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# Physiology of diapause and cold hardiness in the overwintering pupae of the fall webworm *Hyphantria cunea* (Lepidoptera: Arctiidae) in Japan

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## Abstract

The fall webworm *Hyphantria cunea* Drury, which was accidentally introduced to Japan in 1945, overwinters on the ground in pupal diapause. Diapause termination, as indicated by the respiration rate and the period required for adult emergence, began in March and ended in April. Cold hardiness (the ability to survive exposure to  $-15^{\circ}\text{C}$ ) decreased linearly with diapause development from November to the following April under field conditions. Cold hardiness of diapause pupae (DP) decreased as the acclimation temperature decreased from 15 to  $-10^{\circ}\text{C}$ , whereas cold hardiness of non-diapause pupae (NDP) remained high as the acclimation temperature decreased from 5 to  $-5^{\circ}\text{C}$ . However, *H. cunea* in Japan can survive exposure to  $-5^{\circ}\text{C}$  for two weeks, whether it is in a diapause or non-diapause state. Trehalose was the main sugar detected in the body, but its level was less than 0.8%. Trehalose levels increased in field-collected pupae from January to March. DP accumulated less trehalose than NDP, as the acclimation temperature was decreased from 5 to  $-5^{\circ}\text{C}$ . The alanine content in field-collected pupae increased from November to February. Both diapause and low temperature caused an accumulation of alanine. These results suggest that under field conditions, overwintering pupae of *H. cunea* in Japan do not accumulate high levels of sugars and polyols and do not develop a high level of cold hardiness. Furthermore, DP do not accumulate high levels of sugars and polyols and their ability to survive exposure to  $-15^{\circ}\text{C}$  is not greater than that of NDP. The physiological and biochemical bases of diapause in *H. cunea* from Japan are discussed. © 2001 Elsevier Science Ltd. All rights reserved.

**Keywords:** *Hyphantria cunea*; Exotic insect; Diapause; Non-diapause; Trehalose; Cold hardiness

## 1. Introduction

The fall webworm, *Hyphantria cunea* Drury (Lepidoptera: Arctiidae), a native insect in North America, has colonized many areas in the temperate northern hemisphere since 1940 (Warren and Tadic, 1970). In North America, the habitats of *H. cunea* range from Canada ( $45^{\circ}\text{N}$ ) to the Gulf of Mexico ( $30^{\circ}\text{N}$ ), with the number of generations varying from only one in the north to more than four in the south (Oliver, 1964; Morris and Fulton, 1970). This species has been accidentally introduced to some areas of Europe and Asia (Warren and Tadic, 1970). *H. cunea* was accidentally introduced to Japan in 1945. It was initially established in the northern

areas of the country ( $34^{\circ}\text{N}$ – $39^{\circ}\text{N}$ ) where its life cycle was predominantly bivoltine (Masaki, 1975). In the following three decades, as it moved farther south, the southern populations developed a trivoltine life cycle (Masaki, 1975; Gomi, 1996a). Recently, multivoltine populations (more than three generations a year) were found on the Ogasawara Islands (ca.  $27^{\circ}\text{N}$ ) (Takeuchi and Ohbayashi, 1996). However, the factors that control the expansion and the life cycle transition of *H. cunea* in Japan are poorly understood.

The distribution of insects is determined by environmental conditions, especially by low temperatures, which are the major environmental obstacles for insects to survive in the temperate zones. Many overwintering insects inhabiting the temperate zones endure environmental stresses by entering diapause and/or developing cold hardiness via the accumulation of cryoprotectants, such as low molecular weight sugars and polyols

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(Tauber et al., 1986; Danks, 1987; Denlinger, 1991; Storey and Storey, 1991). Although cold hardiness and diapause are essential components for the survival of most overwintering insects, the relationship between these two features is sometimes unclear (Denlinger, 1991). *H. cunea* is a species with a facultative diapause (Oliver, 1964). It enters pupal diapause under the joint control of photoperiod, temperature, relative humidity and food quality in Canada (Morris, 1967). The bivoltine Japanese population of *H. cunea* enters pupal diapause primarily in response to the decreasing photoperiod in autumn and overwinters as a diapause pupa on the ground until the following year (Masaki et al., 1968; Masaki, 1977; Gomi, 1996a,b). However, little is known about the physiological bases of diapause and cold hardiness in this species, and the relationship between these two phenomena. The present study was undertaken to investigate diapause and cold hardiness as a first step in understanding the factors controlling the adaptation of *H. cunea* to new areas in Japan.

Accordingly, the main objectives of our study were to:

1. describe the physiological and biochemical bases of diapause and cold hardiness in field-collected pupae over the winter;
2. compare the level of cold hardiness and biochemical changes between diapause and non-diapause pupae after cold acclimation;
3. discuss the physiological and biochemical bases of diapause in the Japanese population of *H. cunea* based on the overwintering characteristics.

## 2. Materials and methods

### 2.1. Insects and acclimation

Pupae of *H. cunea* were collected from a mulberry field at Yamagata University in Tsuruoka, Yamagata prefecture on the 15th of every month from November 1998 to April 1999. Diapause pupae (DP) and non-diapause pupae (NDP) were cultured from first generation eggs, which were produced by culturing field-collected larvae from the same mulberry field in June 1998. The hatched larvae were reared on an artificial diet of 'Insecta LF' (Nihon Nosan Kogyo Co. Ltd, Japan) in a controlled chamber at different photoperiods. DP and NDP were obtained by rearing larvae at 20°C under 12 h light–12 h dark (12L–12D) and 16 h light–8 h dark (16L–8D) photoperiods, respectively. Newly emerged pupae were used for acclimation. DP and NDP were gradually cooled to 15 and 5°C, respectively, and then successively acclimated to –10°C by decreasing the temperature 5°C every two weeks in the dark. DP and

NDP were analyzed under the same conditions immediately after acclimation.

### 2.2. Days before adult emergence

Field-collected male ( $n=10$ ) and female pupae ( $n=10$ ) were incubated at 25°C under long (16L–8D) and short (12L–12D) photoperiods. The emergence of adults was recorded daily for 90 days.

### 2.3. Supercooling point (SCP) determination

An individual to which a copper–constantan thermocouple was attached was placed in a microcentrifuge tube (1.5 ml). The closed cap was sealed with parafilm. The tube was immersed in a refrigerated ethanol bath that was cooled at a rate of 0.5°C per minute. The thermocouple was connected to a recording potentiometer. The SCP was taken as the lowest temperature before freezing (indicated by the release of latent heat of crystallization).

### 2.4. Respiration determination

For each replicate, a single pupa was placed in a 10-ml vial. The field samples were kept in the dark at 20°C or under light and temperature conditions similar to those in the field, and the acclimated samples were kept in the dark under the corresponding acclimation temperatures. After 24 h, a gas sample (1 ml) was withdrawn with a needle and syringe by poking the vial through the cap. The volume of CO<sub>2</sub> released by the individual was analyzed by a gas–liquid chromatograph equipped with a thermal conductivity detector (GL Sciences GC-320) as described by Tanaka and Saito (1984). The respiration rate was calculated as the amount of CO<sub>2</sub> released per fresh weight per hour ( $\mu\text{l CO}_2/\text{g/h}$ ).

### 2.5. Survival rate before and after exposure to –15°C

The field and acclimated pupae ( $n=15–20$ ) were transferred to a temperature-controlled chamber, whose temperature was lowered slowly (5°C/6 h) to –15°C, held at –15°C for 24 h, and then slowly raised to 20°C and held at that temperature for three days. To determine the survival rate, pupae were cut in the middle of the body and the dorsal vessel was examined under a stereomicroscope. Pupae showing normal pulsation of the dorsal vessel were judged to be alive. The survival rates of acclimated DP and NDP during acclimation before exposure to –15°C were determined by the same method.

### 2.6. Chemical analyses

Trehalose was measured by gas liquid chromatography as described by Goto (1995). Glycogen was

determined by the phenol/sulfuric acid method (Goto et al., 1993). Free amino acids were assayed with an amino acid analyzer (Hitachi L-8800) as described by Goto et al. (1998). Lipid was measured as described by Goto et al. (1998).

## 2.7. Statistical analysis

All data were analyzed with the SigmaStat software (Jandel Scientific, CA, USA). Data were tested with one-way analysis of variance, followed by Tukey's multi-range test.

## 3. Results

### 3.1. Seasonal changes under field conditions

#### 3.1.1. Ambient temperature, adult emergence, respiration rate

The average ambient temperature in the Shonai district ranged from 8.5°C in November to 1.0°C in February to 10.6°C in April. The minimum daily temperature was -2~-3°C in January and February, whereas the maximum daily temperature was nearly 8°C higher (Fig. 1A).

When the pupae were incubated at 25°C, the period required for adult emergence was not significantly different between the long (16L-8D) and short (12L-12D) photoperiods, nor between male and female pupae. There was a significant difference among the experiment months ( $F=318.92$ ,  $P<0.0001$ ) (Fig. 1B). The period for adult emergence decreased from 75.8 days (73~80 days) in November to 17.6 days (16~19 days) in March, and then to 12.6 days (12~13 days) in April.

The rates of CO<sub>2</sub> release at 20°C were higher than those under field condition in the months in which the experiments were conducted (Fig. 1C). Under field conditions, the rate of CO<sub>2</sub> release remained constant at a low value from November to March, but it increased significantly in April ( $P<0.05$ ). The CO<sub>2</sub> release at 20°C also remained constant from November to February, then showed a slight increase in March, and a significant increase in April (Fig. 1C).

#### 3.1.2. SCP and cold hardiness

SCP remained near -22.9°C from November to March, and then slightly, but significantly, increased to -20.2°C in April ( $P<0.05$ ).

The survival rate of field-collected pupae decreased linearly from 84.2% in November to 33.3% in April (Fig. 1D).

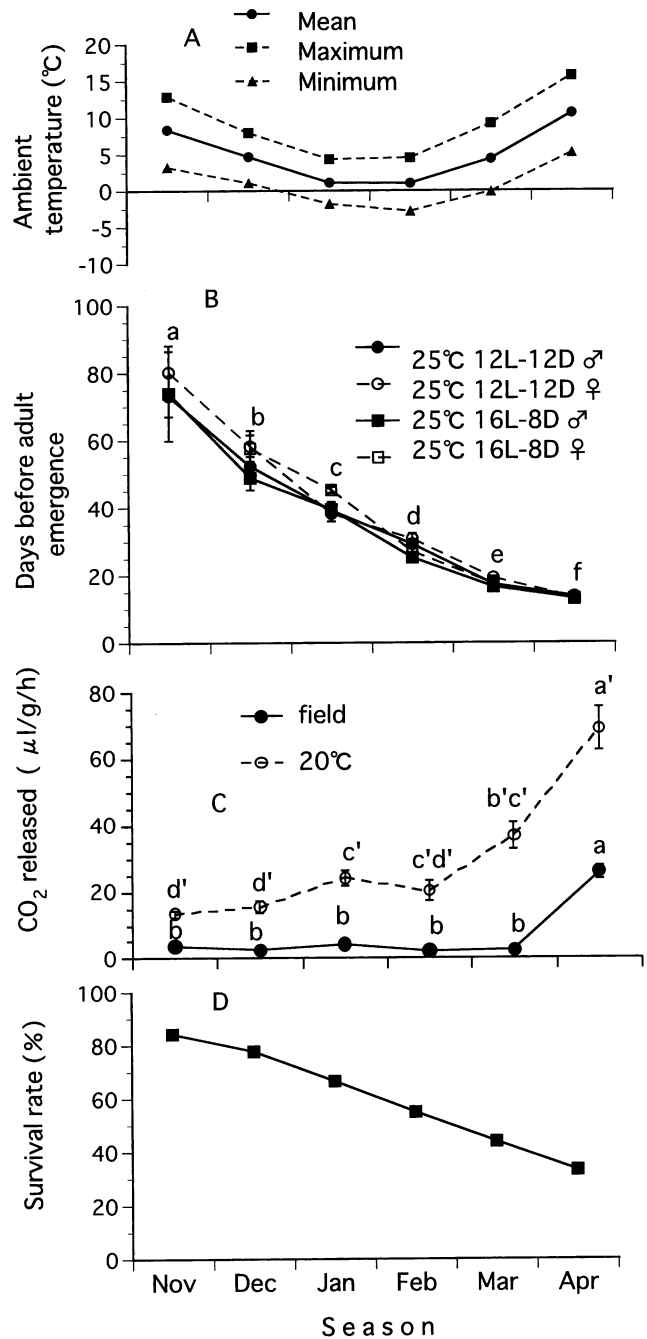


Fig. 1. Seasonal changes in average, minimum and maximum ambient temperatures at Shonai, Yamagata (A), the period required for adult emergence ( $n=6-10$ , B), the respiration rates at 20°C and under field conditions (CO<sub>2</sub> release,  $n=6$ , C) and the survival rates after an exposure to -15°C for 24 h ( $n=15-20$ , D) in pupae of the fall webworm, *H. cunea*. Pupae were sampled on the 15th of every month. Values labeled with the same letters on the same line are not significantly different at the 5% level by Tukey's test after ANOVA.

#### 3.1.3. Trehalose, glycogen and lipid contents

The major carbohydrate in *H. cunea* pupae was trehalose, with trace amounts of glycerol, glucose and inositol. The trehalose content remained constant from November to December, gradually increased to a peak of 3.2 mg/g

in March, and then dropped to 0.7 mg/g in April (Fig. 2A).

No significant difference in glycogen content was detected from November to April ( $F=2.41$ ,  $P>0.05$ ; Fig. 2A). The glycogen content was relatively high from November to December, dropped to low levels from January to March, and then partially recovered in April.

The lipid content remained constant with a mean value of 92.6 mg/g among the experiment months (data not shown).

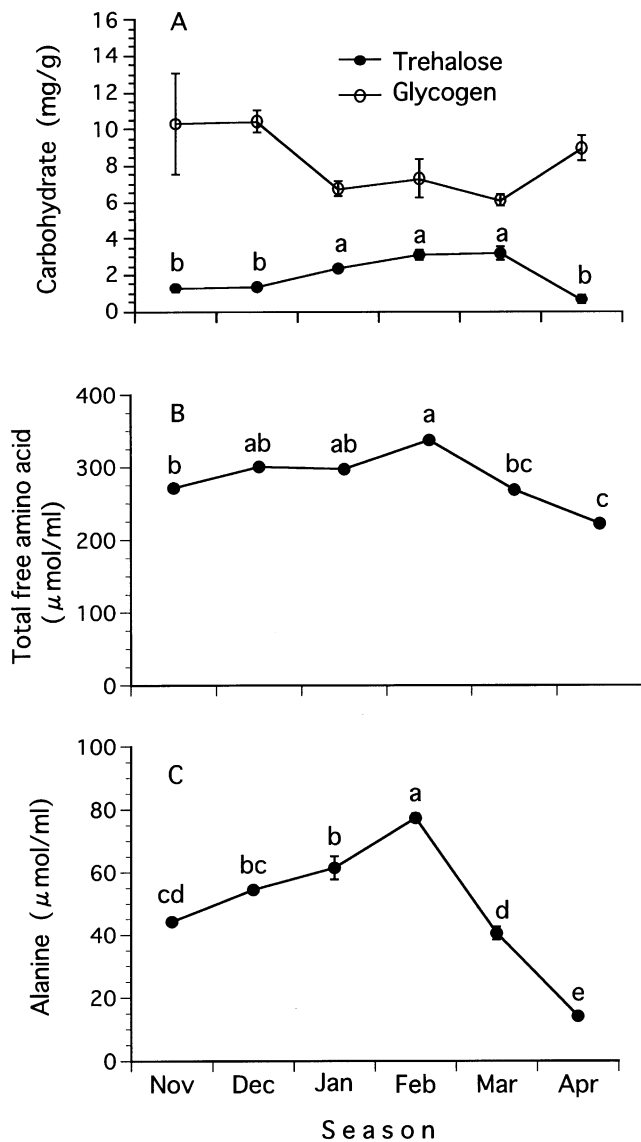


Fig. 2. Seasonal changes in the contents of trehalose and glycogen in whole bodies ( $n=8$ , A), and total free amino acids ( $n=6$ , B) and alanine ( $n=6$ , C) in haemolymph of the fall webworm *H. cunea*. Pupae were sampled on the 15th of every month. Values labeled with the same letters on the same line are not significantly different at the 5% level by Tukey's test after ANOVA.

### 3.1.4. Free amino acid and alanine contents

Thirty-seven free amino acids were detected in the hemolymph of *H. cunea* (data not shown). A significant difference in the total concentration of free amino acids was found from November to April ( $F=57.07$ ,  $P<0.001$ ). The total concentration of free amino acids was unaltered from November to January, then peaked in February, and thereafter decreased gradually in March and April (Fig. 2B).

Alanine, the major amino acid in the hemolymph of the pupae, also showed a significant change over the winter season ( $F=13.47$ ,  $P<0.0001$ ), increasing linearly from 44.3  $\mu\text{mol/ml}$  in November to 77.4  $\mu\text{mol/ml}$  in February, and then dropping to 14.1  $\mu\text{mol/ml}$  in April (Fig. 2C).

## 3.2. Effect of acclimation temperature on DP and NDP

### 3.2.1. Respiration rate

Significant differences in  $\text{CO}_2$  release in both DP and NDP were detected among the various acclimation temperatures (DP:  $F=2.79$ ,  $P<0.05$ ; NDP:  $F=5.76$ ,  $P<0.05$ ; Fig. 3A). The rate of  $\text{CO}_2$  release in acclimated DP decreased linearly from 15 to 5°C, then remained low as the acclimation temperature decreased. In the acclimated NDP, the rate of  $\text{CO}_2$  release decreased as the acclimation temperature decreased from 5 to -5°C.

### 3.2.2. SCP and cold hardiness

SCP values in DP and NDP (with mean values of -22.5 and -24.1°C, respectively) were not affected by the acclimation temperatures.

Under cold acclimation, the survival rate of DP decreased gradually from 93.3% at 15°C to 16.7% at -10°C, whereas the survival rate in NDP remained high (75.0–90.0%) after cold acclimation from 5 to -5°C (Fig. 3B).

### 3.2.3. Trehalose and glycogen contents

Trehalose content in whole-bodies was significantly affected by acclimation temperature in both DP ( $F=40.7$ ,  $P<0.001$ ) and NDP ( $F=51.4$ ,  $P<0.001$ ) (Fig. 4A). Trehalose content in acclimated DP remained low from 15 to 5°C, and increased at 0 and -5°C. In acclimated NDP, trehalose levels increased significantly from 1.6 mg/g at 5°C to 6.3 mg/g at -5°C ( $P<0.05$ ). NDP accumulated higher contents of trehalose than did DP under the same acclimation temperatures (5, 0 and -5°C) (Fig. 4A).

Glycogen content in whole-bodies was not significantly affected by acclimation temperature in DP ( $F=2.14$ ,  $P=0.096$ ), but it was in NDP ( $F=25.3$ ,  $P<0.001$ ) (Fig. 4A). Glycogen content in acclimated DP was lower at -5°C than it was at the other temperatures. Glycogen content in NDP decreased from 13.4 mg/g at

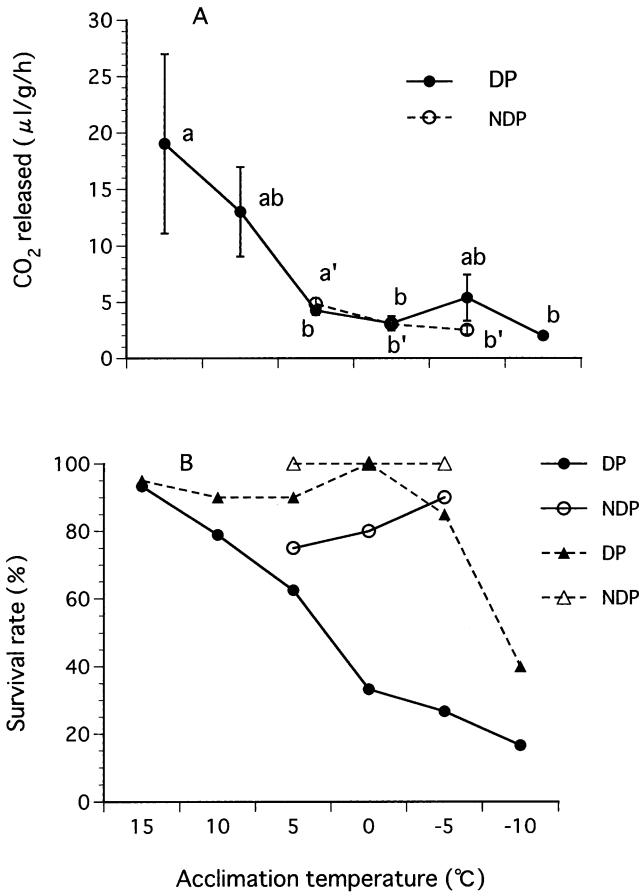


Fig. 3. Effect of acclimation temperature on the respiration rate at the corresponding acclimation temperature (CO<sub>2</sub> release,  $n=6$ , A), and the survival rates ( $n=15-20$ , B) of DP and NDP of the fall webworm *H. cunea*. The dashed lines show the survival rates at each acclimation temperature for two weeks during the acclimation protocol, and the continuous lines show the survival rates at  $-15^{\circ}\text{C}$  for 24 h. DP were successively acclimated at 15, 10, 5, 0,  $-5$  and  $-10^{\circ}\text{C}$  for two weeks. NDP were successively acclimated at 5, 0 and  $-5^{\circ}\text{C}$  for two weeks. Values labeled with the same letters are not significantly different at the 5% level by Tukey's test after ANOVA.

$5^{\circ}\text{C}$  to 9.1 mg/g at  $0^{\circ}\text{C}$ , and then decreased to 8.2 mg/g at  $-5^{\circ}\text{C}$  (Fig. 4A).

### 3.2.4. Free amino acid and alanine contents

Total concentration of free amino acids changed significantly under temperature acclimation in DP, but not in NDP (Fig. 4B). The total concentration of free amino acids in DP did not change as the acclimation temperature was lowered from 15 to  $10^{\circ}\text{C}$ , but then it increased to a high value at acclimation temperatures from 5 to  $-5^{\circ}\text{C}$ . DP accumulated a higher content of free amino acids than did NDP under the same acclimation temperatures (5, 0 and  $-5^{\circ}\text{C}$ ) (Fig. 4B).

Levels of alanine were significantly affected by acclimation temperature in both DP and NDP (Fig. 4C). The alanine level in acclimated DP was unaltered as the acclimation temperature was decreased from 15 to  $5^{\circ}\text{C}$ , and increased abruptly at  $0^{\circ}\text{C}$ , and then increased

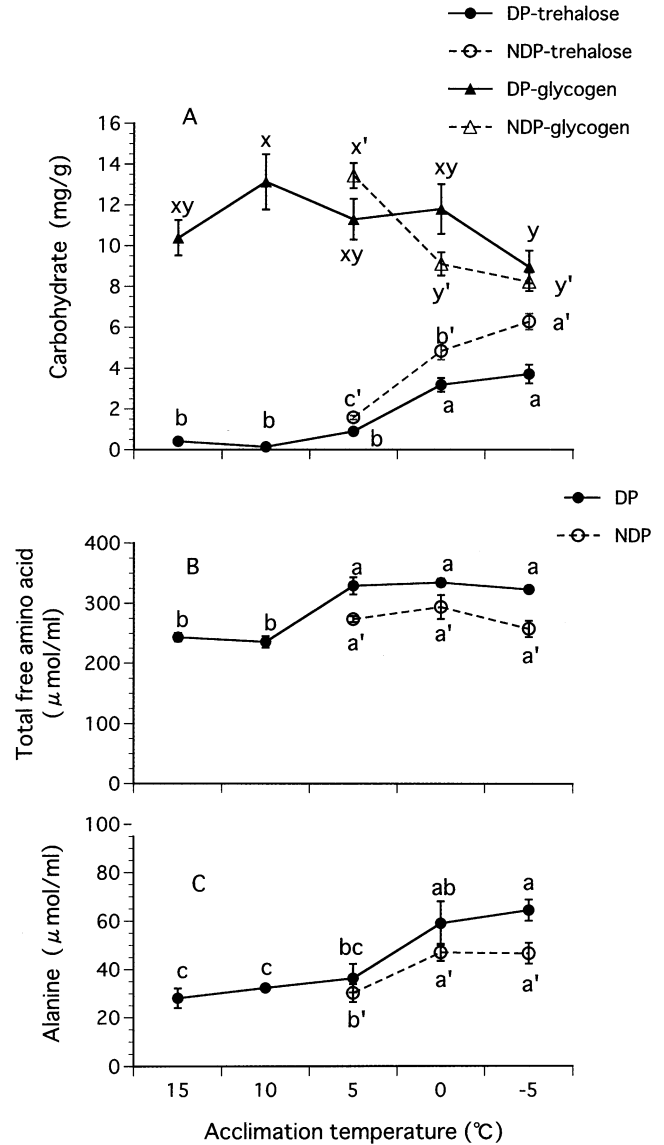


Fig. 4. Effect of acclimation temperature on the content of trehalose and glycogen in whole bodies ( $n=8$ , A), and total free amino acids ( $n=6$ , B) and alanine ( $n=6$ , C) in haemolymph of DP and NDP of the fall webworm *H. cunea*. Pupae were acclimated as described in the legend of Fig. 3. Values labeled with the same letters on the same line are not significantly different at the 5% level by Tukey's test after ANOVA.

slightly at  $-5^{\circ}\text{C}$ . In acclimated NDP, the alanine level increased significantly as the acclimation temperature was decreased from 5 to  $0^{\circ}\text{C}$  ( $P<0.05$ ), and then remained constant at  $-5^{\circ}\text{C}$ . DP accumulated higher levels of alanine than did NDP under the same acclimation temperatures (5, 0 and  $-5^{\circ}\text{C}$ ) (Fig. 4C).

## 4. Discussion

The respiration rate in *H. cunea* at  $20^{\circ}\text{C}$  started to increase in March and peaked in April, suggesting that

diapause termination begins in March. Diapause intensity in *H. cunea*, expressed as the period required for adult emergence, decreased gradually from 75.8 days in November to 12.6 days in April. The period required for adult emergence of NDP is 14.0 days (Ito et al., 1968). This suggests that *H. cunea* has terminated diapause in April. The respiration rate under field conditions remained very low before March, which is a distinct diapause symptom. This indicated that the metabolism is not active before diapause termination, and that the respiration rate is not related to diapause intensity.

Many overwintering insects increase their cold hardiness over the winter (Lee, 1991; Li et al., 2000; Goto et al., 2001). However, cold hardiness in *H. cunea* decreased linearly with diapause development from November to April under field conditions. In this study, the survival rates ranged from 80 to 90% in DP at each sequential acclimation temperature from 15 to  $-5^{\circ}\text{C}$  for two weeks, and the survival rate was 100% in NDP from 5 to  $-5^{\circ}\text{C}$ . This suggests that *H. cunea* in Japan can survive exposure to temperatures near  $-5^{\circ}\text{C}$ , whether it is in diapause or in non-diapause, and overwintering pupae of *H. cunea* can avoid cold stress by selecting specific locales. On the other hand, the ability of DP to survive exposure to  $-15^{\circ}\text{C}$  decreased linearly as the acclimation temperature decreased from 5 to  $-5^{\circ}\text{C}$ , whereas the ability of NDP to survive exposure to  $-15^{\circ}\text{C}$  remained great and did not decrease as the acclimation temperature decreased from 5 to  $-5^{\circ}\text{C}$ . This means that the ability of DP to survive exposure to  $-15^{\circ}\text{C}$  is not greater than that of NDP. However, the decrease in the survival rate at  $-15^{\circ}\text{C}$  in DP after acclimation could be partially due to the different acclimation protocols used for DP and NDP.

Trehalose and glycerol are major cryoprotectants in overwintering insects. In *H. cunea* pupae, the main sugar is trehalose, and glycerol is scarcely detected. Under field conditions, the trehalose content started to increase in January and peaked in March. This suggests that low temperature and diapause termination facilitate trehalose production. The trehalose content in *H. cunea* pupae was much lower than the levels reported in other cold hardy insects. The trehalose content in field-collected *H. cunea* pupae was less than 0.8%. Trehalose accumulation was not closely related to cold hardiness and trehalose appears to have no real role as a cryoprotectant in field pupae. As the acclimation temperature was decreased from 5 to  $-5^{\circ}\text{C}$ , DP accumulated less trehalose than NDP. A positive correlation was found between trehalose accumulation and cold hardiness in NDP. These results suggest that *H. cunea*, when not in diapause, has an inherent ability to accumulate trehalose in response to cold acclimation, and the diapause program is not necessary to achieve a greater level of cold hardiness under cold acclimation from 5 to  $-5^{\circ}\text{C}$ . A clear

interconversion between glycogen and trehalose was shown in acclimated NDP, but not in acclimated DP.

Low aerobic conditions and low temperature contribute to high alanine accumulation in some overwintering insects (Goto et al. 1998, 2001). The patterns of change of alanine level in *H. cunea* pupae imply that the diapause state and lower temperatures enhance alanine accumulation. Lipid content in field-collected pupae remained constant, indicating that lipid has no function as a cryoprotectant in *H. cunea* pupae.

Our results indicate that, under field conditions, overwintering pupae of *H. cunea* in Japan do not accumulate high levels of sugars and polyols, and cold hardiness decreases with diapause development over the winter. After cold acclimation, diapause pupae do not accumulate high levels of sugars and polyols and do not develop a high level of cold hardiness at  $-15^{\circ}\text{C}$  in comparison with non-diapause pupae. However, *H. cunea* in Japan can endure  $-5^{\circ}\text{C}$  exposure for two weeks, whether it is in a diapause or non-diapause state. The physiological and biochemical bases of diapause in *H. cunea* in Japan are similar to those of some summer diapause species. One example is the Mediterranean tiger moth *Cymbalophora pudica*, which is distributed in northwestern Africa through the Iberian peninsula to southern Europe (de Freina and Witt, 1987). Summer diapause prepupae of this species do not accumulate sugars or polyols and have a high level of drought tolerance but a low level of cold hardiness. However, cold acclimation of this species caused a significant increase in cold hardiness, but without an accumulation of sugars and polyols (Košťál et al., 1998). This suggests that the ability of this insect to become adapted to a wide variety of habitats is due to its inherent ability to increase cold tolerance in summer after cold acclimation or to acclimatization following its accidental introduction from warmer areas to cold areas. Although *H. cunea* pupae in Japan enter diapause in autumn and survive cold exposure from the southern areas to the northern areas of Japan, it still remains unclear whether the Japanese population represents a summer diapause population or a winter diapause population.

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## References

- Danks, H.V., 1987. Insect Dormancy: An Ecological Perspective. Biological Survey of Canada, Ottawa.
- Denlinger, D.L., 1991. Relationship between cold hardiness and diapause. In: Lee Jr., R.E., Denlinger, D.L. (Eds.), *Insects at Low Temperature*. Chapman and Hall, New York, pp. 174–198.
- de Freina, J.J., Witt, T.J., 1987. Die Bombyces und Sphinges der Westpalaearkt. Edition Forschung und Wissenschaft, Munchen.
- Gomi, T., 1996a. A mechanism for the decrease in developmental period of a trivoltine population of *Hyphantria cunea* (Lepidoptera: Arctiidae). *Applied Entomology and Zoology* 31, 217–223.
- Gomi, T., 1996b. Mixed life cycles in the transitional zone between voltinisms in the fall webworm, *Hyphantria cunea*. *Experientia* 52, 273–276.
- Goto, M., 1995. Ecology of barnyard grass stem borer *Enosima leucotaeniella* (Ragonot) (Lepidoptera: Pyralidae)? Seasonal changes in trehalose concentration and volume of haemolymph in overwintering larvae. *Japanese Journal of Applied Entomology and Zoology* (in Japanese with English abstract) 39, 264–266.
- Goto, M., Fujii, M., Suzuki, K., Sakai, M., 1998. Factors affecting carbohydrate and free amino acid content in overwintering larvae of *Enosima leucotaeniella*. *Journal of Insect Physiology* 44, 87–94.
- Goto, M., Sekine, Y., Oota, H., Hujikura, M., Suzuki, K., 2001. Relationships between cold hardiness and diapause, and between glycerol and free amino acid contents in overwintering larvae of the oriental corn borer, *Ostrinia furnacalis*. *Journal of Insect Physiology* 47, 157–165.
- Goto, M., Takahashi, K., Suzuki, C., 1993. Ecology study on barnyard grass stem borer *Enosima leucotaeniella* (Ragonot) (Lepidoptera: Pyralidae)? Seasonal changes of carbohydrate contents in overwintering larvae. *Applied Entomology and Zoology* 28, 433–437.
- Ito, Y., Miyashita, K., Yamada, H., 1968. Biology of *Hyphantria cunea* Drury (Lepidoptera: Arctiidae) in Japan. VI. Effect of temperature in development of immature stages. *Applied Entomology and Zoology* 3, 163–175.
- Koštál, V., Sula, J., Simek, P., 1998. Physiology of drought tolerance and cold hardiness of the Mediterranean tiger moth *Cymbalophora pudica* during summer diapause. *Journal of Insect Physiology* 144, 165–173.
- Lee, R.E., 1991. Principles of insect cold hardiness. In: Lee Jr., R.E., Denlinger, D.L. (Eds.), *Insects at Low Temperature*. Chapman and Hall, New York, pp. 17–46.
- Li, Y.-P., Gong, H., Park, H.-Y., 2000. Biochemistry and physiological overwintering in the mature larvae of the pine needle gall midge, *Thecodiplosis japonensis* (Diptera: Cecidomyiidae) in Korea. *Cryo-Letters* 21, 149–156.
- Masaki, S., 1975. Biology of *Hyphantria cunea* Drury (Lepidoptera: Arctiidae) in Japan: a review. *Review of Plant Protection Research* 8, 14–28.
- Masaki, S., 1977. Life cycle programming. In: Hidaka, T., (Eds.), *Adaptation and Speciation in the Fall Webworm*. Kodansha, Tokyo, pp. 31–60.
- Masaki, S., Umeya, K., Sekiguchi, Y., Kawasaki, R., 1968. Biology of *Hyphantria cunea* Drury (Lepidoptera: Arctiidae) in Japan? Photoperiodic induction of diapause in relation to the seasonal life cycle. *Applied Entomology and Zoology* 3, 55–66.
- Morris, R.F., 1967. Factors inducing diapause in *Hyphantria cunea*. *The Canadian Entomologist* 99, 522–528.
- Morris, R.F., Fulton, C.W., 1970. Models for the development and survival of *Hyphantria cunea* in relation to temperature and humidity. *Memoirs of the Entomological Society of Canada* 70, 1–60.
- Oliver, A.D., 1964. A behavioral study of two races of the fall webworm, *Hyphantria cunea* (Lepidoptera: Arctiidae) in Louisiana. *Annals of the Entomological Society of America* 57, 192–194.
- Storey, K.B., Storey, J.M., 1991. Biochemistry of cryoprotectants. In: Lee Jr., R.E., Denlinger, D.L. (Eds.), *Insects at Low Temperature*. Chapman and Hall, New York, pp. 64–93.
- Takeuchi, K., Ohbayashi, T., 1996. First record of *Hyphantria cunea* (Lepidoptera: Arctiidae) in Ogasawara (Bonin) Islands. Abstracts of the 40th Annual Meeting of the Japanese Society of Applied Entomology and Zoology, pp. 84 (in Japanese).
- Tanaka, K., Saito, T., 1984. A method for the determination of respiratory quotient by gas chromatography. *Applied Entomology and Zoology* 19, 456–460.
- Tauber, M.J., Tauber, C.A., Masaki, S., 1986. *Seasonal Adaptations of Insects*. Oxford University Press, New York, Oxford.
- Warren, L.O., Tadic, M., 1970. The Fall Webworm, *Hyphantria cunea* (Drury). *Arkansas Agricultural Experiment Station Bulletin* 759, 1–106.