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The ecdysone titer in last instar larvae of *Cydia pomonella* (L.) (Lep., Tortricidae)

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Last instar larvae (L_5) of the codling moth, *Cydia pomonella,* reared under a constant light regime pupate at the end of the instar, whereas larvae reared under short day conditions fall in diapause and do not moult. The titers of α-ecdysone and ecdysterone (= β-ecdysone) have been determined in the haemolymph of such L_5 during the first four days after the last larval moult, using a very sensitive radioimmunoassay. The larvae with uninterrupted development showed α-ecdysone and ecdysterone peaks of similar height at 36 h after the last larval moult, whereas the diapause-induced L_5 had a much smaller hormone peak at 72 h after the last moult.
According to SIEBER & BENZ (1980a) a much higher second moulting hormone level is reached on the 7th to 8th day of uninterrupted L_5 development (shortly after the larvae have spun their cocoons), but not in diapause-induced L_5. The second hormone peak causes moulting. Since the early hormone peaks in subitane developing and diapause-induced L_5 coincide with the changes in the larval-pupal commitment of their epidermal cells, it is suggested that the primary ecdysteroid peak induces the change in commitment.

INTRODUCTION

As in other insects, moulting in *Cydia pomonella* depends on the secretion of moulting hormone by the prothoracic glands, which are stimulated by the brain hormone or prothoracotropic hormone (PTTH). Investigations by SIEBER & BENZ (1980a) and JANS (1982) showed that neck-ligation of last instar larvae (L_5) up to 5 days after the last larval moult prevents larval-pupal ecdysis. If the brain of less than 4-day-old L_5 is extirpated, the larvae will never moult, due to the lack of PTTH. However, neck ligated or debrained larvae moult after injection with ecdysterone (= β-ecdysone = 20-hydroxyecdysone). Debraining of the L_5 on the 5th day led to 10%, on the 6th day to 80%, and on the 7th day to 100% moulting, indicating that only 6 to 7 days after the last larval moult the PTTH titer is high enough or has acted long enough to stimulate the ecdysteroid production in the larvae.

The ecdysteroid titers have been determined in last instar larvae of the tortricid *Choristoneura fumiferana* (CLEM.) by LAGUEUX *et al.* (1976) and in *C. pomonella* by SIEBER & BENZ (1980a). They found a large ecdysterone peak 1–2 days before pupation. In *C. pomonella* at 26 °C this time corresponds to the second day after spinning the cocoon. Several authors found two ecdysteroid peaks in last instar larvae: a small first ecdysteroid peak early in the instar and a large second peak shortly before ecdysis, as mentioned for the two tortricid species above (BOLLENBACHER *et al.*, 1975; NIJHOUT, 1976; LAFONT *et al.*, 1977; MARÓY & TARNÓY, 1978).

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Pupation involves not only moulting but a change in the commitment of the epidermal cells. In *C. pomonella* this change takes place within the first two days of the last larval instar and depends on the activity of the brain. If younger and older than two-day-old L_5 were debrained and then forced to moult by injecting them with ecdysterone on the fourth day, the L_5 debrained before the second day moulted to supernumerary larvae, whereas the others formed pupae (JANS, 1982). It is not known how the brain induces the new programming of the epidermal cells. It has been shown, however, that the larvae of *C. pomonella* must feed before the reprogramming can take place. If starving L_5 are forced to moult by injecting them with ecdysterone, they moult larval (JANS, 1982).

NIJHOUT (1976), LAFONT *et al.* (1977), and others considered the first ecdysteroid peak to be responsible for the change in larval-pupal commitment. Since SIEBER & BENZ (1980a) using the *Musca*-test for their determinations of the ecdysteroid titres did not find a small primary peak in *C. pomonella,* the hypothesis above seems not to fit with this species. However, since it was possible that the *Musca*-test was not sensitive enough for the detection of the small primary ecdysterone peak, we reinvestigated the ecdysteroid titer in young L_5 of *C. pomonella,* using a much more sensitive radioimmunoassay (RIA).

MATERIAL AND METHODS

Insects

C. pomonella larvae were reared singly at 26 °C and 70% RH on an artificial medium according to the method described by HUBER *et al.* (1972). Different groups of larvae were kept under either constant light (CL) or short day conditions (SD), i. e. 8 h light to 16 h dark. The first group was destined for uninterrupted development with pupation at the end of the instar, whereas the second group was destined for diapause development (SIEBER & BENZ, 1977).

Haemolymph samples

Last instar larvae were collected at 0–2, 12, 24, 36, 48, 60, 72, 84, and 96 hours after the last moult. The insects were cooled to 0 °C on ice and bled in order to obtain haemolymph samples.

Extraction, purification, and quantification of ecdysteroids

For the quantification of ecdysteroids in the haemolymph 200 μl samples were extracted with 65% methanol and purified by thin-layer chromatography after the method described by ZHU *et al.* (1983). α-ecdysone and ecdysterone were thus separated and could be quantified separately, using a RIA described by the same authors. The concentrations of the two ecdysteroids were calculated as ng/ml haemolymph.

RESULTS

The results in Fig. 1 show the titers of α-ecdysone and ecdysterone in the haemolymph during the first 84 h after the L_4–L_5 moult of *C. pomonella* reared under CL conditions, i. e. larvae that would start spinning a cocoon on the 5th

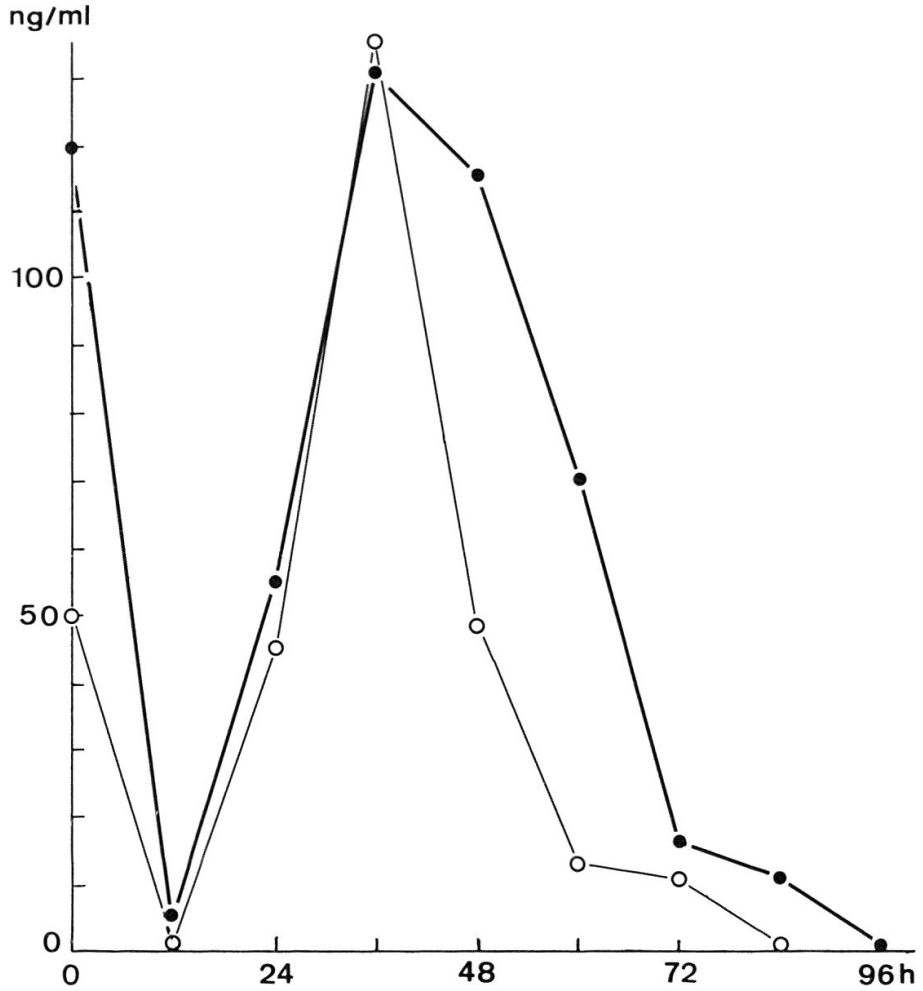

Fig. 1. Titers of α-ecdysone (○) and ecdysterone (●) in ng/ml haemolymph of zero to four-day-old *Cydia pomonella* last instar larvae reared under constant light conditions.

day and pupate 2–4 days later (SIEBER & BENZ, 1977, 1980a). It is evident that the titer of ecdysterone is at least twice as high as that of α-ecdysone during the moulting. The titers of both ecdysteroids then fall within 12 h to almost zero, but rise again in the one-day-old L_5, reaching a maximum at 36 h. The titer of α-ecdysone then drops rapidly, whereas the concentration of ecdysterone stays high for another 12 h and then decreases.

The ecdysteroid titer in larvae grown under SD conditions is much lower than in L_5 reared under CL (Fig. 2). Since the SD conditions induce diapause in the full-grown last larval instar (SIEBER & BENZ, 1977, 1980b), these larvae will not moult in the near future. Nevertheless, there is a small peak of α-ecdysone detectable on the third day, i. e. about 36 h after the ecdysteroid peak in the larvae determined for direct pupation.

DISCUSSION

The results with uninterrupted developing larvae of *C. pomonella* show that in this species an early peak of moulting hormone occurs at 36 h after the last lar-

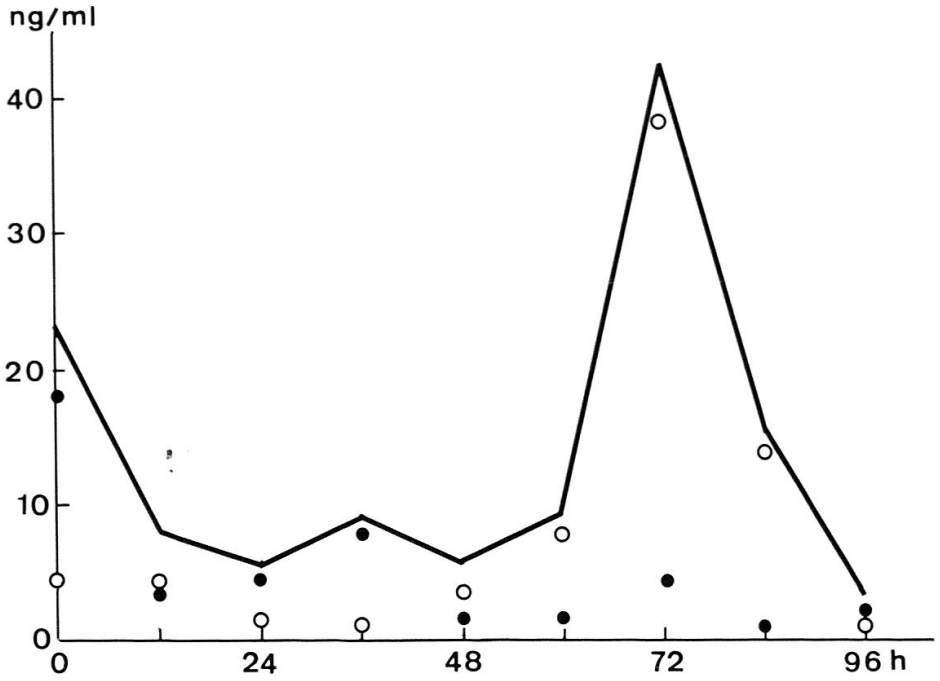

Fig. 2. Ecdysteroid titers of zero to four-day-old diapause-induced *Cydia pomonella* last instar larvae reared under short day conditions. Curve = sum of ecdysteroids, ● = ecdysterone, ○ = α-ecdysone.

val moult, i. e. shortly before the commitment of the epidermal cells changes from the larval to the pupal expression of the genes (JANS, 1982). α-ecdysone and ecdysterone increase at the same time, but the titer of ecdysterone decreases more slowly after the peak has been reached. This might indicate that α-ecdysone is converted into ecdysterone, as demonstrated by LAGUEUX *et al.* (1976) in *Ch. fumiferana*. These authors found that injected radioactive labelled α-ecdysone was for the most part converted into ecdysterone when the endogenous level of moulting hormone was high, but degraded to 3-dehydroxyecdysone and conjugates, when the endogenous level of moulting hormone was low in the larvae. The ecdysone-20-mono-oxygenase for converting α-ecdysone into ecdysterone was found to be positively correlated with the hormone titer.

If, as in Fig. 3, the results of the present paper are combined with the moulting hormone titers determined by SIEBER & BENZ (1980a), a moulting hormone curve with two distinct peaks results. Such curves have been found in Lepidoptera from other families (e. g. *Manduca sexta* by BOLLENBACHER *et al.*, 1975; *Pieris brassicae* by LAFONT *et al.*, 1977; *Galleria mellonella* by MARÓY & TARNÓY, 1978) as well as in other insects (e. g. *Aeschna cyanea* by SCHALLER *et al.*, 1975). In *C. pomonella* the second peak rises shortly after the larvae begin to spin their cocoons. There can be little doubt that this hormone peak causes ecdysis (SIEBER & BENZ, 1980a).

Concerning the first peak of moulting hormone, it has been mentioned before that some authors considered it to be responsible for the change in larval-pupal commitment. However, SCHALLER *et al.* (1975) concluded from data of *Aeschna* that the small first peak induces apolysis, moulting gel secretion, and cuticulin deposition, and that the larger second peak regulates the digestion of the old endocuticle, the deposition of the new procuticle, ecdysis, and sclerotiza-

tion. MARÓY & TARNÓY (1978), on the other hand, suggested that the first peak in *Galleria* causes moulting, whereas the second peak is dispensable for pupation, having some as yet undetermined developmental significance. Since the first hormone peak in *C. pomonella* appears shortly before the commitment of the epidermal cells changes, it seems probable that the change is caused by the early ecdysteroid peak. This hypothesis is strengthened by the fact that, although much smaller, an α-ecdysone peak is also found in diapause-induced larvae. These larvae will not moult until diapause is terminated. Therefore, the small hormone peak is certainly not related to the induction of moulting. According to CAMPONOVO (1980) and SIEBER & BENZ (1980a, b) the commitment change in diapause-induced *C. pomonella* larvae occurs one to two days later than in uninterrupted developing larvae, i. e. at the time when the moulting hormone peak is found in the diapause-induced larvae.

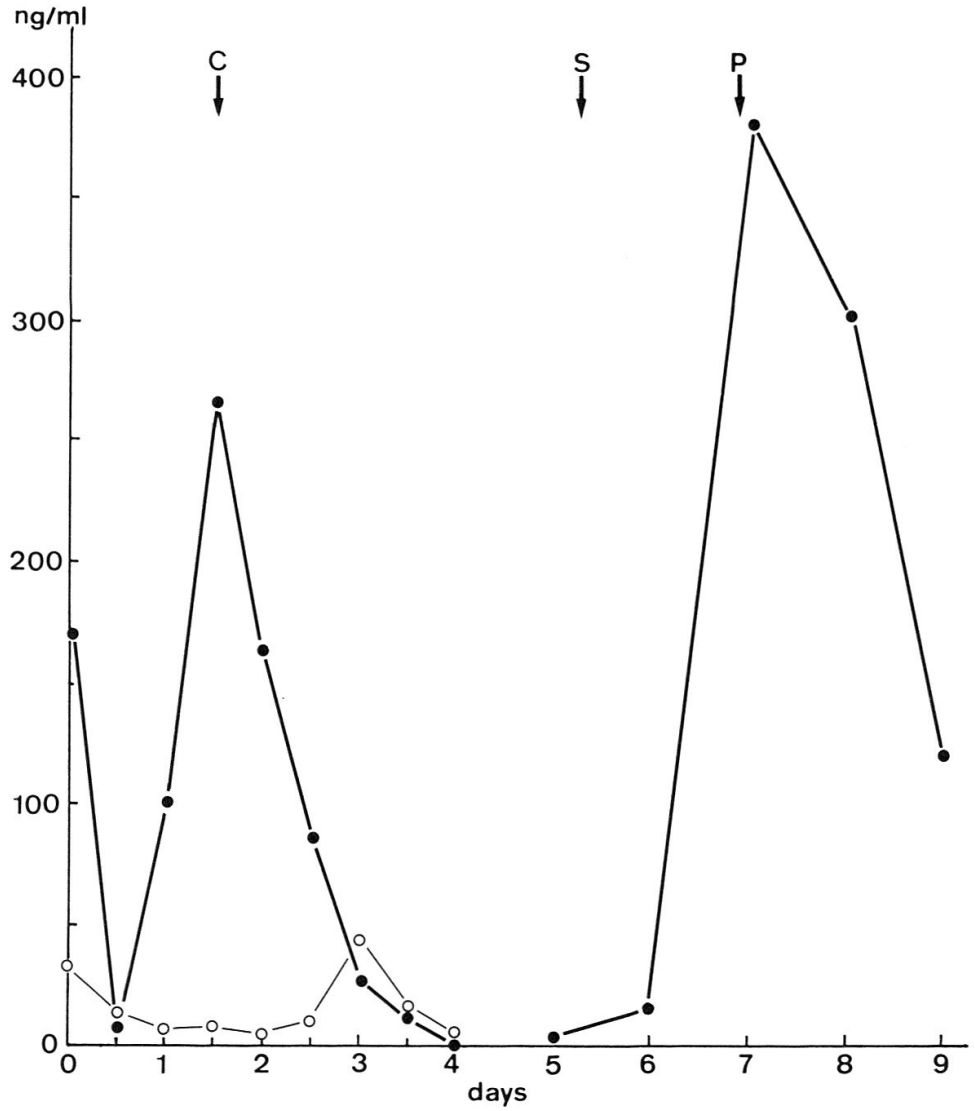

Fig. 3. The ecdysteroid titers in the course of the last larval instar of uninterrupted developing (●) and diapause-induced (○) *Cydia pomonella*. Hormone titers from 5th to 9th day according to SIEBER & BENZ (1980a). Arrows show time of commitment change (C), spinning of cocoon (S), and beginning of pupation (P).

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ZUSAMMENFASSUNG

Der Häutungshormontiter (α-Ecdyson und Ecdysteron) während der ersten vier Tage des letzten Larvenstadiums (L_5) des Apfelwicklers *(Cydia pomonella)* wurde mit Hilfe eines sehr empfindlichen Radioimmunoassays (RIA) bestimmt. Bei Dauerlicht gehaltene subitan sich zur Puppe entwickelnde L_5 wiesen 36 h nach der letzten Häutung eine Häutungshormonspitze auf, während bei Kurztag gehaltene diapause-induzierte L_5 (die sich nicht häuten) ebenfalls eine, allerdings viel kleinere Hormonspitze 72 h nach der Häutung zeigten. Sieber & Benz (1980a) hatten mit dem wenig empfindlichen *Musca*-Test nur bei den subitan sich entwickelnden Raupen kurz nach dem Spinnen des Kokons und vor der Puppenhäutung (7.–8. Tag) einen starken Häutungshormonspiegel gemessen, der ohne Zweifel die Puppenhäutung auslöst. Da die hier festgestellten frühen Hormonpulse zeitlich mit der Induktion des larval-pupalen Bestimmungswechsels der Epidermiszellen zusammenfallen, nehmen wir an, dass der kleine primäre Hormonstoss diesen Bestimmungswechsel bewirkt.

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