Ecological Study on the Barnyard Grass Stem Borer, *Enosima leucotaeniella* (RAGONOT) (Lepidoptera: Pyralidae)
VIII. Seasonal Changes of Carbohydrate Contents
in Overwintering Larvae

Michiyo GOTO, Koetsu TAKAHASHI and Chiaki SUZUKI

*Faculty of Agriculture, Yamagata University, 1-23, Wakaba-cho,
Tsuruoka, Yamagata 997, Japan*

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The seasonal changes of carbohydrate contents in overwintering larvae of *Enosima leucotaeniella* were investigated to understand the relationship between carbohydrate contents and diapause. More than eight kinds of sugars and polyols were detected in the haemolymph and body by gas-liquid chromatography. Trehalose was a dominant substance and trace levels of other sugars and polyols appeared during hibernation. From September to January, trehalose content increased with decreasing glycogen in spite of diapause termination in late November, but total carbohydrate (trehalose plus glycogen) content stayed at an approximately constant level. In February, however, both trehalose and glycogen contents decreased.

Key words: diapause, *Enosima leucotaeniella*, glycogen, seasonal change, trehalose

INTRODUCTION

The species which had been misidentified as *Emmalocera* sp. in a series of our papers (GOTO, 1985; GOTO, 1989; GOTO et al., 1991) was identified recently as *Enosima leucotaeniella* (YOSHIIYASU, 1993).

E. leucotaeniella is a stem borer which only infests barnyard grass (*Echinochloa*). Both the stem borer and its host plant tolerate severe winter in the Shonai district of Yamagata Prefecture, northern Japan. Barnyard grass undergoes withering from late summer to early autumn and its seeds do not germinate until late spring. Consequently, the overwintering larvae of *E. leucotaeniella* may have to tolerate not only the cold winter but also a deficiency of their host plant in this prolonged period.

The life history traits of cold hardiness and diapause in most insects are critical for surviving severe winters in the temperate zone (DENLINGER, 1991), but in many cases, it is not clear whether the two traits are related or independent events (MANSHINGH, 1971; RING, 1972). Diapause in the overwintering larvae of *E. leucotaeniella* terminates in late November, and the larvae then pass the winter in a post-diapausing stage (GOTO et al., 1991).

It has been reported that several insect species accumulate low-molecular-weight substances as cryoprotectants in the haemolymph to enhance their cold hardiness

under low temperatures (SALT, 1961; ASAHINA, 1969), and those substances are converted from glycogen (TSUMUKI and KANEHISA, 1981).

To clarify the relationship between the carbohydrate contents and diapause of *E. leucotaeniella*, we investigated the low-molecular-weight substances in the larvae and the seasonal change in the major substance and glycogen content.

MATERIALS AND METHODS

Insects used. Overwintering larvae of *E. leucotaeniella* were obtained from infested barnyard grass *Echinochloa crus-galli* var. *crus-galli* that was harvested in late August and stored outdoors. Twelve overwintering larvae were sampled just before experiment once a month from September to March.

Haemolymph and body samples. Four μl of haemolymph was collected from the abdominal end of larvae by cutting and squeezing. A precise volume of the haemolymph was collected by using a disposable micro glass pipette with ring marks (μl) (Hirshmann Laborgerate Co.). The remainder of the larva was used for carbohydrate analysis.

Measurement of sugar content. The haemolymph and remainder of the larva was homogenized with 0.4 ml and 1 ml of 80% (v/v) ethanol with 0.01% erythritol as an internal standard, respectively. The supernatants (200 μl) of the homogenates were dried at 80°C in a water bath. TMSI-C (Trimethylsilylating reagent; GL Sciences Co.) (40 μl) was added to the residues and then the solutions were heated at 65°C for 40 min (SHIMADA et al., 1984). The resulting TMS-C derivative (2 μl) was injected into a gas-liquid chromatography (Yanagimoto Co.) using a glass column (3 mm $\times$ 3 m) packed with 5% (w/w) OV-1. The column was heated from 150 to 280°C at 8°C/min and then kept at 280°C. The elution profile was followed using a flame-ionization detector. Sugar content in the body was calculated from the values of the haemolymph and the larva remainder.

Measurement of glycogen content. After extraction of sugars with 80% ethanol, the resultant insoluble residues were washed three times with 2.5 ml of 80% ethanol to remove the remaining sugars and then 2 ml of 10% (v/v) trichloroacetic acid was added to the residues. The mixture was boiled in water for 15 min, and cooled and centrifuged at 3,000 *g* for 15 min. The supernatant was used for glycogen measurement. Glycogen content was determined by the phenol and sulfuric acid method (DUBOIS et al., 1956). A 5% (v/v) phenol solution (0.5 ml) and 2.5 ml of sulfuric acid were added to 1 ml of the glycogen extract diluted 2-fold with distilled water. After 10 min, the mixture was incubated at 30°C for 15 min. The absorbance was determined at 490 nm on a spectrophotometer (Beckman Co.).

RESULTS

Identification of sugars

The standard reagents are shown in Fig. 1A. Sugars and polyols in the haemolymph and body samples were identified by the comparison of retention times of the reagents on gas-liquid chromatograms. As shown in Fig. 1B, trehalose ($R_t=25.9$ min) and minor quantities of unidentified substances, c ($R_t=23.3$) and d ($R_t=28.6$) were detected in the haemolymph sample. Trehalose, glycerol ($R_t=2.9$), glucose (two

Fig. 1. Gas-liquid chromatograms of sugars and polyols in the haemolymph and body of *Enosima leucotaeniella*. A: standard reagents, GLY: (glycerol), ERY (erythritol), GLU (glucose, 2 peaks), SOR (sorbitol), INO (inositol), SUC (succhaloze), TRH (trehalose), unidentified substances, a, b, c and d. B: haemolymph. C: body.

peaks, $R_t=12.4$ and 13.8), inositol ($R_t=15.7$) and unidentified substances a ($R_t=9.7$), b ($R_t=19.4$), c and d (Fig. 1C) were detected in the body samples. Trehalose was a dominant substance and trace levels of other sugars and polyols were detected in the haemolymph and body.

Changes of ambient temperature

Monthly mean ambient temperatures from September to March are shown in Fig. 2A. The average ambient temperature in the Shonai district decreased (20° to 1°C) from September to January and reached its lowest point in February.

Fig. 2. Seasonal changes in ambient temperature (Shonai, Yamagata) and carbohydrate contents in overwintering larvae of *E. leucotaeniella* from September to March in 1991–92. Each value is mean (mean $\pm$ S.E.) of 12 replicate samples. A: average ambient temperature, B: trehalose content in the haemolymph and C: trehalose ($\circ$), glycogen ($\times$) and total carbohydrate (trehalose plus glycogen) ($\blacktriangledown$) contents in the body. Same letters on the same line are not significantly different at 1% level by DUNCAN's multiple range test.

Changes of trehalose content

Changes of trehalose contents in the haemolymph and body samples are shown in Figs. 2 B and C. Trehalose content in both samples increased from September to January. Its level increased by almost three times from 15.0 to 33.8 mg/ml in the haemolymph (Fig. 2B) and 7.0 to 21.9 mg/g in the body (Fig. 2C). However, trehalose content of both samples decreased remarkably from February to March.

Changes of glycogen and total carbohydrate content

Changes of glycogen content in the body samples are shown in Fig. 2C. Glycogen content decreased from 25.8 mg/g in September to 2.5 mg/g in March. The amount of total carbohydrate (trehalose plus glycogen) remained almost constant within the range of 31.0 to 32.9 mg/g from September to January, but significantly decreased to 25.5 and 13.2 mg/g in February and March, respectively.

DISCUSSION

More than eight kinds of sugars and polyols were detected in the body of *E. leucotaeniella* by using gas-liquid chromatography. Trehalose was a dominant substance in both the haemolymph and body during hibernation. Consequently, *E. leucotaeniella* is categorized as a trehalose-accumulating insect, such as the diapausing pupa of the silkworm *Philosamia cynthia pryeri* (HAYAKAWA and CHINO, 1981) and the overwintering pre-pupa of a poplar sawfly *Trichiocampus populi* (TANNO, 1970).

In many insects, increase of low-molecular-weight substances such as trehalose and glycerol occurs only during the diapausing stage (ASAHINA, 1969). However, increase of trehalose content in larvae of *E. leucotaeniella* continued despite the diapause termination in late November, and the content reached a maximum in January (Figs. 2 B and C). Consequently, larvae of *E. leucotaeniella* can accumulate trehalose independently with such a diapause process.

From September to January, trehalose content increased with decreasing glycogen and a high total carbohydrate content (trehalose plus glycogen; mg/g) (HAYAKAWA and CHINO, 1981) was maintained at almost the same level (Figs. 2 B and C). These results show that the conversion of glycogen to trehalose occurred. The conversion of glycogen to low-molecular-weight substances has been discussed in regards to temperature (HAYAKAWA and CHINO, 1981), but rarely in regards to diapause and post-diapause (TSUMUKI and KANEHISA, 1981). The conversion in the larva of *E. leucotaeniella* is characterized by maintenance of total carbohydrate content not only in the diapausing stage but also in the early post-diapausing stage (November to January). Since ambient temperature decreased successively during these periods, the conversion occurs closely in correlation with ambient temperature.

Both trehalose and glycogen contents decreased in February irrespective of the lowest ambient temperature. Larvae of *E. leucotaeniella* entered the post-diapausing stage in late November (GOTO et al., 1991). TSUMUKI and KANEHISA (1981) have demonstrated that metabolic rate of glycerol in *Chilo suppressalis* is higher in post-diapausing larvae than in diapausing larvae. Metabolic rate of trehalose may also be accelerated in late post-diapausing larvae of *E. leucotaeniella*. Decrease of trehalose content may be due to metabolic changes of carbohydrates in late post-diapausing larvae of *E. leucotaeniella* in February: metabolic rate of trehalose may be accelerated or

conversion of glycogen to trehalose reduced. However, details of those metabolic changes remains equivocal. It is necessary to investigate the cause inducing metabolic changes of the carbohydrates in February.

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