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Studies on the sterile-male technique as a means of control of Adoxophyes orana (Lepidopt., Tortricidae). 1. Problems of mass rearing (crowding effects)

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Abstract

Almost all larvae of *Adoxophyes orana* F.R. mature if they are individually confined in vials. In gregarious culture, 2 or 4 larvae per vial, growth is retarded and many larvae die because of frequent fighting. This aggression results from the biggest larvae holding other larvae off the food. Retarded development was not due to optical or olfactory causes, but to food deprivation. In mass rearing the setback can be partly overcome by adding plastic strips that increase the cover for the larvae in rearing boxes.

Introduction

We often observed cannibalism in cultures of *Adoxophyes orana* F.R. on a semisynthetic wheat germ diet (Ankersmit, 1968). When two or more larvae shared a vial, one grew fast while the others were retarded until the biggest had pupated. Then the smaller larva started growing and sometimes killed the pupa. The larvae often fought and many were killed. In mass rearing, necessary for release trials for the sterile-male technique, this cannibalism and aggression diminishes output. We have evaluated this problem and sought ways of avoiding this aggression.

Materials and methods

A parental stock of *Adoxophyes orana* was kept on the wheat germ diet at 20 °C, 70 % relative humidity and a cycle of 18 h light and 6 h darkness (LD 18:6). Newly hatched larvae from this stock were transferred to vials in numbers as stated in tables and reared at 25 °C, 70 % relative humidity and LD 18:6. Unless otherwise stated, each test was with 100 vials, 14 mm diam. and 50 mm high, and 3 ml diet per vial. Larvae were weighed in samples of 20 vials at 4, 8, 12 and 15 days of age. Weighed larvae were discarded, as any handling affected their growth rate. Pupae were weighed the day after pupation. Only larvae or pupae from vials without mortality were weighed. The amount of food was always amply sufficient.

Results

Relation between larval density and weight, survival, duration of development, and weight of pupae

The growth rate of the biggest larva in gregarious culture resembled that of a solitary larva (Fig. 1 and 2, Curve A and B). There was a significant difference in growth rate between solitary larvae and the average of the two groups (Fig. 1, Curve B and C) of gregariously reared larvae. (Respective values for probability, *P*, were at 4, 8, 12 and 15 days 0.012, 0.039, 0.005 and 0.001). The average weight was here used, as the larvae were not sexed and females tend to be heavier than males. When four larvae shared one vial, one larva grew fast and three others developed slowly. Their

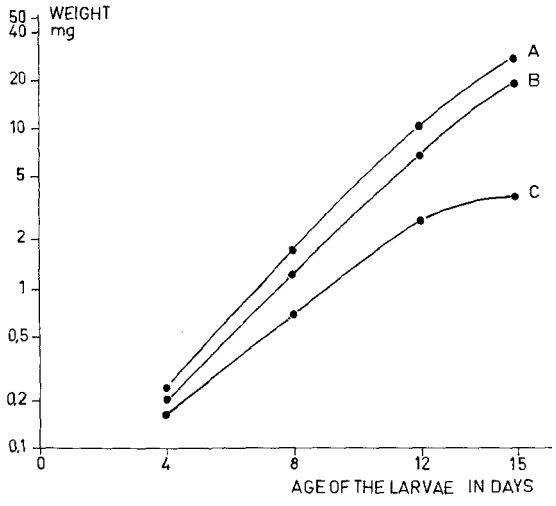

Fig. 1. Growth rate of solitary (A) and gregarious (B and C, 2 per vial) larvae. B is the average for the heavier in each vial and C for the lighter.

Fig. 1. Groeisnelheid van solitaire (A) en in groepjes van twee gekweekte larven. B is het gemiddelde van de zwaarste larve in ieder buisje en C van de lichtste.

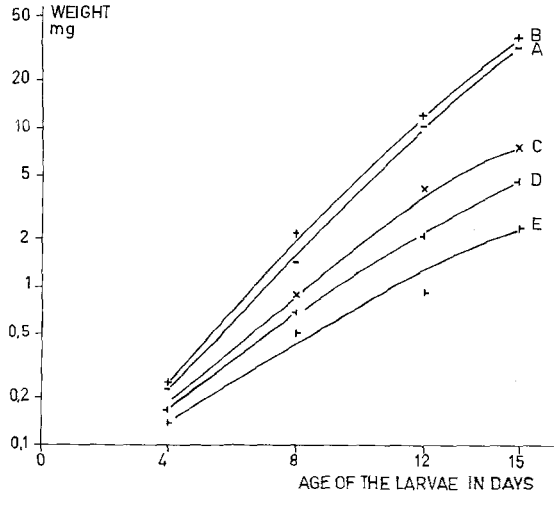

Fig. 2. Growth rate of solitary (A) and gregarious (B, C, D, E; 4 per vial) larvae.

Fig. 2. Groeisnelheid van solitaire en in groepjes van vier gekweekte larven. B, C, D en E geven de groei van de gewichtsklassen in de buisjes.

Table 1. Weight of pupae and development period of larvae reared alone or in groups of 2 or 4 per vial.

Number of larvae	Weight ♂ pupae (mg)	Development of ♂ larvae (days)	Weight ♀ pupae (mg)	Development of ♀ larvae (days)	% survival
1	20.1 a	20	35.3 a	21	85
2	20.3 a	27	29.8 b	30	80
1	22.6 a	20	31.4 a	23	85
4	18.2 a	23	26.9 b	27	50

Values followed by letter b are significantly different from those followed by an a ($P \leqslant 0.05$).

Tabel 1. Gewicht van de poppen en de ontwikkelingsduur van larven gekweekt alleen of in groepen van 2 of 4 per buis.

average weight was significantly lower than that of the solitary larvae ($P = 0.001$, 0.04, 0.01 and 0.001 at 4, 8, 12 and 15 days, respectively). The weight of the pupae was less informative (Table 1). After 15 days, the first larva in the gregarious cultures started pupating. It completed development on the freshest diet. The other larvae lived on the gradually drying diet and weight tended to be lower. Differences in weight were significant only for female pupae where growth retardation was more severe. Mortality was high when four larvae were reared together.

Causes of the effect

Deleterious effects of aggregation may be explained by lack of space, optical effects, odour, secretion of saliva, excrement, and contact.

Lack of space. Trials were with vials of different sizes and with different amounts of diet (Table 2). Mortality decreased with increase in size of the vial when four larvae were confined (Table 3). Growth rate did not increase with increased space (Tabel 4). These results indicate that the larvae could escape attack by the larger larvae in the large vials but they still could not grow so fast.

Table 2. Size of vials, amounts of diet, and available space in the trials on effects of space.

Type	Diam. (mm)	Height (mm)	Diet surface (cm^2)	Quantity (ml)	Available space (ml)
1	14	50	1.5	2.2	4.5
2	17	70	2.2	6.7	6.7
3	23	100	4.5	13.5	13.5
4	40	80	12.5	25.0	60.0
5	60	120	28.0	56.0	280.0

Tabel 2. Afmetingen van de buizen, hoeveelheid dieet en beschikbare ruimte in de buis bij de proeven over ruimteeffect.

Table 3. Percentage of pupae obtained from cultures in vials of sizes described in Table 2, 20 vials per test.

Type of vial	Number of larvae/vial	% pupated
1	1	95
	2	90
	4	56
2	1	95
	2	85
	4	52
3	1	95
	2	85
	4	61
4	1	95
	2	95
	4	75
5	1	100
	2	97
	4	91

Tabel 3. Percentage poppen verkregen in kweken met buizen van verschillende grootte, als beschreven in Tabel 2, 20 buizen per toets.

Table 4. Weight in mg of larvae 15 days old reared in vials of different sizes. For explanation see Table 2.

Number of larvae per vial		Type of vial 1	2	3	4	5
1	♂ larvae	22.3	32.0	30.7	22.7	23.3
	♀ larvae	37.9	63.6	46.5	25.0	28.0
2	Larva 1	31.2	45.9	39.9	29.6	33.1
	2	14.1	11.4	12.6	14.7	14.1
4	Larva 1	27.2	35.8	29.6	30.7	36.6
	2	13.7	34.8	28.2	13.1	21.1
	3	10.4	4.5	24.2	6.2	11.4
	4	3.2	2.0	4.5	3.7	6.2

Tabel 4. Gewicht in mg van 15 dagen oude larven gekweekt in buizen van verschillende grootte (zie tabel 2).

Optical and odour effects. Vials of Type 1 were divided in two with a Perspex partition. Larvae bred in these compartments (one per compartment) could see each other but otherwise had no contact. Despite the smaller space per larva, there was no difference in weight, growth rate or mortality from solitary larvae. When the Perspex was replaced by a phosphorbronze gauze, the larvae could also smell each other; the same results were obtained.

Saliva and excrements. When two larvae met, they usually attacked each other, and produced a fluid. In *Bupalus piniarius* L., Gruys (1970) found an effect of the fluid contents of the intestine on larval growth. Larvae of *Adoxophyes* showed no response in growth rate when reared alone in vials with filter paper impregnated with artificially obtained regurgitated gut contents from 4th or 5th instar larvae. Perhaps other methods should be used, to test the hypothesis. But any handling of the larvae had pronounced effects on growth (Table 5 and 6).

Excrements of fourth or fifth instar larvae did not affect growth of solitary larvae when they were added to the vials.

Table 5. Average weight of ♀ larvae in mg when disturbed by weighing (indicated with weight) or removal of the web (plus sign). No handling is indicated with minus sign.

Group	Age in days						Weight of pupae
	4	6	8	10	12	15	
1	0.2	–	1.7c	–	9.4c	24.0c	26.5c
2	0.2	+	1.9c	+	11.6c	29.4c	27.9b
3	–	–	3.8a	+	17.1a	48.5b	35.7
4	0.2	+	2.7b	+	–	–	30.9b
5	–	–	–	–	14.9a	32.9	30.7
6	–	–	–	–	–	34.3a	29.9
7	–	–	–	–	–	–	34.7a

Values in each column followed by the letter b are different from those followed by a at $0.01 \leqslant P \leqslant 0.05$.
Values followed by the letter c are different from those followed by a at $P \leqslant 0.01$.
Values without a letter were not significantly different from the others.

Tabel 5. Gemiddeld gewicht van ♀ larven in mg na verstoring door wegen (ogenblik aangegeven door gewicht) of door verwijdering (aangegeven door + teken). Een – teken betekent geen behandeling.

Table 6. Average weight of ♂ larvae in mg when disturbed by weighing (indicated by weight) or removal of the web (plus sign). No handling by minus sign.

Group	Age in days						Weight of pupae
	4	6	8	10	12	15	
1	0.2	–	2.0	–	10.4	24.5b	20.4b
2	0.2	+	1.9c	+	10.7b	19.8c	19.0b
3	–	–	2.5a	+	12.3	22.9c	22.6
4	0.2	+	1.3c	+	–	–	18.4c
5	–	–	–	–	12.9a	22.2	20.0
6	–	–	–	–	–	30.7a	22.0
7	–	–	–	–	–	–	24.0a

Values in each column followed by the letter b are different from those followed by a at $0.01 \leqslant P \leqslant 0.05$.
Values followed by the letter c are different from those followed by a at $P \leqslant 0.01$.
Values without a letter were not significantly different.

Tabel 6. Gemiddeld gewicht van ♂ larven in mg na verstoring door wegen (ogenblik aangegeven door gewicht) of door verwijdering (aangegeven door + teken). Een – teken betekent geen behandeling.

Contact. Each encounter resulted in aggression. The smaller larva took flight and as they are always living in a silken web they had to make a new one. It could not reach the food. The disturbance effect was imitated by removing the larvae from the vials and weighing them, or simply by removing their webs at various moments (Table 5 and 6). The results showed that in many cases weighing of the larvae or removal of their webs had a pronounced effect on larval weight. The effect was larger in females than in males and seemed strongest in the first part of the larval development.

Starvation. Besides this disturbance, the resulting starvation could also be a cause of growth retardation. This was simulated by depriving 30 larvae of food by placing them in the vial in a small gauze cage for periods from 4th-6th day, 8th-10th day and 12th-15th day (Table 7, Group 1). Thirty other larvae were simply removed and returned to their vial on the 4th, 6th, 8th, 10th and 12th day (Group 2); Group 3 served as a control. Starved larvae grew less than undisturbed larvae.

No starved larvae died and they resumed growth immediately on return to the food. This resulted in a longer period of development. The weight of gregarious larvae at the age of 12 days (Fig. 1 and 2) except for the biggest in each vial was always less than 5 mg. The disturbed and starved larvae (Table 7, Group 1) did not feed between the 12th and 15th day, so their weight on the 12th day was certainly no less than that on the 15th day, i.e. almost 5 mg. But the gregarious larvae were even more retarded in growth than those starved for 7 of the 15 days.

Consequences for mass rearing. The results have indicated that neither optical nor olfactory factors are responsible for growth retardation. Refuges for the larvae seem more important for protection from bigger ones and for undisturbed eating. Folded plastic strips 60 cm long and 2 cm high added to rearing boxes of the type described by de Jong (1968) significantly increased number of pupae from 134 to 192 per box ($0.02 < P < 0.05$).

The diet was given in small cubes (175 g per box) to give extra refuges to the larvae but no significant difference in yield of pupae was found from that with a method where the diet was given in one layer (yield 177 pupae).

Table 7. The effect of periods of starvation on the weight of 15-days-old larvae, pupal weight and duration of larval period.

Group	Weight of larvae (mg)		Weight of pupae (mg)		Larval period in days	
	♂	♀	♂	♀	♂	♀
1	4.6	5.0	19.7	29.5	25	25
2	11.9	15.5	22.6	35.1	21	22
3	20.6	21.1	23.9	36.5	19	21

Tabel 7. Het effekt van hongerperioden op het gewicht van 15 dagen oude larven, het popgewicht en de duur van de larvale ontwikkeling. 1 Hongerperiode van 4–6; 8–10; en 12–15e dag; 2 verstoring op de 4, 6, 8, 10 en 12e dag; 3 controle.

Discussion

Adoxophyes oviposits in egg masses of about 50–100 eggs. When the larvae hatch, they disperse rapidly, some by walking, others by hanging on a long thread attached to the leaf. On this thread the larvae may be dispersed by wind or they may reach other leaves. These mechanisms result in a solitary habit of life. Mass rearing leads to the unnatural situation of crowding. Under these crowded conditions, we see one larva guarding the food and chasing off all intruders.

For *Adoxophyes*, the area of food must be more than 28 cm^2 (Table 2, 3 and 4), vial Type 5, or 280 ml space. With the codling moth, *Laspeyresia pomonella* L., Howell (1970) kept larvae in cups of 18.5 ml. He also found an adverse effect on growth rate and mortality with increasing larval density (4 larvae per cup). The average yield per cup did not increase when more than 2 larvae were confined to a cup. The codling moth larvae burrow in the medium, so limiting the number of encounters and the chance of larvae noticing each other. Only when burrows intersected effects were noticed.

Windrich (1965), working with apples as food for codling moth larvae, observed a higher mortality. Usually only one larva per apple completed development. In this case the larvae burrow towards the core, and therefore have a greater chance of meeting.

As a whole the behaviour of the codling moth larva resembles that of the *Adoxophyes* larva. They are aggressive towards any intruders that come within range of their sense organs, so that a certain territory around each larva is kept free from potential competitors. Limitation of this territory and an increase of refuges should therefore improve the production of a mass culture. It would be worthwhile studying the effect of further increase in length or number of the strips used in mass rearing. The strips increased the total surface in the boxes by almost 30%, and yield of pupae also increased by 30%.

Samenvatting

Onderzoek over de steriele-mannetjestechniek als bestrijdingswijze van Adoxophyes orana (Lepidoptera, Tortricidae). 1. Massakweek (dichtheidseffecten)

Massakweken van *Adoxophyes orana* zijn noodzakelijk voor de ontwikkeling van een steriele-mannetjesprogramma ter bestrijding van deze plaag. In kweken in buisjes werd een agressief gedrag van de opgroeiende rupsen waargenomen wanneer zich meer dan 1 rups per buis bevond. Hierdoor werd de groeisnelheid vertraagd en trad bij 4 larven per buis veel sterfte op (Fig. 1 en 2, Tabel 1). Deze groeieffecten hadden geen optische of olfactorische oorzaak. Wanneer de larven meer ruimte kregen nam wel de mortaliteit af (Tabel 3) maar niet de groeivertraging (Tabel 4). Verstoring van de larven beïnvloedde hun groei nadelig (Tabel 5 en 6). De groeivertraging is echter voor het grootste deel te wijten aan hongeren (Tabel 7) doordat in de kweek de grotere larve de toegang tot het voedsel ontzegt aan de kleinere.

In massakweken kan de hieruit voortvloeiende groeivertraging en mortaliteit worden verminderd door plastic strips in de kweekbakken te plaatsen waardoor het aantal schuilplaatsen voor de larven toeneemt.

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