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ScienceDirect
Journal of Insect Physiology 53 (2007) 392–397
Journal of Insect Physiology
www.elsevier.com/locate/jinsphys

Seasonal changes in glycerol content and cold hardiness in two ecotypes of the rice stem borer, *Chilo suppressalis*, exposed to the environment in the Shonai district, Japan

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Received 10 November 2006; received in revised form 28 December 2006; accepted 28 December 2006

Abstract

The rice stem borer, *Chilo suppressalis*, is divided into at least two ecotypes in Japan, the Shonai ecotype (SN) which is distributed in the northern part of Japan, and the Saigoku ecotype (SG) which is distributed in the southwestern region. Cold hardiness is positively correlated with the level of glycerol in both ecotypes. To investigate whether ecological distribution affects glycerol accumulation and cold hardiness development in these two ecotypes, overwintering larvae of the SN and SG ecotypes were concurrently exposed to the Shonai district. Obvious differences in the progress of glycerol accumulation and cold hardiness development in SN and SG larvae were found in early winter in the Shonai district. The levels of glycerol content and cold hardiness were low in October and high in January in both ecotypes, but those levels were different within this period (November and December) between ecotypes; the levels in SN larvae quickly reached their maximum, whereas, in SG larvae levels increased slowly. Under controlled conditions, the effect of the period of acclimation at 10 °C and subsequent low-temperature (5 °C) exposure on glycerol accumulation was investigated. These results indicated that glycerol accumulation in SN was stimulated by the progression of diapause termination, whereas a higher cumulative effect on glycerol production in SG was found when diapause was in a deep state.

Keywords: *Chilo suppressalis*; Cold hardiness; Diapause intensity; Ecotype; Glycerol

1. Introduction

The rice stem borer, *Chilo suppressalis*, which is one of the most important pests of rice plants, is distributed throughout most of Japan (31–43°N) (Kishino, 1974). In autumn, the final instar larvae enter diapause in the stem near the soil surface and overwinter. The borer has a bivoltine life cycle in most parts of Japan (Ishikura, 1955; Fukaya and Mitsuhashi, 1961; Kishino, 1974). There is local variation in the diapause duration, and the bivoltine borer is divided into at least two ecotypes, the Shonai ecotype which is distributed in the northern part of Japan, and the Saigoku ecotype which is distributed in the southwestern region (Fukaya and Mitsuhashi, 1961). The Shonai ecotype has a shorter diapause duration than the Saigoku ecotype. Larvae of the Shonai ecotype enter diapause in early September and require only a short period of low temperature for diapause termination, which occurs at the end of October before the coldest months. However, larvae of the Saigoku ecotype require a longer duration (about 4 months or more), and remain in diapause during the coldest months until February (Fukaya and Mitsuhashi, 1961; Tsumuki and Kanehisa, 1978; Goto et al., 2001). The overwintering larvae are freezing tolerant (Tsumuki, 1990; Tsumuki and Konno, 1991) and accumulate enormous amounts of glycerol in winter (Tsumuki and Kanehisa, 1978; Goto et al., 2001; Li et al., 2002a, b). Cold hardiness in the Shonai ecotype is low during diapause, but is relatively high during the

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0022-1910/$ - see front matter
doi:10.1016/j.jinsphys.2006.12.014

post-diapause stage, whereas it is high in the Saigoku ecotype during the diapause stage and relatively low during the post-diapause stage (Tsumuki and Kanehisa, 1978; Goto et al., 2001; Li et al., 2002a, b). Larvae of both ecotypes accumulate glycerol during the coldest months in the winter to increase cold hardiness. Glycerol accumulation in the Shonai ecotype occurs at low temperatures and at low diapause intensity (late diapause), while in the Saigoku ecotype it occurs at low temperatures and at high diapause intensity (Tsumuki and Kanehisa, 1978; Tsumuki, 1990; Goto et al., 2001; Li et al., 2002a, b). It seems that the local variations of diapause duration and cold hardiness are synchronized with field conditions during the coldest seasons, respectively (Kishino, 1974; Tsumuki and Kanehisa, 1978; Goto et al., 2001; Li et al., 2002b).

Overwintering larvae of the Shonai ecotype of *C. suppressalis* terminate diapause before winter, suggesting that the insects are exposed to severe subzero temperatures during the winter. On the other hand, overwintering larvae of the Saigoku ecotype of *C. suppressalis* terminate diapause in February, and glycerol content is maintained at a high level until February (Tsumuki and Kanehisa, 1978), hence the timing of diapause and cold hardiness overlap in the Saigoku ecotype, but it does not overlap in the Shonai ecotype. Previous studies suggested that both progressively decreasing temperatures in the field, and diapause development are responsible for the maximal glycerol production and fully developed cold hardiness in the overwintering *C. suppressalis* larvae of both ecotypes (Tsumuki and Kanehisa, 1978; Goto et al., 2001; Li et al., 2002a, b). However, the sensitivity of both ecotypes to the same field condition should be different. In the present study, we used acclimation experiments to distinguish the effects of these two factors on larvae of the Shonai ecotype and the Saigoku ecotype of *C. suppressalis*, focusing on the mechanisms regulating glycerol accumulation by diapause development and cold acclimation.

2. Materials and methods

2.1. Insects

The overwintering larvae used in the experiments were reared on rice seedlings at 25 °C under short photoperiod (12 L:12 D) for 65 d with the second generation progeny of locally collected overwintering larvae of *C. suppressalis*. Larvae of the Shonai ecotype of *C. suppressalis* were collected from a paddy field in Tsuruoka city in the Shonai district of Yamagata Prefecture in autumn of 2001 and larvae of the Saigoku ecotype were collected from a paddy field in Kurashiki city of Okayama prefecture in autumn of 2001.

2.2. Field-sampled larvae vs. acclimated larvae

Overwintering larvae of the two ecotypes were transferred to the field of Tsuruoka city of Yamagata prefecture on October 2, 2002. Cold hardiness, glycerol, trehalose and glycogen were analyzed once or twice a month until March 2003 (October 21, November 21, December 9, December 23, January 11, January 25, February 25, March 18). Field air temperatures were automatically recorded with a metrological instrument (NAKAASA, M806), which was set at the sampling location. For the acclimation experiment, overwintering larvae of the two ecotypes were acclimated beginning on day 0 at 10 °C for 10, 30 and 50 d. Then, some ($n = 30$) were used to measure diapause intensity, and the others were incubated at 5 °C under short photoperiod (12 L:12 D) for 0, 7, 15 and 30 d, respectively, to determine the content of glycerol in whole bodies ($n = 8$).

2.3. Chemical analysis

Glycerol and trehalose were measured by gas-liquid chromatography ($n = 8$) as described by Goto et al. (1993), and glycogen was determined by the phenol/sulfuric acid method ($n = 8$) as described by Goto et al. (1993).

2.4. Cold hardiness (survival rate after exposure to −15 °C)

Field-collected larvae ($n = 15$) were transferred to a temperature-controlled chamber, in which the temperature was lowered at a rate of 1 °C/h to −15 °C, held at −15 °C for 24 h, and then raised to 20 °C. After 24 h, the numbers of living and dead larvae were counted. Larvae showing no movement and loose body segments were considered as dead.

2.5. Adult emergence

Overwintering larvae ($n = 30$) acclimated at 10 °C were transferred to 20 °C under a photoperiod of 12 L:12 D, and adult emergence was observed every day for 30 d.

3. Results

3.1. Seasonal changes under the same field conditions (Shonai district)

3.1.1. Ambient temperature and cold hardiness

The average ambient temperature in the Shonai district ranged from 18.4 °C in October to 0.6 °C in January. The minimum daily temperature was approximately −2 to −3 °C in January and February, whereas the maximum daily temperature was nearly 5 °C higher (Fig. 1A).

Overwintering larvae of the Shonai and Saigoku ecotypes of *C. suppressalis* were transferred from the laboratory to the same field (Shonai, Yamagata prefecture) in early October 2002, and seasonal changes of cold hardiness, glycerol, trehalose and glycogen contents were investigated through the middle of March 2003.

Overwintering larvae were exposed to −15 °C for 24 h to examine their cold hardiness (Fig. 1B). The survival rate