Corn earworm (*Helicoverpa zea* Boddie), cold hardiness, and climate change: Implications for future distributions and IPM

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Dedication

This thesis project is dedicated to *Helicoverpa zea* (Boddie); without you, this work would not exist.

Abstract

The cold hardiness of *Helicoverpa zea* (Boddie) pupae was assessed using three laboratory methods: supercooling point (SCP), lower lethal temperature (LL*Temp*), and lower lethal time (LL*Time)* determination. Mean SCPs for pupae ranged between -16.4°C and -19.5°C, depending on whether pupae were in diapause or had been acclimated. The LL*Temp* at which 50% mortality occurred (LT_{50}) for diapausing and non-diapausing pupae was -8.8°C and -12.4°C, respectively, though the LL*Temp* mortality curves were not significantly different. The time until 95% mortality for non-diapausing pupae held at -10°C, -5°C, 0°C, and 5°C was 7.2, 81.6, 502.3, and 1073.4 hrs, respectively. Time until 95% mortality for diapausing pupae held at 0°C and 5°C was 2660.19 and 2796.92 hrs, respectively. Sex did not have an influence on cold hardiness. Diapause greatly enhanced cold hardiness in pupae as indicated by a significantly lower mean SCP and longer time to reach mortality at a given temperature compared to non-diapausing pupae. However, given mean SCP comparisons, acclimation of non-diapausing pupae had a cold hardening effect comparable to diapause.

In-field evaluation of overwintering *H. zea* survival in southern Minnesota showed that temperature was a severely limiting factor in overwintering success, though likely not responsible for complete mortality. Laboratory data, coupled with the field results suggest that a small proportion of pupae may be able to survive in Minnesota. However, field observations also suggest that sufficient degree days may not be available during autumn in southern Minnesota to allow for substantial pupation before the onset of winter, thus eliminating the potential for an overwintering population.

Using the cold hardiness data generated for diapausing pupae, the present and future distributions of *H. zea* in North America were calculated with the modeling software CLIMEX. The resulting maps depicting the current distribution of *H. zea* from CLIMEX did not agree with what is currently understood for *H. zea* overwintering distributions and overall geographic suitability; contrary to convention, cold stress is shown to not be a significant constraint to *H. zea* suitability for most of the U.S. Despite the discrepancies in current projections, the present study corroborated Diffenbaugh *et al.* (2008) in illustrating a northern expansion of suitability for *H. zea*, under future climate

change. The implications of potential northern expansion in the geographic range of *H. zea* are discussed within the context of future Integrated Pest Management (IPM) needs for sweet corn, as well as other vegetable and field crops throughout North America.

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CHAPTER I.
Review of the Literature
a. Corn Earworm (*Helicoverpa zea)*

PHYLOGENY/ Nomenclature

Helicoverpa (Lepidoptera: Noctuidae), as established by Hardwick (1965), is a globally distributed genus of 18 described species occurring in tropical and warm-temperate regions. It is represented in North America only by *zea*, which is considered one of the most derived species of the genus (Hardwick 1965). Prior to the work of Hardwick and others (e.g. Common 1953), present day *H. zea* was considered conspecific to numerous other species so historical references should be interpreted with caution; *Bombyx obsoleta* Fabricius, *Noctua amigera* Hübner, *Chloridea obsoleta*, *Heliothis obsoleta*, *armigera*, and *umbrosus* can all refer to *Helicoverpa zea* if the origin is in the New World (Ditman & Cory 1931, Pogue 2004). Additionally, *H. zea* was previously included in the genus *Heliothis* until being placed into *Helicoverpa* by Hardwick (1970) based on morphological characters of the male genitalia. For in-depth phylogenic reviews of *H. zea* and related Heliothines, see also Hardwick (1966, 1970) and Mitter *et al*. (1993).

Due to its highly polyphagous nature, *H. zea* is known by many common names, such as corn earworm, tomato fruitworm, tobacco bollworm, sorghum headworm, false tobacco budworm, cotton bollworm, vetchworm, and shatter-worm (e.g. Sherman 1914, Laffoon 1960, Burkett *et al.* 1983, Dowd & Lagrimini 1997). In early literature, it is also simply referred to as 'the worm' (Quaintance & Brues 1905).

BIOLOGY

Life cycle

Female *H. zea* moths oviposit their eggs at night and have a total fecundity range of 500-3000 (Ditman *et al.* 1931, Hardwick 1965). Eggs appear as ivory domes (becoming brown with age), laid singly and typically near the flowers and fruit of the host plant (Hardwick 1965). In corn, the preferred oviposition host, eggs are most often found on fresh silks, but also on leaf tissue and among tassels in earlier and later stage plants (McColloch 1920). Larvae hatch from the eggs about two to six days following oviposition in the summer months and go through five to six instars. Though early

instars appear pale, they develop myriad colors over time, from pale green to red to pink to almost black (Flood *et al.* 2005). Features distinguishing *H. zea* larvae from other common caterpillar pests include a light-colored head capsule and many black micro-spines along the body. Alternating dark and light lines run lengthwise down their backs. Late instars of *H. zea* can also be distinguished from *Heliothis virescens*, a species similar in appearance that also occurs in cotton, by an extra tooth-like structure on the mandible of the latter species (UC-IPM 1996). Mature corn earworm larvae cease feeding ca.16 days (at 25°C) after hatching and then drop to the ground to pupate in the soil. The average pupal burrow length is 8.9 cm, but can range from 2.5 to 17.8 cm depending on the soil conditions and if the pupae are diapausing (Hardwick 1965). Once fully sclerotized, pupae are a mahogany-brown and average 18-19 mm in length and 0.4 g in weight (Quaintance & Brues 1905). As pupae, they either eclose into adults ca.13 days after pupation, or they enter into diapause and remain in the soil for multiple months to overwinter; in either case, female moths emerge about a day before males (Hardwick 1965, Hogg & Calderon 1981). Moths are a buff to greenish-color with the most conspicuous wing marking being a dark comma-shaped spot on the forewings. They have a wingspan of about 3.8 cm and are most active at night, usually surviving between 5 and 17 days after emergence (Quaintance & Brues 1905, Hardwick 1965, Flood *et al*. 2005).

Developmental times of *H. zea* are influenced by a number of environmental factors, but temperature is the primary driver. While the calendar day approximations stated above are often used for convenience, a measure of "physiological time" is also essential to accurately describe developmental time in terms of temperature dependence. The total amount of heat required, between the lower and upper thresholds, for an organism to develop from one point to another in its life cycle is calculated in units of degree-days (Pedigo & Rice 2006). Table 1 summarizes many of the experimentally determined degree-day requirements for *H. zea*, both as individual stages and total (from egg hatch to adult emergence).

Host plants

As larvae, *H. zea* are highly polyphagous and feed on over 100 cultivated and non-cultivated (wild) host plants. The preferred larval host is corn (*Zea mays* L.), but

many other cultivated crops can be attacked including high-value vegetables, such as tomatoes (*Solanum lycopersicum* L.), peppers (*Capsicum* spp.), lettuce (*Lactuca sativa* L.), and snap beans (*Phasiolus vulgaris* L.), as well as important field crops such as cotton (*Gossypium hirsutum* L.), tobacco (*Nicotiana tabaccum* L.), sorghum (*Sorghum bicolor* L.), and soybeans (*Glycine max* Merr.) (Stadelbacher 1980, Tillman & Mullinix 2004, Foster & Flood 2005, Hutchison *et al.* 2007). At least 76 uncultivated host plants have been documented for *H. zea* including crown and black vetch (*Coronilla varia* L.), common mallow (*Malva neglecta* Wallroth), velvetleaf (*Abutilon theophrasti* Medikus), sunflower, wild geranium (*Geranium dissectum* L.), crimson clover (*Trifolium incarnatum* L.), and toadflax (Neunzig 1963, Harding 1976, Stadelbacher 1981, Sudbrink & Grant 1995, Blanco *et al.* 2007). The ability to feed on a variety of wild host plants allows *H. zea* to extend its multivoltine populations before and after the growing season; late season wild hosts may also be important sites for overwintering populations (Sudbrink & Grant 1995). As late instars, *H. zea* are also highly cannibalistic and predaceous to other lepidopteran larvae (Boyd *et al.* 2008, Dorhout & Rice 2010). Adults collect nectar and water from many plants, often trees and scrubs such as *Citrus*, *Quercus*, *Betula*, clover, and milkweed (Capinera 2008).

Geographic distribution and migration

Helicoverpa zea is found throughout much of North and South America (including Hawaii), as well as the West Indies (Hardwick 1965). It is multivoltine and active year–round in tropical and subtropical climates. However, its voltinism becomes progressively more restricted with increasing latitude in North America due to inclement weather. *H. zea* pupae can enter a winter diapause (discussed below) that facilitates persistence of resident overwintering populations in temperate regions, but overwintering is limited to latitudes <40°N (Hardwick 1965, Lindgren *et al.* 1994). Luttrell *et al.* (2010) present a map to illustrate the current understanding of *H. zea*'s overwintering range in North America (Figure 1), using known or inferred life history parameter estimates important for population growth and persistence. Despite this apparent overwintering boundary, the migratory behavior of *H. zea* allows them to annually infest crops beyond this limit, reportedly up to the 52nd parallels of Saskatchewan in the north

and the Falkland islands in the south (Hardwick 1965); in the northern U.S., local populations from the south and Mexico have been implicated as the sources of annual infestations (Sandstrom *et al.* 2007, Luttrell *et al.* 2010).

Migration of *H. zea* is highly dependent on weather and wind patterns, and is known to be driven by surface and upper wind currents (Sandstrom *et al.* 2007). Based on visual observations and imaging, aerial collection, and radar monitoring, specific information regarding *H.zea* migration is increasingly being described. Moth flight is initiated shortly after sunset and begins with a spiral ascent to at least 50m above ground level, at a rate of 1 m s^{-1}. Average flight speeds of individuals reach ~4.5 m s^{-1} and orientations are frequently distributed downwind (Westbrook 2008). Following ascent, migrants redistribute vertically into one or more layers at altitudes between 300 and 900m (Beerwinkle *et al.* 1994, Westbrook 2008). Radar and pollen markers have indicated that individual moths are capable of travelling several hundred kilometers in a single night (Lingren *et al.* 1994, Westbrook 2008). Sandstrom *et al.* 2007 developed a risk forecasting scheme for pest managers in the U.S. to identify potential *H. zea* infestations throughout the growing season, based on weather patterns. They found that major *H. zea* flight events strongly correlated in areas with an eastern high-pressure cell, a western low-pressure cell, and a frontal boundary associated with the low-pressure cell. Moths are lifted into the lower atmosphere by convective cells and are then transported northwards through the low-level jet-stream created by the pressure cells, eventually dropping out of transit at the frontal boundary, or "Drop Zone" (Sandstrom *et al*. 2007).

In northern states, migrating adults may arrive as early as May or as late as August due to the variations associated with weather; thus, their population biology following migration is variable. The number of generations in northern areas such as southern Canada, Minnesota, and Wisconsin is one (Capinera 2007); two-three in northeastern and –western states such as Maryland and Oregon (Ditman & Cory 1931, Coop *et al.* 1993), three to four generations in regions such as Arkansas and Kentucky (Garman & Jewett 1914, Isely 1935), and between four to seven in southern states like Louisiana, Florida and Texas (Capinera 2007).

There is indirect evidence that shows *H. zea* undergoes a reverse migration from northern regions in the fall (Beerwinkle 1994, Westbrook 2008) but details of this occurrence are still being investigated.

Diapause

The general phenomenon of diapause and its relation to cold hardiness will be discussed in the next section. Here, I describe diapause as it relates to *H. zea*, specifically, how it is initiated and maintained, how it is terminated, and some of its effects on *H. zea* life history.

Initiation

Helicoverpa zea pupae are known to enter facultative diapause (Phillips & Newsom 1966, Meola & Adkisson 1977). That is, diapause that is mediated by environmental stimuli rather than being genetically predetermined, or obligatory (Klowden 2007). The interaction between temperature and photoperiod is vital to initiate pupal diapause, with the photoperiodic response being temperature dependent (Roach & Adkisson 1970). Phillips and Newsom (1966) demonstrated that diapause may be induced in *H. zea* pupae by holding penultimate and late larval instars at photoperiods of 10 hours and prevented with photoperiods of 14 hours. They also noted that progeny of adults that underwent diapause had reduced incidence of diapause. But others (Wellso & Adkisson 1966, Roach & Adkisson 1970) argued that the photoperiodic requirements for induction of diapause in *H. zea* are more complex. They found that sensitivity to photoperiod resides not only in all larval instars, but also the parents and eggs, with the parents and eggs requiring longer photoperiod cues than their resulting larvae. This photoperiod reduction from adult to larva must also be concomitant with a temperature reduction (Roach & Adkisson 1970).

For the subsequent work, I found that slight revision to the larval regimes described in Eger *et al.* (1982) and Meola & Adkisson (1977) produced equal or higher incidence of diapause in my lab-reared colonies (Table 2).

To ensure diapause is being maintained, there are some physiological characters unique to the diapause state that can be used as indicators. In many Lepidoptera,

pigments from larval ocellar stemmata persist into the pupal stage after leaving the ocelli; they can be seen externally as a series of dark spots in the postgenal region (Figure 2). These pigments eventually shift position and degrade away during pharate adult development, an event termed stemmatal pigment retraction (SPR) (Phillips & Newsom 1966, Meola *et al.* 1983). If these "eyes pots" are retained, however, this indicates a lack of adult development and is an easy, reliable indicator of diapause in *H. zea* and in other species (Phillips & Newsom 1966, Shumakov & Yakhimovich 1955). The age of the pupa and/or temperatures at which it was maintained must be known for the use of eyespots to be accurate. Based on parameters from Wellso (1966), and assuming a 12.6°C threshold (Hogg & Caldren 1981), developing pupae can be reliably distinguished from diapausing pupae about 34-42 DD (3-4 days at 25°C) after pupation, as the former will have retracted stemmata by this point. Many studies wait until at least 6 days after pupation for greater certainty (Phillips & Newsom 1966, Roach & Adkisson 1970, Akkawi & Scott 1984). Additional physiological characteristics of diapause in *H. zea* include nearly a 3-fold reduction in oxygen consumption, and cessation of reproductive development (spermatogenesis in males and gondal development in females) (Phillips & Newsom 1966).

Termination

Unlike its initiation, termination of pupal diapause is driven by temperature, with photoperiod having little effect. Diapausing pupae held under no light (0:24 L:D), though, terminated diapause faster than pupae held under similar temperatures with varying photoperiods, with no differences in termination times seen among those with photoperiod (Wellso 1966, Roach & Adkisson 1971). In contrast to many other insects, chilling also does not show an apparent effect on diapause termination in *H. zea* and a refractory phase of diapause does not exist (Wellso 1966, Roach & Adkisson 1971). *H. zea* diapause is also not due to a deficiency of the prothoraciotropic hormone (PTTH) as in many other insects, but rather PTTH potentiates the prothoracic gland to secrete α-ecdysone in a temperature-dependent manner, which then initiates adult development. These characteristics allow *H. zea* to potentially terminate diapause within just a few days after initiation if held at 27°C (Meola & Adkisson 1977). Similar to the emergence

pattern of non-diapausing pupae, diapausing females usually terminate diapause faster than males (Roach & Adkisson 1971). While conducting field studies in Texas, Wellso (1966) found that diapause termination in the spring appears to be dependent, in part, upon the conditions encountered when entering diapause the previous year; the first pupae to enter diapause in the fall are the last to break it in the spring, and conversely, those last to diapause are the first to break.

Life history effects

Akkawi and Scott (1984) evaluated the effect of diapause in *H. zea* on subsequent progeny and found that diapausing females laid fewer eggs, laid eggs with longer developmental periods, and produced larvae with longer development periods compared to non-diapausing females. The authors calculated that larvae of diapausing parents required ca. 50 more degree days (>12ºC, < 35ºC) to develop than larvae of non-diapausing parents. Moreover, as stated previously, the incidence of diapause in successive F_1 and F_2 generations from diapausing parents was shown to be significantly reduced (Phillips & Newsom 1966).

Like with many other insect species (e.g. Mansingh 1974, Lee & Denlinger 1985, Lefevere *et al.* 1989), diapause has been implicated in enhancing the cold hardiness of *H. zea* pupae, as this is the overwintering stage (Ditman *et al*. 1940, Eger *et al.* 1982). It is not surprising then that the incidence of diapause in *H. zea* increases with increasing latitude (Fitt 1989).

ANTHROPOGENIC IMPACTS and MANAGEMENT

The impact of *H. zea* on humans lies primarily in the conflict of overlapping appetites. Included among the list of *H. zea*'s numerous host plants (see above) are many that humans also bestow high value to (e.g. corn, tomato, cotton, peppers, beans), but only in the absence of insect protein. The relationship of *H. zea* as a major pest to humans is well over a century old with the first recorded report of corn earworm as a pest in corn (then *Heliothis obsoleta*) from Illinois in 1842 (Quaintance & Brues 1905). By the early 1900s, it was cause for serious concern as noted by the dismay of researchers that it was "depriving the citizens of the South of [their] favorite vegetable [sweet corn]"

(Quaintance & Brues 1905). Today, it is considered by some to be the most costly crop pest in North America (Capinera 2008).

Fitt (1989) attributes the pest status of *H. zea* (and many other Heliothines) to a suite of physiological, behavioral, and ecological characteristics: polyphagy, mobility, high fecundity, and facultative diapause. These characters combined enable them to successfully exploit numerous agroecosystems across many regions. How much of an impact *H. zea* exerts as a pest is dependent upon the synchrony of oviposition with susceptible (attractive) host plant stages (Johnson *et al.* 1975). Therefore, cultural control practices, such as timing the planting of crops to keep the most attractive oviposition stage out of synch with the presence of moths, were recognized early on as effective measures (Hardwick 1965). The use of naturally resistant host plant varieties (e.g. Matthews *et al.* 2007), as well as trap-cropping (e.g. Capinera 2008) also afford some level of control.

Several natural enemies, including *Trichogramma* species (Hymenoptera: Trichogrammatidae) parasitize *H. zea* eggs, and can inflict significant mortality in some environments (Vargas & Nishida 1980). Larval parasitism is less common, but can be caused by *Cotesia* spp., *Microplitis cropeipes* (Hymenoptera: Braconidae), *Campoletis* spp. (Hymenoptera: Ichneumonidae), *Eucelatoria armigera*, and *Archytas marmoratus* (Diptera: Tachinidae). Generalist predators such as lady beetles (e.g. *Harmonia axyridis*, *Hippodamia convergens*, *Coleomegilla maculata*) lacewing larvae (*Chrysoperla* spp. and *Hemerobius* spp.), minute pirate bugs (*Orius* sp.), and damsel bugs (*Nabis* sp.) also feed on *H. zea eggs* and small larvae (Quaintance & Brues 1905, Vargas and Nishida 1980). The entomopathogenic nematode, *Steinernema riobravis*, is a natural cause of pupal mortality in the southern US and has shown potential as an inundative biocontrol agent (Feaster & Steinkraus 1996). The ubitquitous soil mite, *Tyrophagus putrescentiae* (Shrank), was observed to cause mortality in *H. zea* pupae in the laboratory, though additional research is required to verify field interaction (Appendix A). In the upper Midwest region, generalist predators are more likely to have significant impact on *H. zea* than parasitoids. But even then, efficient and effective management using biological control agents are only possible when used in conjunction with alternative products such as Dipel™ (*Bt*), oils, or transgenic crops (Morey *et al.* 2010). Ultimately, foliar-applied

synthetic insecticides are the most effective and commonly used control measure for *H. zea* across systems, but are being replaced by transgenic crops in many regions, including *Bt* sweet and field corn (e.g. Burkness *et al.* 2002).

Minnesota sweet corn

Minnesota is the leading producer of processing sweet corn in the United States with 122,400 acres harvested in 2009, yielding a production value of $97.5 million (NASS 2010). Minnesota also contributes ~9,900 acres to the high-value fresh market industry (Hutchison & O'Rourke 2002). *H. zea* has become one of the most consistent and damaging pests in upper Midwestern sweet corn; damage being the presence of larvae, damaged kernels and/or larval frass in the ear (Hutchison *et al.* 2007). Moreover, given the dramatic suppression of European corn borer (*Ostrinia nubilalis* Hübner) following *Bt*-corn adoption (Hutchison *et al.* 2010, *in press*), *H. zea* has become the primary insect concern for this crop.

Use of foliar insecticides, primarily synthetic pyrethroids has historically been the most common and effective means of control (Hutchison *et al.* 2007). Action thresholds based on moth catches (using pheromone or black light traps) have been developed to aid growers in deciphering when the first insecticide application is most appropriate (e.g. Flood *et al*. 2005). More recently, Burkness *et al.* (2009) found that a pyrethroid-based program for *H. zea* can be optimized by delaying the first spray until 90-100% silk, providing an alternative to the conventional method of applying multiple sprays during silking.

Managing *H. zea* larvae with conventional foliar-applied insecticides, though, can be difficult due to the variability of adult flights (within and among years) and dynamic weather and field conditions. Additionally, foliar-applied insecticides are ineffective once the larvae have moved from the silks into the ear because they become protected by husk tissue (O'Rourke & Hutchison 2004, Hutchison *et al*. 2007). With the advent of transgenic corn, such as the *Bt*-11, this is no longer an issue because the toxin is expressed throughout the plant tissue (Sims *et al.* 1996). Though early Bt-corn genetics were somewhat problematic in their efficacy against *H. zea* (Storer *et al.* 2001, Burkness

et al. 2002), recent advances have produced "stacked" hybrids that offer more effective control for larvae (Hutchison & Storer 2010)

Because of very low tolerances for damage in sweet corn, sole reliance on biological control of *H. zea* is not realistic for most growers, especially commercial. However, work involving a similar agricultural pest, *O. nubilalis,* indicates that biocontrol could be enhanced when combined with other tactics, such as host plant resistance, transgenic sweet corn (e.g. *Bt*), or foliar sprays with little or no risk to non-targets (e.g. *Bt*, Rynaxypyr) (Hutchison *et al*. 2004).

These control tactics for Minnesota and the upper Midwest are all based on the fact that *H. zea* is currently only economically damaging in late-planted crops; its presence is limited by the timing of conditions necessary for it to migrate and inflict damage to the area. As will be discussed in the coming sections, alterations to these conditions, specifically temperature, could greatly impact the timing and infestation levels of *H. zea* to the region. Consequently, these infestation changes could then demand reevaluation of any management tactic if control is to be maintained.

CHAPTER I.
Review of the Literature
b. Cold Hardiness

Ectotherms, such as insects, are greatly affected by daily and seasonal temperature cycles. With minimal ability to regulate their own body temperature, processes from cell membrane permeability (*e.g.* Kostal 2010) to population dynamics and distribution (*e.g.* Bale *et al.* 2002, Kingsolver 1989) to directions of evolution (*e.g.* Taylor 1981), can all be directly influenced by an insect's thermal environment. For insects inhabiting temperate regions, such as *H. zea*, low temperature can be particularly limiting to their life history, most frequently during the winter months. The capacity of an insect to survive cold temperature exposure is referred to as *cold hardiness* and can be affected by a number of factors, including developmental stage, nutritional status, thermal history, and genetic potential (Lee 1991). "Cold" and "low" temperatures are of course relative terms, and vary depending on the species; many tropical insects may become highly impaired at temperatures of 10°C, whereas some arctic and alpine insects are still fully functional below -10°C (Lee 2010). In relation to *H. zea* and this review, low temperatures will generally refer to those below its lower developmental threshold, or <12°C.

A search for distinct categories of insect responses to cold temperature can lead to a number of definitions and proposed terminology (Ring 1982, Lee 1991, Bale 1996, Worland *et al.* 1997, Ramløv 1998, Sinclair 1999, Sinclair *et al.* 2003). The most recent definitions provided by Lee (2010) will be used for this review and are as follows: chilling intolerant, freezing intolerant, and freezing tolerant. *Chilling intolerance* refers to insects that are fatally injured, directly or indirectly, by the effects of low temperature before internal ice formation occurs. *Freezing intolerance* (or avoidance) refers to insects that can survive cold as long as ice does not form within their bodies. *Freezing tolerance* refers to insects that can survive freezing within their bodies, but typically do so only over a limited temperature range (Lee 2010).

For this review, I will first provide information on key physiological concepts involved with insect cold hardiness, followed by a description of common methodologies used to assess it in the laboratory. Then, I will briefly discuss implications of cold

hardiness for pest management and end with a summary of the work done so far concerning the response of *H. zea* to low temperatures. The focus of this review will be on freezing intolerance/avoidance and chilling intolerance as *H. zea* likely falls into one of these categories due to its known inability to withstand freezing (Eger *et al*. 1982). For comprehensive reviews of additional aspects of insects and low temperatures, see Lee and Denlinger (1991), Duman *et al.* (1991b), and Denlinger and Lee (2010).

PHYSIOLOGICAL EFFECTS OF COLD

Damage

Low temperature damage in insects is often described in terms of freezing. Numerous mechanisms of freeze injury have been identified, such as cellular dehydration and shifts in cellular pH due to solutes becoming more concentrated in the hemolymph. Mechanical damage can result from tissue separation caused by the growing ice lattice as well as the phenomenon of recrystallization, a process in which the size and distribution of ice crystals change after initial freezing (Lee 1991). Though not as well understood, damage can also occur from non-freeze injury, as is evidenced by the mortality seen in chilling intolerant species and mortality seen from prolonged exposure to non-freezing temperature (e.g. Turnock *et al.* 1983). Most often, this injury is attributed to cell membrane failure from phase transitions in membrane phospholipids; as temperature decreases, the viscosity of lipids increases, altering membrane permeability of solutes and active transport systems (Kostal 2010, Holmstrup *et al.* 2010).

Prevention of damage

To overcome the various deleterious effects of cold, insects employ an equally diverse array of physiological and biochemical coping mechanisms. *Supercooling*, or the ability to depress hemolymph temperatures below the melting point (MP) without freezing, is a critical component of sub-zero tolerance in insects, be they freeze-tolerant or freeze-avoidant. Physical studies have shown that supercooling capacity (MP minus SCP) is inversely related to the water volume of the sample (Angell 1982). Most insects have an innate tendency to supercool at least a few degrees, in part because of this phenomenon; they "behave as small vessels of water". Immediate internal freezing following exposures below 0°C is also staved off because of circulating hemolymph

solutes (*e.g.* proteins, sugars, ions) that colligatively depress the melting point (Lee 2010).

In freeze-avoidant insects, the supercooling capacity can be enhanced by the presence of *cryoprotectants*. Though this term has been used in the cold hardiness literature to describe numerous substances, cryoprotectants are defined by Lee (2010) as compounds that enhance cold- and freezing-tolerance, thus, protecting against both chilling and freezing injury. Using this definition, freeze-avoidant insects primarily utilize the cryoprotectants known as *antifreezes*. Antifreezes are most commonly known as low-molecular weight solutes, such as polyhydroxl alcohols and sugars, that depress the freezing point of water by strictly colligative means (Dunman *et al.* 2010). Glycerol is the most common polyol seen in insects, with sorbitol, mannitol, ribitol, erythritol, threitol, and ethylene glycol also occurring. Sugars used include trehalose, sucrose, glucose, and fructose (Storey & Storey 1991). Antifreezes have also been recognized to decrease the freezing point of water through non-colligative means. These antifreezes are high-molecular weight molecules, namely proteins, that prevent inoculative freezing and inhibit ice nucleators in the gut, hemolymph, and urine (Duman *et al.* 2010). Antifreeze proteins (AFPs) are also referred to as thermal hysteresis proteins (THPs) because of their ability to depress the equilibrium melting/freezing point to a lower, hysteric freezing point (Duman *et al.* 1991a) Though detailed structural information for insect AFPs is limited, cysteine appears to be a major component (Duman *et al*. 1991b).

In species that are freeze-tolerant, *ice nucleators* (INs) can also be considered cryoprotectants. They help to catalyzes heterogeneous nucleation of water to promote freezing at temperature higher than that resulting from homogenous (spontaneous) nucleation (as high as -2°C). This allows for a slower rate of extracellular freezing, reducing the potential for osmotic shock in the cell as it is dehydrated, and the potential for lethal intracellular freezing (Zachariassen & Hammel 1976). However, for freeze- and chill-intolerant species, ice nucleators are unwanted (as they initiate freezing) and are either selected against on an evolutionary timeframe or removed/masked seasonally (Duman *et al.* 2010). For freeze-avoidant insects, simply eliminating these nucleators can depress the SCP to around -20°C (Zachariassen 1991). Most often, INs are proteins or lipoproteins evolved specifically for ice nucleation (PINs). Alternatively, they can be

elements of proteins that can cause nucleation but whose primary functions are unrelated to cold hardiness ("incidental INs"). Recently, both bacteria and fungi have been implicated in the ice-nucleating activity of insects. The bacterial genera *Pseudomonas* sp., *Erwinia* spp., and *Enterbacter* spp, have shown such activity, as well as the fungus *Fusarium* sp (Keneko *et al.* 1991, Lee *et al.* 1991, Tsumuki *et al.* 1992). These microbes can occur as natural gut flora or externally on the body surface. (Lee 2010).

Cryoprotectants, whether ice-nucleators, antifreeze proteins, or polyols, have all been shown to seasonally increase or decrease in some insects, depending on their cold survival strategy (Asahina 1969, Sømme 1982, Duman *et al.* 2010). While most species predominately use only one cryoprotectant, multiple cryoprotectant systems have been observed (Storey & Storey 1991, Sømme 1999).

Lastly, simple behavioral adaptations are often crucial to the prevention of cold damage and overwintering. Common examples include migration, micro-habitat selection, and feeding changes (Duman *et al.* 1991b). While some insects escape winter conditions by physically migrating to more favorable areas (e.g. Wells & Wells 1992), many others remain local, but seek protected overwintering microhabitats such as in the soil and leaf litter (e.g. Carrillo *et al.* 2005) or other protected structures (e.g. Koch *et al.* 2004). Usually, these sites provide thermal buffering from the fluctuating and severe ambient conditions, enough so as to not require the extensive physiological adaptations of a species in a more exposed site (Geiger 1965, Duman *et al.* 1991b)

Diapause

A final physiological effect often associated with low temperature survival is diapause. Diapause is an endocrine-mediated dormancy that occurs not as a result of, but rather in anticipation of cold and other unfavorable conditions (Denlinger 1991). It is not a developmental arrest but rather a dynamic process; a stereotypic progression of biochemical, physiological, and endocrinological changes occurs during diapause, coined "diapause development", that ultimately result in the resumption of normal development at a specific time of year (Andrewartha 1952, Denlinger 1991, Klowden 2007). Specific cues are associated with both the initiation and termination of diapause (Sehnal 1991) and those known specifically for *H. zea* were discussed in the previous section.

Characteristics frequently associated with diapause include greatly decreased metabolic rate and food intake (Storey & Storey 2010), reduced respiration and water loss (Holmstrup *et al.* 2010).

Much like cold hardiness, diapause can be an essential component of winter survival in insects. An excellent review of the relationship between cold hardiness and diapause is provided in Denlinger (1991) and should be referred to for more detail on the subject. However, an important point to reiterate is that the relationship between these two phenomena cannot be generalized. Diapause is most often implicated in enhancing cold hardiness, but studies exist that describe the whole range of possible relationships, including cold hardiness not associated with diapause, cold hardiness coincidental to diapause, cold hardiness as a component of diapause, and diapause occurring independently of cold hardiness (Denlinger 1991). This diversity of interactions then implies that, if an insect is known to diapause, incorporating this stage along with the nondiapausing stage into any cold hardiness assessment is vital; diapause could dramatically change the perceived cold hardiness.

Many questions are beginning to be raised concerning the impact of climate change on insect diapause. This will be discussed in more detail in the following section on climate change, but one implication is that vernal development of some diapausing species will not advance as expected with warmer winter temperatures, and in some cases may even be delayed or stopped; the required low temperatures to terminate diapause may never be attained (Bradshaw & Holzapfel 2010).

LABORATORY MEASUREMENTS of COLD HARDINESS

In-field evaluation is undeniably important for an accurate and comprehensive assessment of an insect's response to low temperature. The impact of microclimate in the overwintering site, the effect of predation, parasitism, and disease can all alter how an insect ultimately interacts with cold. Also important, however, are controlled laboratory studies. These allow for known individual factors or interactions to be examined, and are often more logistically feasible to perform and analyze than a field study. Lab-based studies dominate much of the cold hardiness literature and the following are some of the common methods used

Supercooling point (SCP)

As stated before, the SCP is the temperature at which spontaneous freezing of the body fluids occurs when cooled below the melting point. This is physically indicated by the release of an exotherm, or latent heat of crystallization (Lee 1991). Typically, SCPs are determined using contact thermocouple thermometry; a thermocouple is attached to the surface of an insect and records its body temperature as it is cooled below 0°C. Temperatures are then digitally graphed to visualize the SCP, seen as the lowest point reached right before an exotherm, or abrupt increase in temperature (Carrillo *et al*. 2004). Usually, programmable alcohol baths (e.g. ethylene glycol; Andreadis *et al.* 2005), liquid nitrogen (Cárcamo *et al.* 2009), or cooling blocks (Beerwinkle *et al.* 1978) are used as the cooling apparatus.

Recently, Carrillo *et al.* (2004) developed a method using calibrated polystyrene cubes that allows for multiple simultaneous measures of SCPs to be made under constant cooling rates. Here, contact thermocouple thermometry is still used, but the insects in question are placed inside the polystyrene cubes which have been designed to cool at a constant, predetermined rate when put inside a -80°C freezer. The SCPs are then read from computer-generated temperature plots as before.

Lower Lethal Temperature (LL*Temp*)

Though SCP is an important aspect of cold hardiness in insects, the temptation to use this measurement as the primary proxy to describe a given insect's response to cold must be avoided. Early cold hardiness studies often equated the SCP to a measure of the lower lethal temperature for freeze avoidant species (Baust & Rojas 1985). Both field and laboratory observations, though, have shown that significant mortality often occurs well above the SCP (Lee 1991). Moreover, cold hardiness can markedly vary just within a single developmental stage, despite a static SCP; Lee and Denlinger (1985) found that although the mean SCP of diapausing *S. crassipalpis* pupae was -23°C for all pupae 10-80 days old, less than 30% of 10-day old pupae survived exposure to -17°C, whereas over 85% of 45-80-days old survived for the same exposure time. While the SCP could more accurately be considered an absolute measure of lethality for chill- and freeze-intolerant

insects, even then, these values are sometimes highly variable just within a population (Cannon & Block 1988).

Therefore, a procedure to specifically determine the true lower lethal temperature (LL*Temp*), and consequently the freezing status of the insect, is needed. This typically involves cooling the insect down to various temperatures above and below the mean SCP, exposing them for a very brief period, and then immediately returning them to a favorable temperature regime for survival assessment (Bale 1991). The LL*Temp* is commonly described as a dose lethal to 50% or 95% of the population, or LT_{50} and LT_{95}, respectively.

In the subsequent work, LL*Temp* is more specifically defined as an instantaneous measure of lethality, one that minimizes the effect of time on a temperature's lethality. This distinction is not often made in the cold hardiness literature and can be somewhat confusing for the researcher seeking to provide a comparable assessment to others. Exposure times for supposed LL*Temp* procedures have been reported anywhere from one minute (Pullin and Bale 1989, Koch *et al.* 2004), to two hours (Andreadis *et al.* 2005), to 24 hours (Duman 1984), to not stating it at all (Fields & McNeil 1988, Sinclair 1999). Variable times address different questions regarding the effect of a temperature (as discussed in the proceeding paragraphs), so the length should always be stated to accurately represent what the study is addressing. My work will use an exposure of three minutes at a given temperature, one that was deemed long enough to be confident the insect is experiencing the chosen temperature, but short enough to avoid incorporating a confounding effect of time.

Lower Lethal Time (LL*Time*)

Echoing the pioneering observations of Salt (1966), Bale (1991) states that the most significant factor determining mortality at low temperatures in nature is period of exposure. Both adults and nymphs of *Myzus persicae* have SCPs below -20°C, but 50% of the populations can still survive for an hour at temperatures around -14°C (Clough *et al.* 1988). More dramatically, *Cephus cinctus* larvae freeze (and die) after one second at -30°C, but survive exponentially longer at higher temperatures; one minute at -26°C and over a year at -17°C (Salt 1966).

Though relatively ignored in historical studies, intentionally including the factor of time, termed lower lethal time (LL*Time*), is receiving increased attention in laboratory cold hardiness assessments. For freeze-and chilling-intolerant insects, once a SCP and/or LL*Temp* has been determined, groups of individuals can be exposed to constant temperatures at various points above these, for increasing periods of time and assessed for survival (Bale 2010). Similar to the LL*Temp* notation, LL*Time* is described as a lethal dosage (in time) to some percentage of the population for a specified temperature. For example, the $LL*Time*_{50}$ at -5°C for *Noctua pronuba* larvae is nine days (Bale & Walters 2001). While LL*Time* is often measured at a constant temperature, cycling between different low temperature/time increments (e.g. Turnock *et al.* 1983) as well as rearing insect groups at different acclimating regimes, can mimic more closely a temporal pattern of temperature change similar to the natural environment (Bale 1991)

Additional points of consideration

Bale (1987) and Baust & Rojas (1985), among others, have questioned many of the experimentally convenient, but possibly biologically inappropriate methods of measuring the effects of cold stress in insects, such as assessing morality from exposure too soon (24hrs or less), not accounting for sub-lethal effects, not accounting for the effect of exposure time on cold stress, the biological significance of the SCP, and general skepticism in assigning distinct labels of freezing tolerance/intolerance. It is virtually impossible to precisely reproduce the fluctuating conditions of nature in a laboratory, placing implicit ecological limitations on laboratory studies (Salt 1950). However, particular considerations can be made to increase their utility. Bale (1991) lists cooling/warming rate, periods of exposure, changes in temperature, interactions of low temperature with other climatic factors, and lethal and sub-lethal effects measured after extended periods following return to favorable conditions as key areas to address for maximum applicability.

Warming and, especially, cooling rates have been shown to critically influence whether or not an insect survives freezing (Miller 1978, Bale *et al.* 1989). The rate of cooling may also have an effect on the supercooling point: however, cooling rates in the 0.1-5°C min^{-1} range generally alter values by no more than 1-2°C (Lee 1991) and Trån *et*

al. (2007) detected no significant difference in the SCP of *Dendroctonus frontalis* larave when using a cooling rate of either -0.2°C min^{-1} or -0.04°C min^{-1}. The ecological relevance of these rates, however, is still questioned by some (Baust & Rojas 1985, Bale *et al*. 1989, Sinclair 2003), and many cold exposure studies fail to mention what, if any, rate was used for cooling and/or warming (Watanabe 2002, Andreadis *et al.* 2005, Jing & Kang 2003, Beerwinkle *et al.* 1978, Johnson 2007).

Most survival assessments following cold exposure are made within a few hours (e.g. Cárcamo *et al.* 2009), or at most a few days (e.g. Pullin & Bale 1989, Watanabe 2002). However, observations made too soon after exposure have been shown to be inaccurate and do not account for sub-lethal effects that ultimately affect fitness (e.g. adult malformations, decreased reproductive potential) (Bale 1991).

For the subsequent work, cooling/warming rates between 0.3-0.5°C min^{-1} were used for the various procedures, and pupal survival assessments were made based on adult emergence. The effect of numerous time periods on various temperatures was also measured. Fluctuating temperature exposures and interactions with other climatic factors were not examined in this work due to logistical constraints, but Bale (1991) suggested that those insects that pupate and/or remain in the soil (as is the case in *H. zea*) may be less impacted by such influences.

My research in the subsequent chapters will focus on the three methods of assessment just described, so detail on additional laboratory methodologies is beyond the scope of this review. However, it should be noted that other physiological and biochemical mechanisms are increasingly being examined as technologies advance, including: identification and quantification of cryoprotectants, molecular analysis of proteins involved with cold tolerance (aquaporins, dehydrins, and heat shock proteins), evaluating the functional roles of genes and metabolites in cold responses, and exploring newly recognized strategies of winter survival (rapid cold-hardening, cryoprotective dehydration, and vitrification) (Lee 2010, Michaud & Denlinger 2010).

COLD HARDINESS and INTEGRATED PEST MANAGEMENT

Though the insect species that comprise the anthropocentric role of "pest" are numerically dwarfed in comparison to the class Insecta as a whole (less than 1% of the

approximately 1 million described species) (Pedigo & Rice 2006), the amount of money, energy, and resources expended annually on these pest species is far more than their fair share. Agriculture in particular, requires significant funding and resource use in managing insect pests, with the U.S. applying insecticides to over 90 million acres during the last agricultural census (NASS 2007).

Vital to the integrated pest management (IPM) of such pests is the ability to make accurate predictions of seasonal infestations (Pedigo & Rice 2006). In temperate and colder climates, the infestation levels on crops during the most susceptible growth stages are mainly determined by the level of overwintering survivorship (Bale 1991). Based on this knowledge, the most important application of insect cold hardiness studies to pest management is in understanding how overwintering survivorship is determined, thus, allowing more accurate preparations to be made for control. This predictive utility of cold hardiness information was highlighted early on in the founding work of R.W. Salt (1936) and continues to be a focus of applied researchers. Bale (1991 and 2010) provides two excellent reviews on current and past IPM applications with insect cold hardiness, including use in forecasting, biological control establishment, and quarantine control. Therefore, further detailed review will not be provided here.

Increasingly, attention among IPM applications is the expansion of the aforementioned seasonal predictions in the context of global climate change (Bale 2010). Given that climate change often connotes a warming trend it could seem counterintuitive to use cold tolerance data to predict how an insect population may respond to such a change. However, understanding the limits imparted by cold on the abundance and distribution of a species is precisely what is needed to predict how those dynamics may change if such limits are no longer present. Climate change and cold hardiness will be discussed further in the final review section.

H. zea **and cold hardiness**

The primary factor limiting *H. zea* overwintering is presumed to be temperature, with areas never experiencing four or more continuous days below 0°C (or south of the 40th parallel) being approximated as the suitable limit (Flood *et al.* 2005).

Diapause has been implicated in enhancing the cold hardiness of *H. zea* pupae and therefore as a necessary component of successful overwintering (Ditman *et al.* 1940, Eger *et al.* 1982). Ditman *et al*. (1940) noted that diapausing pupae (those that did not have evident development of wing pads after 17 days at 18.9°C) have increased cold hardening capacity compared to non-diapausing pupae, as evidenced by their lower undercooling (supercooling) points when exposed to alternating periods of cold and warm temperatures.

As an insect of great agricultural importance, many studies have been conducted in the last century that involve the winter survival, and therefore cold hardiness, of *H. zea* pupae. However, the majority of these are observational field studies conducted throughout the U.S. in the early to mid 1900s (Quaintance & Brues 1905, Ditman & Cory 1931, Barber & Dicke 1939, Dicke 1939, Mangat 1965) leaving a paucity of studies done under current field conditions, or under controlled laboratory environments. Some information exists on the supercooling points of pupae. Barber and Dicke (1939) concluded the freezing point of pupae in dry soil to be -12.2°C, whereas Ditman *et al.* (1940) concluded SCPs ranged from -14.8°C to -27.4°C and Roberts *et al.* (1972) measured SCPs of -22.15°C and -22.31°C from pupae collected at two geographic locations. These results illustrate that, even though some laboratory data exists for *H. zea*, the results are often highly variable, or they lack consistency (and often specification) of how and what was being measured.

Eger *et al*. (1982) provides the most comprehensive assessment of *H. zea* cold hardiness to date, but leaves ample room for additional investigation, including need for a current, complementary study involving *H. zea* populations from different locations to verify or challenge their findings.

CHAPTER I.
Review of the literature
c. Climate Change

Regardless of the perceived cause of, or necessary response to, the earth's climate is changing and it is doing so at a magnitude, rate, and geographic pattern beyond the realm of human reference (e.g. MacDonald 2010). Global temperatures have shown sharp increases in the last 100 years, with that 1990s being the warmest decade in the past 1000 years (Figure 3). Though pockets of controversy still manage to circulate in the public sector concerning its validity, the volumes of data illustrating the global change are undisputed among the scientific community. The most recent summary from the Intergovernmental Panel on Climate Change (IPCC) should be referred to for reputable and current descriptions of such data, as well as expert projections of future scenarios. Regardless of the scenario, however, the earth's global atmospheric and oceanic temperature is expected to increase by the end of the century, with the atmospheric temperature rising anywhere from c.a. 2-6°C (Figure 4).

Most relevant to this review are those projections for North America, where warming is likely to be largest in winter in northern regions and largest in the summer for more southern regions (Christensen *et al.* 2007). Exact projections of this increase vary depending on the climate scenario used, but for the Upper Midwest (i.e. MN, IA, WI) annual mean surface temperatures are expected to rise 1-4.5°C between the beginning and end of this century (Figure 5). Additionally, the snow season length and snow depth are very likely to decrease as a result of delayed autumn snowfall and earlier spring snowmelt (Christensen *et al.* 2007).

Many other climatic factors will be affected by climate change, including synoptic weather patterns, precipitation, severe weather events, and ultra-violet penetration (Houghton *et al.* 2001, IPCC 2007); however, the focus here will be on temperature. When considering the impacts of climate change on insects, current studies suggest that direct effects of temperature are likely to be more important and larger than any other factor (Bale *et al*. 2002). This influence comes at no surprise when the multitude of intertwined effects between insects and temperature are considered.

TEMPERATURE, INSECTS, and CLIMATE CHANGE

The impacts of temperature on insect life histories are well known. For herbivorous insects in particular, the effects of temperature can act directly on physiological and behavioral traits, or indirectly by mediation through the host plant or other factors (Bale *et al.* 2002). Such traits include rate of development, voltinism, physical size, population density, gene expression, geographical range, overwintering, host plant synchronization, dispersal, and migration (Porter *et al.* 1991, Bale *et al.* 2002). Because of these many relationships, slight changes in temperature alone could dramatically alter an insect's life history.

There is much support that climate change will lead to greater winter survival and prolonged favorable summer conditions for insects (Bale *et al.* 2002, Sinclair 2003). However, with these general temperature shifts will come increased climatic variability and changes in precipitation patterns (e.g. snow cover, rain events) that may temporarily augment or override long-term trends (Helmuth *et al.* 2010). Snow cover on the soil surface is frequently an important factor in survival of insects overwintering in the soil (like *H. zea*); even a relatively shallow snow can significantly buffer the subterranean temperature and enhance survival (Leather *et al.* 1995). Therefore, its presence or absence can directly influence insect survival. For this research, temperature still remains the focal influence of climate change on the overwintering range of *H. zea,* but microhabitat temperature, as dictated by soil depth and snow cover, will be considered for the results to remain realistic. First, the consideration of soil depth on buffering temperatures is taken into account, using the average *H. zea* pupal burrow depth of 8.89cm (3.5 in; Hardwick 1965). A relationship between soil and atmospheric temperature was determined based on regression analysis of bare soil and air temperature from various states and years after 2000. Comparing r^2 values and lack-of-fit statistics (SYSTAT 2002), the polynomial $y = a + bx + cx^2$ was chosen. From this equation, an average adjustment value was obtained for the use of ambient temperature as an estimate of soil temperatures (see Chapter III for further detail).

Second, data of surface and soil temperatures and snow cover during the winter of 2009/2010 in Rosemont, MN were overlaid (Figure 12) to determine if a general relationship could be assumed between snow cover and soil temperature. Based on these

data, it was assumed that when snow cover was 10.16 cm or greater, soil temperatures would not drop below 0°C. These assumptions allow transformation of atmospheric temperature data (which are more widely available) such that they become relevant measures to incorporate in a climate model for a species that overwinters in the ground.

In a recent review of cold hardiness and pest management, including studies that integrate cold tolerance data and climate change scenarios to predict future insect responses, Bale (2010) concurs with the views expressed by Bale *et al.* (2002) and Cannon (1998): it is difficult to reach a general consensus on future insect distributions and abundance. Species-specific differences in life cycles (e.g. diapause and voltinism), host plants, feeding guild and natural enemies can dramatically affect their response to climate change. Nonetheless, the trends that can be deciphered based on current studies generally suggest that warmer climates will favor most pest species; their abundance will increase, suitable ranges will expand and natural control measures will not be sufficient to curtail such changes (Bale 2010). Diffenbaugh *et al.* (2008) voices the more specific expectation of some researchers that insect pests will become more abundant in mid- to high-latitudes, putting many regions of major agricultural production at risk of new or increased exposure to highly damaging insects.

In integrated pest management (IPM), being able to predict the timing and degree of infestation for an insect species is crucial to effective and decision-making for sustainable agriculture (Pedigo & Rice 2006). For insects in temperate regions (such as *H. zea*), annual infestation dynamics are closely linked to overwintering survival (Logan *et al.* 1979); therefore, understanding the factors influencing this survival are of great interest to a pest manager. Long term forecasts, too, are particularly important for major pest species, such as *H. zea,* whose management is increasingly difficult due to complications with insecticide resistance (Zalucki & Furlong 2005, Hutchison *et al.* 2007). The value of predictive models is further amplified when pest management is viewed within the context of climate change. Climate warming could advance the time of year at which flight thresholds are first reached, thus increasing the possibility of early immigration/migration (Bale *et al.* 2002). This can lead to more and new crops becoming susceptible to damage, as well as increased insect generations within a growing season. Furthermore, with warming projected to be the greatest during winter, less mortality may

be incurred in overwintering populations and such populations may exist further and further north. For a region such as Minnesota that is currently only targeted by late-season, migrating *H. zea,* such changes in infestation dynamics could dramatically impact how this pest is successfully managed.

Pest Risk Maps and Modeling

The rapid advancements in technology and information sharing capabilities have made it increasingly easy to produce visual models of potential species distributions. Greater computational power, geographic information systems, global environmental databases, enhanced information on species' geographic distributions, and numerous models of ecological niches all make possible the creation of risk models and maps (Venette *et al.* 2010). Mechanistic, or ecophysiological, models are often used to forecast pest and disease populations based on climate (Ulrichs & Hopper 2008). These require detailed physiological data on the target species, but such information that is not often available. Therefore, purely statistical models are also used based on inferred relationships or climate and habitat data (Ulrichs & Hopper 2008). Regardless of the model type, important to keep in mind is that variation in modeling assumptions and approaches, as well as in underlying objectives and data, can lead to dramatically different maps (Venette *et al.* 2010)

Venette *et al.* (2010) provides an excellent summary of current advancements in pest risk mapping, followed by recommendations from the International Pest Risk Mapping Workgroup for improving the utility of such technology. Based on these recommendations and available resources, I have chosen to use the model CLIMEX for my research with *H. zea*. Therefore, details on other models will not be provided here. However, it is noted that, if possible, comparisons of different models should be made with the same dataset so as to better articulate the merits of each approach and, hence, justification for the final choice (Venette *et al.* 2010). While it was not feasible to include an additional model in my current research objectives, Diffenbaugh *et al.* (2008) produced projected risk maps for *H. zea* using biological data, a RegCM3 regional climate model, and the A2 emission scenario. This will serve as comparison to my

results, and differences between the two approaches will be discussed therein (see Chapter III).

CLIMEX

The CLIMEX model enables the estimation of the potential geographic distribution and seasonal abundance of a species in relation to climate. It does so by attempting to mimic the mechanisms that limit species' distributions and seasonal abundance based on the species' response to temperature, moisture, and light (Sutherst *et al.* 2007). It assumes that populations experience one season that is favorable for growth, and one that is unfavorable to the extent of affecting the ability to persist in an area (Yonow & Sutherst 1998). An annual population growth index (GI) describes the potential for growth during favorable periods, and four stress indices (cold (CS), heat (HS), wet (WS), dry (DS)) describe the probability of survival during unfavorable conditions. These indices are then combined to generate a single annual measure of overall climatic suitability, the ecoclimatic index (EI). The EI is scaled from 1 to 100 and calculated as follows:

$$\mathbf{EI = [100\,\Sigma\,(GI_w/52)]\ x\ [(1 - CS/100)(1 - HS/100)(1- WS/100)(1- DS/100)]\ x\ SX},$$

where GI_w is the weekly growth index interpolated from the product of monthly temperature (TI) and moisture (MI) indices (with 52 weeks in a year). The TI and MI reflect the likely rate of population growth under current temperature and moisture conditions. SX reflects the interaction between the four stress indices, and set to one in this study, as in Venette & Hutchison (1999). Sutherst *et al.* (2007) provides further detail on the theory and mathematics behind the software's calculations.

Indices are calculated on a weekly basis for each location using a meteorological database of monthly climate normals from 1961 – 1990 for 2218 location worldwide of which 175 occur in the U.S. Each location contains a monthly-mean maximum and monthly-mean minimum air temperature, minimum air temperature, precipitation, and relative humidity in the morning and afternoon (Venette & Cohen 2006). The meteorological database within CLIMEX consists of long-term monthly averages that involve considerable smoothing of daily values. Therefore, laboratory or instantaneous

field values cannot be directly equated to the model (Yonow & Sutherst 1998). The transformations applied to the laboratory data in this study to overcome this are described in Chapter III.

To make future predictions in CLIMEX, the user is free to input the projections of their choice. In this study, data from the Hadley Centre coupled atmosphere-ocean general circulation model (HadCM3) and the IPCC Special Report on Emission Scenarios (SRES) B2a emission scenario was used. The B2a scenario describes a more environmentally conscious society, with regionalized solutions to economic, social and environmental sustainability; consequently, the assumed emission of greenhouse gases in this scenario are relatively low compared with other scenarios (IPCC 2001). The HadCM3 is composed of both an atmospheric (HadAM3) component and an ocean (HadOM3) component, and is one of the major models used by the Intergovernmental Panel on Climate Change (IPCC 2007). The HadAM3 is run with a horizontal grid spacing of 2.5° latitude by 3.75° longitude, and 19 vertical levels. The HadOM3 component is run on a 1.25° latitude by 1.25° longitude grid, with six ocean grid boxes to each atmospheric model grid box and 20 vertical levels (Gordon *et al.* 2000). For additional information on the Hadley models, see also Johns *et al.* (1997) and Collins *et al*. (2001).

CLIMEX has been used extensively to predict the potential range of pest species, including weeds, insects, and pathogens (Sutherst *et al.* 2006). It is one of the few pest risk models that is flexible enough to use deductive (laboratory determined) and/or inductive (interpolation from known distributions to climatic preferences) methods in determining relationships between species and environmental conditions (Venette *et al*. 2010). Ulrichs & Hopper (2008), though, described some of the limitations of CLIMEX, as well as other ecophysiological models, in that it relies on data about physiological tolerances to predict distributions. This can be problematic because sufficient detailed tolerances are not available for most species, and if they are, often do not reflect the complex interactions outside of the laboratory (Ulrichs & Hopper 2008).

With the above considerations in mind, CLIMEX was chosen as appropriate modeling software to use for this study, but the results should be compared directly with other models employing the same input data in the future.

Table 1: Mean degree days, with corresponding lower developmental thresholds, required for life stage development of *Helicoverpa zea.*

	male	female	Sex unspecified	Lower Threshold (°C)
Egg			43[2]	12.5
			76[4]	12.2
			40.5[5]	12.6
			75.6[9]	12.2
Larva	239[1]	239		12.6
			200[2]	12.5
			202.3[5]	12.6
			285[6]	12.3
			238.1[7]	12.6[*]
			289.6[9]	12.2
Pupa	230[1]	206		12.6
			179.5[5]	12.6
			170.6[6]	12.4
			162.4[7]	12.6[*]
			351[8]	12.8
			354.9[9]	12.2
Total			690.2[3]	12.6
			484.9[5]	12.6
			787.2[10]	6.1

[1]Hogg & Caldren 1981
[2]Coop *et al.* 1993 (sweet corn diet)
[3]Mangat & Apple 1966a
[4]Luckmann 1963
[5]Hartstack *et al.*1973, 1979
[6]Butler 1976 (corn diet)
[7]Hardwick 1965; [*]*(12.6°C threshold assumed for DD calculation)*
[8]Miller 1967
[9]Mangat 1965
[10]Quaintance & Brues 1905

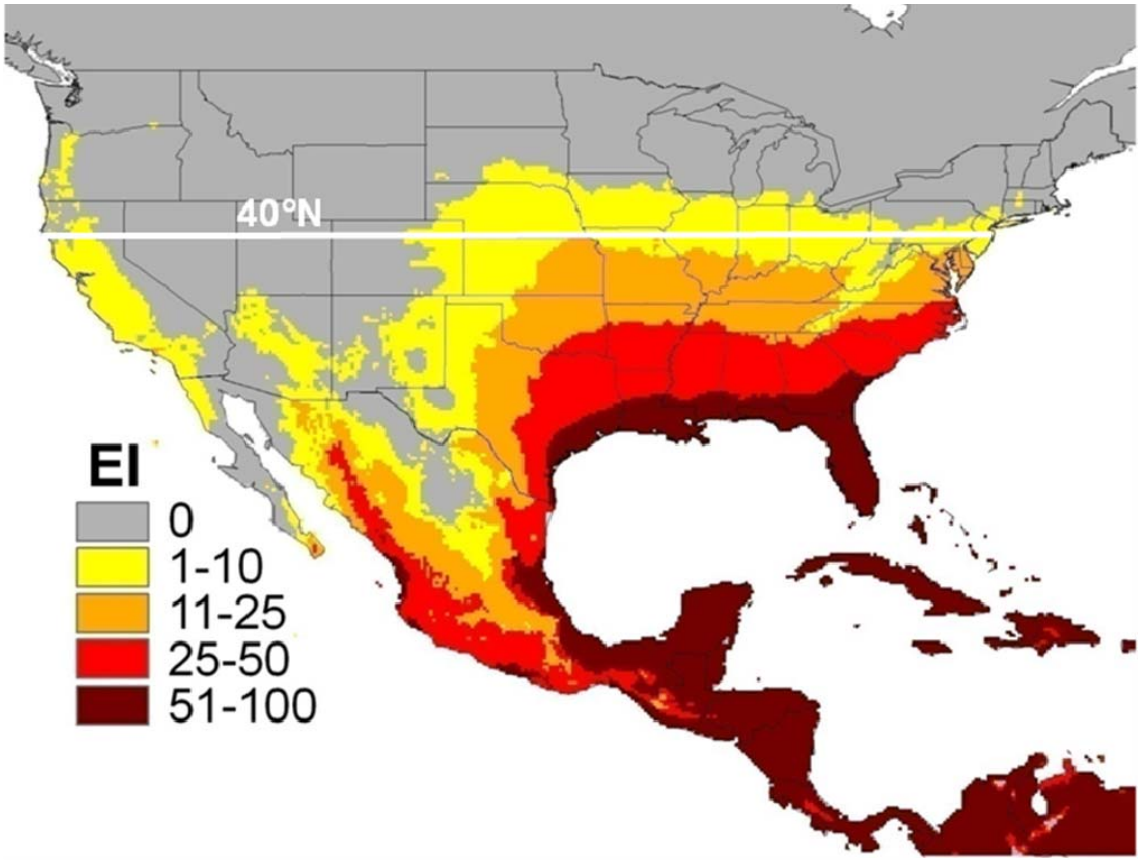


Figure 1: [Adapted from Luttrell *et al*. (in press)] Current overwintering range of *Helicoverpa zea* as estimated by CLIMEX using the parameters of R.W. Sutherst (unpublished data). The Ecoclimatic index (EI) reflects combined potential for population growth during favorable periods and persistence during stressful periods. EI values of 0 are unsuitable, 1-10 marginal, 11-25 favorable, and ≥26 very favorable.

Table 2: Temperature and photoperiod regime used in the present study (Chapter II) to induce diapause in *H. zea*

Stage	Temperature (°C)	Photoperiod (L:D)
Adults	25	14:10
Eggs	25	14:10
Larvae:		
first nine days	21	12:12
next four days	20	11:13
remainder	18	10:14

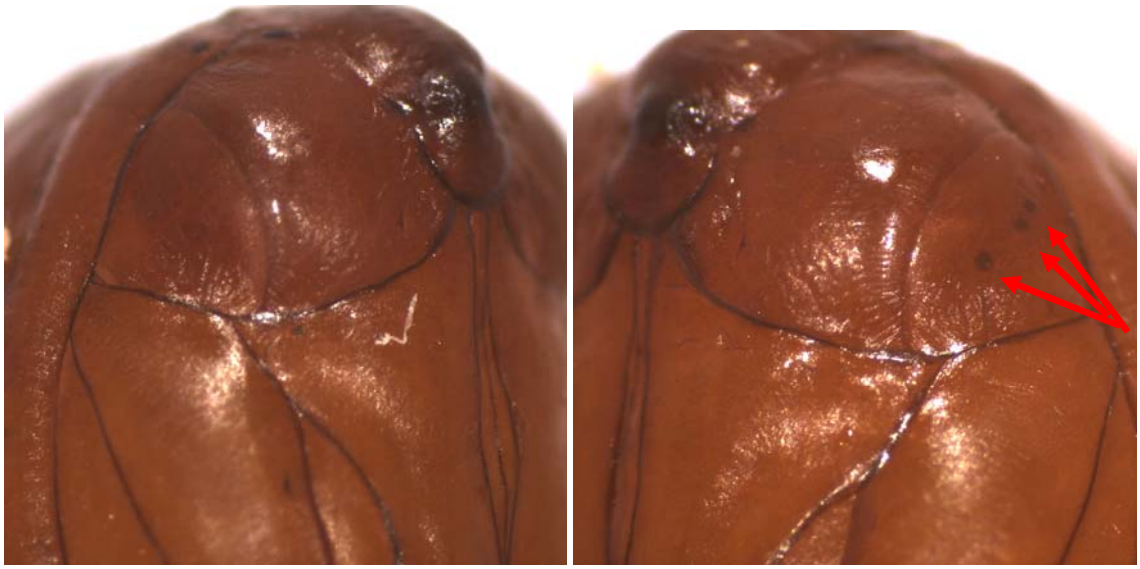
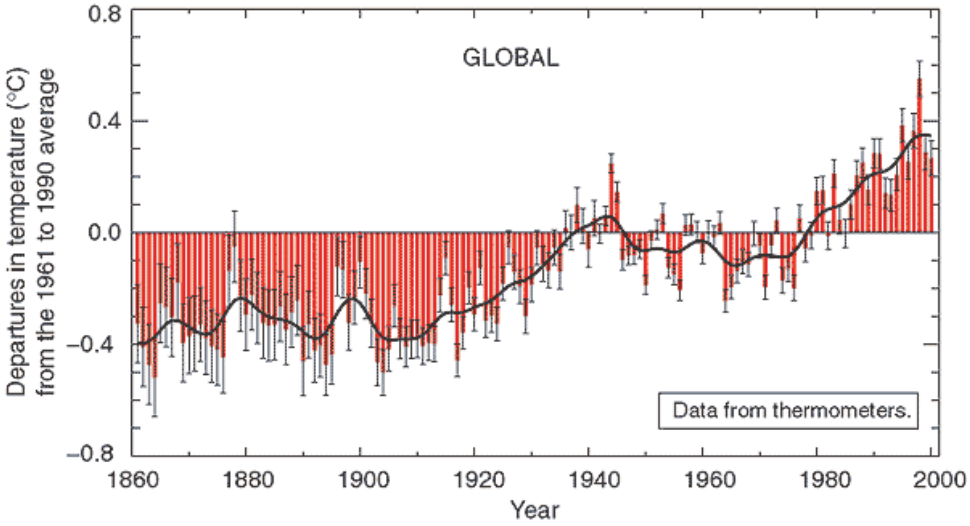


Figure 2: Distinction of diapause in *H. zea* pupae, made six days following pupation. The absence of stemmatal pigments in the postgenal region (*left*) indicates lack of diapause, whereas the retention of such pigments (*right*) indicates diapause.

(a).



(b).

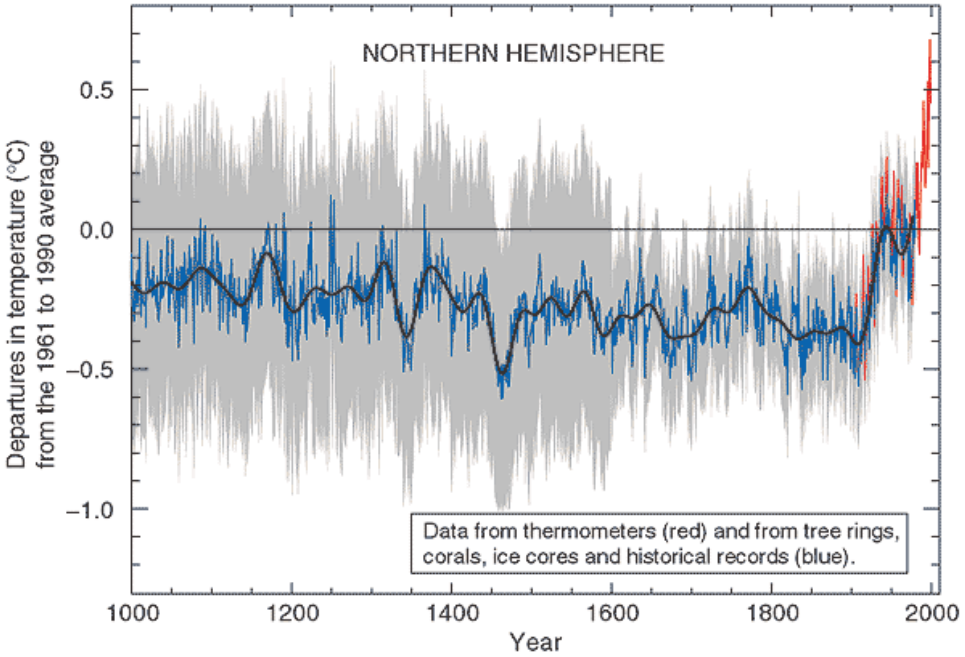


Figure 3. [Image from Folland *et al.* (2001)] Variation of the earth's surface temperature for (a) the past 140 years and (b) the past 1,000 years.

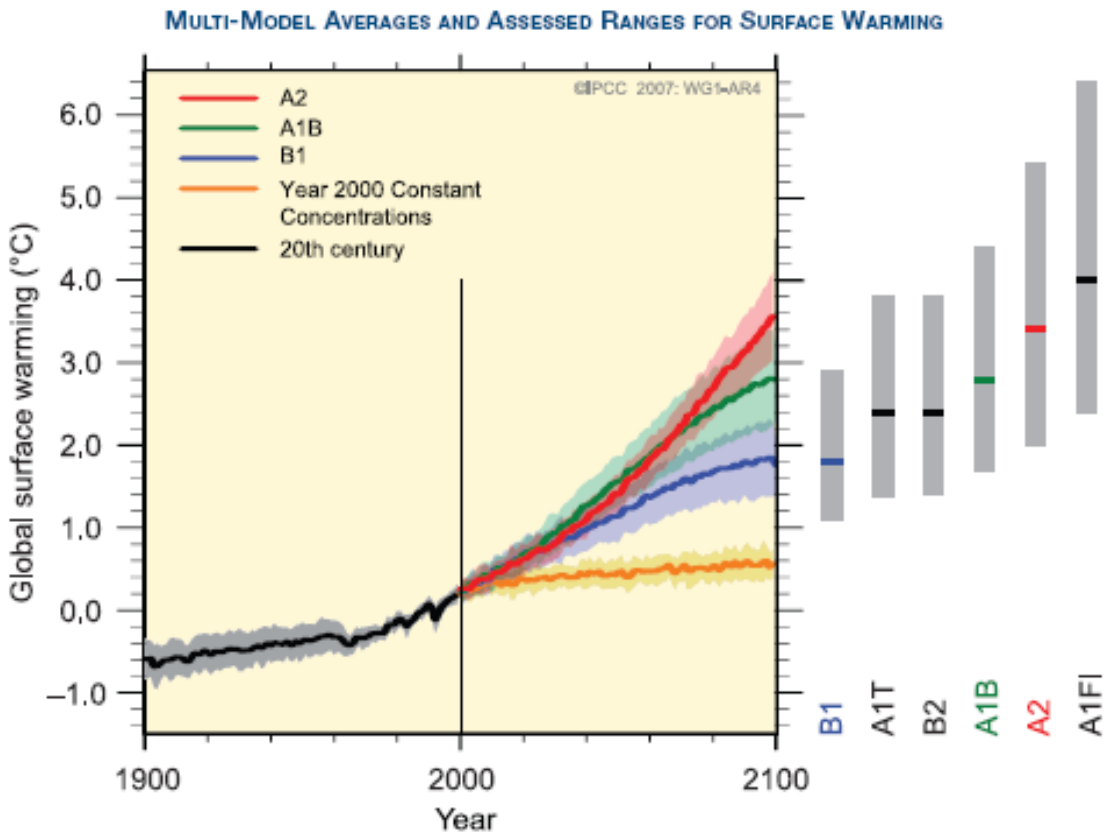


Figure 4. [Image and caption taken from IPCC (2007)] Solid lines are multi-model global averages of surface warming (relative to 1980-1999) for the scenarios A2, A1B, and B1, shown as continuations of the 20th century simulations. Shading denotes ± 1 SD range of individual model annual averages. The orange line is for the experiment where concentrations were held constant at year 2000 values. The grey bars at right indicate the best estimate (solid line within each bar) and likely range assessed for the six SRES marker scenarios. The assessment of the best estimate and likely ranges in the grey bats includes the AOGCMs in the left part of the figure, as well as results from a hierarchy of independent models and observational constraints.

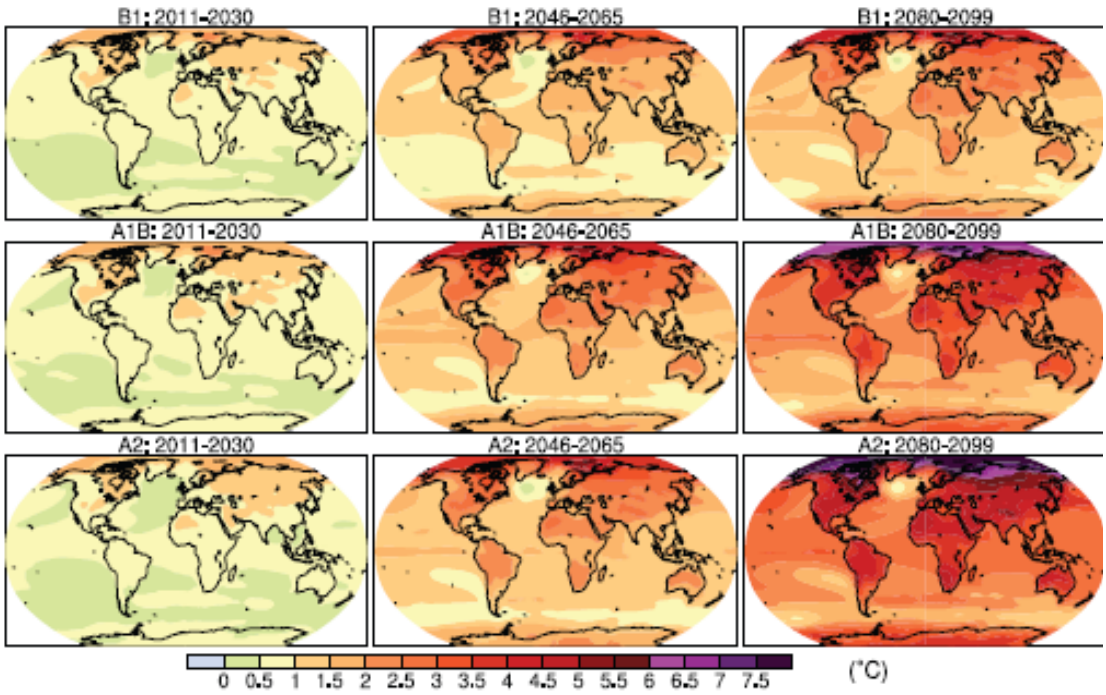


Figure 5. [Image and caption taken from IPCC (2007)] Multi-model mean of annual mean surface warming (surface air temperature ºC) for the scenarios B1 (top), A1B (middle) and A2 (bottom), and three time periods, 2011 to 2080 (left), 2046 to 2065 (middle) and 2060 to 2099 (right). Stippling is omitted for clarity. Anomalies are relative to the average of the period 1960 to 1999.

CHAPTER II.
Cold Hardiness of the Corn Earworm, *Helicoverpa zea* (Lepidoptera: Noctuidae)

INTRODUCTION

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 (Flood *et al.* 2005). *Helicoverpa zea* migrates throughout most of the eastern and midwestern United States and southern Canada during the growing season, but it is not currently known to overwinter north of the 40th latitude (Hardwick 1965). Northern growers of many crops targeted by *H. zea* can presently avoid most infestations by planting high-value crops, such as sweet corn, earlier in the season before the migrating moths arrive. However, early planting is not feasible for all crops, and shifts in the overwintering range can have major impacts on the timing and magnitude of *H. zea* infestations in northern latitudes; these shifts could have important implications for the damage potential, and therefore successful management, of *H. zea* in these areas. Many questions remain regarding its response to low temperatures and, consequently, potential to expand its overwintering range given future climate change scenarios.

Cold hardiness is the capacity of an organism to survive low temperatures (Lee 1991). Insects commonly employ three strategies to do so: freeze tolerance, freeze avoidance/intolerance, and chill intolerance (Lee 2010). To determine the temperatures at which an insect's internal fluid freezes, the supercooling point is measured (Lee 2010). To determine if most mortality occurs above the SCP (chill intolerance), the lower lethal temperature can be measured by exposing insects to temperatures above the SCP and measuring mortality. The 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 assessment of cold hardiness (Bale 1987). This dogma has more recently come under continued scrutiny for largely ignoring other deleterious effects of low temperatures, such as pre-freeze mortality and length of exposure time. More comprehensive and ecologically

relevant assessments are needed to accurately describe the mechanisms of an insect's cold hardiness (Bale 1987, 1991).

Many earlier studies looked at the interaction between *H. zea* pupae and low temperature exposure (Quaintance & Brues 1905, Ditman & Cory 1931, Barber & Dicke 1939, Dicke 1939, Mangat 1965) but most are observational overwintering studies that do not include detail on specific factors, such as thermal history of pupae and exact microclimatic conditions. Other studies included more detailed and controlled assessments (Ditman *et al.* 1940, Miller 1967, Slosser *et al*. 1977, Eger *et al.* 1986), but lacked consistency in how and what aspects of cold hardiness were measured. Luttrell *et al.* (2010) provided a map depicting *H. zea*'s current geographic range based on known or inferred stress parameters, of which cold was included. However, they mention the need for further validation of the parameter estimates of reliable information for *H. zea* cold stress. Additionally, no studies have been conducted in recent decades. Consequently, there is a need not only for verification of any past conclusions, but for more comprehensive analysis of *H. zea* that incorporates recent advances in the understanding of, and assessment techniques for, insect cold hardiness.

In this study, I 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 determination. Additionally, I examine some of the important factors that could influence cold hardiness of *H. zea* pupae such as sex, acclimation, and diapause. The ecological relevance and effects of microclimate on our data are also considered; in-field observations of winter soil temperatures and pupal mortality were conducted and compared to the laboratory results. Finally, the potential future application of this work to aide growers affected by *H. zea* will also be discussed.

MATERIALS and METHODS

Colony source

Early instar *H. zea* larvae, infested on artificial wheat-germ diet modified from Shaver and Raulston (1971) (Blanco *et al*. 2009), were obtained from a laboratory colony in Stoneville, MS (adults and eggs reared at 27.5°C 14:10 L:D). Larvae were reared in

individual cups to pupation in programmable growth chambers in St. Paul, MN. Rearing conditions were manipulated to produce either non-diapausing pupae (25ºC ± 1ºC, 65-80% RH, 14:10 L:D photoperiod,) or diapausing pupae (first nine days after hatch held at 21ºC, 65-80% RH, 12:12 L:D; next four days held at 20ºC, 11:13 L:D; remaining time until treatment held at 18ºC, 10:14 L:D). Diapause was confirmed by observing the retention of larval stemmatal pigments in the postgenal region at least six days after pupation (Phillips & Newsom 1966).

SCP determination

Pupae were attached to thermocouples and cooled at a rate of 0.5ºC min^{-1} in calibrated Styrofoam cubes placed inside a freezer held at -80ºC (Carrillo *et al*. 2004). Temperatures were recorded every second 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 -23ºC, so temperatures were logged until -30ºC. Six pupal groups were used: non-diapausing and unacclimated pupae (held at 25ºC (14:10 L:D) until the time of testing); non-diapausing and acclimated pupae (cooled 0.5ºC/day from 25ºC to either 20ºC, 18ºC, 15ºC, or 10ºC, beginning one day 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 0.3ºC min^{-1} in a freezer as in the above to temperatures below -10ºC, or a programmable growth chamber (Percival Scientific) for temperatures above -10ºC. Once the desired temperature treatment was reached, insects were held at this temperature for approximately three minutes and then returned to 25ºC at a rate of 0.3ºC min^{-1}. Pupae were held at 25ºC (± 1ºC, 65-80% RH, 14:10 L:D) until eclosion or death. Two pupal groups were used: non-diapausing and diapausing. Acclimation treatments were not included in this procedure due to limited resources. Thrity pupae were used for each temperature above -10°C, whereas sample sizes for temperatures below -10°C ranged

from 2 to 24 based on the ability to reach the desired temperature without the pupa supercooling.

*Lower Lethal Time (*LL*Time) determination*

Diapausing and non-diapausing pupae were cooled at a rate of 0.3ºC min^{-1} in a programmable growth chamber to either -10ºC, -5ºC, 0ºC, or 5ºC (0:24 L:D) and held for at least three different time periods per temperature; three or four replications were used per treatment, with a sample size of 25 or 15 pupae, respectively (Table 3). Acclimation treatments were not included in this procedure due to limited resources. Following low temperature exposure, pupae were returned to 25º-27ºC (14:10 L:D) at a rate of 0.3ºC min^{-1}, and held until adult eclosion or death.

In-field evaluation of winter survival

To monitor winter soil temperatures, four temperature probes attached to a caged HOBO data logger (Onset Computer Corporation, Bourne, MA) were placed within rows of a unharvested sweet corn field at UMORE Park (Rosemount, MN; Dakota County; 44º42'27.26"N and 93º06'01.53"W) on October 9, 2009 (Figure 6a). One probe remained exposed to ambient conditions above ground, whereas the remaining three were placed in the soil at depths of 10.16, 5.08, or 2.54 cm. These depths were chosen based on the average pupal burrow depth of 3.5 inches (Hardwick 1965). Data recording ceased on June 11, 2010. Snow depth data was obtained from the Minnesota Climate Working Group (http://climate.umn.edu) for Dakota County beginning October 2009 though June 2010. Snow depth was overlaid with the soil temperatures to illustrate any additional buffering effect to the subterranean microclimate.

To document the overwintering survival of *H. zea* pupae in Minnesota, 19 cages containing 3-12 late instar larvae were placed in the same unharvested sweet corn field as above, on September 26, 2010. Untreated corn ears containing naturally-infested larvae (as verified by gently pulling away the husk tissue near the ear tip for observation) were placed on end in the soil inside mesh cages (Figure 6b). Cages were either circular (24" wide, 32" high), containing 3 infested corn ears, or rectangular (38'x 17"x13") and contained 9 or 12 infested corn ears.

Cage netting was removed October 9, 2009 to prevent unnatural microclimate effects during the winter. Also at this time, one cage area was excavated to confirm larvae had entered the soil. Following this confirmation, each corn ear was examined for the presence or absence of larvae. If absent, it was assumed the larva exited the ear and burrowed into the ground to pupate. If present, they were removed from the trial and the given cage sample size adjusted. Cage nettings were put back out on 16, April, 2010 to capture any emerging moths.

Analysis

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

LL*Temp* data were analyzed using Proc LIFETEST (SAS 2005) to compare the survivor functions between diapausing and non-diapausing pupae, while adjusting for sex. The LT_{50} for diapausing and non-diapausing pupae were linearly estimated based on their respective survivor functions.

LL*Time* data were fit with regression models using TableCurve (SYSTAT 2002) to estimate the relationship between time and mortality for each temperature treatment. Models were fit within non-diapausing 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 & Smith 1998). The associated times to 25%, 50%, and 95% mortality ($LLTime_{25,\ 50,}$ or $_{95}$) were then calculated for each treatment based on these functions. When possible, the curves were compared between diapausing and non-diapausing pupae for each temperature, while adjusting for sex, using Proc LIFETEST (SAS 2005) to describe any differences in time until mortality.

The controls for each procedure showed no mortality, so a correction (Abbott 1925) was not necessary.

RESULTS

Supercooling point (SCP)

Sex did not significantly affect pupal SCP ($P = 0.721$, $df = 185$) so data were pooled for further analysis. Acclimation of non-diapausing pupae and diapause had a significant effect on the SCP as compared to unacclimated, non-diapausing pupae ($p<0.0002$, $df = 191$). All acclimated treatments were significantly lower than the unacclimated treatment, except for 10ºC (Figure 7). Diapausing pupae also differed significantly from the unacclimated pupae, with diapause producing a lower mean SCP (-19.3ºC versus -16.4ºC). There were no differences among the acclimated treatments, or between the acclimated and diapausing pupae (Figure 7). It should be noted that due to limitations in the experimental design, the chosen analysis could not rule out the possibility that time of procedure or pupal age confounded the significance (or lack thereof) seen between treatments. However, for logistical reasons, this could not be avoided and ANOVA was still deemed the most appropriate course of analysis.

*Lower Lethal Temperature (*LL*Temp)*

There was no significant difference between the LL*Temp* morality curves of diapausing and non-diapausing pupae (Log-rank; $\chi^2 = 1.832$, $df = 1$; $p = 0.176$). Similarly, sex did not have a significant effect on mortality (Log-rank; $\chi^2 = 0.402$, $df = 1$, $p = 0.530$). The estimated LT_{50} for non-diapausing and diapausing pupae were -12.2ºC and -8.8ºC, respectively (Figure 8).

*Lower Lethal Time (*LL*Time)*

With the exception of 0°C and 5°C, mortality never exceeded 53% in -10°C and -5°C during the times chosen for diapausing pupae (Figure 9a and b). Therefore, a regression model could only be fit to the 0°C and 5°C data for this pupal type (Figure 9c and d); the Lorentzian cumulative curve was used for both (Table 4). For non-diapausing pupae, however, mortality over time could be modeled for all the temperature treatments; -10°C, -5°C, and 5°C were all fit with a nonlinear logistic dose response curve, and 0°C was fit with a Lorentzian cumulative curve (Table 4; Figure 10). Estimated times until 25%, 50%, 75%, and 95% mortality based on these models are presented in Table 5. The

mortality curves for 0°C were shown to be significantly different between diapausing and non-diapausing pupae (Log-rank; $\chi 2 = 7.810$, $df = 1$, $p = 0.005$), 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 due to the aforementioned low mortality in diapausing pupae for these groups.

In-field evaluation

While ambient temperatures during the testing period fluctuated greatly between 35ºC and -32ºC, all of the soil depths had much less daily variation and the lowest point ever reached during winter was -1.5ºC, which occurred at 2.54 cm. (Figure 12). Most striking was the relative plateau of all soil temperatures beginning shortly after this lowest temperature was reached and remaining at 0ºC ± 1ºC for nearly 4 months (2464 hr), beginning ca. December 2nd, 2009. The occurrence and persistence of the plateau correlated almost exactly with the presence of an estimated ≥ 10.16 cm snow depth (Figure 12).

Larvae in the caged plots were verified to be capable of exiting the ears and burrowing into soil; two out of a potential three in each cage were found during the check excavation on October 9th. All late-instar larvae found had not yet pupated, but were still alive and had constructed burrows ca. 3.8 cm below the surface (Figure 11). Following the examination of all corn ears, a total of 45 larvae were assumed to have entered the soil to overwinter as pupae. Of these, none were captured as emerging moths the following spring. Two plots were excavated on March 31, 2010 to search for unemerged pupae; zero out of a potential two were found in the first plot, and one dead, late-instar larvae out of a potential 11 was found in the second. While the body size and coincidental location of dead larvae suggested it may be *H. zea*, the high level of decomposition prevented positive identification. Regardless, 100% overwintering mortality was concluded due to lack of adult emergence and lack of evidence suggesting live, unemerged pupae.

DISCUSSION

Helicoverpa zea pupae were confirmed to be chill intolerant insects; the LT_{50}s of both diapausing and non-diapausing pupae were higher than their respective SCPs, which indicates *H. zea* is unable to withstand freezing and is also subject to much pre-freeze mortality. Sex does not appear to influence the cold response of pupae, which agrees with previous findings (Ditman *et al*. 1940). Acclimation of non-diapausing pupae to any temperature below 20ºC appears to impart an increased cold hardening equivalent to diapause. The 10ºC acclimation group did not show a statistical difference from the unacclimated control, but this is likely due to 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 acclimation was only demonstrated through SCP comparisons. Due to limited resources, acclimation treatments were not included in other procedures, but future study should further examine its affect, both as the potential rapid cold hardening-like effect (Lee *et al.* 1987) shown here for pupae, and as a conditioning experienced in the larval stage of non-diapausing pupae. The ecological relevance of 4these types of non-diapausing acclimations would not likely be important for considering the overwintering capabilities of pupae (non-diapausing 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 fall within the range of previously published mean SCP values for *H. zea* (Barber & Dicke 1939, Roberts *et al.* 1972, Ditman *et al.* 1940), though 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 all affect the SCP.

The apparent lack of increased cold hardiness between non-diapausing and diapausing pupae seen in the LL*Temp* studies was contradicted by the SCP and LL*Time* studies; the significantly lower SCP with diapause and the greatly extended time until mortality at various temperatures with diapause clearly shows an increased low temperature tolerance in diapausing pupae. The LL*Temp* study lends itself more to being an empirical, instantaneous observation of cold response, rather than one reflecting natural, gradual cooling. Therefore, the conflicting results could be a laboratory artifact,

and/or an illustration of the need to assess cold hardiness using numerous approaches. LL*Temp* may be important to articulate the true lower lethality of temperature to an insect, as both diapausing and non-diapausing *H. zea* pupae die prior to their SCPs, and do so because of mechanisms other than freezing. However, LL*Temp* may not be useful in portraying the impact of ecologically relevant factors, such as diapause or the lethality of warmer temperatures over time. The importance of diapause may not be apparent until situations like those naturally experienced are mimicked. For example, Figure 12 illustrates that the pupal behavior of burrowing in the soil likely protects them from rapid declines to subzero temperatures (as is measured in the LL*Temp* procedure), leaving them to more likely to experience long bouts of cold, but above zero, temperatures (as is measured in the LL*Time* procedure); evolutionarily, there may be no reason for diapause to enhance a response for situations like the LL*Temp* procedure so its utility to singly describe cold hardiness is limited. The same can be said for the SCP in that its use as a single predictor of cold hardiness should be avoided. While 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) largely overestimates the temperature tolerances of this insect. Despite their limitations, though, these procedures should also 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.

While a common model could not be fit to all the LL*Time* results for statistical comparisons across temperatures, it is still quite evident that 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. My data could only demonstrate this completely with non-diapausing pupae, but it is reasonable to assume the same pattern applies to diapausing pupae only on a much longer time scale. This is evident when comparing the LL*Time* data for 0°C between the two groups. Both diapause and non-diapause mortality could be described with a 3-parameter Lorentzian Cumulative (LorCum) curve, but with diapause requiring significantly more time (2660 hr) to reach the same 95% mortality achieved with non-diapausing pupae (502 hr) (Table 5).

The above comparison, showing a nearly 5-fold difference in time until the 95% mortality, also highlights the enhanced cold hardiness gained through diapause in *H. zea* pupae. This conclusion agrees with previous findings of Eger *et al.* (1982) and Ditman (1940). The specific degree to which I found mortality to change with time differs from Eger *et al*. (1982), with their study generally showing more time needed for 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 could likely be due to differences in methodology and equipment used. Eger *et al*. (1982), for example, used a single model for all temperature treatments between -10º and 10ºC to calculate the various time-mortality relationships, but it was based only on data collected for temperatures between -6ºC and 6ºC. 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 some instances (data not shown). There was also no mention of cooling and warming rates with their procedure, a detail that can be quite influential in measuring a cold response (Miller 1978).

Insects that persist in temperate regions must contend with the harsh conditions of winter. Temperature is most often implicated as the driving force of overwintering survival, which, for a pest species, is then a reliable indicator of spring infestation dynamics (e.g. Trån *et al.* 2007). Therefore, cold hardiness measurements can offer important insights into overwintering dynamics. Based on cold hardiness measurements, Figure 13 illustrates that winter temperatures in southeastern Minnesota can prevent significant survival of *H. zea* pupae. Strictly looking at atmospheric temperatures, one may ascribe this to the presence of temperatures well below the LT_{50} (and also mean SCP). However, when the pupal overwintering behavior of burrowing is considered, soil temperatures are a more appropriate focus and as seen, and can be markedly different. Not only are the daily temperature fluctuations dampened from extreme values (as seen by the soil measurements up until December), but once there is constant snow cover in early December (Figure 12) any decrease below 0°C is halted until spring melting. While 0°C is not immediately lethal to pupae, it can be over time. When comparing this measured lethality to the time of soil temperatures spent at 0°C, it is seen that enough time passed to inflict at least 90% mortality in both non-diapausing and, more

importantly, diapausing pupae. Therefore, it may not be the extreme temperatures experienced during the winter that prevent overwintering survival in *H. zea* in this area, but rather the length of time spent at slightly warmer ones.

Interestingly, the in-field observations presented here may suggest an alternative influence on perceived winter mortality. Based on the late instar larvae found during both the fall and early spring excavations, these observations suggest that there may not have been adequate degree days in this area during autumn of 2009 to allow for pupation in the overwintering generation before the onset of winter, thus killing the less cold-hardy larvae early on. However, this is based on only one year of data. Furthermore, the study was designed to monitor potential moth emergence and not to track the point at which mortality was occurring, so future trials should be designed to address this.

If in most years, a significant level of pupation occurs in southern Minnesota it important to note that other factors in addition to temperature likely contribute to the mortality of overwintering *H. zea.* Moisture, soil type, predation, disease, cultivation, and burrow destruction have all been cited as potentially detrimental to overwintering pupae (Barber & Dicke 1939, Ditman *et al.* 1940, Blanchard 1942). Temperature may account for at least 90% of mortality, but as shown in Table 5, 95% mortality for diapausing pupae at 0°C (ca. 2660 hr) is in fact *after* soil temperatures have risen to warmer levels (ca. 2464 hr); other mortality factors aside, my laboratory results suggest diapausing pupae may be capable of overwintering in southeastern Minnesota in some years. However, this conclusion does not match the current understanding of *H. zea* distributions, one based on regular, long-term monitoring programs across the U.S. (e.g. Sandstrom *et al.* 1997). Therefore, three scenarios are likely for the remaining 5% of the population unaccounted for by temperature stress alone; any of the aforementioned mortality factors may prevent that percentage (if not more) from surviving, 5% of the overwintering population is so negligible that these survivors simply go unnoticed, or significant adult mortality may also occur between adult eclosion and moth emergence from the soil in the spring (Slosser *et al.* 1975, Caron *et al.* 1978).

To maximize the utility of these data for grower application, additional studies should be done to complete the LL*Time* profile for diapausing pupae since this is the most cold-hardy and overwintering stage. Inclusion of additional microclimate factors,

such as moisture and soil type, that may interact with the effects of temperature in pupal burrows is also important. As stated previously, the field observations presented here only represent one year of data so further field validation is necessary. Lastly, the base overwintering population for a given area should be determined to verify that winter temperatures, and not fall temperatures, are the main determinant of (or lack of) spring populations. This could be accomplished by identifying the latest date in the fall that diapause-destined larvae need to be burrowed and pupated in the soil before it becomes too cold, and then back calculate in degree days to see if enough time is available for the summer migrants to produce such offspring before this point is reached.

For northern growers of crops adversely affected by *H. zea*, such as sweet corn, the additional information from cold hardiness studies can assist with producing more accurate forecasts of seasonal infestations. 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 (e.g. Sandstrom *et al.* 2007). This allows for more informed management decisions regarding how and when treatment may be warranted. Similarly, the same cold stress data could be incorporated with climate change scenarios to model long-range shifts in the overwintering range of this insect (e.g. Venette *et al.* 2010).

Table 3: Treatments used in the lower lethal time procedures for *H. zea* pupae.

	Temp (ºC)	Time (hours)	Reps	n
non-	-10	1	3	25
diapausing		3	3	25
		5	3	25
		10	4	15
	-5	24	3	25
		48	3	25
		96	3	25
	0	120	4	15
		264	4	15
		480	4	15
		979	4	15
		1218	4	15
	5	593	4	15
		883	4	15
		1048	4	15
		1456	4	15
diapausing	-10	1	3	25
		2	3	25
		3	3	25
		5	3	25
		24	4	15
	-5	24	3	25
		48	3	25
		96	3	25
	0	788	4	15
		1032	4	15
		2659	4	15
	5	840	4	15
		1725	4	15
		3335	4	15

(a)

(a)

Figure 6: Site of in-field overwintering study which included (a) monitoring of atmospheric and soil temperatures (at 10.16, 5.08, and 2.54 cm below the surface), and (b) monitoring of spring moth emergence from late instar *H. zea* larvae collected the previous fall and placed inside mesh cages to burrow and pupate naturally. UMORE Park, Rosemount, MN.

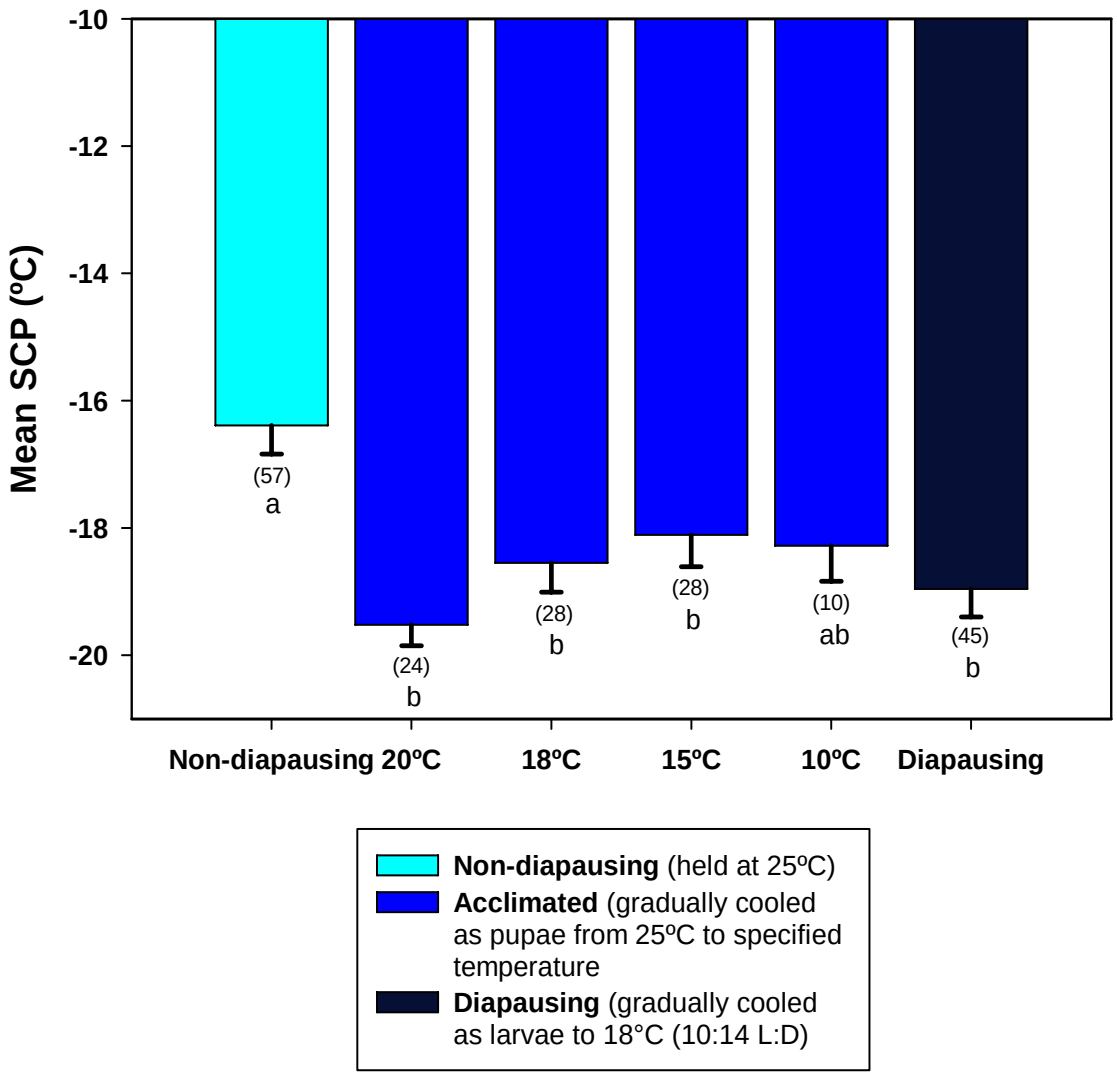


Figure 7: Mean supercooling points (SCP) for non-diapausing, non-diapausing and acclimated, and diapausing *H. zea* pupae. Columns with the same letters are not significantly different ($\alpha<0.05$). Numbers in parentheses indicate sample size. Error bars represent standard error.

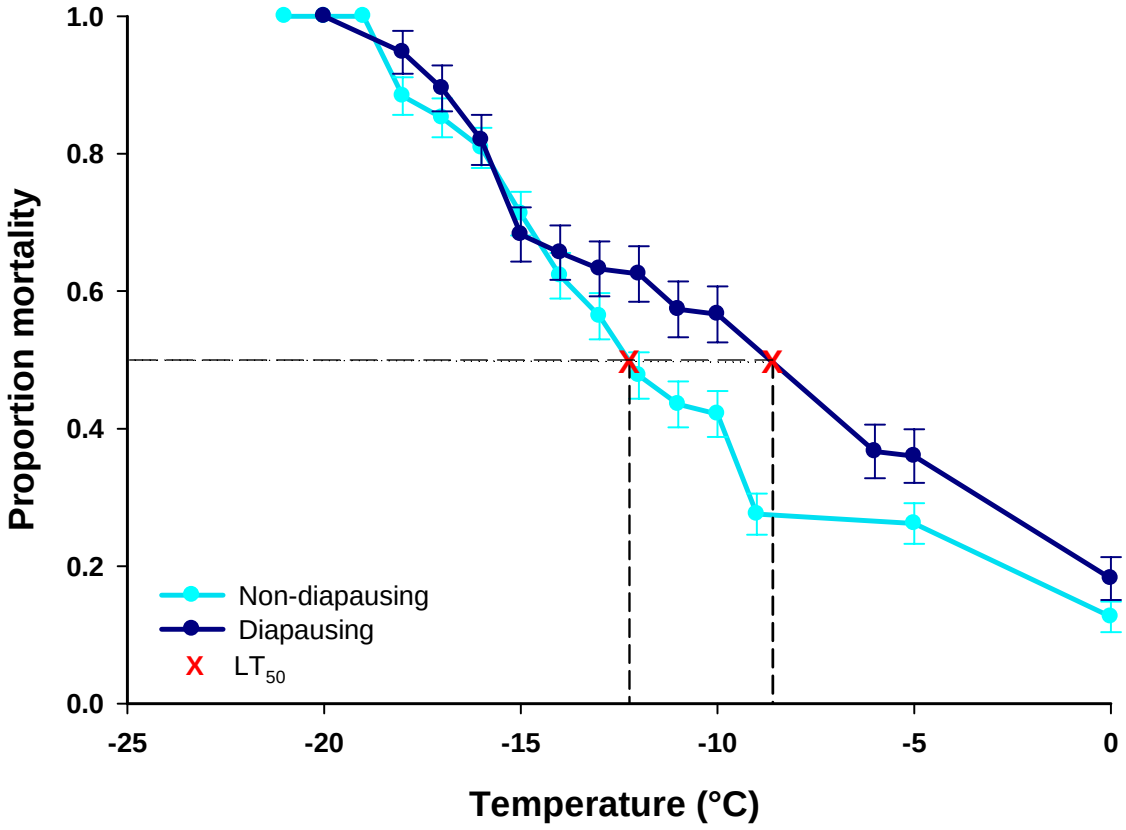


Figure 8: Lower lethal temperature mortality responses for *H. zea* pupae. Error bars represent standard error.

Table 4: Model parameters from regression analysis relating temperature and time of exposure in non-diapausing and diapausing *H. zea* pupae.

	Temperature (°C)	Parameter estimates (S.E.)			r^{2*}	maximum r^2	F-value	P>F
		a	*b*	*c*				
non-diapausing	-10[a]	98.38 (6.14)	3.66 (0.23)	-4.97 (1.15)	0.94	0.96	138.02	<0.0001
	-5[a]	96.76 (10.91)	38.03 (5.11)	-5.23 (2.29)	0.84	0.88	36.82	<0.0001
	0[b]	100.25 (1.98)	474 (16.32)	4.65 (13.50)	0.99	0.99	947.87	<0.0001
	5[a]	1.00 (0.02)	717.90 (13.45)	-7.23 (0.61)	0.99	0.99	878.10	<0.0001
diapausing	0[b]	102.26 (3.50)	1130.95 (54.30)	106.13 (48.96)	0.97	0.98	275.03	<0.0001
	5[b]	98.91(4.20)	2062.72 (4.22)	91.87 (105.93)	0.99	0.99	543.01	<0.0001

[a] Logistic dose equation, $y = a / \{1 + (x / b)^c\}$.

[b] Lorentzian cumulative equation, $y = a / \pi \{\arctan[(x-b) / c] + \pi / 2\}$

* adjusted for degrees of freedom

Table 5: Percent mortality for non-diapausing and diapausing *H. zea* pupae, calculated from the equations in Table 4. Mortality included incomplete adult eclosion and dead pupae. Models could not be fit to the other temperature treatments for diapausing pupae due to insufficient data.

	Time until mortality (hours)					
	non-diapausing				diapausing	
Mortality	-10°C	-5°C	0°C	5°C	0°C	5°C
25%	2.94	31.08	478.92	616.54	1021.07	1972.41
50%	3.68	38.52	474.31	717.55	1127.27	2064.30
75%	4.62	48.18	469.66	834.83	1226.59	2159.45
95%	7.15	81.56	502.32	1073.38	1599.17	2769.92

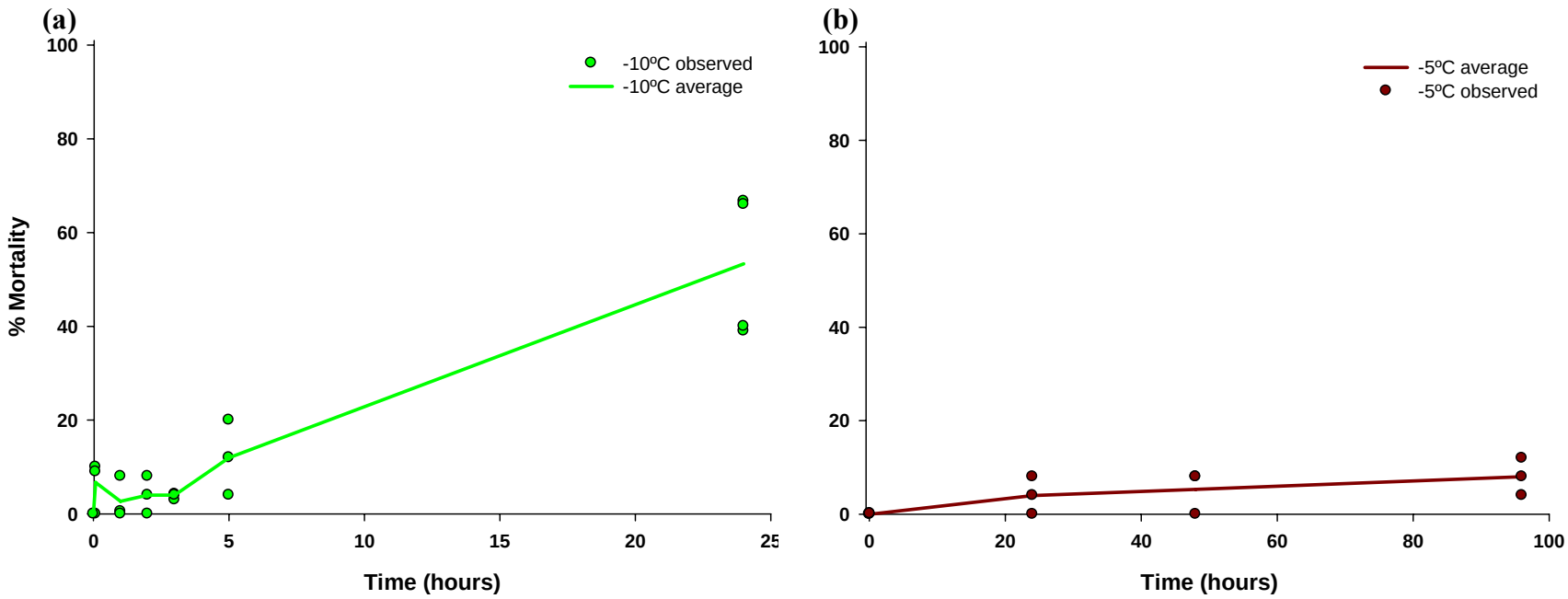


Figure 9: Percent mortality of diapausing *H. zea* pupae held at (a) -10°C, (b) -5°C, (c) 0°C, and (d) 5°C. The equations used to describe the predicted mortality trends of 0°C and 5°C are given in Table 4. Models could not be fit to the -10°C and -5°Cdata, so the average mortality was used for illustration purposes. Dots represent observed mortality for each time treatment replication; treatments are listed in Table 3.

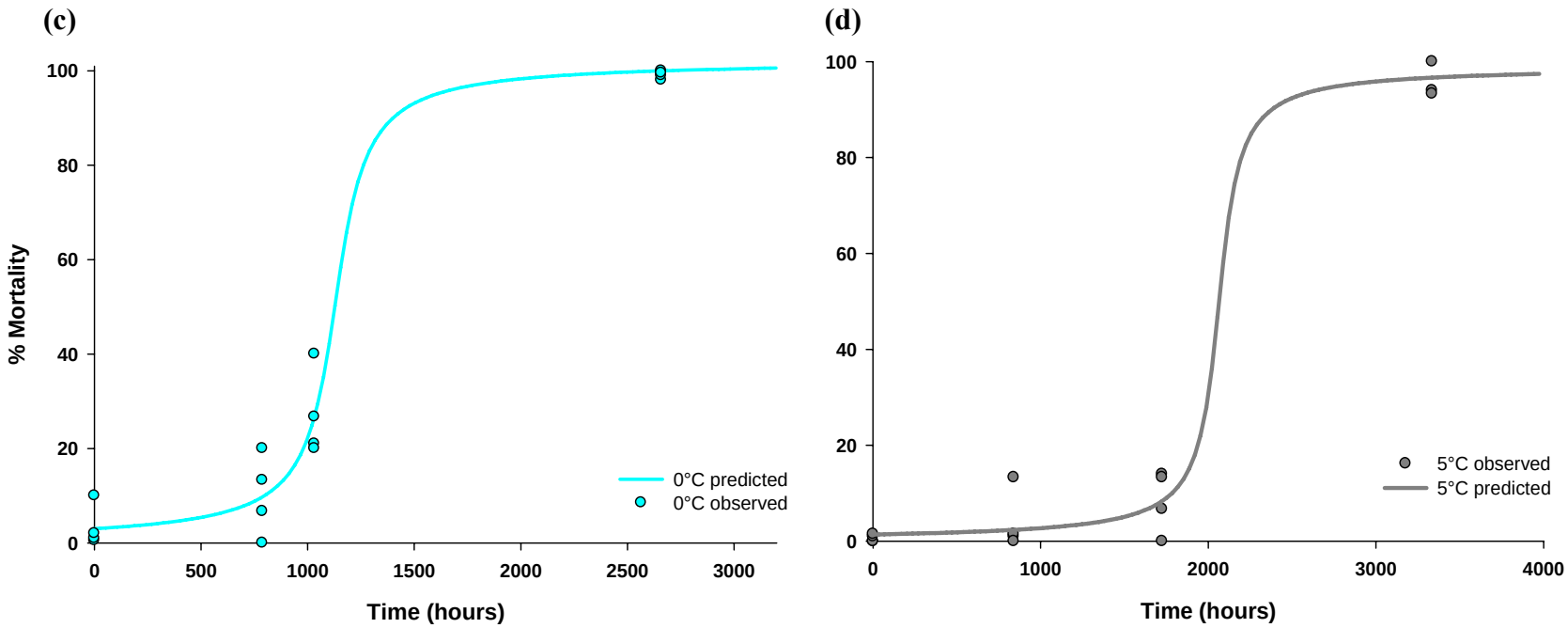


Figure 9: [continued]

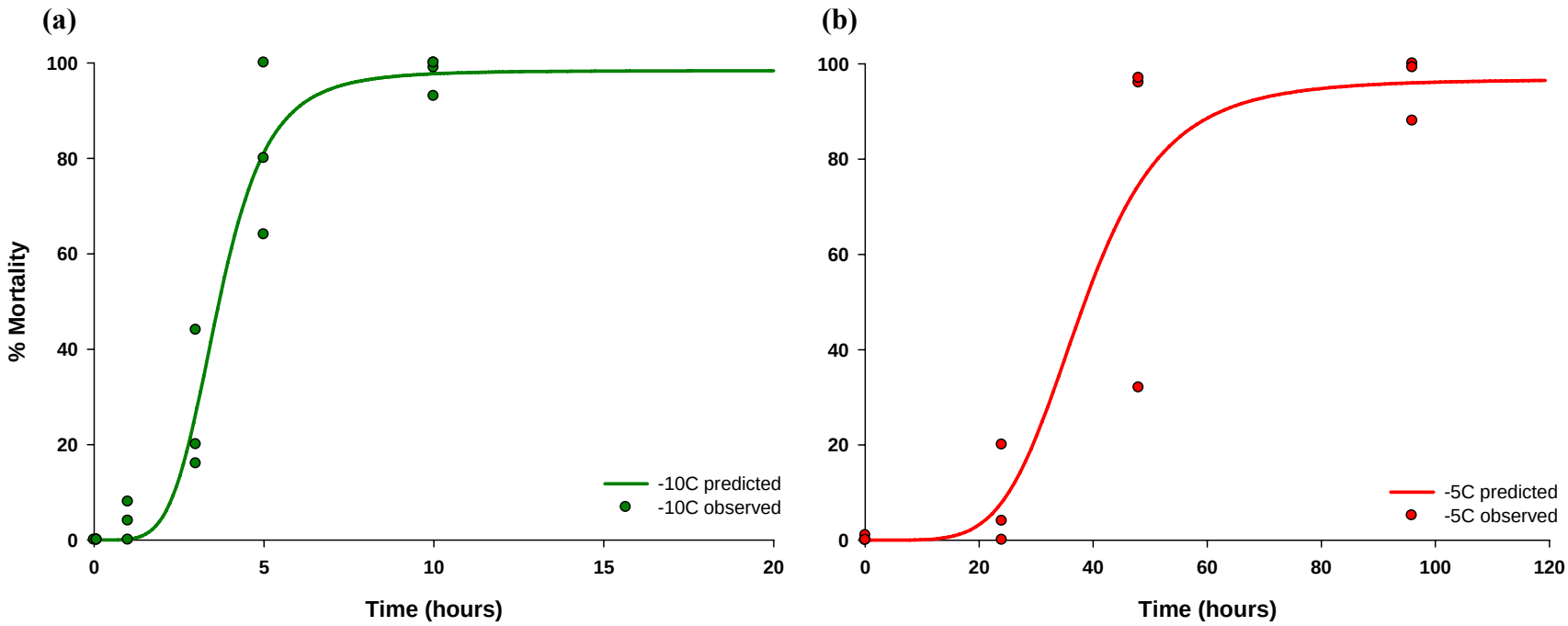


Figure 10: Percent mortality of non-diapausing *H. zea* pupae held at (a) -10°C, (b) -5°C, (c) 0°C, and (d) 5°C. Equations used to describe the mortality trends are given in Table 4. Dots represent observed mortality for each time treatment replication; treatments are listed in Table 3.

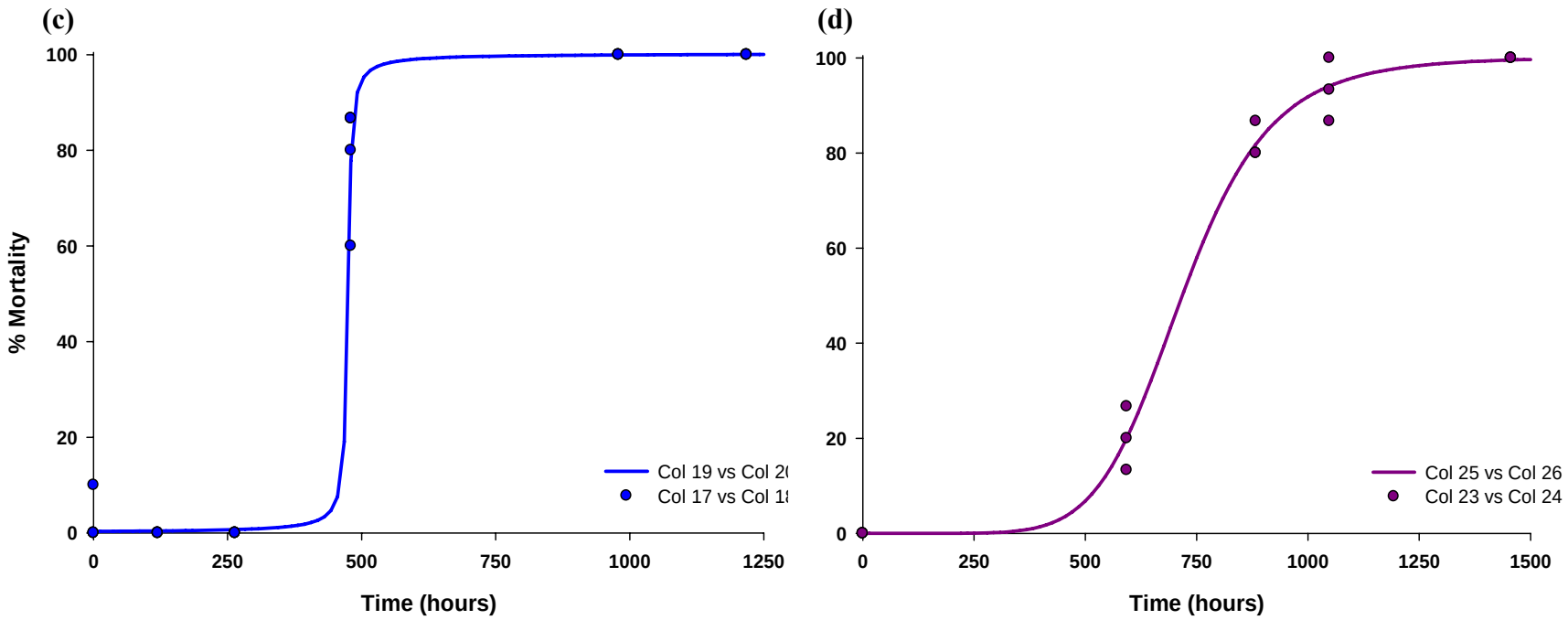


Figure 10: [continued]

Figure 11: A live, late-instar *H. zea* larva excavated from a caged plot area October 9th, 2009 Rosemount, MN (Dakota, Co.). The burrow extended ca. 3.8 cm below the soil surface.

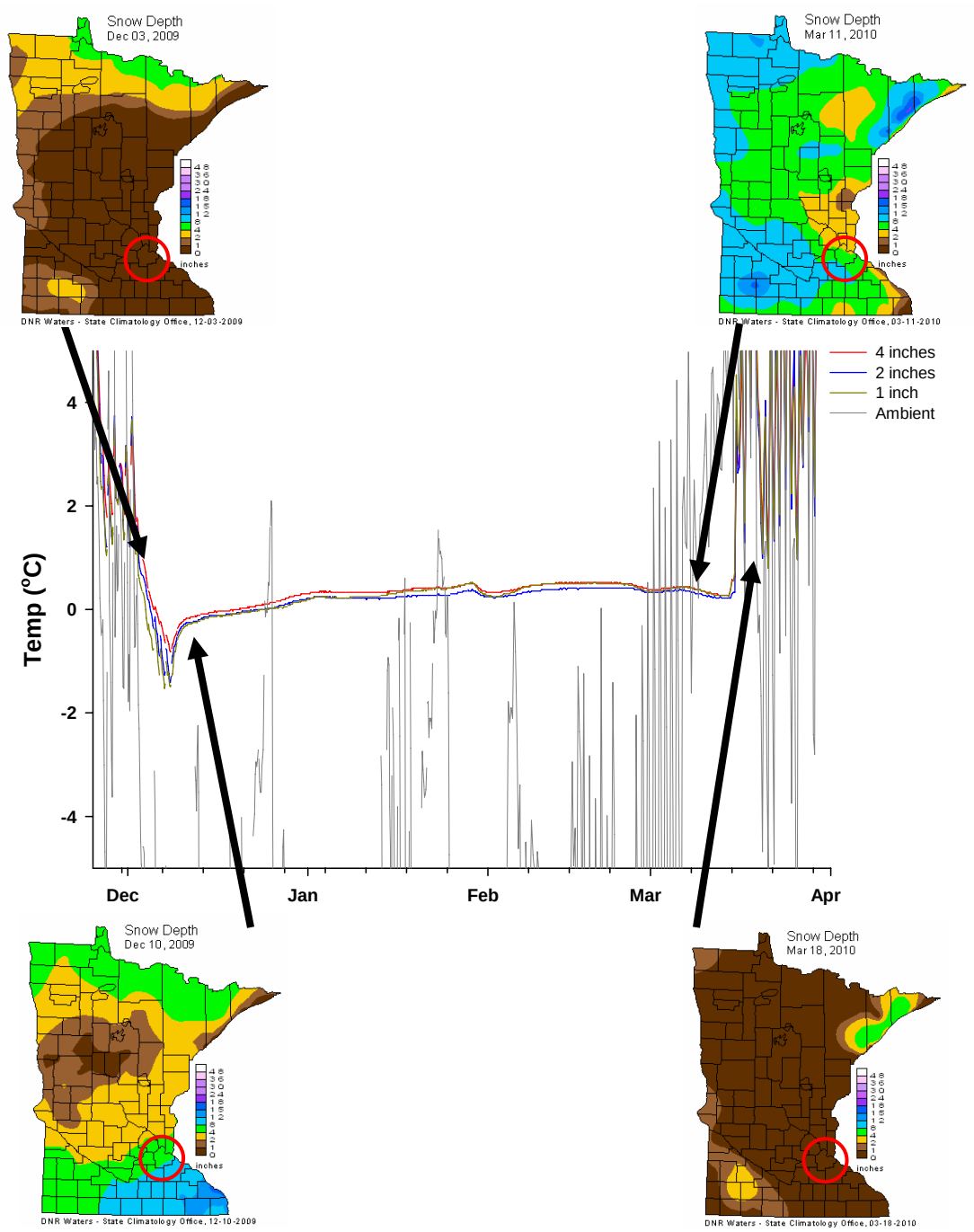


Figure 12: Atmospheric and soil temperatures (at 2.54, 5.08, and 10.16 cm) recorded during the winter of 2009/2010, overlaid with corresponding snow depth measurements from the Minnesota Climate Working Group. Snow depths remained ≥ 10.16 cm between December 9 and March 11 (data not shown). Red circle indicates location of temperature data (UMORE Park, Rosemount, MN, Dakota Co; 44º42'27.26"N and 93º06'01.53"W).

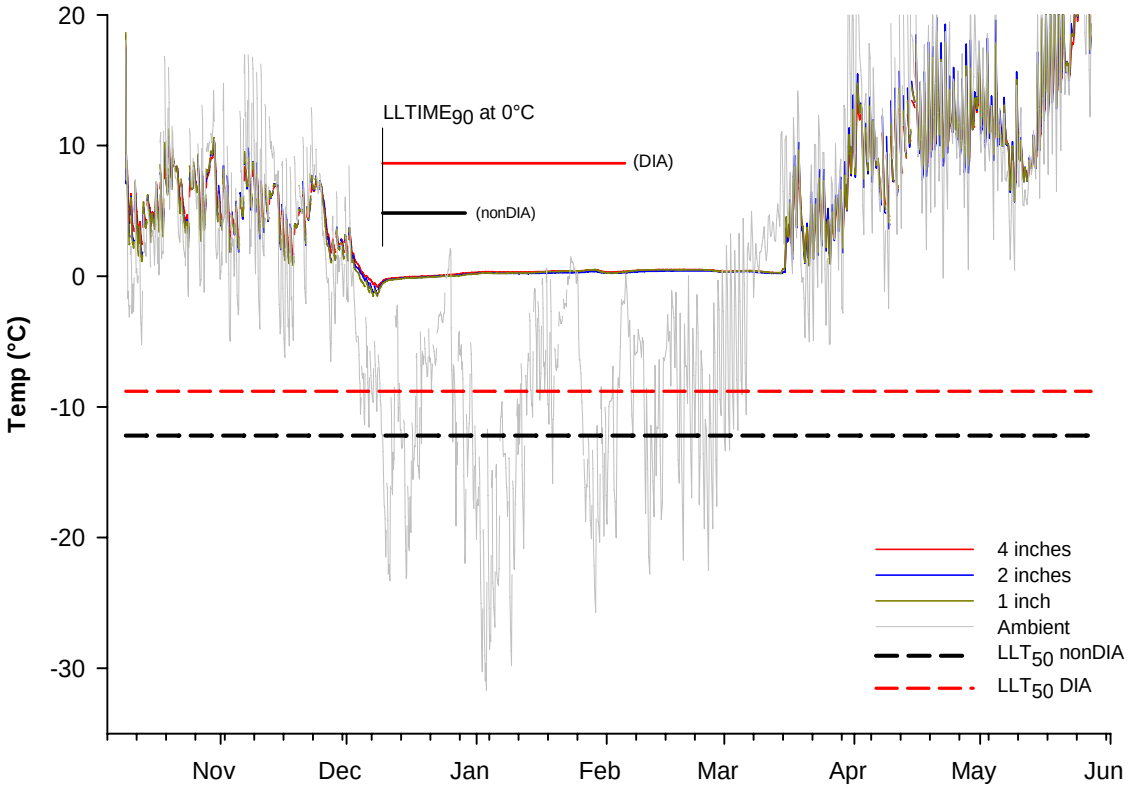


Figure 13: Atmospheric and soil temperatures (at 2.54, 5.08, and 10.16 cm) recorded from October 9, 2009 through May 27, 2010 (UMORE Park, Rosemount, MN, Dakota Co.), overlaid with laboratory-assessed cold hardiness measurements of *H. zea* pupae.

CHAPTER III:
Modeling the Potential Distribution of Corn Earworm in Response to Cold Stress and Climate Change

INTRODUCTION

The corn earworm, *Helicoverpa zea* (Boddie), is a major pest of numerous agricultural crops in North America, including sweet corn, tomatoes, cotton, and soybeans (Flood *et al.* 2005). *Helicoverpa zea* migrates throughout most of the eastern and midwestern United States and southern Canada during the growing season, but it is not currently known to overwinter north of 40-degrees latitude (Hardwick 1965, Sandstrom *et al.* 2007). Consequently, northern growers of many crops targeted by *H. zea* can presently avoid most infestations by planting high-value crops, such as sweet corn, earlier in the season before the migrating moths arrive. However, shifts in the overwintering range can have major impacts on the timing and magnitude of *H. zea* infestations in northern latitudes. Such changes could have important implications for the damage potential, and therefore successful management, of *H. zea* in these areas. Many questions remain regarding its response to low temperatures and, consequently, potential to expand its overwintering range given future climate change scenarios.

Despite criticisms of misrepresentation (e.g. Davis *et al.* 1998, Lawton 1998), climatic mapping through the use of ecophysiological models is the principle, and currently most reliable, method for predicting potential distributions of pest species under current and future climates (Baker *et al.* 2000). The ability to predict how a species is temporally and spatially dispersed is crucial for successful integrated pest management (IPM) strategies and tactics (Zalucki & Furlong 2005, Bale 2010). Ecophysiological models do this by using known or inferred physiological data for the target species and describe its response to climate, as represented by stored meteorological data of either historical trends or future projections (Ulrichs & Hopper 2008). The ecophysiological model CLIMEX has been used extensively to estimate the distributions of pest and disease populations (e.g., Yonow & Sutherst 1998, Zalucki & Furlong 2005, Venette & Cohen 2006, Sutherst *et al.* 2007). CLIMEX allows the user to estimate the suitability of regions for facilitating the establishment and persistence of a species either by direct

entering of parameter values, or through interpolation of values based on known geographical distribution data.

Using the cold hardiness data generated for diapausing pupae (the overwintering stage) in the previous chapter (Chapter II), I calculated the present and future distributions of *H. zea* in North America with the modeling software CLIMEX. The effect of overwintering microclimate, such as temperature buffering from soil depth and snow cover, were incorporated when appropriate. Graphical illustrations of overwintering suitability (cold stress) and overall climatic suitability (ecoclimatic index; EI) were each generated. Future projections were made in the context of predicted climate change to 2080 using the general circulation Hadley model (HadCM3) and B2a emission scenario. These distributions were compared to the current understanding of *H. zea's* geographic range (Hardwick 1965, Luttrell *et al. in press*) and to the projected future range shifts presented in Diffenbaugh *et al.* (2008). The implications of potential changes in the geographic range of *H. zea* are discussed within the context of future IPM needs for sweet corn, as well as other vegetable and field crops throughout North America.

MATERIALS and METHODS

CLIMEX software and climate data

CLIMEX v2 (Sutherst *et al.* 2007) was used to express the overall climatic suitability for a particular location within North America by an ecoclimatic index (EI). The EI was produced using the "compare locations" function and reflects the combined potential for population growth during favorable periods (measured with an annual growth index, (GI)) and persistence during stressful periods (measured with indices for cold (CS), heat (HS), drought (DS), and wet (WS) stress). The GI is the product of a temperature index (TI) and moisture index (MI). The growth and stress indices were calculated on a weekly basis. Sutherst *et al.* (2007) provides further detail on the theory and mathematics behind the software's calculations.

For simulations of future CS and EI distributions with climate change, a downscaled Hadley Centre coupled atmosphere-ocean general circulation model (HadCM3) was used, with the SRES B2a emission scenario.

CLIMEX parameters

The aforementioned parameters used by CLIMEX were estimated using data generated from the preceding chapter (i.e. cold stress), known literature values (e.g. developmental thresholds from Hogg & Calderon (1981) and Hartstack *et al.* (1976)), or from inferred values provided by R.W. Sutherst (Luttrell *et al.* in press). These values are presented in Table 6.

Cold stress in the CLIMEX model is described in terms of a threshold (i.e. the point at which mortality increases due to cold) and rate of stress accumulation. Using the data generated in Chapter II of this thesis, these values were calculated from the time needed to reach 95% mortality at a given temperature for diapausing pupae (Table 5). To conform with assumptions in CLIMEX, these data were first converted from an hourly to a weekly basis. Following this, a second transformation was applied, based on the quadratic equation, to account for the assumed linear relationship between temperature and mortality:

$\text{Mortality}_{\text{transformed}} = 2/(n/(n+1))$, where n is in weeks

Following these transformations (Table 7), mortality was then expressed as a function of temperature by linear regression (Microsoft® Excel 2002). From this regression, the cold stress threshold and rate of accumulation were produced and input into CLIMEX (Table 6); the threshold being the y-intercept coefficient divided by the x-variable coefficient, and the rate being simply the x-variable coefficient.

To compensate for the potential discrepancy between the temperature of soil where the average *H. zea* pupa overwinters and the ambient air temperatures used by the CLIMEX weather database, the cold stress threshold value was adjusted. This was done rather than changing each individual temperature point as justified in Venette and Hutchison (1999). The adjustment value was based on a regression model fit in TableCurve (SYSTAT 2002) of mean daily air temperatures with mean bare soil

temperatures; a similar method was used in Lam and Pedigo (2000) to relate air temperatures with corresponding leaf litter temperatures. The temperature measurements were taken from various states and years using the databases from the United Stated Department of Agriculture Natural Resources Conservation Service Soil Climate Analysis Network (USDA-NRCS SCAN) or the Minnesota Climate Working Group. Soil depths of 10.16 cm were used as they were the closest value measured to the average pupal burrow depth of 8.89 cm. Temperature datasets were chosen if they experienced soil temperatures at or below the cold stress threshold (-7.58°C). This resulted in four years (2004-2007) being used from St. Paul, Minnesota and one year (2008) being used for one location in Colorado and Montana. For each year and state, mean daily air and soil temperature were regressed using the polynomial model, $y = a + bx + cx^2$, chosen based on r^2 values and lack-of-fit statistics (SYSTAT 2002). The air temperature corresponding to a soil temperature of -7.58°C was then calculated for each. The resulting six air temperature estimations were averaged to give a final value of -21.12°C, representing the new cold stress threshold, adjusted for the soil microclimate, to be used in CLIMEX.

Additionally, data of surface and soil temperatures and snow cover during the winter of 2009/2010 in Rosemont, MN were overlaid (Figure 12) to determine if a general relationship could be assumed between snow cover and soil temperature. Based on these data, it was assumed that when snow cover was 10.16 cm or greater, soil temperatures would not drop below 0°C. Areas that historically experienced snow depths of at least this depth for periods shorter than the LL$Time_{95}$ at 0ºC (<67 days) for diapausing pupae were removed from the CLIMEX simulation and assumed to not experience cold stress. Historical snow depth data were obtained from the National Climatic Data Center Climaps database (http://cdo.ncdc.noaa.gov/cgi-bin/climaps/climaps.pl).

Ecoclimatic (EI) maps were produced to depict the current distribution of *H. zea*, as well as three projections of future distribution under climate change in 2020, 2050, and 2080. Cold stress (CS) maps of the same time periods were also produced separately since this was a direct reflection of the cold hardiness data contributed by Chapter II of this thesis.

All CLIMEX results were exported to the geographic information system, ArcView 3.2 (ESRI, Redlands, CA) and isoclines were generated using ArcView Spatial Analyst (ESRI).

RESULTS

The cold stress analysis indicated that cold stress is not a limiting factor in the current distribution of *H. zea* in the contiguous United States, as shown by index values of 0 for everywhere but northern Minnesota (Figure 14a). Consequently, cold stress influence continued to recede further into northern Canada when interpolated with future climate change projections (Figure 14b-d). Because cold temperature estimates in bare soil were not shown to be stressful, the need to incorporate the additional buffering effect of snow cover was no longer deemed necessary.

The EI for the current northern range of *H. zea* (Figure 15a) reflected the limited cold stress seen in Figure 14. Interestingly, based on known *H. zea* distributions in the sourther U.S. (Hardwick 1965, Luttrell *et al*. in press), the EI showed a limited southern range of the most suitable areas for *H. zea*, with northern boundaries barely stretching into Texas, Oklahoma, and North Carolina (Figure 15a). Contrastingly, the regions of favorable suitability extended well into the Midwest and Northeast, and areas of marginal suitability ranged over most of Canada and much of the West. This projection did not change dramatically under climate change, but the regions of favorable suitability did push further north to encompass all of the Northeast, and most of Minnesota, Wisconsin, and Michigan (Figure 15b-d). CLIMEX predicted a slight receding of the northern boundary of highly favorable areas in the south and the western boundary of favorable areas, which its parameter estimates showed was being driven by low moisture stress (data not shown).

DISCUSSION

The results from the above CLIMEX model do not depict what is currently understood of *H. zea* overwintering distribution and overall geographic suitability. In this

study's current cold stress map from CLIMEX (Figure 14a), cold temperatures are clearly not interpreted to be a significant constraint to *H. zea* suitability for most of the U.S.. Combining this with the other stress and growth indices, the resulting current EI map depicts highly suitable areas to be limited to the very southern portions of the U.S. and throughout Mexico. In addition, as could be expected given the lack of cold stress, the EI shows most of the Midwest (including southern Minnesota) and northeast to be favorable (EI 11-25) and areas well into Canada, Alaska, and the northwest to be marginal (EI 1-10).

These results are somewhat surprising, as they differ substantially from what is currently assumed about *H. zea* overwintering and cold tolerance. The dogma for many decades, built and supported on continuous monitoring and sampling programs throughout North America, has been that areas beyond approximately the 40th parallel are not suitable for *H. zea* to persist year round due to lethal winter temperatures (Quaintance & Brues 1905, Hardwick 1965, Sandstrom *et al.* 2007). In a CLIMEX model based on predominately inductive parameter estimates, Luttrell *et al.* (in press) presented a "current" EI map for *H. zea* that follows reasonably well to this convention, showing only marginal suitability in a few areas that extend beyond the 40th parallel estimate (Figure 1, Chapter I).

The reasons for the discrepancy seen with the present study are not entirely known, but at least three options are possible: First, the input data for CLIMEX could be erroneous, i.e. the raw data for time-temperature morality relationships (Chapter II), or either of the transformations of these data to meet the assumptions of CLIMEX (Table 6) and accommodate soil temperatures. Second, the CLIMEX model itself may not be sufficiently flexible, and thus, invoke errors with regards to the assumptions of what parameters are driving the geographic distribution of *H. zea* are not accurate or complete. Or, lastly, the conventional thought behind the factors influencing the *H. zea* distribution, i.e. cold stress experienced by the overwintering stage, are not accurate or complete. The first option of experimental error is a valid possibility and difficult to completely discount. But given the author's confidence in how the data were collected and sufficient justification for what transformations were applied to the data, this is assumed not a significant influence. Therefore, the discrepancy is left to the assumptions made in

CLIMEX, the assumptions made by convention, or some combination thereof. The latter is most likely true. Overwintering mortality of *H. zea*, or more specifically lack of fall larvae surviving to spring adults, is shown to keep *H. zea* from being a local pest of economic importance in areas beyond the 40th parallel. Because the pupa (diapausing) is the overwintering stage and most cold hardy, it is reasonable to use its response to cold as a proxy for how the species will present in the spring. However, if the pupa is actually not the stage receiving the majority of mortality between fall and spring, then it could incorrectly represent survival. This could be the case in this study, where essentially no cold stress was modeled to occur, but field observations indicate mortality occurs following the cold conditions of winter. There is some evidence to suggest that a large portion of apparent winter mortality occurs after pupae survive winter, but before adults emerge from the ground. In a study of overwintering *H. zea* in Arkansas Slosser *et al.* (1975) found that only 1/4-1/3 the number of pupae that survive the winter actually emerge as moths. Similarly, Caron *et al*. (1978) found high levels of spring pupa- to-emerged adult mortality in North Carolina fields, with nearly 50% of pupae surviving winter but less than 3% of those surviving to emerged adults. Reasons for adult spring mortality were hypothesized as lack of diapause termination, deformed eclosion, or disruption of the emergence tunnels (Slosser *et al.* 1975, Caron *et al.* 1978). Unfortunately, CLIMEX is not able to account for lifestage-specific variation in the parameter estimates it uses, allowing only for one value to represent the species. Because the Luttrell *et al*. (in press) model used inductive estimates, meaning those that were interpolated based on current distribution records for *H. zea*, it may indirectly represent an average value of all the lifestages for each parameter, as well as potential microhabitat effects, such as soil conditions, not directly included in CLIMEX.

Therefore, it is likely a combination of factors, namely CLIMEX using only one value to describe a species' stress response and the pupal stage being used as the representative for cold stress mortality in *H. zea*, resulted in the discrepancies mentioned above. For studies using a model such as CLIMEX, these results suggest that amore coarse climate-matching, inductive approach (e.g. Luttrell *et al.* 2010, in press) may be a valid method for initial understanding of species distributions. Alternatively, to more specifically account for stage-specific overwintering mortality (and other stressors), a

more detailed, process-orientated simulation model (e.g. Storer *et al.* 2003) may be necessary.

Concerning the maps of predicted *H. zea* distributions under climate change (Figure 15b-d), comparisons to those produced in Diffenbaugh *et al.* (2008) show a similar overall northern expansion of suitability for *H. zea*. The specific degree and locations at which this occurs clearly differ, however; Diffenbaugh *et al.* (2008) limited the most northern expansions into the Northeast and upper Midwest states, but showed a much broader base of highly suitable areas in the south, whereas the present study extended marginal suitability well into the northern and western states, as well as Canada, and had a much shallower suitable base in the south. Two considerations should be made, though, when making these comparisons. First, the models depicted different "current" distributions, so comparisons of future predictions must be relative to those. Venette *et al.* (2010) cautions that within the context of pest risk mapping, it is important to keep in mind that variation in modeling assumptions and approaches, as well as in underlying objectives and data, can lead to dramatically different maps. This leads to the second consideration of recognizing differences in the model inputs. Though the reference climate periods were similar (1961-1989 vs. 1961 -1990), two different climate change models were used; Diffenbaugh *et al.* (2008) used RegCM3 and the present study used HadCM3. Among other things, such as the HadCM3 being coupled with oceanic data, the models act with different spatial resolutions (Gordon *et al.* 2000, Diffenbaugh *et al.* 2008). The criteria for screening suitability are also quite different, with Diffenbaugh *et al*. (2008) focusing solely on temperature. Even with this focus, they do not include an upper limit to this effect (i.e. upper developmental threshold) and do not account for the buffering effects of soil on temperature. Despite these differences in approach, though, both the present study and Diffenbaugh *et al.* (2008) illustrate a northern movement of suitability for *H. zea,* compared to current distributions, under future climate change.

These projections carry important implications for the successful management of *H. zea,* particularly in northern regions like Minnesota that are currently only affected by late-season migrants. Any northern expansion of suitability, whether or not it encompasses Minnesota, could bring about major changes to the infestation dynamics of *H. zea* in the area. Migrants could arrive earlier, and in larger numbers, consequently

increasing the number of potential generations. Earlier plantings of crops previously affected only late in the season, such as sweet corn, could be at risk of damage. Similarly, crops whose susceptible stage was previously out of synch with most infestations, such as field corn, could become susceptible. These issues all highlight the need for continued IPM practices. In the short term, tactics of persistent local scouting and sampling are important. More long-term strategic planning should involve development and use of alternative *H. zea* control measures to mitigate the development of resistance currently observed with some conventional insecticides and single gene *Bt* crops (Hutchison *et al.* 2007, Tabashnik *et al.* 2008). For example, recent advances in transgenic technology have made possible the use of pyramided *Bt* corn and cotton varieties that employ the use of multiple genes with higher efficacy than previously attained against *H. zea*, and differ in their modes of action (e.g. Hutchison & Storer 2010).

None of the models mentioned here explicitly account for effects of climate change on the synoptic weather patterns across North America. To our knowledge, no currently available model does so, but it has been shown that such weather patterns will be affected by climate change (Westbrook & Lopez 2010). This consideration is important for a highly migratory species like *H. zea* since changes in these weather events could cause directional shifts in infestations, as well as changes to the magnitude, timing, and duration of infestations, including potential reinfestations due to reverse migrations (Beerwinkle 1994, Westbrook 2008). These infestation changes have the same management repercussions as those described previously.

In conclusion, this study provides useful insights into the modeling of *H. zea* distributions, both presently and in the context of climate change. Specifically, additional models using a variety of parameters and assumptions are needed to more confidently depict distributions and that the weight previously given to some factors dictating these patterns, like the cold tolerance of overwintering pupae, may need to be reevaluated. It also further supports the general theory that insect distributions will increase in mid to high latitudes under current climate change projections (Bale *et al.* 2002, Diffenbaugh *et al.* 2008). This support emphasizes the need for vigilant IPM practices, such as local

monitoring of populations and utilizing resistance management strategies, to best respond to the consequences of shifts in *H. zea* distributions.

Table 6: Parameter values used in the CLIMEX model to characterize growth and stress responses of *Helicoverpa zea.*

Parameter	Description	Value
Temperature[a]		
DV0	Lower threshold for growth	12.6
DV1	Lower optimum for growth	25.0
DV2	Upper optimum for growth	33.0
DV3	Upper threshold for growth	40.0
Moisture[b]		
SM0	Lower threshold for growth	0.05
SM1	Lower optimum for growth	0.5
SM2	Upper optimum for growth	2.0
SM3	Upper threshold for growth	4.0
Cold stress		
TTCS[a]	Cold stress threshold	-21.22
THCS[d]	Stress accumulation rate	-0.00263
DTCS[c]	Degree-day threshold	0.0
DHCS[d]	Degree-day stress accumulation rate	0.0
Heat stress		
TTHS[a]	Heat stress threshold	39.5
THHS[d]	Stress accumulation rate	0.01
DTHS[c]	Degree-day threshold	100.0
DHHS[d]	Degree-day stress accumulation rate	0.0
Dry stress		
SMDS[b]	Dry stress threshold	0.20
HDS[d]	Stress accumulation rate	0.002
Wet stress		
SMWS[b]	Wet stress threshold	2.0
HWS[d]	Stress accumulation rate	0.002

[a] °C

[b] expressed as a proportion of soil moisture holding capacity, where 1 = saturation.

[c] weekly sum of degree-days (above threshold) required to sustain the population

[d] rate per week

Table 7: Transformations of LL*Time* data for diapausing *Helicoverpa zea* pupae to conform with the assumptions of CLIMEX.

	Time until mortality	
Mortality	0°C	5°C
95% (hours)[a]	1599.17	2796.92
95% (weeks)[b]	9.52	16.65
Transformed[c]	0.02	0.0068

[a] as generated from laboratory studies in Ch. 2 (Table 5)
[b] weeks = (h/24)/7, where h is in hours
[c] transformed = 2/(n/(n+1)), where n is in weeks

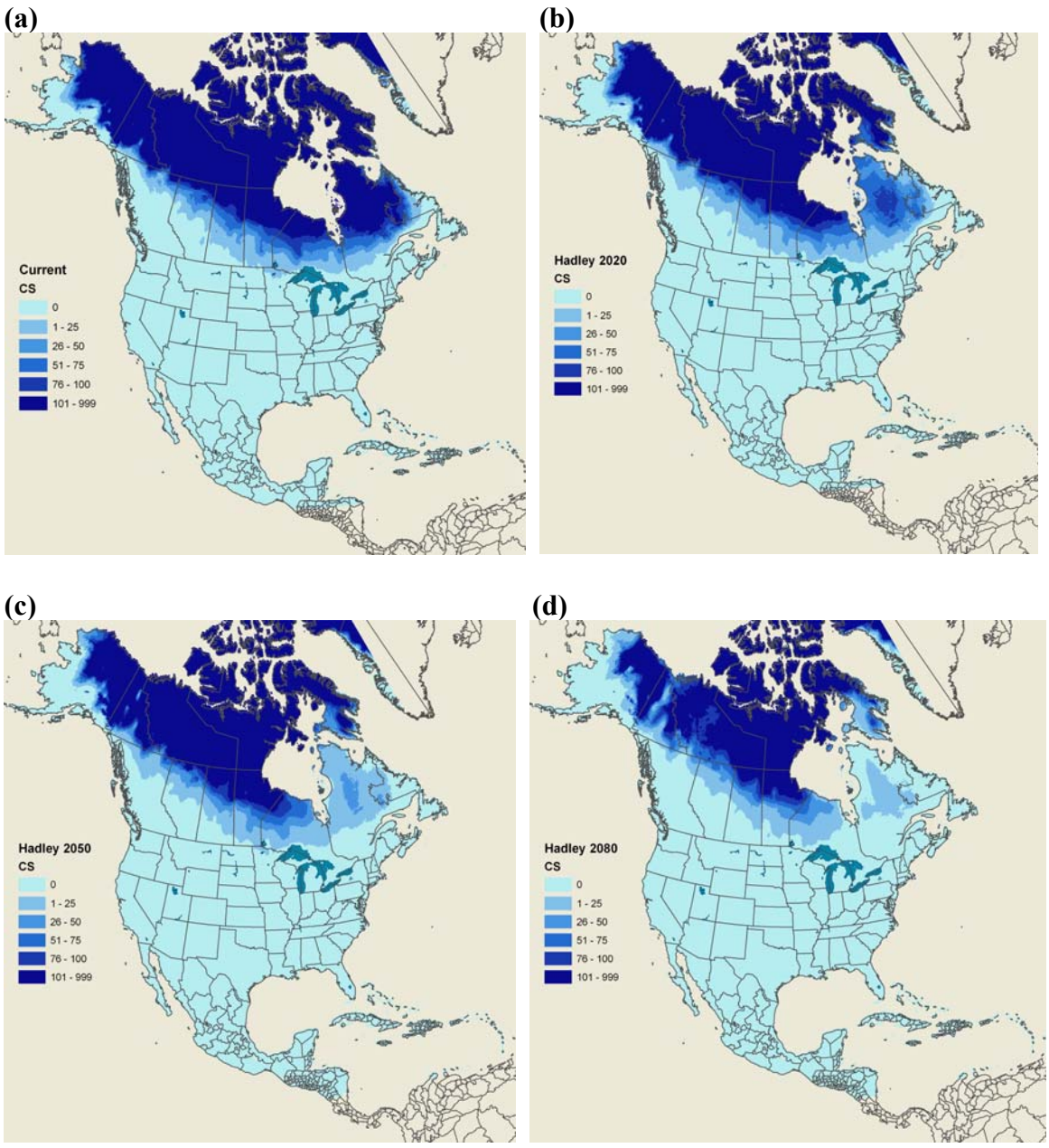


Figure 14: CLIMEX index of cold stress experienced by *Helicoverpa zea* in North America as estimated from the cold hardiness data presented in Ch. 2. Cold stress values >100 are lethal, whereas values <25 are minimally stressful. Future cold stress projections are made using downscaled output from the coupled general circulation model HadCM3 (Hadley Centre, Exeter, United Kingdom). (a) current cold stress (b) 2020 cold stress (c) 2050 cold stress (d) 2080 cold stress. An estimation of the 40th parallel is included for reference of the conventionally understood overwintering limit.

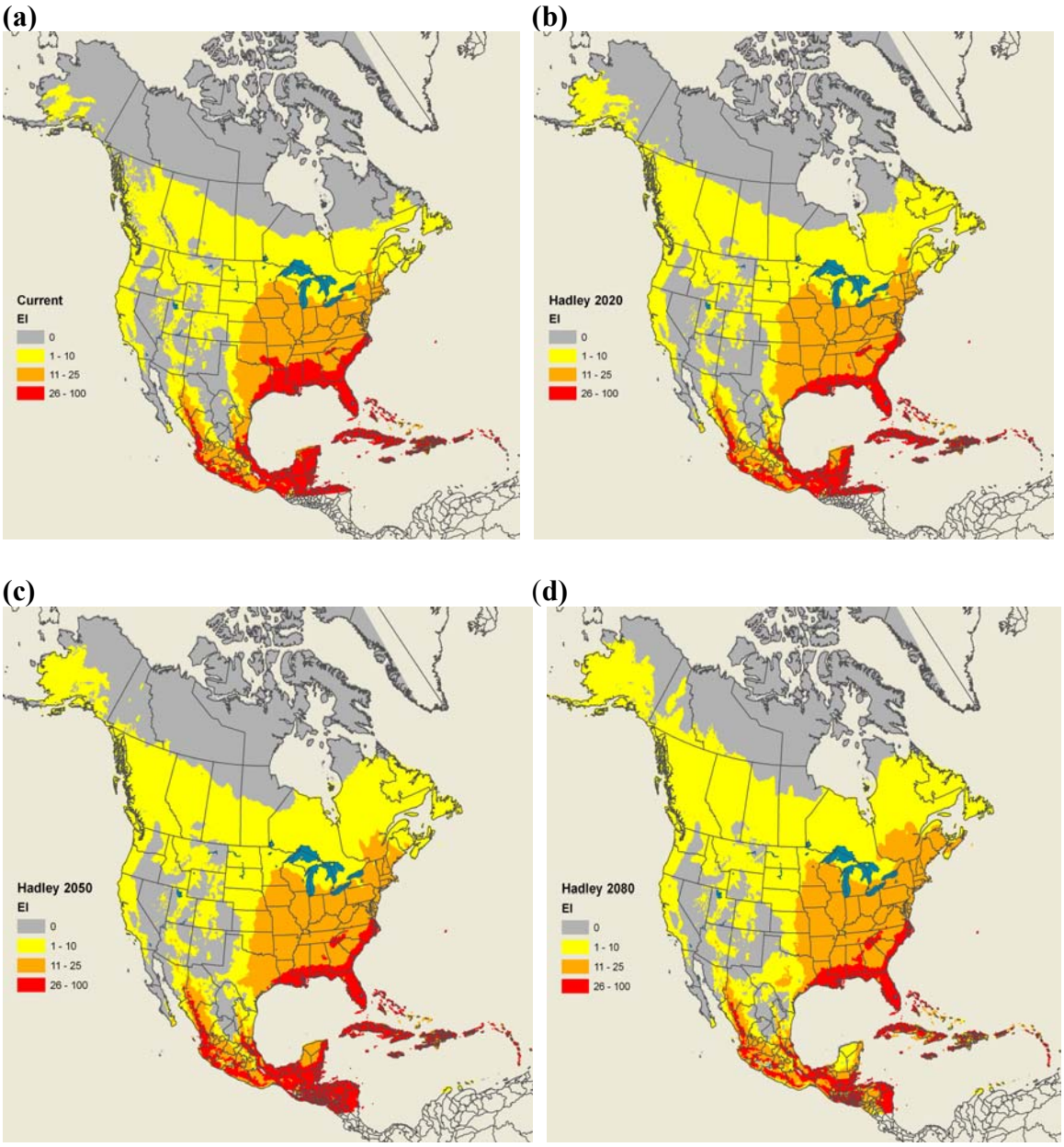


Figure 15: CLIMEX ecoclimatic index (EI) of the combined annual potential for growth during favorable periods and persistence during stressful periods of *Helicoverpa zea* populations in North America. EI values of 0 are unsuitable, 1-10 marginal, 11-25 favorable, and ≥26 very favorable for establishment. Future EI projections are made using downscaled output from the coupled general circulation model HadCM3 (Hadley Centre, Exeter, United Kingdom). (A) current EI (B) 2020 EI (C) 2050 EI (D) 2080 EI. An estimation of the 40th parallel is included for reference of the conventionally understood overwintering limit.

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APPENDIX A
First report of association between corn earworm (*Helicoverpa zea*) pupae and mold mites (*Tyrophagus putrescentiae*) in the laboratory

Amy C. Morey, William D. Hutchison, and Stephen A. Kells

Abstract

Corn earworm, *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae), pupae and mold mites, *Tyrophagus putrescentiae* (Shrank) (Astigmata: Acaridae), both occur within the top 5 cm of soil throughout the U.S., but interactions between these two species have not been documented. Historically, *T. putrescentiae* has been considered strictly fungivorous, though later findings indicated it is also predaceous. This study sought to determine if *T. putrescentiae* could cause mortality to apparently healthy or malformed *H. zea* pupae, and if the pupae were an adequate diet for mite population growth. Completely sclerotized and incompletely sclerotized pupae were individually infested with 0, 10, or 100 adult mites and observed after 5-8 or 9-14 days. Statistically significant pupal mortality was only seen between sclerotization levels, but numerical trends showed the greatest mortality when 100 mites were applied to a pupa with incomplete sclerotization. Total mite population growth was statistically significant for this treatment group after 9-14 days. Additionally, mites were observed entering pupal cuticles with incomplete sclerotization and feeding on internal tissue. These results suggest that the mites may increase the mortality of pupae with incompletely sclerotized cuticles, and can significantly increase in population density when in the presence of such pupae. This is the first study to document feeding activity by *T. putrescentiae* on *H. zea* pupae and provides preliminary data to warrant further laboratory and field investigations of this interaction.

Keywords: Acaridae, Noctuidae, fungivorous mite, natural enemy, incomplete sclerotization
Abbreviations: CS: completely sclerotized, IS: incompletely sclerotized

INTRODUCTION

The mold mite, *Tyrophagus putrescentiae* (Schrank), is a ubiquitous soil mite and important global pest of stored products, such as cheese (Robertson 1952), grain (Hughes 1976), dry-cured ham (Arnau & Guerrero 1994), and commercial dog food (Brazis *et al.* 2008). Originally thought to be exclusively saprophagous and fungivorous (Hughes 1976; Krantz 2009; O'Connor 1982), *T. putrescentiae* has also been observed to prey on small and relatively stationary organisms, such as soil nematodes (Walter *et al.* 1986; Bilgrami & Tahseen 1992) and eggs of southern corn rootworm *Diabrotica undecimpunctata howardi* Barber (Brust & House 1988). More recently, *T. putrescentiae* were observed preying on much larger organisms, such as larvae and pupae of the cigarette beetle, *Lasioderma serricorne* F., both in laboratory settings (Kumar 1997) and *in situ* in a tobacco warehouse (Papadopoulou 2006).

Following an unintentional infestation of *T. putrescentiae* in a laboratory colony of *Helicoverpa zea* (Boddie) pupae, adult mites were seen feeding and reproducing on live and dead *H. zea* pupae; increased pupal mortality was also observed in this colony (Morey, unpublished data). It was unclear, however, if the mites were the primary source of mortality or if the pupae were weakened by previous malformations. To our knowledge, no previous studies have documented the feeding of this mite on *H. zea* pupae.

Corn earworm is an economically damaging pest in many agricultural systems throughout the U.S., such as corn, tomatoes, and cotton, and is becoming increasingly difficult to control due to insecticide resistance (Hutchison *et al.* 2007; Jacobson *et al.* 2009). Overwintering mortality is an important factor influencing the annual infestations of *H. zea*, which overwinter as pupae in the soil (Hardwick 1965). Given the potential habitat overlap in subterranean populations of *T. putrescentiae* and *H. zea*, investigating the dynamics of their interactions could have important implications for local population development of *H. zea.*

The objectives of this study were to determine if *T. putrescentiae* could cause direct mortality to *H. zea* pupae, either as healthy or previously malformed individuals, and if the pupae were an adequate food source for mite population growth.

MATERIALS and METHODS

Colony source

A laboratory colony of *T. putrescentiae* was maintained at the University of Minnesota on semi-moist dog food (25% crude protein, 10% crude fat, and 14% moisture) at 24ºC and 85% RH (Eaton & Kells 2009). Adult *T. putrescentiae* were randomly obtained from the "wandering" population, those found on the lid of the colony container. Wandering populations were chosen because they colonize new food resources (Eaton & Kells 2009). We did not determine, or sort by, the sex of adults prior to the study because the additional handling may have increased stress and mortality of the mites. Post-hoc determination of control sample sex ratios yielded 74% females and this is not significantly different than the average 70% (S.E. ±2.7%) female-male biased sex ratio for this sampling method (Kells, unpublished data).

Early instar larvae of *H. zea* were obtained from a lab colony in Stoneville, MS (USDA-ARS) and reared on artificial diet to non-diapausing pupation in programmable growth chambers (25°C; 14:10 L:D) in St. Paul, MN. Four days following pupation, pupae were separated into two groups; those with normal, completely sclerotized cuticles (CS; Figure 16a) and those with incompletely sclerotized cuticles (IS; Figure 16b). Incomplete sclerotization was characterized by visible spaces or breaks in the pupal cuticle, such that small areas of soft tissue were exposed.

Study design

Eight treatments were used in this study, with 10 replications each. The treatments were: 10 mites only (10M), 100 mites only (100M), a completely sclerotized pupa only (CS), an incompletely sclerotized pupa only (IS), 10 mites per pupa having a completely sclerotized cuticle (10M+CS), 10 mites per pupa having an incompletely sclerotized cuticle (10M+IS), 100 mites per pupa having a completely sclerotized cuticle (100M+CS), and 100 mites per pupa having an incompletely sclerotized cuticle (100M+IS).

Each replication was placed in a 20ml glass vial sealed with medium porosity filter paper placed between the vial and cap to contain the mites while permitting equilibration of temperature and humidity within the vials (Eaton & Kells 2009). Vials were then placed in humidity chambers at 74% RH and 23°C (7.3 mBar vapor pressure deficit) and 14:10 L:D. Desiccators with a saturated salt solution placed in programmable growth chambers maintained these conditions (Winston & Bates 1960).

Mite populations were monitored over time by freezing half of all treatment replications five days from study initiation. The remaining vials were frozen at 14 days from study initiation, or upon moth emergence. For analysis, these freezing times were grouped into two periods, 5-8 days and 9-14 days from infestation, because sample sizes were unequal per day due to variation in moth emergence.

Pupae were checked daily for survival by gently rotating the vial to stimulate pupal movement. Following trial completion, the total number of mite eggs, juveniles (larvae and nymphs), and adults were then counted in each vial. Pupae were dissected to account for any mite activity under the cuticle. When moth emergence occurred, the moth surface was examined for mites.

Analysis

Analysis based on binary logistic regression (PROC GLMMIX, SAS 2004) was used to detect differences in pupal survival among treatments. Least-squares means estimates were calculated for the effect of pupal health (sclerotization level), mite infestation level, and their interaction to test the null hypothesis that the associated treatment population effects equal zero (i.e. no effect on mortality).

Changes in mite populations were analyzed over time as combined counts of all stages observed (adults, juveniles, and eggs). Counts from the pupal treatments (no pupa, CS, IS) were separated by termination periods (5-8 or 9-14 d) within each mite infestation level (10 or 100 per pupa). Each set of counts was analyzed by ANOVA (PROC GLM, SAS 2004) for differences across pupal treatments; where significant differences were found ($P \leq 0.05$), means were separated by protected LSD (PROC

GLM, SAS 2004). Counts were log-transformed where appropriate to meet the assumptions of ANOVA.

RESULTS

Pupal mortality

Pupal sclerotization had a significant effect on mortality (P = 0.001, *df* = 59, F = 11.79), with IS being significantly different from zero (P = 0.005, *df* = 59, F = 11.79). However, no significant differences in pupal mortality were detected from mite density (P= 0.74, *df* = 58, F = 0.31), or the interaction of sclerotization and mite density (P = 0.46, *df* = 55, F = 0.95). Numerically, the highest mortality was observed in the 100M+IS treatment (70 ±13.8%) (Figure 17).

Mite populations

Mites were observed exploring both CS and IS pupae, but appeared unable to penetrate the cuticle unless there was a previous weakening or natural deformity (as seen in all IS pupae). If the IS deformity was present (Figure 16b), mites quickly aggregated in these areas and entered the pupal integument, where their highest densities would thenceforth be maintained (Figure 18). Apparent feeding inside the integument was seen in both live and unresponsive pupae.

Numerically, mite populations in the presence of an IS pupa were on average higher for all infestation levels and termination dates, compared with those placed on a CS pupa or no pupa at all (Figure 19). Statistically, only the high density of 100 mites on IS pupae (100M+IS), during the second termination period, was significantly different ($\alpha = 0.05$; *df* = 13; *P* = 0.009) from the corresponding 100M control and 100M+CS treatment (Figure 19).

DISCUSSION

Helicoverpa zea occurs throughout much of North America, including overwintering populations in the eastern and Midwestern U.S. below the 40th latitude, or by annual migrations to northern crops in the U.S. and southern Canada (Hutchison *et al.* 2004; Westbrook 2008). Prior to pupation, *H. zea* larvae construct small burrows between 2.5 and 13 cm into the soil (Hardwick 1965). *Tyrophagus putrescentiae* generally live in the top 5 cm of soil and are known to inhabit agroecosystem soils in corn-producing areas throughout the U.S. (Brust & House 1988). Given this habitat overlap and recent evidence to suggest this mite may also be predaceous (e.g. Papadopoulou 2006), there is potential for *in situ* interaction between these species.

The results of our study suggest that *T. putrescentiae* is capable of preying upon and surviving on *H. zea* pupae, but that consistent mortality to the pupae may be limited to situations involving those with a pre-existing cuticular deformity. Sclerotization alone has clear influence on the mortality of pupae; all incompletely sclerotized (IS) pupae had significantly greater mortality than completely sclerotized (CS) pupae. Moreover, mortality among the IS treatments was not statistically different (Figure 2). However, it is notable that IS pupae in the presence of a high mite density experienced the highest numerical mortality of all treatments (Figure 17), particularly because the increase in

mite population density was only significant for the 100M+IS treatment (Figure 19). Additionally, mites that entered the pupal integument were observed to be feeding on internal tissue (Figure 18). Though statistical support of numerical difference in IS mortality was not confirmed, this may be due to insufficient replication, causing apparently high variation among treatments. Increasing the initial mite infestation levels or the study duration may also reveal differences in pupal mortality. The mites were able to use pupae as a resource to survive and reproduce, but a significant increase in population density was not observed until near the end of the study. With the average developmental time of *T. putrescentia*e being 8-10 days at 23ºC from egg to reproductive adult (Sánchez-Ramos & Castañera 2001), using non-diapausing pupae did not allow for the measurement of the effects of multiple mite generations; *H. zea* adults began eclosing after ca. 8 days. Therefore, mite densities may never have been allowed to reach levels capable of inflicting significant mortality. Diapausing pupae were not examined here, but these could be an important stage to include because of their prolonged time spent in the soil. Such pupae are often in the soil for 4-6 months (Hardwick 1965), and may therefore be more vulnerable to mite injury by allowing multiple mite generations to occur.

While mites appeared to be causing direct mortality by feeding on the pupa, indirect mortality effects could also be implicated and merit further investigation. Once the cuticle is breached by mites, the pupae may become more vulnerable to pathogens and infection. Also, additional disruption of the cuticle by the mites may result in critical water loss to the pupa.

Mite populations were only found to significantly increase, compared to the controls, when in the presence of an IS pupa (Figure 19). Control populations did increase slightly from their original infestation level, but this was likely due to matings of the female-biased wandering population prior to being placed in treatment vials; only one gravid female would be necessary to produce the estimated daily fecundity (~20 eggs at 23ºC; Sánchez-Ramos & Castañera 2005) needed to cause the increase observed in the 10M treatments, and four to five females would be needed to produce the increase seen in the 100M treatment (Figure 19). Mites seemed unable to penetrate a healthy cuticle on their own, but if it was weakened or absent in an area, they could exploit this softer tissue. It is unknown, however, what parts of the pupae they are feeding on. Given recent works documenting predatory and scavenging behavior with food sources of high protein and fat (Brust & House 1988; Sánchez-Ramos & Castañera 2001; Papadopoulou 2006), these mites may be feeding directly on the pupae itself, or scavenging protein and lipids from underneath the pupal cuticle.

Our results indicate that mold mites can survive and reproduce on *H. zea* pupae with incompletely sclerotized cuticles, and in doing so, may increase the mortality of such pupae. The natural occurrence of incompletely sclerotized *H. zea* pupae is not known, but it has been observed in other insect pupae as a result of nutritional deficiencies (e.g. Navon *et al*. 1985) or genetic mutations (e.g. Bishop *et al.* 1989). This is the first study to document feeding activity by *T. putrescentiae* on *H. zea*, and these results warrant further investigation to better quantify this interaction. Ultimately, field observations will be required to confirm the influence of this mite on *H. zea* pupae *in situ*.

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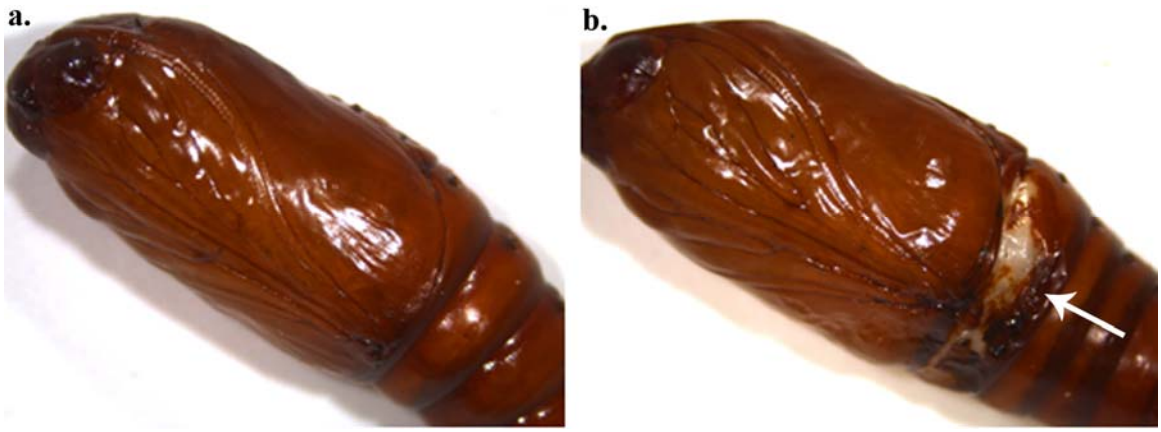

Figure 16: *Helicoverpa zea* pupa with **a.** completely sclerotized (CS) cuticle, and **b.** incompletely sclerotized (IS) cuticle.

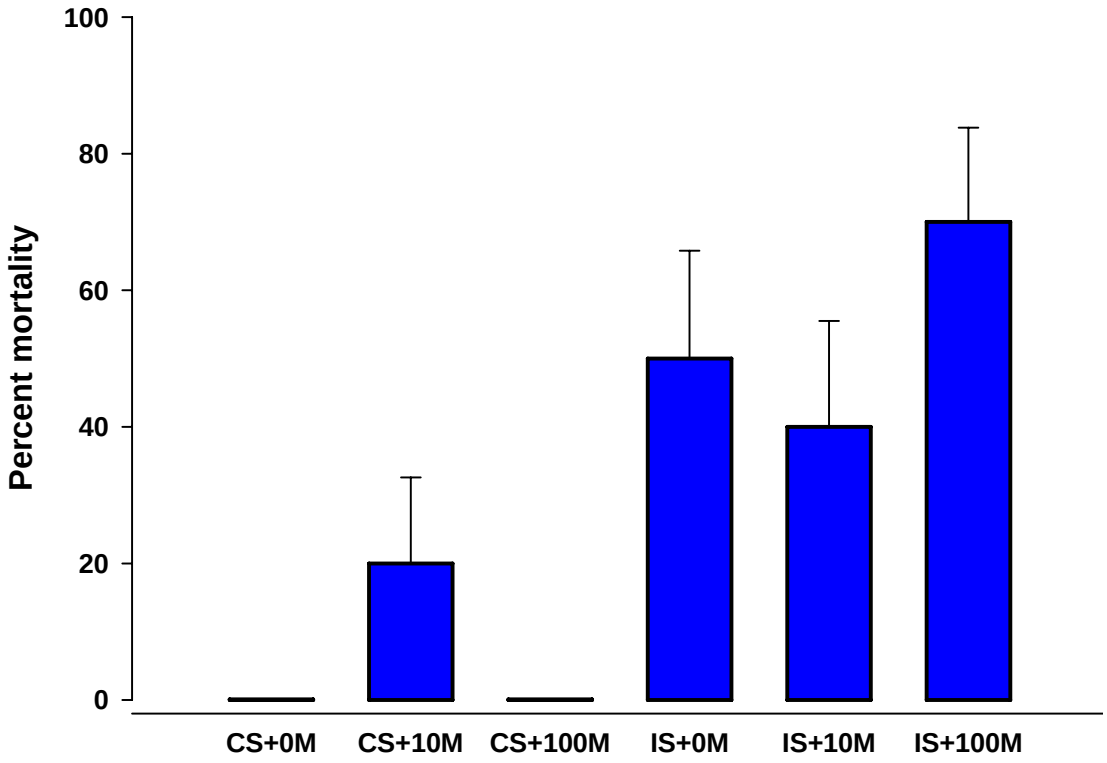

Figure 17: Total percent mortality (± S.E.) of completely sclerotized (CS) and incompletely sclerotized (IS) *H. zea* pupae in the presence of initial mite (M) infestation levels (0, 10, and 100). Untransformed mean and S.E. values are presented here. Significant differences (P <0.05) were only seen as a function of sclerotization level (IS vs. CS).

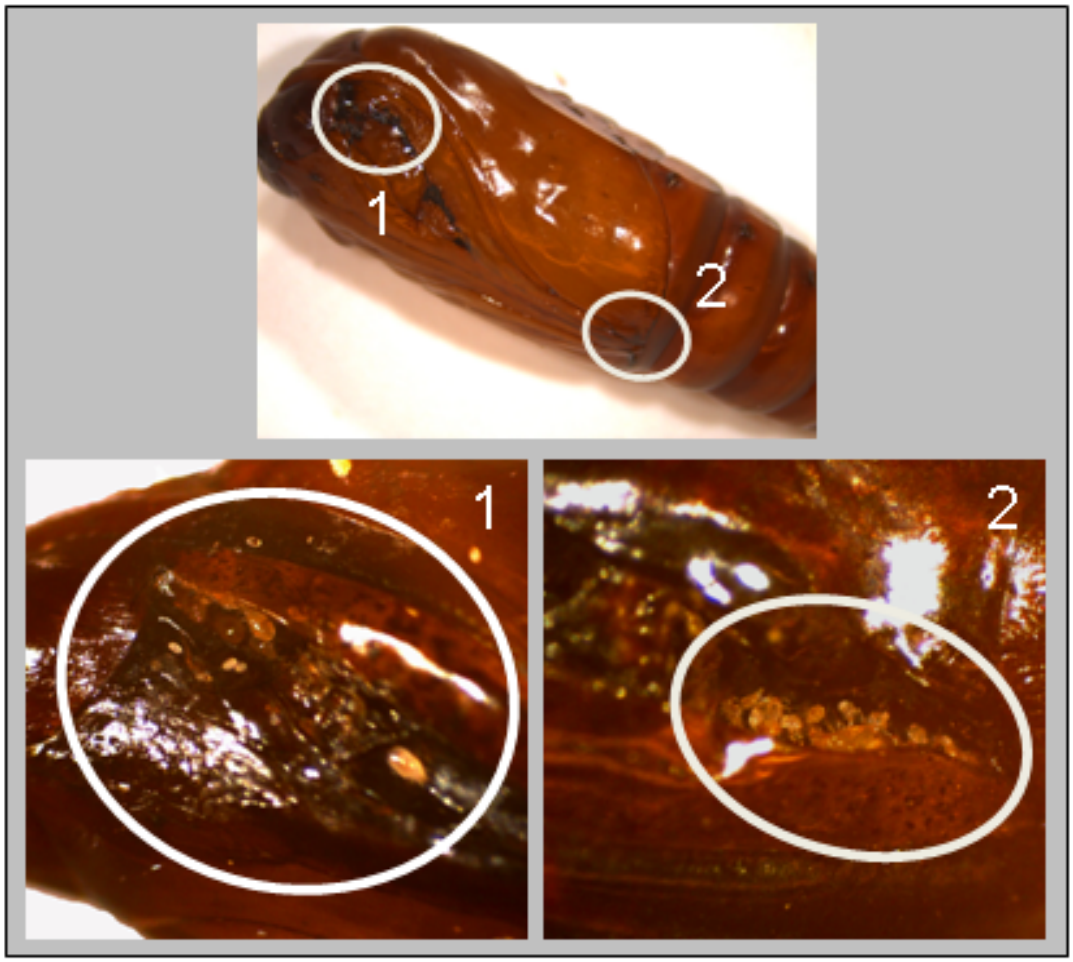

Figure 18: *Helicoverpa zea* pupa with an incompletely sclerotized (IS) cuticle before *(top)*, and after mite infestation *(bottom)*. This pupa was infested with 100 adult mites, many of which burrowed inside the pupal cuticle near the head *(1)* and midline *(2)* where prior weakenings in the cuticle were observed.

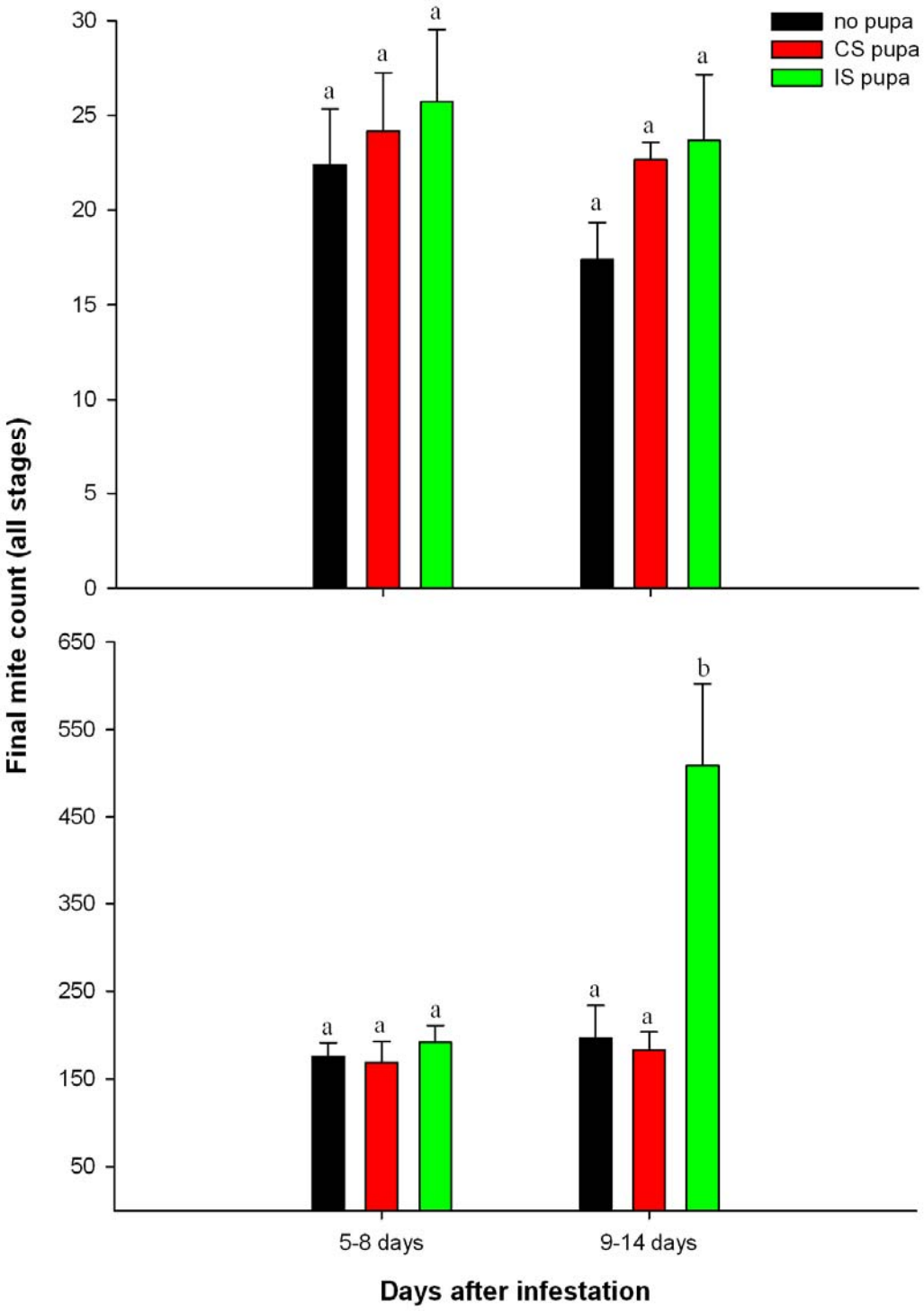

Figure 19: Total mold mite counts (± SE) for *(top)* 10 mite initial infestation level and *(bottom)*100 mite initial infestation level in the presence of completely sclerotized (CS), incompletely sclerotized (IS), or no *H. zea* pupae over time. Bars within a time interval with different letters are significantly different ($P \leq 0.05$, protected LSD).

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