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## Supercooling Point of Western Bean Cutworm (Lepidoptera: Noctuidae) Collected in Eastern Nebraska

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### Abstract

Western bean cutworm, *Striacosta albicosta* (Smith) (Lepidoptera: Noctuidae), is a pest of maize and dry beans that has recently undergone a northeastern range expansion in North America. In order to assess the cold tolerance of *S. albicosta*, we determined the supercooling point of lab and field collected late instar larvae and pre-pupae. Individuals were attached to fine contact thermocouples and cooled at 1°C per minute to detect heat released due to freezing of body fluids. Mean supercooling points decreased as larvae developed into later life stages. Pre-pupa collected in late fall had a mean supercooling point of -12.63°C. This research is the first documentation of cold tolerance measures for *S. albicosta* and will aid in designing future cold tolerance experiments and predicting *S. albicosta* population densities based on winter temperatures.

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Western bean cutworm, *Striacosta albicosta*, (Smith) (Lepidoptera: Noctuidae), is a native, univoltine pest of maize and dry beans in North America (Michel et al. 2010). In recent years, significant *S. albicosta* infestations in maize have been observed beyond the previously described range in the west-central United States (Hoerner 1948, Miller et al. 2009). The pest was recently documented in several northern, Midwestern states, including Iowa (Rice 2000), Minnesota (O'Rourke and Hutchison 2000), and eastward into Michigan, Ohio and Ontario, Canada (Michel et al. 2010). *S. albicosta* females emerge and oviposit during mid-summer, and larvae feed within the maize ear causing damage to kernels (Holtzer 1983). Sixth instars mature in late summer, burrow into the soil, and construct a soil chamber approximately 12-25 cm from the soil surface (Michel et al. 2010). Pre-pupae are non-mobile sixth instars that undergo a reduction in size, overwinter within the chamber, and emerge as adults the following summer (Antonelli 1974, Seymour et al. 2010).

Cold stress can often limit the distribution of insect species (Denlinger and Lee 2010, Morey et al. 2012). Determining the temperature at which fluids freeze within an insect, i.e., the supercooling point, is a basic primary measurement of cold tolerance (Carrillo et al. 2005). The supercooling point of an insect can be detected by the release of heat from water crystallization as the water within the insect freezes. The supercooling point is the lowest temperature reached before this increase in temperature as the insect cools. The temperature increase can be detected by attaching the insect to a thermocouple connected to a data logger recording the temperature of the insect as it cools. Some insects can survive freezing (i.e., freeze-tolerant), while others may die upon freezing (i.e., freeze-intolerant) or mortality may even occur before freezing (i.e., chill-intolerant) (Sømme 1982, Bale 1987, Denlinger and Lee 2010). Several cold

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tolerance studies have examined only supercooling points as a measure of cold tolerance to determine the effects of feeding, body size, and life stage on cold tolerance (e.g., Suh et al. 2002; Hahn et al. 2008; Jones et al. 2008). The supercooling point is typically the basis for further measures of insect cold tolerance to determine mortality due to exposure temperature and time (e.g., Eaton and Kells 2011, Soudi and Moharrampour 2011, Morey et al. 2012).

The intent of this research was to determine the temperatures at which *S. albicosta* individuals freeze and assess the freeze-tolerance of *S. albicosta* as the cold tolerance for the species had not previously been assessed. Determination of the supercooling point will aid in designing future cold tolerance measures for *S. albicosta* that may be used to determine its potential distribution based on cold tolerance.

## Methods

**Rearing and Collection.** Larvae and pre-pupae were obtained from field collection and lab colonies in 2010 and 2011 from multiple rearing conditions (Table 1). The insects, in each year, came from the same generation of egg masses collected in the field or in cages with moths. In 2010, the fifth and sixth instars larvae were obtained from an experimental field at the Northeast Research and Extension Center Haskell Agricultural Laboratory, Concord, NE. Neonates were infested 21- 23 July on maize hybrid DKC 61-72 (YieldGard, Monsanto, St Louis, MO) expressing *Bacillus thuringiensis* Berliner (Bt) protein Cry1Ab, which is not toxic to western bean cutworm (Catangui and Berg 2006, Eichenseer et al. 2008). Larvae were collected approximately 25 days after infestation. The larvae were kept at room temperature (~ 25°C) and 10:14 (L:D) h photoperiod, inside jelly cups with a mixture of equal portions soil, sand, and vermiculite until supercooling measurement of fifth and sixth instars on 19 August.

In 2011, pre-pupae were laboratory reared, and collected in the field. Neonates were lab reared in growth chambers to pre-pupae at Concord, NE. Supercooling points were measured for four different groups of individuals from a lab reared cohort of neonates. Three groups were reared from neonates to pre-pupae 18:6 h (27:20°C) thermoperiod and (L:D) photoperiod. Supercooling points were measured for one group on 3 August and the second on 23 August. The third group was also reared to pre-pupae under the same conditions as the previous two groups, but placed in 15°C and 0:24 (L:D) h conditions 24 days before supercooling point measurements on 6 October to simulate conditions the pre-pupae may experience after burrowing into the soil. The fourth group of neonates was reared at 25°C at 0:24 (L:D) h to pre-pupae and also held at 15°C and 0:24 (L:D) h conditions 24 days before supercooling point measurements on 6 October. Field populations of pre-pupae were obtained by infesting plots at Concord and Clay Center, NE with 4<sup>th</sup> instars infested on 5 August in the maize ear (hybrid DKC 61-72 RR) during the blister or milk stage (Ritchie et al. 1993). The larvae were confined in the ear with flat mesh (25 × 30 cm) pollination bags (Paula-Moraes, 2012). The insects at Clay Center were collected 12 September and reared at 25°C and 0:24 (L:D) h photoperiod. The pre-pupae were then held at 15°C and 0:24 (L:D) h photoperiod for 24 days before supercooling point measurements on 6 October. Pre-pupae were also collected from the field in Concord, NE between 20–23 October at the Northeast Research and Extension Center Haskell Agricultural Laboratory. After collection, the insects were reared at 15°C 18:6 (L:D) h followed by 9°C 0:24 (L:D) h for three days before supercooling point measurements on Oct. 28.

**Supercooling Point Measurement and Freeze Mortality.** Individuals were shipped overnight in insulated coolers to the University of Minnesota in Saint Paul, MN before supercooling point measurements. Individual larvae and pre-pupae were placed on a 32-gauge copper-constantan thermocouple inside a 30cc plastic syringe. Pre-pupae were removed from the soil chamber

Table 1. Life stages and rearing conditions of tested *S. albicosta* larvae and pre-pupae.

Life Stage	Origin and Conditions	SCP Measurement Date	Rearing
5th Instar	field collected <sup>1</sup>	8/19/2010	Rearing at 25°C and approximately 10:14 (L:D) h after collection.
6th Instar	field collected <sup>1</sup>	8/19/2010	Rearing at 25°C and approximately 10:14 (L:D) h after collection.
Pre-pupa	field collected mid-September <sup>2</sup>	10/6/2011	25°C and 0:24 (L:D) h. 15°C and 0:24 (L:D) h 24 days before supercooling point measurement.
Pre-pupa	field collected late-October <sup>3</sup>	10/28/2011	Held at 9°C 0:24 (L:D) h three days before cold exposure.
Pre-pupa	lab colony	8/3/2011	18:6 h (27:20°C) thermoperiod and (L:D) photoperiod.
Pre-pupa	lab colony	8/23/2011	18:6 h (27:20°C) thermoperiod and (L:D) photoperiod.
Pre-pupa	lab colony	10/6/2011	18:6 h (27:20°C) thermoperiod and (L:D) photoperiod. 15°C and 0:24 (L:D) h photoperiod 24 days before supercooling point measurement.
Pre-pupa	lab colony	10/6/2011	25°C and 0:24 (L:D) h. 15°C and 0:24 (L:D) h 24 days before supercooling point measurement.

<sup>1</sup> Collected approximately 17 August 2010.<sup>2</sup> Collected 14 September 2011.<sup>3</sup> Collected 20-23 October 2011.

if a chamber had been built. The larvae were cooled at 1.0°C per minute in a -80°C freezer by placing the syringes inside calibrated polystyrene cubes (Carrillo et al. 2004). The thermocouple wire was attached to a multi-channel data to monitored temperature changes with TracerDAQ Pro 2.1.6.1. Fifth and sixth instars were cooled past the supercooling point and mortality was not assessed. All pre-pupae were removed from the cube and warmed to room temperature after detection of an exotherm when the temperature decreased below the supercooling point to assess freeze-tolerance. Control specimens from all groups did not undergo the supercooling point measurement to compare mortality of individuals experiencing ice formation with unexposed individuals. Individuals with an observed supercooling point and unexposed individuals were reared at 25°C and 50-60% relative humidity. Mortality was assessed as lack of movement and blackening of specimens five days after exposure.

**Statistical Analyses.** Data were analyzed in SAS 9.3 (SAS Institute 2012). Normality of supercooling point distributions were assessed with PROC UNIVARIATE and transformed with a Box-Cox transformation to fulfill assumptions of normality and homogeneity of variance (Box and Cox 1964). Individual absolute supercooling point temperatures were transformed with a Box-Cox transformation ( $\lambda = -1.9$ ). ANOVA and orthogonal contrasts ( $\alpha = 0.95$ ) of rearing treatments were performed with PROC GLM. The proportion of mortality in control groups for each rearing treatment was used to correct the proportion mortality of those that froze using a modified Abbot's correction (Abbott 1925, Rosenheim and Hoy 1989).

## Results

ANOVA indicated a significant effect between the tested groups on mean supercooling point ( $F = 3.77$ ,  $df = 7$ ,  $P = 0.001$ ) (Fig. 1a and b). Orthogonal contrasts ( $\alpha = 0.95$ ) indicated mean supercooling points of fifth and sixth instars were significantly different (Table 2). Mean supercooling points were not significantly different between active sixth instars and pre-pupae from larvae collected in September and October (Table 2). Non-acclimated, 18:6 h (27:20°C) thermoperiod and (L:D) photoperiod, lab reared pre-pupae did not have significantly different supercooling points (Table 2). Since the two non-acclimated lab reared groups were not significantly different, further comparisons for the two groups were pooled. The mean supercooling point of the lab-reared cold acclimated group that was reared under the same conditions as the non-acclimated group was significantly different than the non-acclimated mean supercooling point, but the cold acclimated group reared under 0:24 (L:D) h conditions was not significantly different than the non-acclimated group (Table 2). All individuals with an observed supercooling point no longer moved or had blackened cuticle, excluding lab reared pre-pupae exposed 23 August 2011 and field collected pre-pupae exposed 6 October, 2011 (Fig. 1c and d). All treatment and control individuals failed to emerge as adults, so adult emergence could not be used as a measure of mortality.

## Discussion

The significantly higher supercooling point for fifth instars than sixth instars may indicate that the supercooling point decreases as larvae develop to pre-pupae, but the temperatures experienced as a pre-pupa in late summer and fall do not further decrease the supercooling point. However, the supercooling points were significantly different between acclimated and unacclimated lab reared pre-pupae reared at 18:6 h (27:20°C) thermoperiod and (L:D) photoperiod. This difference could be explained by either the length of the cold acclimation period, or time spent as a pre-pupa. Since the cold acclimated group spent more time as pre-pupae than any other group in this study, the lower mean supercooling point

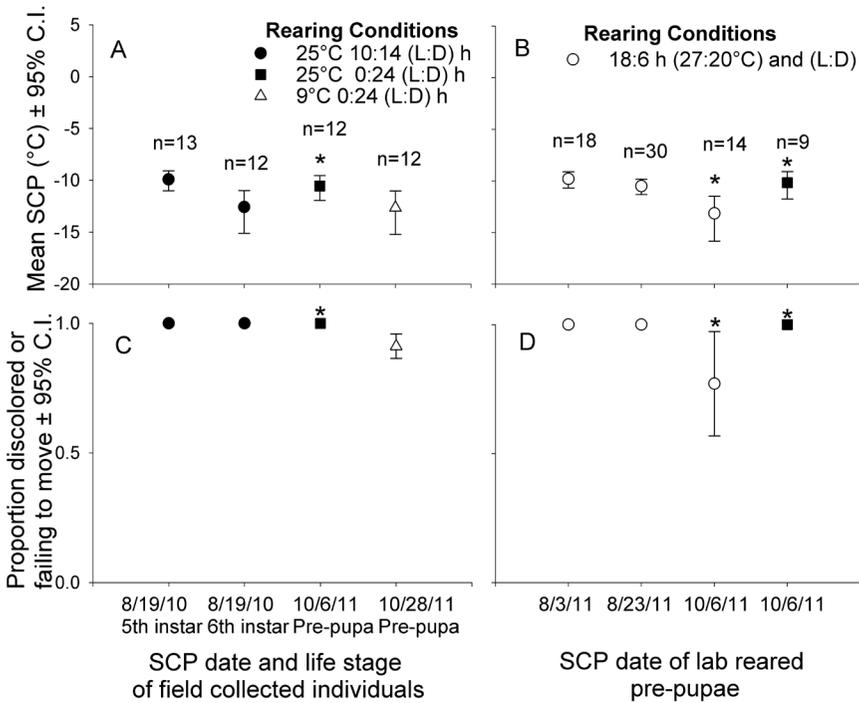


Figure 1. Back-transformed mean supercooling points ( $\lambda = -1.9$ ) of *S. albicosta* from (A) field collected larvae and prepupae and (B) lab reared pre-pupae, as well as proportion of individuals showing discoloration or no movement five days after freezing from (C) field collected larvae and prepupae, (D) lab reared pre-pupae. \* 15°C and 0:24 (L:D) h photoperiod 24 d before SCP measurement.

could be the result of additional time to develop cryoprotection measures, or initiate diapause (Pullin 1996). However, the group reared at 25°C 0:24 (L:D) h was not significantly different than the unacclimated group even after the group reared in total darkness underwent a cold acclimation period. These results suggest that complete lack of daylight throughout development through early instars to prepupal stages does not decrease the supercooling point of *S. albicosta*. However, these results should be interpreted with caution, since continuous darkness is an artificial lab condition that early instars would not experience in the field.

Another possible interpretation of the differences between the cold acclimated group with the unacclimated group is the difference in rearing temperature regimes experienced as early instars. The group reared at 27 to 20°C had a lower supercooling point than the unacclimated group, while the group reared at 25°C did not. These differences in temperature regimes could suggest that temperatures experienced by instars before burrowing in to the soil could drive changes in the supercooling point. Further cold tolerance studies would need to control for both photoperiod and temperature prior to the pre-pupal stage to determine how the supercooling is affected. Cooler temperatures below 15°C could also be used for cold acclimation to determine the effects on supercooling point and mortality.

Table 2. Contrasts of mean supercooling point for *S. albicosta* larvae and pre-pupae.

Contrast	df	F Value	Pr > F
Field collected: 5th vs 6th instars <sup>1</sup>	1	7.10	0.0089
Field collected: 6th instars <sup>1</sup> vs. Sept. collected pre-pupae <sup>2,5</sup>	1	3.31	0.0717
Field collected: 6th instars <sup>1</sup> vs. Oct. collected pre-pupae <sup>3</sup>	1	0	0.9682
Lab reared: 8/3 <sup>4</sup> vs 8/23 <sup>4</sup>	1	1.51	0.2211
Lab reared: Non-acclimated <sup>4</sup> vs 0:24 (L:D) h photoperiod <sup>2</sup>	1	0	0.9593
Lab reared: Non-acclimated <sup>4</sup> vs Cold acclimated <sup>4,5</sup>	1	12.69	0.0005

<sup>1</sup>Collected from Concord, NE. Reared at 25°C and approximately 10:14 (L:D) h.

<sup>2</sup>25°C and 0:24 (L:D) h. 15°C and 0:24 (L:D) h 24 d before supercooling point measurement.

<sup>3</sup>Held at 9°C 0:24 (L:D) h 3 d before supercooling point measurement.

<sup>4</sup>18:6 h (27:20°C) thermoperiod and (L:D) photoperiod.

<sup>5</sup>15°C and 0:24 (L:D) h photoperiod 24 d before supercooling point measurement.

Individuals within two groups did show movement 5 days after cold exposure. This could potentially be an indication of freeze-tolerance (Sinclair 1999). However, freeze injury could have also occurred that did not immediately cause mortality within five days. These potentially freeze-tolerant individuals were both in rearing treatments where temperature was decreased to 15° or 9°C for a period of time, which could be an indication of increased cold tolerance due to acclimation. However, field collected pre-pupae that were also acclimated to 15°C did not survive freezing. Since many individuals turned black and no movement occurred after cold exposure in all other groups, most *S. albicosta* primarily appear freeze-intolerant or chill-intolerant, but we cannot exclude that a portion of *S. albicosta* populations may be freeze-tolerant. We could not use adult emergence as a measure of mortality because very few unexposed individuals developed into pupae, and no adults eclosed. Since most unexposed individuals were either moving or had no noticeable damage, while individuals that experienced ice formation typically were blackened five days after the treatment date, unknown factors other than handling, such as rearing, may have affected development. At the very least, high mortality occurs when overwintering *S. albicosta* pre-pupae freeze. The most likely group from our data to represent overwintering *S. albicosta* would be pre-pupae collected from the field, late in the year (20 and 23 Oct. 2011), as they likely received cues to enter diapause by being exposed to declining fall photoperiod and temperatures. The back-transformed mean supercooling point of -12.63°C can be used to determine target exposure temperatures for further analyses of cold tolerance in *S. albicosta*.

Only using the supercooling point as a measure of cold tolerance, however, has its limitations (Jones et al. 2008). For instance, in areas where *S. albicosta* has been documented, minimum annual soil temperatures are typically well above -12.63°C (Fig. 2). However, cold mortality can occur by mechanisms other than freezing. Cold mortality could occur above the supercooling point if *S. albicosta* is chill-intolerant. Other noctuid maize pests that overwinter in the soil, such as *Agrotis ipsilon* (Hufnagel) and *Helicoverpa zea* (Boddie), have mean supercooling points of -20.7°C and -19.3°C, respectively, which are lower than those for *S. albicosta* (Beck 1988, Morey et al. 2012). *H. zea* also experiences soil temperatures warmer than its mean supercooling point. However, extended periods of cold exposure between 5°C and 0°C can lead to significant *H. zea* mortality (Morey et al. 2012). The effect of exposure time may also be an important factor in *S. albicosta* cold tolerance. In summary, our results indicate that when soil temperatures drop to -12.63°C, approximately 50% of *S. albicosta* will freeze. High mortality was associated with freezing, although freeze-tolerance within the population could not be ruled out by using movement after freezing as a measure of cold mortality.

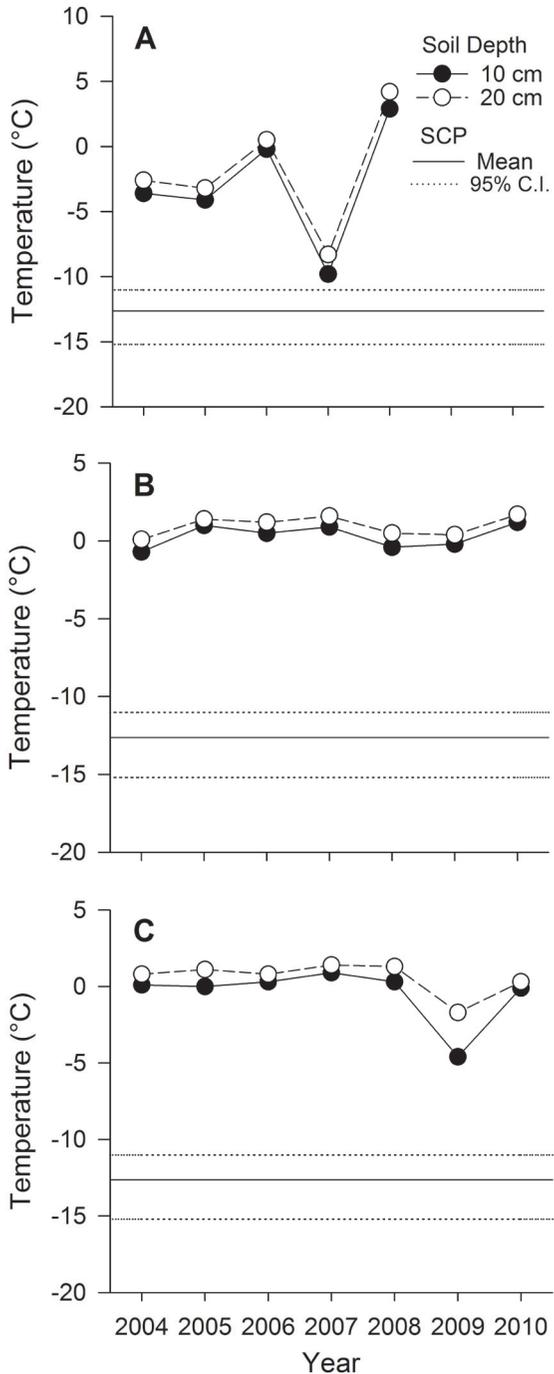


Figure 2. Minimum annual winter soil temperatures in: (A) Minnesota (47° 43' N 96° 16' W), (B) Iowa (42° 1' N 93° 44' W), and (C) Nebraska (40° 51' N 96° 28' W). Each year represents the minimum soil temperature between 1 May of that year and 1 September of the previous year (available at [www.wcc.nrcs.usda.gov](http://www.wcc.nrcs.usda.gov)). Horizontal solid and hashed lines represent back-transformed mean supercooling point and 95% confidence intervals, respectively, of field collected pre-pupae in late October.

Since *S. albicosta* commonly are exposed to winter soil temperatures above the mean supercooling point of  $-12.63^{\circ}\text{C}$ , further cold tolerance studies should focus on mortality at temperatures above the supercooling point for chill mortality, effects of exposure time, and environmental effects that may lead to further cold acclimation. Future studies should also include long term measures of mortality, such as adult emergence, to determine if some pre-pupae are freeze-tolerant or if freeze mortality is not always apparent five days after freezing.

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