Physiological Entomology (2007) **32**, 219–224 DOI: 10.1111/j.1365-3032.2007.00564.x

Influence of soil moisture on egg cold hardiness in the migratory locust *Locusta migratoria* (Orthoptera: Acridiidae)

XIAN-LEI QI[1,2]*, XIAN-HUI WANG[1]*, HONG-FU XU[2] and LE KANG[1]

[1]State Key Laboratory of Integrated Management of Pest Insects and Rodents, Institute of Zoology, Chinese Academy of Sciences, Beijing, China and [2]College of Plant Protection, Shandong Agricultural University, Tai-an, China

Abstract. The present study investigates the influence of environmental moisture on cold hardiness of the migratory locust, *Locusta migratoria*. The water content of locust eggs kept in soil at 30 °C varies according to the moisture content of the substrate. In turn, it can significantly affect the supercooling point of locust eggs (range from –26 to –14.8 °C) and the mortality when exposed to subzero temperatures. Environmental moisture influences the supercooling capacity of eggs and their survival at low temperature. When locust eggs of the same water content are exposed to subzero temperatures under different soil moistures, their mortality varies between short-time exposure and long-time exposure at subzero temperatures. Given a short-time exposure, mortality in wet soil is lower than in dry soil due to the buffering effect of soil water against temperature change. The pattern of egg mortality is reversed after long-time exposure at low temperature, suggesting that inoculative freezing may be an important mortality factor. It is suggested that interactions between soil moisture and low temperature can influence the cold hardiness of locust eggs, and partial dehydration is beneficial to over-wintering eggs of the migratory locust.

Key words. Cold hardiness, *Locusta migratoria*, supercooling point, soil moisture.

Introduction

Outbreaks of the migratory locust, *Locusta migratoria* (L.), which is an important agricultural pest in parts of the world, are related to climatic events, such as drought (Ma, 1958), El Niño (Zhao, 1989; Zhang & Li, 1999) and sunspot activities (Uvarov, 1966). Ma (1962) suggested that, according to historical records, locust outbreaks occur when wet grassland on large river deltas dries up as a result of prolonged drought and the survival of locust eggs laid in the soil increases. El Nino also affects locust outbreaks by decreasing precipitation and increasing winter temperature in northern China (Zhang & Li, 1999). Although, climatic variation can influence outbreaks of the migratory locust, the mechanisms underlying such effects are unknown.

Correspondence: Dr Le Kang, Institute of Zoology, Chinese Academy of Sciences, 25 BeiXisihuan Road, Haidian, Beijing, China, 100080. Tel.: +86 10 62554979; fax: +86 10 62565689; e-mail: lkang@ioz.ac.cn

*These authors contributed equally to this work.

Cold and drought are regarded as two potential evils for over-wintering insects (Block, 1996) and play an important role in population dynamics (Bale *et al.*, 2002). Cold hardiness of insects is influenced by their body water content and its activity at low temperatures that affects cells, tissues and the function of the whole organism (Lee & Denlinger, 1991; Block, 2003). In the majority of insects, total body water content varies between 40–90% of fresh weight (Hadley, 1994). Many studies show that increased solute concentration, pH changes and physical shrinkage, which occur as water leaves cells during freezing, may injure the cells, especially by membrane damage (Meryman, 1974; Steponkus *et al.*, 1993). The reduction in insect water content helps to enhance supercooling capacity and may prevent additional water from freezing onto extracelluar ice once freezing has been initiated (Ring & Danks, 1994; Costanzo *et al.*, 1997). Comparative data for 15 species of adult mites from temperate and Antarctic coastal habitats demonstrate a general depression of the supercooling point (SCP) with a reduction in both total live weight and body water content (Pugh, 1994). In the Antarctic springtail, *Cryptopygus antarcticus*, there is also a good correspondence between body water and supercooling ability, although other factors (gut contents and cryoprotectants) may have influenced their cyclicity independently (Block, 1996).

In winter, environmental moisture is a crucial factor influencing the survival of insects that over-winter in terrestrial habitation (Danks, 1991). Environmental moisture can promote the formation of internal ice by inoculation and influence organismal water balance (Danks, 2000). Over-wintering larvae of the goldenrod gall fly, *Eurosta solidaginis*, freeze by inoculation in wet galls, but they supercool in dry galls (Layne, 1991). Because the vapour pressure of ice is less than that of water, insects in soil may lose water to their environment at subzero temperatures with the presence of ice, leading to desiccation (Lundheim & Zachariassen, 1993; Worland & Block, 2003). Dehydration apparently takes place in this way for the supercooled larvae of the beetle *Pterostichus brevicornis*, which loses water from approximately 70–30% of fresh weight (Miller, 1978). Otherwise, water in the soil moderates the thermal environment of burrowing insects by buffering against rapid changes and extremes of air temperature owing to the high specific heat of water (Costanzo *et al.*, 1998).

In northern China, eggs of the migratory locust over-winter in the soil for 6 months from autumn to the next spring. The cold hardiness of locust eggs appears to have both geographical and seasonal variations (Jing & Kang, 2003b, 2004). Such variations have a complex genetical basis (Wang & Kang, 2005a). The first-instar nymphs have a rapid cold hardening response (Wang & Kang, 2003). Photoperiod, thermoperiod and high-temperature interruption can affect the cold hardiness of locust eggs (Jing & Kang, 2003a; Jing *et al.*, 2005; Wang *et al.*, 2006). Indeed, the minimum winter temperature experienced by locust eggs is only –10 to –18 °C (Ma, 1958), which is substantially higher than the mean SCP value of –22 °C reported for locust eggs (Jing & Kang, 2003b). Although the risk of freezing may appear negligible, winter mortality is typically high (e.g. 50%; Ma, 1958). Apparently, other environmental factors could be involved in mortality of locust eggs above their SCP.

The role of the hydric microenvironment on the susceptibility to inoculative freezing in insects has received little study (Gehrken *et al.*, 1991). Climate changes will alter the balance of water and thermal capacity of the soil, which impact on the over-wintering survival of locust eggs. Therefore, in the present study, a series of controlled experiments is designed to investigate the cold hardiness of locust eggs in response to different combinations of soil moisture and temperature.

Materials and methods

Insects

Egg pods of the migratory locust, *L. migratoria*, were collected in April 2003 from Huanghua County (38.25°N; 117.20°E), Hebei Province, China. The egg pods were incubated in wet sand in an environmental chamber at 30 ± 0.5 °C. The water content of the sand was maintained at 15% of gross mass, which is an optimal condition for the eggs. After hatching, 50 first-instar nymphs were reared in a two-layer floor box (50 × 70 × 80 cm) with corn leaves and wheat bran at 30 °C (LD 14 **:** 10 h photoperiod, 80% RH). Wet sand with 15% water content was used as the medium of oviposition; and newly-laid eggs were collected daily.

Experimental substrates

The experimental substratum was collected from a native habitat, at the same location, where nesting soil was a well-drained and loamy (sand, 48%; silt, 1%; clay; 51%). The soil was passed through a 2-mm^2 sieve and oven-dried at 180 °C for 12 h. The soil was rehydrated with different water contents shortly before the experiments. The soil water content was maintained at 7, 11, 15, 19 or 23% of gross mass. The eggs can develop successfully in such a range of water contents (Guo *et al.*, 1989).

Effects of substrate moisture on water balance and supercooling capacity

Thirty newly-laid eggs were placed individually into plastic cups (inner diameter 7.5 cm, height 8.5 cm) containing 200 g of soil of different water contents. The cups were sealed with parafilm. The eggs were incubated at 30 °C for about 7 days. The SCPs of individual eggs were determined after fresh body mass was measured. For SCP determination, each egg was fixed to a thermocouple with a narrow piece of plastic tape, which was linked to an automatic recorder (uR100, Model 4152, Yologawa Electric Co., Korea) via a bridge. The thermocouple with the egg protected by a plastic tube was lowered into a freezing chamber held at –35 °C. Cold exposure started at room temperature, resulting in a nonlinear cooling rate of approximately 1 °C min^{-1} from 0 to –35 °C. The SCP was indicated on the recorder by a sudden rise in the egg temperature. Details of the methods used are described in Zhao & Kang (2000) and Chen & Kang (2002). After measurement of the SCP, the dry weight of eggs was determined from its gross weight after drying at 65 °C for 12 h in an air oven. The water content of the egg was then calculated.

Effects of the egg water content on viability

Newly-laid eggs were separately reared in the soils of different water contents at 30 °C for approximately 7 days. The eggs were allowed to develop until they were in the early balstokinesis stage to establish different egg body water contents. This experiment employed a factorial design, consisting of 25 treatments. Forty eggs of each treatment were randomly selected and placed in plastic tubes (20 × 50 mm) without soil, and were exposed to –5 °C for 6, 24, 72, 120 or 168 h. The tubes were then removed from the low temperature.

Next, the eggs were removed to plastic cups with 15% water content soil at 30 °C. Hatching eggs at 30 °C were regarded as eggs that had survived. In the control, 40 eggs of each treatment were incubated at 30 °C in soil with 15% water content without low temperature treatment. Each treatment had four replicates.

Mortality of locust eggs in different soil moistures at subzero temperature

Newly-laid eggs were reared in soil with 15% of water content in plastic cups for approximately 7 days. Next, 40 eggs from each group were transferred into cups containing soils of different water content. The eggs were positioned in the cups in such a way that each was isolated from others and the cup wall. Each preparation was acclimated in a chamber held at 5 °C for 24 h. Each cup was then chilled at –5 °C for 6, 24, 72, 120, or 168 h. Then, the eggs were transferred to other cups containing soil with optimal water content (15% of gross mass) at 30 °C. Egg mortality of each group was calculated. In the control, 40 eggs in the cups were acclimated at 5 °C for 24 h, and were not chilled at subzero temperature. Each treatment had four replicates.

Statistical analysis

The survival and corrected mortality of eggs were calculated as described by Zhao & Kang (2000). A general linear model (GLM) analysis of variance (analysis of variance; SPSS, version 10.0; SPSS Inc., Chicago, IL) was used, where appropriate, to test for effects of temperature and exposure time on egg mortality. Where a significant difference was observed, the effect of treatments was determined by the Tukey's honest significant difference test. The amount of time necessary to achieve 50% mortality at a specific temperature (Lt_{50}) was estimated by Probit analysis (SPSS, version 10.0).

Results

Effects of substrate moisture on water balance and supercooling capacity of eggs

The water content of locust eggs (from 75–80% fresh weight) varied significantly when they were maintained in soils of different water content for 7 days ($F = 24.44$, d.f. = 72, $P < 0.001$). The mean water content of eggs in soils with a high moisture content was higher than that of eggs in soils with low moisture content. The mean water content of eggs kept in soils with 7, 11 and 15% water contents did not differ significantly ($P > 0.05$). There were no significant differences in the water content of eggs between 19–23% soil moisture (Fig. 1a). However, there were significant differences in egg water content between the three groups (kept at 7–15%) and the two with 19–23% soil moisture.

The mean SCPs of locust eggs (from –19.5 to –24 °C) kept in different soil water contents were compared. Significant differences of mean SCPs of locust eggs appeared among various treatments ($F = 9.40$, d.f. = 4,78, $P < 0.001$) (Fig. 1b). The correlation between individual SCPs (from –14.8 to –26 °C, $n = 68$) of eggs and reduction in their body water content was analysed by pooling all data from all treatments together. A strong correlation was found ($P = 0.00035$)(Fig. 2).

Effects of the water content on egg viability

Because the soil moisture content influenced the water balance of locust eggs, the effects of water balance changes induced by substrate moisture on egg viability at subzero temperature were investigated. Generally, cold hardiness of the locust eggs was inversely related to soil moisture in which the locust eggs were incubated for approximately 7 days at 30 °C (Fig. 3). Mortality of locust eggs was strongly time dependent for various soil moistures at low temperature. The times of mortality causing 50% mortality of eggs at –5 °C in soil with 7, 11, 15, 19 and 23% water contents were 156, 132, 126, 111 and 70 h, respectively. Notably, the mortality of eggs exposed to –5 °C for 168 h in soil with 23%

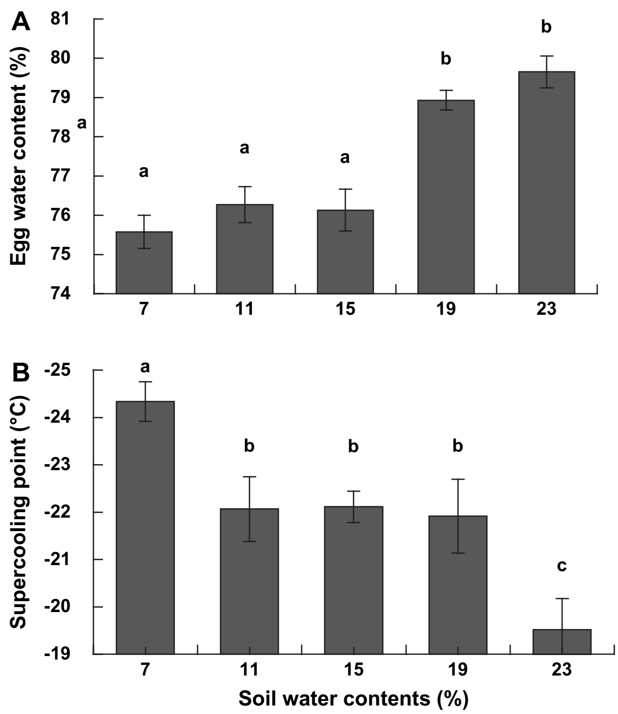

Fig. 1. Changes in (a) water content (% ± SE) and (b) mean supercooling point (± SE) of migratory locust eggs (*Locusta migratoria* L.) in soils of different water content. The eggs were all incubated at 30 °C for 7 days. Data with different letters above their corresponding bars are significantly different (Tukey's honest significant difference, $P < 0.05$).

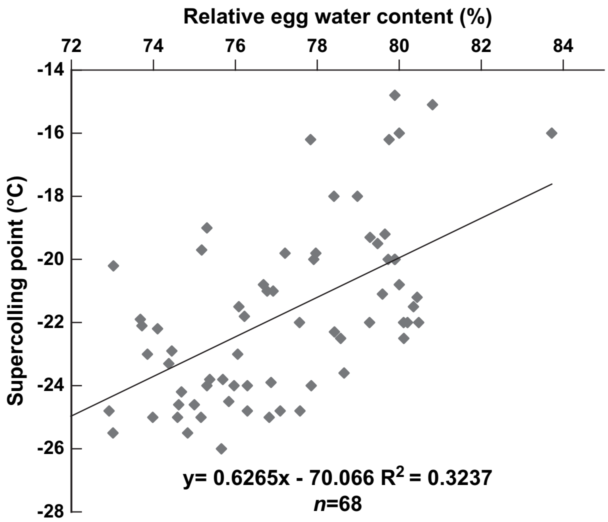

Fig. 2. Correlation between the water content of individual eggs (% fresh weight) and supercooling points of eggs. The eggs were incubated in soils of different water content at 30 °C for 7 days.

water content was approximately two-fold higher than that in soil with 7% water content.

Effects of soil moisture under cold exposure on egg viability

The mortality of the eggs maintained at the same soil moisture content varied greatly at low temperatures in soils with different water contents (Fig. 4). The egg mortality divided into two patterns. When chilled for short-time exposure (6–24 h) at –5 °C, the mortality of eggs in dry soils (7, 11 and 15%) was higher than that in wet soils (19 and 23%). By contrast, the mortality of the eggs in dry soils was lower

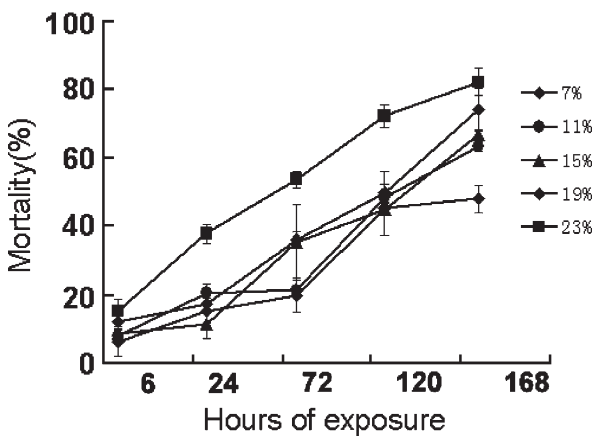

Fig. 3. Mortality (mean percentage ± SE) of migratory locust eggs chilled at –5 °C for 6–168 h without soil. The eggs were incubated in the soils of different water content ($P < 0.01$) for 7 days at 30 °C before cold exposure.

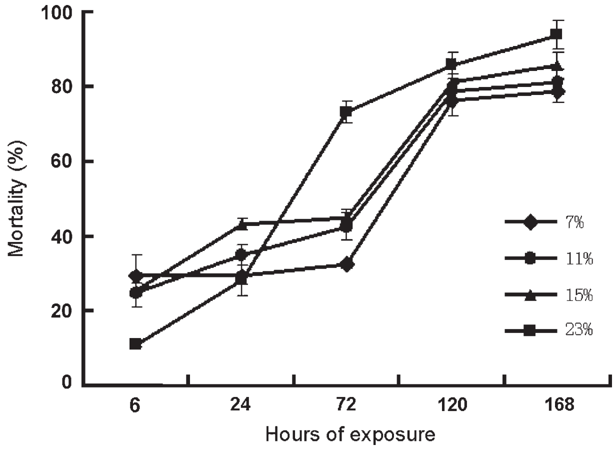

Fig. 4. Mortality (mean percentage ± SE) of migratory locust eggs chilled at –5 °C for 6–168 h in soils of different water content. The eggs were incubated in soil with 15% water content for 7 days at 30 °C before different cold treatments.

than that in wet soils after long-period exposure (72–168 h) at –5 °C. For example, the mortality of eggs chilled at –5 °C for 6 h decreased when the soil water content increased. However, after exposure for 72 h, changes in egg mortality were converse with the soil moisture.

The times needed for soil with different water contents to freeze at –10 °C and to reach –10 °C were determined. The wetter soil needed a longer time to freeze than drier soil (Table 1).

Discussion

The migratory locust is found in moist and low-lying habitats (e.g. lakeshores, river flood plains, coastal areas and inland flood plains) (Uvarov, 1977). In these habitats, frequent change of water level is a key factor that affects population abundance of the migratory locust (Ma, 1962). Soil moisture plays an important role in the life cycle of the migratory locust, and there are significant geographical and seasonal variations in the cold hardiness of locust eggs (Jing & Kang, 2003b, 2004), indicating that an ability to withstand cold in the egg stage is crucial to the distribution and abundance of the migratory locust. Environmental factors, such as photoperiod (Jing & Kang, 2003a), thermoperiod (Wang *et al.*, 2006) and acclimation to low temperature (Jing & Kang, 2003b; Wang & Kang, 2005a), have significant effects on cold hardiness of the eggs. In many invertebrates, soil moisture can have a strong influ-

Table 1. Time needed by soil with different water contents to freeze at –10 °C, and the time needed to reach –10 °C.

Soil water content (% gross weight)	7	11	15	19	23
Time needed to freeze (h)	0.85	1.35	1.6	1.98	2.5
Time needed to reach –10 °C (h)	1.6	2	2.6	2.94	3.5

ence on cold hardiness (Costanzo *et al.*, 1997, 2001). For over-wintering eggs of the locust, field studies reveal that soil moisture content affects egg survival directly or indirectly; for example, anoxic suffocation and infection of parasitic fungus in high moisture conditions, or causing the termination of development of the embryo in drought conditions (Ma, 1958). The present study demonstrates that there are interactions between the effects of soil moisture and low temperature on over-wintering eggs of the migratory locust even within the range of suitable soil moisture (7–23%).

Soil moisture influences the water content of locust eggs, with eggs exposed to wet soil having significantly more water than those in dry soil, which is similar to the results obtained by Guo *et al.* (1989). Liquid water is essential for locust egg development (Qing *et al.*, 1958), and the optimum soil moisture content for oviposition lies within the range 10–20%. When soil moisture is lower than 5% or higher than 25%, oviposition is greatly reduced (Yu *et al.*, 1958).

The hydration state of locust eggs influences their supercooling capacity. Notably, the mean supercooling point of eggs maintained in soil with a low water content is significantly lower than that of eggs exposed to soil with high water content. The reason why the hydration state influences supercooling capacity in insects may relate to changes in solution concentration of body fluids (Worland & Block, 2003). Dehydration can strongly affect the metabolism of many terrestrial invertebrates; for example, cryoprotectants increase (Hadley, 1994; Block, 1996). These cryoprotectants, such as glucose and trehalose, may have diverse roles in providing energy storage and cryoprotection (Bayley & Holmstrup, 1999). Thus, some invertebrates exhibit partial dehydration, in preparation for winter (Ring & Danks, 1994). In locust eggs, cold acclimation significantly increases the concentration of cryoprotectants (myo-inositol, trehalose, mannitol, glycerol, and sorbitol), indicating that these components play important roles in their cold tolerance (Wang & Kang, 2005b). However, there is no direct evidence that dehydration can influence the level of cryoprotectants in locust eggs.

The present study suggests that high soil moisture content is deleterious to over-wintering eggs. In outbreak areas of migratory locust in north China, the range of soil moisture is 8–22%. The mean soil temperature is approximately –0.5 °C in winter at the depths (3–5 cm) at which eggs are laid (Ma, 1958). According to the results obtained in the present study, the mortality of eggs kept in wet soil is higher than that of eggs kept in dry soil. Therefore, if precipitation is high in autumn, the mortality of over-wintering eggs should increase because soil moisture is high. On the other hand, the locust eggs may be threatened by lethal desiccation in their natural habitats in winter (Guo *et al.*, 1989).

Hydrated conditions within the microevironment may influence strongly the susceptibility of an insect to inoculative freezing (Costanzo *et al.*, 1997). The present data suggest that environmental moisture may be an important factor. Given a short-time exposure, mortality in wet soil was lower than in dry soil. The pattern of egg mortality is reversed after long-time exposure. Owing to the high specific heat of water, wet soil needs a longer time to freeze than dry soil (Costanzo *et al.*, 1997, 1998). The water in the soil buffers against the ambient subzero temperature, and thus the eggs are protected. As the soil moisture increases, this protection would be strengthened, whereas eggs may make direct contact with external ice and the freezing of the body haemolymph may be accelerated by the ice crystal after the hydrated soil freezes in longer exposures (Danks, 1971; Humble & Ring, 1985). Salt (1963) provided the first theoretical discussion of inoculative freezing in insects but the role of hydric microenvironment on the susceptibility to inoculative freezing has received little attention (Gehrken *et al.*, 1991).

Water is regarded as the key ecological factor for outbreaks of the migratory locust (Ma, 1958), and it is clear from the present study that interaction between soil moisture and low temperature can influence the cold hardiness of locust eggs. Partial dehydration is apparently beneficial to over-wintering eggs of the locust. Therefore, drought in the previous autumn and winter may contribute to over-wintering survival of locust eggs. Climate change is occurring (Houghton *et al.*, 2001) and, from 1951–90, mean global temperatures rose, especially during winter: average winter minimum temperature rose 2.9 °C in the Northern Hemisphere (Karl *et al.*, 1993). Precipitation and extreme events (e.g. flooding, storms and drought) are also predicted to increase (Bale *et al.*, 2002). Climate change will alter the composition of water in the soil and its thermal properties, which impacts on the over-wintering survival of locust eggs. Therefore, outbreaks of the migratory locust may become more frequent in the future with increasing droughts and warm winters in Northern China.

Acknowledgements

We are most grateful to Dr Tong-Xian Liu for his useful comments. We also thank Dr Shu-guang Hao for assistance with the statistical analysis. This research project was supported by National Natural Science Foundation of China (NSFC-30240017) and the Chinese Academy of Sciences (KSCX2-SW-317).

References

Bale, J.S., Masters, G.J., Hodkinson, I.D. *et al*. (2002) Herbivory in global climate change research: direct effects of rising temperature on insect herbivores. *Global Change Biology*, **8**, 1–16.

Bayley, M. & Holmstrup, M. (1999) Water vapor absorption in arthropods by accumulation of myoinositol glucose. *Science*, **285**, 1909–11.

Block, W. (1996) Cold or drought, the lesser of two evils for terrestrial arthropods?. *European Journal of Entomology*, **93**, 325–339.

Block, W. (2003) Water or ice? – the challenge for invertebrate cold survival. *Science Progress*, **86**, 77–101.

Chen, B. & Kang, L. (2002) Cold hardiness and supercooling capacity in the pea leafminer *Liriomyza huidobrensis*. *Cryo-Letters*, **23**, 173–182.

Costanzo, J.P., Litzgus, J.D., Iverson, J.B. *et al*. (1998) Soil hydric characteristics and environmental ice nuclei influence supercooling capacity of hatchling painted turtles *Chrysemys picta*. *Journal of Experimental Biology*, **201**, 3105–3112.

Costanzo, J.P., Litzgus, J.D., Larson, J.L. *et al*. (2001) Not geographic origin, influence cold hardiness of hatchling painted turtles. *Journal of Thermal Biology*, **26**, 65–73.

Costanzo, J.P., Moore, J.B., Lee, R.E. Jr. *et al*. (1997) Influence of soil hydric parameters on the winter cold hardiness of a burrowing beetle, *Leptinotarsa decemlineata*. *Journal of Comparative Physiology (B)*, **167**, 169–176.

Danks, H.V. (1971) Over-wintering of some north temperate and arctic *Chironomidae* in the winter environment. *Canadian Entomologist*, **103**, 589–604.

Danks, H.V. (1991) Winter habitats and ecological adaptations for winter survival. *Insects at Low Temperature* (ed. by R.E. Lee and D.L. Delinger), pp. 231–259. Chapman & Hall, New York, NY.

Danks, H.V. (2000) Dehydration in dormant insects. *Journal of Insect Physiology*, **46**, 837–852.

Gehrken, U., Stromme, A., Lundheim, R. *et al*. (1991) Inoculative freezing in over-wintering tenebrionid beetle, Bolitophgagus Reticulatus Panz. *Journal of Insect Phsiology*, **37**, 683–687.

Guo, F., Chen, Y.L. & Lu, B.L., eds. (1989) The biology of the migratory locusts in China, pp. 60–514. Shandong Science and Technology Press, China.

Hadley, N.F. (1994) *Water Relations of Terrestrial Arthropods,* pp. 356. Academic Press, San Diego, CA.

Houghton, J.T., Ding, Y., Griggs, Noguer, D. J. *et al*. (2001) *Climate Change 2001: The Scientific Basis*. Cambridge University Press, U.K.

Humble, L.M. & Ring, R.A. (1985) Inoculative freezing of a larval parasitoid within its host. *Cryo-Letters*, **6**, 59–66.

Jing, X.H. & Kang, L. (2003a) Egg cold hardiness of the migratory locust *Locusia migraloria* L. under different photoperiod conditions. *Zoological Research*, **24**, 196–200.

Jing, X.H. & Kang, L. (2003b) Geographical variation in egg cold hardiness: a study on the adaptation strategies of the migratory locust *Locusta migritoria* L. *Ecological Entomology*, **28**, 151–158.

Jing, X.H. & Kang, L. (2004) Seasonal changes in the cold tolerance of eggs of the migratory locust, *Locusta migritoria* L. (Orthoptera: Acrididae). *Environmental Entomology*, **33**, 113–118.

Jing, X.H., Wang, X.H. & Kang, L. (2005) Chill injury in the eggs of the migratory locust, *Locusta migratoria* (Orthoptera: Acrididae): the time-temperature relationship with high-temperature interruption. *Insect Science*, **12**, 171–178.

Karl, T.R., Jones, P.D., Knight, R.W. *et al*. (1993) A new perspective on recent global warming: asymmetric trends of daily maximum and minimum temperatures. *Bulletin of the American Meteorological Society*, **74**, 1007–1023.

Layne, J.R. Jr (1991) Microclimate variability and the eurythermic nature of gall fly (*Eurosta solidaginis*) larvae. *Canadian Journal of Zoology*, **69**, 614–617.

Lee, R.E. Jr & Denlinger, D.L., eds. (1991) *Insects at Low Temperature*. Chapman & Hall, New York, NY.

Lundheim, R. & Zachariassen, K.E. (1993) Water balance of over-wintering beetles in relation to strategies for cold tolerance. *Journal of Comparative Physiology (B)*, **163**, 1–4.

Ma, S.J. (1958) The population dynamics of the oriental migratory locust (*Locusta migratoria manilensis* Meyen) in China. *Acta Entomologica Sinica*, **8**, 1–40.

Ma, S.J. (1962) The structure and transformation of the oriental locust breeding areas. *Acta Entomologica Sinica*, **11**, 17–30.

Meryman, H.T. (1974) Freezing injury and its prevention in living cells. *Annual Review of Biophysics and Bioengineering*, **3**, 341–363.

Miller, L.K. (1978) Freezing tolerance in relation to cooling rate in an adult insect. *Cryobiology*, **15**, 345–349.

Pugh, P.J.A. (1994) Supercooling points and water content in Acari. *Acta Oecologica*, **15**, 71–77.

Qing, J.D., Guo, F., Zai, Q.H. *et al*. (1958) Studies on the locust egg II. Developmental changes of the locust egg during incubation and their possible physiological significances. *Acta Entomologica Sinica*, **6**, 37–60.

Ring, R. & Danks, H.V. (1994) Desiccation and cryoprotection: overlapping adaptations. *Cryo-Letters*, **15**, 181–190.

Salt, R.W. (1963) Delayed inoculative freezing of insects. *Canadian Entomologist*, **95**, 1190–1202.

Steponkus, P.L., Uemura, M. & Webb, M.S. (1993) Membrane destabilization during freeze-induced dehydration. *Plant Responses to Cellular Dehydration During Environmental Stress* (ed. by T.J. Close and C.L. Bolis), pp. 37–47. American Society of plant Physiology, Rockville, MD.

Uvarov, ed. (1966) *Grasshoppers and Locusts*, Vol. I, pp. 1–45. Cambridge University Press, U. K.

Uvarov, ed. (1977) *Grasshoppers and Locusts*, Vol. II, pp. 445–523. Cambridge University Press, U. K.

Wang, X.H. & Kang, L. (2003) Rapid cold hardening in young hoppers of the migratory locust *Locusta migratoria* L. (Orthoptera: acridiidae). *Cryo-Letters*, **24**, 331–340.

Wang, X.H. & Kang, L. (2005a) Differences in egg thermotolerance between tropical and temperate populations of the migratory locust *Locusta migratoria* (Orthoptera: Acridiidae). *Journal of Insect Physiology*, **51**, 1277–1285.

Wang, H.S. & Kang, L. (2005b) Effect of cooling rates on the cold hardiness and cryoprotectant profiles of locust eggs. *Cryobiology*, **51**, 220–230.

Wang, H.S., Zhou, C.S., Guo, W. *et al*. (2006) Thermoperiod acclimations enhance cold hardiness of the eggs of the migratory locust. *Cryobiology*, **53**, 206–217.

Worland, M.R. & Block, W. (2003) Desiccation stress at sub-zero temperatures in polar terrestrial arthropods. *Journal of Insect Physiology*, **49**, 193–203.

Yu, C., Guo, F., Chen, Y. *et al*. (1958) Habits of *Locusta migratoria* manilensis (Meyen). *Acta Entomologica Sinica*, **8**, 119–135.

Zhang, Z.B. & Li, D.M. (1999) A possible relationship between outbreaks of the oriental migratory locust (*Lcousta migratoria manilensis* Meyen) in China and the El Nino episodes. *Ecological Research*, **14**, 267–270.

Zhao, Z.G. (1989) The El Nino phenomena and temperature of China. *Journal of Meteorology*, **15**, 26–30.

Zhao, Y.X. & Kang, L. (2000) Cold tolerance of the leafminer *Liriomyza sativae* (Dipt., Agromyzidae). *Journal of Applied Entomology*, **124**, 185–189.

Accepted 30 November 2006
First published online 8 May 2007

