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Studies on the sterile-male technique as a means of control of Adoxophyes orana F.R. (Lepidoptera, Tortricidae). 2. Dose–response curves after irradiation of the moths with X-rays or fast neutrons

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Abstract

Every degree of sterility can be induced in young moths (up to 24 h old) of *Adoxophyes orana* F.R. with ionizing radiation. Measurements under experimental conditions did not reveal any effect of the dosages used on life-span, mating frequency and fecundity. The same doses of radiation induced a higher degree of sterility in female moths than in male ones.

The relative biological effectiveness of fast neutrons to X-rays is 2.5–5.0, as indicated by the parameter induction of dominant lethal factors in 90% of the reproductive cells.

Introduction

Because the control of *Adoxophyes orana* F.R. (summer fruit tortrix moth) causes problems in an integrated control programme (De Jong et al., 1971) attempts were made to develop the sterile-male technique as a means of controlling this pest. Other genetic manipulations, such as the use of translocated strains (Curtis, 1968; Laven, 1968) and semisterility (North and Holt, 1968; Knipling, 1970) also offer a means of selective control.

The first step towards any genetic control is to collect data on the effect of a sterilizing treatment by chemo-sterilants or radiation using fertility, life-span, mating activity and fecundity as parameters.

This publication gives data on the effect of radiation on male and female moths.

Materials and methods

The moths were taken from a culture of *Adoxophyes orana* that has been reared in the laboratory since 1965 on a wheatgerm diet (Ankersmit, 1968) at 20°C, 70% relative humidity (r.h.) and 16.5 h light per day. Unless otherwise stated, the experimental conditions were: 25°C, 85% r.h. and 16 h light per day. At the time of irradiation, the moths had emerged less than 24 h and had been given no opportunity to mate.

Ten moths of the same sex were irradiated, without anaesthetic in a plastic Petri dish (height about 1 cm, diam. about 8 cm) at 20°C and 60% r.h. They were transferred, immediately after irradiation, together with 10 untreated moths of the opposite sex, to jars (volume about 2 litres) for oviposition. Egg deposition was mainly on thin plastic sheets that sealed the jars. Eggs laid on the sides of the jar were ignored because of poor hatching.

The moths were irradiated with fast neutrons (n_f) in the irradiation facility of the Biological and Agricultural Reactor the Netherlands (BARN) at Wageningen (Chadwick and Oosterheert, 1969). The dose rate of the fast neutrons during the experiments was 7200 rad/h (using acetylene and muscle tissue equivalent ionization chambers). The gammacontamination was 420 rad/h (measured with a magnox-argon ionization chamber) (information from K. J. Puite, Association Euratom-ITAL).

A Van de Graaff electron generator (High Voltage Engineering, type GS) was used for the X-ray irradiations. The 1.5 MeV electrons were transformed into Bremstrahlung (a type of X-rays) by a gold target. The target and the object were 6 cm apart. The object passed through the beam of X-rays in 17.5 s. In that time, the dose was 0.5 krad (measured with a Fricke ferrous sulphate dosimeter).

Male and female moths were irradiated with X-rays and fast neutrons. The relationship between dose and percentage egg hatch (% e.h.) from the fertilized eggs was determined for the four combinations (male X-rays, male n_f, female X-rays, female n_f), as well as the relationships between dose and number of matings per female moth, life-span of the moths and egg production per 10 moths. The number of replicates was four for each dose.

Results

Egg hatch (e.h.). The dose-response curves for % e.h. after irradiation with X-rays (Fig. 1) and with fast neutrons (Fig. 2), have been plotted semi-logarithmically. The % e.h. of the controls was taken as 100%. The % e.h. of the treatments were then multiplied by the same factor as the particular control. The vertical lines in Fig. 1 and 2 show the highest and the lowest % e.h. in the four replicates. It was found that % e.h. of the controls vary between 55 and 95 for unknown reasons, most values lying between 70 and 80.

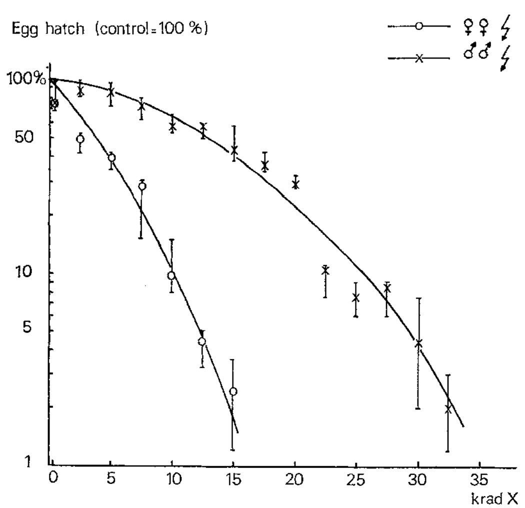

Fig. 1. Effect of dose of X-rays on percentage egg hatch (% e.h.). Vertical lines: highest and lowest value of % e.h.; x and o average % e.h., Flash means: irradiated.

Fig. 1. Verband tussen dosis X-stralen en percentage eiuitkomst. Verticale lijnen: hoogste en laagste percentage eiuitkomst; x en o gemiddeld percentage eiuitkomst. De pijl (rechtsboven in figuur) betekend: bestraald.

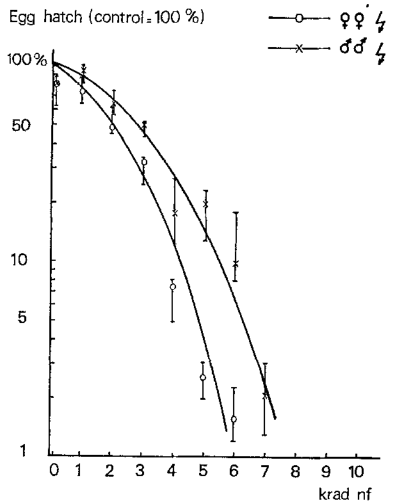

Fig. 2. Effect of dose of fast neutrons (n_f) on percentage egg hatch (% e.h.). Vertical lines: highest and lowest value of % e.h.; x and o average % e.h.

Fig. 2. Verband tussen dosis snelle neutronen (n_f) en percentage eiuitkomst. Verticale lijnen: hoogste en laagste percentage eiwituitkomst; x en o gemiddeld percentage eiuitkomst.

More than 95% sterility is induced in male moths with a X-ray dose of 30 krad or more and in female moths with a dose of 12.5 krad or more (Fig. 1); for fast neutrons these doses are 6 and 5 or more, respectively (Fig. 2). The doses that result in 50% e.h. (HD_{50}) are: for ♂♂, X-rays 12.5 krad; ♂♂, n_f 2.5 krad; ♀♀, X-rays 3.5 krad and ♀♀, n_f 2 krad. This shows that female moths are 1.3 times as sensitive as male moths to fast neutrons, while this factor for X-rays is 3.5, and that fast neutrons are more effective than X-rays in inducing dominant lethal factors.

Preliminary experiments under somewhat different conditions (20°C, 70% r.h. and 16.5 h light), in which moths had also been irradiated with gamma-rays (^{60}Co) and Röntgen-rays (a type of X-rays), had systematically shown a 20–25 units lower % e.h. than in the experiment of Fig. 1. It could not be established whether this was due to the slightly different conditions, to the different kinds of ionizing rays or to a change in radiosensitivity of the moths (Proshold and Bartell, 1972).

The dose range of interest for genetic control of Adoxophyes orana is up to 32.5 krad for X-rays and up to 10 krad for fast neutrons. Therefore the parameters life-span, mating frequency and fecundity have been investigated to see whether irradiation in those ranges causes directly measurable damage.

Life-span. Fig. 3 gives the percentages of male and female moths that live a certain time after irradiation of the female moths with X-rays. No clear effect of irradiation can be seen. It is remarkable that the percentages of irradiated females with a certain life-span, vary in a similar way to the percentages of the corresponding unirradiated males. Hence the life-span is mainly governed by factors other than irradiation. The same conclusion follows from irradiation of males with X-rays and from irradiation with fast neutrons. This has been confirmed by comparing the life-span of irradiated and unirradiated males and females, respectively. It has been demonstrated by experiments using higher doses that 50 krad or more of X-rays or fast neutrons is needed to reduce the life-span of the irradiated moths clearly.

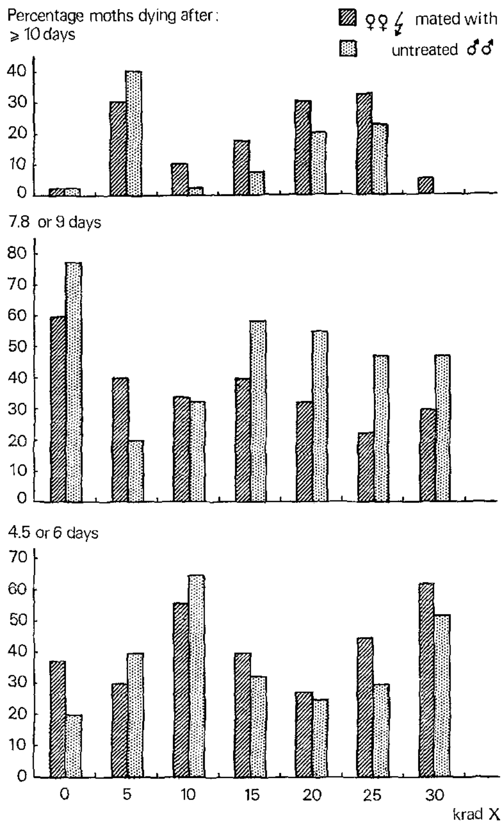

Fig. 3. Effect of dose of X-rays on life-span of irradiated females mated with untreated males.

Fig. 3. Verband tussen dosis X-stralen en levensduur van bestraalde vrouwtjes gepaard met onbehandelde mannetjes.

Mating frequency. This was determined by counting the number of spermatophores in the bursa copulatrix. As far as we know is one spermatophore transferred at a pairing. Fig. 4 shows the percentage of females with a certain number of spermatophores plotted against X-ray dose. The females irradiated with more than 12.5 krad seem to mate less frequently only once but more often thrice, than unirradiated females mated with irradiated males. Irradiation of the males has little or no influence on mating frequency. This has been confirmed by experiments with higher doses of radiation: the mating frequency decreases only after the irradiation of males with more than 50 krad of X-rays or fast neutrons. For females, the doses were 75 krad of X-rays or 25 krad fast neutrons.

Fecundity. In the control, number of eggs laid varies between 1500 and 3000 per 10 females. Only after irradiation of females with doses above 50 krad of X-rays and 25 krad fast neutrons the number of eggs laid, decreased to less than 1000/10 females.

Irradiation of the males influences the egg production at doses that clearly reduce mating activity. Sperm or secretions of accessory glands apparently stimulate oviposition in one way or another (North and Holt, 1972).

Fig. 4. Effect of dose of X-rays on mating frequency. Sp: number of spermatophores/female.

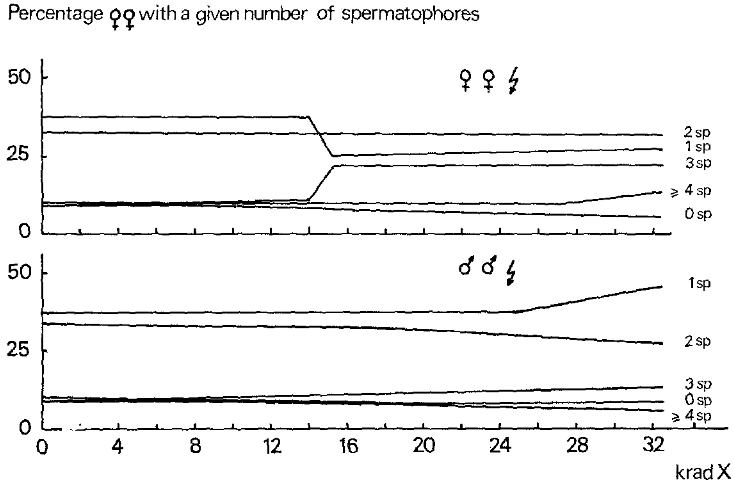

Fig. 4. Verband tussen dosis X-stralen en paringsfrequentie. Sp: aantal spermatoforen/vrouwtje.

Dose rate and relative biological effectiveness (RBE). The dose rate (radiation absorbed dose per unit of time) influences the effects of irradiation. Decreasing the dose rate within the range 0.6–100 rad/min usually increases the chance of repair of sublethal damage during irradiation (Hall, 1972). In our experiments, % e.h. should increase by this mechanism. Hence, if the effect of different types of ionizing radiation be compared, care must be taken that either the dose rates are the same or that there is no effect of dose rate on the parameter used. An effect of dose rate is traced by either (a) changing the dose rate or (b) by splitting the dose. The effect of dose rate has been investigated in order to compare the RBE of fast neutrons to X-rays on the basis of induced dominant lethal factors in the reproductive cells.

In Table 1, the effect of a dose rate of 7200 rad/h is compared with that of a dose rate of 720 rad/h fast neutrons. It can be concluded that, in spite of a decreased dose rate by a factor of 10, no clearly increased % e.h. was observed.

Table 1. % e.h. in relation to the control at a dose rate of 7200 rad/h and of 720 rad/h fast neutrons.

Dose (krad)	% e.h.			
	7200 rad/h		720 rad/h	
	♂♂	♀♀	♂♂	♀♀
0	100	100	100	100
2	65.7	53.2	58	40
4	14.4	7	18	5
6	9.8	1.7	12.1	0.8
8	1.2	0.3	0.7	0.1
10	0.1	0	0.2	0

Tabel 1. Percentage eiuitkomst ten opzichte van de controle bij een doseringssnelheid van 7200 rad/uur en van 720 rad/uur snelle neutronen.

Table 2. % e.h. from (a) fractionated and (b) acute doses of X-rays.

Dose (krad)	% e.h.	
	♂♂	♀♀
2 + 2		40
4		56.5
7.5 + 7.5	40	
15	45.6	

Tabel 2. Percentage eiuitkomst na bestraling met (a) gefractioneerde en (b) acute doses X-stralen.

Table 2 shows that also fractionated irradiation with a certain dose of X-rays (time interval between first and second irradiation is 6 h) does not increase the % e.h. In these cases possible repair mechanisms do not noticeably increase the % e.h. (Hall, 1972; Chadwick and Leenhouts, 1973).

However, Table 3 shows that a difference in % e.h. is found when this percentage is determined for eggs laid on the 2nd and 5th day after irradiation of the males. In the control, there is no difference between % e.h. of samples produced on the 2nd and 5th day after irradiation. Recovery processes may therefore be of some importance, but in calculating the RBE we have assumed that dose rate had no influence.

Table 3. % e.h. on the 2nd and 5th day after irradiation of the males with X-rays.

Time after irradiation	% e.h. 0 krad	15 krad	20 krad	25 krad
2nd day	77.3	7.8	7.8	0.8
5th day	73.4	17.2	6.4	4.1

Tabel 3. Percentage eiuitkomst op de 2e en 5e dag na bestraling van de mannelijke motten met X-stralen.

Discussion

The results look promising for research into the use of a sterile-male technique for *Adoxophyes orana*: a high degree of sterility can be induced in moths without causing somatic damage which would reduce life-span, mating frequency and fecundity under experimental conditions. Consequently, there is at the moment no reason to determine similar dose-response relationships for other developmental stages.

Fast neutrons, having a higher linear energy transfer (LET), are often reported to be superior to X-rays in inducing sterility with a low degree of somatic damage (Offori, 1971). This has not been confirmed by our results and since the use of X-rays takes less time and costs less than an irradiation with fast neutrons, X-rays seem preferable for induction of sterility.

The experiments using two different dose rates and dose fractionation to determine the RBE of fast neutrons to X-rays did not raise the % e.h. Often % e.h. was even reduced for unknown reasons.

The % e.h., various days after irradiation, indicated a recovery from the damage incurred. An explanation could be, that damaged sperm is less viable and dies after some time. In the course of time, the fraction of normal or nearly normal sperm then rises.

It seems that the RBE of fast neutrons to X-rays for the induction of dominant lethal factors in 90% of the reproductive cells of *Adoxophyes orana* is 2.5 for females and 5 for males.

A large variation in % e.h., life-span and fecundity was observed. This variation depended neither on the type of radiation nor on the dose and also appeared in the controls. Consequently, it must be due to differences in rearing conditions since the life-span and egg production within a group of moths from the same rearing box were rather constant. Thus, in spite of controlled climatic conditions, large variations were possible. These were probably mainly correlated with differences in larval density in the rearing box, with possible bacterial and mould infection and with quality of the materials used.

On the basis of the data, a decision can be made about the dose levels to be used in the induction of useful translocations, for research on transmission of semisterility (Bauer, 1967; LaChance, 1970) and the application of semisterility in control of pest populations (North and Holt, 1968; Knipling, 1970).

Acknowledgments

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Samenvatting

Onderzoek over de steriele-mannetjestechniek als bestrijdingswijze van Adoxophyes orana F.R. (Lepidoptera, Tortricidae). 2. Dosis-effectkrommen na bestraling van de motten met X-stralen of snelle neutronen

Uit bestralingsexperimenten met motten (hooguit 24 uur oud) van *Adoxophyes orana* F.R. is gebleken dat zowel met X-stralen als met snelle neutronen iedere gewenste graad van steriliteit geïnduceerd kan worden, zonder een, onder de proefomstandigheden te meten effect op levensduur, paringsfrequentie en aantal gelegde eieren (Fig. 1, 2, 3 en 4). Vrouwelijke motten blijken gevoeliger voor inductie van steriliteit door beide soorten ioniserende straling dan mannelijke motten (Fig. 1 en 2).

Proeven ter bepaling van het effect van de doseringssnelheid en van gefractioneerde bestraling (Tabel 1 en 2) geven aanwijzingen dat enzymatische herstelprocessen geen grote rol spelen. Wel zijn aanwijzingen gevonden dat na verloop van enkele dagen, misschien ten gevolge van afsterven van beschadigd sperma, het percentage steriliteit afneemt (Tabel 3). De RBE van snelle neutronen ten opzichte van X-stralen met dominant lethale factoren in 90% van de voortplantingscellen als parameter, blijkt 2,5 voor vrouwelijke motten en 5 voor mannelijke motten te bedragen.

De verzamelde gegevens vormen een basis voor verder onderzoek naar praktische mogelijkheden van een steriele-mannetjestechniek en semisteriliteit en voor meer fundamenteel onderzoek naar bijv. de wijze van overerving van semisteriliteit en naar stralingsbiologische aspecten in het algemeen.

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