Cold Hardiness of *Helicoverpa zea* (Lepidoptera: Noctuidae) Pupae

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ABSTRACT An insect's cold hardiness affects its potential to overwinter and outbreak in different geographic regions. In this study, we characterized the response of *Helicoverpa zea* (Boddie) pupae to low temperatures by using controlled laboratory measurements of supercooling point (SCP), lower lethal temperature (LT_{50}), and lower lethal time (LL*Time*). The impact of diapause, acclimation, and sex on the cold hardiness of the pupae also were evaluated. Sex did not significantly affect the SCP, LT_{50}, or LL*Time*. However, the mean SCP of diapausing pupae (−19.3°C) was significantly lower than nondiapausing pupae (−16.4°C). Acclimation of nondiapausing pupae to constant temperatures from 10 to 20°C before supercooling also produced a significantly lower SCP than nondiapausing pupae held at 25°C. The LT_{50}s of nondiapausing and diapausing were not significantly different, but confirmed that *H. zea* pupae are chill-intolerant because these lethal temperatures are warmer than the corresponding mean SCPs. Diapausing pupae survived longer than nondiapausing pupae at the same, constant, cold temperatures, a finding consistent with the SCP results. Both of these results suggest enhanced cold hardiness in diapausing pupae. When laboratory results were compared with field temperatures and observed distributions of *H. zea* in the contiguous United States, the laboratory results corroborated what is currently perceived to be the northern overwintering limit of *H. zea*; approximately the 40th parallel. Moreover, our research showed that areas north of this limit are lethal to overwintering pupae not because of low temperature extremes, but rather the length of time spent at near-zero temperatures.

KEY WORDS corn earworm, supercooling point, lower lethal temperature, lower lethal time

The corn earworm, *Helicoverpa zea* (Boddie), is an endemic, but highly mobile and damaging lepidopteran pest of many agricultural crops in North America, including sweet corn, tomatoes, cotton, and sorghum (Kennedy and Storer 2000, Flood et al. 2005). *Helicoverpa zea* overwinters as a diapausing pupa in the soil and, after eclosion, migrates throughout most of the eastern and midwestern United States and southern Canada during the growing season; however, it is not currently known to overwinter north of the 40th parallel (Hardwick 1965). In northern regions, growers of many crops targeted by *H. zea* presently can avoid most infestations by planting high-value crops earlier in the season before the migrating moths arrive. However, early planting is not always feasible. In addition, shifts in the overwintering range could lead to significant changes in the timing and magnitude of *H. zea* infestations in northern latitudes. Accurate projections of the current and future overwintering distribution of *H. zea* depend on assessments of its response to low temperatures.

Cold hardiness is the capacity of an organism to survive low temperatures (Lee 1991). Insects commonly respond to cold in three ways: with freeze-tolerance, freeze-avoidance or intolerance, or chill-intolerance (Lee 2010). The supercooling point (SCP) is the temperature at which an insect's internal fluid freezes. In conjunction with the SCP, the lower lethal temperature can be used to categorize an insect's response to cold by briefly exposing the insect to temperatures around the mean SCP and measuring mortality. If most mortality occurs at the SCP, the insect is freeze-intolerant, below the SCP it is freeze-tolerant, and above the SCP it is chill-intolerant (Lee 2010). Duration of exposure can greatly affect the mortality at low temperatures, so the lower lethal time can be measured for a given temperature (Sinclair 1999). Most of the early work on insect cold hardiness centered around the physiological and biochemical mechanisms of surviving or avoiding freezing as the primary determinant of cold hardiness (Bale 1987). This emphasis recently has been scrutinized for largely ignoring other deleterious effects of low temperatures, such as prefreeze mortality and length of exposure time (Sinclair 1999). More comprehensive and ecologically relevant assessments are needed to accurately describe the mechanisms of an insect's response to cold stress (Bale 1987, 1991).

The effect of low temperature on *H. zea* pupae has been explored previously (Quaintance and Brues 1905, Ditman and Cory 1931, Barber and Dicke 1939,

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Dicke 1939, Mangat 1965), but most of this research was limited to field studies in the early and mid-20th century that did not explicitly account for important factors, such as thermal history of pupae or temperature-time interactions. Other studies were more detailed and controlled (Ditman et al. 1940, Miller 1967, Slosser et al. 1975, Eger et al. 1982), but lacked consistency in how and what aspects of cold hardiness were measured. Most recently, Eger et al. (1982) provided a comprehensive analysis of *H. zea* mortality at 0, −4, and −10°C, but soil temperatures do not always fall below 0°C, particularly in northern areas with snow cover (Groffman et al. 2001, Decker et al. 2003). Consequently, there is a need to conduct further analysis of *H. zea* cold hardiness that explicitly includes the effects of prefreezing temperatures, exposure time, and cold acclimation on pupal mortality.

In this study, we characterize the response of *H. zea* pupae (the overwintering stage) to low temperatures by using controlled laboratory measurements of supercooling point, lower lethal temperature, and lower lethal time. In addition, we examine important factors that could influence cold hardiness of *H. zea* pupae, including sex, acclimation, and diapause. The ecological relevance of our data and potential future applications of this work within the context of climate change and management of *H. zea* also are discussed.

Materials and Methods

Colony Source. Early instar *H. zea* larvae, infested on artificial wheat-germ diet modified from Shaver and Raulston (1971) as per Blanco et al. (2009), were obtained from a laboratory colony in Stoneville, MS (adults and eggs reared at 27.5°C, with a photoperiod of 14:10 [L:D] h). Larvae were reared in individual cups to pupation in programmable growth chambers (Percival Scientific, Inc., Perry, IA) in St. Paul, MN. Rearing conditions were manipulated to produce either nondiapausing pupae (25°C ± 1°C, 65–80% RH, and a photoperiod of 14:10 (L:D) h or diapausing pupae (first 9 d after hatch held at 21°C, 65–80% RH, and a photoperiod of 12:12 (L:D) h; next 4 d held at 20°C, 65–80% RH, and a photoperiod of 11:13 (L:D) h remaining time until treatment held at 18°C, 65–80% RH, and a photoperiod of 10:14 (L:D) h. Diapause was confirmed by observing the retention of larval stemmatal pigments in the postgenal region at least 6 d after pupation (Phillips and Newsom 1966).

Supercooling Point (SCP) Determination. Pupae were attached to thermocouples by using high vacuum grease (Dow Corning, Dow Corning Corp., Midland, MI) and cooled at a rate of 0.5°C min^{-1} in calibrated Styrofoam cubes placed inside a freezer held at −80°C (Carrillo et al. 2004). Temperatures were recorded every second by using a multi-channel data logger. The SCP was identified as the lowest temperature reached before an abrupt spike in temperature, indicating the release of latent heat of fusion. Preliminary results (data not shown) indicated that pupae did not supercool below −23°C, so temperatures were logged until −30°C. Six pupal groups were used: nondiapausing and unacclimated pupae (held at 25°C [14:10 (L:D) h]) until the time of testing), nondiapausing and acclimated pupae (cooled 0.5°C/d from 25°C to 20, 18, 15, or 10°C beginning 1 d after pupation), and diapausing pupae induced into diapause as described above.

Lower Lethal Temperature (LL*Temp*) Determination. Pupae were attached to individual thermocouples and cooled at a rate of 0.3°C min^{-1} in a freezer (as in SCP determination) to temperatures below −10°C, or a programmable growth chamber (Percival Scientific Inc, Perry, IA) for temperatures above −10°C. Once the desired temperature treatment was reached (either 0°C, −5°C, −10°C, or each one degree increment from −10 to −21°C, inclusive), insects were held at this temperature for approximately 3 min and returned to 25°C at a rate of 0.3°C min^{-1}. Two pupal groups were used: nondiapausing and diapausing. After cold exposure, nondiapausing pupae were held at 25°C (±1°C, 65–80% RH, and a photoperiod of 14:10 [L:D] h) until eclosion or death. Diapausing pupae, however, were held at 27°C (±1°C, 65–80% RH, and a photoperiod of 0:24 [L:D] h) until eclosion or death to terminate diapause (Wellso 1966). Thirty pupae were used for each temperature above −10°C. For temperatures below −10°C, 2–24 pupae were used based on the ability to reach the desired temperature without the pupa supercooling.

Lower Lethal Time (LL*Time*) Determination. Diapausing and nondiapausing pupae were cooled at a rate of 0.3°C min^{-1} in a programmable growth chamber to either −10, −5, 0, or 5°C (±2°C, with a photoperiod of 0:24 [L:D] h) and held for at least three different time periods per temperature. These times varied between temperature treatments and pupal groups, but ranged from 0 to 5 h for −10°C, 0–96 h for −5°C, 0–1,218 hr for 0°C, and 0–1,456 hr for 5°C. Three or four replications were used per temperature per time treatment, with a sample size of 25 or 15 pupae, respectively. After low temperature exposure, pupae were returned to 25°C (±1°C, 65–80% RH, and a photoperiod of 14:10 [L:D] h) at a rate of 0.3°C min^{-1}, and held until adult eclosion or death. Death included incomplete adult eclosion or blackened pupae showing no response to gentle probing with forceps after 1 wk. A control for nondiapausing pupae was held at a constant 25°C (±1°C, 65–80% RH, and a photoperiod of 14:10 [L:D] h) until eclosion or death. The diapausing control pupae were held at 17–18°C (65–80% RH, and a photoperiod of 10:14 [L:D] h) until the end of cold exposure in the treatment groups, at which time they were warmed to a constant 27°C (±1°C, 65–80% RH, and a photoperiod of 0:24 [L:D] h) and monitored for eclosion or death.

Forecasting Field Survival by Latitude. To explore the potential meaningfulness of our laboratory results, we compiled soil temperature data from the U.S. Department of Agriculture-Natural Resource Conservation Service (USDA-NRCS) National Water and Climate Center (www.wcc.nrcs.usda.gov) for five states across the United States. Based on decades of field observations, *H. zea* pupae are known to successfully

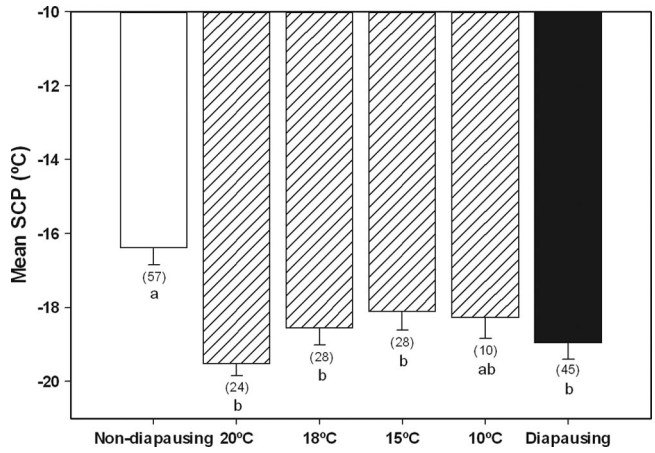

Fig. 1. Mean supercooling points (SCP) for nondiapausing, nondiapausing and acclimated, and diapausing *H. zea* pupae. White bar indicates nondiapausing (held at 25°C), striped bars indicate nondiapausing and acclimated pupae (gradually cooled as pupae from 25°C to specified temperature, and black bar indicates diapausing pupae (gradually brought to 18°C and a photoperiod of 10:14 [L:D] h). Columns with the same letters are not significantly different ($P < 0.05$). Numbers in parentheses indicate sample size. Error bars represent standard error.

overwinter in areas south of approximately the 40th parallel, and do so at an average soil depth of 10.16 cm (Hardwick 1965, Flood et al. 2005). Therefore, we used soil temperature profiles (at 10.16 cm) for states across a latitudinal gradient from ≈45° N to 30° N and compared their lowest annual temperature, as well as total time spent at 0°C and 5°C (±2°C), two of the LL*Time* temperatures measured for diapausing pupae. Data for at least 5 of the 6 yr between 2004 and 2010 were obtained for each state.

Analysis. SCPs were analyzed using analysis of variance (ANOVA) (ANOVA; PROC GLM, SAS Institute 2005), after application of an $x^{0.5}$ transformation as recommended by the Box Cox procedure (PROC TRANSREG, SAS Institute 2005). Means were separated where significant treatment effects were seen (protected least significant difference (LSD); PROC GLM, SAS Institute 2005).

LL*Temp* data were analyzed using Proc LIFETEST (SAS Institute 2005) to compare the survivor functions between diapausing and nondiapausing pupae, while adjusting for sex. The LT_{50} for diapausing and nondiapausing pupae were provided by Proc LIFETEST.

LL*Time* data were fit with regression models by using TableCurve (SYSTAT Software 2002) to estimate the relationship between time and mortality for each temperature treatment. Models were fit within nondiapausing and diapausing groups for each temperature. Selection of the models was based on r^2 values, lack-of-fit tests, and distribution of residuals (Draper and Smith 1998). The associated times to 25, 50, and 95% mortality ($LLTime_{25, 50, \text{or } 95}$) were then calculated for each treatment based on these functions. Curves were compared between diapausing and nondiapausing pupae for each temperature, while adjusting for sex, using Proc LIFETEST (SAS Institute 2005) to describe any differences in time until mortality.

Results

Supercooling Point (SCP). Sex did not significantly affect pupal SCP (df = 185; $P = 0.72$) so data were pooled for further analysis. Diapause and acclimation of nondiapausing pupae had a significant effect on the SCP as compared with unacclimated, nondiapausing pupae (df = 191; $P < 0.0002$). Supercooling points for all acclimated treatments were significantly lower than the unacclimated treatment, except for 10°C (Fig. 1). Diapausing pupae also differed significantly from the unacclimated pupae, with diapause producing a lower mean SCP (−19.3°C versus −16.4°C). There were no significant differences among the acclimated treatments, or between the acclimated and diapausing pupae (Fig. 1).

Lower Lethal Temperature (LL*Temp*). There was no significant difference between the LL*Temp* mortality curves of diapausing and nondiapausing pupae (Log-rank; $\chi^2 = 1.832$, df = 1; $P = 0.18$). Similarly, sex did not have a significant effect on mortality (Log-rank; $\chi^2 = 0.402$, df = 1, $P = 0.53$). The estimated LT_{50} for nondiapausing and diapausing pupae were −13.0°C (95% CI: −14 to −11°C) and −10.0°C (95% CI: −12 to −10°C), respectively (Fig. 2), but were not significantly different based on overlapping 95% confidence intervals.

Lower Lethal Time (LL*Time*). For nondiapausing pupae, mortality over time was modeled for all the temperature treatments; −10, −5, and 5°C were all fit with a nonlinear logistic dose–response curve, and 0°C was fit with a Lorentzian cumulative curve (Table 1; Fig. 3). Estimated times until 25, 50, 75, and 95% mortality based on these models are presented in Table 2. For diapausing pupae, however, a regression model could only be fit to the 0°C and 5°C data, with the Lorentzian cumulative curve used for both (Table 1). Mortality never exceeded 53% in −10°C and −5°C during the times chosen for diapausing pupae (Fig. 4),

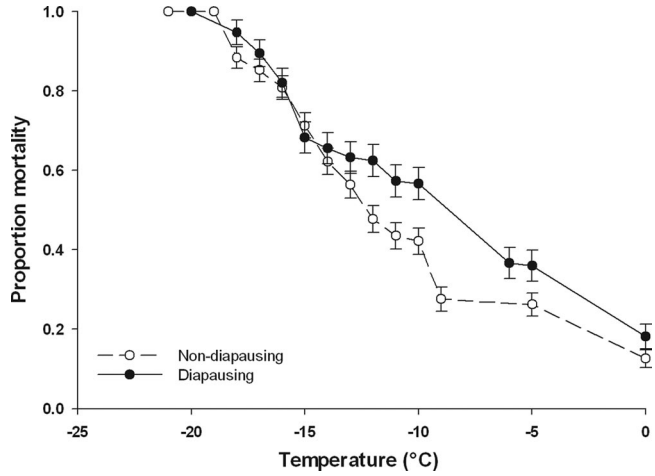

Fig. 2. Lower lethal temperature mortality responses for nondiapausing and diapausing *H. zea* pupae. Error bars represent standard error. Response curves did not differ (Log-rank; $\chi^2 = 1.832$, df = 1; $P = 0.18$). LT_{50} estimates from Proc LIFETEST for nondiapausing and diapausing pupae were −13°C and −10°C, respectively.

so a model could not be reliably fit. The mortality curves for 0°C were shown to be significantly different between diapausing and nondiapausing pupae (Log-rank; $\chi 2 = 7.81$, df = 1, $P = 0.005$), with sex still having no significant effect on survival (Log-rank; $\chi 2 = 0.26$, df = 1, $P = 0.61$). A comparison between the 5°C curves could not be made because they were described with different models. Comparisons for −10°C and −5°C could also not be calculated because of the aforementioned low mortality in diapausing pupae of these groups.

Forecasting Field Survival by Latitude. The temperature profiles for a soil depth of 10.16 cm at selected sites in Minnesota, Iowa, Kansas, Missouri, and Texas are presented in Table 3. None of the sites reached the LT_{50} for diapausing pupae during 2004–2010. The time spent at 5°C never reached the LL$Time_{50}$ for diapausing pupae (2064.30 h) at any of the sites. However, the time spent at 0°C was sufficient to reach the LL$Time_{50}$ for diapausing pupae (1127.37 h) during some years, with the number of lethal years increasing with latitude; none of the years in the most southern sites (Texas and Missouri) experienced lethal exposure times, half of the years were lethal in Kansas, two-thirds of the years were lethal in Iowa, and all of the years were lethal at the most northern site (Minnesota).

Discussion

Previous studies have concluded that *H. zea* pupae are freeze-intolerant (Hardwick 1965). We confirmed this result but believe it is more accurate to consider the pupae chill-intolerant. Chill-intolerance indicates that the LT_{50}s of both diapausing and nondiapausing pupae were warmer than their respective SCPs, demonstrating that *H. zea* pupae are unable to withstand freezing and are subject to much prefreeze mortality. In our study, sex did not influence the cold response of pupae, which corroborate the previous findings of Ditman et al. (1940). Acclimation of nondiapausing pupae to any temperature below 20°C immediately preceding subzero exposure appeared to impart an increased cold hardening equivalent to diapause. The 10°C acclimation group did not show a statistical difference from the unacclimated control, but this was likely because of insufficient sample size in this treatment group; only ten pupae were still alive for testing by the time 10°C was reached. This increase in cold hardening with most acclimation was only demon-

Table 1. Model parameters from regression analysis relating temperature and time of exposure in nondiapausing and diapausing *H. zea* pupae

	Temperature (°C)	Parameter estimates (± SEM)			r^{2a}	max r^2	*F*-value	*P*>*F*
		a	*B*	*c*				
Nondiapausing	−10[b]	98.38 (6.14)	3.66 (0.23)	−4.97 (1.15)	0.94	0.96	138.02	<0.0001
	−5[b]	96.76 (10.91)	38.03 (5.11)	−5.23 (2.29)	0.84	0.88	36.82	<0.0001
	0[c]	100.25 (1.98)	474 (16.32)	4.65 (13.50)	0.99	0.99	947.87	<0.0001
	5[b]	1.00 (0.02)	717.90 (13.45)	−7.23 (0.61)	0.99	0.99	878.10	<0.0001
Diapausing	0[c]	102.26 (3.50)	1,130.95 (54.30)	106.13 (48.96)	0.97	0.98	275.03	<0.0001
	5[c]	98.91 (4.20)	2062.72 (4.22)	91.87 (105.93)	0.99	0.99	543.01	<0.0001

[a] adjusted for degrees of freedom.
[b] Logistic dose equation, $y = a / \{1 + (x / b)^c\}$.
[c] Lorentizian cumulative equation, $y = a / \pi\{\arctan[(x-b)/c] + \pi/2\}$.

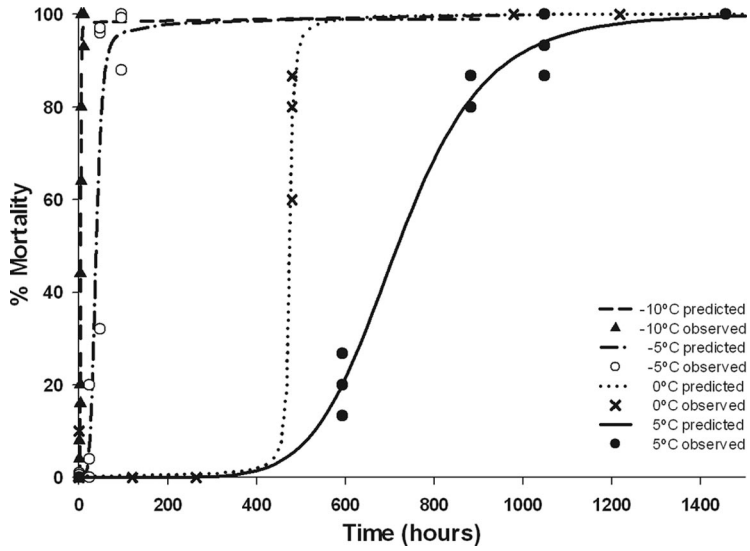

Fig. 3. Percent mortality of nondiapausing *H. zea* pupae over time held at −10, −5, 0, and 5°C. Equations used to describe the mortality trends are given in Table 1. Dots represent observed mortality for each time treatment replication.

strated through SCP comparisons. Because of limited resources, acclimation treatments were not included in other procedures, but future study should continue to examine its effect, both as a potential rapid cold hardening-like effect (Lee et al. 1987) in pupae, and as a conditioning experienced in the larval stage of nondiapausing pupae. Ecological relevance of these types of nondiapausing acclimations would not likely be important for considering the overwintering capabilities of pupae (nondiapausing pupae would develop long before winter ends), but they could be influential in maintaining early fall or spring populations that may be subject to brief cold spells not preceded by the necessary cues to enter diapause. The SCPs found here fell within the range of previously published SCP values for *H. zea* (Barber and Dicke 1939, Roberts et al. 1972, Ditman et al. 1940), although direct comparison is difficult because of high variability between experimental conditions (e.g., food source, humidity, equipment, age, and thermal history of the pupae), which can affect the SCP.

Time can have a dramatic effect on the lethality of a given temperature. Moreover, the time necessary to cause mortality at one temperature can be orders of magnitude different with just a few degrees change in temperature. Our data demonstrated this for four different temperatures with nondiapausing pupae, but it is reasonable to assume the same pattern applies to diapausing pupae. This was evident when comparing the LL*Time* data for 0°C between the two groups. Both diapause and nondiapause mortality were described with the same model, but with diapause requiring significantly more time (2,660 hr) to reach the same 95% mortality achieved with nondiapausing pupae after 502 hr (Table 2).

The comparison between diapausing and nondiapausing pupae, showing nearly a five-fold difference in time until the 95% mortality, highlights the enhanced cold hardiness gained through diapause in *H. zea* pupae. The significantly lower SCP of diapausing versus nondiapausing pupae further supports this conclusion. Enhanced cold hardiness with diapause is frequently seen in other insect systems (e.g., Carrillo et al. 2005, Régnière and Bentz 2007). This pattern also agrees with previous findings of Eger et al. (1982) and Ditman et al. (1940) for *H. zea*, although the SCPs and specific degree to which we found mortality to change with time differs. Eger et al. (1982) generally showed more

Table 2. Estimated percent mortality for nondiapausing and diapausing *H. zea* pupae, calculated using regression equations (Table 1)

Mortality	Time until mortality (hours)					
	Nondiapausing			Diapausing		
	−10°C	−5°C	0°C	5°C	0°C	5°C
25%	2.94	31.08	478.92	616.54	1,021.07	1,972.41
50%	3.68	38.52	474.31	717.55	1,127.27	2,064.30
75%	4.62	48.18	469.66	834.83	1,226.59	2,159.45
95%	7.15	81.56	502.32	1,073.38	1,599.17	2,769.92

Mortality included incomplete adult eclosion and dead pupae. Models could not be fit to all temperature treatments for diapausing pupae due to insufficient data.

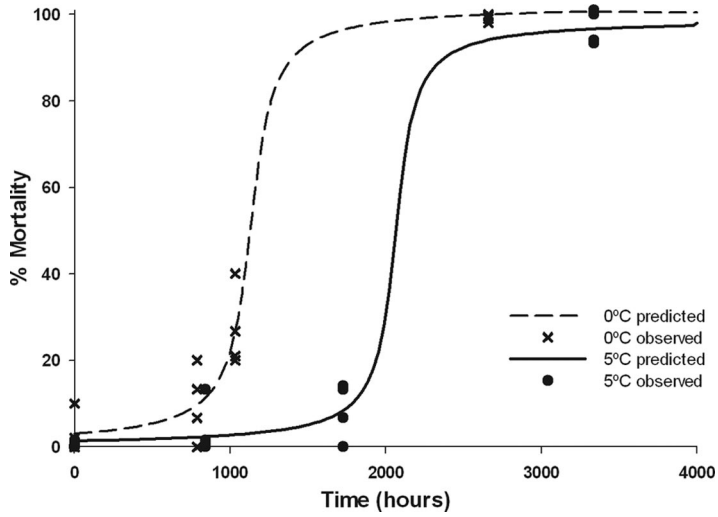

Fig. 4. Percent mortality of diapausing *H. zea* pupae over time held at 0°C, and 5°C. Equations used to describe the predicted mortality trends are given in Table 1. Dots represent observed mortality for each time treatment replication.

time needed for a given level of mortality. The SCPs in Ditman et al. (1940) were similar to those presented here, but on average a few degrees lower. Reasons for the discrepancies are unknown, but likely were because of differences in methodology and equipment used. Eger et al. (1982), for example, used a single model for all temperature treatments between −10 and 10°C to calculate the various time-mortality relationships, but it was based only on data collected for temperatures between −6°C and 6°C. Attempts in the current study to describe all time–temperature relationships with a single regression model led to large discrepancies in the predicted and observed values in many instances (data not shown). There also was no

Table 3. Temperatures experienced at soil depths of 10.15 cm for years between September 2004 and September 2010

		Lowest temp (°C) reached	Time spent at 0°C ± 2°C (h)	Time spent at 5°C ± 2°C (h)
Minnesota	2004–2005	−6.4	*2,006	927
45° 25′ N	2005–2006	−4.3	*3,070	653
93° 57′ W	2006–2007	−8.5	*2,703	1,058
	2007–2008	−6.5	*2,460	—
	2008–2009	—	—	—
	2009–2010	−0.4	*2,679	1,028
Iowa	2004–2005	0.9	*1,813	949
42° 1′ N	2005–2006	0	1,011	1,401
93° 44′ W	2006–2007	0.8	*1,443	1,726
	2007–2008	−0.4	*2,443	768
	2008–2009	−0.3	*2,575	1,138
	2009–2010	0	429	1,170
Kansas	2004–2005	−3.8	1,058	1,488
39° 42′ N	2005–2006	−0.3	840	1,675
96° 10′ W	2006–2007	−2.6	*1,352	1,391
	2007–2008	0.1	*1,837	1,174
	2008–2009	−0.6	*1716	1,314
	2009–2010	0.5	958	977
Missouri	2004–2005	0.5	264	1,400
37° 4′ N	2005–2006	1.1	191	1,671
93° 53′ W	2006–2007	0.4	799	1,109
	2007–2008	0.5	468	1,504
	2008–2009	—	—	—
	2009–2010	0.3	1,028	964
Texas	2004–2005	7	0	3
30° 5′ N	2005–2006	5.7	0	45
95° 59′ W	2006–2007	4.3	0	134
	2007–2008	—	—	—
	2008–2009	8.5	0	0
	2009–2010	4.8	0	94

Asterisked values indicate years where the predicted time to reach 50% mortality of diapausing *H. zea* pupae was reached. Coordinates indicate the weather station site used for a given state. Data obtained from (www.wcc.nrcs.usda.gov).

mention of cooling or warming rates with their procedure, a detail that can be quite influential in measuring a cold response (Miller 1978).

Lack of differences in cold hardiness between nondiapausing and diapausing pupae seen in the LL*Temp* studies appears to contradict the conclusion of cold hardiness enhancement through diapause. However, it is important to consider the utility of this type of experiment. The LL*Temp* study lends itself more to being an empirical, instantaneous observation of cold response, rather than one reflecting natural, gradual cooling. LL*Temp* is important to articulate the true lower lethality of temperature to an insect, as both diapausing and nondiapausing *H. zea* pupae die before their SCPs, and do so because of mechanisms other than freezing. However, it may not be useful in portraying the impact of ecologically relevant factors, such as diapause or the lethality of above-zero temperatures over time. The importance of diapause may not be apparent until conditions like those naturally experienced in the field are mimicked, so the use of LL*Temp* as a single descriptor of cold hardiness is limited. The same can be said for SCP. Although in this experiment the trends of SCPs concur with LL*Time* in indicating enhanced cold hardiness with diapause, the specific degree of cold hardiness (i.e., the SCP itself) largely overestimated the long-term temperature tolerances of this insect. Despite their limitations, though, these procedures also should not be rejected. LL*Time* is the most direct, ecologically relevant procedure, but is greatly enhanced when the SCP and LL*Temp* can serve as proxies for its design and provide additional insight into the physiology of a cold response.

Temperature is most commonly implicated as the driving force of overwintering survival, which, for insect pest species, often is a reliable indicator of spring infestation dynamics (Trˊan et al. 2007). Cold hardiness measurements can offer important insights into overwintering dynamics, as long as they reflect ecologically meaningful conditions. When our cold hardiness results for diapausing pupae are compared with the time and temperature data compiled from the USDA-NRCS database (Table 3), it is interesting to note that at no point in any of the five states did the soil temperatures reach the mean LT_{50} (−8.8°C), and certainly not the SCP (−19.3°C). This implies that it is not the extreme temperatures that prevent overwintering *H. zea* survival in a given area, but rather the length of time spent at slightly warmer ones. This is likely because of the insulating properties of its subterranean overwintering microhabitat, the effect of which has been noted in other insect systems (Lam and Pedigo 2000, Carrillo et al. 2005, Cárcamo et al. 2009). Bale (1991) echoes the pioneering observation of Salt (1966) in stating that the most significant factor determining mortality at low temperatures in nature is period of exposure. Our results further support this, showing that SCP and LL*Temp* (measures of extreme response) may not be as ecologically relevant as the LL*Time* procedure (a measure of long-term response) for characterizing insect cold hardiness. Our study also corroborates previous field observations for *H. zea* (Flood et al. 2005); there is a decreasing incidence of survival with increasing latitude, and the shift from predominate survival over time to predominate mortality occurs very near 40° N (Table 3). In conclusion, the combined effects of temperature and time on *H. zea* mortality seen in the laboratory appear to provide relevant information regarding the consequences of cold exposure under some field conditions for this insect.

To increase the utility of these data for grower application, such as assessing the impact of climate change on the overwintering range, additional studies should be done to complete the LL*Time* profile for diapausing pupae because this is the most cold-hardy and, therefore, the overwintering stage. Inclusion of additional microclimate factors, such as moisture and soil type, that may interact with the effects of soil temperature in pupal burrows also is important.

Given the dominating influence of temperature on overwintering *H. zea* populations, cold stress thresholds can be coupled with the monitoring of weather data to alert growers of areas most conducive to potential source populations for migrating infestations and where to optimize monitoring of moth flights (Sandstrom et al. 2007). This allows for more informed management decisions regarding integrated pest management (IPM) strategies, such as the use of transgenic corn hybrids in selected production areas (Burkness et al. 2010). Similarly, cold stress data could be incorporated with climate change scenarios to model long-range shifts in the overwintering range of this insect (Venette et al. 2010).

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