYPE AND QUANTITY OF SPERM TRANSFERRED AS RELATED TO THE OVIPOSITIONAL RESPONSE IN THE CABBAGE LOOPER

Irradiated males of many species of Lepidoptera often fail to elicit a normal ovipositional response from unirradiated females. The reason may be related to sperm transfer per se, or to the accessory secretion of the male. If a male fails to transfer sperm, he may therefore fail to transfer the needed accessory fluid to elicit a normal ovipositional response. Leopold (1970) in his studies on the house fly, *Musca domestica* L., showed that a basic protein in the accessory secretion of the male is probably responsible for eliciting the ovipositional response in the female. Holt and North (1970) showed that though irradiated male cabbage loopers transferred a spermatophore, they often fail to transfer sperm and accessory secretions in which the sperm are embodied. Perhaps this secretion is responsible for eliciting the ovipositional response in the female cabbage looper. Tests were conducted to determine whether sperm per se or a particular type of sperm were responsible for eliciting the ovipositional response. Unirradiated 3-day-old males and females of the same age were mated. The females were allowed to oviposit for 2 nights, the spermathecae were dissected, and the amount and type of sperm were recorded. The individuals were then divided into three classes: those that received a normal complement of sperm; those that received largely apyrene sperm; and those that mated but received no sperm. These results were then compared with the ovipositional response of virgin females — the average number of eggs per oviposition (Table VI).

Females which received a normal sperm complement or largely apyrene sperm oviposited at the same rate. Mated females which received no sperm had a higher oviposition response than virgin females. Caution must be taken in interpreting these data, because females classified as having received largely apyrene sperm may have used a great percentage of their complement of eupyrene sperm and may actually be no different than those that were recorded as having a normal ratio of eupyrene: apyrene sperm.

Also a female receiving a normal amount of sperm, even if largely apyrene, would more than likely receive an adequate amount of accessory secretion in the spermatophore. These data are therefore not conclusive as to whether sperm per se or accessory secretion elicits the ovipositional

response. A search is underway to determine the effects on oviposition of various male accessory secretions. Four distinct accessory secretions can be found in the male cabbage looper reproductive tract. Through development of a method of artificial insemination we hope that these secretions can be bioassayed for their ability to elicit a normal ovipositional response. Although it will be difficult to find, a chemical that would induce normal oviposition in a virgin female could be a future control mechanism.

The data presented in Table VI indicate that females that received no sperm had a higher oviposition rate than virgin females. Therefore, it seems plausible that the probability of the oviposition response is caused by accessory secretion of the male incorporated into the spermatophore, rather than by the sperm per se. As shown by Holt and North (1970), the spermatophore bulb is filled early in the copulatory act with a clear fluid, and even females that receive no sperm from the spermatophore may receive small amounts of accessory fluid that could elicit normal oviposition. This is also borne out by examining the data of individual matings in this class of individuals. Although the average ovipositions are different, mated females that received no sperm either oviposited large numbers of eggs or oviposited no more than virgin females. This could be validated by determining if the spermatophore bulb was sufficiently filled with an accessory secretion at the time of mating.

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INHERITED STERILITY AND ITS USE IN POPULATION SUPPRESSION OF LEPIDOPTERA*

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Abstract

INHERITED STERILITY AND ITS USE IN POPULATION SUPPRESSION OF LEPIDOPTERA.

Although the progeny of irradiated male Lepidoptera are more sterile than their male parents, this result does not occur when females are irradiated. The progeny of irradiated female cabbage loopers are only partially sterile if the female parent received at least 20 krad of gamma radiation. Over 90% control can be obtained by releasing only males that received 15 krad of gamma radiation. This is presently the most effective means of population suppression for the cabbage looper when the sterility principle is used, and is more effective than the release of fully sterile moths. Preliminary studies show that the release of both irradiated males and irradiated inseminated females at a 9:1 ratio can give over 90% control over two generations from a single release. A plan is proposed to irradiate and release the colonies. This would afford an efficient means to handle mass releases with no increase in handling or sexing. Preliminary data show that fast neutrons have added advantages over gamma radiation in both sterilizing and inducing inherited sterility in moths.

1. INTRODUCTION

Many research workers have been trying to emulate the success of the sterile-male release program with the screwworm fly, Cochliomyia hominivorax (Coquerel), with sterilized Lepidoptera. These efforts have not proved nearly as successful as those with the dipteran species. The only release of sterile moths to achieve population control that can be enthusiastically cited to date was recorded by Proverbs et al. (1969). These authors released sterile male codling moths, Laspeyresia pomonella (L.) over a period of 3 years in a commercial orchard in the Okanagan Valley in Canada. The results of these releases appear promising since good control was obtained for all 3 years.

Lepidopterous insects include some of the most destructive pests in agriculture. The need for a successful means of biological control has become increasingly more urgent with the mounting concern over insecticide use. The approach to date has been primarily the release of sterile moths into the natural population. Proverbs (1962) first noted that the progeny of irradiated codling moths were sterile. Cogburn et al. (1966) said the progeny of irradiated Indian meal moths, Plodia interpunctella (Hübner), and the Angoumois grain moth, Sitotroga cerealella (Olivier) were also sterile. North (1967) and North and Holt (1968a and b) demonstrated the same phenomenon in the cabbage looper, Trichoplusia ni (Hübner) and

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found complex chromosomal rearrangements to be the probable primary cause of the inherited sterility; they suggested the use of this type of sterility for control of lepidopterous populations. Bauer (1967) has described the genetic behaviour of chromosome translocations in *Pieris brassicae* L. Since then, inherited sterility has become an accepted phenomenon in Lepidoptera; it has been found in at least a half dozen other species. North and Holt (1969) reported that in laboratory populations of the cabbage looper a single release of partially sterile males gave 92% control over two generations. Knipling (1970) thoroughly discussed releasing partially sterile moths to achieve population suppression. He projected moth population trends under many various release schemes. Research has been continued to determine the most efficient way to achieve inherited sterility in Lepidoptera.

2. MATERIALS AND METHODS

The cabbage looper stocks used in these experiments have been maintained in our laboratory for over 70 generations. The larvae were reared on a semisynthetic diet (Ignoffo 1963). Twenty-five first instar larvae were placed on 100 ml of diet in plastic-covered paper cups of 260-ml capacity (90 mm in diameter, 50 mm in depth). The immature insects were held at 27.5°C, 35% relative humidity, and a 14-h light period per day. The adults were held at 27°C, 65 - 75% relative humidity and also at a 14-h light period per day. The adults were fed 10% sucrose solution.

The population cages used were the same as described by North and Holt (1969). The laboratory populations and the resulting larvae were handled the same as previously described. The insects were irradiated using a ⁶⁰Co gamma radiation facility without anaesthetization. All irradiations were done at a dose rate of 5490 rad/min as determined by thermoluminescent dosimetry (TLD) using LiF.

3. BASIC CHARACTERISTICS OF INHERITED STERILITY IN LEPIDOPTERA

The phenomenon of progeny inheriting more sterility than their irradiated male parents is peculiar to species having holokinetic chromosomes. Therefore, the use of this phenomenon for economic control of insect populations is restricted to Lepidoptera and at least to some species of Hemiptera and Hemiptera. In all studies with lepidopteran species, the progeny from irradiated males were more sterile than their male parents. The cabbage looper (North 1967, and North and Holt 1968a and b), the sugarcane borer, *Diatraea saccharalis* (F.), (Walker and Quintana 1968), the tobacco budworm, *Heliothis virescens* (F.), (Proshold and Bartell 1970), the corn earworm, *Heliothis zea* (Boddie), (North and Holt, unpublished), are examples of the delayed sterility that have already

been found. Studies using the Hemipteran, the large milkweed bug, Oncopeltus fasciatus (Dallas), showed that sterility is inherited by the progeny of irradiated parents (LaChance et al. 1970, North, unpublished data), but the sterility in the F_1 generation is not as great as that exhibited by the parents. It is suspected that the F_1 generation from irradiated males in Lepidoptera may be sterile because of chromosomal aberrations and deleterious effects on quantity and quality of the sperm since they definitely have complications in achieving successful sperm transfer. We have known for some time of the inability of F_1 male cabbage loopers to transfer sperm (North and Holt, unpublished). This inability was also described by Proshold and Bartell (1970) in their work on the tobacco budworm. A detailed account of the mechanisms of sperm transfer is presented by North and Holt (1970). They found, in their work on the tobacco budworm, that F_1 males from a male parent which had received 15 krad were only 5% fertile; however, only approximately 30% of the females placed with these males received sperm. It is not presently known whether this is a problem of sperm transfer or of an inadequate production of sperm by the F_1 male, but is not considered to be a hindrance to the use of inherited sterility for population suppression.

The F_1 progeny in tobacco budworms from a cross involving an irradiated male and an unirradiated female have a longer developmental period than is considered normal and larval and pupal mortality are increased (Proshold and Bartell 1970). The authors also found a higher ratio of males to females. This sex distortion and the increased mortality in progeny from irradiated males were also observed in the codling moth by Proverbs and Newton (1962). Sex distortion is apparently a phenomenon that occurs in progeny from irradiated males in Lepidoptera. It has been observed in the codling moth (Proverbs 1962); the navel orangeworm, Paramyelois transitella (Walker), by Hussein and Madsen (1964); the cabbage looper (North and Holt 1969); as well as in the tobacco budworm. The reasons for sex distortion are not known at this time. Experiments involving crosses with both male and female cabbage loopers (F_1 's through F_5 's) and showing sex distortion have shed very little light on the subject. Since in Lepidoptera the female is the heterogametic sex (see Mittwoch 1967 for references), sex distortion may well be caused by the expression of lethals induced on the X-chromosome; the resulting female progeny therefore succumb and this results in a sex ratio in favour of the male. Female progeny from a cross involving an unirradiated female and an irradiated male have greater fertility than do their male sibs. For example, when the progeny are examined of a cross in the cabbage looper where the male parent received 15 krad, the F_1 males times normal females have an egg hatch of 2.3%, whereas the F_1 females have an egg hatch of 19.5% (North and Holt 1968b). Egg hatch can be somewhat misleading in predicting the effect semisterile individuals will have on population suppression. F_1 females from such a cross will oviposit only half as many eggs as will normal females; therefore even though they have 20% fertility in terms of their relationship to normal production, they are 90% sterile. Therefore, it is felt by the authors that all considerations in assessing the value of releasing sterile and partially sterile moths should be based on the number of larvae produced per female. The fact that F_1 females from irradiated male parents oviposited fewer eggs has also been substantiated in the tobacco budworm by Proshold and Bartell (1970).

TABLE I. STERILITY INHERITED BY CABBAGE LOOPER PROGENY FROM AN IRRADIATED FEMALE
MATED TO AN UNIRRADIATED MALE

Dose to P ₁ ♀♀ (krad)	1 P ₁ ♀♀ × N ♂♂		1 F ₁ ♂♂ × N ♀♀		1 F ₁ ♀♀ × N ♂♂	
	No. of eggs	Per cent hatch	No. of eggs	Per cent hatch	No. of eggs	Per cent hatch
0 (control)	10 352	85.9	4 463	96.6	2 538	90.7
10	9 531	38.2	3 171	81.2	2 914	72.8
15	7 882	14.7	1 480	65.7	3 186	82.8
20	5 108	6.6	2 017	41.3	1 006	83.4

TABLE II. SUPPRESSION OF LABORATORY CAGE POPULATIONS OF CABBAGE LOOPERS BY RELEASING 3-DAY-OLD IRRADIATED MALES AND/OR MALES AND FEMALES IN 9 : 1 RATIO

	Avg. eggs per ♀ per day	Per cent hatch	No. larvae per ♀ per day	Reproductive potential (% control)
<u>F₁ larval population</u>				
Control	43.5	89.7	39.0	100.0
15 krad ♂♂ only	25.2	68.6	17.3	44.4
20 krad ♂♂ only	39.8	77.0	30.7	78.7
Control	45.8	72.0	31.0	100.0
15 krad ♂♂ and ♀♀	38.5	34.8	13.4	43.2
20 krad ♂♂ and ♀♀	30.2	49.4	14.9	48.1
<u>F₂ larval population</u>				
Control	87.7	58.8	51.6	100.0
15 krad ♂♂ only	31.8	26.5	8.4	16.3
Control	64.7	55.4	35.8	100.0
20 krad ♂♂ only	46.8	33.0	15.5	43.3
Control	83.4	63.7	53.1	100.0
15 krad ♂♂ and ♀♀	58.5	46.3	27.1	51.0
Control	78.0	68.9	53.7	100.0
20 krad ♂♂ and ♀♀	48.7	66.2	32.2	60.0

4. IRRADIATED FEMALE MOTHS AS A SOURCE OF INHERITED STERILITY

All of the basic studies and theoretical considerations for the use of inherited sterility for population suppression in Lepidoptera have been done on the basis of releasing only males. Because of the high cost of rearing and handling Lepidoptera, a more economical approach would be the release of both sexes. There are two approaches to this method: (1) a dose where the inherited sterility in the irradiated female is equal to that of the male; or (2) to use treatments that render the female fully sterile but not the male.

To test these hypotheses, 3-day-old adult cabbage loopers were administered doses of 0, 10, 15, and 20 krad of gamma radiation and mated to unirradiated moths. The F₁ progeny from these crosses were then mated to unirradiated moths of the opposite sex, and fertility of the cross was determined. When adult male cabbage loopers are irradiated, the F₁ progeny are always more sterile than the P₁ parent, and the F₁ progeny from irradiated adult females are partially sterile but are not as sterile as the irradiated female parent (Table I). Although progeny from

TABLE III. THEORETICAL SUPPRESSION BASED ON LABORATORY DATA OF A POPULATION OF LEPIDOPTERA OVER TWO GENERATIONS BY A SINGLE RELEASE OF PARTIALLY STERILE MALES AND/OR MALES AND FEMALES AT A 9:1 RATIO

Generation	Uncontrolled population ^a	15 krad ♂♂ only	% control	20 krad ♂♂ only	% control	15 krad ♂♂ and ♀♀	% control	20 krad ♂♂ and ♀♀	% control
Initial	5 000	5 000	-	5 000	-	5 000	-	5 000	-
1	25 000	11 000	55.6	19 500	22.0	22 000	12.0	24 000	4.0
2	125 000	9 030	92.8	42 900	65.7	57 200	54.2	72 000	42.4

^a Uncontrolled population assumed to increase at 5× rate for the several generations and the per cent control achieved in the other populations was based on this figure.

TABLE IV. SUPPRESSION OF LABORATORY POPULATIONS OF CABBAGE LOOPERS BY A SINGLE RELEASE OF IRRADIATED (15 KRAD) INSEMINATED FEMALES AND MALES AT A 9:1 RATIO

Population	No. of eggs	No. of replications	% hatch
Control	1st generation after release 14 638	4	88.5
Population where irradiated inseminated ♀♀ and irradiated ♂♂ were released	19 477 2nd generation after release 9 399	11 5	9.0 91.9
Control	30 048	10	53.5
Population resulting from release of irradiated ♂♂ and inseminated ♀♀			

irradiated female cabbage loopers are not as sterile as progeny from irradiated males, the male progeny are still the most sterile of the two sexes. It is obvious from these data that a dose cannot be used where the sterility of the progeny of the irradiated female is equal to that of the male. Though the female cabbage looper is sterilized at a lower dose (20 - 25 krad) than the male, the progeny from parents receiving this dose would be, from all that is known presently, quite noncompetitive. This would apparently preclude the possibility of irradiating and releasing both sexes.

Increased benefits would be incurred if both sexes could be released. Therefore studies were conducted in laboratory population cages to determine the comparative values of releasing (1) only partially sterile males and (2) irradiated males and females. Table I shows that though the percentage is high, the number of eggs oviposited per female (at 20 krad) is half of that of the control. Therefore, the number of progeny actually being placed into the population by the irradiated female possibly would not be sufficient to have a deleterious effect on suppression.

Table II shows the results of releasing into laboratory population cages at a 9:1 ratio, males that received 15 or 20 krad, and males and females that received 15 or 20 krad. Since it is felt that egg hatch alone in competition cages is not an accurate measure of the population suppression capacity of the treated insects, the number of larvae per female per day was used as the basis to calculate the reproductive potential in per cent of control. In the first generation following release, suppression from the release of only males that had received 15 krad was equal to that obtained by the release of males and females that had received 15 and 20 krad. However, in the assessment of the F_2 populations, the reduction gained by the release of only males that had received 15 krad was far superior to that of any other group.

Table III shows a projected estimate of population suppression based on the experimental data represented in Tables I and II. The basis for the calculations was that males and females were released in a 9:1 ratio into a natural population of 5000 moths and that the normal reproductive rate of the uncontrolled population was five-fold for each generation (Knipling 1970). It appears that after a single release of males that received 15 krad the population is suppressed for two generations and this is by far the most efficient method of suppressing a lepidopterous population. It should be remembered, however, that in small laboratory population cages all males are given a nearly equal chance to mate. Under natural field conditions, this most likely represents a gross overestimate of the capabilities of partially sterile males to suppress the population. On the other hand, in a small population cage, the fact that the males are in close proximity to the females could well mean that the advantage could be to the normal male, whereas in nature where there are not as many matings, the sterile or partially sterile male would have more impact. In recent tests in field cage populations at the Entomology Research Station at Riverside, California, it was shown that with one release of males receiving 15 krads, 92% control was achieved over two generations (Toba, Kishaba, and North, 1970). The two experiments with two replications each in the field cages at Riverside are most encouraging because they indicate that the data obtained in population cages in a laboratory are valid.

5. LABORATORY POPULATION SUPPRESSION BY THE RELEASE OF IRRADIATED INSEMINATED FEMALES AND MALES

To achieve maximum economic efficiency of delayed sterility for the suppression of lepidopterous populations, the release of both sexes is essential. Preliminary studies have just been completed which show that females inseminated before irradiation are sterile after they receive a dose of 15 krad. (This means that the criteria of Hypothesis 2 stated in Section 4 "to use treatments that render the female fully sterile but not the male" can be successfully met.) These tests involved laboratory populations of 10 pairs into which irradiated, inseminated females and irradiated males were released at a 9:1 ratio.

The results of these studies are given in Table IV. Only cages where there were no deaths of the natural population for a 5-day period are represented in these data. The released moths were marked with an artist's acrylic paint. By counting only population cages where there was no mortality of normal moths within the 5-day test period, the results are weighted toward the unirradiated moths. We feel this weighting in laboratory populations gives a more valid assessment in planning field cage tests. Table IV shows that by releasing inseminated females and males irradiated with 15 krad, the first generation following release had an average hatch in 11 population cages of only 9%. This is in contrast to the fertility (88.5%) of the four control population cages.

The larvae were seeded on Ignoffo's media in 260-ml containers (20 larvae/container). The resulting pupae were sexed, and when all moths emerged, selections were made at random, and the population cages were set up 10 pairs/cage. The second part of Table IV shows that the fertility of the F_1 adult was higher than that of the irradiated released moths (53.5%). However, the magnitude of the reductions in both generations is great enough to realize over 90% control from a single release in two generations.

Not only does the release of irradiated inseminated females and irradiated males give impetus to the use of delayed sterility for population suppression but this method may also, upon further refinement, prove to be ideal for releasing both sexes which have received a substerile dose. This could result in better suppression than when sterile insects are released. This technique would involve setting up large colony cages, allowing the moths to mate, collecting the eggs for one night, and then irradiating the moths and releasing them. A continuous progression of cages would be in use; thus there would be no need for separate colony cages, and the ease and cost of rearing would also be reduced. This continuous line of production would be an efficient method for a program of mass release in the field.

6. INDUCTION OF DELAYED STERILITY IN THE CABBAGE LOOPER BY FAST-NEUTRON IRRADIATION OF PUPAE

If the basic causes for delayed sterility in Lepidoptera are gross chromosomal rearrangements, it would be logical to assume that radiation of a high LET would be the most effective per unit of absorbed energy for induction. On this premise, male cabbage looper pupae (72 h before

TABLE V. STERILITY INHERITED BY THE PROGENY OF MALE CABBAGE LOOPER PUPAE EXPOSED TO 0.43-MeV FAST NEUTRONS^a

Dose to P ₁ generation (krad)	% hatch in % control (F ₁ larvae)	% hatch in % control (F ₂ larvae)	
		♂♂	♀♀
0 (control)	100.0	100.0	100.0
1	97.9	92.2	100.0
3	92.6	44.1	69.3
5	89.1	39.4	-
10	56.9	0.0	1.7
15	15.3		
20	12.9		

^a Pupae were irradiated 72 hours before eclosion.

emergence) were irradiated with various doses of fast neutrons (0.43 MeV), and the amount of sterility in the first and second generations was measured. Irradiations were conducted through the courtesy of the Brookhaven National Laboratory, Upton, Long Island, New York. The control group was shipped and handled in the same manner as the irradiated group. The pupae left our laboratory in the morning by air freight and arrived in Brookhaven that afternoon. They were irradiated the following morning and were back in our laboratory that evening. The results from these tests are presented briefly in Table V. Since the previous studies using gamma irradiation to produce delayed sterility in the cabbage looper were done with adults, a comparison between the two irradiations of different LET cannot be made at this time. We irradiated the same stage of pupae with gamma irradiation so that accurate comparisons between the two types of irradiation can be made. These results are not complete at the time of writing this report and will therefore be included in a later publication.

It is interesting, however, to note that with fast-neutron irradiation, the relationship between the amount of sterility induced in the P₁ generation and the F₁ generation is not entirely the same as was found for adults irradiated with gamma radiation. The male F₁'s are more sterile than the female F₁'s when their male parents were given the same dose. The doses, however, at which the offspring of fast-neutron irradiated pupae became completely sterile are lower than those of gamma irradiation used to sterilize adults. In the previous studies with adults, 15 krad of gamma radiation was considered the minimum dose to achieve full sterility. As can be seen in Table V, 10 krad of N_f gives essentially complete sterility when pupae are irradiated.

One characteristic that is different between the delayed sterility pattern achieved when pupae are irradiated with fast neutrons and when adults are given gamma rays is the difference in the fertility of the irradiated individuals and their F₁ progeny. When pupae were irradiated with neutrons

TABLE VI. DEPRESSION OF FERTILITY IN THE F₁ GENERATION RESULTING FROM CROSSES OF MALES IRRADIATED WITH 10 KRAD OF 0.43-MeV FAST NEUTRONS AND UNIRRADIATED FEMALES

	Irradiated ♂♂ × unirradiated ♀♀		F ₁ irradiated moth × unirradiated moth of opposite sex	
	No. eggs	Per cent hatch	No. eggs	Per cent hatch
Control	1 959	98.1	1 570	93.2
Irradiated ♂♂ line	12 481	95.5	8 349	55.8

TABLE VII. CORRELATION BETWEEN F₂ FERTILITY AND F₃ LARVAL DEVELOPMENT TIME FROM PROGENY WHOSE P₁ MALE PARENT RECEIVED 10 KRAD OF 0.43-MeV FAST NEUTRONS

Fertility of F ₂ generation ^a	No. of F ₃ individuals	Mean days to pupation	Range
0 - 25	57	15.1	13.9 - 15.7
26 - 50	260	14.1	12.7 - 14.8
51 - 75	312	13.4	12.9 - 14.9
76 - 100	1 158	13.5	12.0 - 14.9
Control	233	13.3	-

^a Based on per cent egg hatchability.

(0 - 10 krad) their hatch was higher than that achieved when gamma radiation was used, and there was more sterility in the F_1 progeny. This could be a benefit in a release program since the higher fertility in the original irradiated parent would mean that a larger number of F_1 's from the irradiated parent could be placed into a population. This would mean that the population suppression in the first generation would not be as great but should be increased in the second generation.

When adult male cabbage loopers (0 to 24 hours old) are exposed to 0.43 MeV neutrons and given a dose of 10 krad, there is very little sterility induced in these individuals. There is, however, increased sterility the next generation (Table VI). These irradiations were also done through the courtesy of the Brookhaven National Laboratory. It is planned to determine whether neutron irradiation is advantageous.

As these crosses were made as individual pairs, it was possible to correlate larval development time with fertility. This pertained directly to the data presented by Proshold and Bartell (1970). Table VII demonstrates that the few progeny coming from the highly sterile crosses required significantly longer time to reach pupation than those that were obtained from crosses of lesser sterility. Although the data presented are only for F_3 progeny, these data have been found to be true for F_1 and F_2 progeny. It is suspected that the chromosomal imbalance which leads to sterility also has its somatic position effects which disrupt the continuity of development and lengthen it.

7. CONCLUSIONS

The ability of partially sterilized males to suppress a population in which they have been released has been successfully demonstrated in both laboratory and field cage populations. In both of these types of test populations, a release of 9 irradiated to 1 unirradiated moth was sufficient to give 92% control over two generations. With the high cost of rearing and handling lepidopterous species, the ability to suppress populations to this degree with only a single release is advantageous. The use of delayed sterility in regions where there is migration from an overwintering population could well protect the outlying areas from ever being infested. This concept is discussed in detail by Knipling (1970).

The preliminary results from releasing irradiated inseminated females and males are most encouraging. The possibility of being able to release moths after they have mated and have been allowed to oviposit at least once (so that there is no need for the maintenance of separate colonies) in a mass rearing program has many economic advantages. The further refinement of the irradiation of an inseminated female could well lead to more efficient means of controlling Lepidoptera.

The use of neutrons as a source of radiation to induce delayed sterility is most encouraging, particularly because the level of sterility in the F_1 is far greater than in the irradiated P_1 generation. The single most important factor in obtaining population suppression through the use of delayed sterility is the percentage of individuals obtained in the F_1 generation that are from an irradiated parent.

From previous calculations (North and Holt 1969) to get an effect of economic advantage, at least 60% of the F_1 population needs to come from the irradiated parent.

We believe that the concept of releasing partially sterile moths for population control on a large scale is not only plausible but possible, particularly with the use of irradiated inseminated females.

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CURRENT STATUS OF THE CO-ORDINATED RESEARCH PROGRAM ON USE OF STERILE-INSECT TECHNIQUE AGAINST THE RICE STEM BORER

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Abstract

CURRENT STATUS OF THE CO-ORDINATED RESEARCH PROGRAM ON USE OF STERILE-INSECT TECHNIQUE AGAINST THE RICE STEM BORER.

The co-ordinated research program on control and/or eradication of rice insects is presently limited to Chilo suppressalis, Tryporyza incertulas, Sesamia inferens and Chilo traxa polychrysa. There are presently seven research contractors conducting investigations on rearing and nutrition, ecology and radiation effects. Progress to date is presented.

The co-ordinated program of research for rice insect control and/or eradication is presently limited to the rice stem borers, Chilo suppressalis, Tryporyza incertulas, Sesamia inferens and Chilo traxa polychrysa. The objective is to determine whether the sterile-insect technique can be utilized against them. There are seven research contractors and their research includes investigations on ecology, radiation effects, and rearing and nutrition of rice stem borers.

Of all the rice stem borer species, the ecology of C. suppressalis is probably the best understood. However, as related to the sterile-insect technique, more information is required. For example, light-trap data are abundant and based on them there is lots of information on when the first spring emergence is expected, the approximate number of generations per year, relative numbers of moths through the season, etc. However, attempts to correlate light-trap catches with actual population estimates in the field have been limited. To determine if the sterile-insect technique will be practical against rice stem borers, it is important to know the population density in the field. Nickel (1964) stated that the "chief deterrent to the eradication of rice stem borers by male sterilization is the relatively high numbers of adults normally present per unit area in the field". He assumed a 5% infestation of tillers, 10 tillers per hill, 25 cm × 25 cm spacing, development time of 4 weeks, male longevity of 1 week and calculated that the number of male adults emerging per week would be 1 000 000 per square kilometre of rice. Obviously, if this were the real field situation, releasing sterile moths would not be practical and I doubt whether one could even control such a population. One must first determine whether the population can be lowered to much smaller numbers.

One contractor is presently investigating the factors contributing to mortality of the overwintering population so that population estimates of the spring generation can be made by sampling the diapaused population.

He is also trying to determine seasonal abundance based on data from light-traps, field surveys of egg counts, larval counts, and sweeping specified distances with a net. Preliminary data confirm results of other workers who show that destruction of rice stubble would greatly reduce the overwintering population.

Dispersal studies using isotopes have been limited but Hyun et al. (1968) have shown that moths will fly as far as 800 m away from the release point. Unfortunately the number of moths released was small. The only presently available data on the effect of gamma radiation on any of the four species of rice stem borer are those of pupal treatment on C. suppressalis. Most of the results are similar to those obtained from other Lepidoptera, such as:

1. When pupae of different ages are irradiated with the same dosage, less mortality is evidenced with older pupae;
2. The number of eggs per female was reduced with increased dosage;
3. About a 1% hatch of the eggs was obtained when males treated as pupae with 30 krad were mated with UT females;
4. There is some reduction in mating of adults from pupae irradiated with 35 krad.

There are no data as yet on:

1. F_1 sterility following pupal and adult treatments;
2. Competition studies following pupal treatment;
3. Sterile dosage when adults are treated;
4. Comparison of competitiveness between pupal and adult treatments.

Progress on the sterile-insect technique has been slow primarily because mass rearing of them has been difficult. I feel that mass rearing presents the biggest problem in the development of the sterile-insect technique for rice stem borers. That is why at present most of the emphasis of the research contractors is on mass rearing.

Attempts to rear the rice stem borer have been made in Japan since 1930. Because the primary objective in those days was to use the rice stem borer eggs to rear Trichogramma japonica Ashmead, laboratory rearing as contrasted to mass rearing was sufficient and therefore, although tedious, fresh cut rice stems were used. Koyama et al. (1951) and later Ishii (1952) were among the first to rear Chilo suppressalis on a synthetic diet. They modified the European corn borer diet of Beck and Stauffer (1950) and rearing was conducted under aseptic conditions. Subsequently, Ishii and Urushibara (1954) and Ishii and Hirano (1955) and Hirano and Ishii (1957) worked out some of the nutritional requirements of Chilo suppressalis by using the modified casein diet. None of the diets used were fully satisfactory and it was apparent that some nutritional factor was missing. Hamano (1961) improved the diet by adding rice bran or stems and reported the importance of ascorbic acid in protein formation. In addition, he reported a diet which was rich in choline. Kamano (1964) reported that ascorbic acid was necessary for the successive rearing of Chilo suppressalis; 200 - 400 mg of ascorbic acid in 100 g of medium

increased the larval body weight as well as moth emergence. Fukaya and Kamano (1964) reported that rice stem borers could be mass reared in 200-ml Erlenmeyer flasks (30 full-grown larvae each) at a cost of about \$1/100 larvae excluding labour. The disadvantage of this method is that the larvae must be reared aseptically.

Kamano and Fukaya (1965) reported that inbreeding Chilo suppressalis for four generations reduced egg hatch from 88.7% to 37.5%, the percentage pupation from 77.2% to 31.2% and the number of egg masses per female from 3.6 to 0.6. By rotational breeding they avoided the deleterious effects through eight successive generations. Recently, however, Kamano (personal communication) stated that the yield and fecundity are still not comparable to that of field grown moths.

Several contractors have started experimenting with various modifications of the pinto bean diet of Shorey and Hale (1965) and Bowling (1967). Some of the modifications have been to add casein, rice stem or sprout (for "leaf factor"), using different kinds of beans, etc. One contractor reported that rice sprout gave better pupal yield and larval development as compared with rice stems. Some of the contractors have had limited success with the bollworm diet (wheat germ diet plus ascorbic acid). T. incertulas is the only species which has not yet been reared for more than one generation on an artificial diet.

In summary, it is much too soon to state whether or not the sterile-insect technique can be utilized against the four rice stem borer species under investigation. T. incertulas still cannot be reared artificially. However, rearing of C. suppressalis, S. inferens and C. polychrysa shows promise. Data on the effect of radiation on the above four species are almost negligible, but limited results indicate that the effects will be similar to those on other Lepidoptera.

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ORCHARD ASSESSMENT OF RADIATION-STERILIZED MOTHS FOR CONTROL OF Laspeyresia pomonella (L.) IN BRITISH COLUMBIA

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Abstract

ORCHARD ASSESSMENT OF RADIATION-STERILIZED MOTHS FOR CONTROL OF Laspeyresia pomonella (L.)
IN BRITISH COLUMBIA.

Procedures are described for control of the codling moth, Laspeyresia pomonella (L.), in British Columbia by the release of sterile moths. These procedures include: selection and preparation of the test orchard; estimating the absolute population, the commencement and rate of adult emergence, and the rate of increase per generation; methods of adult release; and methods of marking. Results of orchard experiments with fully sterile moths are presented for the period 1962 to 1970. Good to excellent control was achieved, but recent laboratory and field cage experiments indicate that reproduction may be suppressed quicker by release of partially sterile males plus fully sterile females.

INTRODUCTION

In 1962 the first release of sterile codling moths, Laspeyresia pomonella (L.), was made in British Columbia for control or possible eradication of this pest. Although the procedure is still in the experimental stage, it has advanced to the point that it has given excellent control in a 40-hectare orchard and it is expected that an area-wide release involving about 400 hectares will be initiated in 1971. A large program like this should illustrate whether it is economically sound to proceed with commercial implementation of this method of control.

This paper describes the procedures involved for assessing the effectiveness of sterilized codling moths in controlling this insect in British Columbia and reviews the results of field control from 1962 to 1970.

ORCHARD PROCEDURES BEFORE AND DURING RELEASE OF STERILE MOTHS

Selection and preparation of the test orchard

Although it is most desirable to have test orchards completely isolated from outside sources of adult reinfestation, this condition was very difficult to achieve. It is now apparent that, at least in the interior of British Columbia, the male codling moth (and probably also the female though this remains to be investigated) can fly much greater distances than reported in the literature. For example, in an area in which only about 12 scattered host trees (apple and pear) intervened between the

release site and point of recovery, 0.8% of the released male moths (marked and sterilized) were recovered 3 km from the release site. In this and similar experiments male moths were often trapped up to 6 km, and an occasional individual up to 9 km, from the release site. On the other hand, the male moth evidently does restrict its flight in areas that are densely planted to apple and pear trees. For example, when a 4-hectare orchard in a densely planted area received 368 000 marked sterile moths throughout one summer, only three of the male moths were recovered in a sex trap maintained in an unsprayed orchard 2 km away.

Some reinfestation can be tolerated if the objective is control rather than eradication. However, because the moth is capable of flying reasonably great distances, all abandoned host trees within at least 3 km of the test orchards were usually removed 1 year before starting a release program. Where they were not removed the abandoned trees were sprayed regularly, both before and during the release program, with an effective and persistent codling moth insecticide. If permission were denied to remove or to spray the trees, all immature fruit was stripped from the trees and destroyed before the 1st generation larvae had left the fruit.

The importance of removing unsprayed trees cannot be over-emphasized. In the interior of British Columbia it is a common occurrence at harvest time to find 80% or more of the apples injured by the codling moth in unsprayed trees. Because the excess immature fruit is not removed as in commercial orchards, a single neglected tree is potentially capable of producing several thousand codling moths in 1 year. One such tree is enough to jeopardize the success of a program of sterile-moth release.

To avoid overtaxing the rearing facilities, it was essential that the native population be within manageable levels in test orchards before the start of sterile-moth release. The population was sufficiently low in commercial orchards selected for experimentation, but abandoned orchards required treatment. Chemical sprays were used in one orchard, and in another the population was reduced by removing and destroying most of the immature fruit.

One point that must not be neglected in preparing an orchard for sterile-insect release is the possibility that overwintering larvae are cocooned in the tree props. Because props are a favourite site for overwintering larvae, and because orchardists often stack the props together at one end of the orchard, it may be advisable to fumigate them before adult eclosion in the spring. If this is not done a high concentration of moths in spring could start a serious infestation in trees adjacent to the prop pile. This spring (1970), for the first time, infested tree props from one section of a test orchard were fumigated with methyl bromide.

Estimating the absolute population

It is obvious that the numbers of moths that must be reared in the laboratory to overflow the native population at a predetermined ratio depends largely on the size of the native population, though other variables such as rate of adult emergence in the field and rate of moth increase per generation are also very important. In British Columbia the population size for the coming year was predicted largely on the basis of fruit examination at harvest. Each fruit with a larval exit hole represented a mature larva potentially capable of overwintering. (The work was re-

duced by examining only the unmarketable apples at the packinghouses, a procedure evidently first used by U.S. entomologists [1]). To these larvae should have been added the 1st generation larvae that entered diapause. However, the latter were omitted because in British Columbia only about 15% of the usually small population of 1st generation larvae enter diapause. The numbers of overwintering larvae that die without reaching the moth stage must be deducted from the total numbers of larvae that entered diapause, but at present the information required to arrive at an accurate estimate of this mortality is unavailable. It is known that the overwintering mortality is most variable from orchard to orchard and from year to year. It is not uncommon in unsprayed orchards for 40% or more of the overwintering larvae to be destroyed by the hymenopterous parasite *Ascogaster quadridentata* Wesmael. However, this parasite is seldom found in sprayed apple orchards. Bird predation is an important factor in reducing the numbers of overwintering larvae in most abandoned apple and pear orchards but insectivorous birds are not abundant in sprayed orchards and contribute very little there to the destruction of codling moth larvae. Of the abiotic factors, unusually cold winter weather is by far the most important one in reducing the numbers of overwintering larvae. The degree of mortality depends not only on temperature but on where the overwintering larvae cocoon and on the depth of snow cover [2]. In the winter of 1968 the temperature in most orchards in British Columbia fell to -30°C and lower. This, coupled with a scanty snow cover, resulted in great larval mortality and in below normal codling moth infestations during the first generation in most apple orchards. For the present, until more detailed data are accumulated, I rather arbitrarily assume about 20% overwintering mortality in commercially operated orchards and 50% in abandoned orchards, though these percentages must be increased in unusually cold winters.

One method that is sometimes suggested for estimating the overwintering population is based on trapping the larvae in cloth or paper bands surrounding the tree trunks and scaffold limbs. To use this method it is necessary to determine what percentage of the total overwintering larvae cocoon in the bands and this varies largely according to the numbers of natural overwintering sites available [3]. Also, the percentage mortality of overwintered larvae may be very different between those in bands and those spun up in natural sites. This was illustrated in an experiment in which diapausing larvae were confined in sleeve cages to 1-metre-long sections of tree trunk. Where the larvae were permitted to select natural overwintering sites, most of them evidently failed to find a suitable site and 49% died. In contrast, where corrugated paper bands were provided for the larvae, mortality was reduced to 10%. In any event, it was found that in most British Columbia orchards only a small proportion of the diapausing larvae cocooned in trunk bands. Even when considerable time was spent in scraping loose bark scales from the trunks and scaffold limbs, the numbers of larvae trapped in the bands were disappointingly small. Possibly now that growers are planting a smaller type of apple tree with smoother bark it will be possible to use bands as a method of estimating the size of the overwintered codling moth population.

Release-recapture techniques that are often used to estimate population numbers of other insects were not used for the codling moth. The chief reason for this is that we do not know how the behaviour of the released

laboratory insect compares with that of the wild moth. There is some evidence that the laboratory moth, which is reared at a constant high temperature (26°C), is less active in the cool spring weather than the wild insect which has wintered in the orchard.

Estimating the commencement and rate of adult emergence

Several methods were available for determining when eclosion begins in the spring, but a combination of two or more of the methods was likely to give more accurate results than any single procedure. Sex traps baited with virgin female moths were found particularly useful. These captured young male moths, sometimes even on the day of emergence if the prevailing temperature at dusk was not below about 14°C. Ultra-violet light traps were also useful, but in early spring they were not quite so effective as sex traps.

The start of eclosion in spring was also determined by examining natural and artificially-provided cocooning sites for empty pupal cases. Usually this was not done in orchards where sterile insects were being released, but was conducted in similarly located orchards in which the codling moth population was reasonably high. A slight modification of this procedure was used for 3 years. Known numbers of diapausing larvae were caged in the autumn at various locations on the trees, both above and below soil level. In spring, the percentage mortality of the overwintered larvae was first established, and thereafter the numbers of adults that emerged per day were recorded so that it was a simple matter to calculate the rate of eclosion. With this technique it is advisable to collect the diapausing larvae from orchards in the same locality as the experimental orchard for conditions prior to diapause are believed to influence the date of eclosion [4]. For best results it is desirable to establish what proportion of the larvae hibernate in and on the soil and at various locations on the trunk and scaffold limbs, because the site of hibernation has some effect on the date of adult emergence.

The foregoing procedures told us when eclosion started and the rate at which it was progressing, but other methods were needed to forecast these events. More work probably has been done with so-called heat units or developmental units than with any other procedure for predicting the beginning and peak of codling moth eclosion in spring [5, 6]. So far, this procedure has not been used in British Columbia because under controlled conditions in the laboratory it was less accurate than would be expected from most of the published literature.

We found that we could relate the onset of codling moth emergence to the stage of apple bud development. Eclosion did not begin until the McIntosh variety was in the late pink-bud stage of development, and maximum rate of adult emergence was about 3 or 4 weeks later. Continuous temperature records were kept in the experimental orchard. These records coupled with graphs of moth flight in previous years permitted us to make adjustments for any unusually hot or cold weather that was encountered. For practical purposes the following rule of thumb was usually adequate. If the apple bloom is past the petal-fall stage and the maximum daily temperature on 2 or 3 consecutive days is above 29°C, maximum eclosion will occur 1 or 2 days later unless a severe cold period intervenes.

Our procedures for forecasting the peak of adult emergence are admittedly crude and should be improved but over the years they have served us reasonably well.

Estimating rate of increase per generation

This is one of the most important, variable, and difficult to predict of the many factors affecting codling moth control by the sterility procedure. Temperature plays the most significant role. In British Columbia the increase in numbers of the codling moth in the first generation is quite modest, probably seldom greater than 3 or 4 fold. This is attributed to the below optimum temperatures that prevail throughout much of the ovipositional and egg hatching period. During the second generation the prevailing temperatures are appreciably higher, and many more larvae become established in the fruit. A 12-fold increase is probably a realistic figure when there is a large apple crop, though more detailed data must be collected to verify this. The potential exhibited by the codling moth for increasing its numbers was illustrated by the results in two semi-isolated apple orchards in which the population was reduced by sterile-moth release to the point where less than 0.1% of the fruit was injured at harvest. One year after the release program was discontinued, fruit injury at harvest had increased to about 10%.

Other priorities may preclude the possibility of working out detailed life tables for the codling moth, but these tables would help considerably in the successful application of the sterility principle since they would allow much more accurate estimates to be made of such important factors as population size, rate of adult emergence, and rate of population increase per generation.

Methods of adult release

As stated previously, codling moths that were released in densely planted orchard areas tended to remain rather close to the site of release. This was further illustrated in an apple orchard in which about 3000 marked male moths were released from one point. Four days after release, sex traps were set out in every tree up to 95 metres from the point of release. That night the average numbers of males captured per trap were 31, 19, 16, 6 and 3 at 15, 35, 55, 75 and 95 metres, respectively, from the release site. This and other experiments conducted over longer trapping periods indicated that the majority of the male moths did not fly more than about 60 metres from the point of release. Because of the limited area of dispersal, it was essential that the method of releasing the moths should in itself distribute the insects uniformly throughout the test orchards.

Ground release stations were used in the early experiments. At first, moths were released from approximately 120 stations per hectare, but it was soon found that this number was unnecessarily high. On the basis of flight studies and on the severity of apple injury at various distances from the release stations [7], the numbers of stations were eventually reduced to about 12 per hectare.

Many types of stations were tried before the adoption of a plywood box with a hinged lid. One wall of the box was of plywood, and the other three were made of stiff wire screen in which the mesh was large enough

to permit easy exit of the moths. The station was provided with a central, square corral made by nailing four strips of wood to the floor. The sterilized moths, immobilized by cold, were placed in the corral. This structure prevented high winds from blowing the temporarily inactive insects to the ground where, in unsprayed orchards, many would have been destroyed by predators. The lid gave protection from rain, and the screen walls kept out insectivorous birds. The stations were isolated from ants by barriers of Stickem adhesive (manufactured by Michel & Pelton Co., Emeryville, California).

The use of fixed release stations was satisfactory for distributing moths in small orchards, but more rapid procedures were needed for larger areas. In 1969 the sterilized moths were released in 18.5 cm × 11.5 cm × 5.0 cm cardboard boxes from a helicopter using a box ejector that was developed and previously used by the U.S. Department of Agriculture [8]. The aircraft flew about 70 km per hour in flight lanes approximately 30 metres (4 or 5 tree rows) apart, and since one box was dropped every 30 metres this resulted in approximately 11 boxes per hectare. The moths evidently were not injured by this method of release. In three experiments in which the aerial release method was compared with ground release, 58% of the male moths recovered in sex traps were from the aerial releases and 42% from the ground releases.

Although moth release in the boxes resulted in good insect distribution, the method was unsuitable for treating large areas because considerable time was required to prepare and load the boxes with moths. Also, the cargo space in the helicopter was very limited. This year (1970) we are using so-called free insect release in which the moths are discharged directly from the helicopter into the air stream without the protection of boxes or other containers. Essentially, the release mechanism, as developed by Mr. A.D. McMechan of the Summerland Research Station, consists of a vertically mounted funnel in which the end of the spout is about 1.8 cm above the floor of a rapidly vibrating trough (Syntron Vibratory Feeder manufactured by Syntron (Canada) Ltd., Stoney Creek, Ontario). The open end of the trough is in turn located immediately above a vertical discharge tube which extends almost to the level of the helicopter skids. Cold-immobilized moths are added to the funnel as required. The rate at which the moths are delivered by the trough to the discharge tube is regulated by the rate of vibration of the trough and its degree of inclination to the horizontal. Preliminary results indicated that the moths are uninjured by this method of release when the helicopter flies at 70 km per hour.

Preliminary work was done on a ground release system that might be used in small areas or in places where the topography is unsuitable for aircraft. Essentially, the apparatus consists of a 10-cm diameter flexible tube attached to the air discharge orifice of an air-blast orchard sprayer. A rigid pipe, about 5 cm in diameter, is inserted into the flexible tube through a circular hole cut in its wall. The pipe projects about 3 cm into the tube and is at right angles to it. Moths for release are fed into the exposed end of the tube from a vibrating trough as described previously. Moths released by this apparatus evidently were not injured even when the air velocity at the discharge end of the flexible tube was 80 km per hour. When marked moths were discharged a few

metres above the orchard floor, and equal numbers of differently marked males were scattered by hand in the grass in the same area, 48% of the moths recovered in sex traps were released by the air blast method and 52% by the procedure of scattering by hand.

Methods of adult marking

All sterile codling moths were marked before release so that, with the aid of traps, a more or less continuous record could be kept on ratios of sterile to wild moths in and outside the release area, on sterile moth dispersal, and on adult longevity where different colour markers were used.

Many methods are available for marking insects but the ones we tried for mass marking the codling moth all had undesirable features. Spraying the insects with an alcoholic or acetone solution of eosin left the moths so bedraggled that assessment of this technique was discontinued, though it has been used elsewhere with evidently satisfactory results [9]. For 2 years moths were marked with zinc 8-hydroxyquinoline powder applied by a small hand duster [10]. This greenish powder fluoresces strongly in ultra-violet light so that dusted moths can be readily detected. The treatment did not cause any obvious injury, but more detailed work would be needed to establish definitely whether or not the powder affects the response of the male moth to untreated females. The use of this chemical was discontinued in favour of Day-Glo pigments (Switzer Bros. Inc., Cleveland, Ohio, U.S.A.) because the latter are available in several colours and are relatively inexpensive. These dusts, which fluoresce intensely in ultra-violet light, did not affect the longevity, degree of mating, or egg laying in the codling moth, but recent work in a greenhouse suggests that they may affect the response of the male to untreated females if the moths are dusted excessively. The chief disadvantage of the Day-Glo pigments is that the powder will contaminate untreated moths if large numbers of the dusted insects are captured in a confined space such as a small sex trap.

Experiments are in progress on marking adult moths by incorporating calco red and other pigments into the larval diet. If this procedure works it will save appreciable time, but more important, the moths should reach the orchard in better condition since one handling procedure (dusting) would be eliminated.

The use of the so-called golden codling moth [11] was investigated as a genetic marker, but this strain proved more difficult to rear than the common grey strain, and there was also some possibility of misidentification of old moths that had lost most of their scales.

ASSESSMENT OF PARTIALLY STERILIZING DOSES OF RADIATION

Field control by release of partially sterile codling moths was not started in British Columbia until this spring and consequently no results are available. However, in view of the current interest in this procedure for suppressing other lepidopterous pests, I shall present some of our data from laboratory and field cage experiments.

TABLE 1. F₁ PROGENY PER CAGE AFTER STERILE (40 KRAD) OR PARTIALLY STERILE (25 KRAD) CODLING MOTHS^a WERE CONFINED WITH FERTILE MOTHS IN PAPER-BAG CAGES

Moths per cage ^b	Dosage (krad)	1st instar F ₁ larvae	% F ₁ larval entries in apples	% F ₁ larvae to reach moth stage	% established F ₁ larvae to reach moth stage	F ₁ adults
50 I ♂+ 50 I ♀ + 5 N ♂+ 5 N ♀	40	172	85	73	86	128
50 I ♂+ 50 I ♀ + 5 N ♂+ 5 N ♀	25	126	56	40	70	50
5 N ♂+ 5 N ♀	0	930	91	72	79	665

^a Gamma irradiated in air at 2°C.
^b Two cages per treatment.
^c I = Irradiated, N = nonirradiated.

It was shown some years ago [12] that when the male codling moth is exposed to certain partially sterilizing doses of radiation and then mated with an untreated female, the surviving F_1 offspring are predominantly male and mostly sterile. At that time it was decided that the use of partially sterilizing doses was impractical for a high-priced crop like apples, for although most of the F_1 offspring would die in the 1st larval instar many of them would attack and injure the fruit before they succumbed. However, because of the poor competitiveness of fully sterilized codling moths, coupled with the high cost of laboratory rearing, it was decided to reassess the feasibility of using partially sterilizing doses of radiation.

Adult virgin male and female moths, not more than 24 hours old, were chilled to 2°C and exposed in an insulated cannister to 40 or 25 krad. (40-krad males \times nonirradiated females results in about 2% egg hatch and 25-krad males \times nonirradiated females in about 15% hatch. These dosages cause complete sterility in irradiated females.) The insects were then confined in paper-bag cages with nonirradiated virgin moths at a ratio of 10 irradiated male and female moths to 1 nonirradiated male and female. The cages were held at about 27°C and all eggs laid were collected. To avoid cannibalism all hatched larvae were reared individually on immature apples.

Table I shows that there was little difference in numbers of 1st instar F_1 larvae between cages that contained the 40-krad and those that contained the 25-krad moths. However, almost one-half of the larvae (44%) at the lower dosage failed to become established dying some time before or shortly after they entered the apples. Most of the established larvae eventually developed into adult moths, but there was evidently a slightly higher mortality in established larvae from the 25-krad than from the 40-krad treatment. With the 40-krad treatment the numbers of F_1 adults were 2.5 times greater than the number at the 25-krad treatment.

Unfortunately two important points were not cleared up in this experiment. The number of superficial injuries (stings) caused by the 1st instar larvae could not be established because the stored apples used in the experiment were badly shrivelled. Second, the fertility of the F_1 adults was not determined. Almost all eggs laid by the F_1 moths, including the control, failed to hatch, presumably because they were inadvertently subjected to prolonged temperature above 40°C.

In a somewhat similar experiment the radiation doses, conditions of exposure, and ratio of irradiated to normal moths were the same as before, but the insects were confined in 110 cm \times 47 cm \times 12 cm trays containing one layer of immature apples (about 300). The insects readily reproduced on the apples. When all the F_1 adults had emerged, it was evident, as in the previous experiment, that the insects treated with 40 krad were less competitive than those treated with 25 krad, for the numbers of F_1 adults that developed in trays containing 40-krad moths were 1.8 times greater than in trays with 25-krad moths (Table II). As was stated previously, when male codling moths are exposed to partially sterilizing doses of radiation and then mated with nonirradiated females, most of the offspring that reach the adult stage are males. In the present experiment there were twice as many F_1 adult males as females at the 25-krad treatment, which suggests that the male parent of many of the F_1 moths was an irradiated insect. Further work is needed to determine whether the sperm of 25-krad males are competitive with sperm from nonirradiated males.

TABLE II. F_1 ADULTS AFTER STERILE (40 KRAD) OR PARTIALLY STERILE (25 KRAD) CODLING MOTHS^a WERE CONFINED WITH FERTILE MOTHS IN CAGES MADE OF ALUMINIUM TRAYS

Moths per cage ^b	Dosage (krad)	Avg. F_1 adults per tray	% reduction	$F_1 \delta : F_1 \varphi$
50 I ^c ♂ + 50 I♀ + 5 N ^c ♂ + 5 N♀	40	119	61	1 : 0.8
50 I♂ + 50 I♀ + 5 N♂ + 5 N♀	25	59	81	1 : 0.5
5 N♂ + 5 N♀	0	308	-	1 : 0.9

^a Gamma irradiated in air at 2°C.

^b Two cages per treatment.

^c I = irradiated; N = nonirradiated.

TABLE III. F_1 ADULTS PER CAGE AFTER STERILE (50 KRAD) OR PARTIALLY STERILE (30 KRAD) CODLING MOTHS^a WERE CONFINED WITH FERTILE MOTHS OVER DWARF APPLE TREES IN AN ORCHARD

Moths per cage ^b	Dosage (krad)	F_1 adults	% reduction
65 I ^c ♂ + 65 I♀ + 5 N ^c ♂ + 5 N♀	50	21	66
65 I♂ + 65 I♀ + 5 N♂ + 5 N♀	30	12	80
5 N♂ + 5 N♀	0	61	-

^a Gamma irradiated in CO₂ atmosphere at 7°C.

^b Four cages per treatment.

^c I = irradiated; N = nonirradiated.

The competitiveness of sterile and partially sterile moths was also compared in caged trees. The moths were chilled to 7°C and exposed in a carbon dioxide atmosphere to 30 or 50 krad. (Under these conditions of treatment, 30 and 50 krad give about the same degree of sterilization as 25 and 40 krad in air at 2°C.) One hundred immature apples were tied to the branches of each tree, and the irradiated moths introduced into the cages with nonirradiated insects at a ratio of 15 irradiated male and female moths to one untreated male and female. About 4 weeks later, just before the developing larvae had reached maturity, the fruit was removed from the trees and held in the laboratory until the insects had reached the moth stage. It was found (Table III), as in the previous experiments, that the insects irradiated at the higher dosage were less competitive than those treated at the lower dosage. Cages that initially contained moths exposed to 50 krad produced 1.7 times as many F_1 adults as the cages that were loaded with moths exposed to 30 krad.

These preliminary findings with partially sterilized male codling moths are certainly promising, but the truly significant result will come this fall after the moths are released in orchards for 1 year.

FIELD EXPERIMENTS ON CONTROL

Release of sterile males alone in an abandoned orchard

Only male moths were released in this first field experiment on codling moth control by the sterility procedure [10]. The orchard consisted of 20 abandoned apple trees in which at least 80% of the fruit was injured each year by this pest. To reduce the moth population to a manageable level, the orchard was sprayed with DDT 1 year before adult release. The insects were sterilized by exposing the pupae about 2 h before eclosion to 40 krad of gamma radiation from ^{60}Co . They were irradiated in a ventilated cannister at a temperature of about 15°C. Approximately 2 h after eclosion the moths were marked with zinc 8-hydroxyquinoline and taken to the orchard where equal numbers were placed in each of 20 release stations, one station per tree. In this, and in all future experiments, the first moth release each year was made at the early pink-bud stage of McIntosh apple. As a rule three releases were made per week, with the last release of the season occurring during the 3rd week in September when codling-moth flight virtually ceases. When sufficient moths were available, the rate of release was adjusted to the rate of normal moth emergence in the field.

After 1 year of sterile-moth release the codling moth population at harvest, as determined by examination of all harvested and windfall apples, had increased to 957 diapausing larvae from an estimated 400 larvae the previous autumn. The increase was attributed to a shortage of sterile moths during peak emergence of 1st brood adults. On the basis of trap records the ratio of sterile to fertile males during this period was approximately 8 : 1 instead of the intended ratio of at least 20 : 1. The 8 : 1 ratio was not high enough to induce a downward trend in the wild population, but it did suppress reproduction, for the numbers of codling moth in nonsprayed orchards in British Columbia may increase 20-fold or more within 1 year.

During the 2nd year of release the ratio of sterile to fertile males, according to sex-trap records, never fell below about 20 : 1 and was usually much higher. Every effort was made to keep the ratio high during peak emergence of the 1st brood moths, for this is the period when the sterile moths are likely to have the most influence in suppressing reproduction. Dissection of reproductively old moths trapped in British Columbia orchards showed that 1st brood females mate an average of 1.1 times whereas 2nd brood females mate about 2.3 times. Because of the predominantly monogamous habit of 1st brood females, sperm from irradiated males seldom have to compete with sperm from wild males during spring and early summer; competitiveness then is based essentially on mating ability. Later in summer, the effectiveness of sterile males is reduced because of the polygamous nature of most 2nd brood females, for females that mate both with sterile and fertile males produce mostly viable eggs. The effectiveness of sterile moths during the 2nd brood is further reduced because of the normally high reproductive rate of the codling moth during mid summer.

At the end of the 2nd year of release the numbers of larvae capable of overwintering were reduced to 43. During the 3rd (final) year of release more than 2000 male moths were trapped in the orchard, but only one of these was a wild moth. Fruit examination at harvest confirmed this very successful control, for only six apples were found with exit holes from 2nd brood larvae. There is a good chance that the insect would have been completely eliminated if the orchard had been further removed from surrounding sources of reinfestation. Records of moths captured just outside the experimental orchard indicated that fertile moths were moving into the area from other apple orchards about 1 km away.

One year after the release program was discontinued the moth infestation had increased to the point where about 10% of the apples were injured at harvest. The following year the injury had increased to approximately 80%.

Release of mixed sexes in an abandoned orchard

Laboratory and field cage experiments [12-14] indicated that the addition of sterile males to a population of untreated moths suppressed reproduction somewhat more effectively than the addition of sterile moths of both sexes. However, separation of the sexes is very time consuming because no one has developed a good mechanical separator. Segregation of pupae at the correct stage of development for irradiation is another slow procedure. Consequently, it was decided to assess the effectiveness of releasing male plus female moths sterilized as adults.

An abandoned 2-hectare apple orchard with a history of severe codling moth injury was selected [15]. Adult moths for release were prechilled to about 13°C and exposed to 50 krad in a carbon dioxide atmosphere. This treatment induced about the same degree of sterilization as 40 krad in air, the dosage and conditions of irradiation in the 1st field experiment. The methods of marking and release were the same as used previously.

After sterile moths were released for 1 year (2 generations) the numbers of larvae capable of overwintering had declined to 119 from an estimated 5000 the previous year. After the 2nd year of release the numbers of larvae capable of overwintering were further reduced to 55,

which represented 0.09% injured fruit. The reduction from 119 to 55 larvae after the release of 478 000 sterile moths in 1 year does not seem particularly encouraging. However, it must be remembered that when a population reaches a low level it requires only a few fertile females to maintain the population.

Trap records showed that the orchard was being reinvaded by fertile moths from unsprayed apple trees 1.5 km away. Because sperm of sterilized males were not competitive with nonirradiated sperm, wild females that mated with fertile males and subsequently invaded the test area were capable of maintaining the infestation despite the release of exceptionally large numbers of sterile males.

Control was very good, despite reinfestation, indicating that release of sterile males plus sterile females was a satisfactory procedure for codling moth suppression, but there were too many uncontrollable variables to say whether or not this procedure was as effective as release of sterile males alone. One point remains that is of some concern. At the end of the release program, the codling moth infestation was limited almost exclusively to one small section of the orchard. It is possible that where mixed sexes are released the sterile males may tend to mate with the nearby sterile females rather than seek out wild females in isolated pockets of infestation.

Release of mixed sexes in a commercial orchard

The previous field experiments showed that codling moth control could be effected by release of sterile moths in semi-isolated, abandoned orchards. Would this method work equally well in commercially operated orchards in which the usual cultural practices and pesticide programs, with the exception of codling moth sprays, were followed?

A 4-hectare apple orchard was selected which was closely surrounded by other apple and pear orchards [7]. Codling moth injury in these orchards was normally kept at about 0.5% to 1.0% damaged fruit at harvest by the use of insecticides. During the 1st year of the experiment the moths were released in 1.3 hectares of the test orchard; the remainder was sprayed with azinphosmethyl for codling moth control. Adult moths of both sexes were sterilized by exposure to 50 krad in a carbon dioxide atmosphere at approximately 17°C, marked with Day-Glo fluorescent powder, and released three times weekly from the pink-bud stage of apple until the 3rd week in September.

At harvest a sample of 90 700 apples in the release area was examined without finding any codling moth injury, though the fruit pickers did eventually find three infested apples. In contrast, approximately 0.5% of the fruit was damaged in the sprayed area. The exceptionally good control realized in the release area is attributed to the maintenance of a high ratio of sterile to wild males. Only for a few days did the ratio of sterile to wild males drop below 20 : 1; the average for the season was 280 : 1 on the basis of sex-trap records.

It should be noted that sex traps probably do not give the absolute ratio of sterile to wild males because many of the sterile males may be injured or affected in some way so that they are not attracted to females in the traps. It seems more likely that the sex traps reflect the effective ratio of sterile to wild males, and after all this is the important factor,

not the absolute ratio, in estimating the degree of control that will be achieved by the sterile males. With light traps the ratio of sterile to wild males is often different from the ratio obtained with sex traps. Because the stimulus for attraction is light rather than sex, it seems reasonable that some of the moths that are attracted to light may be completely unresponsive to the sex pheromone and consequently would be of little or no value in suppressing reproduction.

During the 2nd year of the program an attempt was made to lower costs by reducing the numbers of sterile moths to the minimum required for control. The numbers of release stations were also reduced from 58 to 12 per hectare. The release area was increased to 4 hectares primarily to make it easier to determine whether the 12 stations per hectare were sufficient to permit adequate dispersal of the sterile insects.

During peak emergence of the 1st generation moths the ratio of sterile to wild males, on the basis of sex traps, was 9 : 1. Shortly after this small numbers of larval entries were observed in the fruit. The 9 : 1 ratio evidently was not quite high enough to prevent some increase in codling moth numbers even at this time of the year when the reproductive rate of the insect is rather modest. For about 2 weeks in mid August, during the second generation, the ratio of sterile to wild males fell to 10 : 1. New larval entries soon appeared so the ratio was increased and maintained at about 40 : 1 for the rest of the season. Examination of all unmarketable apples at harvest showed that control was very good, for only 0.1% of the fruit was injured by the codling moth.

Sterile moth release was continued into the 3rd year, but two-thirds of the orchard had to be sprayed in July because of an accidental release of substerile moths. Release of sterile moths was continued in the remaining one-third of the orchard in sufficiently large numbers to maintain an average ratio of about 50 sterile: 1 wild male. At harvest 0.7% of the fruit was injured by the codling moth, but most of this was caused by larval progeny of the substerile adults released in July.

This accidental release of substerile females reduced the usefulness of the experiment, but it clearly indicated the inherent danger of releasing female insects and emphasized the importance of measuring the radiation dosage received by each lot of insects before they are released.

The results of this 3-year experiment indicate that the sterility procedure can be used successfully for codling moth control in commercial orchards even when chemical sprays are applied against other pests. The selection of the chemicals used for these other pests may influence the effectiveness of the sterile moths, but recent work at this laboratory and in the U.S. has fortunately indicated that irradiated codling moths are more resistant than the nonirradiated insect to some of the commonly used orchard pesticides.

Aerial release of medium-reared moths in a commercial orchard

In 1969 male and female moths, sterilized as chilled adults by exposure to 50 krad in a carbon dioxide atmosphere, were released in about 40 hectares of apple and pear trees and 8 hectares of apricots and cherries in preparation for an area-wide release (about 400 hectares) planned for 1971. The timing and frequency of release were as in previous experiments, but for the first time in British Columbia the moths were

released from a helicopter using small cardboard boxes as explained in an earlier section of this paper. Also for the first time, most (90%) of the released insects were produced on an artificial larval medium rather than on apples, providing an opportunity to determine whether the medium-reared moths would control a wild population of this pest under orchard conditions.

On the basis of sex-trap records, the ratio of sterile to wild males never fell below 35 : 1 except during the 1st 2 weeks of release when the ratio was 5 : 1. Control was very good for less than 0.05% of the fruit was injured by the codling moth at harvest. This represented better control than in adjacent chemically sprayed orchards. Most of the injury in the release area was confined to about 1.5 hectares in the south-west corner of the apple orchard, where approximately 1% of the fruit was damaged. Trap records indicated that this corner was being invaded by wild moths from semi-abandoned apple and pear trees 100 to 200 metres away. These trees were sprayed but control was inadequate because of poor spray coverage.

The release program this year (1970) is being continued in the same orchard, but with some modifications. The moths are discharged from a helicopter but without the protection of boxes or other containers. Instead of using carbon dioxide for immobilization, adults are now handled and irradiated at temperatures of 1-5°C. The orchard has been divided into two blocks. Moths released in the larger north block are treated with 40 krad; those in the southern block (4 hectares) with 25 krad. As stated previously 25 krad causes partial sterility in the male moth and complete sterility in the female.

The effectiveness of partially sterile males plus completely sterile females (25 krad dosage) is also being investigated in two other small blocks of apple and pear trees. Ground release stations are being used in one block and free aerial release in the other.

INFLUENCE OF STERILE CODLING MOTH RELEASE ON OTHER APPLE PESTS

This report would not be complete without a brief look at some of the changes that may occur in the density of certain orchard pests as a result of programs involving the release of sterile codling moths.

A commercially operated apple orchard, in which codling moth sprays were omitted for 6 years, was used to determine some of the good and bad features that could be expected from such programs [16]. Aphids and spider mites were generally held under control by parasites and predators. In some years the European red mite, Panonychus ulmi (Koch), was troublesome, but on some apple varieties it was not numerous enough to cause commercial damage [17]. In contrast, the fruit tree leaf roller, Archips argyrospilus (Walker), the eye-spotted bud moth, Spilonota ocellana (D. & S.), and the white apple leafhopper, Typhlocyba pomaria McAtee, soon increased to injurious numbers, and throughout the 6-year period of observation natural enemies were unable to bring these insects under control. This was not surprising for these three pests together with the codling moth are usually the most injurious species in abandoned apple orchards throughout the interior of British Columbia.

As might be expected, somewhat similar changes occurred in insect and mite populations in two commercial orchards under 1- or 3-year programs of sterile moth release. The apple aphid, Aphis pomi DeG., and woolly apple aphid, Eriosoma lanigerum (Hausm.), were of no significance. The European red mite remained at low population levels in one orchard; in the other it was necessary to apply one spray in each of three consecutive years. The McDaniel spider mite, Tetranychus mcdanieli McG., increased to harmful numbers in one orchard during the 1st year necessitating the application of a miticide; in later years it was held at noninjurious levels by predators, particularly Typhlodromus occidentalis Nesbitt. Rust mites were observed throughout each summer but were not numerous enough to require treatment. The fruit tree leaf roller quickly increased in both orchards so that one spray was required to control this pest during the 2nd year of sterile-moth release in both orchards. However, the eye-spotted bud moth and the white apple leafhopper did not increase as rapidly as anticipated. Chemical sprays were not specifically required for these pests during the 3 years of moth release in one orchard, and so far, after 1 year in the 2nd orchard, these insects remain at noninjurious levels.

There can be little doubt that the chief advantage in controlling the codling moth by the sterility procedure is the opportunity that it provides for the study and eventual adoption of nonchemical methods of control for other orchard pests. In the interim we must learn more precisely how to use chemicals for control of these other pests without causing unnecessary harm to beneficial species and without interfering with programs of release of sterile codling moths.

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CHEMOSTERILIZATION OF POTATO TUBERWORM MOTH, Phthorimaea operculella (Zeller)

II. Factors modifying the sterilizing effect of metepa *

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Abstract

CHEMOSTERILIZATION OF POTATO TUBERWORM MOTH, Phthorimaea operculella (Zeller). II. FACTORS MODIFYING THE STERILIZING EFFECT OF METEPA.

The influence of temperature, female sex pheromone and diurnal rhythm during treatment on sterilization of male potato tuberworm moths with metepa were investigated. The sterilizing effect of the sterlant could be considerably enhanced if the treatment is given in the presence of female sex pheromone and during periods of high biological activity. Complete sterility could also be induced even at low dosages by giving the treatment at high temperatures.

INTRODUCTION

Chemosterilants are gaining widespread acceptance as an inexpensive and mobile source for sterilizing insects. Among the chemicals that hold promise as potential insect sterlants, the alkylating agents like apholate, tepa, metepa, etc. have proved very effective in inducing sexual sterility in males. Toxicological information on the effects of insect chemosterilants on mammals and other life is, however, of vital importance before these chemicals can be used for sterilizing naturally occurring populations. Although the biochemistry and acute toxicity of some of the alkylating agents to higher animals is now known, the cumulative effects of chronic doses is not known. The unfavourable toxicity, qualitative and quantitative, of many alkylating agents calls for a close surveillance at the site of distribution and on the amount the insects carry. There is little doubt that only a small portion of the administered alkylating agent is involved in the actual sterilization process and the excess quantity is rapidly metabolized [1-9]. It would, therefore, be desirable that the insects be sterilized using the minimum possible chemosterilant concentration.

The sterilizing efficiency of a chemosterilant could be improved by modifying treatment conditions. With dry mixing, 0.1% apholate reduced viability of eggs to a level comparable to 0.25% apholate added to the diet in solution [10]. Effectiveness of apholate in sterilizing boll weevil was

* Chemosterilization of potato tuberworm. I. Effect of metepa on the fertility of males, to be published in the Journal of Economic Entomology.

improved when the sterilant solution was mixed with corn oil before preparing residual films [11]. Higher efficiency of fractionated treatment over a single-dose treatment was observed in the case of boll weevil [11] screw-worm fly [12] and housefly [13]. Extending the intervening period between treatment and mating enhanced the sterilizing effect of chemosterilants in carpenter worm [14] and bean weevil [15]. Prolonged exposure of adult gypsy moths to low concentrations of tepa or metepa was more effective than short exposure at higher concentration [16].

The uptake of a chemosterilant from residual film would be governed by its concentration in the film and the frequency with which the insects come in contact with fresh residue sites, the latter being largely dependent on the physical activity of the insect during treatment. It is well known that environmental and biological factors appreciably regulate insect activity. We, therefore, investigated the influence of temperature, female sex pheromone and diurnal rhythm on sterilization of male potato tuberworm moths with metepa.

MATERIALS AND METHODS

Moths were reared in the laboratory as described by Finney et al. [17]. To prevent mating before the treatment, insects were sexed as pupae and held in separate containers till adult emergence. In all the experiments freshly emerged moths were used. The residual films of metepa were obtained by placing, in 12-dram vials, 1 ml of acetone containing a requisite concentration of technical grade (92%) metepa and rotating the vials during evaporation of the solvent. The residues are expressed as microgram quantities of technical material per unit area of the treated surface. In each vial only 10 insects were kept during treatment.

Treated males were crossed, 4-6 hours after treatment, with untreated virgin females of the same age. Individual pairs were placed in glass specimen tubes (7.5 cm × 2.5 cm) containing a cotton wad soaked in 10% aqueous sucrose solution as a source of food. A piece of coarse cloth secured over the open end of the specimen tube provided a surface for oviposition. Eggs laid by each female and the number hatched were recorded. In all the experiments post-treatment temperature was $25 \pm 1^\circ\text{C}$.

EXPERIMENTAL

The influence of temperature during treatment was investigated by exposing males to metepa residues of 10.8 and 21.7 $\mu\text{g}/\text{cm}^2$ at 25, 30, 34 and 38°C. Moths used in these treatments were preconditioned for 16 hours to the respective temperatures.

For studies on the effect of female sex pheromone, male moths were exposed to metepa residue of 43.4 $\mu\text{g}/\text{cm}^2$ for periods ranging from 10 to 60 min during which a filter paper disc treated with 0.05 ml of ether extract of the pheromone was suspended in the vial after evaporating the ether. Female sex pheromone extract was obtained by homogenizing in 1 ml ether 50 abdominal tips of newly emerged virgin females. After filtration the supernatant, which contained pheromone, was used.

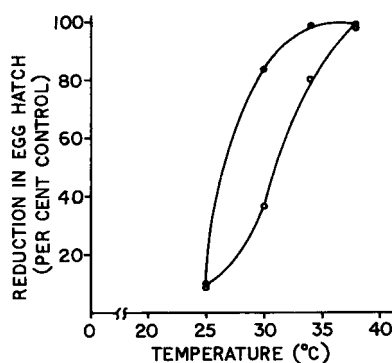


FIG. 1. Influence of temperature during treatment on the sterilizing effect of metepa on potato tuberworm moth. Males exposed for one hour to sterilant residues of 10.8 (o) and 21.7 (●) $\mu\text{g}/\text{cm}^2$.

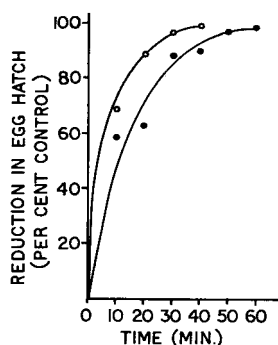


FIG. 2. Sterility induced in male potato tuberworm moth after exposure to metepa residue of 43.4 $\mu\text{g}/\text{cm}^2$ in the presence (o) and absence (●) of female sex pheromone.

The influence of biological activity was evaluated by exposing male moths for 1 hour at 5 a.m. and 7 p.m. to metepa residues of 10.8 and 21.7 $\mu\text{g}/\text{cm}^2$.

RESULTS

Higher temperature during treatment increased sterility at both metepa concentrations (Fig. 1). Exposure to the residue of 21.7 $\mu\text{g}/\text{cm}^2$ at 34°C induced near complete sterility in all the treated males and the same result was evident at one half this concentration when treated at 38°C. There was no difference in the degree of sterility when exposed at 25°C to either of the metepa residual concentrations. However, the increase in sterility at 30°C was many-fold, particularly with 21.7 $\mu\text{g}/\text{cm}^2$.

Exposure of males for any period to metepa residue in the presence of female sex pheromone resulted in increased sterility. Treatment for 30 min was needed to obtain near-complete sterility in the presence

TABLE I. STERILITY INDUCED IN MALE POTATO TUBERWORM MOTH AFTER EXPOSURE TO METEPA RESIDUES FOR 1 HOUR EARLY IN THE MORNING AND EVENING

Time of exposure	Reduction in egg hatch (per cent control)	
	Metepa residue ($\mu\text{g}/\text{cm}^2$)	
	10.8	21.7
5 - 6 a.m.	25.43	31.79
7 - 8 p.m.	3.08	20.94

TABLE II. EFFECT OF AGITATION DURING TREATMENT ON STERILIZATION OF MALE POTATO TUBERWORM MOTH
Males exposed for 1 hour to the metepa residue of $10.8 \mu\text{g}/\text{cm}^2$

Treatment condition	Reduction in egg hatch (per cent control)
Disturbed	51.44
Undisturbed	8.17

of the pheromone while in its absence double the period of exposure was required (Fig. 2). Treatment of males in the morning hours resulted in greater sterility as compared to the same treatment when given in the evening (Table I).

DISCUSSION

The increase in sterility induced following treatment at higher temperatures could be attributed to two factors: greater uptake of the sterilant and/or increased reactivity of target site. The former may be caused by either the direct effect of temperature on the permeability of cuticle or the enhanced physical activity of insects and consequent contact with fresh residue sites, or both. Both these factors have been shown to operate during enhanced insecticidal uptake at high temperatures [18-22]. Although literature on insect chemosterilants is lacking in information on the effect of temperature on their uptake, it would be reasonable to assume that the same factors operate in the case of chemosterilants also.

It is well known that males exposed to the female sex pheromone exhibit greater excitation and activity. This would explain the increased sterility when males were exposed to the sterilant residues in an atmosphere containing sex pheromone. Males of many insect species, particularly Lepidoptera, become active and take to flight only during certain hours of the day or night. Differences in sterility induced in potato tuberworm moth when treatments were given during morning and evening hours could, therefore, be attributed to differential activity.

In all these cases, increased insect activity was probably the major factor modifying the sterilizing effect of metepa. Evidence for this was provided by an experiment in which two groups of male moths were separately exposed to a metepa residue of $10.8 \mu\text{g}/\text{cm}^2$ for 1 hour during which insects of only one group were kept constantly active by gently rolling the vial. This treatment resulted in 51.4% sterility as compared to 8.17% in the moths left undisturbed (Table II).

The activity of an insect following treatment has been shown to enhance the metabolism of injected or absorbed chemosterilant. In male cabbage looper, *Trichoplusia ni* (Hübner), flight immediately after treatment with tepa markedly reduced the sterilizing effect, thus requiring a higher dose to produce the same degree of sterility [9]. This could be due to rapid metabolism of the sterilant absorbed. In the housefly, 50% of the ingested tepa was recovered after 8 hours when the flies were kept in narrow space, while the same amount was recorded even after 5 hours when the flies were provided enough space for normal activity [6]. In the present studies, increase in insect activity was only during the treatment and their post-treatment activity was normal. Therefore, any enhancement in sterilant metabolism as a result of increased activity would occur during the period of treatment. Since the duration of treatment was never more than one hour, the quantity of the sterilant metabolized would be expected to be considerably low. Furthermore, during this period uptake of the sterilant was also taking place simultaneously.

These studies clearly show that by enhancing physical activity during exposure to a sterilant residue, moths could be sterilized using a lower concentration than would normally be required. Although oral administration could be a convenient method for treating insects in large numbers, its inadequacy in obtaining uniform level of sterility has been demonstrated in a number of cases [15, 23-26]. In Lepidoptera, besides being inadequate, deleterious effects, such as reduction in mating frequency and adult survival, abnormal matings, reduced response to female sex pheromone, etc., have been shown to occur [27-30]. It would, therefore, be of interest from the practical point of view to investigate whether the efficiency of the feeding method could be improved by manipulating the treatment conditions.

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AN ATTEMPT TO ERADICATE
THE CORN EARWORM,
Heliothis zea (Boddie),
FROM ST. CROIX, U.S. VIRGIN ISLANDS,
BY THE STERILE-MALE TECHNIQUE

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Abstract

AN ATTEMPT TO ERADICATE THE CORN EARWORM, Heliothis zea (Boddie), FROM ST. CROIX, U.S. VIRGIN ISLANDS, BY THE STERILE-MALE TECHNIQUE.

Ecological studies as well as eradication attempts of the corn earworm, Heliothis zea (Boddie), were conducted on St. Croix, U.S. Virgin Islands. Population size and distribution for the species were determined by labelling a cornfield with ^{32}P and capturing adults emerging from the field in a series of traps. Population estimates indicated St. Croix as a suitable location for suppression programs. Two eradication attempts were made and both failed in the primary objective, but valuable biological and ecological information was obtained. Many problems were encountered in the 1968 attempt which led to the unsuccessful program. In 1969, these problems were minimized except for periodic slumps in our rearing and release programs. The result of high ratios of sterile to natural males was the elimination of oviposition rather than the production of sterile eggs. The overall effect was a cycling of the natural population of St. Croix with our Tifton rearing program.

INTRODUCTION

The successful eradication of the screw-worm, Cochliomyia hominivorax (Coquerel), from the southeastern United States created an intense desire to adopt the sterile-male technique to Lepidoptera. The Southern Grain Insects Research Laboratory has been involved with this concept for a number of years at our permanent location at Tifton, Georgia, as well as on St. Croix, U.S. Virgin Islands. The island work has involved an actual large-scale eradication attempt of the corn earworm, Heliothis zea (Boddie). This paper attempts to summarize the entire program with emphasis on those phases which may aid others involved in similar work.

GENERAL ISLAND INFORMATION

St. Croix is located 80 miles south-southeast of San Juan, Puerto Rico. Figure 1 shows its location in relationship to the United States and South America. St. Croix has a land area of 84 square miles (about 22 miles long with a maximum width of 6 miles), and the nearest land mass, St. Thomas, U.S. Virgin Islands, is about 40 miles to the north.

As a result of wide variations in annual rainfall and elevations, St. Croix offers a range of habitats. Mountains abundant with trees and shrubs,

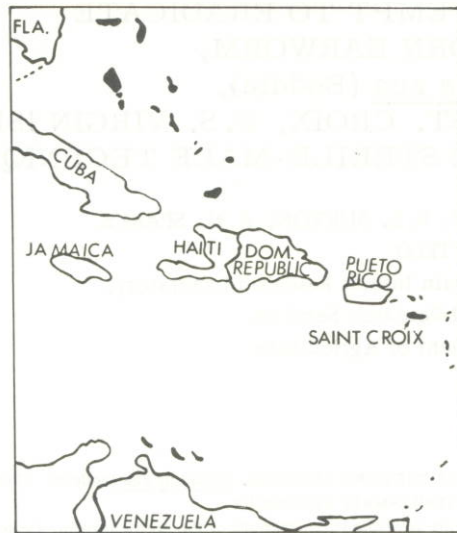


FIG.1. Location of St. Croix, US Virgin Islands, in relationship to the United States and South America.

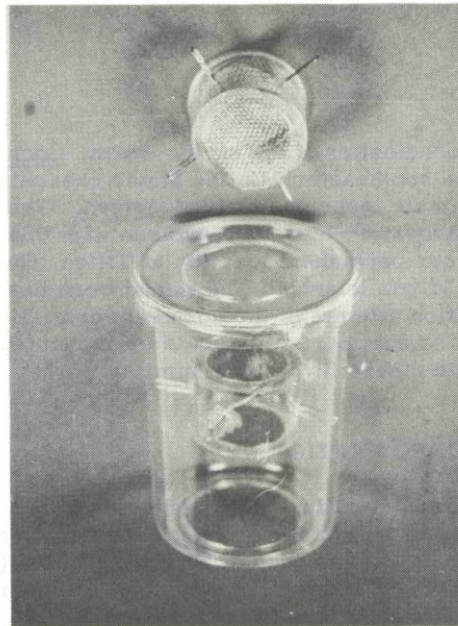


FIG.2. Virgin-female trap used to capture adult male corn earworms on St. Croix.

mountains covered with cactus and thorny bushes, and several rain forests are present as well as a vast plains area once rich in sugarcane production.

The concept of research on island locations is of such importance as to warrant some discussion. Many islands, and particularly St. Croix, have the unique characteristics of a small land area and, for all practical purposes, an ecologically closed population. These factors permit types of research that can never be accomplished under mainland conditions. The ocean on four sides provides a useful and meaningful boundary. In this situation, sampling of the entire ecosystem and observations of man-made or natural phenomena affecting insect populations, make interpretation of results more feasible.

The greatest disadvantages with remote research locations are the difficulties in procurement and lack of technical skills. Some caution must also be taken in applying data collected from islands to mainland locations. However, the assets of island research far outweigh the disadvantages, and the probability for the future is more work on island locations.

Active work has taken place on St. Croix during the last three summers for periods of 3 to 5 months. The first summer was devoted to ecological studies and the last two were actual eradication attempts using the sterile-male release concept. Much of the published data concerning the induced sterility method of insect control has been theoretical or speculative. In an actual eradication attempt many problems of gross ecology and method development were encountered that needed practical answers. As known by those with experience, theories, once they enter the field, often become subject to change. In this paper these areas will be emphasized.

DISTRIBUTION AND POPULATION LEVEL

The principal target species in all our eradication work has been the corn earworm and the island ecology of this insect was totally unknown. The immediate problem was to determine if it was present on the island and if so, what host plants, distribution patterns, population levels, etc., the species had under the island situation. The methods used in answering these questions, with modification, will also answer the same questions for other species in other locations.

The corn earworm was definitely established on the island as shown by the collection of larvae from corn. An adult survey system was immediately established consisting of 32 traps baited with virgin females and placed randomly over the island. The type of trap used (Fig. 2) consisted of a quart plastic container with holes cut in each end and lined with a thin layer of Stikem. Live females were held in a plastic cage secured in the trap with paper clips. Comparative data collected at Tifton had shown these traps to be up to five times more efficient than light traps in capturing males during periods of low natural populations (Snow 1970). The adult trap also had the advantage of low cost, easy operation, small size, and no dependency on electricity. This system was operated in all years and provided readily available data on trends in the natural population. Details of this survey may be obtained by consulting a paper by Snow et al. (1968).

While survey-type information is useful, it does not answer the question of population levels. This was accomplished by radioactive labelling a

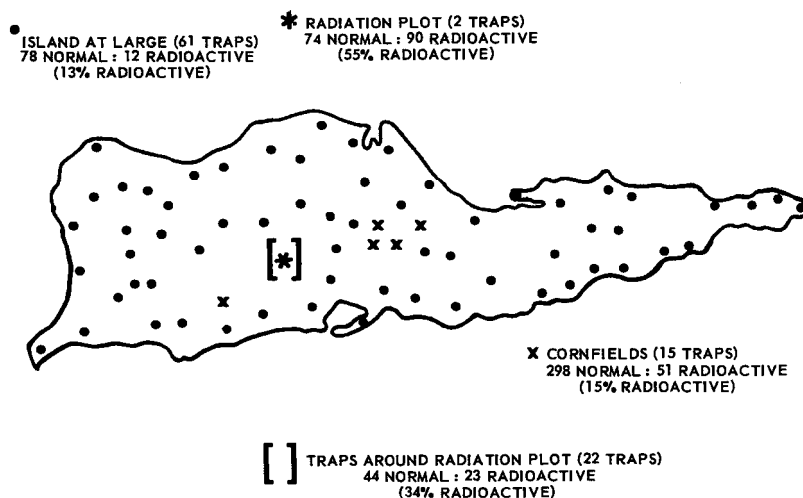


FIG. 3. Captures of radioactive and natural corn earworm males from various trap locations on St. Croix.

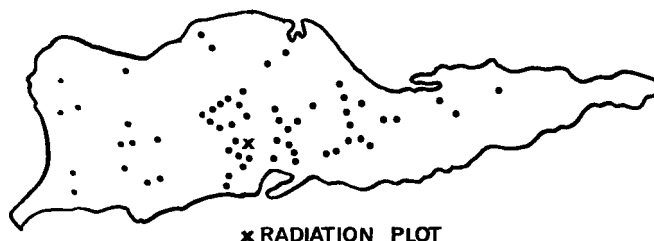


FIG. 4. Distribution of radioactive corn earworms collected from light traps on St. Croix. Approximately three traps per square mile with only positive capture locations plotted.

small cornfield (58 ft X 220 ft) located in the south central portion of the island. Each corn plant was injected with 82.5 microcuries of ^{32}P delivered with a microsyringe. The natural earworm population subsequently developing on the corn was radioactive. Based on sampling, 6384 adults emerged from the field during a 32-day period. One hundred virgin-female traps were maintained during this period in cornfields, around and in the radiation plot and over the general island terrain. General trap locations and captures are shown in Fig. 3. Based on these data, an island population of 12 768 to 19 152 males was calculated, for the 37-day period of radioactive male captures. On a day basis, this reduces to 345 to 518 males entering the population daily. The estimate represents the emerging or potential natural population since mortality factors were not taken into account.

A surprising finding was the high degree of concentration of adults around cornfields which is also shown in Fig. 3. Only five fields,

representing about 6 acres, were available other than the radiation plot, but 79% of all normal male captures and 87% of all radioactive male captures were in these small fields (exclusive of capture in the radiation plot and adjacent traps). This high level of concentration around a single host species, if true of the United States situation, may offer the only hope of eventual eradication.

A better estimate of the distribution of the corn earworm from the radiation plot can be obtained by viewing captures from 250 light traps (approx. 3 miles square) operated by the Vegetable Insects Research Branch, Agr. Res. Serv., USDA, with the major purpose of trapping tobacco hornworms, *Manduca sexta* (Johannson). These data are shown in Fig. 4 and a total of 128 radioactive earworms were collected from all areas of the island except the east end and portions of the northwest mountains. This distribution pattern indicates that the earworm is capable of moving throughout the island without regard to wind direction or terrain.

In summary, the island was found to be an excellent location for an attempted eradication program. The earworm was established on the island at a low level and concentrated heavily around a single host species. In addition, a similar pattern of distribution was shown for the sugarcane borer around cane fields while the fall armyworm and tobacco budworm were found to have wide distribution patterns and to attack many hosts. The latter is also true for the earworm at other times of the year. Complete details may be obtained in a paper by Snow et al. 1969.

Special considerations

With these data collected, our next steps were to prepare for an eradication attempt during the same months of the following year. Several things became obvious at this time. In projecting rearing needs, our question was not the natural population level, but rather the numbers of laboratory insects needed to achieve the desired ratio. The sterilization process itself is well established in most Lepidoptera as affecting competitiveness. Equally great, but more often forgotten, is the variation in competitiveness due to the colonization and subsequent rearing under laboratory conditions. Thus, our method, while it did prove correct, did not utilize the real situation of laboratory-reared sterile insects.

The system of release is equally important and affects the numbers of insects needed to do a particular job. In our original estimate, the radioactive insects distributed themselves from one point throughout the island. The furthest capture from the radioactive cornfield was approximately 11 miles, so these insects received maximum pressure from mortality factors working in the environment. In our release program, up to seven release sites were utilized with more releases in area of high natural population, which resulted in minimizing mortality factors. Thus, while our original estimate proved correct because of the offsetting factors, from a purely eradication standpoint, estimates would be more reliable and quickly obtained based on laboratory-reared sterile insects released in a similar manner as used in the actual program.

The dosage of radiation necessary to achieve sterilization of males was equally affected by verification with a natural population. The realistic situation was laboratory sterile males mating with native females. In this combination, it was necessary to increase the dosage by approximately

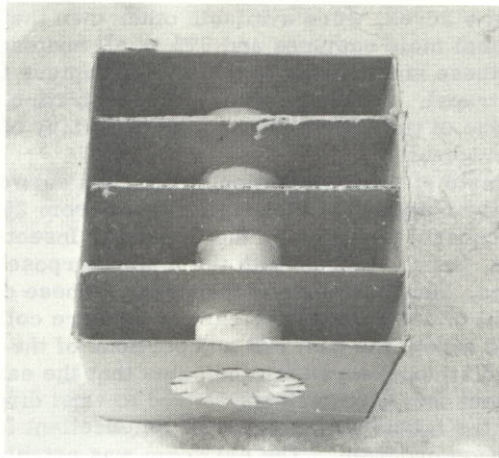


FIG. 5. Container used to ship corn earworm pupae to St. Croix.



FIG. 6. Release cage used for sterile males of the corn earworm on St. Croix.

3000 rads to achieve comparable results with laboratory sterile males × laboratory female pairings. This alternation of dosage could be particularly important in consideration of substerile release programs.

ERADICATION ATTEMPTS

Methods

The insects to be released on St. Croix were produced at Tifton and reared individually in 1-ounce plastic cups using CSM diet (Burton 1970). The cups of diet were infested with eggs, and pupae were machine-collected after 20 days. Production was to be maintained by infesting 30 000 cups per day with the expectation of releasing 5000 males per day on St. Croix. After collection, pupae were packed in cardboard boxes (2000 or 2500 pupae per box), filled with woodshavings and shipped via air mail to St. Croix. The shipping container is shown in Fig. 5 and had a hollow tube in the centre for dissipation of pupal metabolic heat.

Upon arrival, the pupae were placed in an emergence room and the moths were collected each morning, treated with 33 000 rads of radiation, marked with Rhodamine B, and taken to release sites. Our data showed that pupae could not be treated because the range of emergence for insects isolated on the same age was 9 days. This would have necessitated treating many young pupae, which are extremely sensitive to radiation.

The release cage shown in Fig. 6 was constructed of $\frac{1}{2}$ -in. outdoor plywood with two sides having a double thickness of wire screen. The inside screen was permanently fastened $\frac{1}{2}$ -in. hardware cloth, and the outside screen was a 18 × 24 mesh wire hinged door.

Treated insects were placed in the release cage in the early morning to become acclimated to the outside environment and were released between 7 and 8.30 each evening. Seven release sites were established and most areas of the island were within a 3-mile radius of a release cage. More moths were released in areas of high natural population.

The importance of release site location was shown to us on the north shore of St. Croix. This area is isolated by a series of mountains and ratios of sterile to fertile moths were always lower. To adjust the ratio by releases on the south side of the island (approximately 3 miles away) would require thousands of moths daily while a few hundred accomplished the task with a release cage established in the area. The 32 virgin-female traps previously mentioned as well as 10 to 20 additional traps in cornfields were maintained during the sterile-male releases.

The former traps were permanently located whereas the cornfield traps were rotated as a field of corn matured and other fields became attractive. They were serviced, and data collected on Monday, Wednesday and Friday. Captures were used to determine the ratio of marked sterile males to natural males.

Results

Details of the eradication attempt on St. Croix have been prepared for publication and will be released as a USDA Production Research Report in the near future (Snow et al. 1970a). The 1968 attempt was doomed almost

in the beginning to failure in that a late start and insect production and shipment problems developed. Significant ratios of sterile to natural males were never obtained on the island. This conclusion was easily accepted, since natural females were readily caught in light traps and shown to be fertile. Large samples of eggs were obtained from silks and they hatched. Therefore, the discussion will be limited to the 1969 work.

The ecological habitat of St. Croix was more conducive to an eradication program in 1969. The survey with virgin female traps was begun on April 1, and the first sterile releases were made on April 16. Maturity of a 10-acre cornfield in late March resulted in high captures (St. Croix standards) of natural males in the early part of April. After mid-April the population appeared lower than at any time during the previous 2 years. No more than 3 to 4 acres of corn were available at any one time from this date until late July. The striking concentration of adults within the limited corn acreage was noted again.

Most of the shipment and production problems encountered in the first year had been solved except for periodic slumps in insect production. During these slumps, the insects that were produced had a high mortality in all stages and were low-quality insects that produced very few eggs. The rearing slumps generally lasted 3 to 4 days but affected the numbers of males released 1 month later for 7 to 10 days. They occurred at approximately 1-month intervals and the final one was so serious that it became impossible to continue the project. The cause of the decline in production was not determined, but it may have been related to a non-inclusion virus or a particular virulent strain of *Nosema*.

Other than during the slump periods, 1100 to 5000 males were released daily and those were sufficient to produce ratios ranging from 20:1 to 50:1 sterile to natural males. The result of the high ratios, except for the monthly slump, resulted in cycling of the natural St. Croix population with our Tifton insect production. When production was going well at Tifton, the island population was controlled. During the slumps, the natural population reproduced unmolested. However, an interesting phenomenon was noted that caused much concern when first observed. The collection of eggs from corn plants and subsequent observations of viability were planned as the evaluation system. However, the observed phenomenon was the reduction or total lack of eggs during high ratio periods and a resumption of egg production during the slump.

Ratios of 20:1 appeared to be the breaking point. Below this level eggs were collected from silks while they quickly disappeared if ratios reached and exceeded this level. Evaluation proved extremely difficult because of the low natural population and lack of oviposition. Additional female traps were operated to check the validity of ratio estimates established by the survey system and these were found correct. Battery-powered light traps and virgin-female traps were operated in the same cornfields to compare their ratio estimates. If our females used as bait were not attractive to the native populations, then the estimates would have disagreed. Results showed similar ratios.

The real proof of the low natural population was established through operating the 250 light traps previously mentioned. Approximately three traps per square mile were located throughout the island. None were in or adjacent to cornfields. During a 1-week period the light traps captured 3 natural males, 125 sterile males, and 2 females, whereas virgin female

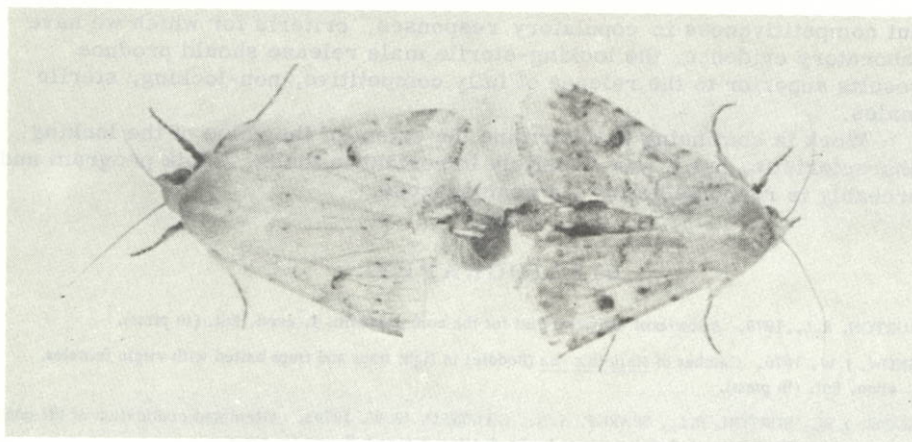


FIG. 7. Male and female moth that failed to separate after copulation.

traps caught 2 natural males and 172 sterile males. The ratios were 42:1 and 86:1, respectively. If catches in virgin-female traps near release cages are eliminated, the ratio reduced to 47:1, which is comparable to 42:1 established with the light traps. The low numbers of natural insects captured in the two types of traps adequately proved the validity of ratio estimates and the low density of the natural population.

However, these data did not completely account for the lack of oviposition during periods of high ratios. Natural females for mating to laboratory-reared sterilized males were obtained by collecting ears of corn with final instar larvae. In this manner, 7 natural virgin females were obtained and caged with 5 laboratory-reared sterile males. Four of the females locked in copulation and produced no eggs, 1 female did not mate, 1 mated but did not lay eggs, and the 7th mated and laid 200 eggs, which did not hatch. Figure 7 shows a pair of moths that failed to separate after mating. If these results occurred in the field, then the sudden termination of egg production rather than production of sterile eggs is understandable. Actually, such a phenomenon works for, rather than against, an eradication attempt.

In a later test at Tifton, it was determined that over 60% locking was occurring in crosses between sterile males and natural females (Snow et al. 1970b).

From a rearing standpoint, this causes great problems in that rearing costs rise. However, from an eradication standpoint, the characteristic may be very beneficial in the multi-mating corn earworm. Competitiveness of the species is greatly reduced because of radiation damage and rearing procedures, but the characteristic of locking partially overcomes this barrier. Locking destroys a reproductive female so that sperm level competition is no longer a factor.

Thus, when males of a multi-mating species are released at a 9:1 sterile to normal ratio and 60% of these males lock during mating, a tremendous advantage is gained. Assuming noncompetitiveness of sperm

but competitiveness in copulatory responses, criteria for which we have laboratory evidence, the locking-sterile male release should produce results superior to the release of fully competitive, non-locking, sterile males.

Work is continuing to determine the extent of the value of the locking characteristic, but it was definitely important in the St. Croix program and probably is responsible for the near success.

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CURRENT WORK ON GENETIC CONTROL OF Carpocapsa pomonella

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Abstract

CURRENT WORK ON GENETIC CONTROL OF Carpocapsa pomonella.

Reasons are mentioned which justify the study of the sterile-insect release method for codling moth under local conditions. Main topics of the work are: Rearing procedures on agar-free diets, exclusion or suppression of granulosis, quality control by recapturing males with sex traps, study of time of activity of adults, and population assessments in experimental orchards. Exchange of information is maintained by a working group of OILB.

INTRODUCTION

A limiting factor for the success of integrated pest-control systems in our apple orchards is certainly the codling moth. Apart from apple scab and apple mildew this species remains a key pest, although populations are lower than in warmer climates. (In the eastern part of Switzerland untreated trees have usually not more than 15% and seldom more than 30% of fruit injuries due to codling moth.)

By combined efforts of extension entomologists and growers it has been possible to limit considerably the number of insecticide treatments. However, the sampling of the different stages of the pest is so difficult that some preventive sprays have to be maintained and a search for alternative methods to chemical control becomes imperative (Wildbolz 1970). Because of these reasons interest for the sterile-insect release method is growing also in our country (Fossati et al. 1968, Murbach 1970).

The sterile-male technique has been studied with promising results in the northwest of North America (Proverbs et al. 1969, Butt 1967). How do the conditions of this area compare with ours? In British Columbia and Washington State the weather is favourable for the species during the summer months, whereas it is often marginal in our region. As a consequence, oviposition is not so favoured and there is practically just one generation per year. Population levels remain comparatively low and do not rise fast from one year to the other, facts which are positive in our context. During rainy periods adult mortality is high and the activity of surviving moths is interrupted. In a release program the demand for sterile moths will become very uneven and will not be predictable in a useful way over the season. It is not known if there are differences in the behaviour of moths between our region and the study areas of North America which will influence release programs. We just mention dispersal, searching of the other sex and copulation. All these

points are reason enough for studying genetic control of the codling moth under local conditions. By-products of these investigations will be so important to justify the exercise.

Similar considerations have led other European institutes to the same conclusion. Genetic control of the two tortricids Carpocapsa pomonella and Adoxophyes reticulana is at present being studied in Austria, France, Germany, Hungary, Italy, the Netherlands and Switzerland. A regular exchange of information is maintained by a working party of OILB with the help of the Joint FAO/IAEA Division of Atomic Energy in Food and Agriculture. In 1969 a meeting of about 30 investigators was held in Wädenswil and Changins where preliminary results and current programs were discussed (Working Group 1969).

REARING

The codling moth has been reared in our laboratory since 1963 (methods in Wildbolz and Riggenbach 1969). Genetically fixed diapause, which amounts to about 50% in field-collected material, was practically eliminated by selection.

At the beginning, larval food consisted of apples; since 1968 artificial diets have also been used. Under the conditions of our laboratory the best results were obtained with agar-free media, especially a modification of the formula of Brinton et al. (1969). In order to lower mortality of young larvae we had to reduce the water content of the formula by about 20%. Addition of peptone or safflor-oil resulted in adults of larger size.

Care had to be taken that the sawdust included in the diet was free of insecticides which originate from chemical control of bark-beetles by foresters. Moulds practically do not appear on the diets in the first two weeks of rearing due to the antimicrobials included in the formula. This danger becomes much more acute at the end of the four-week period of larval and pupal development. (Most larvae pupate inside the diet.) Better sanitation and superficial spraying of antimicrobials helped to reduce such infections.

A few months ago we were faced with a serious problem, too familiar to other laboratories, of the introduction of the granulosis virus. Losses were heavy, especially on artificial diets. We hope to master this difficulty, but we have learned that a regular production of codling moth will depend a great deal on the possibility to exclude or to suppress this agent.

In the oviposition chamber the air humidity has to be kept high, especially for field-collected material not yet adapted to laboratory conditions. Feeding liquid water also proved to be important. Experiments on the influence of nutrients on adults were started based on the data of Navon (1968). Sucrose as well as honey increased oviposition, whereas the vitamins used by the author did not result in an additional increase (Ulrich 1970).

DIAPAUSE

A good knowledge of the diapause conditions of field stock and laboratory strains is important for continuous rearing and for stockpiling of full-grown larvae. Results of earlier experiments have been

published (Wildbolz and Riggenbach 1969). In further experiments the effect of different cool temperatures on the termination of diapause was studied. It could be shown that keeping larvae for 2-4 months at 2°C was ineffective, whereas 4, 6, and 9°C terminated diapause.

STERILIZATION

Sterilization of adults was accomplished by standard procedures, i.e. irradiation with gamma rays of 40 krad. The moths were inactivated by temperatures of 6-8°C.

QUALITY CONTROL

Competitiveness of laboratory reared and eventually also of irradiated moths with material from the field was compared by different methods. Experiments in field cages with a ratio of 1:10 normal to sterile moths gave a reduction of fertile eggs. Results were, however, difficult to interpret because bad weather often inhibited oviposition.

Flight activity of males and their attraction to females was checked by recapturing released moths in sex traps. In an apple orchard traps were located in circles of 25 metres (4 traps) and of 50 metres (8 traps) around the central release point. The recapture ratio was always high (more than 20%, mostly between 50 and 80%) and was practically equal for the field stock and for the laboratory strain reared on apple or on the diet.

A modified flight mill (Boller, in preparation) will be used for estimating the fitness of the moths. Preliminary results showed high distances travelled by adults. However, comparisons between different material were not yet possible.

BEHAVIOUR

Earlier investigations were centered on the orientation of females before oviposition (Wildbolz 1958) and on the dispersal of adults (Wildbolz and Baggiolini 1959). Further work is planned on both these investigations.

In 1969 the above-mentioned experiments for the recapture of released males by sex traps enabled us to make additional observations on flight behaviour. In the experimental orchard captures were checked every hour on six evenings in 1969 (27/30 June, 1/28 July, 4/5 August). The results of five evenings were quite similar. A definite peak occurred during twilight between 20 and 21 h with a total catch of 302 males (85% of the total catch). On 27 June, however, 71 males (93%) entered the traps between 19 and 20 h. One or the other of two factors might have influenced this early peak. On 27 June evening temperatures were lower than on the other days (20 h: 16.5°C compared with 19.2 - 25.7°C). Furthermore, weather was characterized by an imminent thunderstorm. However, rain did not start before early in the morning of 28 June.

Direct observation of traps showed that flight activity was even more concentrated than indicated by hourly records of catches. In some cases

90% of the males arrived within 30 min at a particular trap. These moths alighted onto the traps upwind in a straight line. Other moths leaving the orchard flew directly to a huge pear tree, obviously by visual orientation. The usual flight pattern of the adults is quite different. Most of the moths could be observed in their well-known zigzag flight with a tendency to follow the lines of the trees. Breezes had no obvious influence on flight direction. When the wind became stronger flying adults preferred the sheltered sides of the trees.

The observed peak of males caught at sex traps is certainly important information. However, further data are needed to understand this phenomenon and to judge how far it reflects normal events among adults in the orchard. Are these results really due to a maximum of activity of the males or to a concentrated pheromone production of the females? Active flights of males to light traps are certainly taking place in the later hours of the evening.

POPULATION ASSESSMENTS

Codling moth populations were sampled in three orchards which did not receive insecticide sprays during summer (2 or 1 year). This work was aimed to get information on population trends and immigration. In two of the orchards it serves also as a preparation for future release trials.

Sampling was concentrated on larval populations in summer and autumn. Sampling units consisted of four trees (10 - 26 units/orchard).

The orchard Uetwilen (1.3 ha) is quite isolated with the nearest other host plants in a well-sprayed orchard 200 m to the north. Larval populations in autumn amounted to 1100 in 1968 and to 1640 in 1969, i.e. below the economic threshold of about 1% of the apples harvested. The relative stability of the population in the absence of insecticide sprays shows clearly that conditions did not favour a numerical build-up and that immigration was negligible. If we tentatively assume an autumn and winter mortality of 50% (full-grown larvae searching cocooning sites and overwintering), adult populations will amount to 500 - 700.

The orchard Grabs (0.8 ha) is surrounded by unsprayed apple and pear trees. Upon ceasing insecticide treatments the codling moth population increased sharply from 470 larvae in 1968 to 2360 in 1969. This increase is obviously, to a large extent, due to immigration. Importance of neglected trees is illustrated by population counts. In 1968 such a tree had 1027 larvae, in 1969 it had 549 larvae (a decrease due to lower fruit number). In 1969 another high-yielding tree had 1022 larvae, about the same number as half of our study orchard!

The Marschlins orchard (0.5 ha) is well isolated to the east and south (nearest host-plants more than 300 m). The orchard is sheltered to the northwest by a 5-m high dam and to the north by a narrow band of woodland. Higher numbers of larval attacks in the west corner of the orchard indicate that immigration occurred from unsprayed pear trees behind the woodland at a distance of 150 m. The larval population amounted to 2950 in 1969. It was higher than in the other younger plantations. However, the crop was so important that the economic threshold was reached but not exceeded.

ACKNOWLEDGEMENTS

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RECOMMENDATIONS OF THIS PANEL

GENERAL RECOMMENDATIONS

Lepidopterous insects include many of our most important agricultural and forest pests. Their economic importance results from losses of agricultural commodities in the field and in storage. Even in highly developed countries vast amounts of food and fibre are lost each year because of lepidopterous infestations. These losses occur despite the best conventional control methods currently available.

The increasing concern of scientists and the public about the possible adverse effects of conventional chemical control measures such as (1) insect resistance to chemicals, (2) environmental pollution, (3) detrimental effects on beneficial arthropods and (4) legal restrictions limiting international trade of agricultural commodities with objectionably high pesticide residues, all demand increasing research on developing alternative methods of insect control.

The panel notes that investigations on induced sterility in numerous insect species during the past 15 years indicate that the sterile-insect release method is one of the most promising alternative control methods for obligate sexually reproducing insect species. Despite its theoretically general applicability to insect control the panel fully realizes that major research developments are required to refine the technique to make it economical for many insect species.

The panel believes that the use of the sterile-insect release method (SIRM) for the control and suppression of lepidopterous populations is potentially feasible and economical for many species. Research on the sterility principle for lepidopterous species is conducted in many countries and ranges from preliminary laboratory studies to pilot field release programs.

Pilot research programs in which sterile insects have been released into field populations of Laspeyresia pomonella (L.), Heliothis zea (Boddie), Manduca sexta (Johannson), Pectinophora gossypiella (Saunders) and Porthetria dispar (L.) have all resulted in measurable reductions in the rate of increase of these pests in their natural environment. However, the results also show that further studies are necessary for the commercial implementation of the sterility technique in these species.

The panel agrees that there is sufficient information on other species of Lepidoptera to justify the evaluation of the sterile-insect release method in small-scale field trials. It is our opinion that such tests should be conducted as soon as possible because of the urgent need for alternative methods of controlling lepidopterous pests.

In addition to the five species noted above, research on the application of the sterility principle is currently being conducted on many other lepidopteran species, such as:

Grapholitha molesta (Busck)
Adoxophyes orana (F. R.)
Cadra cautella (Walk.)
Diatraea saccharalis (F.)
Ostrinia nubilalis (Hübner)

Spodoptera littoralis (Boisduval)
Heliothis virescens (F.)
Trichoplusia ni (Hübner)
Spodoptera frugiperda (J. E. Smith)
Chilo suppressalis (Walk.)
Tryporyza incertulas (Walk.)
Eucosma schistaceana (Snellen)

In most cases these are important or so-called "key" insect pests for which effective control techniques are unavailable or populations are currently prevented from developing to devastating numbers by multiple chemical applications.

The panel stresses the importance of concentrating research facilities on such species which seem to be most appropriate for the method. Sufficient data on the economic status as well as on the ecology have to be present before extensive research programs are started. Sound ecological methods have to be developed during the preparation of a release program. They are essential for the proper execution of a project and for an objective evaluation of the results.

The release of sterile insects to suppress and control natural populations of lepidopterous insect pests is compatible and complementary with many other weapons available to entomologists working on control methods for these insects. In many cases high insect populations may demand integration of the sterility method with the use of chemicals, pathogens, attractants, parasites, predators and other cultural, mechanical and bio-environmental methods to achieve efficient systems of insect population management.

The panel notes that mass rearing of lepidopterous insects (see further below) represents one of the most difficult obstacles to the immediate field testing of the sterility principle for a great many of these pests. However, it strongly emphasizes that some insects affecting stored products are easy to mass rear and, therefore, should be ideal candidates with which to study the application of the sterility principle. Further, these insects may be appropriate species in which to test modifications of the sterility method. The information gained in these studies, for example with Cadra cautella, would be extremely useful for the future application of the sterility principle for the control of other lepidopterous species.

Based on these factors and on the scientific data presented and discussed during the meeting the panel recommends that:

- A. The Joint FAO/IAEA Division should continue to make its varied resources available to scientists in all member states especially in the area of advising on the feasibility of application of the sterility principle for insect control.
- B. The present FAO/IAEA Joint Division's projects on the Heliothis complex, the rice stem borers, the codling moth, and the gypsy moth, be continued. Basic information gained on the biology of these insects would provide information that would be useful for a wide range of integrated control methods including the sterility technique.
- C. The FAO/IAEA support the program for the control of Cadra cautella in Ghana using the sterile-male technique (see paper by Amuh, these Panel Proceedings). This insect appears to be a very favourable

- candidate as there is already a considerable amount of information on its biology, and the simplicity of the storage practice for cacao beans suggests that control of the species by the sterile-male technique would be comparatively simple and economical. If successful in cacao storage this species might also be controlled in date storage.
- D. In view of the economic importance of the rice stem borers, as soon as laboratory handling of these species permits, research on sterilization should be further developed as rapidly as possible. The panel notes and endorses the current FAO/IAEA sponsored research work on rice stem borers.
 - E. Recognizing the valuable role of the Seibersdorf Laboratory in providing training in the principles of sterilization of lepidopterous species, we recommend that for training purposes, the Laboratory maintain also a species of Lepidoptera that is relatively easy to rear.
 - F. Radiation sources be loaned to scientists in developing countries in order to stimulate and facilitate their investigations in insect control and suppression.
 - G. The Agency maintain a contingency fund of \$1000/year to be used to supply small amounts of special materials to researchers attempting insect rearing and ecological studies in developing countries where there may be special problems of supply.
 - H. National commissions concerned with the application of atomic energy and agricultural research institutions should co-operate closely in support of studies leading to developing sterility methods for the control of serious agricultural and forest pests.
 - I. Economic data which are required to assess the various control methods and plan long-range programs should be assembled. These data on many crops in various countries are desirable because the choice between various methods of insect control always depends on the comparative economics and associated environmental effects of these methods.
 - J. The professional and financial support of the FAO/IAEA should be on a long-term basis in order to enable scientists to progress to field trials when laboratory experimental results indicate that the technique can be applied on an area-wide basis.

SPECIFIC SUBCOMMITTEE RECOMMENDATIONS

Nutrition and mass rearing of Lepidoptera

The panel has identified disease problems associated with mass rearing as one of the major impediments to the successful application of the sterile-insect release method with many species of Lepidoptera. Frequently, the seriousness of this problem is not apparent while only small-scale rearing is conducted. Consequently, we recommend that:

- A. Area-wide suppression programs requiring large numbers of insects not be undertaken until serious disease and production problems are solved.

- B. The Joint FAO/IAEA Division act as a clearing house for all information pertaining to disease control, particularly viruses, in lepidopteran cultures.
- C. Research into insect disease prevention be encouraged and that the Joint FAO/IAEA Division support this research by providing research contracts and research agreements.
- D. The Joint FAO/IAEA Division schedule a panel of experts to consider the control of insect diseases and mass rearing of insects as soon as is convenient. This panel should be encouraged to consider how to disseminate information on the design and construction of insect-rearing facilities and the basic design (including specifications, mechanical equipment, wall and floor finishes, special equipment for diet handling and preparation) should be made available to all interested scientists.
- E. Scientists be encouraged to use a standardized and explicit statement when quoting the costs of rearing insects. We suggest that the cost analysis be based on one million acceptable insects and report separately (1) the cost of ingredients (detailed breakdown per ingredient) assuming efficient bulk purchase, (2) formulation costs (labour and clean-up) and (3) storage costs. This procedure would permit comparison of the economy and efficiency of various methods which is not possible at the present time.
- F. Wherever possible, concerted research efforts involving insect nutritionists and insect pathologists be initiated and directed toward the solution of insect mass-rearing problems.

Radiation sterilization of Lepidoptera

The panel recognizes that induced sterility in insects is intimately related to genetic damage in reproductive cells and that, at the chromosomal level, Lepidoptera differ significantly from most other insect orders.

Relatively high radiation doses are required to induce sterility in Lepidoptera. This increases the probability of physiological and somatic damage which may seriously alter the competitiveness of sterile insects. The degree of reduced competitiveness may often be much greater than that produced in the irradiation of insects of other orders. However, many of the causes of reduced competitiveness after irradiation have now been identified. Future studies with other species of Lepidoptera should progress much more rapidly.

The use of partially sterilizing doses of radiation to obtain more competitive insects and to take advantage of the phenomenon of inherited sterility in progeny of irradiated parents could be an important factor in population suppression programs involving Lepidoptera. Under certain circumstances the introduction of inherited sterility factors into a lepidopterous population (by releasing partially sterile males which produce totally sterile progeny when outcrossed to wild females) may be more advantageous than the release of completely sterile insects. This modification of the sterility principle has already been tested with several species in laboratory and small field cages with promising results.

We recommend that:

- A. Research efforts be expended on reproductive physiology, morphology of the reproductive tracts and behaviour of irradiated Lepidoptera.
 - B. The possibility of releasing partially sterile moths to achieve population suppression be actively studied. Release of partially sterile moths utilizing the sterility inherited in the next generation could provide an economical method of keeping a natural population at a level below which the species becomes an economic pest.
 - C. Investigators be guided by the following assessment: Lepidopterous species can generally be divided into three groups based on the amount of information available about the induction of sterility with radiation.
1. Species ready for field trials

Enough radiobiological information is available on the following species to warrant their use in sterile-release studies under field conditions:

Laspeyresia pomonella (L.)
Heliothis zea (Boddie)
Manduca sexta (Johannson)
Pectinophora gossypiella (Saunders)
Trichoplusia ni (Hübner)
Porthetria dispar (L.)
Heliothis virescens (F.)

Additional research recommended for these species is:

- (a) Studies of the competition in the field between irradiated and native moths;
 - (b) Determination of the ability of F₁ progeny from released partially sterile males to further suppress the population;
 - (c) Development of more efficient methods of releasing irradiated moths in the field.
2. Species requiring further study

Cadra cautella (Walk.)
 Sugar cane borer spp.
Spodoptera littoralis (Boisduval)
Phthorimaea operculella (Zeller)
Adoxophyes orana (F. R.)
Eucosma schistaceana (Snellen)

Essential additional studies are recommended as follows:

- (a) Competitive ability of sterile and/or partially sterile moths with normal unirradiated moths in laboratory and/or field cage populations.
- (b) Ability of laboratory-reared irradiated insects to compete with unirradiated field-reared insects.

3. Species of economic importance for which basic data are required

There are many species of Lepidoptera on which basic radiological information is not known. The panel feels that, in terms of economic importance, the rice stem borer complex has the greatest priority for initiating such research. The types of research needed on these insects, besides nutritional and ecological studies, are as follows:

- (a) Studies on reproductive biology including gametogenesis, morphology of reproductive tracts, sperm utilization and mating ability of treated males;
- (b) Sterilization procedures and suitable partially sterilizing doses to give F_1 sterility;
- (c) Determination of the somatic damage incurred at sterilizing and partially sterilizing doses.

Ecological studies

One of the main requirements for the successful application of the sterile-insect release method for the control of lepidopterous pests is a sufficient understanding of their ecological relationships. This information is necessary:

- (a) for the choice of a target insect
- (b) for the execution of the program
- (c) for the evaluation of the results

It should be emphasized that laboratory-reared insects are usually used in the sterile-insect release method. These must effectively compete with a natural population. Therefore, field behaviour of such insects has to be included in ecological studies.

The panel recommends that:

- A. The taxonomic position and the bionomics of the target species be known. Special consideration should be given to such aspects as reproduction rate, diapause, aestivation and host-plant relationships.
- B. Information be obtained on important aspects of behaviour, such as short and long distance movements and sexual behaviour.
- C. Appropriate methods be used for estimating numbers of the target insect in an area.
- D. Knowledge of the abundance of the insect in space (distribution) and time (fluctuation) be obtained.
- E. Efforts be made to understand the main factors determining and/or regulating insect populations in a given area. In this context appropriate meteorological records are essential.
- F. The initial and ultimate effect on the ecosystem produced by sterile-insect release programs must be considered.
- G. The FAO/IAEA support ecological studies of lepidopterous pests in relation to the sterile-insect technique.

RECOMMENDATIONS OF THE PANEL
AND RESEARCH CO-ORDINATION MEETING
ON ECOLOGY AND BEHAVIOUR
OF THE Heliothis COMPLEX AS RELATED TO
THE STERILE-MALE TECHNIQUE
Held in Bogotá, Colombia, 18-21 May 1970

The following recommendations from the Bogotá meeting are included here because of their particular relevance to the subject of the present panel.

I. The taxonomy of the Heliothis-Helicoverpa complex

Considering that the taxonomy of the Heliothis-Helicoverpa complex has not been studied in several countries in Latin America, the participants recommend the following: All present and future research contract and agreement holders on this pest complex, as well as all other entomologists interested, should collect material (adults and last instar larvae) and send it to the following two specialists: D.T. Hardwick (Entomology Research Institute, Dept. of Agriculture, Ottawa, Canada), and E.L. Todd (U.S. Dept. of Agriculture, Systematics Entomology Laboratory, U.S. National Museum, Washington, D.C.). It was further recommended that Heliothis be used as the generic name for all species within the Heliothis-Helicoverpa complex as long as U.S. National Museum specialists recognize this name.

II. Sampling methods

One of the most important aspects for the successful application of the sterile-insect technique as a method for control or eradication of insect pests is adequate information on the population size of a given species when it is at its lowest point. Sampling techniques have to be worked out or improved for this purpose. In addition, all true hosts (where the species can complete its life cycle) have to be known. It is therefore recommended that the distribution of true hosts be mapped in an area so that the sampling system can be determined. General recommendations for the best sampling technique will be drafted and should be followed by the group. All entomologists working within the co-ordinated research program on Heliothis will try to adopt this method at their institutions and/or in their countries. The results of its applicability will be discussed at the next research co-ordination meeting.

III. Population dynamics and seasonal abundance in all major hosts in large geographic areas

For any type of effective integrated control or pest management program on Heliothis, population dynamics and seasonal abundance should be determined by taking records of larval and adult populations in a given area. Larval population fluctuation on different true hosts with special

emphasis on the preferred hosts should be determined all year round. It was agreed that all data on larval and/or egg counts should be presented on a per hectare basis, even when determining population density on wild hosts (for sampling method, see point II). By studying these levels of population density for a number of years (at least for 5 years) the most appropriate time for sterile-insect releases and other methods of control can then be ascertained.

Light traps and other trapping devices (virgin female and sex attractant traps) should be employed for population fluctuation and migration studies. Moth flights in accordance with lunar cycles can then also be established for the Heliothis species (Heliothis zea is, for instance, more influenced by the moon in its flight behaviour than the Heliothis virescens species). When light traps are used it is recommended that more than one such trap be employed in the area of population fluctuation studies, as a certain trap in a section with insecticide-protected crops, for example, can influence to a great extent the moths captured. The following parameters should be taken into account as factors in evaluating and/or influencing catches with light traps: mean wind velocity and dominance, rain, relative humidity, temperature, accumulated solar radiation (heliograph) and lunar face cycle. Furthermore, light-trap catches should be correlated with egg counts on host plants. In the case of H. zea it should be emphasized that light traps catch only a small percentage of moths (probably only 5%) and should therefore not be used as a control measure for Heliothis species.

IV. Dispersal studies

There is little doubt that Heliothis can migrate or disperse long distances away from their original breeding grounds. Dispersal studies are of great importance in a sterile-insect release program as a method of suppression or eradication. During 1971 tests will be initiated by the Brownsville Laboratory, USA, releasing large numbers of colour-marked sterilized H. virescens adults in north-eastern Mexico in an attempt to follow the population into Texas and probably through the south-eastern United States. It is recommended to study the possibility of marking 1 or 2 hectares of corn using ^{55}Zn (corn seed to be soaked in a solution with this isotope) in El Salvador or in another Latin American country (southern Mexico, Guatemala, or Nicaragua) where cotton is protected against Heliothis by repeated applications of insecticides. Light traps should be put up in several strategic locations in Central America, Mexico and south-west United States, taking into account the dominant wind currents and other climatological factors during the time of the study. The collected biomass of traps can easily be scanned with Geiger counters for presence of radioactive material. Therefore, Heliothis dispersal or migration could be monitored. The data obtained would be important in a sterile-insect release program and in other aspects of Heliothis studies.

It could be of great advantage if sterility were incorporated into the dispersing population by the release of partially sterile males. It is recommended to study (a) if F_1 sterile males disperse as readily as normal males, (b) if they compete with them and can effectively mate with normal wild females, and (c) the effect of F_1 sterile females on the population.

V. Diapause and aestivation

It is recommended that all members of the co-ordinated research program on Heliothis, as well as other interested entomologists, ascertain if diapause exists in their country and when it appears. Very simple methods can be followed but care must be taken in keeping field-collected pupae under similar light, temperature and humidity conditions as in the field.

After discussing the seasonal Heliothis population variations in different countries, it was found that there seems to be an indication that aestivation in the form of a "summer diapause" occurs. It is recommended that a study in this respect be initiated in Peru, Venezuela and in Mexico.

VI. Rearing of the Heliothis complex

For several of the recommended studies rearing of Heliothis species is required (in order to obtain adults from field-collected material, for taxonomy, diapause studies, etc.). It would therefore be very useful if in each country a rearing facility were set up. The method to be used will be outlined pointing out the main precautions to be taken. An isolated room for mixing the diet is absolutely necessary, but with other precautions and cleanliness it is quite easy to rear Heliothis.

VII. Studies on parasites and predators

It is recommended that data on predators and parasites of larvae and/or eggs be taken whenever field inspections to evaluate population increase are carried out, or when live material is brought into the laboratory. Ecological conditions should be recorded. These data are of value for possible translocations of Heliothis enemies to other countries and for integrated control programs in a given area, including the sterile-insect technique. Data should be reported in percentage parasitism or predatism. In doubtful cases of predatism, live predators should be brought into the laboratory and fed on eggs or larvae in order to determine their habits.

VIII. Studies on plant resistance

Since plant resistance to insects is part of integrated control, and since the use of such varieties could be very useful in reducing the population of Heliothis or in follow-up programs after having used the sterile-insect technique, it is recommended that research contractors exchange material of corn, cotton and other Heliothis hosts.

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