Nymphal Diapause in *Laodelphax striatellus* (Hemiptera: Delphacidae)[1]

Yifan Zhai[2], Xiaoyan Zhang[3], Zhenjuan Yin, Qisong Zhu[4], Mei Tao[3], Yi Yu, and Li Zheng

Institute of Plant Protection, Shandong Academy of Agricultural Sciences, Jinan 250100 China

J. Entomol. Sci. 53(2): 107–122 (April 2018)

Abstract Diapause, an important developmental process in insects, is a physiological adaptation for avoiding adverse environmental conditions. *Laodelphax striatellus* Fallén is an important graminaceous crop pest in East Asia, and there are few reports regarding the nymphal diapause from fields with rice–wheat rotation. In the present study, we determined the fourth-instar nymph as the main diapause stage through investigation under field and laboratory conditions. Developmental duration of the third and fourth instars was longer during the short day-length (10 h light:14 h dark [10L:14D]) at 20°C than during other photoperiods. The third-instar nymph is the most sensitive stage to changes in the photoperiod. The optimal environmental factor for diapause termination was long day-length (16L:8D) at 25 to 28°C. The supercooling point was significantly reduced in diapause nymphs, and activities of trehalase, pyruvate kinase, and sorbitol dehydrogenase were significantly decreased (46.46, 37.90, and 17.64%, respectively). The information obtained in this study may be beneficial to the development of control strategies for this pest.

Key Words *Laodelphax striatellus*, nymphal diapause, cold hardiness

Insects have multiple strategies to adapt to environmental changes. Diapause, a key physiological adaptation for avoiding adverse environmental conditions, is regulated through genetic and environmental factors (Denlinger 2002). Among the many external factors, photoperiod and temperature are the most important environmental signals. Most insects rely on photoperiod and/or temperature cues to reach their diapause with decreased metabolism, arrested development, and increased stress resistance (Kostal 2006). The diapause stages usually accumulate and utilize some cold-tolerance substances, and the process is precisely controlled by a series of enzymes of intermediary metabolism. In addition, diapause typically occurs at a specific developmental stage for each species, including the embryo, larvae/nymph, pupae, or adult stages (Baker and Russell 2009, Jiang et al. 2010, Kobayashi et al. 2014, Liu et al. 2010). Knowledge of diapause is essential for understanding pest control, occurrence prediction, and economic insect domestication (Denlinger 2008).

[1]Received 11 November 2016; accepted for publication 03 April 2017.
[2]Corresponding author (zyifan@saas.ac.cn).
[3]College of Plant Protection, Yunnan Agricultural University, Kunming 650000 China.
[4]Shandong Rice Research Institute, Jinan 250100 China.

The small brown planthopper, *Laodelphax striatellus* Fallén (Hemiptera: Delphacidae), is an economically important pest insect in East Asia, where it attacks a wide range of graminaceous crops, such as rice, wheat, corn, etc. (Kisimoto 1989). This pest not only consumes plant nutrients but also transmits viral plant diseases, such as rice stripe virus and rice black-streaked dwarf virus (Otuka 2013). *Laodelphax striatellus* exhibits more cold resistance than other rice planthopper species, *Nilaparvata lugens* (Stål) and *Sogatella furcifera* (Horváth), and *L. striatellus* undergoes nymphal diapause during the winter in northern China (Xu 2012, Zhang 2012). *Laodelphax striatellus* diapause has been studied under different laboratory conditions (Kisimoto 1989, Wang et al. 2014); however, Hou et al (2016) reported that the diapause traits of *L. striatellus* were different among its geographic populations, and the most northern populations showed the highest diapause incidence and a longer critical photoperiod.

A study of *L. striatellus* diapause under a rice–wheat rotation on the plains of northern China has not been conducted. Here, we report an investigation of *L. striatellus* overwintering biology under field and laboratory conditions. The information obtained in this study may be beneficial to the development of control strategies for *L. striatellus*.

Materials and Methods

Field experiment. To characterize the seasonal cycle of *L. striatellus* under field conditions, we sampled a field population for two full winter seasons. The sampling was conducted in a rice–wheat rotation field at Ji'nan (N 36°41′, E 116°54′), Shandong Province, China, from September 2012 to April 2014. Five different fields were selected for the experiment. Within each field, five sites (1 m^2 per site) were selected to examine the plants (e.g., rice stubbles, wheat seedling, and weeds). Air temperature, day length, and instar stage of *L. striatellus* were recorded.

Insect origin and insect rearing conditions. For laboratory studies, a *L. striatellus* colony was obtained from the Shandong Rice Research Institute (Shandong, China) in 2010. These insects were collected in rice fields at Ji'ning (N 35°33′, E 116°15′, Shandong, China) and maintained in a laboratory for 6 yr. These insects were reared on fresh rice seedlings and maintained in the laboratory at 25 ± 1°C under a 16 h light:8 h dark (16L:8D) photo regime and 70 to 80% relative humidity.

Diapause induction. Based on data obtained from the field experiment, the original colony of *L. striatellus* was reared on fresh rice seedlings at 25°C under a 16L:8D photo regime for several generations before use, and fresh rice seedlings with newly laid eggs were incubated at different temperatures in phytotronic environments (20 ± 1°C, 23 ± 1°C, 25 ± 1°C, and 28 ± 1°C). Different photoperiods (4L:20D, 8L:16D, 10L:14D, 12L:12D, and 16L:8D) were obtained by adjusting an opaque black cloth. One neonate first-instar nymph was placed in a glass tube (length: 200 mm; diameter: 25 mm) on fresh rice seedlings for further observation. During the entire experimental period, nymph molting, developmental duration, death, and emergence were recorded at the same time every day. Approximately 70 to 90 individuals were tested in each of the four replicates for the treatments.

Table 1. The classification standard of the diapause nymph population in *Laodelphax striatellus* at 20°C.

Diapause type	Developmental Duration of 1st–5th Instars (d)
Nondiapause	≤26
Incomplete diapause	27–36
Complete diapause	>36

Developmental delay often characterizes nymph population diapause (Hansen et al. 2011). The nymphal diapause of *L. striatellus* was further divided into three categories: nondiapause, incomplete diapause, and complete diapause, depending on the length of delay. Diapause was scored as complete diapause if all the individuals did not emerge for over 10 d after the nondiapause control had completed the emergence, and incomplete diapause if some individuals emerged for less than or equal to 10 d after the nondiapause control had completed the emergence (Table 1). The quadratic parabolic regression equation of diapause critical photoperiod was tested with R statistical software.

Determination of the sensitive stage to photoperiod. To determine the photoperiodically sensitive stage of *L. striatellus*, a test was conducted according to the methods of Spieth (1995), with some modifications. Different developmental stages of *L. striatellus* from newly laid eggs to fifth-instar nymphs were grown under different combinations of long (16L:8D) and short (10L:14D) day lengths at 20 ± 1°C. Neonate nymphs were collected daily and placed in a glass tube for further testing. Approximately 35 to 45 individuals were tested in each of the three replicates for all treatments.

Diapause termination. Diapausing *L. striatellus* were obtained by maintaining newly laid eggs to third-instar nymphs under short day-length (10L:14D) at 20 ± 1°C on fresh rice seedlings. Subsequently, the freshly molted fourth-instar nymphs were transferred to a variety of temperature and photoperiodic conditions: three temperatures (20 ± 1°C, 25 ± 1°C, and 28 ± 1°C) and three photoperiods (10L:14D, 12L:12D, and 16L:8D). The time to emergence was recorded. Between 30 and 40 individuals were tested in each of the three replicates for all treatments.

Measurement of cold hardiness. Newly laid eggs were cultivated to third-instar nymphs on fresh rice seedlings under different photoperiods (10L:14D, 12L:12D, 16L:8D) and temperatures (20 ± 1°C, 25 ± 1°C), and the supercooling points (SCP) of the fourth to fifth instars under different treatments were measured. The abdomen of each nymph was fixed with plastic tape to the tip of a thermocouple attached to a multichannel automatic recorder (TOPTEST TP9024, Top Electric Co., Shenzhen, China). The thermocouple with the nymph was placed into an insulation tube in a cryogenic refrigerator to insure that the temperature of the nymph decreased at a cooling rate of approximately 1°C min^{-1}. The SCP was identified as the lowest inflexion point and peak point in temperature, respectively. Between 45 and 55 individuals were tested in each of the three replicates for all treatments.

Measurement of metabolic enzyme activities. To clarify the physiological adaptation of diapause (10L:14D) or nondiapause (16L:8D) nymphs (the first-day fourth-instar nymphs) of *L. striatellus* at 20 ± 1°C, activities of some cold tolerance-related enzymes, such as trehalase (TRE), sorbitol dehydrogenase (SDH), pyruvate kinase (PK), and alkaline phosphatase (ALP), were measured in diapause and nondiapause nymphs whole bodies using testing kits (Suzhou Comin Biotechnology Co., Ltd., Suzhou, China). The absorbance of TRE, SDH, and PK was measured at 340 nm, and ALP was measured at 510 nm. Between 35 and 40 individuals were tested in each of three replicates for all treatments.

Statistical analysis. The statistical analyses were performed using SPSS17.0 software. The effects of photoperiod, temperature, and their interactions on the induction of diapause were tested using a general linear model. Differences between treatments were tested using analysis of variance, followed by Tukey's test.

Results

Field experiment. To identify the stages of overwintering diapause in *L. striatellus* and to determine the factors for diapause induction, we investigated the rice–wheat rotation fields at Ji'nan from 2012 to 2014. Fig. 1 shows that the proportions of third- and fourth-instar nymphs increased from mid-September to mid-October. During that period, the day length gradually reduced to less than 12 h, and the mean temperature gradually decreased to below 20°C. Results showed that third- to fifth-instar nymphs were the overwintering stages, and the fourth-instar nymphs accounted for more than 50% of the insect population from mid-November to mid-March of the following year (Table 2). As the day length and temperature gradually increased with time, the proportion of fifth-instar nymphs and adults gradually increased, and the proportion of adults was greater than 50% on 11April 2013 and 15 April 2014.

Photoperiodic and temperature responses for diapause induction. The photoperiodic response curves were clearly different at 20°C, and the duration of nymphal development at this temperature is obviously different from those at other temperatures (Fig. 2A). Specifically, during a short day-length (10L:14D), the mean developmental duration for the nymph was 55.07 ± 1.40 d. The nymphs developed more rapidly under the long day-length (16L:8D), with mean development duration of 23.45 ± 0.34 d (Table 3). The different developmental durations for nymphal instars at 20 ± 1°C are shown in Fig. 2B. The developmental duration of third and fourth instars were longer during the short day-length (10L:14D) than during other photoperiods, particularly the developmental duration of the fourth instar, which reached 30.92 ± 2.69 d (Table 3). The diapause induction results were consistent with the findings from the field experiment.

Diapause occurred under short day-length at 20°C, and the incidence of diapause at 20°C was related to the photoperiod (Table 1). The photoperiod range of diapause induction was narrow, and the incidence of diapause reached 100% under the short day-length (10L:14D). Once the illumination time was beyond a certain range, the incidence of diapause decreased. When the photoperiod reached 16L:8D, a nondiapause phenomenon was observed (Fig. 3). The photoperiod

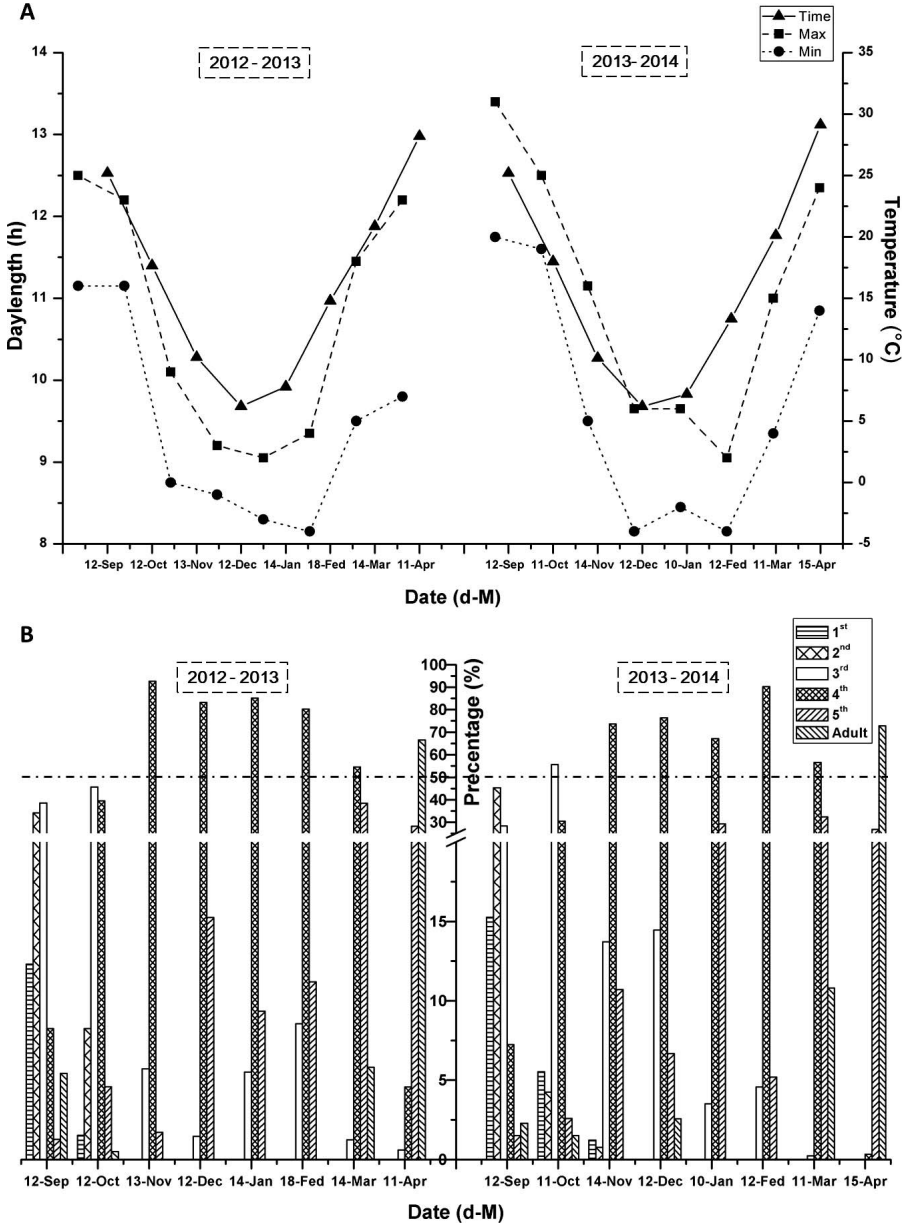

Fig. 1. Investigation of the overwintering generation of *Laodelphax Laodelphax striatellus* in the rice–wheat rotation field at Ji'nan measured once a month. (A) The day length and temperature recorded on different survey dates under various field conditions. "Time" means day length (see the left y-axis), "Max" and "Min" are temperature (see the right y-axis). (B) The percentage of nymphs and adults of *L. striatellus*.

Table 2. Numbers of *Laodelphax striatellus* under overwintering investigation in the rice–wheat rotation field at Ji'nan once a month.

		Nymphal Stage						
Year	**Data**	**1st**	**2nd**	**3rd**	**4th**	**5th**	**Adult**	**Total**
2012	12 September	30	83	94	20	3	13	243
	12 October	3	16	89	77	9	1	195
	13 November	0	0	3	50	1	0	54
	12 December	0	0	1	57	10	0	68
2013	14 January	0	0	2	32	4	0	38
	18 February	0	0	2	19	3	0	24
	14 March	0	0	1	46	32	5	84
	11 April	0	0	1	7	46	109	163
	12 September	20	61	38	10	2	3	134
	11 October	7	6	73	40	3	2	131
	14 November	2	1	17	92	13	0	125
	12 December	0	0	11	60	5	2	78
2014	10 January	0	0	1	19	9	0	29
	12 February	0	0	1	18	1	0	20
	11 March	0	0	1	36	21	6	64
	15 April	0	0	0	2	41	110	153

response in *L. striatellus* was typical for long-day species, with a diapause critical photoperiod of 13.54 h at 20°C, according to the regression equation $Y = -2.043X^2 + 38.607X - 98.7$ ($R^2 = 0.904$).

Most sensitive stage to the photoperiod response. The incidence of diapause increased with increasingly short day-length, and decreased with decreasing photoperiod, suggesting that short day-length had a cumulative effect on diapause induction in *L. striatellus* (Table 4). No diapause was observed at the egg stage during the short photoperiod or the nymph stage during the long photoperiod (Table 4, Treatment No. 1). In contrast, the incidence of diapause reached 100% at the egg stage during the long photoperiod and nymph stage during the short photoperiod (Table 4, Treatment No. 7). The incidence of diapause increased from 38.0 to 93.8% at the third nymph stage during the short photoperiod (Table 4, Treatment Nos. 3, 4), and the incidence of diapause decreased from 72.5 to 26.3% at the third nymph stage during the long photoperiod (Table 4, Treatment Nos. 9, 10). These results suggest that every instar reacted to changes in the photoperiod throughout the nymphal development, whereas the thirdinstar nymph

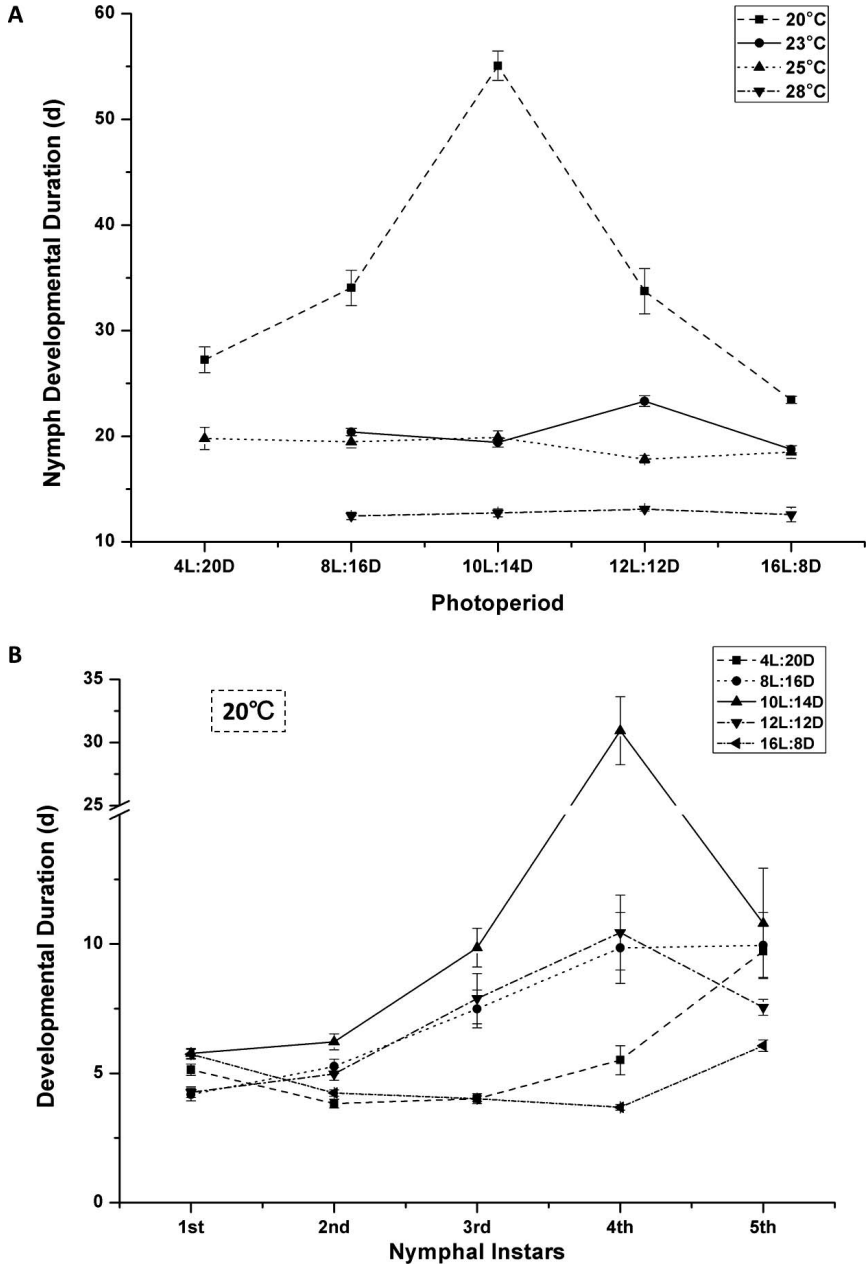

Fig. 2. Photoperiodic and temperature responses for diapause induction in *Laodelphax striatellus*. (A) Nymph developmental duration measured at different photoperiods (4 h light:20 h dark [4L:20D], 8L:16D, 10L:14D, 12L:12D, and 16L:8D) and temperatures (20 ± 1°C, 23 ± 1°C, 25 ± 1°C, 28 ± 1°C). (B) Different nymphal instars' developmental duration measured at different photoperiods at 20°C. The data represent the means ± SEM of four biological replicates.

Table 3. Nymphal developmental duration of *Laodelphax striatellus* at different temperatures and photoperiods.

Temperatures (°C)	Photoperiods	Nymphal Developmental Duration (d)*					
		1st	2nd	3rd	4th	5th	1st–5th
20	4L:20D**	5.14 ± 0.22 b	3.83 ± 0.17 c	4.02 ± 0.19 c	5.51 ± 0.56 c	9.72 ± 1.01 a	28.22 ± 1.22 c
	8L:16D	4.18 ± 0.24 c	5.27 ± 0.28 b	7.49 ± 0.73 b	9.85 ± 1.38 b	9.55 ± 1.28 b	36.34 ± 1.67 b
	10L:14D	5.77 ± 0.19 a	6.22 ± 0.31 a	9.86 ± 0.75 a	30.92 ± 2.69 a	10.80 ± 2.13 a	55.07 ± 1.40 a
	12L:12D	4.27 ± 0.21 c	4.98 ± 0.25 b	7.89 ± 0.97 b	10.44 ± 1.45 b	7.55 ± 0.31 c	33.73 ± 2.15 b
	16L:8D	5.74 ± 0.19 a	4.24 ± 0.12 c	4.02 ± 0.11 c	3.69 ± 0.11 c	6.07 ± 0.22 c	23.45 ± 0.34 c
23	8L:16D	4.05 ± 0.15 B	3.96 ± 0.12 A	3.48 ± 0.09 B	3.78 ± 0.09 A	5.46 ± 0.20 B	20.41 ± 0.34 B
	10L:14D	3.65 ± 0.09 C	2.93 ± 0.11 B	3.14 ± 0.13 B	3.68 ± 0.11 A	6.35 ± 0.39 A	19.43 ± 0.45 C
	12L:12D	4.27 ± 0.12 A	4.92 ± 0.16 A	4.92 ± 0.20 A	4.10 ± 0.21 A	5.83 ± 0.20 A	23.33 ± 0.51 A
	16L:8D	3.78 ± 0.0.9 C	2.92 ± 0.11 B	3.24 ± 0.09 B	3.78 ± 0.12 A	5.23 ± 0.16 B	18.77 ± 0.35 C
25	4L:20D	5.00 ± 0.16 a	2.94 ± 0.1 a	2.63 ± 0.1 a	3.17 ± 0.2 b	6.17 ± 0.7 a	19.83 ± 1.05 a
	8L:16D	4.36 ± 0.16 b	3.05 ± 0.20 a	2.73 ± 0.19 a	3.19 ± 0.16 b	6.19 ± 0.41 a	19.48 ± 0.58 a
	10L:14D	4.24 ± 0.19 b	2.71 ± 0.16 a	2.76 ± 0.18 a	3.90 ± 0.32 a	6.29 ± 0.26 a	19.90 ± 0.61 a
	12L:12D	4.17 ± 0.19 b	2.83 ± 0.13 a	2.66 ± 0.11 a	3.14 ± 0.15 b	5.03 ± 0.21 b	17.83 ± 0.36 b
	16L:8D	4.73 ± 0.20 a	2.82 ± 0.21 a	2.77 ± 0.16 a	3.27 ± 0.18 b	4.91 ± 0.20 b	18.50 ± 0.59 a
28	8L:16D	2.82 ± 0.07 B	2.11 ± 0.06 A	2.19 ± 0.04 A	2.29 ± 0.06 A	3.19 ± 0.12 B	12.47 ± 0.35 B
	10L:14D	2.60 ± 0.07 C	2.21 ± 0.04 A	2.26 ± 0.07 A	2.32 ± 0.08 A	3.47 ± 0.12 A	12.75 ± 0.37 B
	12L:12D	3.11 ± 0.05 A	2.07 ± 0.04 B	2.14 ± 0.07 A	2.35 ± 0.09 A	3.64 ± 0.14 A	13.11 ± 0.17 A
	16L:8D	2.98 ± 0.07 A	2.00 ± 0.06 B	1.88 ± 0.06 B	2.25 ± 0.06 A	3.64 ± 0.17 A	12.59 ± 0.69 B

* Data represent the mean values ± SD, and the different capital letter and lowercase indicate significant difference for instar respectively among different photoperiod ($P < 0.05$, Tukey's post-hoc test).

** 4L:20D indicates 4 h light:20 h dark. This style is followed for all photoperiods.

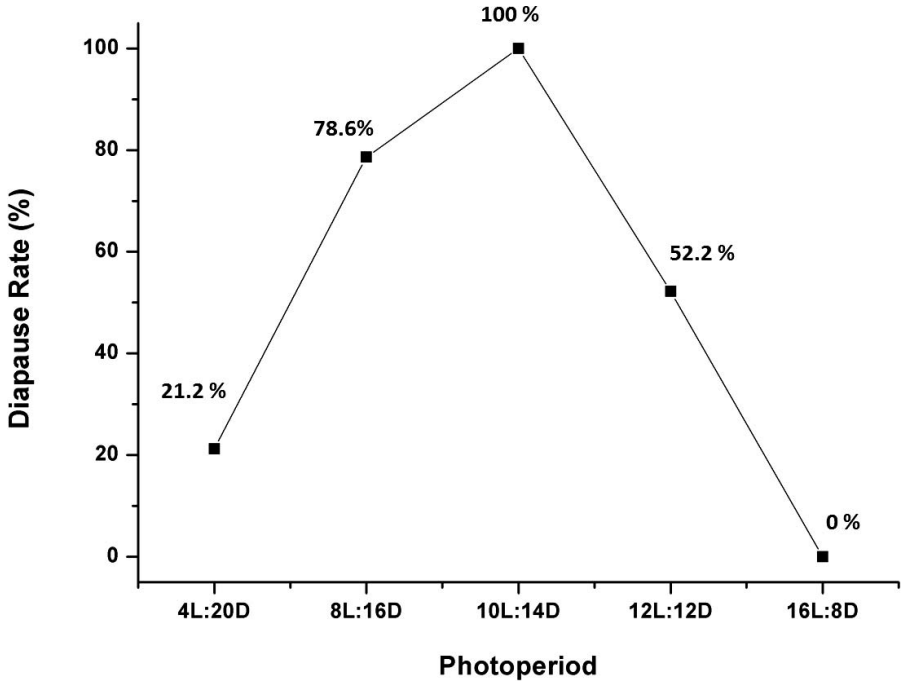

Fig. 3. The effects on the incidence of diapause during different photoperiods at 20°C. The data represent the mean values ± SEM ($n = 3$).

was most sensitive to photoperiod (Hou et al. 2016, Wang et al. 2014), and the incidence of diapause of the second-instar nymph increased significantly.

Effect of photoperiod and temperature on diapause termination. Different combinations of photoperiod and temperature significantly affected diapause termination (Fig. 4). An increase in temperature or photoperiod terminated nymphal diapause. The photoperiod significantly affected diapause termination at low temperatures (20 ± 1°C), and the developmental duration of fourth and fifth instars decreased with increasing photoperiod. Increases in temperature did not apparently affect the photoperiod. Temperature was a key factor in nymphal diapause termination, and the effect of temperature was greater than that of photoperiod, indicating that low temperature (20 ± 1°C) and short day-length (10L:14D) maintained the nymphal diapause state. The optimal combination of photoperiod and temperature for diapause termination was long day-length (16L:8D) at 25 to 28°C.

Effect of photoperiod and temperature on cold hardiness. SCP is the measure of cold hardiness for insects. Fig. 5 shows that the SCP was not significantly different between different photoperiods at high temperature (25 ± 1°C), while decreasing temperature affected SCP at different photoperiods. A significant difference was observed between the SCPs of diapause (10L:14D) and nondiapause (16L:8D) populations at low temperatures (20 ± 1°C), suggesting that the cold hardiness was greater in diapause nymphs compared with the non-

Table 4. The incidence of diapause after photoperiods of 10 h light:14 h dark (10L:14D) and 16L:8D at different developmental stages in *Laodelphax striatellus* at 20°C.

Treatment No.	Different Developmental Stages						No. of Individuals	Incidence of Diapause (%)
	Egg	1st	2nd	3rd	4th	5th		
1	S*	L	L	L	L	L	106	0.0 ± 0.0 f**
2	S	S	L	L	L	L	110	15.4 ± 1.6 e
3	S	S	S	L	L	L	105	38.0 ± 0.8 d
4	S	S	S	S	L	L	130	93.8 ± 2.2 b
5	S	S	S	S	S	L	124	100.0 ± 0.0 a
6	S	S	S	S	S	S	107	100.0 ± 0.0 a
7	L	S	S	S	S	S	116	100.0 ± 0.0 a
8	L	L	S	S	S	S	124	90.2 ± 2.1 b
9	L	L	L	S	S	S	132	72.5 ± 0.8 c
10	L	L	L	L	S	S	118	26.3 ± 1.3 d
11	L	L	L	L	L	S	121	12.8 ± 1.2 e
12	L	L	L	L	L	L	132	0.0 ± 0.0 f

* L represents the long photoperiod (16L:8D), and S represents the short photoperiod (10L:14D).
** The data represent the mean values ± SD, and the different letters denote a significant difference ($P < 0.05$, Tukey's post-hoc test).

diapause nymphs, and that the effect of photoperiod on SCP weakens as the temperature increases.

Changes of metabolic enzyme activities in diapause and nondiapause nymphs. The enzymatic activities of TRE, PK, and SDH significantly decreased (by 46.46, 37.90, and 17.64%, respectively), in diapause nymphs compared with nondiapause nymphs (Fig. 6); ALP activity did not decrease.

Discussion

For most insect species, diapause occurs during a specific stage or instar; however, diapause occurs in several nymphal stages of *L. striatellus* and varies with geographic location. The overwintering stage is reported in first- to fifth-instar nymphs in the Nanchang region of China (Wang et al. 2014), third- to fifth-instar nymphs in the Nanjing region (He 2010), and second- to fifth-instar nymphs in northeastern China (Lin et al. 2004). In this study, we discovered that the overwintering diapause occurred in third to fifth instars, with the fourth-instar nymph being the predominant diapause stage. Indeed, the photoperiod-induced diapause in many insect species is affected by latitude (Wang et al. 2012). Generally, there

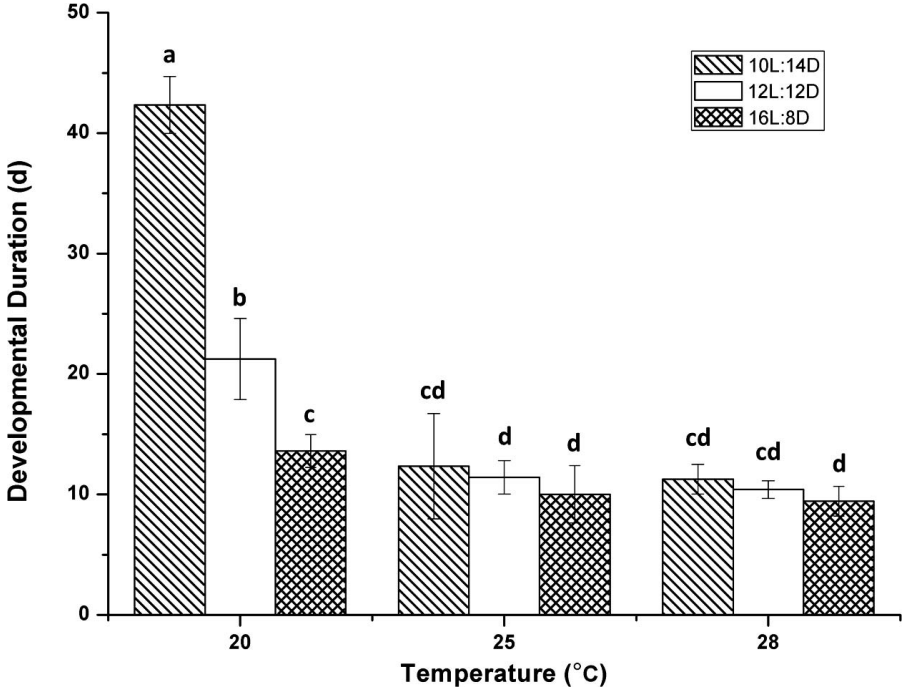

Fig. 4. Photoperiodic and temperature responses for diapause termination in *Laodelphax striatellus*. Developmental duration of fourth and fifth instars measured at different photoperiods (10 h light:14 h dark [10L:14D], 12L:12D, and 16L:8D) and temperatures (20 ± 1°C, 25 ± 1°C, 28 ± 1°C). The data represent the mean values ± SEM ($n = 3$), and the values in the columns followed by different letters denote a significant difference ($P < 0.05$, Tukey's post-hoc test).

are higher diapause incidences in higher-latitude populations than in lower-latitude populations (Hou et al. 2016).

There are some morphological indications for diapause in many insect species, such as eye spots in the postgenal region of the pupa during movement in *Helicoverpa armigera* (Hübner) (Zhang et al. 2012) and the mature larva forming oblate-shaped cocoons in *Carposina niponensis* F. (Huang et al. 1976). However, there is no morphological standard to differentiate the diapause and nondiapause nymphs in *L. striatellus*, which is instead based on the development duration (He 2010). Kisimoto (1958) suggested that the *L. striatellus* individual diapause standard was a nymphal development duration greater than 20 to 21 d at 20 to 22°C. Wang (2014) suggested that diapause determination should be based on the proportion of nymphs remaining as third and fourth instars 7 d after comparable control cultures had completed emergence. This study showed that at 20 ± 1°C, 26 d was the longest nymphal development duration in the long day-length (16L:8D) treatment groups, and nondiapause was considered to occur when the develop-

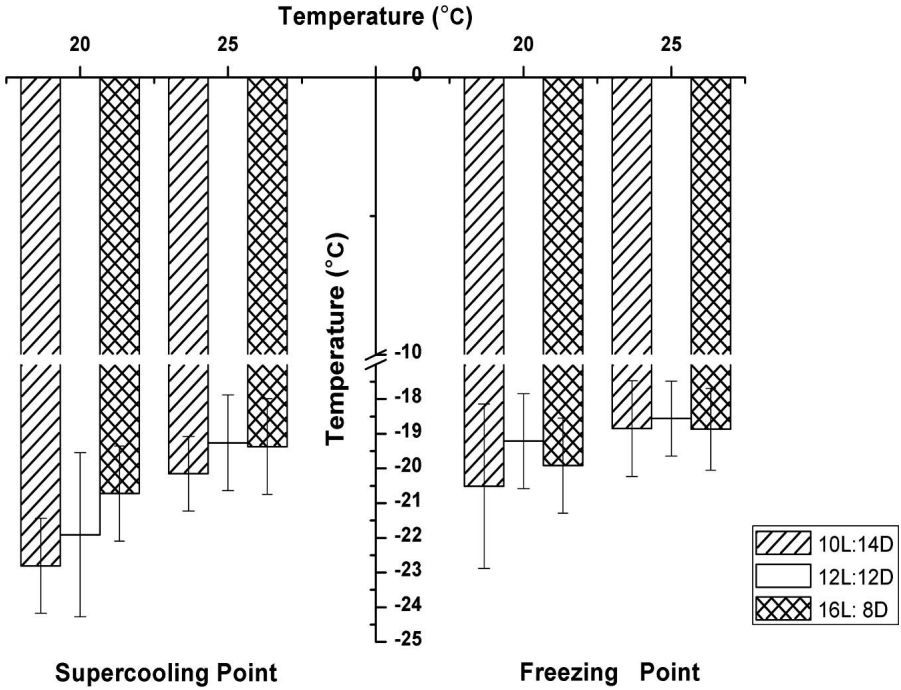

Fig. 5. Photoperiodic and temperature responses for supercooling point (SCP). The SCP of fourth- and fifth-instar nymphs measured at different photoperiods (10 h light:14 h dark [10L:14D], 12L:12D, and 16L:8D) and temperatures (20 ± 1°C, 25 ± 1°C). The data represent the mean values ± SEM ($n = 3$), and the values in the columns followed by different letters denote a significant difference ($P < 0.05$, Tukey's post-hoc test).

ment duration of all individual nymphs in the treatment group was <26 d. Complete diapause was considered when the nymphs remained in third and fourth instar stages for more than 36 d. The development duration between 26 and 36 d was considered as incomplete diapause.

In temperate zones, photoperiod and temperature are the two key diapause-inducing environmental factors. Decades of research on insect diapause suggests that the relative importance of photoperiod and temperature in diapause regulation is widely variable and highly species-specific, and that temperature often enhanced or inhibited the induction of diapause through photoperiod variability (Hodek and Hodkov 1988). Based on results from the field experiment, the fourth-instar nymph was the major diapause stage. Nymphal diapause induction during different photoperiod and temperature combinations are reported (He 2010, Wang et al. 2014, Zhang 2012). In the present study, only variations in the developmental duration were tested at 20°C under different photoperiods, and no obvious diapause was observed above 20°C. As shown in Fig. 2, temperature inhibited the induction

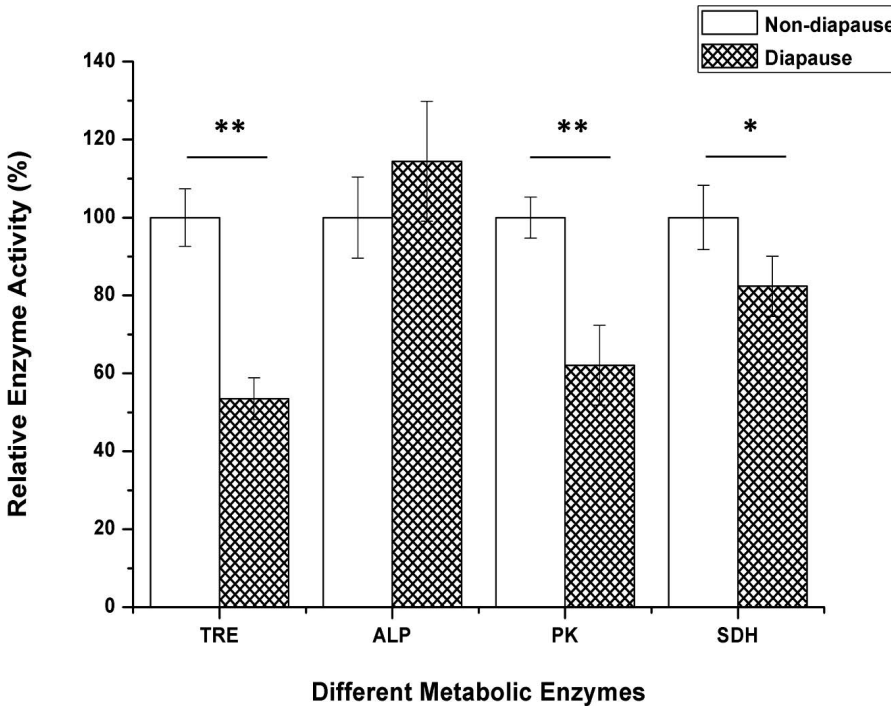

Fig. 6. Relative activity of some metabolic enzymes. Relative enzyme activity in first-day diapause and nondiapause fourth-instar nymphs. The data represent the mean values ± SEM ($n = 3$); * $P < 0.05$, ** $P < 0.01$, *t* test.

of diapause through photoperiod variability. At 20°C, the developmental duration was longer during the short days and shorter during the long days. The responses to photoperiod variability indicated that a critical photoperiod of diapause for typical long-day species was 13.54 h at 20°C in late August in the Ji'nan region.

Different insect species exhibit different sensitivity to photoperiod, but one or two instars before diapause induction are observed for most insect species in their larval/nymphal stage (Xiao et al. 2013). Results of the present study revealed that the third-instar nymph was the most sensitive stage to the photoperiod, consistent with the findings of Wang (2014). The *L. striatellus* nymphal diapause was terminated through increases in the temperature or photoperiod (Fig. 4), and photoperiod had a significant effect on diapause termination at a low temperature (20 ± 1°C). However, the effect of photoperiod was not apparent with increasing temperature. These results suggest that temperature is strongly correlated with nymphal diapause induction and termination. These results are consistent with other studies, such as those of *Manduca sexta* (Cantelo 1974), *Battus philenor* (Sims and Shapiro 1983), and *Trichogramma cordubensis* (Ventura Garcia et al. 2002).

Cold hardiness and diapause are essential components of winter survival for most insects in the temperate zone. Insect cold hardiness is influenced by various

factors, and different insect species adjusted the cold hardiness in different ways, such as reduction of SCP, freezing point, and water content; accumulation of cold hardiness substances; export of ice nucleating substances; and the regulation of metabolic enzyme activity (Stephens et al. 2015, Teets and Denlinger 2013, Zachariassen 1985). The SCP was below −20°C (Fig. 5), reflecting the fact that *L. striatellus* could cope with cold environments. Some reports indicated that insect diapause was not associated with cold hardiness, and many species can have low SCPs and not be tolerant to temperatures close to the SCP (Denlinger 1991, Pullin 1996). Some cold-tolerance substances, such as trehalose, sorbitol, and glucose, can increase cold tolerance. TRE is a key enzyme in trehalose hydrolysis, and changes in the activity of this enzyme directly affect energy metabolism (Kamei et al. 2011). Sorbitol, through SDH, is converted to glycogen and subsequently utilized as energy for embryonic development (Rubio et al. 2011). PK is a major glycolysis enzyme mediating the conversion of phosphoenolpyruvic acid and adenosine diphosphate into pyruvic acid and adenosine triphosphate (Rider et al. 2011). In the present study, activities of TRE, PK, and SDH significantly decreased, indicating that the diapausing nymphs enhanced their cold tolerance through the accumulation of cold-tolerance substances.

To our knowledge, this is the first study to ever report the *L. striatellus* nymphal diapause under a rice–wheat rotation. Furthermore, we conducted a field investigation and determined the impact of different combinations of photoperiods and temperatures on nymphal diapause induction and termination, and performed a preliminary examination of the physiological and biochemical mechanism of nymphal diapause. Understanding the diapause characteristics of *L. striatellus* may be useful for improving strategies of pest management. Additional studies will reveal the molecular mechanisms of nymphal diapause at the level of omics in *L. striatellus*.

Acknowledgments

This work was financially supported through a grant from the National Natural Science Foundation of China (31401803) and the Shandong Provincial Natural Science Foundation, China (ZR2014CQ014). The authors thank Jianlong Bi (University of California) for the manuscript language revision.

References Cited

Baker, D.A. and S. Russell. 2009. Gene expression during *Drosophila melanogaster* egg development before and after reproductive diapause. BMC Genomics 24: 242.

Cantelo, W.W. 1974. Diapause in a tropical strain of the tobacco hornworm. Ann. Entomol. Soc. Amer. 67: 828–830.

Denlinger, D.L. 1991. Relationship between cold hardiness and diapause, Pp. 174–198. *In* Lee, R.E. and D.L. Denlinger (eds.), Insects at Low Temperature. Chapman & Hall, New York.

Denlinger, D.L. 2002. Regulation of diapause. Annu. Rev. Entomol. 47: 93–122.

Denlinger, D.L. 2008. Why study diapause? Bull. Entomol. Res. 38: 1–9.

Hansen, E.M., B.J. Bentz, J.A. Powell, D.R. Gray and J.C. Vandygriff. 2011. Prepupal diapause and instar IV developmental rates of the spruce beetle, *Dendroctonus rufipennis* (Coleoptera: Curculionidae, Scolytinae). J. Insect Physiol. 57: 1347–1357.

He, Y. 2010. The studies on population dynamic and diapause of small brown planthopper. MS Thesis, Nanjing Agricultural Univ., Nanjing, China.

Hodek, I. and M. Hodkov. 1988. Multiple role of temperature during insect diapause. A review. Entomol. Exp. Appl. 49: 153–166.

Hou, Y.Y., L.Z. Xu, Y. Wu, P. Wang, J.J. Shi and B.P. Zhai. 2016. Geographic variation of diapause and sensitive stages of photoperiodic response in *Laodelphax striatellus* Fallén (Hemiptera: Delphacidae). J. Insect Sci. 16: 1–7.

Huang, K.X., Y.Z. Wang, Z.X. Ye, N.X. Zhang, L.Y. Zhang and Z.Q. Shu. 1976. The effect of photoperiod and temperature on the induction of diapauses in the peach fruit borer (*Carposina niponensis*). Acta. Entomol. Sinica 2: 149–156 (in Chinese).

Jiang, X.F., S.H. Huang, L.Z. Luo, Y. Liu and L. Zhang. 2010. Diapause termination, post-diapause development and reproduction in the beet webworm, *Loxostege sticticalis* (Lepidoptera: Pyralidae). J. Insect Physiol. 56: 1325–1331.

Kamei, Y., Y. Hasegawa, T. Niimi, O, Yamashita and T. Yaginuma. 2011. Trehalase-2 protein contributes to trehalase activity enhanced by diapause hormone in developing ovaries of the silkworm, *Bombyx mori*. J. Insect Physiol. 57: 608–613.

Kisimoto, R. 1958. Studies on diapause in the planthopper. Appl. Entomol. Zool. 2:128–134.

Kisimoto, R. 1989. Flexible diapause response to photoperiod of a laboratory selected line in the small brown planthopper, *Laodelphax-Striatellus* Fallén. Appl. Entomol. Zool. 24: 157–159.

Kobayashi, N., M. Takahashi, S. Kihara, T. Niimi, O. Yamashita and T. Yaginuma. 2014. Cloning of cDNA encoding a *Bombyx mori* homolog of human oxidation resistance 1 (OXR1) protein from diapause eggs, and analyses of its expression and function. J. Insect Physiol. 68: 58–68.

Kostal, V. 2006. Eco-physiological phases of insect diapause. J. Insect Physiol. 52: 113–127.

Lin, Z.W., Y. Liu and H.P. Xin. 2004. A primary study of *Laodelphax striatellus* (Fallén) bio-character in cold region rice. J. Heilongjiang August First Land Reclamation Univ. 16: 15–18 (in Chinese).

Liu, Z., P. Gong, D. Li and W. Wei. 2010. Pupal diapause of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) mediated by larval host plants: Pupal weight is important. J. Insect Physiol. 56: 1863–1870.

Otuka, A. 2013. Migration of rice planthoppers and their vectored emerging and novel rice viruses in East Asia. Front. Microbiol. 4: 309.

Pullin, A.S. 1996. Physiological relationships between insect diapause and cold tolerance: Coevolution or coincidence? European J. Entomol. 93: 121–129.

Rider, M.H., N. Hussain, S.M. Dilworth, J.M. Storey and K.B. Storey. 2011. AMP-activated protein kinase and metabolic regulation in cold-hardy insects. J. Insect Physiol. 57: 1453–1462.

Rubio, R.O., A. Suzuki, K. Mitsumasu, T. Homma, T. Niimi, O. Yamashita and T. Yaginuma. 2011. Cloning of cDNAs encoding sorbitol dehydrogenase-2a and b, enzymatic characterization, and up-regulated expression of the genes in *Bombyx mori* diapause eggs exposed to 5°C. Insect Biochem. Mol. Biol. 41: 378–387.

Sims, S.R. and A.M. Shapiro. 1983. Pupal diapause in *Battus philenor* (Lepidoptera: Papilionidae). Ann. Entomol. Soc. Amer. 76: 407–412.

Spieth, H.R. 1995. Change in photoperiodic sensitivity during larval development of *Pieris brassicae*. J. Insect Physiol. 41: 77–83.

Stephens, A.R., M.K. Asplen, W.D. Hutchison and R.C. Venette. 2015. Cold hardiness of winter-acclimated *Drosophila suzukii* (Diptera: Drosophilidae) adults. Environ. Entomol. 44: 1619–1626.

Teets, N.M. and D.L. Denlinger. 2013. Physiological mechanisms of seasonal and rapid cold-hardening in insects. Physiol. Entomol. 38: 105–116.

Ventura Garcia, P., E. Wajnberg, J. Pizzol and M.L. Oliveira. 2002. Diapause in the egg parasitoid *Trichogramma cordubensis*: role of temperature. J. Insect Physiol. 48: 349–355.

Wang, L., K. Lin, C. Chen, S. Fu and F.S. Xue. 2014. Diapause induction and termination in the small brown planthopper, *Laodelphax striatellus* (Hemiptera: Delphacidae). PLoS ONE 9: e107030.

Wang, X.P., Q.S. Yang, P. Dalin, X.M. Zhou, Z.W. Luo and C.L. Lei. 2012. Geographic variation in photoperiodic diapause induction and diapause intensity in *Sericinus montelus* (Lepidoptera: Papilionidae). Insect Sci. 19: 295–302.

Xiao, L., S. Fu and F.S. Xue. 2013. Characters of insect diapause stage and photoperiod sensitive stage. Biol. Disaster Sci. 36: 1–8 (in Chinese).

Xu, Y.B. 2012. The study on occurrence and detriment regularity of small brown planthopper in Shandong. PhD Dissertation, Nanjing Agricultural Univ., Nanjing, China.

Zachariassen, K.E. 1985. Physiology of cold tolerance in insects. Physiol. Rev. 65: 799–832.

Zhang, H.Y. 2012. Study on the occurrence regularity in Shandong and Jiangsu province and ovarian development of small brown planthopper. PhD Dissertation, Nanjing Agricultural Univ., Nanjing, China.

Zhang, Q., Y.X. Lu and W.H. Xu. 2012. Integrated proteomic and metabolomic analysis of larval brain associated with diapause induction and preparation in the cotton bollworm, *Helicoverpa armigera*. J. Prot. Res. 11: 1042–1053.