**Mechanisms Mediating the Descent into Diapause: The relationship between stored resources and diapause timing.**

**James T. Brown**

**MS Thesis Proposal**

**Advisor: Dr. Dan Hahn**

**Committee Member: Dr. John Beck**

**Changing Climate:** Earth’s climate is warming. According to the National Oceanic and Atmospheric Administration, 2016 was the warmest year on the record with global surface temperatures and North American land surface temperatures averaging 0.94°C and 1.86°C above the 20th century averages respectively (NOAA National Centers for Environmental Information 2017). Additionally, conservative projections of future temperatures estimate at least a 1.5°C increase in global surface temperature by the end of the 21st century that will continue to increase thereafter (DeLucia et al. 2008, Stocker et al. 2015). Seasonal temperature averages in the United States during 2016 echoed this upward trend and average temperatures for spring, summer, fall, and winter all surpassed 20th-century temperature averages (NOAA National Centers for Environmental Information 2017). In north temperate regions of the continuous United States, for example in Maine, annual temperatures can peak in the summer around 24°C and in the winter temperatures frequently dip below freezing. As annual temperatures continue to increase, warmer days will begin earlier in the year and end later in the year, reducing the number of cool days in the spring and fall, effectively increasing the number of warmer days and the duration of summer (Bradshaw and Holzapfel 2006, Hahn and Denlinger 2011, Scriber 2014). For many organisms, warmer temperatures generally increase development and for these organisms, more frequent warmer days during the year could favor development during these warmer seasons. As it relates to insects these longer, warmer growing seasons could provide more time for development that could be directed towards more resource gathering, mate finding, or reproduction possibly leading to increased population sizes and even greater numbers of generations each year (Bale et al. 2002, Bradshaw and Holzapfel 2006, Hahn and Denlinger 2011, Scriber 2014). For insect pests, managing the potentially damaging effects caused by larger insect pest populations that last longer into the growing season will require an integrated approach and likely increased use of chemical insecticides.

Insecticide use can manage insect pest populations, but even under strict application regimens insects can significantly reduce crop yields. Under current climate conditions, yield reductions in chemically managed, pre-harvest crops due to arthropods is estimated between 13%-16% annually (Culliney 2014). Crop loss due to insect pest insect damage here in the United States from 1945 to 2000, has nearly doubled from 7% to 13% while insecticide use has increased 10-fold (Pimentel and Burgess 2005). As warmer temperatures begin earlier in the year and end later, larger pest insect populations could lead to lower crop yields and the cost to manage these potentially larger and earlier occurring pest populations using chemical insecticides is likely to increase. As temperatures continue to rise, lower crop yields due to insect damage will endanger access to safe nutrient-rich foods for people in developed and developing countries around the world. Here in the United States, our population is predicted to exceed 450 million by the year 2100 and this population increase will demand sustained or even increased crop yields (Melorose et al. 2015). Investigating the responses of pest insect populations to increases in temperature is an opportunity to better understand and predict how climate change could affect these pests, and use those predictions to mitigate their damaging effects and ensure the security of our nation’s food as populations increase.

**Responses to Climate Change:** Because the performance of all animals is influenced by the thermal conditions they experience in their environments, increased temperatures could affect animals either positively or negatively (Huey and Stevenson 1979, Chown and Terblanche 2006). As seasonal temperatures increase in temperate regions, the duration of the warm growing season will increase with warmer days that arrive earlier in the spring and end later into fall. In effect, warmer seasonal temperatures in northern latitudes will resemble the seasonal temperatures of adjacent southern latitudes increasing the geographic distribution of warmer environments (Parmesan et al. 1999, Breed et al. 2012). Insects whose populations are impacted negatively by climate change can be colloquially termed “losers” and those impacted positively can be termed “winners”. The direct and indirect interactions between temperature and the resulting winners could lead to temperature tolerance, increasing populations or expanding ranges (Hughes 2000, Williams et al. 2008). Winning insects could adjust to warmer temperatures through plasticity or adaptation. Understanding how climate change might increase insect populations, expand population distributions or how insects could adjust to warmer temperatures could help predict some of the damaging effects these winning pest insects could have on agricultural crops.

An insect’s body temperature directly affects its performance, and the effect of body temperature on performance can be described using a thermal performance curve (Huey and Stevenson 1979). At the peak of this curve is an insect’s thermal optimum, this is the temperature where performance is maximized. The range of temperatures where the performance of an insect is half of the thermal optimum, represents the thermal breadth. Finally, the range of temperatures where performance is positive is an insect’s thermal tolerance range. Those temperatures at the edge of an insects thermal tolerance are termed the critical thermal maximum and critical thermal minimum, respectively (Bale et al. 2002, Huey et al. 2012, Sinclair et al. 2016). As warmer days begin earlier in the year and last longer, losing insects could be unable to tolerate these changes due to narrow thermal breadth. For these losing insects, warmer daily and seasonal temperatures could reduce their performance by exceeding their thermal breadth earlier in the day or earlier in the season. Continued increases in temperatures for these insects could be lethal by exceeding their critical thermal maximum. Winning insects, in contrast, could tolerate warmer temperatures due to a wider thermal breadth. Additionally, some winners whose thermal environment is currently below their thermal optimum experience increased performance as temperatures increase towards their thermal optimum. In a review of population fitness (with fitness defined as the intrinsic population growth of r-strategy insects) and average thermal conditions, Deutsch et al. (2008) tracked and compared population size between 38 representative insect species from temperate and tropical latitudes. For those representative species across temperate latitudes, the thermal breadth of these insects tended to be wider and the thermal conditions experienced in these locations, on average, tended to be further away from their critical thermal maximum, compared to the representative taxa from tropical latitudes (Deutsch et al. 2008). In the tropics, environmental temperatures vary little relative to temperatures in temperate regions and insects in tropical regions experience temperatures that tend to be closer to their optimum temperature relative to temperate insects whose environment tends to be cooler than optimum. This work suggests that tropical insects already exist near their thermal limits and thus could quickly become losers as climate warms.

As temperatures rise, the growing season in northern latitudes will resemble adjacent southern latitudes with growing seasons that begin earlier in the year and end later. For losing insects that cannot tolerate increasing temperatures in their current environment, occupying these north-shifting thermal conditions through shifts in the geographic range of the population could allow them to win and those insects unable to shift their geographic rage could lose. Winning insects could experience a net increase in both population size and geographical distribution with more individuals spread across more geography. Winning insects might also experience a northern shift of their entire geographical distribution with no change in population size. In Europe, changes in range distributions have been observed in 35 species of non-migratory butterfly species. Of these butterflies, 63% were observed to have a distribution shift northward and 3% were observed to have a distribution shift southward (Parmesan et al. 1999). As favorable thermal conditions for winning insects shift farther north and warmer days increase in frequency and duration, the spatial distribution of winning insects could track those favorable temperatures. Warming northern latitudes do offer winning insects the opportunity to shift their population distributions. However, those insects that experience shifted distributions will be exposed to environmental cues, like photoperiod, that are intrinsic to these northern latitudes. Photoperiod, like temperature, is an important environmental cue that insects use to make life history decisions. Failure to adjust to the photoperiods of these warmer northern latitudes could negatively impact the timing of life history events for those shifted populations, turning winners into losers.

Seasonal changes in temperature are cyclic and correspondingly can delimit the availability of resources (like host plants for phytophagous insects). Being able to reliably predict seasonal changes is probably one of the most important challenges all organisms encounter. For plants and animals alike, temperature has a strong influence on their growth and performance, but daily temperatures can fluctuate from year to year. To prepare for seasonal changes in temperature, many plants and animals synchronize their development using other environmental cues that consistently cycle with these changes in seasons. In the temperate regions farther from the equator, photoperiod consistently changes incrementally by latitude and season (Hut et al. 2013). During the summer, photoperiod is long and increases as latitude increases; while in the winter, photoperiod is short and decreases as latitude increases. Insects in temperate regions use these consistent, incremental changes in photoperiod at specific latitudes to synchronize their life histories with the availability of resources in their environment. With growing seasons beginning earlier and ending later in each year with climate change, a hypothetical photoperiod of 13 hours that previously indicated the average beginning of the growing season could, as temperatures increase, indicate on average the second week of the growing season instead. As temperatures increase, photoperiod will become uncoupled from seasonal changes in temperature and resource availability. Those insects that depend on photoperiod to make life history decisions, but are unable to adjust to the warmer temperatures approximated by photoperiod, could lose. Winning insect populations could be pre-adjusted to warmer temperatures or, as temperatures rise, they could gain the ability to adjust to the warmer predictions of photoperiod (Williams et al. 2008). The capacity to adjust to these photoperiodic changes could be the result of phenotypic plasticity or evolutionary adaptations.

As temperatures rise, it can directly affect the performance of insects. When environmental temperatures are too high, they can exceed the thermal maximum of insects by inhibiting activity, development and eventually causing mortality. However, warmer and less predictable seasonal temperatures can also have indirect effects on insect performance by increasing environmental stress. Environmental stress, induced by warmer temperatures, may reduce resources which can lead to starvation. Winning insects could adjust to these cyclic and acyclic changes in their environment through phenotypic plasticity. Phenotypic plasticity is defined as the capacity of a single genotype to express multiple, different phenotypes as a function of the environmental conditions that genotype encounters (Agrawal 2001). These phenotypically plastic could mediate the effects reduced resources as temperatures rise by expressing phenotypes better suited to tolerate this environmental stress. In a recent survey of phenotypic plasticity, researchers investigated the response of eight clinally distinct *Drosophila melanogaster* populations to determine if phenotype plasticity could increase their resistance to starvation. These populations were reared under temperature regimes that fluctuated daily, similar to average daily summer and winter temperatures for 6 days with a spike in temperature for 5 hours on day 7. After day 7, the flies were treated under starvation conditions and mortality was tracked. In each of the populations, starvation resistance was significantly increased in those fly treatments exposed to summer temperature regimens compared to winter (Hoffmann et al. 2005). For these flies, resistance to starvation increased as a function of their environment. As seasonal temperatures become less predictable and the availability of resources could sporadically change and adjusting to those atypical changes could increase survival of winning insects.

In temperate regions, insect phenology tracks seasonal changes in temperature because these seasonal changes determine the availability of resources. To approximate seasonal changes in resource availability, many insects depend on photoperiod to synchronize their life history and maximize their development. As temperatures rise, the thermal conditions experienced in northern latitudes will begin to resemble adjacent southern latitudes, however, photoperiod in these latitudes will remain relatively consistent. If insects are to win, they will need to adjust how they respond to the warmer temperatures being approximated by photoperiod. Some insects could adjust to these changes through evolutionary adaptation. Evolutionary adaptation can be described as a product of natural selection changing the average genetic frequency of traits within a population whereby genotypes within a population that are better suited for a given environment increase (Lee 2002). As temperatures warm, insects with genotypes that enable them to adjust to the warmer temperatures predicted by photoperiod could migrate into these changing northern regions, with southern thermal conditions, and win. The pitcher plant mosquito, *Wyeomii smithii,* illustrates how increasing temperatures can lead to evolutionary changes within populations over time. Pitcher plant mosquitos spend their entire larval developing the water-filled pitcher of a pitcher plant. As the growing season ends these larvae experience a reduction in available resources. To synchronize their life history decisions with seasonally available resources, these larvae use photoperiod. For these mosquitoes, when resources decline they enter a state of dormancy, induced by a critical photoperiod. Critical photoperiod is the number of light hours required to induce dormancy in 50% of a population. Within *W. smithii* photoperiod is highly heritable and genetically determined. To investigate the possibility of insects adapting to temperature, populations of *W. smithii* were sampled from different latitudes between Florida and Canada in the years 1972, 1988, 1993 and 1996. After collecting samples, mosquito larvae were reared and the critical photoperiod determined. Reportedly, their critical photoperiod has decreased form 15.79 hours of day light in 1972 to 15.19 hours in 1996 (Bradshaw and Holzapfel 2001). This decrease in the number of daylight hours required to induce the dormancy correlates to a shift in the dormancy timing of these mosquitoes by about 9 days and is indicative of an adapting genotype towards a more southern phenotype where growing seasons are longer.

The relatively consistent nature of photoperiod makes it a reliable cue insects can use to approximate the changes in their environments. If insects are to win as temperatures warm, they will need to adjust to these changing photoperiods to take advantage of these increased temperatures, and longer growing seasons. Phenotypic plasticity and evolutionary adaptations in important traits that predicate life history decisions, like critical photoperiod, could allow populations to adjust to changes in their environment by delaying the onset of dormancy by adjusting the critical photoperiod. A warmer climate means growing seasons will become longer and it will be those insects that are synchronized with these extended growing seasons who will have the advantage and could win.

**Adjusting through Dormancy:** To ensure their survival, organisms must monitor their internal and external environments and they be able to avoid or take advantage of those changes that are stressful or suitable to their survival, respectively. Insects specifically ectothermic and readily susceptible to thermal changes in their environment both acute and chronic. Acute thermal stresses include the atypical and unpredictable changes in temperatures. While chronic thermal stresses include the cyclic and predictable changes in temperatures. Dormancy is a strategy used by most insects to mediate the negative effects of acute and chronic stress they experience in their environments. Quiescence is a transient state of reduced activity that insects use to protect themselves from acute environmental stress (Koštál 2006). Acute stress, like unpredictable temperature fluctuations, can increase water loss and lead to desiccation. As environmental stresses are detected quiescence can be induced in direct response to those stresses. Once the stress is relieved, quiescence is completely reversed and insect activity can resume, immediately. Diapause is an endogenously regulated type of dormancy used by insects in response to cyclic and chronic stress encountered in their environment (Koštál 2006). Seasonal changes in temperature and available resources are common stresses insects typically encounter. For most temperate insects, as temperatures decrease their physiology struggles to maintain a metabolic rate suitable for continued development and as resource availability declines, insects struggle to acquire enough resources to fuel their metabolism. Diapause protects insects from these chronic stresses through profound changes in an insects behavior and physiology. However, unlike quiescence, diapause is generally initiated preemptively before an environmental stress is encountered. Insects monitor predictable cues, like photoperiod in temperate environments, to approximate periods of prolonged environmental stress. By taking advantage of environmental cues that signal changes in their environment and escaping those changes through diapause. Insects that experience adjusted diapause phenology, allowing them to track the warmer temperatures approximated by photoperiod could win as climate temperatures continue to increase.

Diapause is a genetically determined, environmentally influenced phase of development and generally during which metabolic activity can be suppressed, development blah…. This life history phase can be “obligatory” as observed in univoltine insect species (Tauber and Tauber 1981, Koštál 2006). These insects exist in environmental conditions that permit the growth and reproduction of one generation of offspring during a single growing season. Or diapause can be “facultative” as observed in multivoltine insect species. Multivoltine insects live in environments that allow the successful production and reproduction of more than one annual generation of offspring during a single growing season. Depending on the species of insect, the life stage when diapause is initiated, the intensity of diapause, and even the cues used that herald environmental changes can be and are tremendously variable. However, within a single species the environmental cue that stimulates diapause, the life stage sensitive to that cue and the resulting diapause phenotype all operate under strict genetic regulation and are entirely consistent within a single species (Bale and Hayward 2010). Diapause development occurs across four distinct stages; pre-diapause, diapause, and post-diapause. Before pre-diapause can even be induced the insect must reach a species specific developmentally sensitive period. During an insects sensitive period, they are sensitive to the environmental factor that initiates diapause and they are physiologically competent to respond to the environmental cue by shifting away from direct development and towards diapause (Koštál 2006, Bale and Hayward 2010). Once the environmental cue is received by an insect during its sensitive period, diapause is initiated. During diapause, many insects suppress their metabolism, reduce their respiration, and suspend development to conserve energy and reduce consumption. Those insects that diapause feed very little or not at all during diapause and many do not feed after diapause ends, thus it is imperative that these insects begin diapause with enough resources to both survive the stressful diapause period and meet the energy requirements for metamorphosis and reproduction after diapause ends (Hahn and Denlinger 2007, Sinclair 2015). Because pre-diapause occurs before an insect’s environment becomes stressful it is during this stage that insects prepare for their time in diapause and accumulate all the resources they will need during diapause (and in many cases after diapause) prior to prolonged environmental stress (Koštál 2006).

Diapause initiation and development is a genetically controlled Synchronizing insect development with environmental changes using diapause could be how some insects will win as climate temperatures continue to rise.

Diapause, across different insect species, can occur at almost any life stage, however within a single species, the timing of diapause is genetically determined and tends to be fixed along with the stage that is sensitive to the external cues that commit an insect to diapause. It is unclear wheather the genetic architecture of diapause responses is similar or different among species that differ in their diapause life history. Weather predicts short term changes in rain, humidity, temp, etc across short periods of time. Climate is an average weather factors across relatively longer periods of time. Organisms acoss latitudes use predictable cues to sync their life history with the environ. Those

As temperatures rise, those insects who depend on photoperiod to make life important history decisions will need to adapt to the changing photoperiod as they begin to colonize more northern environments and they could do so through plasticity in the vary traits that link their life histories to changes in their environment. The pitcher plant mosquito, *Wyeomii smithii,* illustrative how increasing temperatures have permitted northern range expansion and how plasticity can function to maintain synchrony with a novel environment. Pitcher plant mosquitos spend their larval growing phase entirely in the leaves of a pitcher plant. As photoperiod decreases, these insects enter a state of programmed dormancy in preparation for lower temperatures and declining resources. Rising temperatures have allowed these mosquitos to colonize more northern latitudes and plasticity in their response to photoperiod have resolved to allow these mosquitos to fully utilize these more northern resources. Between the years 1972 and 1996, the critical photoperiod (that is the threshold day length required to induce photoperiod among 50% of a population) has reportedly decreased form 15.79 hours of day light to 15.19 hours. This decrease in the number of daylight hours required to induce this dormancy response correlates to an increase in this insects growing season by an average of 9 days (Bradshaw and Holzapfel 2001). While photoperiod remains a crucial proxy for annual resource availability, the genotype controlling when this dormancy programming is initiated is phenotypically plastic and this plasticity allows these mosquitos to respond to changes in the environment.

As temperatrues rise, diapause may be too tightly controlled and may lead some insects out of synchrony with their environemt. may lose the ability to diapause changes are initiated when seasonal changes is strictly regulated at the genetic level. As conditions conducive to their survival

Bradshaw 2001 paper: mosquito pops monitored over time. Winter is shrinking, important photoperiods are becoming more southern like. That is to say mosquitoes in the north that used to enter diapause at longer day lengths (northern fall/winter) are now entering diapause at shorter day lengths (southern fall/winter) because the growing season is longer. This is important because photoperiod in these mosquitoes is genetically determined fixed within populations. This change in diapause timing points towards selection acting on this population.

**Stored Resources:** In preparation for diapause, many insects selectively store large amounts of triglycerides to meet the energetic demands of diapause and later metamorphosis (Hahn and Denlinger 2011). Diapause is a multistage biological state consisting of pre-diapause, diapause, and post-diapause development. Pre-diapause is demarked by the perception of some external cue like photoperiod or temperature by an insect during a genetically determined period during its life history known as the sensitive period (Koštál 2006). The perception of this external cue induces the genetic programming that destines an insect for diapause. Once diapause is induced, some diapause destined insects enter a preparation phase, and it’s during this phase when some insects can experience differences in physiology and behavior to promote diapause survival (Koštál 2006). During autumn, diapause is induced in adult monarch butterflies, *Danaus plexippus*, and diapause induction alters their behavior. As part of the diapause program in this species, they become migratory and begin their journey to overwintering sites in California and central Mexico where they will complete diapause before flying back to the southern US to begin reproduction the next spring (Goehring and Oberhauser 2002). In other insects like the mosquito *Culex pippens* or the Colorado potato beetle, *L. decemlineata*, diapause induction can dramatically change an individual insect’s physiology and in preparation for diapause, these insects accumulate large quantities of lipid compared to their non-diapausing conspecifics and storing these lipids as a source of energy during diapause (Hahn and Denlinger 2007, Bale and Hayward 2010, Sinclair 2015). In contrast, for some insects like the (insect citation), diapause preparation does not alter the amount of resources accumulated from its environment, instead consumed resources are directed away from reproductive tissues and somatic tissue development and toward storage (citation). The genes controlling the initiation of traits related to the diapause phenotype in most species represent a black box that requires more investigation to understand the mechanisms by which these genes function to initiate the many aspects of the diapause phenotype, downstream of their activation by photoperiod. The resulting phenotype generated from these genes is, generally, a combination of external and internal changes in character state, a phenotype that exists as a spectrum but is specific within a single species (citation). For some insects, diapause is a protective state where metabolic rates are drastically reduced to conserve energy and maintain physiological processes necessary to surviving diapause and thriving post-diapause (citation). For these insects, high energy biological molecules are the substrate that power the biological reactions allow these insects to thrive both during and after diapause, and they must be stored prior to the onset of diapause (citation). In preparation for diapause, some insects experience a steep increase in the stored amounts of lipids and proteins, specifically triglycerides and multimeric proteins, stored and produced by the fat body. While these molecules are biologically multifunctional, they also serve as energy reservoirs. Triglycerides, and other lipids, are used to stabilize membranes, slow or prevent desiccation, can be degraded into carbohydrates for energy. Stored proteins can serve as a reservoir of amino acids that can be reconfigured, under the right conditions, into other metabolically metabolic tools. These molecules have been observed to occur in high concentrations at the outset of diapause in (insect, insect, insect) (citation). Tracking the movement of these molecules using radiolabeled atoms, researchers show triglyceride carbons incorporated into (tissue, tissue, tissue) and amino acids from stored proteins incorporated into (tissue, tissue, tissue) (citation). Diverting resources away from direct development and into storage is a risky endeavor. Diapause preparations, in some species, is initiated during times when environmental resources are abundant. If seasonal temperatures vary away from historical averages and towards a warmer winter, physiologically switching away from direct development and preparing for diapause could be detrimental to the survival of a species (citation). Photoperiod is generally the proximate cue that insects use (within their specific latitudes) to initiate these changes in physiology because of its annual consistency and inherent relationship with changes in temperatures. Excluding the poles and the equator; as latitudes increase, photoperiods shrink and temperatures reduce gradually setting up a gradient of daylight hours during the growing season such that the photoperiod and temperatures experienced during the season becomes shorter and cooler (Hut et al. 2013). Historically, the relationship between photoperiod and temperature has predictably cycled from season to season, and it is in this way that insects, and other animals, have evolved to alter their phenotype to protect themselves from stressful changes in their environment. Ecologically, this regular pattern in changes in daylight hours sets up predictable species gradients of insects that are optimized to respond appropriately to the proximate stimulus in preparation for the eventual changes in their environments (Hut et al. 2013). In the context of increasing temperatures, higher latitudes experience the same photoperiod but the temperatures experienced during these photoperiods more resembles lower latitudes. Effectively leading to the uncoupling of photoperiod and temperature (Bale and Hayward 2010). understand the degree to which this uncoupling will disrupt species diversity and how pests are managed will require a model organism sensitive to these changes not unlike *Ostrinia nubilalis* (European corn borer).

If climate is causig longer longer and shorter, animals should increase their growing and initiate dormancy later

Agricultural systems are currently under perennial pressure from a throng of phytophagous pests that damage crops and reduce yields and to manage these populations, growers utilize a combination of, cultural, biological, and chemical tools to minimize the effects of these pests. However, as temperatures increase and insect pests “win” the tools we currently use to manage these pests will be additionally stressed. An unlucky scenario would be that increased temperatures could distribute insect pests into new agricultural geography or those warmer temperatures would increase the phenology of these pests, producing more crop damaging generations annually.

**European corn borer:** European corn borer, *Ostrinia nubilalis,* is an important agricultural pest here in the United States, its range extends from the Atlantic coast to the Rocky mountain range, as far north as Canada and as far south as Florida. its diapause phenotype is facultative induced by both photoperiod and temperature. During its ultimate larval stage, and photoperiod. seasonal climates and global food security are tenuously bound making a comprehensive approach to dealing with these changes imperative. Farmers and growers must be able to make short-term and long-term management decisions concerning methods, timing, and tools to utilize when planning pest control strategies and climate patterns are an important part of that calculus. with based upon how the climate affects those populations.

The consequences of increased temperatures on insect phenotypes can be estimated by understanding the direct relationship between latitudinal changes in temperature, photoperiod, and how insect respond to these changes physiologically.

Here in the United States, 92 percent of all the corn acreage is planted with a genetically engineered corn crop that expresses *Bacillus thurengensis* (Bt) crystalline protein toxin. Bt toxin was developed agriculturally to assist in managing European corn borer corn pest. pressure manage the that can be done to corn by an infestation of European corn borer. For this technology to be effective, farmers need to predict European corn borer infestations (Fernandez-Cornejo et al. 2014). “Studies detailing diapause-associated changes in intermediary metabolism and feeding physiology are needed across taxa with different diapause strategies to expand our understanding of the metabolic processes underlying prediapause reserve accumulation. The goal in this area is to under- stand the underlying neurological and endocrine signaling mechanisms that regulate diapause-associated shifts in feeding patterns and intermediary metabolism.” (unfinished)

**OBJECTIVE**

The objective of this study will be to quantify and compare energy stores between two genotypically different strains of *Ostrinia nubilalis,* the European corn borer. Further, European corn borer (ECB) destined for diapause and ECB avoiding diapause will be compared within each strain. Characterizing the energy stores of ECB destined for diapause, has yet to be capitulated in ECB and is necessary to understanding diapause biology and usefulness as a model system, managing it as an agricultural pest, and predicting its behavior as seasonal climates become less predictable (Denlinger 2008).

These Higher than average temperatures can lead to increased feeding, mating, and generation output. (example in corn) With climate being unpredictable and allow some insect pests to produce more generations during the season and Crop pests are able to produce more generations not only extend the growing season for plants it also extend the amplify the destructive effects of insect pests can is amplified and insect move into new regions or as especially those invasions that hold ecological or agricultural importance. (define invasions in significant terms and provide an agricultural example in corn). The largest threat posed by corn insect pests is in part a function of population turnover.

I hypothesize that the amount of lipids the European corn borer stores in preparation for the additional stress of diapause, can be a direct proxy in understanding how it performs during diapause. Specifically, diapause destined individuals will increase their storage of triglycerides and storage proteins at a specific rate, in relation to the length of time they will spend in diapause. The ECB strain preparing for a long period of diapause will store more energy than their shorter diapausing and diapause avoiding counterpart. Diapausing ECB may be storing energy in different ratios than diapause avoiders. Higher levels of triglycerides may be used to supplement water and protect against desiccation while higher protein stores could be used to rebuild damaged or depleted enzymes.

European corn borer model is well suited for this type of study. Within the species there are of 2 distinct genotypes that differentially express the diapause phenotype. The univoltine-Z (UZ) genotype expresses a long diapause phenotype, while the bivoltine-E (BE) genotype expresses a shorter diapause phenotype. Further, the expression of the diapause phenotype is facultative. When exposed to a photoperiod of 12h:12h (light hours:dark hours) the diapause phenotype is expressed, photoperiods of 16:8 suppress the diapause phenotype. The strains of ECB persist as inbred siblings, originating from naturally occurring populations genotype can either express or avoid. ECB preparing for a longer period of diapause will store more energy in the form of fats and protein, while ECB preparing for shorter period of diapause should store relatively less energy in the form of fats and proteins. there is a direct relationship between the amount of energy stored when feeding ends and the length of time an individual spends in the diapause state. Such that ECB destined for a longer period of diapause will store more energy than ECB destined for a shorter diapause. Further, the larvae destined for diapause will differentially store more energy than those larvae that are avoiding. To that end I will

Characterizing these metabolic intermediates is intended to approximate the amount of energy an individual has reserved after feeding ends. European corn borer was chosen as the model for these experiments due to their facultative diapause life history strategy, differing genotypes and physiologies, and their different phenotypes. When either strain is exposed to the same photoperiodic and thermal cues in the laboratory, their specific response can be reproducibly observed but the physiological link between genotype and phenotype has not yet been described.

Approximately, $10 billion dollars is spent annually on chemical insecticides to control the damaging effects of insect pests (Pimentel 2005). Corn is an incredibly valuable crop in the United States and protecting it from actively feeding phytophagous insects includes the dynamic use of chemicals and biotechnology. To control the ephemeral outbreaks of ECB, farmers in the US spend approximately $10 billion dollars on chemical pesticides*.* In 2016, 92% of the corn acreage in the US was planted with BT corn. This type of pest management is a very powerful tool due to its specificity for phytophagous insects. However, the widespread use of this toxin pressurizes competition in the population. Those individuals in that can survive the toxic effects are given a mating advantage over its less advantaged, or dead, peers. Combined with the ability of ECB to produce one or two large generations a year, resistance across populations can be quickly amplified.

Investigating the physiological requirements of this tenuous life history decision will expose diapause phenology to being controlled. Strategies that can precisely affect the progression of the ECB through diapause could be valuable. Perturbing the corn borers ability to survive diapause by affecting how it allocates resources could be used as an added layer of pest management.

***PRELIMINARY DATA***

**PROPOSED METHODOLOGY**

**Origin and Husbandry of European Corn Corer:** The Univoltine-Z (UZ) and Bivoltine-E (BE) strains of European corn borer (ECB) that will be used in this experiment were collected by members of the Dr. Dopman laboratory at Tufts University. Strain identity was determined genotypically using the pgFAR autosomal gene, this gene codes an important enzyme involved in determining the female sex-pheromone blend, and is partly responsible for the strain differences (Lassance et al. 2010). Both strains were collected as larvae, pupae and adults from New York state prior to 2015 (Wadsworth et al. 2015). For the duration of the experiment, each strain will be continuously mass reared at 26°C under a 16-hour photoperiod. To compare the differences in stored triglycerides and storage proteins between diapause and non-diapause larvae, newly hatched larvae from each strain will be reared at 23°C under conditions that either induce diapause or non-diapause. Those larvae treated under diapause inducing conditions from the UZ and BE strains will be labeled UZ12 and BE12 respectively and those treated under diapause avoiding conditions will be labeled UZ16 and BE16 respectively.

**Sampling Wandering Larvae.** European corn borer eggs, intended for treatment, from the UZ and BE strains will be hatched at 23°C and 65% relative humidity. These hatched larvae will be provided European corn borer diet, purchased from Frontier Agricultural Sciences, ad libitum. Non-diapause treatment larvae will experience a photoperiod of 16-hours, while diapause treatment larvae will experience a 12-hour photoperiod. The regime experienced by each treatment will be held constant throughout the duration of the experiment. At the beginning of the fifth instar, larvae will be separated into 32-well bioassay trays purchased from Frontier Agricultural Sciences, these trays will serve as individual arenas. Once larvae reach the end of the fifth instar, they will be assayed to determine if they have entered the wandering phase. At the beginning of the wandering phase, larvae discontinue feeding, empty the contents of their gut and after clearing their gut larvae no longer produce frass. The wandering phase is a necessary step all European corn borer larvae undergo in preparation for either diapause or pupation and adult metamorphosis (Gelman and Hayes 1982). Because the termination of frass production is indicative of the wandering phase, it will be used to diagnose putative wandering larvae. To diagnose late fifth instar larvae as wandering, larvae will be removed from their individual arenas and held in a clean, empty petri dish and monitored for three minutes. Those larvae whose gut is not clear will produce frass will be placed back into their arenas and those that do not produce frass will be characterized as wanders, 30 individuals from each treatment will be collected, accessioned, and tracked for the duration of the experiment. Hemolymph and lipid extractions from sampled larvae will be analyzed for storage protein and triglyceride content, respectively.

**Protein Extraction and Quantification:** A portion of hemolymph will be drawn from larval samples and the total protein concentration in the extracted hemolymph will be measured. To extract hemolymph, a small incision will be made through the cuticle of the larval proleg (Gelman and Woods 1983). Using a micropipette, lymph fluid will be gathered and stored in a microcentrifuge tube. Extracting lymph from live larvae exposes the lymph fluid and the contained proteins to degradation from proteolytic enzymes. To reduce the activity of these enzymes, extracted hemolymph samples will be stored in microtubes containing 500 µL of 1x PBS and 5 µL of Halt™ Protease Inhibitor Cocktail with EDTA and kept at -80°C. After collecting lymph from larvae across each of the four treatments, samples will be grouped into cohorts and total protein concentration will be quantified. A cohort will consist of equal numbers of larvae from each strain, and from each photoperiod treatment. Hemolymph proteins will be quantified in relation to a standard curve of bovine serum albumin (BSA) using the Pierce™ Coomassie (Bradford) Protein Assay. When bound to protein, the coomassie-dye molecule experiences a shift in its conformation that changes the wavelength of light absorbed by the molecule from 465nm to 595nm. The total amount of light absorbed by this molecule when bound to a protein standard at known concentrations can be quantified using a spectrophotometer. The relationship between the wavelength of light absorbed by coomassie-dye bound to known protein concentrations can be used to infer the concentration of proteins in the hemolymph sample when bound by coomassie-dye given its measured absorbance.

**Storage Protein Separation and Quantification:** Insect hemolymph contains proteins that range in size and contained in that mixture of lymph proteins are insect storage proteins. Storage proteins are multimers composed of six identical or similar subunits and each subunit weights approximately 80kDa each (Burmester 1999, Pick et al. 2009). The relative quantity of storage proteins in each larval sample will be determined by comparing the optical density of the larval samples to optical density of a known protein standard. To make this comparison, larval hemolymph and the protein standard will be separated by size using gel electrophoresis. 100ng/mL aliquots of each hemolymph sample will be mixed with sodium dodecyl sulfate, giving each protein in the mixture a net negative charge. The protein standard, containing a mixture of proteins of known size and concentration, will then be loaded onto a polyacrylamide gel, along with the larval lymph samples. Polyacrylamide is a synthetic matrix of composed of differently sized openings that selectively allow molecules to pass through the openings based on the size of the molecules. When a positive charge is applied to the gel, it attracts the negatively charged proteins and pulls them through the pores of the gel matrix based upon size. To visualize the ending location of the protein on the gel, Bio-Safe™ Coomassie Stain will bind proteins nonspecifically and the resulting color can be photographed and analyzed using the NIH Imagej software.

**Lipid Extraction, Separation and Quantification:** The total lipid content from each larva will be extracted and quantified individually. First, larval dry mass will be determined by removing water from the larval sample by freeze-drying them in a vacuum at -80°C until their dry weight varies by less than 1% over a 24-hour period. Once dry, lipids will be separated from the larval tissues using a slightly modified Folch method (Folch et al. 1957). This method takes advantage of the polarity and density differences between chloroform and methanol that allow each solvent to selectively solubilize molecules of similar polarity and to produce distinct layers when mixed together. When a larval sample is solubilized in this solvent mixture, the less polar lipids are captured in the less polar chloroform layer. This layer can be decanted away from the remainder of the sample, the solvent removed and the total amount of lipids extracted from the sample can be quantified gravimetrically. The total lipid content extracted from each larval sample contains a mixture of different lipid classes from which triglycerides will need to be separated and quantified. Separating and quantifying triglycerides in the total lipid extract will be accomplished using Liquid Chromatography (LC) coupled with an Evaporative Light Scattering Detector (ELSD). LC takes advantage the physical properties of lipid molecules in the sample to adsorb to a C18 silica column, this strength of this interaction changes as solvent flows through the column. The solvent concentration is graded mixture of 0.01% Acetic Acid in Methanol and 40% Hexanes in 2-Propanol. As the solvent gradient changes the lipid molecules in the sample desorb from the column flow into the ELSD where they are nebulized, the solvent is evaporated and the amount of light scattered is computed into a response peak. The response peak output of the ELSD can then be quantified by comparing it to the response peak of a standard concentration of triglycerides.

**Lipid Identification:** To identify the fatty acid components of the triglycerides quantified by LC-ELSD, the triglycerides in the total lipid extract will need to first be converted into fatty acid methyl esters (FAMEs). Cohorts of 4 lipid samples from each strain and from each photoperiod treatment will be esterified and 4 blank samples will be used to characterize the extent to which any background lipid contaminants may be present in our extraction method. The efficiency of the esterification will be determined using triheptadecanoic acid, a spike-in standard obtained from Sigma Millipore. Triglycerides in the total lipid extract will be methylated via base-catalyzed esterification with an acid catalyzed work-up (Christie and Christie 1993, Liu 1994). Extracted lipids will be mixed in a solution of 10M methanolic potassium hydroxide at 55°C for thirty minutes in a capped vial. The capped and heated solution will be vortexed for two minutes, then cooled on ice for five minutes. While still on ice, the vial will then be uncapped and 12M sulfuric acid will be added to neutralize the KOH and terminate the reaction. After the reaction is terminated 3 mL of hexanes will be added into the reaction vial to solubilize the FAMEs. The hexane layer will then be decanted and any water species formed by the esterification procedure will be precipitated out of solution using sodium sulfate. Identification of the methyl ester species will be accomplished using Gas-Liquid Chromatography (GC) coupled with a Flame Ionization Detector (FID). GC-FID separates each FAME by taking advantage of the specific interactions between different FAMEs and the packing material in a DB-WAX capillary column. The FAMEs in the sample adsorb onto the column and inert gas flows through the column. Over time, the column temperature increases and the FAME molecules desorb from the column based on their molecular composition and the inert gas carries them to the detector. At the detector, retention time is recorded and each FAME molecule is ionized and the intensity of ionization is recorded as a peak area. FAMEs will be identified in comparison to a 37 Component FAME Mix purchased from Sigma Millipore.

**Data Analysis:** Storage protein and triglyceride quantification will be expressed as a concentration in comparison to a protein standard and a triglyceride standard, respectively. The initial hemolymph protein concentration and putative storage protein concentrations will be determined relative to an external standard of known proteins and at known concentrations. Total lipid concentration will be determined as the total sum of the triglyceride peak areas in relation to the peak area of an external standard of known triglycerides at known concentrations. A multivariate analysis of accumulated lipids and storage proteins will be used to explore the interactions between different experimental observations and used to determine if there are interesting patterns.

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