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Change in Protein and Carbohydrate Contents in Diapausing and Non-diapausing Pupae of the Oriental Tobacco Budworm, *Heliothis assulta* Guenee*

담배나방 휴면용과 비휴면용의 단백질과 탄수화물의 변화

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ABSTRACT Studies were carried out to investigate the change in proteins and carbohydrates from diapausing pupae of the Oriental tobacco budworm, *Heliothis assulta* Guenee. The oxygen consumption rate of non-diapausing pupae through the whole pupal period showed an U-shaped curve, while that of diapausing pupae decreased to a mean level of 20 μl/g/hr. But the rate of oxygen consumption increased in diapausing pupae before their emergence. The body weight of diapausing pupae showed almost no change during 12 days after pupation. The total contents of major carbohydrates and soluble proteins were higher in diapausing pupae than those in non-diapausing pupae and the change in carbohydrate and protein contents was more dynamic in non-diapausing pupae than that of diapausing puape. The electrophoretic band patterns of proteins were similar in both of diapausing and non-diapausing pupae. Diapausing pupae increased their haemolymph osmolarity further when they were exposed to low temperature.

KEY WORDS Oriental tobacco budworm, *Heliothis assulta*, oxygen consumption rate, osmolarity

초 록 정상적으로 발육하는 담배나방 용 및 휴면용의 단백질과 탄수화물의 함량변화, 산소소비율, 몸무게의 변화, 삼투압등을 비교하였다. 정상용의 경우에는 산소소비율의 변화가 전체 기간에 걸쳐 U자 모양의 곡선을 보인 반면, 휴면용의 경우에는 20μl/g/hr의 매우 낮은 수준으로 감소되어 유지되다가 우화직전에 산소소비율이 다시 높아졌다. 또한 휴면용의 몸무게는 용화후 12일동안 변화가 거의 없었다. 주요 탄수화물과 수용성단백질 함량은 비휴면용보다 휴면용에서 더 높았으며, 함량변화는 비휴면용에서 더 급격했다. 휴면용과 비휴면용의 단백질에 대한 전기영동 패턴은 유사했으며 휴면용의 혈림프 삼투압은 저온에 보관되었을 때 더 올라갔다.

검 색 어 담배나방, 휴면용, 탄수화물 함량, 단백질 함량, 몸무게, 산소소비율, 삼투압

Diapause is an important adaptive mechanism for dormancy during periods of unfavorable environmental conditions, such as low winter temperature, extreme summer heat, periods of drought and seasons in which appropriate food is not available (Beck 1980). Usually diapausing insects are biochemically distinct from their non-diapausing counterparts. Fat, carbohydrate and protein reserves are often higher and specific proteins may be unique to di-

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apausing individuals (Denlinger 1985).

The Oriental tobacco budworm, *Heliothis assulta* Guenee, is widely distributed in Korea, Japan, China, Australia and Africa. In Korea, the Oriental tobacco budworm causes serious damage to the tobacco and especially to hot pepper fruits and it is difficult to control *H. assulta* in the field, since its larvae have a feeding habit inside hot pepper fruits and pupate in the underground. In addition, they show resistance against several insecticides. Therefore, to cope with this problems, investigations for controlling this pest have been concentrated on adults and eggs with the sex pheromone and egg parasites. However, there is still a large gap in our information on their basic biology, especially on their overwintering physiology.

This study was conducted to investigate the change in protein and carbohydrate contents in diapausing and non-diapausing pupae of the *H. assulta* Guenee to gain some of the basic information on their winter survival strategy. The species overwinters in the soil as diapausing pupae (Boo et al. 1990).

MATERIALS AND METHODS

Insects

The Oriental tobacco budworm, *Heliothis assulta* Guenee, larvae were collected in the suburbs of Suwon and reared in an insectary at the College of Agriculture, Seoul National University. The larvae were grown in individual plastic containers with an artificial diet (unpublished). The third instar larvae were exposed to diapause-inducing condition (10L/14D, 20±1℃) (Boo et al. 1990) until they pupate for diapause-destined pupae. For non-diapausing pupae, newly hatched larvae were grown at a diapause-free condition (16L/8D, 25±1℃) until pupation.

Measurement of the oxygen consumption

Oxygen consumption during pupal stage was measured with a Warburg manometer (13 positions, circular model, Townson & Mercer, LTD) at 27℃ using a method described by Denlinger et al. (1972).

Daily change of pupal body-weight

Body weights during pupal stage were measured with a chemical balance (Chyo Jupiter SD-160, Chyo Balance Corp.) at 24hr intervals.

Carbohydrate contents of pupae

Five pupae were homogenized with 3 mℓ of 50 % (v/v) ethanol in a glass homogenizer. The homogenizer was washed twice with 1 mℓ ethanol each and the washings were added to the original homogenate. The combined homogenate was centrifuged at 4,000 rpm for 15 min. and the supernatant was separated from the sediment. The supernatant was used for a measurement of soluble sugars and the sediment for glycogen content.

The supernatant obtained from the homogenate was concentrated with nitrogen gas to make a final volume of about 0.5-1 mℓ. After concentration, the supernatant was filtrated with a membrane filter (pore size; 0.45 μm, Gelman Inc.). The filtrate was used for sugar and sugar alcohol determination with HPLC (Column : Nucleosyl 10-NH2, PYE UNICAM PU 4023 refractive index detector, PM 8251A one line recorder, PYE UNICAM PU 4010 pump, Phillips). Acetonitril-water mixture (80 : 20) was used as the elution solvent.

The separated sediment was mixed with 10% (w/v) trichloroacetic acid (TCA). The mixture

was incubated at 100℃ for 15 min. and then centrifuged at 4,000 rpm for 15 min. The supernatant was separated from sediment. The glycogen content in an aliquot of the supernatant was determined by the anthrone/sulphuric acid method (Seifter et al. 1950, Travely & Harrison 1952), using glucose as a standard.

Protein content and bands from pupae

Haemolymph samples were collected from pupae puncfured in the head region. The samples were centrifuged at 4,000 rpm for 10 min. to remove haemocytes. Fat body samples were collected from insects into a tube with its weight known. Insects were dissected in the dissection Ringer solution (NaCl 149.0, KCl 40.0, $MgCl_2$ 9.0 mM, pH 7.0) containing phenylthiourea (0.05%). Fat bodies were homogenized with the normal Ringer solution (D-glucose 34.4, NaCl 129.0, KCl 8.6, $CaCl_2$ 2.0, $MgCl_2$ 8.6, $NaHCO_3$ 10.2, NaH_2PO_4 4.3 mM, pH 6.7). Fat body homogenates of known concentration (g/mℓ) was centrifuged at 4,000 rpm for 10 min. and the supernatant was used in experiments. Protein contents were determined from diluted haemolymph samples and fat body extracts using the Lowry method (Lowry et al. 1951). Haemolymph and fat body protein extracts were analyzed by SDS-polyacrylamide gel electrophoresis (Vertical slab unit 2000, Vocam 2000-300-150 power supply, Shandon) using the method of Wever et al. (1972). The separating gel concentration was 10% and that of stacking gel was 5%. The stacking voltage was constant at 100 mV and separation was carried out at 200 mV.

Haemolymph osmotic pressure

The haemolymph osmotic pressure was determined with an osmometer (Advancved wide range Osmometer 3WⅡ, Advaned Instruments Inc.). Fifty microliters of haemolymph collected from last instar larvae, prepupae and pupae were dissolved in 0.5mℓ Normal Ringer solution. The osmolarity was calculated from the difference between the osmolarity of the sample and the Ringer solution and the dilution factor.

RESULTS AND DISCUSSION

Oxygen consumption rate in pupae

The oxygen consumption rate of non-diapausing pupae through the whole pupal period showed an approximately U-shaped curve (Fig. 1, NP). But diapausing pupae showed a different type of oxygen consumption rate with a relatively stable, but low level of about 20 μl/g/hr (Fig. 1, DP). However, the oxygen consumption rate of diapausing pupae showed a similar type with that of non-diapausing pupae when they resume adult development at the end of diapause (Fig. 2). According to Keeley (1970), Wright (1971) and Denligner et al. (1972), the oxygen consumption rate of diapausing insects was about 10% of non-diapausing counterparts. In *Heliothis assulta*, the oxygen consumption rate of diapausing pupae falls to about 12% of the maximum and 41% of the minimum of that from non-diapausing pupae.

Daily change of pupal body weight

The body wight of non-diapausing pupae declined sharply while that of diapausing pupae showed almost no change during the initial pupal period (Fig. 3). The weight loss of diapausing pupae was only 3% during the early 13 days (the same as the period for normal pupal development) right after pupation (Fig. 3, DP). But the reduction in the body weight of

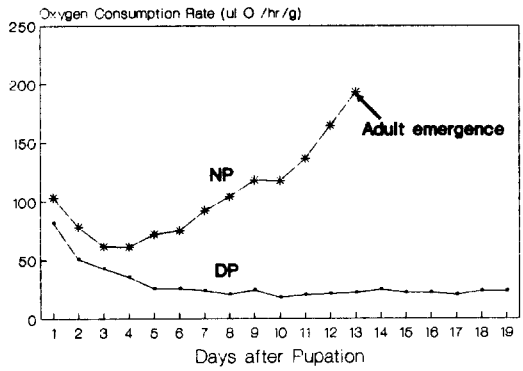

Fig. 1. Comparison of oxygen consumption rate between diapausing (DP) and non-diapausing (NP) *Heliothis assulta* pupae.

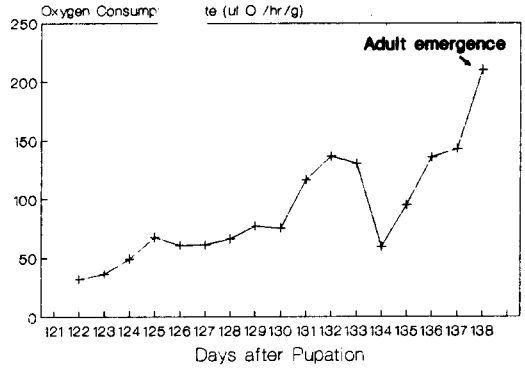

Fig. 2. Recovery of high oxygen consumption rate in diapausing *H. assulta* pupae before adult emergence.

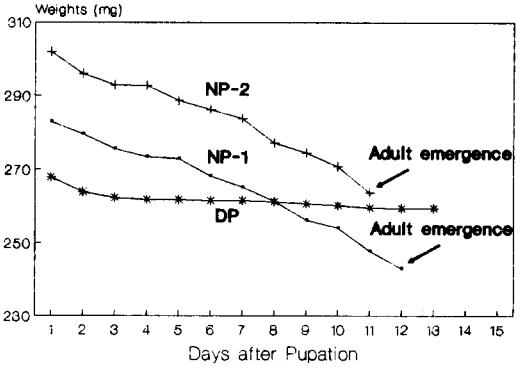

Fig. 3. Decline in body weight of non-diapausing (NP) and diapausing (DP) *H. assulta* pupae (NP1 : non-diapausing pupae which emerged at 12th day, NP2 : non-diapausing pupae which emerged at 11th day).

the non-diapausing pupae was about 13% of its initial wight during the pupal period (Fig. 3, NP -1, NP-2), which is still much smaller reduction when compared to other insects. For example, in the tobacco budworm (*Heliothis virescens)*, a 60% loss in body weight occurred during pupal-adult development (Williams-Boyce & Jungreis 1980) and 20% loss in the *Sarcophaga* flesh flies (Denlinger et al. 1972).

Contents of glycogen, sugars and sugar alcohols

Contents of major carbohydrates (glycogen, glucose, sorbitol and trehalose) were higher in diapausing pupae than those in non-diapausing pupae (Table 1). In the pupae of the fall webworm, *Hyphantria cunea* Drury, eight types of carbohydrates were found (Choi & Boo 1987). But, only six types of carbohydrates were detected in the pupae of Oriental tobacco budworm, *H. assulta* Guenee in this study. But there was no difference in kinds of carbohydrates between diapausing and non-diapausing pupae.

The contents of total carbohydrates in diapausing pupae decreased very slowly with only about 7% reduction in the initial contents during 12 days after pupation, while those of total carbohydrates fell to about 62% of its initial contents in normally developing pupae during the equivalent period. It seems that glycogen and carbohydrates are broken down to supply substrates for the energy metabolism in pupal-adult development. It is a general phenomenon that glycogen is intensively accumulated during the last instar larval stage for the preparation of metamorphosis, and then during the pupal stage (in Holometabola) most of this is consumed as the chief energy source for adult development (Wyatt 1967).

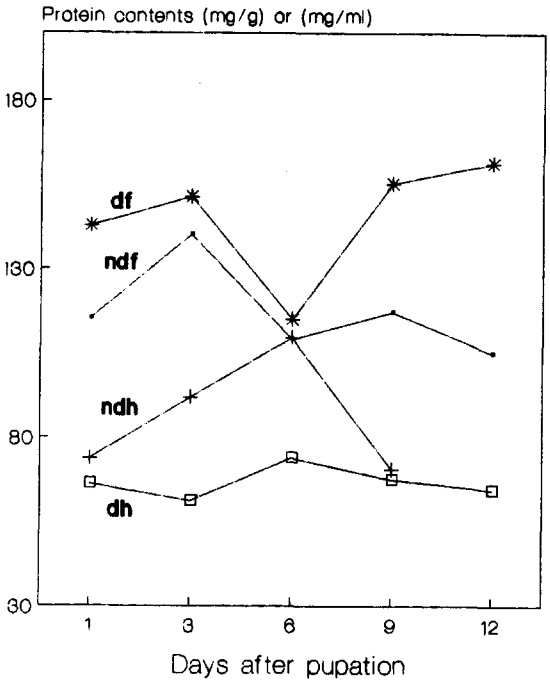

Fig. 4. Change in total soluble protein contents from fat body and haemolymph of diapausing and non-diapausing *H. assulta* female pupae (df: fat body protein of diapausing pupae, ndf: fat body protein of non-diapausing pupae, dh: haemolymph protein of diapausing pupae, ndh: haemolymph protein of non-diapausing pupae).

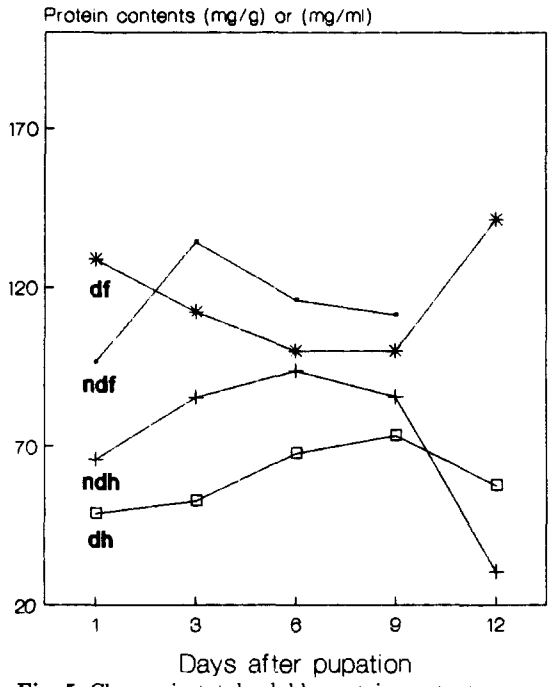

Fig. 5. Change in total soluble protein contents from fat body and haemolymph of diapausing and non-diapausing *H. assulta* male pupae (see fig. 6 for abbreviations).

Change in protein contents

The change in contents of total soluble proteins from haemolymph and fat body in diapausing and non-diapausing pupae is recorded in figs. 4 and 5. In the case of non-diapausing pupae, female and male showed a similar pat-

Table 1. Contents of major carbohydrates in diapausing(DP) and non-diapausing pupae(NP) of *Heliothis assulta*[a]

(mg/5 individuals)

Pupae	Age(days)	Glycogen	Glucose	Sorbitol	Trehalose	Glycerol	Xylose	Total
NP	1	17.7	5.7	1.9	0.8	0.6	0.3	27.0
	3	12.0	4.9	1.2	0.5	0.2	0.2	19.0
	6	10.4	2.7	0.8	0.3	0.2	0.1	14.5
	9	9.5	2.0	0.7	0.2	0.2	0.1	11.7
	12	6.7	0.9	0.5	0.2	0.5	0.3	9.1
DP	1	19.6	6.9	2.1	1.1	0.5	0.6	30.8
	3	19.6	5.1	2.7	1.1	0.8	0.4	29.7
	6	19.5	5.3	2.4	1.2	0.8	0.2	29.4
	9	19.5	5.1	2.5	1.2	0.7	0.1	29.1
	12	19.4	4.7	3.0	0.9	0.4	0.3	28.7

[a] Individual body weight at pupation(NP: 285 ± 12.4, DP: 261 ± 22.17).

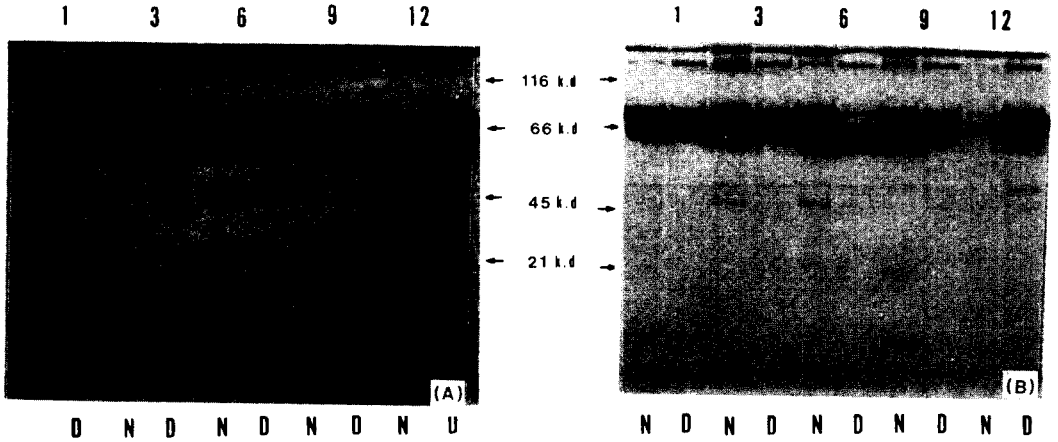

Fig. 6. SDS-PAGE of haemolymph proteins in non-diapausing(N) and diapausing(D) *H. assulta* female(A) and male(B) pupae (separated by SDS-PAGE in 10% acrylamide)(1-12 : pupal age-days).

tern in the change of protein contents. The content of fat body proteins was higher in diapausing pupae than that in non-diapausing pupae soon after pupation, but, in the case of haemolymph proteins, the reverse trend was observed. The total amount of soluble proteins was higher in diapausing pupae than in non-diapausing pupae of *H. assulta*.

Diapausing larvae of *Trogoderma granarium* also showed an enormous increase in the amount of proteins over that present in non-diapausing larvae (Karnarvar & Nair 1969) and the larvae of *Sarcophaga crassipalpis* destined for pupal diapause contain nearly twice as much haemolymph proteins as larvae that do not enter diapause (Adedokun Denlinger 1985).

Electrophoretic separation of proteins

All anodally-migrating protein bands separated by SDS-electrophoresis are shown in figs. 6 and 7. Fig. 6 presented haemolymph proteins

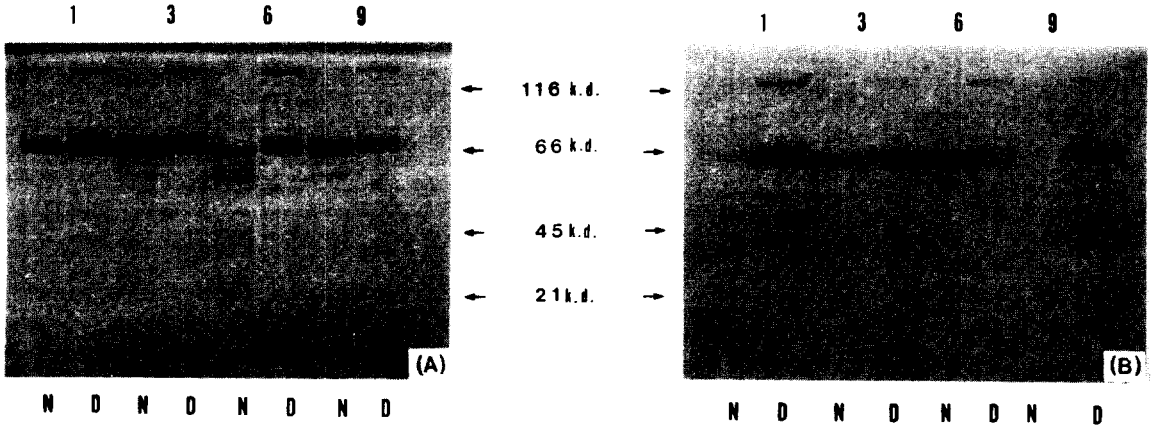

Fig. 7. SDS-PAGE of fat proteins in non-diapausing(N) and diapausing(D) *H. assulta* female(A) and male (B) pupae (separated by SDS-PAGE in 10% acrylamide)(1-12: pupal age-days).

Table 2. Osmotic pressures of prepupal and pupal heamolymph in *H. assulta*

Heamolymph from[a]	Mean (mOsm/kg · H_2O)[b]
NPP	389 ± 37.6
NP 3	293 ± 8.3
DPP	329 ± 19.4
DP 3	333 ± 12.2
DP 45	331 ± 15.0
DP 15C	448 ± 10.3
DP RC	424 ± 12.1

[a] NPP : non-diapausing prepupae.
NP 3 : Non-diapausing 3-day old pupae.
DPP : Diapausing prepupae.
DP 3 : Diapausing 3 -day old pupae.
DP 45- : Diapausing 45day old pupae.
DP 15C : Diapausing pupae kept for 15 days at 2 ±1℃.
DP RC : Diapausing pupae kept for 15 days at 2 ±1℃ after storage for 2 months at 25 ±1℃.

[b] Six pupae were sampled for each treatment and haemolymph from two individuals were pooled for a single measurement.

in diapausing and non-diapausing pupae. Female and male pupae showed silmilar band patterns. There are about sex major bands (2 bands above 116 kd., 2 bands about 66 kd., 2 bands between 66 kd. and 1 about 45 kd.). In diapausing pupae, the band pattern showed no change during initial 12 days after pupation but there were changes of band pattern in non-diapausing pupae.

Fat body proteins showed band patterns different from those of haemolymph in diapausing and non-diapausing pupae(Fig. 7). They presented 2 major bands (1 band above 116 kd., 1 band about 66 kd.). In non-diapausing pupae, the band patterns changed clearly in contrast to diapausing puape. It is noticeable that the predominant bands seen in the fat body of diapausing pupae were negligible in non-diapausing pupae. The similar features were found in the haemolymph proteins of codling moth larvae, *Cydia pomonella* (Brown 1980).

Althogh, there are many insects that have diapause associated-protein (Trunen & Chippendale 1980, Dillwith & Chippendale 1984, Sakurai et al. 1987, Kono 1988, Lefevere et al. 1989), it was not found in *H. assula* in this study. From these results, it is reasonable to predict that the predominant proteins in the fat body of diapausing pupae have no diapause-specific function but may have a role in post-diapause development as in the Colorado potato beetle, *Leptinotarsa decemlineata* (Lefevere et al. 1989). However, it can not be elucidated until each protein band is characterized.

Osmotic pressure

The osmotic pressure of haemolymph in diapausing pupae was a little higher than that of non-diapausing pupae (Table 2). The difference in the osmotic pressure was only about 13 % when diapausing pupae were stored at room temperature. But diapausing pupae, when kept in cold temperature, increased their osmotic pressure about 50% higher than that of non-diapausing pupae. It implies that diapause and higher osmotic pressure are not directly related. Rather the osmotic pressure increased only when insects were exposed to old temperature.

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