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## Weight increase of granulosis virus infected allatectomized larvae of the codling moth, *Cydia pomonella* (L.) (Lep., Tortricidae)

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The weight-increase of both normal and allatectomized codling moth larvae infected with a low dose of a granulosis virus is higher and lasts longer than in uninfected larvae, indicating that the effect of the virus is not due to juvenile hormone synthesis.

Optimization of *Baculovirus* production for insect suppression depends to some extent on a good understanding of the virus/insect interactions (BENZ, 1981) which, in turn, depend on the physiological conditions and the hormonal milieu in the insect (CAMPONOVO, 1980; CAMPONOVO & BENZ, 1984). By treating last instar larvae of the codling moth, *Cydia pomonella* (L.), with the juvenile hormone mimetic Altosid<sup>®</sup>, their time of growth (SIEBER & BENZ, 1978) and susceptibility to infection with a specific granulosis virus (CpGV) could be prolonged (CAMPONOVO, 1980). This led to a higher yield of virus per larva. Prolonged larval growth and better yield of virus per larva were also obtained by infecting the larvae with a dose of the CpGV low enough to allow the insects to survive for several days (CAMPONOVO, 1980). Since both, the treatment with Altosid<sup>®</sup> and the low virus dose, gave the same results, the prolonged weight increase of the infected larvae might be due to endogenous juvenile hormone (JH) produced by the corpora allata (CA), which were possibly stimulated by the virus. In order to test this hypothesis, young last instar larvae of *C. pomonella* were infected with a low virus dose after their CA had been extirpated.

### MATERIAL AND METHODS

The larvae were reared individually in small plastic boxes on a semisynthetic medium at 26°C under continuous illumination (HUBER *et al.*, 1972). Newly moulted last instar larvae (L<sub>5</sub>) were collected every 3 h, narcotized with CO<sub>2</sub>, submersed in a Ringer solution, and allatectomized (FRIEDLÄNDER *et al.*, 1981; JANS, 1982). As the CA are very closely attached to the corpora cardiaca (CC) the CA-CC complexes on both sides of the brain were extirpated. Within 1–2 h after allatectomy the larvae were injected via the mouth into the gut (MARTIGNONI, 1955) with 1 µl of either water (controls) or a CpGV suspension (3.6×10<sup>6</sup> capsules/ml). All larvae were returned to their individual rearing boxes and weighed daily. Of the virus infected group only the insects with visible signs of virogenesis (pale pink skin) were included in the experiment.

The apyrene spermatogenesis in last instar male larvae of *C. pomonella* occurs normally in connection with larval-pupal transformation (FRIEDLÄNDER *et al.* 1981; JANS, 1982) in the absence of JH (FRIEDLÄNDER & BENZ, 1982). To prove the absence of JH in the infected last instar larvae and that larval-pupal transformation occurred in these larvae, the spermatogenesis was studied in a number of male larvae. These larvae

were not allatectomized but infected at 12–24 h after the last larval moult. The testes of the larvae showing signs of infection were extirpated at 120–123 h, 144–158 h or 168–182 h after the  $L_5$  moult. They were fixed and stained in acetic-orcein on microscope slides and gently squashed with a cover glass. The squash preparations were studied by phase contrast microscopy.

#### RESULTS AND DISCUSSION

Fig. 1 shows that for 2 days all allatectomized larvae gained weight. After that time the non-infected individuals lost weight, whereas the infected larvae gained

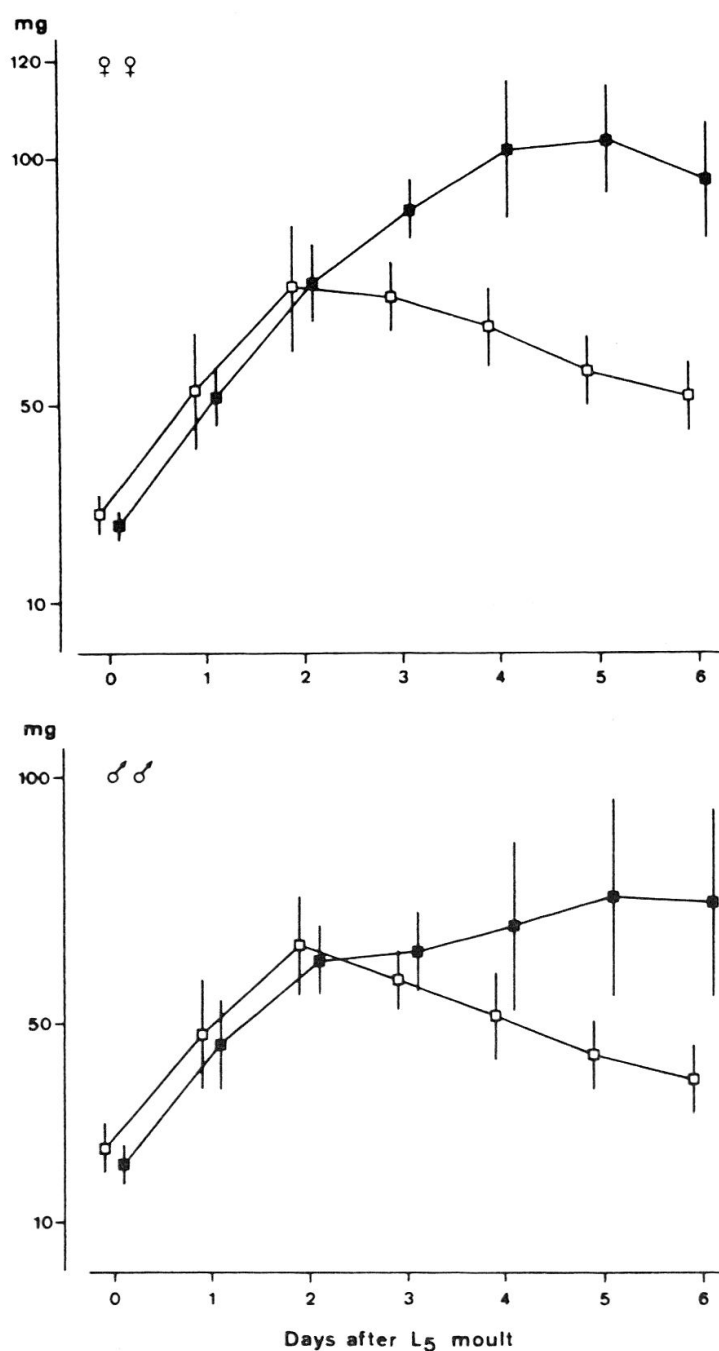


Fig. 1: Weight development of female (above) and male (below) codling moth larvae after allatectomy and virus infection on day 0 of the last larval instar ( $L_5$ ). Open squares = controls injected with water (10 ♀♀, 12 ♂♂); black squares = infected larvae (6 ♀♀, 8 ♂♂); vertical bars = standard deviation.

weight for another 3 days. Infected 3-day-old female and 4-day-old male larvae were significantly heavier than the corresponding uninfected stages ( $P < 0.01$ ).

Table 1 shows that apyrene metaphases appear in the testes of CpGV infected male larvae already at 120–130 h after the last larval moult, i. e. at the same time as in healthy larvae (FRIEDLÄNDER & BENZ, 1981).

Age after the L <sub>5</sub> -moult (in h)	Nr. of larvae tested	Apyrene metaphases	
		N	%
120-134	6	5	83
144-158	4	4	100
168-182	4	4	100

Tab. 1: Apyrene metaphases in larval testes of codling moths infected with a granulosis virus 12–24 h after moulting to the last larval instar and dissected at different ages.

Since allatectomized larvae cannot produce JH, the results prove that the higher weight increase of the infected codling moth larvae does not result of endogenic JH production stimulated by the virus. However, it could not be fully excluded that JH at a low level persisted in the infected individuals as in diapause-induced healthy larvae, where the JH concentration in the haemolymph was found to decrease to a certain level only (SIEBER & BENZ, 1977), making the larvae feed longer and grow heavier than larvae not induced for diapause (SIEBER & BENZ, 1978). In the latter JH is found in freshly moulted L<sub>5</sub> only and then disappears within 24 h (SIEBER & BENZ, 1977) because of metabolic degradation. This degradation of JH might not occur or be slowed down in the infected L<sub>5</sub>, because the GV infects mainly the fat body (WÄGER & BENZ, 1971), which is the main source of JH-esterase (NOWOCK *et al.*, 1976). Even if the remaining JH titer would be comparatively low, it could cause the longer feeding period and higher weight of the infected larvae. However, it has been demonstrated in diapause induced L<sub>5</sub> that the low JH titer of these larvae also suppresses the induction of apyrene spermatogenesis (FRIEDLÄNDER & BENZ, 1982), whereas Tab. 1 shows that apyrene metaphases appear in the testes of CpGV infected male larvae at the same time as in healthy non-diapause larvae. This finding excludes the presence of JH in the infected larvae and demonstrates – at least from the testicular point of view – that the larval-pupal transformation takes place. Therefore, we conclude that the prolonged weight increase of codling moth larvae infected with a low dose of CpGV is caused by the *Baculovirus* itself, independent of JH.

The situation in CpGV infected *C. pomonella* differs markedly from the one in *Spodoptera litura* (F.) infected with a nuclear polyhedrosis virus (subgroup A *Baculovirus*). In the latter the virus causes first a delay in the production of JH and then an abnormally prolonged high JH-level, which inhibits the larval-pupal transformation of the infected larvae (SUBRAHMANYAM & RAMAKRISHNAN, 1980). However, the difference between these two pathophysiological systems is probably not only due to the different types of baculoviruses involved, but to the different hormonal situa-

tions in the two insect species. Whereas the JH concentration in normal instar larvae of *C. pomonella* drops to an immeasurably low level (SIEBER & BENZ, 1977), it becomes very high in the last instar larvae of *S. litura* (SUBRAHMANYAM & RAMAKRISHNAN, 1980).

#### ZUSAMMENFASSUNG

Infektion im letzten Stadium von normalen und allatektomierten Apfelwicklerlarven (*Cydia pomonella* L.) mit einer relativ geringen Dosis des Apfelwickler-Granulosisvirus (CpGV) bewirkt, dass die infizierten Tiere mehr und länger wachsen als nicht-infizierte. Da in den allatektomierten Larven kein Juvenilhormon (JH) synthetisiert werden kann, zeigen die Resultate, dass die Gewichtszunahme der infizierten Larven nicht auf einem erhöhten JH-Spiegel beruht, sondern vom CpGV auf andere Weise bewirkt wird. Die Untersuchung der Spermatogenese in infizierten männlichen Larven zeigt durch das Auftreten von apyrener Spermiogenese, dass in infizierten Larven kein JH vorhanden ist und dass in diesen Larven die Induktion der Larven/Puppen-Transformation normal erfolgt.

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