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Studies on the sterile male technique as a means of control of Adoxophyes orana (Lepidopt., Tortricidae). 3. An evaluation of competitiveness of laboratory-reared moths

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Abstract

Mating competitiveness of an inbred irradiated, mass reared strain of *Adoxophyes* was approximately half that of males collected as nearly fullgrown larvae or pupae in the field. This reduction was not caused by irradiation effects. No indications of genetic inferiority of the laboratory strain were found since the difference in competitiveness disappeared after wild moths were reared for one generation on artificial diet. A diet effect on competitiveness seems most likely.

Introduction

As part of an integrated programme for control of the leafroller *Adoxophyes orana* (F.R.), a serious pest in apple orchards, the sterile male technique is being studied in the Netherlands (Ankersmit and de Jong, 1970; de Jong et al., 1971). The sterile male technique necessitates the production of mass numbers of sterile moths for release in the orchard. But in addition to quantity quality is also important and this is usually evaluated on the basis of laboratory competition experiments. Little is known about the actual competitiveness of sterile insects in the field. In assaying the competitiveness between wild males and laboratory-reared, sterilized males of *A. orana* for available wild females we kept the conditions as close as possible to those in the field by carrying out experiments in caged trees in the orchard. Potential causes of inferior quality of laboratory-produced moths were also studied.

Methods and materials

The laboratory culture of *A. orana* used for mass-rearing was maintained on an artificial diet (Ankersmit, 1968) at 20 ± 1 °C with a light: dark (L:D) regime of 16.5:7.5. The culture originated from wild moths collected in Zeeland, the Netherlands, in 1965.

The adults were sterilized as in a sterile male release programme (radiation with 25 krads from a ^{60}Co source within 24 h after adult eclosion). Dose rate 1.3–1.4 krads per minute.

Moths used in competition experiments were collected as pupae from the mass culture room and transferred to an environmental chamber (20 ± 1 °C) in which the

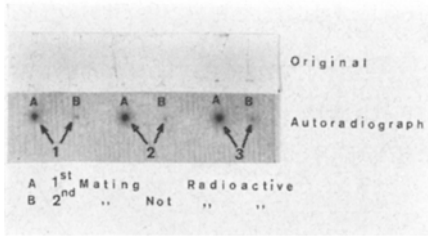

Fig. 1. Radioactive and non-radioactive spermatophores resulting from subsequent matings with ^{32}P labelled and unlabelled males.

Fig. 1. Radioactieve en niet-radioactieve spermatoforen bij achtereenvolgende paringen van met ^{32}P gemerkte en ongemerkte motten.

daily photophase was changed at about 10 day intervals to approximate closely the day length in the field or greenhouse. Females were placed in the competitive situation on the day of adult eclosion and males on the second day. Occasional individuals were stored for a maximum of 3 days at 15 ± 1 °C.

The competitive situation was provided by placing 6 females with 12 males (6 males of 2 types). In each test, 1 of the 2 types of males, and hence the spermatophores it produced, was labelled with ^{32}P by adding 1.5 μCi ^{32}P/g larval diet. After 4 or 6 days the moths were recaptured. The origin of the recovered males and the source of spermatophores dissected from the females were determined by mounting the spermatophores on filter paper and covering them with Kodak Definise X-ray film. The films were stored for an exposure of 3 weeks at 4 °C (Noordink and Minks, 1970). One of the results is given in Fig. 1. The slight blackening of the spermatophores B, originating from unlabelled males, could be attributed to radioactivity transmitted during the first mating.

Laboratory assay of effect of marking and sterilization

The effect of the ^{32}P label used for marking 1 of the 2 types of males in subsequent experiments was tested in the laboratory by matching 6 lab ♀♀ with 6 unmarked, lab ♂♂ and 6 ^{32}P marked, lab ♂♂'s. The moths were placed in 2 litre plastic pots provided with a 20 % solution of sucrose for 6 days at 20 ± 1 °C and L:D 16.5:7.5. The results are described under A in Table 1. As in all of the competition experiments described, the percentage of spermatophores originating from each of the 2 types of males should approximate 50 % if the 2 types are equally competitive. No effect was demonstrated by the ^{32}P labelling technique (Student's *t* test, at a level of $p = 0.05$).

The effect of sterility on competitiveness was examined with tests using 6 lab ♀♀ with 6 nonirradiated lab ♂♂ and 6 irradiated, lab ♂♂. In half of the replicates the nonirradiated males were ^{32}P marked, in the other half the irradiated males. This experiment (Table 1, B) was carried out in the same manner as A. The mating success of sterile and fertile males was not shown to be different (Student's *t* test, at a level of $p = 0.05$).

Field assay of competition between wild and laboratory males

Wild moths were obtained by collecting late larvae and pupae in apple orchards in Zeeland and in the Oostelijk Flevoland Polder. Individuals collected as larvae were

Table 1. Evaluation of competition between two types of males of *Adoxophyes orana*.

Experimental conditions	Origin of moths			Number of replicates	Number of females recovered	% recovered			% ♀♀'s mated	Total number of spermatophores	spermatophores from ♂♂$_1$
	♀♀	♂♂$_1$	♂♂$_2$			♀♀	♂♂$_1$	♂♂$_2$			
A (lab.pot)	lab	lab	lab marked	8	48	100	100	100	79.2	86	46.5
B (lab.pot)	lab	lab	lab sterile	4	24	100	100	100	87.5	44	56.8
C (cage, field)	wild	lab	wild	27	57	35.2	49.7	42.1	94.2	72	29.2[1]
D (cage, green house)	lab	lab heavy	lab light	5	28	93.3	100	96.6	92.2	39	59.6
E (cage, green house)	lab	lab	wild on lab diet	5	26	89.3	100	100	56.0	19	52.3

[1]Values for ♂♂$_1$ and ♂♂$_2$ are significantly different at p = 0.02 (Student's *t* test).

Tabel 1. Resultaat van concurrentieproeven tussen twee typen mannetjes van Adoxophyes orana.

fed on apple leaves in the laboratory. The test matched 6 wild ♀♀ with 6 marked, sterilized, lab ♂♂ and 6 wild ♂♂. The experiments (Table 1, C) were conducted in 1 × 1 × 2 m nylon mesh cages placed over small apple trees in an orchard near Wageningen. No attempt was made to remove spiders and other predators or scavengers from the cage. Experiments were conducted over 4 day periods between 10 August and 13 September 1971; the experimental period was within range of the second flight period for *A. orana*.

In contrast to the laboratory experiments in which all of the moths can be recovered, many individuals die in the field and are not recovered. A difference in the survival rate of the 2 types of males in the field could bias the data on the origin of the spermatophores, but the recovery rates of ♂♂$_1$ and ♂♂$_2$ were not shown to be different (Student's *t* test, p = 0.05).

Instead of spermatophores originating from both types of males in equal proportion, as would be expected if the laboratory males were fully competitive, the results show the laboratory males to account for only 29.2 % of the matings with wild females (t = 2.54, d.f = 21, p = 0.02).

We thought the low competitiveness of the laboratory males in contrast to the wild males might perhaps be traced to genetic inferiority of the laboratory (the strain was reared continuously in the laboratory for 6 years) and/or to the effect of the artificial diet. It was also apparent that wild *A. orana* were smaller than the laboratory-reared moths. This observation suggested the possibility that smaller males may be more active due to an advantage of having both a reduced wing load and possessing the capability for flight at a lower temperature threshold (Dorsett, 1962). The following experiments were designed to examine the potential sources of low competitiveness in laboratory males.

Effect of weight on competition

The mean weight of male pupae collected in the wild was 16.4 $\pm$ 0.4 mg (N = 41), whereas the weight of a representative group of laboratory-reared males was 23.1 $\pm$ 0.6 mg (N = 72). To assess the effect of the moth's size, laboratory males were weighed and categories of 'heavy' and 'light' were established. Mean pupal weight of 'heavy' males was 24.1 mg (range 20.4–32.8); mean pupal weight of 'light' males was 15.0 mg (range 10.6–17.7). In 3 of the replicates the 'heavy' males were ^{32}P marked; in the other 2 replicates the 'light' males were ^{32}P marked. The experiments (Table 1,D) were carried out within a greenhouse in 0.5 $\times$ 0.5 $\times$ 1.0 m cages containing a small tree of Lombardy poplar (*Populus nigra* c.v. italica) and 2 vials of 20% sucrose solution. Moths were left in the cages for 4-day periods between 29 September and 10 October 1971. The results discredit the theory that the smaller size of the wild males is advantageous (Student's t test, at a level of p = 0.05).

In this experiment greenhouse temperature was not controlled and may have exceeded the threshold temperatures for activity in both 'heavy' and 'light' males. Laboratory experiments at constant temperatures suggest that 13 °C is close to the threshold for mating activity; some mating occurs at 13 °C but no mating were ever found at 12 °C. To examine further the effect of male size laboratory-reared moths (ratio of 1♀:2♂♂) were kept for a week in 2 litre pots within an environmental chamber at 13 °C. In the first trial (N = 54♂♂) 'heavy' males (pupal weight > 25 mg) mated 0.20 times/male and 'light' males (pupal weight 15–20 mg) 0.46 times/male. In a second trial (N = 100♂♂) however, 'heavy' males mated 0.72 times/male and the 'light' 0.62 times/male. Although flight activity may not be too important in respect to the mating activity observed in 2 litre pots the results give no evidence that the smaller moths have an advantage.

Effect of genetics and diet on competition

If wild *A. orana* reared as larvae on the artificial diet are compared with the laboratory males it is possible to determine whether the low competitiveness is due to genetical defects or to the laboratory diet. If the competitiveness of the laboratory males remains low the effect can be attributed to genetic inferiority, but if the competitiveness of the laboratory males is increased by rearing the wild larvae on the artificial diet, the results would indicate a deleterious effect from the artificial diet.

Under the experimental conditions (Table 1,E), larvae which hatched from eggs laid by wild females collected in Zeeland were reared on artificial diet used in the mass rearing programme. Laboratory males were reared on the same artificial diet, but to which ^{32}P had been added. Experiments were conducted in cages within a greenhouse as under conditions D. The 4-day experimental periods were between 10 November and 8 December 1971.

Since no significant difference was demonstrated between laboratory males and wild males reared for one generation on the artificial diet, we can conclude that the laboratory-reared males are not genetically inferior, but that the rearing procedure is probably responsible for the decreased competitiveness in the laboratory males in relation to males reared on their natural host plants in the wild.

Comparison with preliminary study in 1970

A similar evaluation of competition was obtained in a preliminary field-cage study made by one of our students (P. Engels). In his tests egg hatchability was used as the criterion for determining if the female mated with an irradiated or a fertile male. Multiple matings were avoided by keeping the moths in the competitive situation for only one night. Irradiation did not effect competitiveness; irradiated males accounted for 54% of the matings (N = 67 ♀♀). Laboratory males accounted for 35% of the matings when in competition with wild males originating from pupae collected in the field (N = 32 ♀♀). After rearing the wild strain in the laboratory for one generation, the competitive advantage of the wild strain was lost (N = 27 ♀♀; 74% mated by laboratory males). In a subsequent test a wild strain collected in the fall and reared on artificial diet was compared with the laboratory strain during April of the following year. In this test the laboratory strain was marked with ^{32}P and the moths were released into a large cage inside a greenhouse. The laboratory strain accounted for 53% of the matings (N = 58 ♀♀). These data again indicate that within the limits of a large cage evaluation there is no demonstrable genetic inferiority of the laboratory strain. Again an effect from the diet seems likely.

Discussion

The assessment of competitiveness in laboratory males of *A. orana* used in the mass release of sterile males for suppressing the natural population resulted in the conclusion that sterilized, laboratory males reared on an artificial diet were only 29/50 = 0.58 × as effective as wild males. The difference could not be accounted for by the act of sterilization, the size difference of wild and laboratory males, or by a genetic inferiority of the laboratory strain. The artificial diet is believed to be responsible for the lower competitiveness because when wild males were also reared on the artificial diet the superiority of the wild males was obliterated. Other aspects of the laboratory rearing procedure may also be involved.

Minks (1971) described a lower pheromone production in our inbred strain of *A. orana* as compared to another laboratory colony which occasionally was interjected with insects from the field. Rearing methods for the two colonies were, however, slightly different; our larvae were reared at a higher density and without ascorbic acid in the diet. Although genetic inferiority of our laboratory strain is suggested by the pheromone data, in view of our results on competitiveness, it appears that a deleterious effect from the diet rather than genetics needs to be our main focus for attention. It seems likely that the major selection for a laboratory strain would occur within the first generation, and unless such a selection can be avoided it does not appear advantageous to introduce periodically a new strain from the field.

If the diet cannot be changed to improve the quality of the laboratory-reared moths, the same final result for control purposes can be obtained by almost doubling the number of sterile moths released; entering a value for competitiveness of 0.58 into the formula proposed by Berryman (1967) permits computation of the number of moths needed for release as a function of the competitive ability of the moths. As the competitiveness of the released moths is reduced the cost of the release programme correspondingly increases.

The technique of assaying competitiveness outside in cages placed over trees incorporates a more natural situation than can be simulated in the laboratory. The large outside cages require more pre-mating flight and permit exposure to natural weather conditions, natural day-lengths, and a limited number of predators – all of which could influence the longevity or effectiveness of 1 type of male more than the other. Yet, the moths are still restricted to a relatively small area in comparison to the true field situation, and the value for competitiveness remains an approximation of reality.

We tried to establish a more competitive situation by providing a 2:1 ratio of ♂:♀ instead of the 1:1 ratio which occurs in the field and which is frequently used in studies on competitiveness. Although little is known about the epigamic behavior of *A. orana* we have observed that the female does not normally mate more than once a night in the laboratory. In laboratory cultures with equal numbers of males and females more than one and often 3 spermatophores are recovered from each female when dead; field collected females likewise contain a mean of between one and two spermatophores (Minks and Noordink, 1971). By contrast, laboratory males are capable of mating 7–8 times (in some tests, a maximum of 12 times). Thus, an overabundance of males should provide a sensitive method for detecting differences in male aggressiveness and mating success.

The technique used for assaying competition between 2 types of males is based on identification of the source of the spermatophores. In the Oriental fruit moth *Grapholitha molesta*, males were found to be capable of inseminating more females than the yield of spermatophores indicated (George and Howard, 1968). Likewise we have occasionally found fertile eggs laid by females of *A. orana* from which we could observe no spermatophore. This was especially so when females had mated with old males. The size of the spermatophore decreases with subsequent matings. But unless the ability to produce spermatophores differs between the 2 types of males tested, the identification of spermatophore source can still serve as a reliable criterion for assaying competitiveness.

This method, however, does not allow an evaluation of sperm quality. In previous tests (Snieder et al. 1973) females mated with males irradiated with x-ray dosages up to 25 krad always laid eggs which showed some development of the embryo. This indicated that at least a part of the sperm produced by the irradiated males was capable of fertilization. Further studies will be needed to evaluate the exact sperm quality.

Samenvatting

Onderzoek over de steriele-mannetjestechniek als bestrijdingswijze van Adoxophyes orana. 3. Bepaling van het concurrentievermogen bij de paring van gekweekte motten

De paringsactiviteit van in massa gekweekte motten werd met behulp van spermatoforenonderzoek (Fig. 1) vergeleken met die van als volwassen rups of pop buiten verzamelde motten (Tabel 1). Hieruit werden aanwijzingen verkregen dat de paringsactiviteit der gekweekte dieren circa de helft was van die van de uit het veld afkomstige. Er werd geen effect van bestraling op de paringsactiviteit gevonden. Wel bleken motten die slechts één generatie in het laboratorium waren gehouden, geen betere

paringsactiviteit te hebben dan de reeds sinds 1965 in het laboratorium gekweekte stam. Geen aanwijzing werd verkregen dat het tussen de gekweekte dieren en 'veld-dieren' bestaande gewichtsverschil (popgewicht resp. 23,1 mg en 16,4 mg) de oorzaak was van het verschil in gedrag. Het waarschijnlijkst lijkt een dieeteffect.

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Erratum

In the article of C. Hiruki and Jeanne Dijkstra 'Light and electron microscopy of Vinca plants infected with mycoplasma-like bodies of the sandal spike disease' (Neth. J. Pl. Path. 79 (1973) No 5) there are some mistakes.

On page 207 the fifth line of 'Introduction' should be: 'and Lee, 1972; Hiruki and Dijkstra, 1973) *Dodonaea viscosa* (Hull et al., 1970) and'.

On page 208 the ninth line of 'Materials and methods' should be: '1/15 M K_2HPO_4, pH 9.5, for 20 min. Adjustment of pH was made by adding a dilute'.

On page 213 the twelfth line from the bottom should be: 'The second possible source of fluorescence in infected phloem cells is the myco-'.