Effects of photoperiod and temperature on endogenous ice nucleus production in larvae of the rice stem borer, *Chilo suppressalis* Walker (Lepidoptera: Pyralidae)

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(Received 1 September 2006; Accepted 26 December 2006)

Abstract

Overwintering larvae of the rice stem borer, *Chilo suppressalis* Walker are known to produce endogenous ice nuclei in the muscle and epidermis as a means of avoiding intracellular freeze damage. However, the nuclei are not produced in non-diapausing larvae. To determine the effects of photoperiod and temperature on ice nucleus production, mature larvae were reared at 25°C under short and long photoperiods and acclimated to low temperatures. The ice nuclei were produced only in the muscle and epidermis of diapausing larvae reared under a short photoperiod and acclimated to low temperatures. The production of endogenous ice nuclei in the muscle and epidermis was sufficiently stimulated by short photoperiod and cold-acclimation to explain the crystallization temperature of the overwintering larvae.

Key words: Ice nucleus production; *Chilo suppressalis*; crystallization temperature; photoperiod; cold-acclimation

INTRODUCTION

Freeze-intolerant insects avoid freezing of their body fluids by depressing the crystallization temperature (CT) through the accumulation of cryoprotectant substances such as polyols (Asahina, 1969; Sømme, 1982; Danks, 2005) and thermal hysteresis or antifreeze proteins (Duman and Patterson, 1978; Duman et al., 1982, 1995; Zachariassen and Husby, 1982). On the other hand, some freeze-tolerant insects do not allow the CT of their body fluids to drop too low, even though they produce high levels of cryoprotectant substances (Zachariassen, 1980, 1982; Lee, 1991). Overwintering larvae of the rice stem borer, *Chilo suppressalis* are freeze-tolerant (Tsumuki, 1990). Although *C. suppressalis* larvae produce high levels of glycerol in the haemolymph (Tsumuki and Kanehisa, 1978), its crystallization is maintained at relatively high subzero temperatures in winter (Tsumuki and Konno, 1991). The CT is regulated by the muscle and epidermis ice nuclei (Tsumuki and Konno, 1991), which seem to be proteinaceous

(Hirai and Tsumuki, 1995). In overwintering larvae, the endogenous ice nuclei on the surface of the muscle cell membranes induce freezing of the haemolymph even though it contains high levels of glycerol (Hirai and Tsumuki, 1995). This prevents intracellular ice formation, which is usually lethal (Izumi et al., 2005, 2006).

Enhancement of insect low temperature tolerance is a seasonal phenomenon that is related to the onset of low temperature in winter. The seasonal changes in photoperiod and temperature may be responsible for the production of polyols and ice nuclei (Zachariassen, 1982; Lee, 1995; Duman, 2001) and antifreeze proteins (Duman, 1979, 2001; Duman et al., 1982). The antifreeze proteins increase in the haemolymph of larvae of *Dendroidea canadensis* (Horwath and Duman, 1983) and *Choristoneura fumiferanae* (Tyshenko et al., 1998) at low temperature and/or short photoperiod. However, no studies have reported to prove the effects of photoperiod and temperature on ice nucleus production in insects.

In the present experiment, we investigated the

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DOI: 10.1303/aez.2007.305

effects of photoperiod and temperature on ice nucleus production in rice stem borer larvae.

MATERIALS AND METHODS

Insect rearing. Rice stem borer larvae reared at 25°C under a short photoperiod entered diapause at last instar larval stage, while most of the larvae reared under a long photoperiod pupated after 35 to 60 days of hatching. To obtain non-diapausing and diapausing larvae, newly hatched larvae were reared on rice seedlings at 25°C under long (16L:8D) and short (10L:14D) photoperiods, respectively (Tsumuki and Kanehisa, 1978). About 26 days after hatching last instar larvae were divided into two groups. The first group (cold-acclimation) was reared at 15°C for 5 days on rice seedlings. Subsequently, the temperature was lowered in a stepwise fashion by 5°C per 5 days until it reached 5°C, and then maintained at the temperature for 25 days to achieve cold-acclimation. The second group (non-acclimation) was continuously reared at 25°C.

Crystallization temperature (CT) measurement. Larvae were dissected in 0.9% NaCl solution at different time intervals for the measurement of CTs of the hemolymph, gut, fat body, muscle and epidermis (Tsumuki and Konno, 1991). Because of the difficulty to separate the muscle from the epidermis completely, the CT was measured in these tissues combined. The dissected tissues were sandwiched between two layers of paraffin oil in capillary tubes attached to 30-gauge copper-constantan thermocouples and cooled at a rate of about 0.5°C/min until frozen (Tsumuki and Konno, 1991; Tsumuki et al., 1992). To measure the CT of the whole body, larvae were directly attached to the topes of thermocouples with a sticky tape and cooled at the same rate as above.

RESULTS

The CTs of the fat body in both non-diapausing and diapausing larvae were not affected by cold-acclimation although they decreased temporarily at the initiation of cold-acclimation (Fig. 1).

The CTs of the gut in non-diapausing and diapausing larvae were the highest of all tissues tested and were almost similar during cold- and non-acclimations except for non-acclimated non-diapausing

Fig. 1. Effects of photoperiod and temperature on the crystallization temperature in the fat body of rice stem borer larvae. Bars represent SE of mean ($n=10$).

Fig. 2. Effects of photoperiod and temperature on the crystallization temperature in the gut of rice stem borer larvae. Bars represent SE of mean ($n=10$).

larvae where the CTs of non-acclimated non-diapausing larvae decreased gradually as larvae grew (Fig. 2).

In the muscle and epidermis, the changes of the CTs during cold-acclimation depended on rearing photoperiod at larval stage (Fig. 3). The CT of these tissues obviously increased in diapausing larvae when acclimated at low temperatures, but no such elevation in the CT of cold-acclimated non-diapausing larvae was observed.

The CTs of the whole bodies of non-diapausing and diapausing larvae ranged from -4°C to -8.5°C during cold-acclimation and non-acclimation, respectively (Fig. 4). However, the CTs in non-diapausing and diapausing larvae were not affected by cold-acclimation.

Fig. 3. Effects of photoperiod and temperature on the crystallization temperature in the muscle and epidermis of rice stem borer larvae. Bars represent SE of mean ($n=8-10$).

Fig. 4. Effects of photoperiod and temperature on the crystallization temperature in the whole body of rice stem borer larvae. Bars represent SE of mean ($n=10$).

DISCUSSION

Ice nucleating activity has been detected in fat body cells of the gall fly, *Eurosta solidaginis* (Mugano et al., 1996). In our studies the CTs of the fat body of both cold-acclimated and non-acclimated larvae of the rice stem borer were in the range of -16°C to -18°C (Fig. 1). Previously, it has been established that potentially endogenous ice nuclei are not produced in the fat body of overwintering *C. suppressalis* larvae (Tsumuki and Konno, 1991). Consequently, potentially endogenous ice nuclei are not produced in the fat body of diapausing and non-diapausing larvae during cold acclimation.

Gut contents generally contain potentially exogenous ice nuclei that regulate the freezing of whole

insects (Salt, 1953, 1968). In non-diapausing larvae of the rice stem borer, potentially exogenous ice nuclei are also present in the gut (Tsumuki and Konno, 1991; Tsumuki et al., 1992). In the present experiment, the CTs of the gut of non-acclimated and cold-acclimated non-diapausing and diapausing larvae (except non-acclimated non-diapausing larvae) were -5°C to -8°C (Fig. 2), which were similar to those of the whole larvae (-4°C to -8.5°C) (Fig. 4). This suggests that potentially exogenous ice nuclei are present in the gut of cold-acclimated non-diapausing and diapausing larvae. However, the CT of the gut in the overwintering larvae collected in midwinter was below -17°C (Tsumuki and Konno, 1991). All of the gut contents were excreted in autumn to winter and thereby the CT of the gut decreased in winter (Tsumuki and Konno, 1991). In the present experiment, because acclimation temperatures were lowered in a short period, not all of the gut contents were excreted during cold-acclimation. Consequently, the CT of the gut might not have decreased during cold-acclimation. However, the CT of non-acclimated non-diapausing larvae decreased 60 days after hatching, because gut contents had been excreted before pupation.

In the rice stem borer, potentially endogenous ice nuclei are produced in the muscle and epidermis of overwintering larvae (Tsumuki and Konno, 1991; Hirai and Tsumuki, 1995). In the present experiment, 9 days after cold acclimation at 5°C (45 days after hatching) the CT of the muscle and epidermis of diapausing larvae rose to about -11°C (Fig. 3), which is almost the same temperature as for the overwintering larvae (Tsumuki and Konno, 1991). However, the CTs of non-acclimated and cold-acclimated non-diapausing, and non-acclimated diapausing larvae ranged from -15°C to -17°C . These results show that the endogenous ice nucleus production of the muscle and epidermis may primarily be activated by both short photoperiod (diapause) and cold temperature exposure. We have already obtained the supporting observations in which ice nucleus production of the muscle and epidermis was activated by JH inducing the larval diapause and inactivated by ecdysone breaking the diapause (Tsumuki and Hirai, 1999). Similar findings with respect to antifreeze protein production in *D. canadensis* have also been reported by Horwath and Duman (1983). However, the production

of antifreeze protein in the beetle is activated by either short photoperiod or low temperature, regardless of diapause.

In summary the production of endogenous ice nuclei in the muscle and epidermis was sufficiently stimulated by short photoperiod and cold-acclimation to explain the CT of the overwintering larvae with the empty gut, even though the excretion of exogenous ice nuclei in the gut might not be completed by the conditions used in the present experiment.

ACKNOWLEDGEMENTS

The authors express their thanks to Dr. M. Ashfaq, Research Institute for Bioresources, Okayama University for critical reading of the manuscript and useful suggestions. This study was supported in part by a Grant-in-Aid for Scientific Research (B) (11460022) from Japan Society for the Promotion of Science and by a grant from the Ohara Foundation for Agricultural Research.

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