Effect of Low Temperature Exposure on the Supercooling Point of Oriental Cockroach (Dictyoptera: Blattidae)[1]

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Abstract A system constructed with thermocouples and a conventional refrigerating bath was used to measure the freezing and melting points of various developmental stages of oriental cockroach, *Blatta orientalis* L., after exposure to different pre-freezing temperature conditions. Low temperature exposure of the oriental cockroach was not consistently effective in enhancing the supercooling capacity at various stages. There were minimal differences among the various developmental stages, and freezing points ranged −6.85 to −11.18°C. A significant lowering of freezing point ($P < 0.05$) was observed only from the first and second nymphal stages; freezing points were below −11°C after exposure at 5°C after 2 wks at 10°C. The melting points were consistently around 0°C (range of −0.19 to 0.31°C). The relatively limited supercooling capacity of oriental cockroaches suggest that freezing avoidance by microhabitat selection is a major mechanism for overwintering of this insect.

Key Words *Blatta orientalis,* supercooling point, overwintering

The oriental cockroach, *Blatta orientalis* L., is a primary household pest in temperate regions of the world (Benson and Zungoli 1997). This species has been reported as one of major domiciliary pests in most urban areas of the United Kingdom (Alexander et al. 1991) and United States, except Florida (Mampe 1972). Also in Nebraska, the oriental cockroach is one of the most important insect pests in households and public places and is considered the most repulsive of all of the roaches because of its strong odor (Kamble and Keith 1993).

It has been suggested that oriental cockroaches are able to overwinter outdoors without movement to an indoor harborage in the temperate region even though they are freeze-intolerant (Solomon and Adamson 1955, Shuyler 1956, Alexander et al. 1991, le Patourel 1993). In addition, they have a limited ability to disperse and move. Only 2% of oriental cockroaches released outdoors subsequently moved indoors (Thoms and Robinson 1987), and mixed stages of the insects are frequently observed under stones and leaf debris (Shuyler 1956). All these facts indicate that many oriental cockroaches may stay outdoors because they are able to utilize harborages to overwinter. Moreover, this species seems to possess some level of cold-hardiness induced by low temperature acclimation (le Patourel 1993) even though they rapidly died at −10°C. It suggests that the outdoor population of oriental cockroach is capable to survive during the cold winter period. The capacity of oriental cockroaches to

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overwinter at outside harborages during winter might enhance the potential infestation in next season, and the management program should involve the annual outdoor population for effective control.

Generally, two main strategies of cold-hardiness have been categorized in the insect overwintering strategies (Baust 1982). Freeze-tolerant species are able to survive the formation of extracellular ice, however, for the freeze-intolerant species the supercooling state is maintained far below the melting point (Salt 1961). The purpose of this study was to identify the involvement of the supercooling phenomenon in the cold-hardiness of oriental cockroaches. The supercooling points were investigated with various developmental stages exposed to different temperature regimes. The supercooling capacity and possible survival strategy in the overwintering oriental cockroach were also examined.

Materials and Methods

Insects. A laboratory colony of oriental cockroaches was obtained from the Department of Entomology, Purdue University, West Lafayette, IN, in 1994 and established at University of Nebraska-Lincoln. The colony was reared on Purina® dog chow (Ralston Purina, St. Louis, MO) and water, and maintained in Plexiglas® containers (W30.3 cm × H30.3 cm × D30.3 cm) with pieces of paper egg carton as harborage. The cockroach containers were maintained at 25 ± 2°C, 60 ± 10% RH, and a photoperiod of 12:12 (L:D) h. Each developmental stage of nymphs and adults were collected from each separated culture that was established for this study with newly-hatched nymphs from oothecae. Nymphal stages were identified according to the descriptions of the ventral aspect of the terminalia reported by Short and Edwards (1991).

Low temperature exposure. Cockroaches were maintained in temperature and light programmable incubators (Model I-35VLX®, Percival Scientific, Boone, IA). The cockroaches were exposed at different temperature treatments: (1) 10°C for 1 wk; (2) 10°C for 3 wks; (3) 10°C for 5 wks; (4) 5°C for 1 wk after at 10°C for 2 wks; (5) 5°C for 3 wks after at 10°C for 2 wks. Temperature and humidity were continuously monitored with a traceable temperature/humidity meter (Fisher Scientific, St. Louis, MO).

Measurement of freezing and melting points. A system consisting of thermocouples and a conventional refrigerating bath was constructed to simultaneously measure the freezing and melting points from 12 cockroaches (Fig. 1). Each cockroach was attached to a thermocouple (Type K, Teflon-FEP insulated probe with 2.3 mm diam, Cole-Parmer Instrument, Vernon Hills, IL) in a microcentrifuge tube (0.5, 1.5, and 2.0 ml depending upon the insect size) with a 2.5 mm-diam hole in the cap. A cotton plug was inserted to maintain contact between the insect and the probe (Fig. 1A). Each microcentrifuge tube was placed in a glass test tube (O.D. 25 × 100 mm long) (Fig. 1B), and a test tube rack holding 12 test tubes was submersed in 50% ethylene glycol solution in a refrigerated circulating bath (Model RTE-210 Neslab®, Fisher Scientific, St. Louis, MO). The temperature of the bath was lowered to –20°C at a rate of 0.45 –1.0°C/min. After reaching –20°C, the bath was switched off to allow slow increase of temperature at a rate of 3°C/h up to ambient temperature.

Temperatures were recorded every 5 s with a 12-channel thermocouple scanner (Model 92000-00, Cole-Parmer Instrument, Vernon Hills, IL). The data were downloaded from the scanner to a personal computer through a serial RS-232 connection

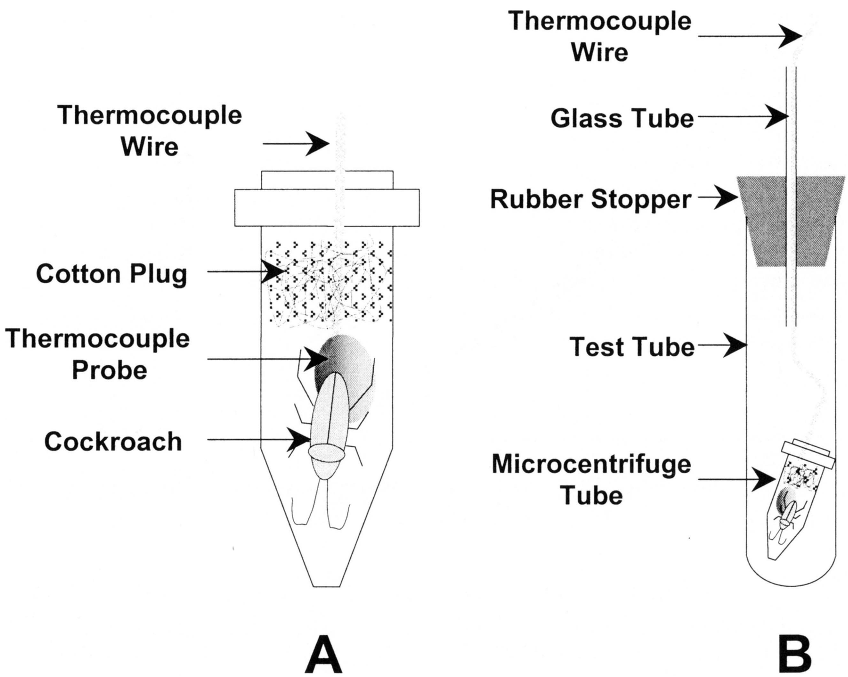

Fig. 1. Assembly diagram of a cockroach with the copper-constantin thermocouple probe in a microcentrifuge tube (A) and arrangement of the assembled microcentrifuge tube within a glass test tube (B).

and further processed with spreadsheet software (Excel 97®, Microsoft Co., Redmond, WA). The freezing point was determined from the temperature curve. It corresponds to a sudden rise of temperature caused by the release of heat of fusion from freezing water. The melting point was determined as a delayed increase of the temperature just after a transition state in the temperature curve (Leather et al. 1993).

Data analysis. The effect of cold temperature exposure on the freezing and melting point data were tested with Student *t*-test ($P = 0.05$) against the same stage of control cockroaches maintained at room temperature. The effect of temperature on the freezing and melting points among different developmental stage data were analyzed by analysis of variance (ANOVA) using PROC GLM (SAS Institute 1996). Differences were compared by the Scheffe multiple comparison procedure.

Results and Discussion

For purposes of this study, the system constructed of thermocouples and a conventional refrigerating bath was sufficient for the measurement of the freezing points for all developmental stages of oriental cockroaches despite the lack of cooling rate control in the water bath. The sudden rise in temperature caused by the latent heat at the beginning of freezing was clearly observed in Fig. 2. The size of the peak repre-

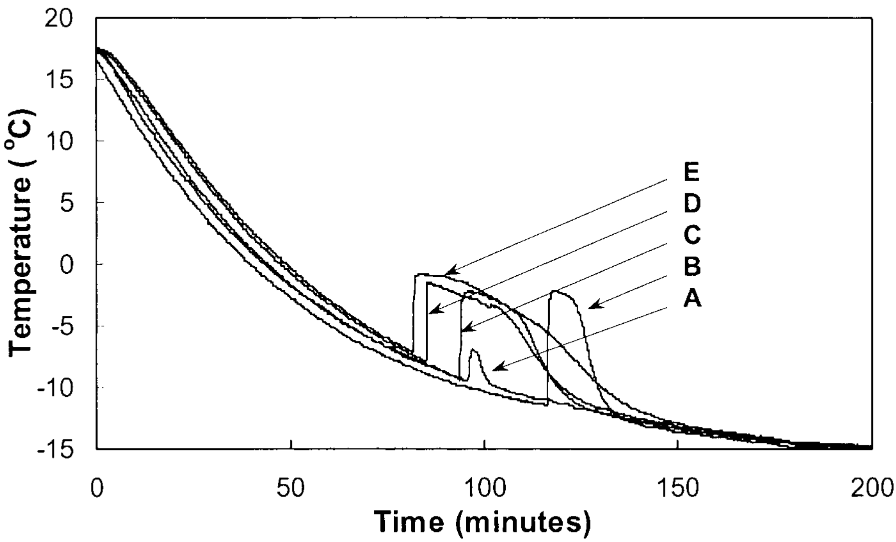

Fig. 2. Freezing points from various developmental stages of oriental cockroach appeared as sudden rises of the temperature during cooling. A: first-instar nymph, B: third-instar nymph, C: fifth-instar nymph, D: adult male, and E: adult female. Each line was selected randomly from each developmental stage and sex.

senting the mass of the latent heat emitted during freezing corresponded to the size of the insect body.

However, this system was not adequate to accurately measure the melting points of first-instar nymphs because of the inability of heating rate control and the relatively small latent heat from the small body size (Fig. 3, line A). The transition states typical for the melting point were easily detected from other older nymphs and adults after turning off the bath and monitoring the change of the temperature to the ambient temperature.

The freezing points measured from different developmental stages were not significantly different between controls and treatments with range of −7.22 (±0.25) to −10.73 (±0.57)°C in all stages examined except for the significant decrease in first and second-instar nymphs exposed to 10°C for 2 wks and then 5°C for 1 wk (Table 1). The significant increase of freezing points was also observed from the third-instar nymphs exposed to 10°C for 3 wks and to 5°C for 1 wk after 2 wks at 10°C (Table 1). These increases of freezing point are not able to explain at present time whether this nymphal stage is critical for the acclimation or not.

The melting points were not significantly different among various stages and exposure conditions (Table 2). All melting points occurred systematically around 0°C within −0.19 (±0.21) and 0.31 (±0.07)°C. Because these melting points were far above the freezing points, this observation implies that the oriental cockroach has some level of supercooling capacity that enable to maintain the body fluid unfrozen far below the melting point regardless of developmental stage. These freezing points

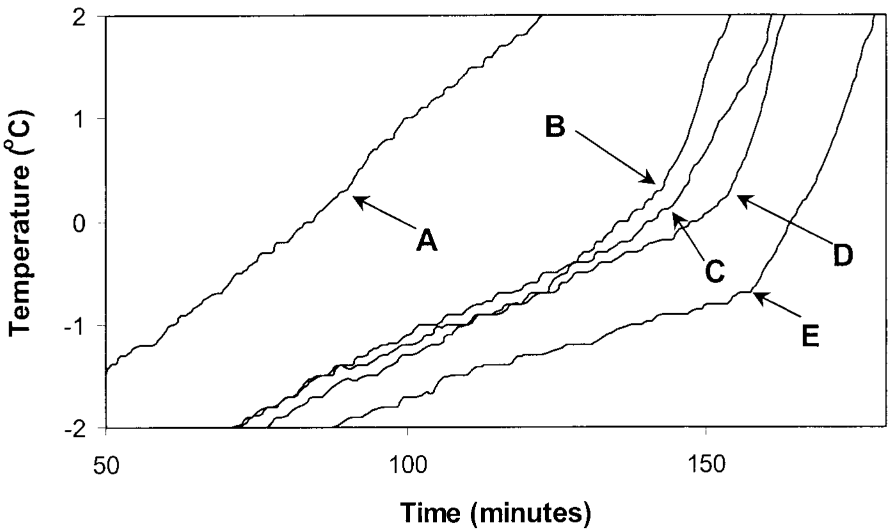

Fig. 3. Melting points from various developmental stages of oriental cockroach appeared as a transition state before the sudden increase of temperature during heating. Each arrow is indicating the possible melting point from each developmental stage. A: first-instar nymph, B: third-instar nymph, C: fifth-instar nymph, D: adult male, and E: adult female. Each line was selected randomly from each developmental stage and sex.

correspond to the actual supercooling points as previously defined (Leather et al. 1993).

The relative consistency of supercooling points among the various stages of oriental cockroach at different temperature regimes suggests that the lowered supercooling capacity is less involved in the overwintering mechanism of this insect. It suggests that the overwintering population of the oriental cockroach would be mixed with various developmental stages. This assumption is supported by the observations of le Patourel (1993) who reported the increase of survival rate from all stages at −5°C after exposure to 10°C for 14 to 28 d.

In addition to enhanced supercooling capacity for overwintering, Leather et al. (1993) described winter avoidance as an alternative strategy for freeze-intolerant insects. Many insects are able to survive by avoiding freezing temperatures when they move to nearby warmer harborage whether they are active or not during winter. Winter survival of *Periplaneta japonica* in Japan was suggested to be based on the microhabitat selection with stage limited freeze intolerance (Tanaka and Tanaka 1997). In temperate regions, with lowest air temperature of around −20°C, there might be a number of thermally-protected sites in urban outdoors even though we do not have good observations from the urban microenvironment. There are abundant examples of freeze-intolerant insects overwintering by microhabitat selection with relatively low supercooling capacity. For example, aphids move to a pile of field-stored barley (Harper and Blakely 1968), collembolan species move to deep and warmer

Table 1. Freezing points measured from various developmental stages of the oriental cockroach exposed to different temperature conditions and periods

Exposure condition	Nymphs					Adults	
	1st	2nd	3rd	4th	5th	Male	Female
Control	−8.87A	−9.37A	−9.37A	−8.73A	−8.07A	−9.63A	−8.35A
	(0.90)	(0.54)	(0.54)	(0.33)	(0.50)	(0.44)	(0.35)
10°C							
1 wk	−10.73B	−9.80AB	−9.80AB	−7.83A	−7.40A	−9.18AB	−8.62AB
	(0.57)	(0.38)	(0.38)	(0.48)	(0.69)	(0.35)	(0.34)
3 wk	−8.97A	−9.05A	−6.85*A	−7.67A	−7.38A	−8.95A	−8.53A
	(0.57)	(0.36)	(0.34)	(0.42)	(0.64)	(0.52)	(0.34)
5 wk	−9.88B	−9.08AB	−8.70AB	−7.75A	−7.95AB	−8.13*AB	−7.67A
	(0.04)	(0.37)	(0.32)	(0.45)	(0.38)	(0.40)	(0.40)
5°C after 2 wk at 10°C							
1 wk	−11.18*C	−11.02*C	−7.82*AB	−8.1AB	−6.85A	−9.53BC	−9.02B
	(0.35)	(0.40)	(0.29)	(0.43)	(0.34)	(0.25)	(0.34)
3 wk	−11.17*B	−9.88AB	−7.92A	−7.22A	−7.30A	−9.13AB	−8.65AB
	(0.59)	(0.91)	(0.41)	(0.25)	(0.50)	(0.41)	(0.51)

Each value is a mean from six replicates with SEM in a parenthesis. Means marked with asterisks (*) are significantly different from the control of each developmental stage ($P = 0.05$; Student *t*-test). Significant differences ($P < 0.05$) among mean values in the same row of each temperature condition are indicated by different letter.

Table 2. Melting points measured from various developmental stages of the oriental cockroach exposed to different temperature conditions and periods

Exposure condition	Nymphs					Adults	
	1st	2nd	3rd	4th	5th	Male	Female
Control	0.02	−0.03	0.06	0.19	0.16	0.04	−0.04
	(0.14)	(0.20)	(0.16)	(0.19)	(0.16)	(0.16)	(0.15)
10°C							
1 wk	0.01	0.04	0.07	0.07	0.14	−0.01	0.02
	(0.17)	(0.18)	(0.12)	(0.08)	(0.14)	(0.20)	(0.20)
3 wk	−0.12	0.12	0.06	0.20	0.02	0.05	0.13
	(0.18)	(0.19)	(0.17)	(0.16)	(0.21)	(0.18)	(0.18)
5 wk	0.31	−0.16	−0.11	−0.17	0.04	−0.02	−0.08
	(0.07)	(0.17)	(0.21)	(0.15)	(0.18)	(0.19)	(0.11)
5°C after 2 wk at 10°C							
1 wk	0.08	0.08	−0.04	0.16	0.00	0.02	0.02
	(0.15)	(0.18)	(0.19)	(0.12)	(0.16)	(0.20)	(0.20)
3 wk	−0.15	0.12	0.12	0.13	−0.18	−0.19	0.19
	(0.17)	(0.18)	(0.17)	(0.18)	(0.17)	(0.21)	(0.19)

Each value is a mean from six replicates with SEM in parenthesis. Means in the same column of each developmental stage are not significantly different from the control (P = 0.05; Student t-test). Values in the same row of each temperature condition are not significantly different (P = 0.05; Scheffe multiple comparison procedure).

place to avoid freezing in the snow (Somme 1976), some hymenopteran gall-formers overwinter beneath the snow but above ground level (Shorthouse et al. 1980), and lady beetles aggregate under rocks and debris (Harper and Lilly 1982). Oriental cockroaches appear to survive the winter in the temperate region without freezing of the body fluid by microhabitat selection. This may explain the observation by le Patourel (1993) that they were able to survive for several weeks at 2°C. Urban outdoor habitats for oriental cockroaches (Shuyler 1956, Mampe 1972, Nixon 1984, Thoms and Robinson 1986), such as sewers, dumpsites, crack-and-crevices of buildings, wall voids, and other outdoor facilities might be a satisfactory refuges to avoid the cold winter temperatures.

In conclusion, supercooling capacity alone does not appear to be the primary overwintering strategy for the freeze-intolerant oriental cockroach. It is more reasonable to believe that the urban outdoor environment provides abundant harborages for cold avoidance by the oriental cockroach. Oriental cockroach seems to benefit from the urban outdoor environment with abundant harborages available for cold avoidance by microhabitat selection.

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