**Triglyceride and Storage Protein Production as an Indicator of Diapause Induction**

**James T. Brown**

**MS Thesis Proposal**

**Advisor: Dr. Dan Hahn**

**Committee Member: Dr. John Beck**

* The European corn borer is another a dull brown colored moth but with big consequences. This species of lepidopteran is responsible for a large amount of damage to a number of agricultural crops and to no small amount corn plants. Once mated, the females of this species will lay their eggs and fix them to the substrate (in this case a host plant), and after the eggs mature, they will hatch and neonate larvae will eclose from the egg and begin feeding on the host plant. They bore a hole into the stem of their host plant and over the course of its life these larvae will consume almost their body weight in food. During this time these larvae are sensing the environment around them and integrating that information as they develop to make important developmental decisions. Light timing plays an important role in how these creatures progress through their life cycle. More specifically, the number of hours of light these insects are exposed to every 24 hours can mean the difference between continuous development from larva to pupa to adult or discontinuous development, where the time between larva and pupa are separated by months. Making these decisions as a larvae are critical life events. Imagine making the wrong decision and diapausing in the middle of winter.
* The decision to diapause modulates different processes that we can use as indirect indicators of diapause. Two of these processes include the upregulation of lipid and protein production by the fat body. These metabolites do some amazing things when given the chance to shine.

The next step I will be taking is to create an experimental design that will assist me in characterizing the

Seasons

Diapause

Diapause induction

Biology of

**Introduction**

**Hypothesis:** If triglyceride and storage protein levels play an important role in supporting the life history step after the larval wandering stage and given the additional metabolic demands of diapause, then larvae preparing to enter diapause and larvae preparing to molt into pupa will differentially express these products. Specifically, UZ and BE larvae preparing for diapause should see a quantifiable increase in the production of triglycerides and storage proteins when compared to UZ and BE larvae that are not preparing for diapause.

Seasonality and organisms

* What are seasons generally and how does its regularity or irregularity effect ecosystems and the organisms in those ecosystems
  + Talk about how phytophagous insects depend on timing their life cycles on food availability
* Talk about the confluence of life history timing and seasonality and resource availability
  + Why are seasons so important for insects to detect?
  + How can insects detect changes in their environment?...hormones
  + What effect does sensing these environmental changes have on the insect
    - Finally discuss diapause as one of those choices

Diapause

* In general, what is diapause
  + Programmed dormancy brought on by: temperature, light, or a combination of the two
  + Occurrence of diapause in across taxa
  + Protective state for when resources are low/ environment is inhospitiable
  + Hallmarks include reduced metabolism/respiration,
  + Can be obligatory or facultative
* What does diapause look like in my model
  + Generally what do ECB face leading up to diapause
  + Cue that induce diapause
  + Life stage that diapause is signaled
  + Life stage that diapause is induced
  + Physiology of my system at diapause induction. Feeding, metabolism, respiration
  + End on a discussion about the proliferation of fat and proteins

Fat Body

* What is the fat body and describe its function
* What is the biology of the fat body
* The role of the fat body leading up to diapause
  + Lipid and protein production
* The overall goal of my project

The European corn borer lends its self to characterizing the role of the fat body in inducing diapause.

**Thesis Objective**

“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 ultimate 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. ”

The objective of this study is to characterize the extent to which the production of TGs and SPs differ across the 4 phenotypes of European corn borer. Specifically, quantifying the production of TAG and SP produced by the fat body across the 4 phenotypes of ECB is intended to approximate either continuous development resulting in pupation or discontinuous development resulting in a diapause phenotype. I hypothesize that there is a direct relationship between triglyceride and storage protein production and the life history direction the larva will take., ECB strain, and the life stage following the 5th instar such that in the strain that spends a longer period of time diapausing will produce more TG and proteins. To chase this hypothesis I intend to characterize the production of TGs and SPs at the point in the ECB life history where TG and protein production is ultimate. produces that directly coorelates with the each strains differning length of diapause. Thus, the univoltine strain UZ spends a longer amount of time diapausing and thus needs a larger supply of fat.

The evidence produced in this study will have two effects. First, it will add to the body science relating to part of the physiological requirements leading up to diapause in related taxa. Second, characterizing TG and protein production and how that production differs between the different phenotypes is something that has yet to be capitulated in *Ostrinia nubilalis*. Further, having a clearer understanding of the physiological requirements of this fragile

The first step will pertain strictly to preliminary trial information gathering. I will use this time to flush out problems with equipment and protocols. I will look for alternative ways to complete experiments and find and use all of that to inform the experimental aspects of my project.

**PROPOSED METHODOLOGY**

**Origin and Husbandry of European Corn Corer.** The univoltine and bivoltine strains of European corn borer we provided courtesy of (\_\_\_\_). The laboratory reared colonies were established in the year (\_\_) as larvae. These larvae have been sibling mated to over the course of (\_\_) and throughout the course of these experiments. Three environmental regimens implemented to accommodate the different requirements of each of the phenotypes. The cohort of individuals, from both strains, used to perpetuate the colony were reared using 16 hours of light and 8 hours of dark at a temperature of 26° C. Those that are to be used as sampling For the duration of these experiments, these colonies were maintained under stable hour light, temperature, and humidity regimens

**Preparing Wandering Larvae.** Sampling larvae when they have produced the ultimate amounts of both triglycerides and proteins will be crucial to accurately characterizing the differences in life history choice. For the purposes of these experiments that ultimate developmental period, the “wandering” stage, will be the point at which samples will be taken from the cohort of larvae. Because there are no other diagnostic characters that indicate a larva will be considered in the “wandering” when all the contents of the gut are cleared. Samples will be selected on day 15 that appear to be in the 5th instar, those larvae will be separated into individual arenas where they will continue to grow and feed for the following 4 days. Upon reaching day 4 the individual arenas will either be cleaned or the larva will be moved into a cleaned arena to continue developing until such point that it does not expell frass over the course of 8 to 12 hours, and thus is in the wandering stage.

Wandering larvae will be divided into two groups for triglyceride or protein analysis. Those larvae destined for protein analysis will be bled through a small incision where approximately 10-12µL of hemolymph will be extracted and used for protein analysis. Those larvae destined for triglyceride analysis will be lyophilized for approximately 48 hours and stored for triglyceride analysis.

**Triglyceride Extraction and Derivatization.** The larvae in the triglyceride group will be extracted in sets of 16. Two larvae from each treatment group, UZ16, BE16, UZ12, and BE12, will be paired with an “empty” tube that does not have a larva but is treated in the same way as the tubes with larvae. These “empty” tubes will function to describe the background effect of the extraction method. To monitor the efficiency of the extraction methodology, undecanoic acid will serve the function of a spike lipid. One larvae and its “empty” counterpart will receive the spike, while the other larvae and its counterpart will not. Each larva will be weighed after lyophilization to obtain a dry weight. A modified version of the Folch method to extract the total lipid content from each sample. To reduce the effects of oxidation, the remainder of the experiment will be performed on ice to reduce oxidation and 0.5mg/ml of BHT (butylated hydroxytoluene) will be added to methanol. Grinding beads (1.1-1.4mm Zirmil.2) will be added to each tube, mechanically homogenized for 30 seconds using a (\_\_\_), BHT in methanol will be added to the tubes, again homogenized for 30 seconds, then hexane will be added to the tubes and the tubes will be homogenized for a third and final time for 30 seconds. After grinding the sample, the next step will be to separate the lipid containing fraction from the rest of the insect matrix. Separating the phases requires the sample be centrifuged at 4°C for 5 minutes, the organic fraction containing lipids is collected, the samples will then be re-suspended in organic solvent and placed on a table shaker for 15 minutes. The process of centrifugation and table shaking will be repeated three times. After the organic layers have all been pooled, the pooled fractions will then be dried using a 0.9% solution of NaCl and that aqueous layer will be removed and the dry pooled fractions will be dried under a stream of N2 to remove the organic solvent from the lipids. Those “dry” lipids will be weighed. The pooled fraction of lipids extracted represents the total lipid content from the insect. To remove some of these additional classes of lipids the pooled, dried lipid fractions will be re-suspended in 4mL of organic solvent and passed through a SPE (solid phase extraction) cartridge. This cartridge will capture the non-target lipids and allowing triglycerides to pass through the matrix and be collected. Once non-target lipids have been removed from the solution dried under N2 and weighed, the triglycerides will be converted into a FAME (fatty acid methyl ester). To accomplish this conversion, the clean lipid solution needs to

**Triglyceride Analysis.**

**Protein Extraction.**

**Protein Analysis.**

**Data Analysis.**

Lyophilization of larvae:

**Refercences**