

REVIEW ARTICLE

Rapid cold hardening process of insects and its ecologically adaptive significance*

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Abstract In contrast to overwintering cold hardening, a rapid cold hardening process has recently been described in insects. This process can rapidly enhance cold tolerance of insects in several hours or even minutes. The characteristics of rapid cold hardening include inducing conditions, cold hardening effects, and fitness costs. Physiological and biochemical mechanisms of rapid cold hardening include rapid changes in the phospholipid composition and the increase in hemolymph osmolalities, polyols production, proline and cold-induced proteins. Moreover, there are different views about the ecologically adaptive significance of rapid cold hardening in insects. This paper reviews research results about rapid cold hardening of insects since 1987, focusing on forms of cold injury, strategies of cold tolerance, and definitions, characteristics, physiological and biochemical mechanisms and adaptive significance of rapid cold hardening.

Keywords: insect, cold injury, cold hardiness, rapid cold hardening, ecological adaptation.

In temperate regions, low temperature during winter is identified as one of dominant abiotic factors affecting development and survival of insects. Therefore, cold hardiness is nearly correlated with life cycle, range, population dynamic, and adaptive evolution of insects^[1-3]. With the ever-increasing concern of global warming, the research on cold hardiness has been focused newly on insect ecology, insect physiology and evolutionary genetics^[4].

Cold acclimation is an important mechanism enhancing insect cold tolerance in insect cryobiology. In contrast to overwintering cold hardening studied over a long time, a rapid cold hardening process was recently described in insects^[5]. This process can rapidly enhance cold tolerance of insects in several hours or even minutes. By over ten years' study, this cold hardening has been rather well understood. Therefore, this paper reviews rapid cold hardening of insects focusing on definitions, characteristics, physiological and biochemical mechanisms and adaptive significance of rapid cold hardening.

1 Categories and mechanisms of cold injury

Based on presence or absence of ice formation

within an organism, cold injury may be divided into two types: nonfreezing injury and freezing injury^[6].

Nonfreezing injury may be subdivided into direct injury and indirect injury. Direct chilling injury, referred to as cold shock, is present in a wide variety of prokaryotic and eukaryotic organisms and is induced by a short-term exposure to low temperature. The mechanism of cold-shock injury may be due to the direct effects of low temperature causing the induction of phase transition in membrane lipids or excessive thermoelastic stress resulting in leakage and a severe loss of membrane function. Indirect chilling injury is induced by a long-term exposure to low temperature. Although few studies have carefully examined this category, it is supposed to cause lethal injury. During extended periods of up to 140 days in the bertha armyworm *Mamestra configurata*, the inability to emerge from the pupal case and malformed adults was observed in the range of -5 to -20 °C, but specific mechanisms of injury are unknown^[7].

Freezing injury is induced by ice-formation of extracellular or intracellular solutes within organisms. Freezing of body solutes can result in protein denaturation because of cellular dehydration, the enhanced

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concentration of ionic solutes and changes in pH. Cellular membrane may be another site of freezing injury due to the action and interaction of hypertonicity and direct effects of low temperature induced by cellular dehydration. Moreover, freezing injury is also induced by loss of osmotic responsiveness, mechanical injury of water channels, histological injuries of recrystallization and so on.

2 Strategies of cold tolerance in insects

All insects have the supercooling capacity depressing the freezing point of the body fluids in general. This depressed freezing point is called a supercooling point. Supercooling capacity plays an important role in insect cold hardiness.

Traditionally, strategies of insect cold tolerance may include two main categories: freezing intolerance and freezing tolerance corresponding to their supercooling ability. Although freezing intolerance species are greatly susceptible to injuries induced by ice-crystal formation, they can greatly avoid freezing of the body water by their supercooling capacity. Most insects are freezing intolerance and the supercooling point is the absolute lower lethal temperature for them. Although their supercooling capacity is generally weak, freezing tolerance insects can survive extracellular ice formation. Most of them can prevent freezing of intracellular fluids through producing initiatively ice nucleation to induce extracellular freezing at higher sub-lethal temperatures, thereby, avoiding the potentially lethal effects of intracellular freezing^[6].

According to the limits of their cold tolerance, i. e. the circumstances under which they are observed to die, Bale^[8] proposed that strategies of cold tolerance could be conveniently classified into five groups: freeze-tolerance, freeze-avoidance, chill-tolerance, chill susceptibility and opportunistic survival. Thus, all insect species can be placed in one of the distinct categories based on clearly defined and ecologically relevant criteria.

3 Definitions of rapid cold hardening

Cold hardening is defined as the phenomenon that insect capacity against chilling injury is increased by exposure to a moderately low temperature above the stress temperature. This process may be an evolu-

tionary phenomenon that insects adapt to adverse environments. Through cold hardening, a series of changes in insect behaviors or physiological conditions occur such as entering dormancy or diapause, decrease in supercooling point, the synthesis of low-molecular-weight cryoprotectants and antifreeze proteins, ice-nucleator formation, and dehydration, thereby, resulting in enhanced resistance to nonfreezing injury and freezing injury^[9]. According to duration of exposure to acclimating temperatures, cold hardening can be divided into two main categories: (i) long-time cold hardening, i. e. the acquisition of enhanced cold tolerance through acclimation at low temperature within days or weeks (for indirect chilling injury and freezing injury mainly), and (ii) short-time cold hardening or rapid cold hardening, i. e. a rapid response that occurs within hours or even minutes (for direct chilling injury and cold shock)^[10].

For long-time cold hardening, inducing conditions and mechanisms enhancing cold hardiness have been discussed in detail in many insect species^[11]. However, research of rapid cold hardening has a rather short history of only over a decade. Lee^[5] proposed a simple definition of rapid cold hardening in 1987, i. e. a rapid protective mechanism against cold injury to respond to changing environmental temperatures on a daily or even an hourly basis in nondiapausing species. Rapid accumulation of glycerol and rapid changes in the phospholipid composition may be physiological basis inducing this cold hardening.

The process of rapid cold hardening was first described in larvae and pharate adults of the flesh fly, *Sarcophaga crassipalpis*, and was then obtained in adults of the elm leaf beetle, *Xanthogaleruca luteola* and the milkweed bug, *Oncopeltus fasciatus*^[5]. Now, rapid cold hardening has been identified in a number of diverse terrestrial arthropods including Diptera, Coleoptera, Lepidoptera, Thysanoptera, Hemiptera, homoptera, Orthoptera, and mites^{[12], 1)}. Compared with over a decade ago, many characteristics of rapid cold hardening have rather detailedly been understood such as inducing conditions, acclimating effects, fitness costs, physiological and molecular mechanisms, and ecologically adaptive significance. Based on these researches, Broufas^[12] and Kelty^[13] extended Lee's definition: In contrast to overwintering cold hardening, the acquisition of which requires an extended period, the rapid

1) Wang, X. H. et al. A rapid cold hardening of the first instar hopper of the migratory locust *Locustia migratoria* L. . CryoLetter, submitted.

cold hardening response is induced by either a brief exposure (from minutes or several hours) to low temperatures or through gradual cooling of the diapause or nondiapause experimental insects (rates at $0.05\text{ }^{\circ}\text{C}\cdot\text{min}^{-1}\sim 0.1\text{ }^{\circ}\text{C}\cdot\text{min}^{-1}$) and results in increased survival after a cold shock exposure. A period of anoxia or a short exposure to heat ($35\text{ }^{\circ}\text{C}$) can also stimulate this response. The rapid cold hardening response is correlated with the accumulation of cryoprotective substances. Through this response, many insect species can rapidly enhance their cold hardiness against cold shock injury due to diurnal changes in temperature or short-time cold exposure in late autumn or in early spring when a sudden drop of temperature could cause devastating effects.

Compared with Lee's definition, diapause was added to states inducing rapid cold hardening; and gradual cooling, anoxia and high temperature hardening were added as inducing conditions in Broufais and Kelty's. The definitions of rapid cold hardening are being developed and amended continually because of rapid research progress in rapid cold hardening. For example, it was recently found that the rapid cold hardening response could be induced by natural thermoperiodic cycles^[14]. A rapid cold hardening response depressing the supercooling point in an Antarctic microarthropod *Cryptopygus antarcticus* has been reported^[15]. In addition, the physiological and biochemical mechanisms of rapid cold hardening have also been further understood. Broufais and Kelty's definition does not include these new contents.

4 Characteristics of rapid cold hardening

4.1 Inducing conditions

4.1.1 Inducing temperatures and duration

Most researches detecting rapid cold hardening followed basically similar protocols designed by Chen and colleagues for studies of the flesh fly^[16]. The supercooling point of insects is first measured in order to determine the lower lethal temperature limit, and then survival percentages are assessed after the direct transfer of insects from their rearing temperature to a series of temperature gradient from $0\text{ }^{\circ}\text{C}$ to this lethal temperature limit. Low temperature resulting in the survival percentage from 15% to 30% may be selected to detect rapid cold hardening response and detecting duration of 2 hours is adopted in general. Insects will be transferred from their rearing temperature to protection-inducing temperature for some times, and

then exposed directly to detecting temperature for 2 hours, and finally survival percentages will be investigated at rearing temperature to evaluate whether cold hardening increases survival percentage as compared with direct transfer. The optimal range of inducing temperatures is between $0\text{ }^{\circ}\text{C}$ and $5\text{ }^{\circ}\text{C}$ and the optimal duration is between 15 minutes and several hours in general. However, the optimal interaction between temperature and duration is different in different insect species. For instance, although exposure to $0\text{ }^{\circ}\text{C}$ for 30 minutes can increase survival significantly in larvae and pharate adults of the flesh fly, exposure to $0\text{ }^{\circ}\text{C}$ for 1 hour is optimal^[16]. In pupae of housefly, *Musca domestica*, the optimal inducing condition is exposure to $0\text{ }^{\circ}\text{C}$ for 4 hours^[17], and yet acclimation effect at $5\text{ }^{\circ}\text{C}$ was more than that at $0\text{ }^{\circ}\text{C}$ in 5 instar larvae of beet armyworm, *Spodoptera exigua*^[10]. In only a few arthropod species, exposure to higher temperatures can induce the rapid cold hardening response. For instance, the rapid cold hardening response could be induced by 2 hours at $10\text{ }^{\circ}\text{C}$ in fruit fly *Drosophila melanogaster*^[13] and predatory mite *Euseius finlandicus*^[12]. In an Antarctic microarthropod *C. antarcticus*^[15], exposure to $11\text{ }^{\circ}\text{C}$ can induce rapid cold hardening response, but has no inducing effects for most insects.

4.1.2 Diapausing and nondiapausing states

Many insect species get through winter or other adverse environments by diapause. The relationship between cold hardiness and diapause has been well discussed^[7]. Diapausing induction for insects is generally correlated with many environmental cues such as temperature, photoperiod, nutrition, and population density. Cold hardening response associated with diapause is a long-time process because low temperature acclimation within days or weeks is required to enhance cold hardiness. In Lee's definition^[5], the rapid cold hardening response is restricted to nondiapausing individuals, and yet diapausing state is not included. New researches demonstrated that diapausing insects could also show the rapid cold hardening response. For instance, diapausing predatory mite showed a similar rapid cold hardening response to nondiapausing state^[12] and diapausing pupae of the flesh fly did so^[16]. These results suggest that diapausing or nondiapause state is not limiting factors inducing rapid cold hardening.

4.1.3 Gradual cooling and natural thermoperiodic cycle

A rapid cold hardening response induced through gradual cooling for insects was first described

in pupae of the housefly^[17]. The significantly increased survival after exposure to low temperatures was accomplished through gradual cooling at rates of $0.1 \sim 0.25 \text{ } ^\circ\text{C} \cdot \text{min}^{-1}$, but no acclimating effect at higher rates than $0.5 \text{ } ^\circ\text{C} \cdot \text{min}^{-1}$. The range of $0.05 \sim 0.1 \text{ } ^\circ\text{C} \cdot \text{min}^{-1}$ is regarded as ecologically relevant rates, at which cold acclimating effect of the fruit fly is higher than that at rates of $0.5 \sim 1 \text{ } ^\circ\text{C} \cdot \text{min}^{-1}$ significantly^[13]. The optimized range of acclimating rates in first instar hoppers of migratory locust is similar to the fruit fly¹⁾. These of the western flower thrips *Frankliniella occidentalis*^[18] and the predatory mite^[12] are $0.5 \sim 1 \text{ } ^\circ\text{C} \cdot \text{min}^{-1}$ and $0.4 \text{ } ^\circ\text{C} \cdot \text{min}^{-1}$ respectively, comparatively higher than migratory locust or fruit fly. An ecologically based thermoperiod can also induce the rapid cold hardening response^[14]. As flies cooled from 23 to $16 \text{ } ^\circ\text{C}$ at an average rate of $1.4 \text{ } ^\circ\text{C} \cdot \text{h}^{-1}$, their survival after exposure to $-7 \text{ } ^\circ\text{C}$ for 1 hour increased significantly from $5.0 \pm 5.0\%$ to $29.6 \pm 6.3\%$. Then, as they cooled from 16 to $9 \text{ } ^\circ\text{C}$ (average rate $0.6 \text{ } ^\circ\text{C} \cdot \text{h}^{-1}$), their survival after sub-zero treatment again increased to $62.5 \pm 7.3\%$; as they were warmed at an average rate of $1.75 \text{ } ^\circ\text{C} \cdot \text{h}^{-1}$ from $9 \text{ } ^\circ\text{C}$ to $23 \text{ } ^\circ\text{C}$, their survival decreased to $48.3 \pm 8.9\%$. Flies subjected to six thermoperiods exhibited an average survival rate of $88.3 \pm 3.0\%$ following 1 hour of exposure to $-7 \text{ } ^\circ\text{C}$. In conclusion, the inducing effect of rapid cold hardening through natural thermoperiod is very significant.

4.1.4 Anoxia and heat acclimation Insects treated through anoxia and high temperature can also show a similar rapid cold hardening response to those acclimated by low temperature, gradual cooling, and natural thermoperiod. After an anoxic acclimation of only 10 minutes, survival in pupae of the housefly from an exposure to $-7 \text{ } ^\circ\text{C}$ for 2 hours increased from 0% to 25.3% , and survival was similar to the controls after 40 minutes of anoxia. Exposure for 1.5 hours at $0 \text{ } ^\circ\text{C}$ was required to produce an equivalent level of survival after 2 hours at $-7 \text{ } ^\circ\text{C}$ as 40 minutes of anoxia^[19]. Therefore, these results suggest that cold acclimating effect of anoxic treatments is very significant. Except for cold acclimating capacity against cold shock injury, insects have also heat acclimating response against heat shock injury. It is very interesting that some insect species can produce rapid cold hardening response in heat acclimating conditions. For instance, survival of pharate adults of the flesh fly exposed to $-10 \text{ } ^\circ\text{C}$ for 2 hours increased sig-

nificantly after treatment at $36 \text{ } ^\circ\text{C}$ for 2 hours. *Drosophila plexippus*^[20], *Drosophila melanogaster*^[21] and *Thecodiplosis japonensis*^[22] also show a similar response.

4.2 Effects of cold acclimation

Effects of rapid cold hardening include increased survival at low temperature, changes of LT_{50} (LT_{50} indicates the temperature that causes 50% mortality after exposure for various time periods ($Ltemp_{50}$) or the duration that it takes to kill 50% individuals ($Ltime_{50}$)), lethal temperature, the critical thermal minimum (or CT_{min}), the supercooling point, and duration of rapid cold hardening.

4.2.1 Survival enhancement at low temperature and changes in LT_{50} Rapid cold hardening response can significantly increase survival of insects exposed to the discriminating low temperature. In optimal acclimating conditions, survival at detecting temperature increases from $15\% \sim 30\%$ to $80\% \sim 90\%$ in general. For example, survival of pupae of the flesh fly successfully emerging after exposure to $-10 \text{ } ^\circ\text{C}$ for 2 hours increased from 20% to 90% through treatment at $0 \text{ } ^\circ\text{C}$ for 2 hours^[16]. Effects of rapid cold hardening induced by gradual cooling, natural thermoperiod, anoxia, and heat acclimation can also increase survival of low temperature exposure^[13,14,19,22]. In addition, $Ltime_{50}$ and $Ltemp_{50}$ are two important criteria indicating whether cold hardness increases or not. For example, the $Ltime_{50}$ of 3rd instar larvae of pine needle gall midge at $-15 \text{ } ^\circ\text{C}$ increased from 1.6 to 5.0 hours through rapid cold hardening at $4 \text{ } ^\circ\text{C}$ for 2 hours^[23]. The $Ltime_{50}$ of stored grain pest *Cryptolestes ferrugineus* at detecting temperature of $-12 \text{ } ^\circ\text{C}$ increased 8.7 times compared with that of unacclimated beetles^[24]. The $Ltime_{50}$ of the western flower thrips could also be enhanced from 2 to 8 hours, but $Ltemp_{50}$ decreased significantly after cold acclimation^[18].

4.2.2 Decline of low lethal temperature and CT_{min} Most insect species possessing capacity for rapid cold hardening are chilling susceptible, i.e. death heavily occurs at low temperatures above the supercooling point and the temperature is higher than the supercooling point. A number of insect species can depress their low lethal temperature through rapid cold hardening. For instance, low lethal temperature of first

1) See footnote 1) on page 642.

instar hoppers decreases from $-10\text{ }^{\circ}\text{C}$ to $-12\text{ }^{\circ}\text{C}$ after rapid cold hardening¹⁾. CT_{\min} indicates the critical thermal minimum at which insects enter a state of cold torpor. At or below the temperature, insects will suffer severe injuries because of inability to seek food or to avoid predation. Therefore, the research on the effect of rapid cold hardening at this temperature has important ecological significance. For instance, cooling rate $0.1\text{ }^{\circ}\text{C}\cdot\text{min}^{-1}$ of *Drosophila melanogaster* can depress CT_{\min} from $6.5\pm 0.6\text{ }^{\circ}\text{C}$ to $3.9\pm 0.9\text{ }^{\circ}\text{C}$ ^[14].

4.2.3 Decline in supercooling point This type of cold acclimating effect is peculiar because the rapid cold hardening process can not generally change supercooling points of insects. An Antarctic microarthropod can produce this particular acclimating effect that leads to a depressed mean supercooling point of its summer population from -10 to $-20\text{ }^{\circ}\text{C}$ over a 4-hour period as the microhabitat temperature changed from $10\text{ }^{\circ}\text{C}$ to $0\text{ }^{\circ}\text{C}$ ^[15,25]. It is very amazing that such large-scale variation in the supercooling point is produced in such a short duration, whereas its mechanisms are not clear.

4.2.4 Duration of rapid cold hardening The protection obtained through a rapid cold hardening response is lost as rapidly as it is developed when insects are transferred directly to a higher temperature environment after acclimation and its duration does not exceed several hours. For instance, survival of the pupae of the housefly is not different from that of the control after its return to rearing temperature of $27\text{ }^{\circ}\text{C}$ for 2 hours following the treatment in the optimal condition of 2 hours at $0\text{ }^{\circ}\text{C}$ ^[17].

4.3 Costs of fitness

Similar to other cold acclimation regimes, the rapid cold hardening response produces adverse influences on other facts of fitness when insect survival is increased after cold shock. For example, the mean longevity of emerging adults decreased after pupae of the housefly was acclimated at $0\text{ }^{\circ}\text{C}$ for 3 hours^[26]. In non-diapause predatory mites, gradual cooling resulted in a lowered total fecundity and survival of offspring compared with the control^[12]. Although fecundity of *Drosophila melanogaster* over the first 5 days was influenced by gradual cooling, it was difficult to draw safe conclusions that total fecundity did not suffer from rapid cold hardening because oviposition was not determined later^[14]. However, it was found in the

flesh fly that rapid cold hardening process could prevent reduction of fecundity caused by cold shock^[27].

5 Physiological and biochemical mechanisms of rapid cold hardening

It is a complex physiological and biochemical process that insect cold hardiness is increased by cold acclimation. Changes in metabolic substances refer mainly to low-molecular-weight cryoprotectants such as glycerol and trehalose, ice nucleations inducing extracellular freezing, antifreezing protein preventing ice formation, and correlative enzymes. Mechanisms underlying a long-time cold acclimation associated with overwintering have been well understood, but those for rapid cold hardening have been not clearly known as yet, and were only reported in several insects. For instance, after exposure to $0\text{ }^{\circ}\text{C}$ for 2 hours for all developmental stages of the flesh fly, hemolymph osmolality increased from 308.7 Osm/kg to 346.3 Osm/kg and glycerol levels rose 2 ~ 3 fold^[16]. A 2-hour treatment at $5\text{ }^{\circ}\text{C}$ increased hemolymph osmolalities from 208.8 Osm/kg to 305.6 Osm/kg , producing two cold-induced proteins (27 kD and 31 kD) for 5th instars of beet armyworm, and elevated glycerol contents^[10]. Rapid cold hardening process had no oxidative stress in pine needle gall midges because of the simultaneously increased content of reduced glutathione and activity of glutathione reductase through cold acclimation^[22]. Through gradual cooling and natural thermoperiod for *Drosophila melanogaster*, concentration of low-molecular cryoprotective substances such as glycerol, trehalose and fructose did not change and Hsp70 was not detected except that concentration of proline increased^[13,14,28]. In addition, rapid cold hardening can prevent neuromuscular injuries induced by cold shock^[29]. Rapid changes in the phospholipid composition of cellular membranes may also be a physiological base for this cold acclimation^[16].

6 Ecologically adaptive significance of rapid cold hardening

The phenomenon that many insect species can enhance their cold hardiness through cold acclimation has obviously ecologically adaptive and evolutionary significance^[30]. However, reports about evolutionary significance of cold acclimation mainly focus on a long-time type associated with overwintering. Be-

1) See footnote 1) on page 642.

cause the research history of rapid cold hardening is rather short, there are different views about its ecologically adaptive significance.

Chen^[20] found that the cold acclimation effects in temperate species were more obvious than that in other natural fruit fly species inhabited in the areas of tropical highland and tropical lowland. Because air temperature fluctuates more frequently in temperate regions than tropic regions, it was speculated that rapid cold hardening was an adaptive response to local environments. Yamamoto^[31] acquired similar results by investigating sixteen species of the fruit fly inhabiting in different ecological environments. In addition, Chen^[32] carried out selection of cold-shock tolerance in *Drosophila melanogaster* in laboratory. Three treatments including a short-time chilling, cold shock, and combined treatment of short-term chilling and cold-shock were designed as selecting stress. After ten generations of selection, rapid cold hardening effect of three selected lines increased significantly, and the greatest increase was found in combined line. The enhanced cold-shock tolerance was relatively stable since no decrease was observed after four generations without selection. Based on these results, it is concluded that rapid cold hardening response has obvious adaptation and environmental temperature plays an important role in evolution of rapid cold hardening response in nature.

For many insect species having no overwintering strategies such as diapause, dormancy or migration, rapid cold hardening responses are very important because a sudden drop in environmental temperature is generally lethal. For instance, migrants of the monarch butterfly, *Danaus plexippus*, is often subjected to subzero temperature, heavy dews and frost, which may cause devastating effects. The monarch butterfly has the capacity for rapid cold hardening to resist this abrupt temperature change^[33]. The western flower thrips, a glasshouse pest, does not generally suffer acute temperature changes. However, when infested plant material moved from glasshouses, they will experience sudden decreases in temperature and rapid cold hardening may be an important attribute to survival^[18].

Early studies designed a lower cold-shock temperature and then detected rapid cold hardening response by exposure to this temperature directly. However, this condition did not easily occur in nature because detecting temperature was relatively low and

cooling rates were very high. Kelty^[13,14] found the rapid cold hardening response induced through simulating cooling rates and thermoperiods in nature could also increase cold-shock tolerance and two inducing treatments could decrease significantly, which suggested the rapid cold hardening response had ecological significance.

However, Coulson^[17] and Bale^[19] considered that rapid cold hardening was not an adaptive strategy of cold hardiness, but rather a by-product of suppressed metabolism based on studies in the housefly. They explained it by four reasons: (1) although cold hardiness is increased by extending the period of acclimation at 0 °C from 30 min to 4 h, mortality increases after 6 h at 0 °C, without any exposure to a sub-zero temperature; (2) the extra cold hardiness conferred is relatively small; (3) the cold hardening response is lost as rapidly as it is developed when insects experience high temperature; and (4) anoxic hardening and high temperature acclimation can also acquire similar effects.

In conclusion, researches on rapid cold hardening play important roles to many fields such as integrated control of medical, glasshouse, stored grain and quarantine pests, cooling storage of valuable and experimental insects, and overwintering survival forecast of agricultural pests^[6]. Particularly in recent years, global climate warming not only increased average temperature but also caused exceptional temperature fluctuation such as sudden drop or rise of temperature in early spring and late autumn, which resulted in enhanced effects of rapid cold hardening on insect populations survival^[4]. However, although the advancements of inducing conditions, cold-acclimating effects, physiological and biochemical mechanisms, and ecologically adaptive significance had been achieved, the research on rapid cold hardening is not enough in depth and extent in contrast to cold acclimation associated with overwintering. Moreover, there is no report about rapid cold hardening in China up to now. Therefore, it is very important to carry out the research on rapid cold hardening not only from theoretical studies but also from practical applications.

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