ABSTRACT

DILLARD, DESHAE. Evaluating Abiotic Factors Influencing *Helicoverpa zea* (Lepidoptera: Noctuidae) Pupae. (Under the direction of Drs. Dominic Reisig and Hannah Burrack).

Helicoverpa zea (Boddie) is one of the most economically important insects in North America, able to feed and reproduce across a wide variety of environmental conditions. Pupation, and the ability to diapause, are one of the key reasons why Heliothinae have become significant insect pests. However, the variety of soils in which Heliothinae pupate has been understudied, and the influence of abiotic and biotic factors on pupation are poorly understood. My study objective was to examine the effect of soil type on *H. zea* during pupation in-season and over the winter. In addition, I wanted to investigate how soil type was interacting with moisture, another key abiotic factor in the environment.

To determine the impact of soil type on *H. zea* pupation in-season and over the winter, three distinct soils representing the North Carolina landscape were collected to be used in experiments in North and South Carolina over two years. *Helicoverpa zea* larvae were used to infest the soils immediately before pupation. Pupal survivorship, depth, and weight varied significantly among soil types for location and year depending on the variable measured. Pupal depth did not correlate with weight. Diapausing characteristics varied significantly by the interaction between location and year.

The influence of soil type and moisture on *H. zea* pupation was measured in paired studies in the field and greenhouse. Soils were infested with larvae and either saturated or left unsaturated with water, this occurred both before and just after pupation. There was a significant difference between wet and dry conditions in adult emergence among the three soils. Pupal depth varied significantly with soil moisture, but the interaction between soil type and moisture was not significant. Pupal weight did not vary among soil types, moisture, or their interaction.

Soil type and moisture both mediate Heliothinae ecology, but the relationship between these two environmental variables and time is still not clear. However, these experiments were the first to document a significant relationship between Heliothinae pupation and soil type, suggesting that additional studies examining the interaction of soil type with moisture are necessary.

Evaluating Abiotic Factors Influencing *Helicoverpa zea* (Lepidoptera: Noctuidae) Pupae

by
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DEDICATION

To Drs. Steven K. Schwartz and Gary C. Chang, extraordinary mentors that ignited and supported my passion for entomological research at the beginning of my scientific career.

To my mom, without your support and sacrifice, none of this would have been possible.

BIOGRAPHY

DeShae Dillard comes from a non-agricultural background growing up in the suburbs outside of Seattle, WA. While pursuing an undergraduate degree at Gonzaga University he had the opportunity to participate in invertebrate research that introduced him to the field of Entomology. After graduating, DeShae wanted to apply his technical knowledge of Entomology towards addressing complex societal problems leading him to pursue the agricultural sciences. An interest in integrating diverse disciplinary studies in agriculture brought him to North Carolina State University to pursue a Master of Science in Entomology.

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CHAPTER 1

Helicoverpa zea (Lepidoptera: Noctuidae) in-season and overwintering pupation response to soil type

Abstract

Soil pupation in lepidopterans has been understudied and changes in interactions with the environment induced by climate change are influencing Heliothinae and other Noctuidae. I observed the pupation of *Helicoverpa zea* (Boddie) in-season and over the winter in a common garden experiment. Three distinct soil types (coarse sand, high organic muck, and fine-textured clay) were brought to two separate locations to assess survivorship, diapause, depth, and weight under identical environmental conditions. Pupal survivorship in fine-textured clay soils was less than coarse sand or high organic muck. Over the winter, pupal survivorship varied significantly by soil type and year. Pupal depth was shallowest in fine-textured clay soils, and pupae recovered were slightly smaller in weight. Diapausing characteristics varied significantly by the interaction between year and location but were difficult to explain given my experimental design. Fine clay soils have a clear negative impact on Heliothinae pupation. Future studies should explore other closely related Noctuidae like *Spodoptera frugiperda* (Fabricius) to understand how each stage of the lepidopteran life cycle is impacted by the environment over time.

Introduction

Heliothinae (Lepidoptera: Noctuidae) are some of the most significant global agricultural pests due to well-documented insecticide resistance, as well as the ability to disperse, undergo multiple generations in a single year, initiate facultative diapause, and develop on a broad range of host plants (Fitt 1989). In North America, *Helicoverpa zea* (Boddie) can undergo facultative diapause during pupation and will overwinter in more southern latitudes while succumbing to lethal temperatures in more northern latitudes (Phillips and Barber 1929, Barber 1939, Barber 1941, Blanchard 1942). In the southeastern United States, *H. zea* typically produces four to five generations in a single season before entering diapause to overwinter as pupae in the soil. Adults from the overwintering generation in the south initiate migration north during the following year (Westbrook 2008).

Helicoverpa zea pupal survivorship, depth, and adult emergence have been studied across varying environmental conditions (Parencia 1964, Slosser et al. 1975, Caron et al. 1978, Roach and Hopkins 1979, Eger et al. 1983, Rummel et al. 1986). Most overwintering *H. zea* pupae survive the winter temperatures south of the 40th parallel (Hardwick 1965), although this may be shifting farther north (Lawton et al. 2022, in review). Soil type can impact overwintering survival, but with considerable variation among studies (Phillips & Barber 1929, Barber 1939, Blanchard 1942, Parencia 1964, Roach and Hopkins 1979). Differences in observations across studies have been attributed to several abiotic and biotic factors that can include but are not limited to, temperature, soil type, rainfall, shelter, and the activity of other soil organisms (Hardwick 1965).

For instance, Barber (1939) reported that overwintering pupae only survived in sandy soils while other studies observed survival in other soil types, such as loam and clay soils

(Phillips and Barber 1929, Blanchard 1942, Parencia 1964, Slosser et al. 1975, Rummel et al. 1986). Survivorship ranged from 0 – 75.1% in sand (Blanchard 1942), from 0 – 22.5% in clay (Phillips and Barber 1929, Blanchard 1942, Parencia 1964, Slosser et al. 1975), and from 0 – 26.5% in loam (Phillips and Barber 1929, Blanchard 1942, Roach and Hopkins 1979, Rummel et al. 1986). Pupal depth also varied among studies. Barber (1941) reported an average pupal depth of 10.1cm in sandy soils while Eger et al. (1983) reported an average depth of 2.8cm. Direct comparisons of pupal depth among soil types under otherwise identical environmental conditions have only been conducted in a single study (Phillips and Barber 1929), reporting a depth of 8.9cm in clay loam and 10.4cm in loam.

Understanding the ecology of an insect pest species is important for informed decision-making for pest management and modeling population dynamics. Targeting the pupal stage of Heliothines using tillage, for example, has been proposed as a pest management tactic (Hopkins 1972, Roach 1981, Downes et al. 2017), but the efficacy of this tactic could vary if the depth of pupation or survival differs among soil types. Moreover, the impact of soil type on survival may also influence proposed areawide management efforts (Knipling and Stadelbacher 1983) targeted toward the first generation just after emerging from overwintering.

Studies to examine North American Heliothinae pupal behavior under varying environmental conditions have not been revisited in recent decades. Periodical observation of distinct life stages is essential for insects with complex life cycles given the varying impact of changing climate on insect ecology (Kingsolver et al. 2011). Current climate change modeling using forecasted temperatures suggests that the successful survival of overwintering pupae will change by geographical region in the coming decades (Lawton et al. 2022, in review). Ongoing observations of *H. zea* ecology will be essential to adapting pest management strategies to the changes induced by insecticide resistance, climate change, the spatiotemporal configuration of agricultural systems, and host availability. Furthermore, observing additional biological parameters like diapausing characteristics could elicit additional strategies for managing Heliothinae.

My experiments were designed to mitigate the impact of confounding environmental variables in previous studies of *H. zea* pupation during the growing season and over the winter. I expand on the current understanding of Heliothinae pupal ecology by demonstrating a significant relationship between several biological parameters (survivorship, depth, weight, and diapause) and soil type. This was accomplished using three types of soils (coarse sand, fine-textured clay, and high organic loam) in a common garden experiment in two separate locations (North Carolina: 35.8º N and South Carolina: 34.3º N) to isolate the impact of soil type on pupation. My main hypothesis was *H. zea* pupal survivorship would differ among soil types. Fine-textured clay soils were expected to reduce survivorship based on anecdotal observations and unpublished studies. In addition, I hypothesized that pupal depth and weight would also be affected by soil type and that there would be a positive correlation between depth and weight. I did not anticipate diapausing characteristics to vary among soil types but hypothesized there may be a significant relationship with location, year, or their interaction. Inconsistent observations in the literature impeded my ability to make specific hypotheses about the relationship between depth, weight, and diapausing characteristics with soil type, but I also hypothesized that they may be different among soil types.

Materials and Methods

Experimental Design

Three distinctive soils representing the diversity of the Coastal Plain, Sandhills, and Piedmont in North Carolina were collected to be used in a completely randomized common garden experiment conducted in two locations (Plymouth, NC, and Florence, SC) from 2020 to 2022. Soils consisted of Candor sand (Sandy, kaolinitic, thermic Grossarenic Kandiudults) from Moore County, NC (35.183643, -79.680189) with 0.5 – 1% organic matter and 1 – 4% clay, Belhaven muck (Loamy, mixed, dysic, thermic Terric Haplosaprists) from Washington County, NC (35.830119, -76.556088) with 20 – 95% organic matter and 0% clay, and Mecklenburg clay loam (Fine, mixed, active, thermic Ultic Hapludalfs) from Davidson County, NC (35.592215, -79.998049) with 0.5 – 1% organic matter and 20 – 35% clay. Soil was hand-collected down to a range of 50cm to reflect the documented depth of the *H. zea* pupal chamber (Quaintance and Brues 1905).

After collection, the soil was divided into 115L polyethylene storage containers (Sterlite, Model: 18188206, 83 x 50 x 44cm) with 0.635 – 1.270cm drainage holes in the bottom. These holes were initially covered with fine wire mesh to prevent larval escape, but the wire was later removed once I observed that larvae rarely burrowed to the bottom of the containers. Each container was filled with soil to a depth of 15cm and placed outside to settle for at least one month before infestation. Each of the three treatments (coarse sand, fine-textured clay, and high organic matter muck) was replicated 20 times at each of the two locations. Roundup PowerMAX (glyphosate, 2% solution, Bayer Crop Science, St. Louis, MO) was applied for weed control as necessary using a hand pump sprayer, and all containers were hand-weeded before infestation. Extinguish Plus (hydramethylnon/S-methopren, 1.68kg/hectare, Wellmark International, Schaumburg, IL) or Amdro Fire Ant Bait (hydramethylnon, 1.12kg/hectare, Central Garden and

Pet, Walnut Creek, CA) was also applied as necessary to the field to prevent *Solenopsis invicta* predation.

In-season

Starting mid-to-late June of 2020 (June 18th; June 24th) and 2021 (June 26th; June 28th), 0.1 hectares of non-Bt corn (hybrid DKC-67-70, Bayer Crop Science) were planted in North and South Carolina. These plantings did not receive foliar insecticide applications and were naturally infested with *H. zea*. Containers with the three soil types were spaced at 0.75m apart in the rows of non-Bt corn using a completely randomized design. When fourth instar larvae were present in corn ears, 25 – 30 corn ears were removed and evenly distributed across containers so that larvae could exit the ears and pupate into the soil in the container. After 7 – 10 days, containers were excavated to assess pupation depth and survival.

Overwintering

During the fall, but before overwintering, *H. zea* population abundance declines. Artificial infestation using colony-reared insects was therefore used to assess overwintering. Larvae from Benzon Research Inc. (Carlisle, PA) were maintained outdoors under ambient conditions on a modified commercial diet (Southland Products, Lake Village, AR) in Raleigh, NC during late September and early October to initiate diapause, as nearly 100% of *H. zea* larvae enter diapause after the last week of September in North Carolina (Stinner et al. 1974). Infestations were made during the first two weeks of October in 2020 and 2021 in the same soils and same locations as the in-season experiment. Containers with the three soil types were placed into the ground, matching the soil level in the containers to the surrounding field using a completely randomized design spaced at 0.75m apart. Late sixth instar larvae were placed in each soil container (n =20 – 22 per container), replicating the method of infestation in previous studies (Phillips and Barber 1929, Blanchard 1942, Roach and Hopkins 1979, Murray and Zalucki 1990). After seven days, larvae that had not pupated were removed and discarded. Larvae that failed to pupate were incorporated in analyses on survivorship as mortality during the process of pupation would contribute to survivorship in the field. Pupae were allowed to overwinter in the soil until the last week of February and excavated before spring adult emergence, which can begin as soon as 1 May in North Carolina (Neunzig 1969), and likely sooner given recent estimates of range expansion due to climate change (Lawton et al. 2022, in review).

Excavation

Helicoverpa zea pupae were recovered in both the in-season and overwintering studies by cutting open containers and carefully excavating soil from the edge of the container while working inward. Upon finding a pre-pupa or pupa, the depth from the soil surface to the middle of the specimen and survivorship were immediately recorded. If alive, the specimen was transported to the laboratory where it was cleaned and weighed. Overwintering pupae were also examined for indicators of diapause through the presence or absence of eyespots, and the fat body (Phillips and Newsom 1966). Some specimens were damaged in the excavation process and mortality, weight, and characteristics of diapause were coded as missing data.

Statistical Analysis

An analysis of variance (ANOVA) approach was applied to much of the data. Non-normal data were transformed before analysis to fit the assumptions of the model. If normality could not be achieved using standard transformations in a general linear mixed model (PROC MIXED; SAS v9.4, SAS Institute, Cary, NC), model fit statistics (i.e., AIC) were evaluated via PROC GLIMMIX (SAS v9.4, SAS Institute, Cary, NC), and data were analyzed using the best fitting distribution, which was lognormal in all cases. Analyses were conducted with all interactions between the fixed effects of soil type, location, and year. Replicate was nested within the interaction between treatment, year, and location as a random effect to account for subsampling in a completely randomized design. Pupal survivorship, weight, and the proportion of pupae with diapausing characteristics were analyzed via PROC MIXED. Pupal depth was analyzed via PROC GLIMMIX. Tukey’s honestly significant difference was used to separate means (Tukey, 1953). A correlation between in-season pupal depth and weight was assessed using Pearson’s coefficient for each soil type, by year and location (PROC CORR; SAS v9.4, SAS Institute, Cary, NC).

Results

In-season

Pupal survivorship was significantly different for the interaction between soil type and location (*F* = 11.67, d.f. = 2, 228, *P* < 0.0001). In South Carolina, survivorship in fine-textured clay (77.7% ± 1.8 SEM) was lower than North Carolina (90.3% ± 1.8). In addition, survivorship in South Carolina fine textured clay was also lower than survivorship in high organic matter muck (NC: 92.1% ± 1.4; SC: 91.1% ± 1.2) and coarse sand (91.8% ± 1.4; 91.8% ± 1.1) in both North and South Carolina.

Pupal depth was significantly different for the interaction between soil type, location, and year (*F* = 12.58, d.f. = 2, 228, *P* < 0.0001; Figure 1.1). During 2020 in North and South Carolina depth was shallower in fine-textured clay (NC: 2.99cm ± 0.10; SC: 2.39cm ± 0.10) than high organic matter muck (3.95cm ± 0.10; 4.73cm ± 0.15) or coarse sand (5.08cm ± 0.10; 5.68cm ± 0.19). The same trend was observed during 2021 in North and South Carolina for fine-textured clay (NC: 3.25cm ± 0.14; SC: 3.26cm ± 0.10), high organic muck (4.54cm ± 0.13; 4.36cm ±

0.10), and coarse sand (4.99cm ± 0.10; 4.62cm ± 0.10). Fine textured clay in South Carolina during 2020 was significantly different from every other combination of soil type, location, and year.

Pupal weight was significantly different among soil types (F = 7.57, d.f. = 2, 227, P = 0.0007), as well as for the interaction between location and year (F = 38.51, d.f. = 1, 227, P < 0.0001). Weight in fine-textured clay (0.454g ± 0.003) was less than high organic matter muck (0.468g ± 0.002) or coarse sand (0.471g ± 0.002). There were two positive correlations between pupal weight and depth by each soil type for location and year, one for coarse sand located in South Carolina in 2020 and another for high organic muck in South Carolina in 2021 (Table 1.1).

Overwintering

Pupal survivorship was significantly different for the interaction between soil type and year (F = 5.38, d.f. = 2, 228, P = 0.0052; Figure 1.2). In 2021, survivorship in the fine-textured clay (3.9% ± 1.0 SEM) was lower than the high organic matter muck (10.9% ± 1.2), but not coarse sand (8.1% ± 1.0). In 2022, survivorship in the fine-textured clay (11.5% ± 1.9) was lower than the high organic matter muck (27.1 ± 2.2) and coarse sand (27.9% ± 2.8).

The proportion of pupae retaining diapausing eye spots was significantly for the interaction between location and year (F = 7.44, d.f. = 1, 158, P = 0.00071). The interaction of year and location also had a significant effect on the proportion of pupae retaining diapausing fat bodies (F = 7.31 d.f. = 1, 158, P = 0.0076). The proportion of pupae retaining diapausing eyespots that were recovered from North Carolina in 2021 (56.1% ± 5.5) was less than 2022 (95.6% ± 1.4), and less than South Carolina in 2021 (81.7 ± 4.6) and 2022 (95.2 ± 1.6). The proportion of pupae with the fat body intact recovered from North Carolina in 2021 (54.7% ±

6.9) was less than 2022 (94.4% ± 2.0), and less than South Carolina in 2021 (82.6% ± 5.3) and 2022 (97.4 ± 1.2).

Pupal depth was significantly different for the interaction between soil type and year (*F* = 3.97, d.f. = 2, 174, *P* = 0.0168), as well as for the interaction between year and location (*F* = 5.83, d.f. = 2, 174, *P* = 0.0168). Depth in fine-textured clay in South Carolina (4.20cm ± 0.22) was shallower than high organic muck (NC: 4.94cm ± 0.18; SC: 4.77cm ± 0.12) and coarse sand (5.18cm ± 0.15; 5.18cm ± 0.17) in North or South Carolina. Depth in fine-textured clay in South Carolina was not significantly different from North Carolina (4.40cm ± 0.28).

Pupal weight was significantly different for the interaction between year and location (*F* = 12.84, d.f. = 1, 158, *P* = 0.0005). During 2021, weight in North Carolina (0.359g ± 0.003) was greater than 2022 (0.316g ± 0.006), as well as South Carolina for both years (2021: 0.312g ± 0.007; 2022: 0.298g ± 0.004). During 2022, weight in North Carolina was also greater than South Carolina.

Discussion

My study adds clarification to the ecology of *H. zea* pupation and overwintering by documenting a statistically significant effect of the interaction between soil type, location, and year on pupal survivorship and depth. Pupal weight was also impacted by the relationship between soil type and location. Fine-textured clay soils restrict the depth of pupation, reduced survivorship, and lower weight. Past qualitative observations have highlighted the key role that soil type can play in pupation and overwintering but were not supported with quantitative analysis (Barber and Dicke 1939). In addition, soil type can interact with year and location to influence diapausing characteristics, biological parameters that have not been previously examined.

In contrast, Roach and Hopkins (1979) reported no impact of soil type on *H. zea* pupal depth or survivorship as indicated by adult emergence. However, the soil types examined in their study consisted of Dothen fine loamy sand, Dunbar fine sandy loam, and Verina loamy fine sand. Despite the variation in sand, silt, and clay content among these soils, they are all sandy and similar in soil series structure. The soil types used in my experiment (Candor sand, Belhaven muck, and Mecklenburg clay loam) reflect a broader range in soil particle content. As a result, I am the first to document a statistically significant relationship between soil type and pupation. Before my study, Phillips and Barber (1929) had observed in-season and overwintering emergence among a range of soils (clay, loam, sandy loam, and clay loam), but only report summary data and do not directly compare soil types.

Throughout the study, there was a noticeable change in the structure of the soils from collection to experimentation that was unavoidable because of the nature of the common garden design. Before collection, the fine-textured clay was impenetrable by hand beyond the top 15cm of soil. Regardless of the length of time the clay was left out in the field, the soil did not regain the initial hard compacted structure. In contrast, despite the structure of the coarse sand and high organic matter muck also being compromised, these soils were quick to recover and were similar to the original state of collection during my experiments. I hypothesize that field observations of *H. zea* in undisturbed fine-textured clay would reveal an even greater negative impact on pupation because of the hard compacted structure. Conversely, for coarse sand and high organic matter muck, I would not expect a significant difference in H. zea pupal survivorship, depth, and weight between soil found at the original site and the soil utilized in my study.

The differences in survivorship, both in-season and during overwintering, have biological relevance for pest management of this species. *Helicoverpa zea* pupae developing in fine-textured clay soils may experience population suppression compared to other sandier or more organic soil types. Other factors, for example, the presence of natural enemies, which were not observed in this study could also interact with soil type to influence survivorship in the field. Survivorship in clay during the growing season (75.6 – 92.5%) was higher than over the winter (3.9 – 12.8%) across location and year. The mortality of pupae in fine-textured clay could be associated with the increased water capacity of clay in comparison to silt or sand (Dexter 1988). Roach and Hopkins (1979) found that soil moisture impacted the adult emergence of *H. zea* suggesting a close relationship between moisture and the success of pupation. The relationship between soil type, moisture, *H. zea* pupation over time should be studied to predict population dynamics more accurately in the landscape. The low survival of overwintering pupae in fine-textured clay soils suggests management involving post-harvest cultivation (i.e., tillage or pupae busting) should be focused on controlling *H. zea* pupae in sandy or high organic matter soils.

After pupation, eclosing adults will rely on the pupal chamber for emergence. If the chamber is compromised by the surrounding soil, the individual may be unable to successfully emerge. Soil particle hierarchy determines the strength of the bonds between compound particles, and soils higher in clay particles will have higher internal strength (Dexter 1988). As a result, pupal chambers formed in fine-textured clay may be more stable than those in coarse sand or high organic muck. However, if the pre-pupa is unable to penetrate fine-textured because of the strength of compound particles, the pre-pupa may pupate near or on the soil surface. Maintaining the integrity of the pupal chamber is potentially important for Lepidopteran defense as the silk lining has been shown to have defensive properties (Lindstedt et al. 2019). Soils lower in shear strength may be impacting Heliothinae pupal mortality through pathogen exposure as the structure of the pupal chamber would be impacted under stress.

Studies on Heliothinae diapausing ecology have been restricted to the greenhouse or laboratory under controlled temperature, humidity, or light conditions (Pullen et al. 1992, Mohammed et al. 1999, Shimizu and Fujisaki 2002, Morey et al. 2012). This study is the first to observe diapausing characteristics of the pest in the field and demonstrates that environmental factors over time and space add additional confounding variables that have not been accounted for in the laboratory. Visible diapausing characteristics like eyespots or fat bodies will disappear as the pupa begins to emerge from overwintering in the spring (Phillips and Newsom 1966). Measuring diapausing characteristics can be difficult because the timing of excavation is essential for observation. Pupae in North Carolina during 2021 had a lower incidence of diapausing eye spots and fat bodies, suggesting an earlier emergence date compared to pupae in North Carolina during 2022, as well as pupae in South Carolina during 2021 and 2022. In all the other treatments at both locations, the presence of both eyespots and fat bodies was higher. In 2022, temperatures in the field had likely not yet triggered a break in diapause for *H. zea* (Phillips and Newsom 1966).

The biological consequences of pupal depth on fitness are not fully understood, but a reasonable hypothesis is that shallower pupae may be more prone to predation or negative abiotic stressors during the winter, such as ice nucleation or lethal temperature events. In contrast, deeper pupae may be more prone to mortality due to excessive moisture or experience greater difficulty emerging. However, the average pupal depth was within 5.0cm of the soil surface, a contrast to previous studies that reported depths greater than 10.0cm (Phillips and Barber 1929, Barber 1939). Shallower pupae could be more accessible to a range of soil cultivation practices (moldboard plow, strip-till, disking, etc.). In North America, cultivation was once a widely employed pest management practice (Hopkins et al. 1972, Roach and Hopkins 1979, Roach

1981), but has been abandoned in favor of conservation agriculture promoting reduced or no cultivation. Although the effects of tillage on pest populations are mixed (Reay-Jones et al. 2020), Heliothinae populations in areas like the southeastern U.S. have been traditionally controlled by cultivating cotton fields (Stinner and House 1990). Cultivation in conservation agriculture (i.e., strip-till) should be explored in the context of pest management to determine if this strategy is environmentally and economically sustainable.

In Australia, post-harvest cultivation of fields at elevated risk for overwintering populations of *Helicoverpa armigera* (Hübner) has been utilized in area-wide management, a tactic known as "pupae busting" (Downes et al. 2017). Post-harvest cultivation has also been effective in managing *H. armigera* resistance to transgenic Bt cotton. *Helicoverpa zea* in North America, unlike *H. armigera* in Australia, is resistant to transgenic Bt Cry toxins in corn and cotton (Dively et al. 2016, Reisig et al. 2018). Cultivation has been suggested to play a vital role in managing ground-dwelling pests, and a scientific advisory panel convened by the US EPA in 2018 recommended "tilling where possible" to reduce the risk of insecticide resistance to Bt traits (US EPA 2018).

The differences in pupal weight among soil types in the in-season trials were small, but potentially biologically meaningful. *Helicoverpa zea* pupal weight has been linked to sublethal effects of Bt corn (Bilbo et al. 2018, Reay-Jones et al. 2020). In South Carolina's coarse sand during 2020, and high organic muck during 2021, there was a significant correlation between pupal depth and weight. No other combination of soil type, location, and location demonstrated a significant correlation between pupal depth and weight. Pupal depth was highly variable, and the only two significant correlations did not have strong relationships, indicating depth is better correlated with factors outside of weight. However, both coefficients for the two significant correlations were positive, and the relationship between pupal depth and weight should be investigated further to identify environmental or biological conditions where weight is driving the depth of pupation.

Study locations and years experienced different abiotic conditions, which influenced much of my observations. For example, I observed a significant difference in overwintering pupal survivorship between 2020 and 2021 among coarse sand (2020: 8.1%; 2021: 27.9%), high organic muck (10.9%; 27.1%), and fine-textured clay soils (3.9%; 11.5%). During the winter of 2020 to 2021, North and South Carolina received more rainfall than during 2021 to 2022. Soil moisture is another abiotic factor that will need to be explored in the context of pupation, as adult emergence in dry soil conditions can be reduced by more than 50% (Roach and Hopkins 1979). Soil moisture may be a primary driver of overwintering mortality in Heliothinae. Additional studies on the relationship between soil moisture and *H. zea* pupation are necessary to separate compounding sources of mortality and understand the basic pupal ecology of Heliothines.

My results demonstrate there is a clear interaction between soil type and pupation, but the mechanism mediating this relationship is still poorly understood. Aggregate soil structure, moisture, and particle content are only a few of the key drivers of successful development that have been proposed and require further observation to isolate environmental factors. However, my study observes how soil type influences *H. zea* pupation using distinct soils found across North Carolina (coarse sand, high organic muck, and fine-textured clay). Additional studies should examine the effect of soil aggregate structure and moisture, as well as the interactions that can occur between all three variables. My observations suggest that soil type has a key role in influencing *H. zea* pupation, a factor that has not yet been considered when modeling population dynamics in the landscape (Murray and Zalucki 1990). Heliothinae pupation and overwintering ecology is an essential component of understanding how these pests will adapt to changing environmental conditions induced by climate change. Our studies quantify a relationship between soil type and the pupal ecology of *H. zea* and demonstrate a need for further observation.

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TABLES

Table 1.1. Pearson's correlation coefficients for in-season pupal depth and weight for each soil type, by year and location. Values indicated by * are significant at $p < 0.001$.**

Treatment	Year	Location	Coefficient Estimate	P
Fine-texture clay	2020	SC	0.0829	0.3890
High organic muck	2020	SC	0.1498	0.0817
Coarse sand	2020	SC	0.4057	0.0002***
Fine-texture clay	2020	NC	-0.0117	0.8656
High organic muck	2020	NC	0.0997	0.1273
Coarse sand	2020	NC	0.0577	0.3733
Fine-texture clay	2021	SC	0.0357	0.5662
High organic muck	2021	SC	0.1811	0.0007***
Coarse sand	2021	SC	0.0289	0.6027
Fine-texture clay	2021	NC	0.0718	0.4563
High organic muck	2021	NC	0.1343	0.0580
Coarse sand	2021	NC	0.0286	0.7401

FIGURES

Figure 1.1. Mean ± SEM in-season pupal depth for each soil type (coarse sand, high organic muck, and fine-textured clay), by each location (North and South Carolina) and year (2020 and 2021).

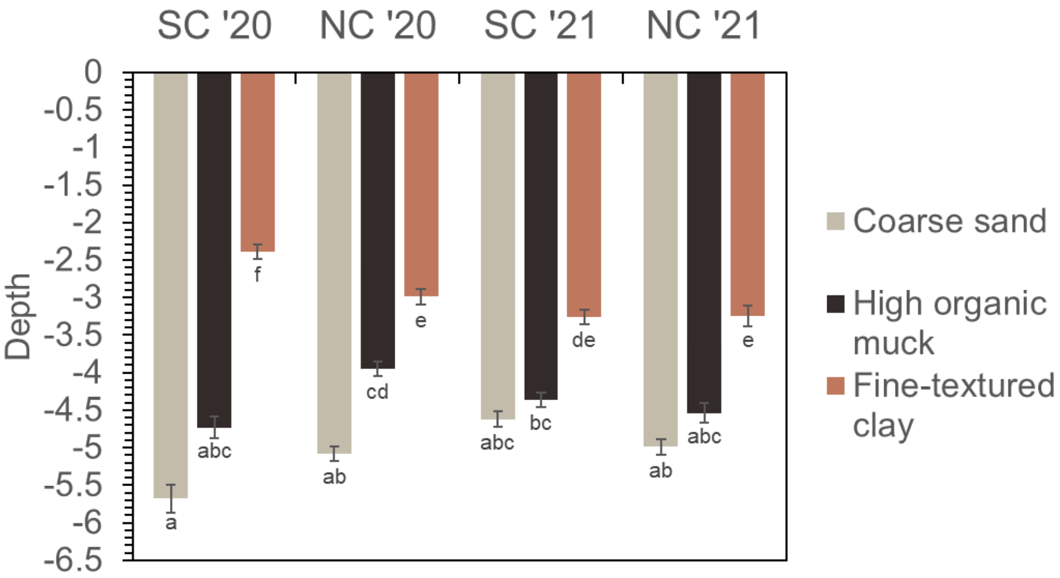
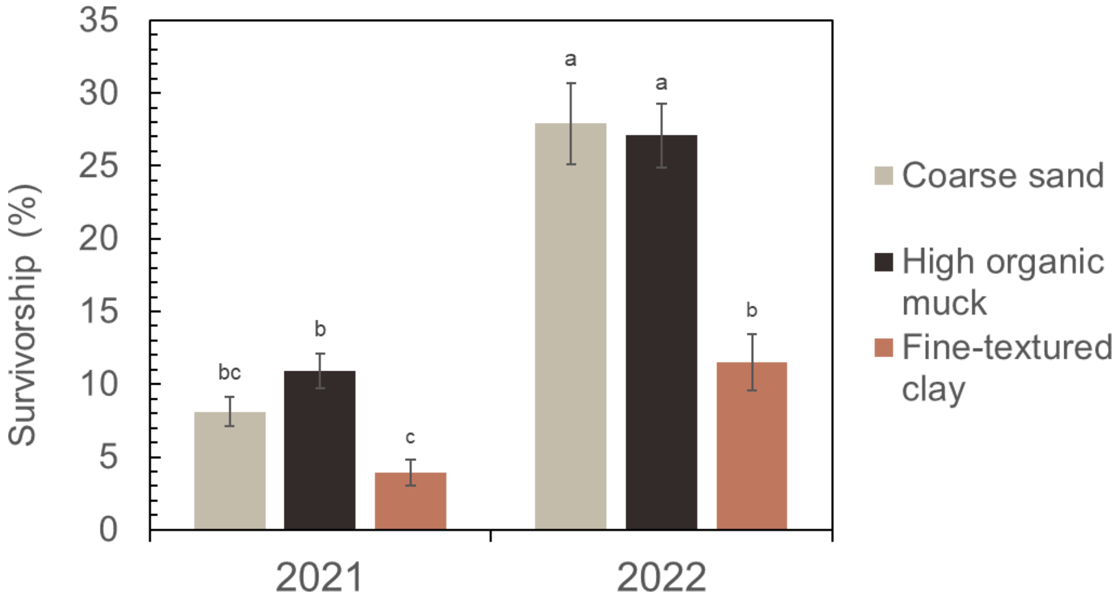


Figure 1.2. Mean ± SEM proportion of pupae surviving overwintering (%) for each soil type (coarse sand, high organic muck, and fine-textured clay), combined by location (North and South Carolina) for each year (2021 and 2022).



CHAPTER 2

Helicoverpa zea (Lepidoptera: Noctuidae) pupation response to soil type and moisture

Abstract

Evaluating the effect of soil moisture and other variables is essential to explaining the environmental factors that are driving Heliothinae pupation. I compared the pupation (mortality, depth, and weight) of *Helicoverpa zea* (Boddie) under differing moistures (wet and dry) and across three distinct soil types (coarse sand, high organic muck, and fine-textured clay) and observed adult emergence, as well as pupal depth and weight. Mortality of emerging adults was greater in dry fine-textured than dry coarse sand. However, there was no effect of soil type, or the interaction between soil and moisture, on pupal depth. Soil moisture was the primary driver of pupal depth, suggesting pre-pupae use moisture to mediate their positioning within the pupal chamber. In addition, there was no effect of soil type, moisture, or their interaction on pupal weight. My study demonstrates that soil moisture can be a greater driver of *H. zea* pupation than soil type, but additional observations are necessary to understand the mechanism by which moisture is impacting pupation

Introduction

Helicoverpa zea (Boddie), and other related Heliothinae, are significant agricultural pests and exploit a variety of agroecosystems (Fitt 1989). In 2020, control costs and yield losses for *H. zea* were estimated to be over $100 million across U.S. cotton alone (Cook and Threet, 2020). Pupation is a vulnerable life stage for lepidopterans such as *H. zea* because they are sessile in the soil and unable to disperse away from threatening conditions. Populations of diapausing, or overwintering, Heliothinae pupae have been managed using cultivation because of insecticide resistance to pyrethroids (Fitt and Daly 1990), and now Bt (Downes et al. 2017). Below ground, Heliothinae pupae can die due to low temperature, moisture, soil compaction, flooding, parasitism, predation, and disease (Fitt 1989); however, mortality frequently goes unobserved in the field since this life stage is not visible. Moisture is an essential component of development that has been understudied in the context of insect seasonal ecology (Tauber et al. 1998), including *H. zea*.

Studies have quantified the impact of low temperatures on mortality of diapausing and non-diapausing *H. zea* pupae (Morey et al. 2012); however, these observations were made under laboratory conditions and may not reflect population dynamics in the field because they are isolated from additional compounding sources of mortality. For example, the winter survival of *H. zea* and *Chloridea virescens* (Fabricius) in the field can be primarily impacted by moisture and not temperature (Eger et al. 1983). Outside of mortality, more detailed studies on *H. zea* pupation, including how pupal depth varies among environmental conditions, are lacking. Understanding the interaction among environmental factors on *H. zea* pupation is essential for accurately modeling the population dynamics of this species.

The effect of soil type on adult emergence of *H. zea* was previously explored and suggested to have no effect (Roach and Hopkins 1979). However, *H. zea* adult emergence in wet soil conditions was increased by 41%. Soil types examined in this study consisted of Dothen fine loamy sand, Dunbar fine sandy loam, and Verina loamy, which were all fine-textured sand and similar in soil series structure. Soil texture and particle size composition can impact moisture retention in the soil, particularly for fine-textured clay soils when compared to soils higher in the composition of sand or silt (Williams et al. 1983). However, the impact of distinctly different soil types and moisture on *H. zea* pupae has not been examined in a single replicated experiment. Prior work suggests a clear relationship between moisture and survivorship of pupae, as well as adult emergence. While data were not analyzed with statistics, Barber and Dicke (1939) described “a tight clay soil with good surface drainage” as often favorable for winter survival.

Soil moisture can affect additional biological parameters of Heliothinae beyond emergence and can vary by species. For example, *Helicoverpa punctigera* (Wallengren) pupate 1.8cm deeper in dry soils compared to wet soils, while *Helicoverpa armigera* (Hübner) dig 0.9cm deeper in dry soils compared to wet soils (Murray and Zalucki 1990). Barber (1941) also observed *H. zea* pre-pupae digging to an average depth of 12.5cm during a dry year and 10.0cm during a wet year. Additionally, pupal mass, survivorship, and adult emergence of *Spodoptera frugiperda* (Fabricius), another Noctuid, are sensitive to relative moisture levels in the soil (He et al. 2021). Finally, soil moisture has been suggested as a key factor to incorporate into the population dynamics modeling for *Helicoverpa* spp. (Murray and Zalucki 1990).

My study observed the effects of soil type, moisture, and simulated rainfall on *H. zea* pupal depth and mortality. I designed field experiments to look at the effect of moisture in a single soil type. However, expanding on Roach and Hopkins (1979), I incorporated a broader range of soils (coarse sand, high organic muck, and fine-textured clay) in greenhouse experiments. I anticipated *H. zea* pupal depth would be deeper in dry soils as the pre-pupa searched for adequate moisture to maintain optimal development. In addition, pupal survivorship and adult emergence were expected to be lower in dry soils because the pupa loses moisture much more readily to the surrounding environment. I hypothesized that fine-textured clay soil would affect these three biological parameters more than coarse sand or high organic muck due to water retention properties.

Materials and Methods

Helicoverpa zea eggs were purchased from Benzon Research Inc. (Carlisle, PA) and reared on a modified commercial diet (Southland Products, Lake Village, AR) until the late-stage sixth instar. Larvae were reared indoors under ambient conditions.

Field Studies

Portsmouth fine sandy loam soil (Fine-loamy over sandy or sandy-skeletal, mixed, semiactive, thermic Typic Umbraquults) was collected from Washington County, NC (35.843085, -76.667466) with 8 – 15% organic matter and 10 – 25% clay. The soil was separated into 115L polyethylene storage containers (Sterlite, Model: 18188206, 83 x 50 x 44cm, Townsend, MA) modified with 0.635 – 1.270cm holes in the bottom to allow for drainage. Each container was filled with 15cm of soil and placed outside in early May to allow the soil to settle before infestation. After two weeks each container was covered with a lid to prevent precipitation and keep the soil unsaturated. The first study to quantify the impact of soil moisture on pupal depth and weight was a completely randomized design with soils that had high or low moisture. The second study to observe the impact of rainfall on pupal survivorship was a completely randomized design with simulated rainfall regimes (0cm, 2cm, 4cm, 6cm, 8cm, and 10cm).

To assess the impact of soil moisture on pupal depth and weight, a high or low moisture treatment was randomly assigned to each of the soil containers. There was a total of 15 replicates (i.e., containers) for each of the two treatments. Low moisture containers received no additional rainfall, and high moisture treatment received a rainfall equivalent (RE) of 4cm, enough to saturate the soil and flow through the bottom of the container. Rainfall was simulated using a secondary polyethylene storage container placed over the soil, water was added and drained on top of the soil. After the simulated rainfall, the soil was allowed to settle for one hour before infestation. Thirty larvae were released into each container. Pupae were excavated 5 – 7 days after entering the soil, which was before adult emergence. Upon excavation, depth and survival were immediately recorded.

To assess the impact of simulated rainfall on pupal mortality, one of six different rainfall treatments (0, 2, 4, 6, 8, and 10cm) was randomly assigned to a soil container. There was a total of five replicates for each of the six treatments. Thirty larvae were released into each of the soil containers. Each treatment was only applied after infestation with *H. zea* larvae. Each container was infested with larvae and left undisturbed for five days to ensure that prepupae in the soil had the opportunity to develop into pupae. Each assigned rainfall treatment was then applied over the soil and the container was covered to prevent adult emergence. Adult emergence was recorded daily for seven days after the first adult appeared and was used to assess survivorship from each of the treatments.

Greenhouse Studies

Three distinctive soils (coarse, fine-textured, and high organic matter) representing the diversity of soils from the North Carolina Coastal Plains and Piedmont regions were collected and arranged in a factorial design to explore the interaction between soil type and moisture. Soils consisted of Candor sand (Sandy, kaolinitic, thermic Grossarenic Kandiudults) from Moore County (35.183643, -79.680189), NC with 0.5 – 1% organic matter and 1 – 4% clay, Belhaven muck (Loamy, mixed, dysic, thermic Terric Haplosaprists) from Washington County (35.830119, -76.556088), NC with 20 – 95% organic matter and 0% clay, and Mecklenburg clay loam (Fine, mixed, active, thermic Ultic Hapludalfs) from Davidson County, NC (35.592215, -79.998049) with 0.5 – 1% organic matter and 20 – 35% clay. The soil was separated into 3.8L polyethylene storage containers (PFS Sales Company, Model: VP-210222, 21.64 x 12.85cm, Raleigh, NC) modified with 0.95cm holes in the bottom to allow for drainage.

To assess the impact of soil type and moisture on pupal depth, high or low moisture treatments were assigned to each of the three soils. There was a total of five replicates for each of the six treatment combinations. Each container was completely saturated with water and left to air dry. The soil was left uncovered in the greenhouse for two weeks before the study to ensure the dry treatment had as little moisture as possible. The simulated rainfall wet soil treatment was saturated with water and allowed to drain immediately before infestation with larvae. Following the application of simulated rainfall, 10 larvae were released into each of the soil containers. Each container was covered with a lid for 48 hours to prevent larvae from escaping. After 3 – 5 days, the container was excavated by hand and the depth and weight of each individual were immediately recorded.

To assess the impact of soil type and moisture on adult emergence, high or low moisture treatments were assigned to each of the three soil types. There was a total of five replicates for each of the six treatment combinations. The soil was left uncovered in the greenhouse for two weeks before the study to ensure the dry treatment had as little moisture as possible. The simulated rainfall wet soil treatment was saturated with water and allowed to drain immediately before infestation with larvae. Following the application of simulated rainfall, 10 larvae were released into each of the soil containers. Each container was covered with a lid for seven days to prevent larvae from escaping and to retain moisture in the soil. After the lids were removed, Supertuff paint strainers (Trimaco, Model: 11311/25, 3.8 L, Morrisville, NC) were used as emergence tents and placed over each container, then checked daily for adult emergence.

Statistical Analysis

Data were analyzed via ANOVA using PROC MIXED (SAS v9.4, SAS Institute). Pupal depth in fine sandy loam in the field was analyzed using soil moisture as a fixed effect, and adult emergence in fine sandy loam in the field was analyzed using the amount of rainfall as a fixed effect. Pupal depth and weight, as well as adult emergence, in the greenhouse were analyzed using soil type, moisture, and their interaction as fixed effects. Replicate was used as a random effect in each model. Tukey's honestly significant difference (HSD) was used to separate means (Tukey 1953). Non-normal data were transformed as necessary before analysis to fit the assumptions of the model. A correlation between depth and weight was assessed using Pearson's coefficient for each soil type and moisture combination (PROC CORR; SAS v9.4, SAS Institute).

Results

Pupal depth was significantly different between wet and dry fine sandy loam in the field ($F = 31.40$, d.f. = 1, 28, $P < 0.0001$; Figure 2.1). Depth was shallower in wet soil (4.25cm ± 0.10

SEM) compared to dry soil (2.86cm ± 0.10). Adult emergence did not vary among rainfall treatments (*F* = 1.15, d.f. = 5, 24, *P* = 0.3608).

Pupal depth also differed between wet and dry soil in the greenhouse (*F* = 24.06, d.f. = 1, 24, *P* < 0.0001). Depth was shallower in wet soil (4.92cm ± 0.18) compared to dry soil (3.48cm ± 0.16). There was no significant effect of soil type (*F* = 3.11, d.f. = 2, 24, *P* = 0.0623) or the interaction between soil type and moisture (*F* = 0.03, d.f. = 2, 24, *P* = 0.9716) on pupal depth. Pupal weight did not significantly differ by soil type (*F* = 3.31, d.f. = 2, 24, *P* = 0.0530), moisture (*F* = 0.82, d.f. = 1, 24, *P* = 0.3749), or their interaction in the greenhouse (*F* = 1.33, d.f. = 2, 24, *P* = 0.2817). There was also no correlation between depth and weight for soil type, by moisture (Table 2.1). An interaction between soil type and moisture influenced adult emergence (*F* = 5.52, d.f. = 2, 24, *P* = 0.0103; Figure 2.2). Adult emergence in dry fine-textured clay (60.0% ± 13.0) was less than dry coarse sand (96.0% ± 4.0).

Discussion

The results of my study contrast with previous observations made by Roach and Hopkins (1979) to show a significant relationship between soil type, moisture, and adult emergence of *H. zea*. In addition, my study expands on Murray and Zalucki (1990) to document a significant relationship between soil moisture and *H. zea* pupal depth, a species closely related to *H. armigera* and *H. punctigera*. However, the interaction between soil type and moisture did not impact pupal depth. Neither soil type, moisture, nor their interaction impacted pupal weight. Finally, I did not observe a significant effect of simulated rainfall on pupal mortality.

When *H. zea* emergence occurred, the fine-textured clay was more compact than the coarse sand or high organic muck due to the finer particle composition. The dry fine-textured clay led to reduced adult emergence in comparison to dry coarse sand, with many of the emerging moths also having noticeable wing deformation which prevented flight. I hypothesize that the rigid structure of the dry clay impacted the pupal chamber and prevented successful adult emergence. Based on the wing deformation I observed, successful emergence may be partially impaired by the soil damaging the wings after eclosion, but before the time when hemolymph is pumped into the wings for expansion. Future studies should consider examining pupae before emergence to confirm mortality does not occur before eclosion.

Several Heliothinae species vary their pupal depth for soil moisture (Barber 1941, Murray and Zalucki 1990), as also shown in this work. However, soil type, and the interaction between soil type and moisture, did not affect pupal depth. Soil moisture is likely a greater driver of pupal depth than soil type as this is a behavior the pre-pupae can mediate to attain optimal moisture for the pupal stage. Depth of the pupal chamber in response to moisture could have ramifications for the integrity of the chamber structure and the damage inflicted on adult wings during eclosion. Pre-pupae that burrow deeper in the soil will have a longer pupal chamber which will potentially increase the likelihood of structural failure and increases the distance adults need to travel to reach the surface. However, deeper pupae may also be less vulnerable to predation, and there may be a balance between the risk of predation and the risk of structural failure.

Soil type and moisture did not affect *H. zea* pupal weight, unlike *S. frugiperda*, which is sensitive to the relative moisture of the soil (He et al. 2021). *S. frugiperda* pupae have a lower mass than *H. zea* which could explain why *H. zea* pupae are not as heavily impacted by changes in soil moisture. Smaller pupae may be unable to regulate internal moisture as effectively as larger pupae. Hydrocarbons play a vital role in the regulation of water for insects and are an essential component of the cuticle and distinct species also vary in pupal hydrocarbon cuticle composition (Nelson 2001). Finally, my previous observations on pupal weight (Dillard, Chapter 1) revealed only minor differences in pupal weight across soil types. There was no correlation between pupal weight and depth, which was not unexpected given that other environmental factors can be a greater influence on depth (Chapter 1).

The effect of rainfall on pupation is important for pest management as irrigation can impact pupal mortality of *H. armigera* and has been suggested as a potential pest management tool for Heliothinae (Yu et al. 2008). Irrigation is commonly used in the rainfed southeastern U.S., but the quantity and timing required to cause significant mortality in *H. zea* are not well understood. Furthermore, moisture is required for pupal development and irrigation could contribute to survival. However, my observations revealed that, even under an extreme amount of rainfall (10cm), pupal survivorship was not affected. In North Carolina, rainfall can accumulate quickly, and it would not be unusual for 12-24cm of rainfall to occur over several days following a tropical weather system. One key difference between these two studies was the methodology employed. Yu et al. (2008) does indicate the soil type and how the soil was used in the study. If the soil did not have the chance to regain soil structure before experimentation, then the impact of saturated soil could be compounded.

Qualitative observations have revealed that *H. zea* pupae have multiple strategies for avoiding mortality during periods of extreme rainfall. For instance, Murray and Zalucki (1990) describe pupae floating near the surface of the chamber under total saturation, thus avoiding oxygen deprivation. Further research is necessary on the effects of moisture and simulated rainfall, the timing of application, rate, and interaction with other biotic variables (e.g., predation, pathogen exposure, etc.) to elucidate the primary cause of mortality due to moisture in pupal populations. For example, soil moisture and the timing of irrigation have been shown to enhance the spread of entomopathogenic fungi that parasitize *H. zea* pupae (Cabanillas and Raulston 1996). I hypothesize that the interaction between soil moisture and pathogen exposure is a key driver of mortality for *H. zea* pupae in the field.

Moisture affects *H. zea* pupation as confirmed in prior studies conducted across a broader range of Heliothinae species. The interaction between soil type and moisture can have a varying effect on pupation. Pupae are limited in their ability to adjust behavioral and physiological responses to detrimental conditions. Pre-pupal response to soil moisture mediates the depth of the pupal chamber and actions taken in this stage will determine the success of the individual. Moisture and soil type have not been observed in tandem to understand what is driving mortality in undisturbed pupal populations. Isolating factors, and establishing clear testable hypotheses, will allow for future studies to layer environmental factors and produce more accurate population models.

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TABLES

Table 2.1. Pearson's correlation coefficients for pupal depth and weight in the greenhouse for each soil type, by moisture.

Treatment	Moisture	Coefficient Estimate	P > |r|
Fine-texture clay	Dry	-0.0344	0.8542
Fine-texture clay	Wet	-0.0933	0.5827
High organic muck	Dry	0.1257	0.4396
High organic muck	Wet	0.01811	0.9230
Coarse sand	Dry	0.2249	0.2010
Coarse sand	Wet	-0.1618	0.3060

FIGURES

Figure 2.1. Mean ± SEM pupal depth by soil moisture (wet and dry) in fine sandy loam in a field trial in North Carolina.

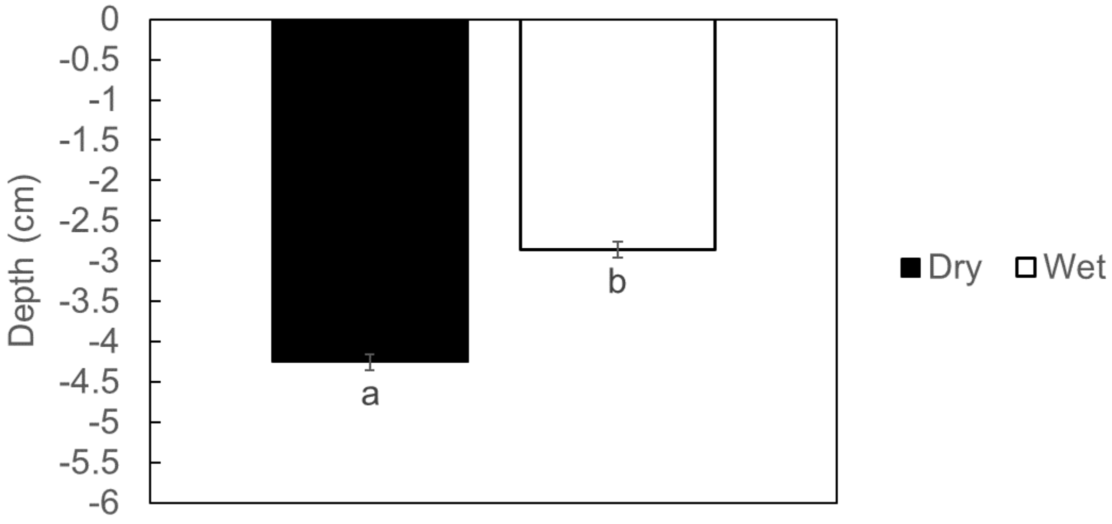

Figure 2.2. Mean ± SEM adult emergence for each soil type (coarse sand, high organic muck, and fine-textured clay) and moisture treatment (wet and dry) in greenhouse conditions.