Sterilisation of Males of the Larch Bud Moth *Zeiraphera diniana* by γ-rays

Since the striking success of the American eradication program for the screw worm by the release of sterilized insects only a few further positive results with this method have been reported. The sterile male technique seems to be useful for the control or eradication of a few selected species of insect pests only. An extremely low population density is a prerequisite of paramount importance.

The larch bud moth *Zeiraphera diniana* (Guenée) is a pest of the alpine larch forests in France, Italy, Switzerland and Austria[1] with regular gradation cycles of 8 to 9 years[2,3]. The high populations defoliate the larch forests and then fall back to low density levels. Since the populations of the different alpine valleys are autonomous to a large extent, and since low density levels of the species occur naturally, *Z. diniana* might be a candidate for the successful application of the sterile male technique. However, the density of the bud moth populations is never so low as in the case of the American screw worm. Additional control measures might therefore be necessary, before the sterile males are released in the field.

The following experiments were made in order to establish the dose of radiation needed for the sterilization of the male moths. Male pupae were irradiated with different doses of γ-rays 1 to 2 days before eclosion of the moths. A source of ^{60}Co (10 kR/min) was used, as described in an earlier paper[4]. Table I shows that irradiation with doses up to 40 kR did not interfere with eclosion and morphological differentiation of the moths. Doses of 60 kR reduced the hatchability and the spreading of the moth's wings only slightly, whereas doses of 80 kR had a strong and statistically significant negative effect. A similar negative effect of these doses on the longevity of male moths has been reported before[4].

For further studies only the normal looking male moths were used. They were paired with untreated females of the same age, each couple being kept individually in a rearing box as described in an earlier paper[5] and in Figure 1. The female abdomina were dissected after death and inspected for the presence of spermatophores in the bursa copulatrix. 10 to 14 days later the eggs laid by each female were counted. Inseminated and unfertilized eggs could be distinguished by their colour.

The number of females containing one or more spermatophores give a measure of the mating performance of the males, whereas the number of females laying at least some inseminated eggs indicate the number of males which were able to transfer at least some active spermatozoa. However, the latter numbers give no indication of the proportion of active spermatozoa transferred. It has been demonstrated in an earlier paper that maturation of eggs and oviposition in *Z. diniana* is largely dependent on the presence of active spermatozoa in the receptaculum seminis of the female[5]. The number of eggs laid per female and the percentage of inseminated eggs may therefore be regarded as the best parameters for the estimation of sperm activity in irradiated males.

Table II shows that the percentage of males transferring a spermatophore is significantly reduced only after irradiation with doses above 60 kR .Up to doses of 20 kR all males transferring a spermatophore also transfer active spermatozoa. This is not true for males irradiated with 40 kR or more. Of the females mated with such males 10% or more contained a spermatophore but laid only unfertilized eggs. Thus some of the irradiated males were able to produce and transfer spermatophores which, however, did not contain viable spermatozoa. The percentage of inseminated eggs was significantly reduced when the males were irradiated with 40 kR and became zero with 80 kR. The mean number of eggs per female, on the other hand, was significantly reduced only when the males had been irradiated with 60 or 80 kR. This result confirms the earlier observation that a small number of active spermatozoa in the female is sufficient for full stimulation of oogenesis and oviposition[5].

Table III shows that only $^2/_3$ of the inseminated eggs from control pairs gave rise to larvae and subsequent mortality was so high that only about 13% of the inseminated eggs developed into adult insects. The sex ratio in the control rearings was not fully normal either, but shifted slightly in favour of the males. The factor of multiplication of the males (number of sons divided by number of fathers) was 5.7, that of females 4.3 only. Factors of population increase as low as these are not rare in nature, but must be regarded as exceptionally low in laboratory rearings. Two facts may account for the bad breeding results of the controls: 1. the parent insects originated from a natural population of high density, 2. the eggs had been kept for longer than the optimal time at 2 °C (7 months instead of 5 months). However, since the mortality of em-

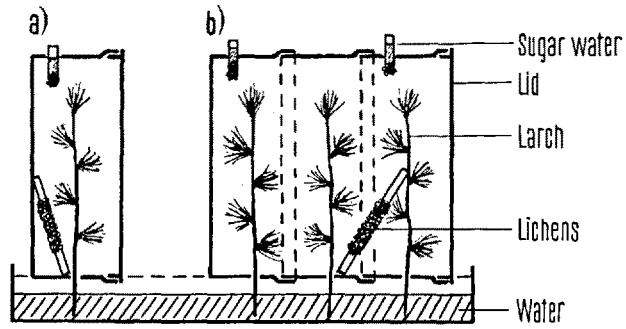

Fig. 1. a) Scheme of polystyrene rearing boxes (18 × 10 × 8 cm) used in experiments with single pairs, and in experiments with 1 female and 2 males. b) Example of rearing box for 1 female and 6 males. The branch with lichens offers the female oviposition sites. Ventilation of the boxes was assured by 5 screened air holes with diameters of 3 cm.

Table I. Influence of different doses of γ-rays on hatchability of pupae, and morphological differentiation of moths

Dose in kR	No. of pupae	Eclosed moths No.	%	Normal looking moths No.	%
0	121	115	95	110	91
10	25	24	96	21	84
20	77	71	92	68	88
40	120	115	96	109	91
60	115	95	83	85	74
80	154	81*	53*	46*	30*

*Significantly different from control at 5% level.

[1] P. Bovey, Bull. Murithienne *83*, 1 (1966).
[2] W. Baltensweiler, Proc. 11th int. Congr. Entomol. (Wien 1960), *2*, 185 (1962).
[3] C. Auer, Z. angew. Entomol. *62*, 202 (1968).
[4] G. Benz, Experientia *26*, 1252 (1970).
[5] G. Benz, J. Insect Physiol. *15*, 55 (1969).

bryos was significantly higher when the parent males had been irradiated with 20 kR and since the percentage of adult offspring was significantly reduced after 40 kR, it may be safe to conclude that these differences were due to dominant lethal factors induced in the irradiated male parents. The results of Table III suggest that irradiation of males with 60 kR induces at least one dominant lethal factor in each sperm. Thus only 4% of the inseminated eggs (i.e. 0.3% of the eggs laid) gave larvae, and none of these developed to the 5th instar. The low ratio of females to males shows further that the irradiation of males causes higher mortality in the female than in the male progeny. The higher mortality may be caused by recessive lethal factors of the sex chromosome which in the heterogametic daughters would be exposed to selection. The detrimental action of recessive lethal factors or other sex limited factors shows best in the progeny of males irradiated with 20 kR. In this group the factor of multiplication of the males was not much lower than that of the controls, but it was drastically reduced to 0.9 for the females.

Irradiation of males with 40 kR reduced propagation of both sexes considerably. Since 100 pairs merely produced 27 sons and 9 daughters, it is probable that this dose would sufficiently sterilize males of *Z. diniana* for a sterile male control program.

A few brother × sister crossings of the F_1 were made in order to get some information on the reproductive capacity of these moths. Table IV shows that about $^2/_3$ of the sons of irradiated males were able to produce and transfer spermatophores (i.e. a similar percentage as in the irradiated parental generation). However, whereas 80% of the control females laid inseminated eggs, only one of 28 daughters of irradiated males laid 19 inseminated eggs and none of these gave larvae. Although the number of F_1 brother × sister crossings was too small to allow definitive conclusions, the results suggest that in the field the release of a high number of males irradiated with 20 kR might already cause a strong reduction of the natural populations of *Z. diniana*. Whether or not this is true can be determined only in field experiments.

Two sets of small laboratory assays were made in order to find out how large the proportion of irradiated to non-irradiated males must be to get a significant reduction of a population. In the first set of experiments, spacious population cages (30 × 44 × 50 cm) furnished with fresh twigs of larch, branches with lichens for oviposition, and a source of sugar water were populated with 10 untreated females and 10 males per cage. Different proportions of the males had been irradiated with 60 kR (see Table V). The results of the different rearings are presented in Table V. Compared with the controls (experiment 1) the number of offspring was significantly reduced in all cages containing a proportion of irradiated males. However, the results show also that, under these experimental conditions, a good control effect can only be obtained by a high proportion of sterile males. When about equal numbers of normal and irradiated males were present, as in the experiments Nr. 2 and 3, the female populations still multiplied by an average factor of 3.5 (control, No. 1 = 8.8). Even at the proportion of 9 irradiated males to 1 normal male (No. 6), 10 females produced 7 daughters, i.e. the factor of female propagation, though reduced to 0.7, was still relatively high. In addition, the fact that in this experiment all inseminated eggs gave L_1 and that 50% of the L_1 became adults suggests that the latter were genetically normal.

In nature one would probably find worse conditions. The number of natural males would about equal the number of females and released sterile males would only be added to these. In order to find out which effect the flooding of a natural population with irradiated males might produce, an additional experiment was made. In this case one male and one female were put in each rearing box and different numbers of irradiated males (1, 2, 4 or 6) were added. Possible crowding effects were avoided by adjusting the volumes of the rearing boxes to the number of individuals present, i.e. the volume of one unit was added for each couple of additional males. Enlargement of the rearing boxes was made by inserting one or more ring like units (i.e. boxes whose bottoms had been cut out) between the first box and the lid (Figure 1). 4 small control experiments were made in order to find out whether or not the presence of different numbers of males per female might influence the mating and oviposition behaviour of females. Each of these experiments comprised only 5 pairs to each of which were given 1, 2, 4 or 6 supernumerary normal

Table II. Reproductive capacity of males, irradiated with different doses of γ-rays, and mated with untreated females

Dose in kR	No. of pairs	Males transferring spermatophores (%)	Females with inseminated eggs (%)	Mean No. of eggs per female	Eggs inseminated (%)
0	110	78.2	78.2	92.4	84.2
10	20	85.0	85.0	99.4	84.1
20	51	70.6	70.6	95.2	64.4
40	100	62.0	52.0*	83.9	24.6*
60	85	60.0	12.9*	30.8*	7.5*
80	46	17.4*	0*	10.8*	0*

*Significantly different from control at 5% level.

Table III. Number of inseminated eggs and adult offspring (F_1) from rearings indicated in Table II. Hatched larvae (L_1) and last instar larvae (L_5) are given in % of inseminated eggs. The multiplication factors of the two sexes were calculated by dividing the number of sons and doughters respectively by the number of parents (= number of pairs in Table II)

Dose in kR	No. of eggs inseminated	L_1 (%)	L_5 (%)	F_1 moths produced				Ratio ♀/♂	Factor of multiplication	
				No. ♂	No. ♀	Sum	%		♂	♀
0	8564	66.1	19.6	631	471	1102	12.9	0.75	5.7	4.3
20	3126	36.1*	11.1	211	48	259	8.3	0.22	4.1	0.9
40	2093	6.6*	2.3*	27	9	36	1.7*	0.33	0.3	0.1
60	196	4.1*	0*	0	0	0	0*	–	–	–

*Significantly different from control at 5% level.

Table IV. Brother × sister crossings of nonirradiated F_1 individuals recorded in Table III, and their offspring

Treatment of father	Pairs No.	Males transferring spermatophore (%)	Females laying inseminated eggs (%)	No. of eggs per female	Eggs inseminated (%)	L_1 (%)
Control	15	80	80	52.5	82.7	61.8
20 kR	27	63	3.7	26.1	2.7	0
40 kR	1	100	0	0	–	–

males. The 4 control experiments gave very similar results, indicating that under conditions of constant male density the behaviour of the females was not influenced by the number of supernumerary males. The data of these experiments were therefore computed and presented in Table VI. The same table presents the data of the experiments with supernumerary irradiated males.

The figures of Table VI show that under these laboratory conditions at least 4 to 6 irradiated males per normal couple must be present to achieve a significantly reduced rate of insemination of the eggs and of hatched L_1. Since the L_1 have not been reared further, nothing can be said about the end effect of the irradiated males. However, it is possible to compare the multiplication factors of the parent generations (10 pairs per experiment and 20 pairs in the control) up to the L_1. In laboratory rearings this factor had usually a value of about 50 for controls, but it reached only the value of 21 in the controls of these experiments. Compared with this value the multiplication factors in the experiments 1 and 2 were not reduced at all (factor 24). Only the experiments 3 and 4 gave reduced factors of 6.6 and 14.2 respectively. Thus with 5 irradiated males per normal couple, one might expect an average reduction of the multiplication factor to half the value of the control. Such an effect would probably not allow proper control of a population in the field.

The experiments, though small, furnish also some indirect evidence that males irradiated with 40 kR might be slightly less competitive for mating than the untreated males. When the percentages of insemination of the eggs of each mated female are plotted as in Figure 2, it can be seen that more than 40% of the eggs of each control female, but less than 20% of the eggs of some experimental females, were inseminated. If all mated females which laid badly inseminated eggs are regarded as having been mated by an irradiated male, one finds in the experiments No. 1 to 4 the respective values of 20%, 20%, 70% and 50% of such matings, instead of 50%, 66%, 80% and 85%, which would be expected, if all males had had the same chances of mating. Because of the small number of 10 females per experiment the difference between the real and the expected value is statistically not significant in the individual experiment. However, the real values show the same tendency of deviation in all experiments. Therefore, if the values of the 4 experiments are computed, the sum of 16 matings of irradiated males (against 24 normal matings) is significantly smaller than the expected 28 ($\chi^2 = 6.11$; $P < 0.02$).

It has been mentioned in an earlier paper[5] that in the laboratory one spermatophore per female is the rule for single pair breedings and that only about 20% of the females carry 2 or more spermatophores. It was therefore interesting to see whether or not this was also true in these experiments, where each single female was caged together with 2 to 7 males (mean = 4.25 per female). However, no obvious correlation was found between the number of females carrying 2 or more spermatophores and the number of males per female. Of a total of 58 mated females 27.6% carried 2 or more spermatophores (5 of 18 control females, and 11 of 40 experimental females) which is not significantly different from the results of single pair breeding. Thus the limited rate of copulation depends on the behaviour of the females rather than that of the males. More than $^2/_3$ of the females seem not to accept more than one male for copulation. Whether or not this is also true in the field has to be verified.

The preliminary experiments presented in this paper show that the sterile male technique might be applicable for the control of *Z. diniana*. Irradiation of the males with

Table V. Experiment with population cages (see text)

Exp. No.	No. of males normal	irradiated	No. of eggs	Eggs inseminated (%)	L_1 (%)	No. of moths ♂	♀
1	10	0	806	90.8	72.7	103	88
2	6	4	845	48.2	40.0	54	43
3	4	6	873	32.8	28.1	35	28
4	3	7	577	39.9	27.7	26	23
5	2	8	406	14.5	0	–	–
6	1	9	506	7.3	7.3	10	7
7	0	10	250	0	0	–	–

Reproductive capacity of females caged with males of which some had been irradiated with 60 kR; 10 females and 10 males per cage. The reproductive capacity of the females may be expressed by the factor of multiplication, which is found by dividing the numbers of the F_1 females by 10. Compared with the control cage (No. 1) the number of offspring in all cages with irradiated males is significantly reduced.

Table VI. Population experiment simulating flooding of a population with irradiated males

Exp. No.	Irradiated males added per normal couple	Females mated (%)	No. of eggs laid per female	Inseminated eggs (%)	L_1 (%)
1	1	100	123.1	57.3	39.1
2	2	100	113.5	62.7	41.8
3	4	100	79.4	24.2[a]	16.6[a]
4	6	100	112.4	43.3[a]	25.4[a]
Control		90	77.9	79.7	53.1

Each experiment comprises 10 identical sets, each with a non-irradiated male and a female to which different numbers of irradiated males (40 kR) were added. The number of individuals per unit volume of rearing box was the same in all sets (Figure 1). The control values are the computed data of 4 experiments with 20 females, as described in the text. [a] Significantly different from control at 5% level.

20 to 40 kR of γ-rays would be necessary to induce a sufficiently high rate of lethal factors or sterility of the males. In order to reduce a natural population of *Z. diniana* noticeably, the released males would have to outnumber the natural males considerably. Thus the technique could only be applied when the natural populations reach a point of minimum density. However, unpublished results of male captures in sex traps by VON SALIS indicate that even at an extremely low population density (1 larva per 400 kilos of larch twigs[6]) the moth populations are higher than one would except from the data of the larval census. Without further control measures it would therefore be very difficult to produce and release such a number of irradiated males as would be necessary for the control of a large area. The application of conventional insecticides being out of question, a further reduction of the minimal populations during the larval stage might be necessary. This coud be achieved by microbiological control methods[7]. Unpublished field experiments by BENZ and AUER indicate that about 90% larval mortality can be achieved by the application of a preparation of *Bacillus thuringiensis* in combination with its exotoxin. However, this method would be expensive. Whether or not a reduction of the natural male populations by precapture in male traps baited with virgin females or the sex pheromone would be practicable in the field has to be investigated.

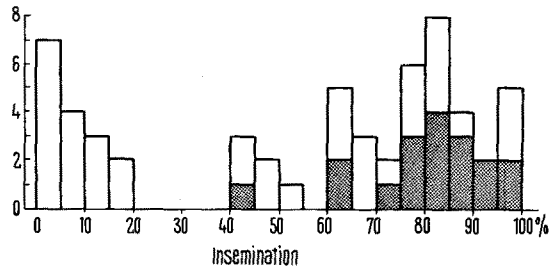

Fig. 2. Histogram of mated females with percentage of insemination (abscissa) of their eggs. Each column represents a class of 5%; the height indicates the number of females per class. Dark columns, females caged with 2 to 7 normal males. White columns, females caged with a normal plus 1 to 6 irradiated males.

Zusammenfassung. Bestrahlung von Männchenpuppen des Lärchenwicklers mit 40 kR induzierte in den Spermien so viele Letalfaktoren, dass nur eine sehr kleine Nachkommengeneration heranwachsen konnte. Bestrahlung mit 20 kR ergab eine relativ grosse männliche und eine prozentual geringe weibliche F_1. Letztere unter sich gekreuzt ergab praktisch keine Nachkommen. Zwei kleine Vorversuche lieferten Schätzwerte über den populationsdynamischen Effekt verschieden hoher Proportionen von bestrahlten zu unbestrahlten Männchen[8].

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[6] C. AUER, Internal Report 1967, unpublished.
[7] P. GRISON and P. BOVEY, C.r. Acad. Sci., Paris *270*, 1261 (1970).
[8] Contribution Nr. 44 of the research team for the investigation of the population dynamics of the larch bud moth, directed by Professor Dr. P. BOVEY. The research was aided by a grant of the Swiss National Funds for Scientific Research.

Sex-Pheromones in *Bradysia tritici*

The sex-pheromones had been described in many orders of insects, mainly in orthoptera, lepidoptera, coleoptera, hymenoptera and some diptera[1–8]. Chemically they were described mainly as unsaturated alcohols and their esthers, aliphatic acids and terpene-like compounds[9]. A number of pheromones have been found to be lipids[5]. In all the cases, the substances must be volatiles. In the majority of the cases, pheromones are produced as liquids and they evaporate into the air and form a cloud of vapor over the animal[5].

In some insects, the production and the response to sex attractants are under hormonal control and are regulated by the corpora allata; they elicit alertness, sexual excitement, antennal waving, and wing raising, as well as courtship[6,7,10–12]. Our purpose was to investigate whether pheromones also occur in the family Sciaridae and, if so, in which sex.

In order to verify the possibility of existence of a pheromone of sexual attraction in *Bradysia tritici* (*Sciara ocellaris*), pupae were individually isolated in culture glass tubes to obtain virgin flies. When the flies hatched, males and females were separated. We also used non-virginal flies to verify whether or not there were differences in behavior between these flies and the virgin ones.

The female flies were placed in little glass tubes for $1^1/_2$ h, while the male flies were placed in other tubes for the same time, and other glass tubes without animals were used as controls.

We used rectangular boxes made of transparent plastic containing food; the boxes had lateral holes, in which the glass tubes could be introduced (Figure).

Males or females were placed into the boxes. The glass tubes, in which the flies remained for $1^1/_2$ h, were introduced into one of the box holes, immediately after removal of the flies. In the other hole, we introduced the control glass tube which had not had flies in it. When the flies in the box were males, those which remained in the glass tubes were females, and vice-versa. We made 5 types of experiments:

Glass tube	Plastic box
virgin males	virgin females
virgin males	non virgin females
non virgin males	non virgin females
virgin females	virgin males
non virgin females	virgin males.

During 10 min we counted the number of flies which entered the glass tube (experimental and control). As the flies were not marked, the counting included also cases in which the same fly entered more than once. The results obtained for *Bradysia tritici* are shown in Tables I and II.

These results show that virgin females of *Bradysia tritici* produce some aromatic substance to attract the males. The odour seems to disappear as a result of the copulation. We repeated the experiment using females (virgins or non-virgins) in the plastic box and glass tubes