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Effects of 20-hydroxyecdysone on Adult Development of Diapausing Fall Webworm (*Hyphantria cunea* Drury) Pupae

휴면중인 미국흰불나방 번데기의 성충발육에 미치는 20-hydroxyecdysone의 영향

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ABSTRACT Studies were carried out to elucidate the efficacy of exogeneous 20-hydroxyecdysone treatment on terminating pupal diapause in the fall webworm, *Hyphantria cunea* Drury And the difference was also investigated between normal adult development and post-diapause development after 20-hydroxyecdysone treatment. In the diapause termination rate of pupae treated with 20-hydroxyecdysone after storage for various periods at 16L·8D and 25±1°C, the highest rate was observed from the group stored for the longest period and the lowest rate from those stored for 1.5 months. The time needed for adult emergence was inversely propotional to the chilling (at 0±1°C) period, and the longer its exposure period at low temperature, the higher its sensitivity to 20-hydroxyecdysone treatment. Pupal diapause duration was almost the same, regardless of storage period in the total darkness or at the photoperiod of 16L. 8D, and also they successfully emerged to adult even without any experience at low temperature. The oxygen consumption rate in normally developing pupae showed nearly a typical U-form. But, that of diapausing pupae treated with 20-hydroxyecdysone slowly increased and jumped 14 days after the treatment. Pupal diapause began before formation of adult tissues, and a timing of adult tissue formation coincided with ascending timing of the metabolic rate in both normally developing pupae and diapausing pupae treated with 20-hydroxyecdysone. The diapausing pupae treated with 20-hydroxyecdysone was similar to normally developing pupae in band patterns of proteins from haemolymph or fat body.

KEY WORDS *Hyphantria cunea*, Diapause Termination, Oxygen Consumption, Cold Temperature, 20-HE, Hemolymph Protein, Fat Body Protein, electrophoresis

초 록 미국흰불나방(*Hyphantria cunea* Drury) 번데기의 휴면 종료에 미치는 탄피호르몬 20-hydroxyecdysone (20-HE)의 처리효과와 또한 호르몬처리로 유기된 휴면 후 성충발육 모습을 정상적인 성충발육과 비교하기 위하여 본실험을 수행하였으며, 그 결과는 다음과 같다 광주기 16L·8D와 25±1°C에서 여러가지 다른 기간 동안 저장했던 휴면번데기에 20-HE를 처리했을 때 가장 높은 휴면 종료율은 가장 오랫동안 처리된 군에서 나타났고 45일 동안 저장했던 휴면번데기에서 가장 낮은 종료율을 보였다. 우화에 필요한 기간은 저온(0±1°C)처리기간에 반비례하였고 또한 저온처리기간이 길어질수록 20-HE에 대한 민감도는 높아졌다. 번데기 휴면기간은 그들이 16L 8D 광조건에 처리되었건 혹은 지속적인 암조건에 저장되었건 간에 거의 같았고, 또한 저온처리없이도 성공적으로 성충이 출현하였다. 정상적으로 우화(휴면없이)하는 번데기의 산소소모율은 전형적인 U자 모양을 보였지만 20-HE에 처리된 휴면 번데기의 산소흡수량은 천천히 증가하다가 처리 후 14일에 급격히 증가하였다. 번데기 휴면은 성충조직을 형성하기 이전에 들어갔으며, 정상적으로 발육하는 번데기나 또는 20-HE에 처리되어 발육하기 시작하는 휴면 번데기 모두에서 성충조직 형성시기와 대사율이 증가하기 시작하는 시기는 일치하였다. 탈피호르몬처리로 발육을 시작한 휴면 번데기의 혈림프와 지방체에서 분리된 단백질종류는 모두 정상적으로 발육하는 번데기의 조직에서와 같았다

검색어 미국흰불나방, 탈피호르몬, 휴면종료, 휴면탈피, 산소소비량, 저온처리, 단백질 종류, 전기영동

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After William's experiments (1946) about a relation of brain and diapause-breaking in the giant silkworm, *Hyalophora cecropia*, the role of brain and prothoracic glands in diapausing pupae has been studied by serial investigators (Williams 1952, Bell *et al.* 1975, Meola and Adkisson 1977, Waker and Denlinger 1980, Browning 1981, Richard and saunders 1987). The exogeneous treatment of ecdysteroids on diapausing pupae has a direct effect on termination or shortening of diapause duration. But the diapausing pupae show a species-specific response to the dose, number and a timing of treatment of the ecdysteroids (Denlinger 1985). And also, the change of ED_{50} (effective dose required for diapause termination in 50% of the individuals treated) in hormone sensitivity (Bodnaryk 1977) illustrates indirectly that diapause can be viewed as a dynamic state.

According to Park and Boo (1988), the treatment of 20-hydroxyecdysone on 2.5% whole body homogenate of diapausing fall webworm pupae increased its respiratory activity, and the injection of this hormone into the diapause-bound pupae caused its emergence as an adult. Separated fat body protein bands of diapause-bound pupae also differed from those of non-diapausing pupae.

In the role of environment as a diapause maintenance and termination factor, the photoperiod is one of two major factors that act to maintain and terminate diapause, and some species exclusively rely on the photoperiod for this function (Beck 1980). But in most hibernal diapause the temperature acts as a major factor of diapause intensification, maintenance and termination. They also decelerate the utilization of metabolites that are essential to diapause development and they serve to synchronize the initiation of post diapause development (Tauber *et al.* 1986).

This study was carried out in a laboratory to investigate the effect of 20-hydroxyecdysone on diapausing pupae and adult development of the fall webworm, *Hyphantria cunea* Drury.

MATERIALS AND METHODS

Effects of 20-hydroxyecdysone on terminating diapause

The experimental insects were reared at a diapause-inducing condition (13L:11D, 25+1°C) (Park and Boo 1988) and pupae were stored at different photoperiods and temperture (the details are in the tables and figures under the section of results and discussion). Injection of 20-hydroxyecdysone (Sigma Chemical Co.) followed the method of Bodnaryk (1985) and Park and Boo (1988). The injection of each dose (in 5 μl) was carefully carried out through the membraneous cuticle between the 1st and 2nd abdominal segments and delivered with a 25 μl Hamilton syringe.

Determination of the oxygen consumption

Oxygen consumption of individual pupa was measured at 25°C with a Warburg manometer (13 positions, circular model, Tawnson & Mercer, LTD) with a method described by Denlinger *et al.* (1972). The experimental insects were non-diapausing pupae or diapausing pupae treated with 20-hydroxyecdysone. The oxygen consumption was monitored for 3 or 4 days from one pupa, and then changed with a different one.

Developmental events

The appearance of various developmental events was examined for the chronology of adult development. The experimental insects were non-diapausing pupae or diapausing pupae treated with 20-hydroxyecdysone. A portion of body from the cuticle at the posterior region of pupal thorax was carefully removed into the plate which was filled with a Ringer's solution (NaCl, 149.0 mM; KCl, 40.0 mM; $MgCl_2$, 9.0 mM; pH 7.0; adjusted with solid $NaHCO_3$) containing 0.05% phenylthiourea (PTU). The developmental events of samples were investigated through this open region, until adult emergence, with a stereomicroscope.

Protein analysis

Haemolymph samples were collected by the method of Park and Boo (1988) with a cool, graduated tube and centrifuged at 5,000g for 10 min. Fat bodies were dissected out from pupae in the normal Ringer's solution containing 0.05% PTU, and fat body homogenates were then prepared in a tube

with the sample solvent for electrophoresis to be centrifuged twice at 5,000g for 10 minutes.

Haemolymph and fat body extracts were subsequently analysed by SDS-poly-acrylamide vertical slab gel (Vertical Slab Unit 2000, Vocam 2000-300-150, Shandon) (10% separating gel, 120 mm and 5% staking gel, 30 mm) by the method of Smith (1984) with marker proteins (Sigma Chemical Co.; molecular weight range: 25-205 Kd). Bromophenol blue was used as the tracer. After separation, the gels were treated with 0.25% Coommassie Brilliant Blue for 24 hours, and then with destaining solution (methanol : acetic acid : distilled water=45 : 5 : 50(v/v)). Finally, the results were scanned by a densitometer (Shandon *Chello* 3) with the green filter.

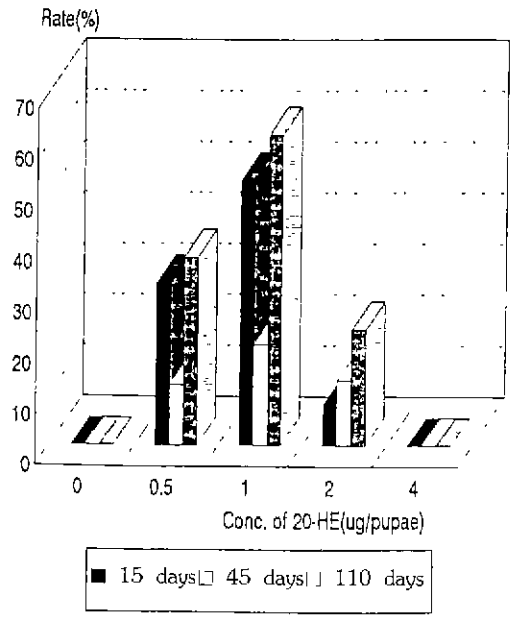
RESULTS AND DISCUSSION

Diapausing *H. cunea* pupae showed different sensitivity to 20-hydroxyecdysone treatment depending on the storage periods under 16L : 8D and 25± 1 °C, showing the lowest sensitivity after storage for 45 days (Fig. 1). Already a similar phenomenon has been reported on the sensitivity of the prothoracic gland to 20-hydroxyecdysone treatment The prothoracic glands changed its sensitivity to 20-hydroxyecdysone treatment during diapause duration in *Mamestra brassicae* (Bodnaryk 1977). This change can be connected with diapause development (induction, intensification, maintenance and termination). But the light condition (long day or total darkness) did not give any effect on diapause duration (Table 1) or on the diapause termination rate by 20-hydroxyecdysone treatment (Fig. 2)

The time needed for adult emergence was inversely proportional to the storage period at 0± 1°C (Table 2). Even one month of storage at the low temperature cut the diapause period into almost the half of the period needed for adult development

Table 1. Pupal diapause duration(days) of *H. cunea* when exposed to 16L : 8D or total darkness (25± 1°C)

Incubation condition	No. of samples	Diapause duration	Emergence rate(%)
16L : 8D	145	124 7± 13	33 8
Total darkness	134	128.4± 11	31.3



Figs. 1. Diapause termination rate (16L · 8D, 25± 1°C) of *Hyphantria cunea* pupae treated with 20-hydroxyecdysone (20-HE) after storage for different periods at 16L : 8D and 25± 1°C.

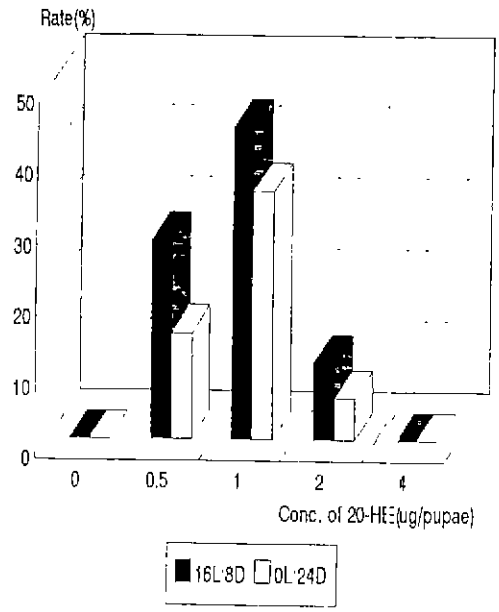


Fig. 2. Diapause termination rate, at 16L 8D and 25 ± 1°C, of *H. cunea* pupae treated with 20-hydroxyecdysone (20-HE) after storage for 110 days at 25± 1°C and 16L : 8D or total darkness

Table 2. Number of days at 25± 1°C needed for adult emergence of diapausing pupae exposed to 0± 1°C for various periods

Exposure periods (months)	No of samples	No of adult emerged	Period to emergence (days)
1	37	3	59± 5
2	30	2	47± 10
5	25	4	21± 2

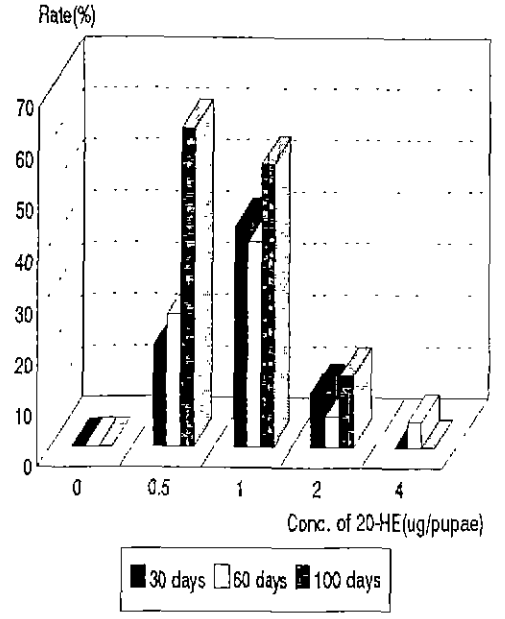


Fig. 3. Diapause termination rate, at 16L 8D, of *H. cunea* pupae treated with 20-hydroxyecdysone (20-HE) after storage for different periods at 16L. 8D and 0± 1 °C.

without any cold treatment (Tables 1, 2). Of course, the overall low rate of emergence prevents from drawing a firm conclusion, which means that further experiments should be carried out more carefully. And the longer its exposure to the low temperature, generally the higher the sensitivity to exogeneous ecdysteroid treatment (Fig. 3). Tauber *et al.* (1986) demonstrated that temperature does not act as a general diapause terminating signal, but as a factor regulating the rate of diapause development and also determining how fast post-diapause development will proceed. Diapause termination experiment in *H. cunea* pupae also revealed a similar result (Choi and Boo 1987).

In the result of Table 1, they successfully emerged even without exposure to the low temperature, and this result was contradictory to that of Choi and Boo's expriment (1987). Perhaps, this contradiction results from the temperature condition: the temperature decreased gradually during diapause and increased beyond a threshold point in Choi and Boo's experiment (1987). On the contrary, the present study carried out at a constant temperature (25± 1 °C). No individuals emerged to adult when they had been treated with solvent only or simply prickled. It is not clear why but the result was observed only for 20 days.

The optimum range of 20-hydroxyecdysone dosage (about 3.3-6.4 μg/g) for diapause termination, regardless of storage conditions, is similar with that of the other lepidopteran species, such as *Heliothis zea* (Meola and Adkisson 1977), *Heliothis punctiger* (Browning 1981) and *Mamestra configurata* (Bodnaryk 1985), but it is higher than that of *Manduca sexta* (Bradfield and Denlinger 1980) and a dipteran species, *Cuterebra tenebrosa* (Baird 1972).

The oxygen consumption rate during pupal-adult development of *H. cunea* showed a typical U-form (Fig 4), as already shown by Park and Boo (1988), just like in many other holometabolous insects, such as *Galleria mellonella*, *Tenebrio molitor* (Slàma 1982) and *Sarcophaga crassipalpis* (Denlinger *et al.* 1972). Diapausing pupae kept for 45 days (25± 1°C) had maintained a low oxygen consumption rate (about 30-70 μl/O_2/g/hr) before 20-hydroxyecdysone treatment. But they started to increase their oxygen uptake rate, about 4 days after 20-hydroxyecdysone treatment, to reach a level, at the day 18, as much as that(about 325 μl/O_2/g/hr) of non-diapausing pupae just before adult emergence (Fig. 4). In Park and Boo's experiment (1988), when diapausing *H. cunea* pupae, kept for 1 month at 0°C, were treated with 20-hydroxyecdysone, the respiratory activity increased to about 292.5% of that from diapausing 15-day old pupa (about 50 μl/O_2/g/hr). And also, the rate of oxygen consumption in the initial stage of diapausing *H. cunea* pupae decreased to the 9-25% range of those from non diapausing

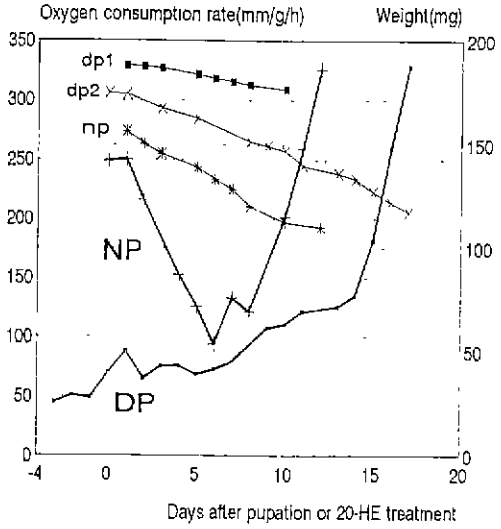


Fig. 4. Oxygen consumption rate (left) between non-diapausing pupae (NP) and diapausing pupae (DP) treated with 20-hydroxyecdysone (20-HE) after storage for 45 days (16L : 8D, 25± 1°C) and body weight change (right) of non-diapausing pupae (np), diapausing pupae treated wit solvent (dp1) or 20-HE (dp2). Each value is a mean of more than 5 individuals.

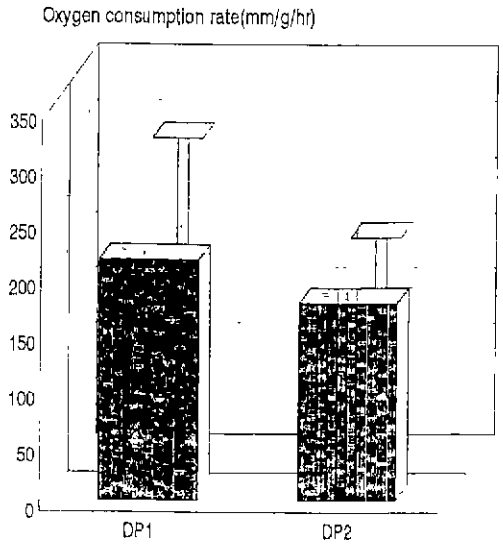


Fig. 5. Comparison of oxygen consumption rate in diapausing *H. cunea* pupae, measured at 25± 1°C, after storage for 120 days at 25± 1°C but at different photoperiodic conditions
*DP1: Stored 16L : 8D
DP2 Stored in total darkness

Table 3. Comparison of developmental events between normal pupae (left) and diapausing *H. cunea* injected with 20-hydroxyecdyson (20-HE) (1 μg/individuals) (right) at 25± 1°C

Days after pupation	Developmental events	Days after 20-HE treatment
0	pupated or 20-HE injected	0
1	pupae tanned or O_2 consumption increased smoothly wing disc evaginated and appeared transparent	1
5	corpora cardiaca and corpora allata filled with some materials	
6	histolysis of larval tissues started. pupal haemolymph filled with cellular debris	11
7	adult thorax started to form. leg discs evaginated	12
8	compound eye pigmentation visible externally	14
9	antennae and palps formed: haemolymph became clear	15
10	wing color became whitish	16
11	adult tissue formation completed or hyperecdysonism	17
12	adult emerged	18

pupae

The oxygen consumption rate was compared between diapausing pupae after storage for 120 days at 25± 1°C but at different photopenodic conditions (Fig. 5). Photoperiod during storage does not seem to differentially affect the rate In *H. cunea* pupae, histolysis of larval tissues initiated about one or two days before the beginning of pigmentation of compound eyes (Table 3). At this time, the larval midgut was almost completly destructed and therefore the haemolymph was filled with cellular debris. An extemally observable cue on adult development was

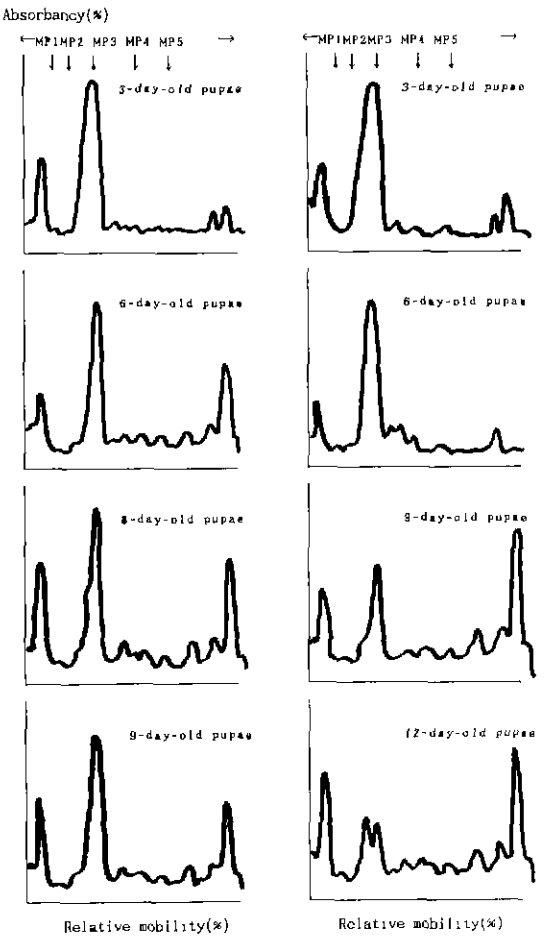


Fig. 6. Densitometric scans of haemolymph proteins from non-diapausing pupae (left) and diapausing pupae treated with 20-hydroxyecdysone (right) (separated by SDS-PAGE in 10% acrylamide). MP marker proteins (1: 116 Kd, 2: 97.4 Kd, 3: 66 Kd, 4: 45 Kd, 5: 25 Kd).

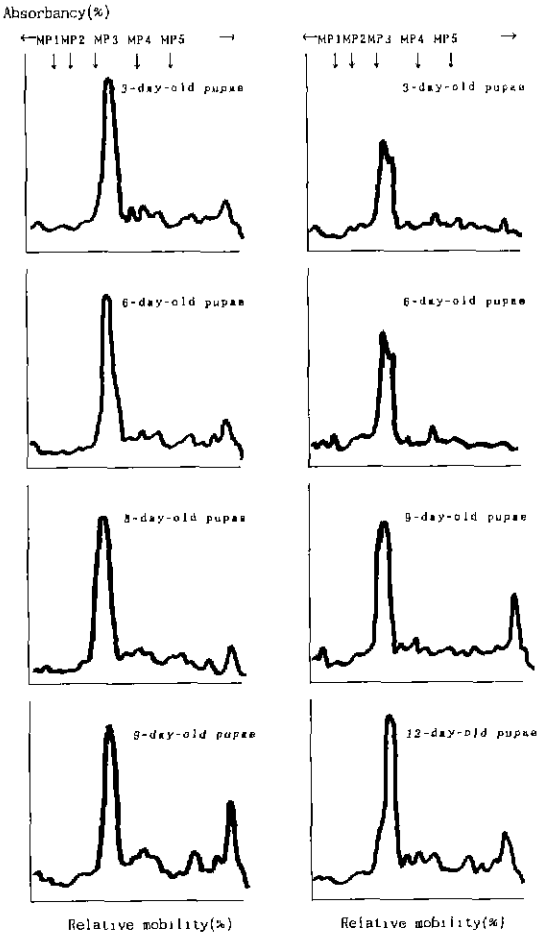


Fig. 7. Densitometric scans of soluble fat body proteins from non-diapausing pupae (left) and diapausing pupae treated with 20-hydroxyecdyssone (right) MP: same as in Fig 6.

only a black pigmentation of compound eyes at day 8 after pupation or day 14 after 20-hydroxyecdsone treatment. Thin and transparent membranes seen under thorax cuticle in the early stage (1 day after the start of development) must be wing discs. The colour of the wing discs became more and more whitish with the progress of development. And a distinction between the fore- and hind-wings was clarified only a couple of days before actual adult emergence (Table 3).

Although pattern of the metabolic rate in diapausing pupae injected with 20-hydroxyecdysone differed from that of normal developing pupae, a timing of adult tissue formation coincided with an ascent timing of the metabolic rate (oxygen uptake rate) in both normally developing pupae and diapausing pupae treated with 20-hydroxyecdysone (Fig. 5, Table 3) And also, there was no significant difference in pattern of haemolyph and fat body protein bands between normally developing pupae and diapausing pupae treated with 20-hydroxyecdysone (Figs. 6, 7).

In male pupae of *M. configurata*, the transitory peaks of ecdysone appeared at day 8 during the post-diapause development when induced by 20-

hydroxyecdysone (Bodnaryk 1985). And when the diapausing pupae was transferred from 0°C to 20 °C, they showed a similar peaks of ecdysone content. The timing of these peaks corresponded to the temporary upsurge in many other lepidoteran pupae during pupal-adult metamorphosis (Holman and Meola 1978, Sehnal *et al.* 1981, Loeb 1982). Therefore, if the ecdysteroids be titrated directly in diapausing and non-diapauaing *H. cunea* pupae; the relation between the acending timing of metabolic rate and the rise of endogeneous ecdysteroids will be clarified.

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