The induction of diapause protects insects from unfavorable environmental changes and for many insects, once diapause begins all metabolic activity is fueled by stored nutrition (Hahn and Denlinger, 2007; Hahn and Denlinger, 2011; Sinclair, 2015). Diapause length for many insects is determined by a highly heritable diapause genotype. European corn borers have at least two different diapause genotypes, each with differences in regulating the response to the environmental cues used to trigger diapause, the physiological changes associated with induction of diapause, and most notably the duration of diapause (McLeod, 1976; Coates et al., 2004; Dopman et al, 2004; Calcagno et al., 2007; Ikten et al., 2011). This research leverages between-strain genetic variation in diapause duration in *O. nubilalis* to test the relationship between diapause length and nutrition storage. I found that European corn borer larvae preparing for diapause had a lower metabolic rate than non-diapause larvae. In diapause programming conditions, larvae with the long-diapause genotype expressed a deep-diapause phenotype. Larvae with the short-diapause genotype could show either a deep-diapause or a shallow-diapause phenotype. Diapause genotype was also associated with differences in lean mass and lipid mass accumulation. Larvae with the long-diapause genotype and had more lean mass and stored more lipids than larvae with the short-diapause genotype. Lean mass and lipid mass depletion during diapause did not differ significantly between the two diapause genotypes.

Previously Submitted

Understanding the relationship between diapause length and nutrient storage has several applications with regard to understanding how European corn borer populations may be affected by climate change. Many insects survive diapause by increasing their nutrition stores and suppressing their metabolic activity to reduce the consumption of those stored nutrients. Warmer fall temperatures resulting from climate change could increase metabolic activity and possibly reduce lipid stores during diapause preparations and/or drain lipid stores during the early portion of diapause before the onset of winter (Adkisson et al., 1963; Williams et al., 2012; Wipking et al., 1995). Similarly, warmer winter temperatures during diapause could prematurely drain stored energy causing insects to die during diapause or emerge from diapause the next spring without enough reserves to restart their lifecycle, including enough energy to support dispersing, mating, and reproducing.

Consistent with the idea that climate change may affect insect diapause, warmer and more variable temperatures at the beginning of diapause have been found to reduce nutrition stores by increasing metabolic activity and draining stored energy before the onset of winter. For example, a study by Williams et al. (2012) on the effect of temperatures on stored nutrition suggests that diapausing insects experiencing temperature variations with greater warm times at the beginning of diapause store less resources and deplete those resources faster than insects in thermally stable environments before the onset of winter. To investigate the relationship between fluctuating warm temperatures and nutrition storage, these researchers reared *Erynnis propertius* (Scudder and Burgess) caterpillars that originated from environments that differed in thermal stability in a reciprocal common garden experiment with stable and fluctuating thermal regimens. Larvae reared in stable conditions stored significantly more lipids and entered dormancy three to four weeks later compared to their counterparts reared in thermally variable environments. European corn borers that do not accumulate enough energy ahead of diapause could fail to enter diapause, terminate diapause prematurely, or sub-optimal nutrition could lead to reductions in post-diapause adult functions.

Previously Submitted

Nutrition storage prior to the onset of diapause has repeatedly been shown to be a pivotal step in diapause preparation and this result has been demonstrated across several taxa (Adkisson et al., 1963; Mitchell and Briegel, 1989; Hahn and Denlinger, 2007). Eventually, climate change is expected to cause summer temperatures to expand and fall and winter temperatures to rise. How insects manage their nutrition during diapause preparation could explain how some insects might adjust to warmer winters. Results of a previous study showed that diapause programming among European corn borers is associated with increased lipid accumulation during diapause preparation compared to continuously developing larvae. Vukašinović et al. (2013) measured lipid stores in European corn borers they collected from maize fields in the fall and found that larvae preparing for diapause accumulated more lipids compared to non-diapause larvae collected in the summer. The results from that study and this current study show an association between diapause programming and increased nutrition accumulation ahead of diapause, however they did not test for a correlation between diapause length and lipid accumulation, as I have shown here. I found that European corn borer larvae of both long and short-diapausing genotypes programmed for diapause stored more lipids when compared to continuously developing larvae of the same strain, but that the long-diapause strain stored more than the short-diapause strain. In nature, long-diapause genotype larvae enter diapause earlier in the fall and exit later in the spring compared to larvae with the short-diapause genotype. Because of the differences in the timing of diapause, long-diapause genotype larvae diapause at higher temperatures and remain in diapause longer than larvae with the short-diapause genotype. Long-diapause larvae experiencing a warmer diapause period may have a higher metabolic rate and, in turn may need larger energy stores to fuel their higher metabolic rate, compared to larvae with the short diapause genotype. The larger lipid stores of European corn borers experiencing longer warmer diapause periods could function to meet that increased demand for metabolic fuel.

Previously Submitted

Increasing seasonal temperatures are expanding the duration of the warm growing season, however the photoperiod cues that insects use to predict seasonality will remain unchanged (IPCC, 2013; NOAA, 2019). The relationship between increasing temperatures, diapause length, and nutrition accumulation could be useful for estimating changes in the seasonality of insect populations. For European corn borer, access to longer growing seasons could provide more time to produce additional generations or to increase nutrition stores before the onset of diapause. The association between increasing seasonal temperatures and the delayed induction of diapause in *W. smithii* (pitcher plant mosquito) shown by Bradshaw et al. (2004) is one example of how insects could adjust to climate change and gain access to longer growing seasons. Researchers monitored the critical photoperiod of pitcher plant mosquitoes for decades. Critical photoperiod for this study corresponds to the number of daylight hours at which diapause is induced among 50% of larvae in laboratory conditions (Bradshaw et al., 2004). After decades of observations, the critical photoperiod of these mosquitoes shifted down from 15.79 light hours hours to 15.19 light hours. The shift in critical photoperiod corresponds to a 9-day delay in the onset of diapause in the fall. This delay in diapause initiation gives mosquito larvae a longer period to grow and accumulate nutrition reserves to get them through diapause.

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A similar shift in critical photoperiod has also been noted in the fall webworm (*Hyphantria cunea* (Drury)). Gomi et al. (2007) collected fall webworm larvae from the same field site in 1995 and 2002, reared them at 25°C, and measured their critical photoperiod in response to a range of photoperiods between 13.75 light hours and 14.5 light hours. The critical photoperiod of larvae collected in 2002 shifted from 14.40 hours to 14.16 hours and the thermal constant for a specific population of fall webworm to complete a single generation was determined to be 725 degree days (Gomi et al. 2007). The researchers then calculated the annual cumulative temperature between the years 1998 and 2005 and found that the number of degree days had increased from 2032 to 2259. The increase in the number of degree days and the shift in the number of daylight hours required to induce diapause implies that the fall webworm population may be shifting from bivoltine to trivoltine. These two studies suggest longer growing seasons provide some insects with increased access to nutrition ahead of diapause (pitcher plant mosquito) and increased voltinism (fall webworm). If European corn borers respond to longer growing seasons with delayed diapause induction, then they would avoid the risk of premature energy depletion associated with diapause induction at higher temperatures, increase nutrition stores ahead of diapause, or possibly experience increases in voltinism (Bradshaw and Holzapfel, 2001; Gomi et al., 2007; Sinclair, 2015; Thompson and Davis, 1981; Williams et al., 2012).

Results

This research shows an indirect association between a longer diapause length and increasing lipid stores. Diapause-programmed European corn borers prepare for diapause by increasing their nutrition stores compared to continuous developing larvae. Additionally, long-diapause genotype larvae preparing for diapause store more lipids than larvae with the short-diapause genotype (Figure 3-8B). The difference in the timing of diapause entry and exit and differences in lipid stores between the two diapause genotypes evidenced in this research suggests that metabolic activity during a longer diapause is met by increasing nutrition stores ahead of diapause. As climate change increases growing seasons, genotypic plasticity in the diapause genotype to the environmental cues that induce diapause could advance the termination of diapause in the short-diapause genotype and the delay of diapause in the long-diapause genotype.

In this study, long-diapause genotype responded to diapause programming with the deep-diapause phenotype while diapause programming for short-diapause genotype individuals led to at least two different phenotypes, a shallow-diapause phenotype and a deep-diapause phenotype (Figure 3-9). Deep-diapause larvae remained in diapause for the entire duration of the 40-day trial while larvae in shallow-diapause terminated diapause at some point prior to the end of the trial (Figure 3-4). The two phenotypic responses to diapause programming observed in the short-diapause genotype could suggest an increased sensitivity to the cues that terminate diapause and it could be one method European corn borers take advantage of growing seasons that begin earlier (McLeod and Beck, 1963). Increased temperatures during early spring will expand growing seasons during the time when diapause is ending among short-diapause genotype larvae. If larvae with the short-diapause genotype are capable of expressing the shallow-diapause phenotype in response to warmer temperatures then these larvae could respond to increased spring temperatures by terminating diapause earlier. Larvae in shallow-diapause that terminate diapause early may have access to a longer growing season, increasing the length of their active period, and possibly increasing the number of generations produced annually if there is enough time and resources to complete that additional generation.

Ahead of unfavorable seasonal change European corn borers integrate changes in photoperiod and temperature. Once these environmental factors reach critical thresholds, diapause is programmed near the end of the larval stage. Long-diapause genotype larvae exposed to increased temperatures at the end of the growing seasons could experience increased voltinism as higher temperatures delay the onset of diapause. Photoperiod will not change as temperatures continue to increase, however increased temperatures have the potential to reduce diapause induction and drive individuals toward a non-diapause developmental trajectory (Ikten et al., 2011; McLeod and Beck, 1963). Larvae with the long-diapause genotype that are able take advantage of longer growing seasons and delay the onset of diapause, could pupate, eclose as adults, and those adults could produce an additional generation of herbivorous larvae capable of economic damage.

Longer and warmer growing seasons have the potential to increase insect feeding, mating, and voltinism. Climate change will affect insect populations, and how insects respond to climate change will determine which insects are losers and which are winners. European corn borer is a major agricultural pest in the United States, accounting for up to $2 billion dollars in costs associated with its management (Hyde et al., 1999). Investigating the energy required for insects to successfully complete diapause could expose mechanisms that could be used to regulate insect pests. Developing strategies to manipulate the mechanisms regulating the progression of European corn borer through diapause could be valuable. Eventually, perturbing the European corn borer larvae’s ability to survive diapause by affecting how it accumulates and stores resources in preparation for diapause could complement existing pest management programs for these pests. Until then, the link between seasonal temperatures and global food security will become more tenuous and finding a comprehensive approach to dealing with the response of pest insects to climate change is imperative.

**REFERENCES**

Bradshaw, W. E., and C. M. Holzapfel. 2001. Genetic shift in photoperiodic response correlated with global warming. Proceedings of the National Academy of Sciences 98:14509-14511.

Gomi, T., M. Nagasaka, T. Fukuda, and H. Hagihara. 2007. Shifting of the life cycle and life-history traits of the fall webworm in relation to climate change. Entomologia Experimentalis et Applicata 125:179-184.

Hyde, J., M. A. Martin, P. V. Preckel, and C. R. Edwards. 1999. The economics of Bt corn: valuing protection from the European corn borer. Review of Agricultural Economics 21:442-454.

Ikten, C., S. R. Skoda, T. E. Hunt, J. Molina-Ochoa, and J. E. Foster. 2011. Genetic variation and inheritance of diapause induction in two distinct voltine ecotypes of *Ostrinia nubilalis* (Lepidoptera: Crambidae). Annals of the Entomological Society of America 104:567-575.

McLeod, D., and S. D. Beck. 1963. Photoperiodic termination of diapause. The Biological Bulletin 124:84-96.

Sinclair, B. J. 2015. Linking energetics and overwintering in temperate insects. Journal of Thermal Biology 54:5-11.

Thompson, A. C., and F. M. Davis. 1981. The effect of temperature on the rate of metabolism of lipids and glycogen in diapausing southwestern corn borer, *Diatraea grandiosella*. Comparative Biochemistry and Physiology - Part A: Physiology 70:555-558.

Williams, C. M., K. E. Marshall, H. A. MacMillan, J. D. Dzurisin, J. J. Hellmann, and B. J. Sinclair. 2012. Thermal variability increases the impact of autumnal warming and drives metabolic depression in an overwintering butterfly. PLoS ONE 7:e34470.