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COLD TOLERANCE CHARACTERISTICS AND OVERWINTERING STRATEGY OF *SESAMIA INFERENS* (LEPIDOPTERA: NOCTUIDAE)

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ABSTRACT

The pink rice stem borer, *Sesamia inferens* (Walker) (Lepidoptera: Noctuidae), is a major rice pest in China and elsewhere in Asia. While low winter temperatures are a major environmental constraint on the survival of most insect species, the mechanism of *S. inferens*' cold tolerance in winter remains unknown. In this study, we elucidated the cold tolerance characteristics of *S. inferens* collected in the field from Oct 2012 to Apr 2013. The cold tolerance of overwintering larvae was found to vary significantly. Maximum *S. inferens* cold tolerance was observed in larvae collected on 30 Jan 2013. However, the SCP (supercooling points) of larvae did not vary insignificantly, with a mean of -6.80 °C. Before 9 Mar 2013, larval water content stabilized at the mean low level of 63.5 %, but subsequently rose significantly, to 75.2%. Low molecular weight sugars and polyols, closely related to freeze tolerance strategy, increased from low levels to their peaks in Jan (glycerol, 359.8 µg/g; trehalose, 20.5 mg/g; fructose, 69.8 µg/g; glucose, 377.3 µg/g; *myo*-inositol, 59.6 µg/g), after which levels declined. This study demonstrates that the Yangzhou population of *S. inferens* is freeze-tolerant.

Key Words: *Sesamia inferens*, cold tolerant strategy, supercooling point, water content, low molecular weight sugars and polyols

RESUMEN

El barrenador rosado del tallo de arroz, *Sesamia inferens* (Walker) (Lepidoptera: Noctuidae) es una de las principales plagas del arroz en China y en otras partes de Asia. Mientras que las bajas temperaturas de invierno son una restricción ambiental importante en la sobrevivencia de la mayoría de especies de insectos, el mecanismo de tolerancia al frío de *S. inferens* hacia el invierno sigue siendo desconocido. En este estudio, hemos encontrado las características de tolerancia a frío de *S. inferens* recolectados sobre el campo de octubre de 2012 hasta abril de 2013. Se encontró que la tolerancia al frío de larvas en hibernación varia significativamente conforme el cambio de estación. Se observó la tolerancia al frío máximo de *S. inferens* en larvas recolectadas el 30 de enero de 2013. Sin embargo, las larvas tuvo un promedio de SCP (punto sobreenfriamiento) de sólo -6.80 °C y el SCP no vario significativamente. Antes del 9 de marzo del 2013, el contenido de agua en las larvas se había estabilizado en el promedio del nivel bajo de 63.5%, pero posteriormente se incrementó considerablemente al 75.2%. Azúcares de bajo peso molecular y polioles, estrechamente relacionados con la tolerancia a congelación, incrementó de niveles bajos en octubre a niveles máximos en enero, como glicerol, 359.8 µg/g; trehalosa, 20.5 mg/g; fructosa, 69.8 µg/g; glucosa, 377.3 µg / g; mio-inositol, 59.6 µg/ g; y subsecuentemente con el acercamiento de la próxima estación de crecimiento, estos niveles se redujeron. Este estudio demostró que la población de Yangzhou de *S. inferens* es tolerante a la congelación en lugar de resistente a la congelación.

Palabras Clave: *Sesamia inferens*, estrategia tolerantes al frío, punto de sobreenfriamiento, contenido de agua, azúcares de bajo peso molecular y polioles

Temperature is a critical abiotic variable, affecting the geographic distribution and seasonal activity patterns of organisms (Salt 1961; Lee & Denlinger 1991; Hoffmann et al. 2003; Chown & Terblanche 2007; Doucet et al. 2009; Stotter & Terblanche 2009). Consequently, the cold tolerance of insects has been frequently investigated. By the 1980s, a largely laboratory-based paradigm of insect cold hardiness had appeared, which proposed that insects survive low temperatures by 2 kinds of strategies. One strategy involves keeping the insect's body fluids in the liquid state below their ordinary melting point (associated with freeze avoidance, increased cold tolerance, significant

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variation in supercooling points, and an increase in low molecular weight sugars and polyols) (Bale 1996; Sinclair et al. 2003). The other strategy involves surviving the formation of ice in the insect's tissues (associated with freeze tolerance, increased cold tolerance, insignificant variation in supercooling points, and a similar increase in low molecular weight molecular sugars and polyols) (Bale 1996; Sinclair et al. 2003). However, the physiological roles of low molecular weight sugars and polyols between these 2 strategies are considerably different. Among freeze-tolerant species, sugars and polyols protect membranes and proteins against phase transitions and control the ice fraction size and minimum cell volume resulting from the freeze concentration and osmotic dehydration that accompanies freezing (Zachariassen 1985; Ramløv 2000; Clark & Worland 2008). This theory has stimulated much recent cold tolerance research.

The pink rice stem borer, *Sesamia inferens* (Walker) (Lepidoptera: Noctuidae), is a major rice pest in China and other Asian countries. This pest has different generation numbers per a year in different areas of China. For example, *S. inferens* has 3-4 generations per year in Jiangsu, Zhejiang and Anhui province (Li et al. 2002), 4 generations in Jiangxi, Hunan, Hubei and Sichuan province (Zhou & Chen 1985; Ding & Su 2002), 4-5 generations in Fujian, Guangxi and Yunnan province (Zhou & Chen 1985; Ding & Su 2002), 6-8 generations in southern Guangdong and Hainan province (Zhou & Chen 1985; Ding & Su 2002). *S. inferens* larvae have six instars, and usually overwinters in the fifth or sixth instar larvae in southern of Jiangsu province (Gu 1985; Ding & Su 2002). But the second to sixth instars of *S. inferens* were also found to overwinter in Jiangxi province (Wang 1983). Based on our investigation, most of overwintering larvae are fifth instars, and fourth and sixth instars were minorities in Yangzhou, Jiangsu province (unpublished data).

Overwintering *S. inferens* have been found not only in rice stubble (Zhang et al. 1954; Lu 1963; Zhou & Chen 1985; Guo et al. 2002; Chen et al. 2010), but also in wild rice (*Zizania latifolia* L.; Poales: Poaceae) (Tan 1954; Zhang et al. 1954), reeds (*Phragmites australis* (Cav.); Poales: Poaceae) (Tan 1954) and nut sedge (*Cyperus rotundus* L.; Poales: Cyperaceae) (Wang 1960). The overwintering larvae of *S. inferens* in the rice planting area mainly overwinter in rice stubble.

Many studies of this pest have been carried out since the middle of the last century. The main aspects studied include population dynamics (Ohtsu & Ikeyama 1978; Tayade 1978; Misra & Hora 1982; Liu et al. 2006; Han et al. 2008), the biology and ecology (e.g., effects of humidity, temperature, and light on growth and development) (Qureshi et al. 1975; Rahman & Khalequzzaman 2004), and the control techniques (Yadava 1978; Fang et al. 2008; Chen et al. 2010).

However, a comprehensive study on the overwintering strategies and cold tolerance of *S. inferens* has not been reported so far. To better understand the cold tolerant characteristics and strategy of *S. inferens* overwintering in the field, we examined several cold tolerance indices of overwintering larvae collected in the field from Oct 2012 to Apr 2013, such as $LTemp_{50}$ and $LTemp_{90}$ (the temperatures at which 50% and 90% of *S. inferens* died in samples of larvae collected only in Oct 2012), the mortality of larvae exposed to low temperatures, their supercooling points (SCPs), water content and the compositions of their low molecular weight sugars and polyols.

Materials and Methods

Insects

Larvae of *S. inferens* were collected from the rural rice fields of Yangzhou (N 32° 39' E 119° 42'), Jiangsu province, China, from Oct 2012 to Apr 2013. Larvae within the rice straw and stubble were placed outdoors where temperatures were similar to the sampling site. Within 12 h of field collection, the larvae were subjected to testing, and these larvae were stripped out of the straws just prior to testing. All larvae used in this study were overwintering fifth instars (0.3 ± 0.05 g body weight), and were assigned randomly to each experimental group.

Determination of Lethal Temperatures, i.e., $LTemp_{50}$ and $LTemp_{90}$

Larvae collected in Oct 2012 were used to study survival under cold stress. Collection of larvae was completed within one week. Larvae within rice straw and stubble were placed outdoors where temperatures were similar to the sampling site. Within 12 h of field collection, the larvae were subjected to testing, and these larvae were stripped out of the straws just prior to testing. Fifth instars (n = 20) confined individually in glass tubes (1.5 mm thickness, 200 mm length, and 20 mm diam) were exposed to constant temperatures of -12, -10, -9, -7, -6, -4, and -2 °C for 2 h in a constant temperature subzero incubator (DC-3010, Jiangnan Equipment, China). Tubes with larvae were then held at 26 °C for 2 h to recover before assessment of larval survival. Control groups were held at 26 °C for both the exposure and recovery periods, and each treatment was repeated 3 times. Larvae were classified as either alive by showing rapid and coordinated crawling or dead or near death by showing slow, uncoordinated movement and no response to stimulation with a fine paintbrush (Lu et al. 2012).

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Seasonal Changes in Cold Tolerance

Larvae were collected on 16 Oct 2012, 30 Oct 2012, 14 Nov 2012, 30 Nov 2012, 14 Dec 2012, 6 Jan 2013, 18 Jan 2013, 30 Jan 2013, 20 Feb 2013, 9 Mar 2013, and 2 Apr 2013. Larvae within rice straw and stubble were placed outdoors where temperatures were similar to the sampling site. Within 12 h of field collection, the larvae were subjected to testing, and these larvae were stripped out of the straws just prior to testing. The temperatures used in this experiment were chosen using the $LTemp_{50}$ and $LTemp_{90}$ values determined above. Experimental groups of 20 fifth instars on each collection date were used, and treatment and survival assessment were as described above (Lu et al. 2012). The daily maximum and minimum temperatures from Oct 2012 to Apr 2013 of the sampling site were obtained from the Yangzhou Bureau of Meteorology. Less than 10 km separated the recording station and the sampling site.

Seasonal Changes in Supercooling Points

Larvae within rice straw and stubble were placed outdoors where temperatures were similar to the sampling site. Within 12 h of field collection, the larvae were subjected to testing, and these larvae were stripped out of the straws just prior to testing. Experimental larvae (n = 20, 0.3 ± 0.05 g body weight, all fifth instars) were collected from the field on the same days as those used for determination of seasonal changes in cold tolerance experiment. SCPs were measured by the thermistor method described by Lv et al. (2010), in which a single larva was held in close contact with a thermistor in a freezer (-40 °C, ramp rate of -2 °C/min). The resulting resistance value was measured by multimeter (Fig. 1). Since the temperature coefficient of resistance (TCR., the relative change of resistance when the temperature changes 1 °C) of the thermistor is negative, the resistance value increased as larval temperature decreased. We recorded the resistance value in the moment of its reduction, and used it to calculate the SCP temperature (*SCP*) by solving equation (1).

$$SCP = t'B/[B + t'ln(R_i/r')] - 273.15 \quad (1)$$

where t' is the maximum absolute temperature in the known temperature range, r' is the resistance value at t', R_i is the resistance value at the SCP, and B is a temperature coefficient provided by the thermistor manufacturer.

Seasonal Changes in Water Content

Larvae within rice straw and stubble were placed outdoors where temperatures were similar to the sampling site. Within 12 h of field collection, the larvae were subjected to testing, and these larvae were stripped out of the straws just prior to testing. Experimental larvae (n = 20, 0.3 ± 0.05 g body weight, all fifth instars) were collected from the field on the same days as those used to determine the seasonal changes in cold tolerance. The fresh weights (*FW*) of individual larval specimens were measured on an electronic scale (sensitivity 0.1 mg); the dry weights (*DW*) were obtained after drying the specimens for 24 h at 60 °C in a ventilated drying cabinet; water contents (*WC*) were then calculated by solving equation (2) (Košťál & Šimek 2000; Qiang et al. 2008).

$$WC = (FW - DW)/FW \times 100\% \quad (2)$$

Seasonal Changes in Low Molecular Weight Sugars and Polyols

Low molecular weight cryoprotectants in the experimental larvae were measured by capillary

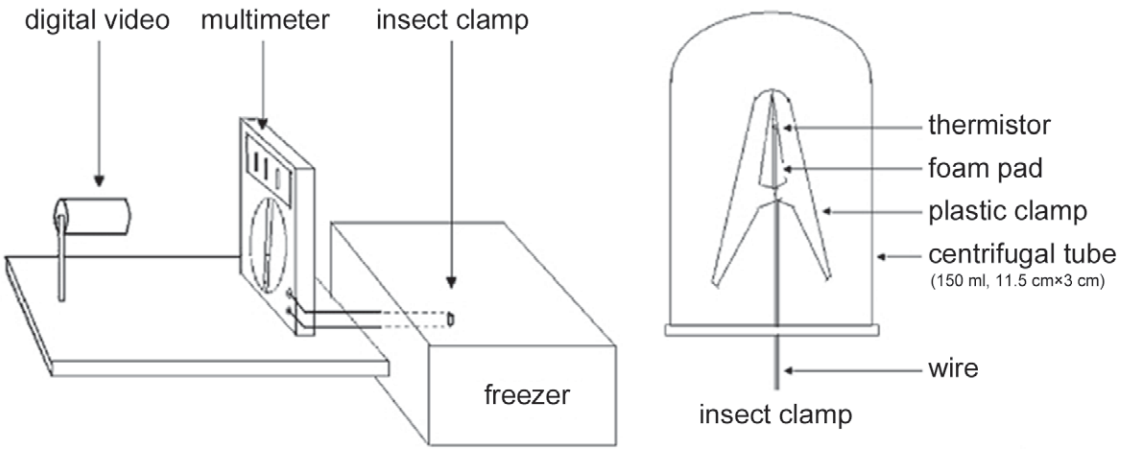


Fig. 1. Diagram of equipment used for measuring supercooling points (SCPs). Based on Lv et al. (2010).

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gas chromatography as their *o*-methyloxime trimethylsilyl derivatives (Wang et al. 2010). Single larva was weighed (fresh weight) and homogenized with 1 mL of 70% (v/v, diluted with ddH_2O) ethanol containing 25 mg dulcitol (an internal standard) in an Eppendorf tube that had been rinsed with 0.2 mL of 70% ethanol. After centrifugation at 10,000 × *g* for 5 min, the supernatant was removed and the process repeated 4 more times. One milliliter of these pooled supernatants was then evaporated until dry under a stream of nitrogen at 40 °C. Fifty microliters of dimethylformamide and 50 µL of pyridine (containing *o*-methylhydroxylamine at 200 mg/mL) were added to the residue for oximation, then heated to 70 °C for 30 min. Silylation was accomplished by adding 150 µL of dimethylformamide and 60 µL of trimethylsilylimidazol to the reaction mixture, which was further heated to 80 °C for 30 min. After re-extraction of the desired derivatives into isooctane using 2 × 75 mL of the solvent, a 1-µL aliquot was injected into the injection port of a gas chromatograph (7890A, Agilent, USA). Separation and quantification of sugars and polyols were achieved on a 25 m × 0.25 mm i.d. BP-5 silica capillary column. The temperature program was as follows: held at the initial temperature of 120 °C for 3 min, then increased at 12 °C/min to 240 °C and held at 240 °C for 2 min, then increased at 20 °C/min to 280 °C and held at 280 °C for 25 min. Identity of the revealed components was established against authentic standards (glycerol, trehalose, fructose, glucose and *myo*-inositol). Larvae within rice straw and stubble were placed outdoors where temperatures were similar to the sampling site. Within 12 h of field collection, the larvae were subjected to testing, and these larvae were stripped out of the straws just prior to testing. Experimental larvae (0.3 ± 0.05 g body weight, all fifth instars) were collected from the field on the same days as those used to determine the seasonal changes in cold tolerance. Eight larvae were measured for each collection date.

Statistical Analysis

Nonlinear regression was used to estimate $LTemp_{50}$ and $LTemp_{90}$ values. Homogeneity of variances among different groups was evaluated by Levene's test. If a dataset had failed Levene's test, the data were arcsine square root transformed before multiple comparisons and then subjected to ANOVA. Significant differences between treatments was identified by Tukey's test for multiple comparisons. Treatment differences were considered significant at $P < 0.05$. Values were reported as means ± SE. Data were analyzed using MATLAB 7.0 (MathWorks, America) and PASW Statistics 18.0 (IBM, America).

Results

Determination of Lethal Temperatures, i.e., $LTemp_{50}$ and $LTemp_{90}$

Larvae of *S. inferens* collected in Oct 2012 were unable to tolerate temperatures below -12 and -10 °C, at which 100% and 90.0 ± 2.9% of larvae died, respectively. But at -4 °C, larvae had a mortality of only 13.3 ± 1.7%, and at -2 °C all larvae survived. Mortalities at -9, -7 and -6 °C were 81.67 ± 4.41%, 70.00 ± 2.89%, and 46.67 ± 1.67%, respectively. Mortalities at -7, -6, -4, and -2 °C was reduced significantly ($F = 251.356$; df = 6, 14; $P < 0.001$). The relation between temperature (T) and mortality (M) was fitted to a curve (3) ($RSS = 0.0418$, $R^2 = 0.946$, $T = [-12, -2]$) (Fig. 2),

$$M = 0.932e^{-0.0354(T+12)-0.0004(T+12)^{-4}} \quad (3)$$

The $LTemp_{50}$ and $LTemp_{90}$ values were calculated as -6.12 and -9.77 °C, respectively.

Seasonal Changes in Cold Tolerance

Maximum cold tolerance (2 h exposure at -6 °C) was exhibited by larvae collected on 30 Jan 2013 with only 10.0 ± 2.9% mortality. However, for larvae collected on 30 Oct the mortality was 54.4 ± 13.7%, which was the maximum mortality. We found that mortality did not vary significantly from 16 Oct 2012 to 14 Dec 2012, but it decreased significantly from 14 Dec 2012 (45.0 ± 2.9%) to 6 Jan 2013 (11.7 ± 11.7%). After decreasing, mortality again varied insignificantly from 06 Jan 2013 to 02 Apr 2013 ($F = 6.55$; df = 10, 22; $P < 0.001$) (Fig. 3). In summary, mortality decreased with decreasing ambient temperature (Figs. 3 and 4).

Larvae were unable to tolerate temperatures of -10 °C for 2 h. No significant differences in mortality were detected between larvae (2 h exposure at -10 °C) collected on each collection date ($F = 1.15$; df = 10, 25; $P = 0.365$). Mean mortality was 92.1 ± 2.0% (Fig. 3).

Seasonal Changes in Supercooling Points

No significant differences in the SCPs of *S. inferens* 5th instars collected on different dates were detected ($F = 0.526$; df = 10, 209; $P = 0.871$), with a mean of -6.80 °C. (Fig. 5).

Seasonal Changes in Water Content

Before 9 Mar 2013, water content of larvae remained at a stable and low level, with a mean content of 63.5% and no significant differences. But after this period, water content increased significantly to 76.5 ± 0.8% on 9 Mar 2013 and 76.0 ± 0.5% on 2 Apr 2013 ($F = 37.03$; df = 10, 215; $P < 0.001$) (Fig. 6).

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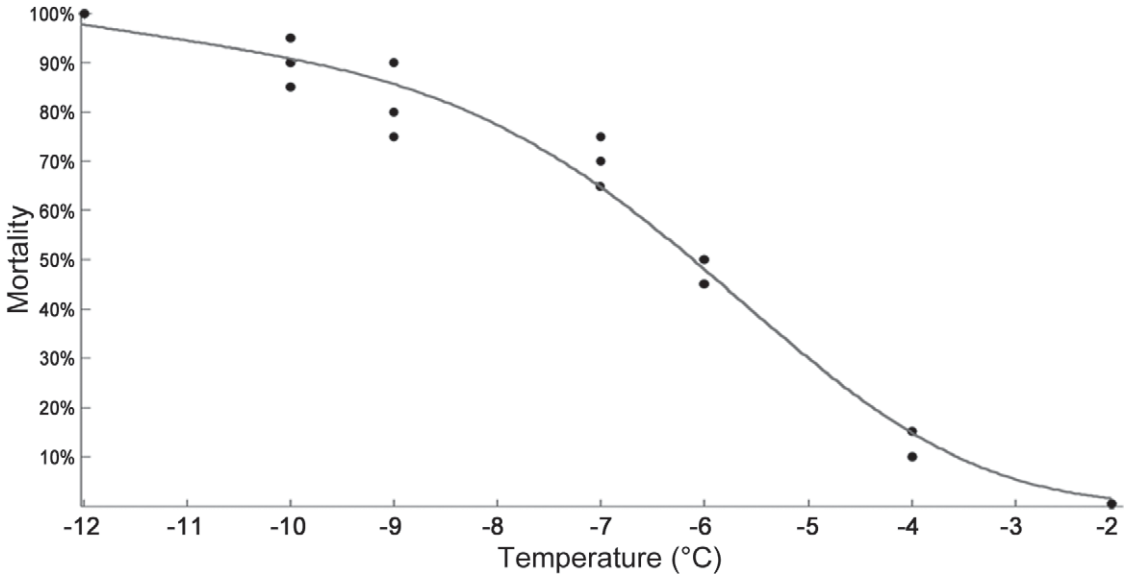


Fig. 2. Percent mortality of *Sesamia inferens* after a 2 h exposure to -12, -10, -9, -7, -6 -4, and -2 °C.

Seasonal Changes in Low Molecular Weight Sugars and Polyols

ANOVA revealed that low molecular weight molecular sugars and polyols accumulated as the ambient temperature declined with the progression of the season toward its coldest period, and subsequently the levels of low molecular weight sugars and polyols declined as the ambient temperatures rose (Figs. 4 and 7).

Glycerol content of larvae increased from 142.26 ± 24.78 µg/g on 16 Oct 2012 to a peak of 324.98 ± 73.63 µg/g on 18 Jan 2013 and 359.79 ± 156.90 µg/g on 30 Jan 2013. It then decreased to 204.47 ± 50.29 µg/g on 2 Apr 2013 (F = 2.05; df = 10, 77; P < 0.05) (Fig. 7: A). Trehalose was much

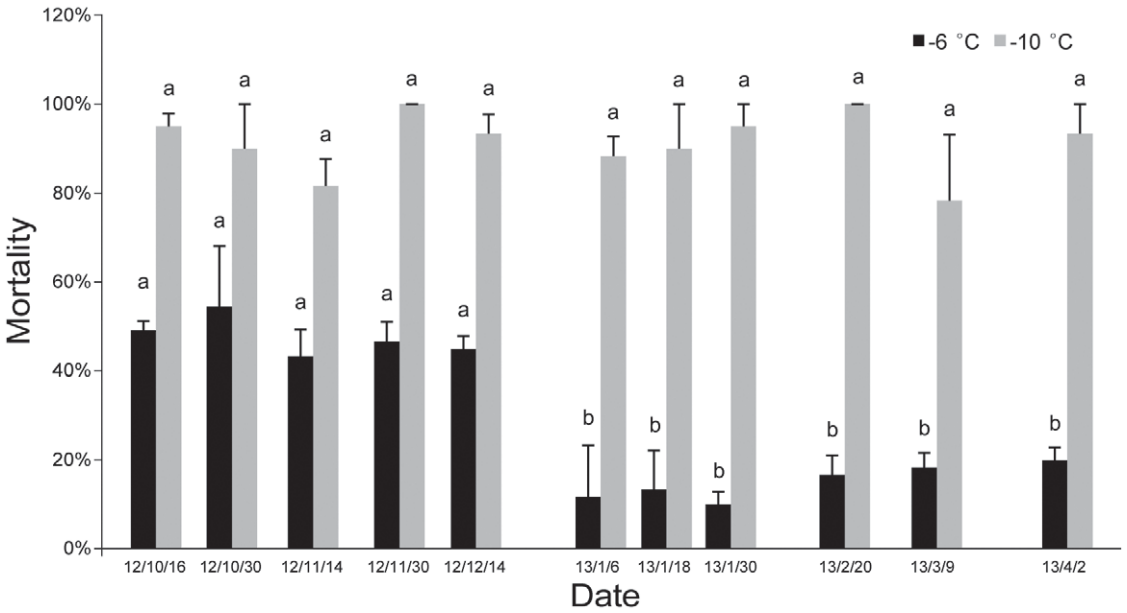


Fig. 3. Percent mortality (mean ± SE) of *Sesamia inferens* collected on different dates and then exposed to -6 or -10 °C for 2 h. The same letter above 2 or more bars indicates no statistical difference (P = 0.05).

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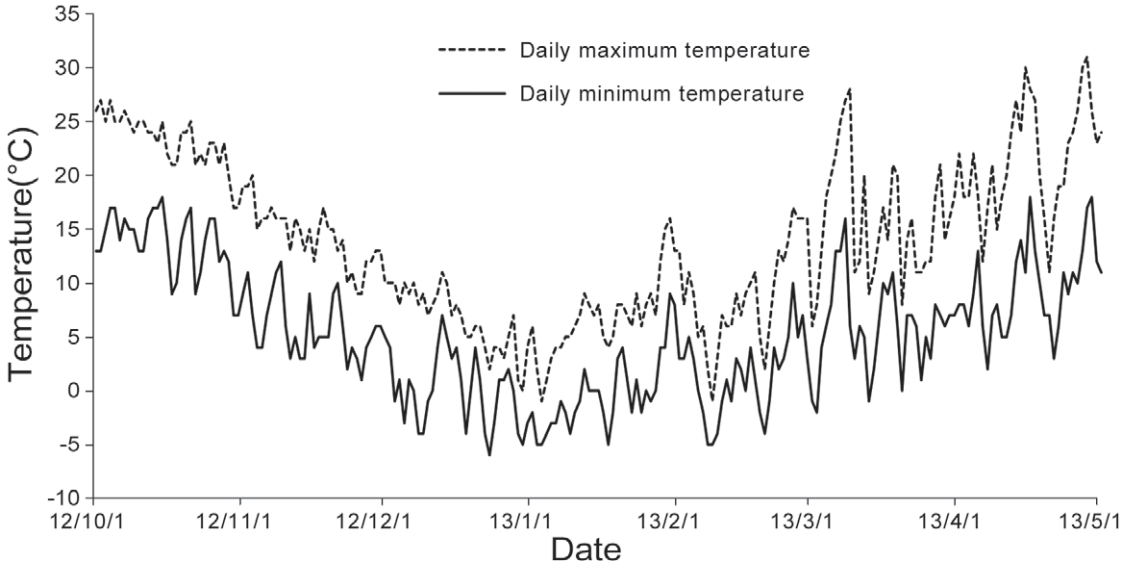


Fig. 4. Daily maximum and minimum temperatures obtained from the Yangzhou Bureau of Meteorology weather station from Oct 2012 to Apr 2013.

more abundant than glycerol in the *S. inferens* larvae. Trehalose content increased continuously from 3.27 ± 0.56 mg/g on 16 Oct 2012 to a peak of 20.46 ± 2.46 mg/g on 18 Jan 2013, and subsequently declined gradually from 17.15 ± 1.78 mg/g on 30 Jan 2013 to 7.70 ± 0.95 mg/g on 2 Apr 2013 (F = 12.50; df = 10, 77; P < 0.001) (Fig. 7: B).

While the other sugars and polyols had less obvious trends than trehalose, the variation patterns of fructose (maximum value of 69.78 ± 18.68 µg/g, F = 6.20; df = 10, 76; P < 0.001) (Fig. 7: C), glucose (maximum value of 377.25 ± 135.90 µg/g, F = 3.03; df = 10, 76; P < 0.05) (Fig. 7: D) and *myo*-inositol (maximum value of 59.55 ± 21.95

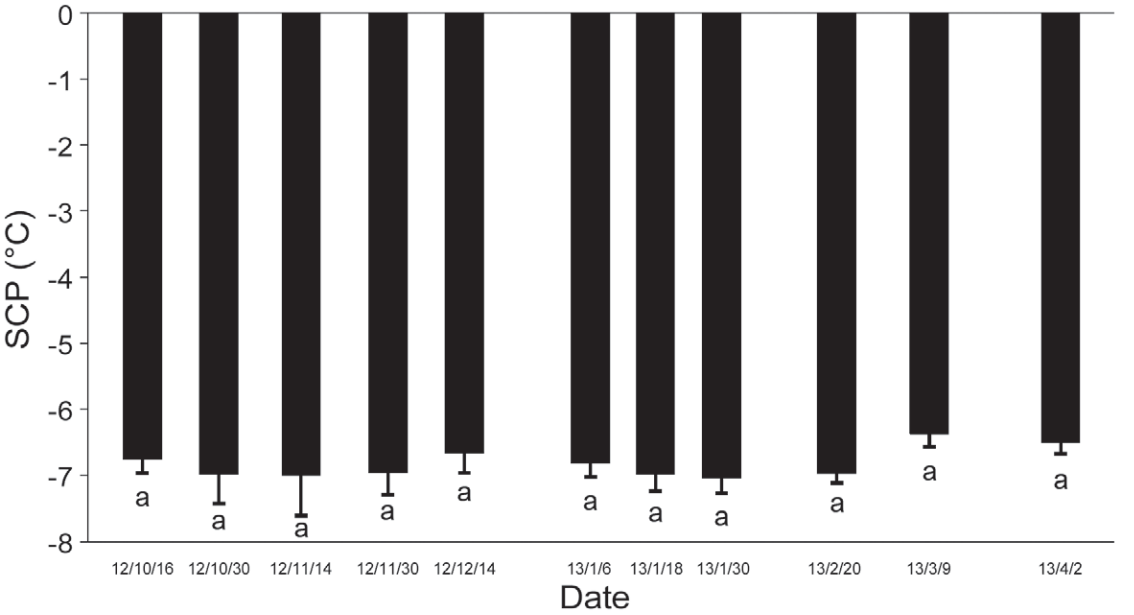


Fig. 5. Soopercooling points (SCPs; mean ± SE) of *Sesamia inferens* 5th instars collected on different dates (year-month-day format) between consecutive rice growing seasons (16 Oct 2012 to 2 Apr 2013). The same letter above 2 or more bars indicates no statistical difference (P = 0.05).

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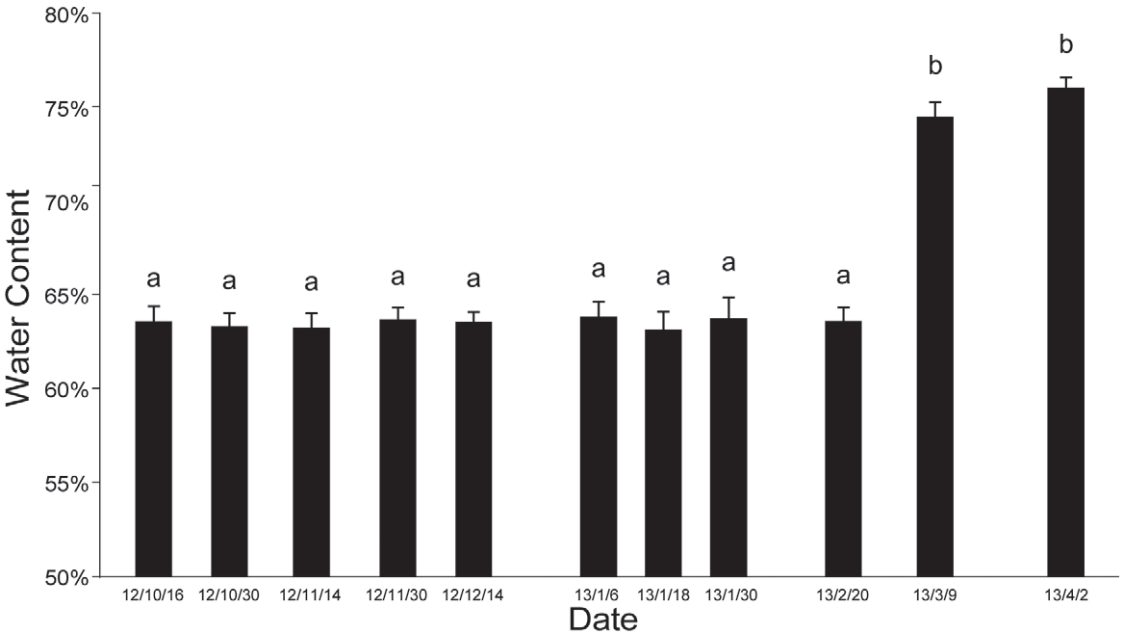


Fig. 6. Percent water content (mean ± SE) of *Sesamia inferens* 5th instars collected on different dates (year-month-day format) between consecutive rice growing seasons (16 Oct 2012 to 2 Apr 2013). The same letter above 2 or more bars indicates no statistical difference ($P = 0.05$).

µg/g, $F = 3.81$; df = 10, 76; $P < 0.001$) (Fig. 7: E) were similar to those of glycerol and trehalose. All sugar and polyol contents were expressed per fresh weight.

Discussion

Since 1990, *S. inferens* populations have been gradually increasing (Fu & Huang 2005). Moreover, with global warming, this pest has expanded to northern regions where it was not present several years ago.

In recent years the Asiatic rice borer, *Chilo suppressalis* (Walker) (Lepidoptera: Pyralidae), another major rice pest in the rice planting area of China, which is biologically similar to *S. inferens*, has been found in rice fields of Yangzhou Prefecture. Lu et al. (2012) studied the seasonal cold tolerance of the Yangzhou *C. suppressalis* population, and found that the cold hardiness of *C. suppressalis* was very low from Aug to Nov, then progressively increased to peak in Jan (the coldest month in Yangzhou). It then declined gradually to its previous level from Apr to May (during which period *C. suppressalis* emerge).

We found that the profile of *S. inferens* and *C. suppressalis* cold tolerance mechanisms differ in that seasonal cold tolerance of *S. inferens* did not vary as dramatically as that of *C. suppressalis*. However, the cold hardiness of *S. inferens* and *C. suppressalis* increased similarly in Jan. In addition, the cold hardiness of *S. inferens* was lower than that of *C. suppressalis*, as the mortality of *C. suppressalis* after a 2 h exposure to -18 °C was only 1% in Jan, while the mortality of *S. inferens* after a 2 h exposure to -10 °C was 78% and higher, even in Jan. This difference could explain why *C. suppressalis* can be found much farther north than *S. inferens*.

For overwintering insects, SCP is an important index of cold hardiness, and the variation of SCP values with the progression of the season is the standard for distinguishing the cold tolerance strategy of insects (freeze-avoidance vs. freeze-tolerance) (Sinclair et al. 2003). Our research in this paper showed only insignificant variation in the SCPs of overwintering *S. inferens* larvae in Yangzhou, with a mean SCP of -6.80 °C in the winter. This phenomenon was not the same for *C. suppressalis*. According to Qiang et al. (2008), the SCP of overwintering *C. suppressalis* in Yangzhou first decreased, then increased, and the variation was significant. These authors reported that *C. suppressalis* in Yangzhou was a freeze-avoiding insect. In contrast, our research suggests that *S. inferens* in Yangzhou is a freeze-tolerant insect.

Chilo suppressalis is divided into at least 2 ecotypes in Japan, i.e., the Shonai ecotype which is distributed in the northern part of Japan, and the Saigoku ecotype which is distributed in the southwestern region (Ishiguro et al. 2007). According to Ishiguro et al. (2007) obvious differences in the progress of glycerol accumulation

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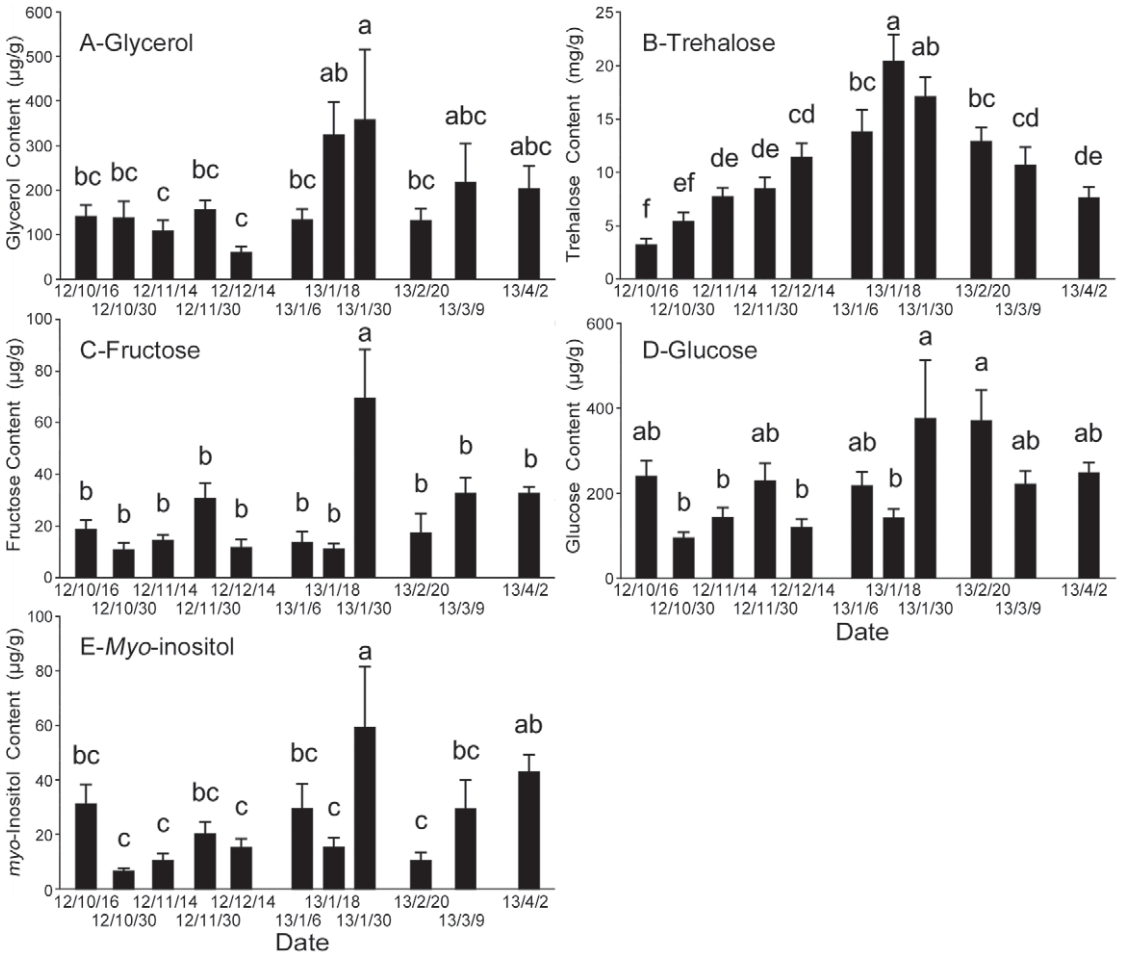


Fig. 7. Low molecular weight sugar and polyol contents (mean ± SE) of *Sesamia inferens* 5th instars collected on different dates (year-month-day format) between consecutive rice growing seasons (16 Oct 2012 to 2 Apr 2013). Low molecular weight sugars and polyols include glycerol (A), trehalose (B), fructose (C), glucose (D), and *myo*-inositol (E). Sugar and polyol contents are based on fresh weight. The same letter above 2 or more bars indicates no statistical difference ($P = 0.05$).

and cold hardiness development between these 2 ecotypes were found. Possibly the overwintering strategies of these 2 ecotypes are different. In other words, *C. suppressalis* may have more than one overwintering strategy. *Sesamia inference* is very widely distributed. We suspect *S. inference* may be divided into several ecotypes in China, and different ecotypes may have different overwintering strategies. This possibility should be investigated. We concluded from our research, that the Yangzhou *S. inference* population is exclusively freeze-tolerant.

According to our research, the content of body water of overwintering larvae is lower than the larvae in the spring, leading us to speculate that the water content of overwintering *S. inferens* has already been reduced before mid-Oct. Both *S. inferens* and *C. suppressalis* in Yangzhou go through a period of body water reduction, but this reduction in water content took place earlier in *S. inferens* than in *C. suppressalis* (Qiang et al. 2008). The water content of *C. suppressalis* declined significantly during the period of 15 Oct to 15 Jan (Qiang et al. 2008). The threat of freezing is overcome if water has been removed from the body (Clark & Worland 2008), and ice formation is usually restricted to extracellular compartments, with dehydration ensuring that water does not freeze within the cells (Ramløv 2000). The water content of *S. inferens* in Yangzhou increased significantly in Mar, which also occurred in *C. suppressalis* in Yangzhou (Qiang et al. 2008).

Storey & Storey (1991) reported that the accumulations of low molecular weight sugars and polyols were associated with cold hardiness in many insects during overwintering. Sugars and

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polyols can alleviate osmotic stress during freezing, colligatively decreasing the probability of water crystallization in the unfrozen cell contents and altering the melting point. However, their function in cold tolerance of insects remains debatable because not all insects accumulate high concentration of low molecular weight sugars and polyols during overwintering (Lee et al. 1987; Meier & Zettel 1997). This study had shown that trehalose and *myo*-inositol exhibited the maximal and minimal concentrations in *S. inferens* larvae, respectively. For insects, trehalose, the main component of blood glucose, had been clearly understood as cryoprotectants (Tanaka & Udagawa 1993; Košťál & Šimek 1995). In the case of dehydration caused by heat shocking or freezing, trehalose could effectively protect and stabilize the cell membranes and protein structure (Williams 1990; Tomos 1992; Li et al. 2000; Wang et al. 2010). *Myo*-inositol contents was very low in arthropods. This was same as in *S. inferens* larvae. *Myo*-inositol was accumulated in *Ceratomegilla undecimnotata* (Schneider), *Achaearanea tepidariorum* (Koch), and *Aulacophora nigripennis* Motschulsky (Tanaka 1995; Košťál et al. 1996; Watanabe & Tanaka 1998) and it may have a role in stabilizing proteins and cell membranes (Košťál & Šimek 1995). Low molecular weight sugars and polyols of *Pyrrhocoris apterus* L. could be induced when insects were exposed to -5 °C (Košťál et al. 2001). The lowest temperature of -5 °C was often measured in Jan in Yangzhou (Fig. 4), and the peaks of overwintering *S. inferens* larvae low molecular weight sugars and polyols were also exhibited in Jan.

Systematic analyses of cold tolerant characteristics of overwintering *S. inferens* larvae are expected to provide important insights into its population outbreak mechanism. We expect that further investigations of the pests overwintering strategy will be a key factor in the integrated management of *S. inferens*.

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