ABSTRACT

SUITS, RACHEL MICHELLE. Establishing a Flowering Threshold and Feeding Behaviors between *Helicoverpa zea* and Soybeans. (Under the direction of Dr. Dominic Reisig and Dr. Hannah Burrack).

Helicoverpa zea (Boddie), corn earworm, can be a damaging insect pest of many crops, including soybeans. In North Carolina, the recommended economic threshold for soybeans in the pod filling stages is 2.4 *H. zea* larvae per row meter which is intended to prevent economic damage to seeds. The impact of flower feeding by *H. zea* larvae on seed yield is poorly understood and currently there is no economic threshold for flowering-stage soybeans. A split-plot experiment was established during 2011 and 2012 to assess the impact of *H. zea* feeding during the flowering stages of determinate soybeans on various yield components. Insect densities were manipulated with insecticides. During both years densities exceeded 2.4 *H. zea* larvae per row meter in some treatments, but yield did not differ among treatments. During 2012, the number of injured flowers by *H. zea* was counted. In one of two locations, there was a positive correlation between *H. zea* abundance and injured flowers, but flower feeding at these levels still did not significantly affect soybean yield. This suggests that the current threshold for podding-stage soybeans may be too conservative. If feeding occurs only on flowers, soybeans may have the ability to compensate for flower loss during pod and seed filling stages.

Helicoverpa zea larvae have been observed feeding on multiple soybean tissue types; however, little is known concerning which tissues are preferred and on which tissue type performance is best. The objective of these studies was to determine larval feeding preferences and to measure performance on different soybean tissue types. Feeding behavior

and resulting performance of second (early) and fourth (late) instar *H. zea* larvae on leaves, flowers and pods was assessed in no-choice and choice assays. We measured survival rates and calculated consumption indices and growth rates from changes in soybean tissue and larval body mass, respectively. Early-instar larvae performed better when fed leaf tissue than when fed other tissue types. In no-choice assays, 32% of early-instar larvae that fed exclusively on newly emerging trifoliates reached the pupal stage, and 50% exclusively fed on fully emerged leaf trifoliates survived to pupation. Early instar survival was poor (ranging from 0-3%) on all other tissue types, including flowers, stems, and pods. However, when given a choice of tissue types throughout their larval lifetime, early-instar larvae preferred to feed on newly emerging trifoliates and early developing pods, consuming an average of 51% and 38%, respectively, of each tissue type. Late-instar larvae performed best on pods with fully developed seeds, however, when presented with a choice throughout their lifetime, late instars did not feed at a higher rate on any one tissue type. If *H. zea* exhibits similar behavior under field conditions, information on preference can be used to inform management practices and may aid in the development of conventionally-bred and transgenic varieties.

Establishing a Flowering Threshold and Feeding Behaviors between *Helicoverpa zea* and Soybeans

by
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BIOGRAPHY

Rachel grew up in the central part of California, in the medium-sized town of Hanford, CA. She grew up on a small hobby farm with an abundance of vegetables and fruits that supplemented the household with fresh food. She attended college as an undergraduate at University of California, Berkeley where she continued to foster her love of biology and entomology. She majored in Integrative Biology with an emphasis on natural history and ecology courses.

While at UC Berkeley, Rachel had the opportunity to work as a curatorial assistant in the herpetology lab at the Museum of Vertebrate Zoology and in the Essig Museum of Entomology. It was during her last semester in college where she first discovered the exciting world of insects. Although she loved insects, it was a few years later that she could call herself a true entomologist. When she graduated from UC Berkeley, she traveled up to the beautiful Pacific Northwest in pursuit of living near the mountains and water. After attending graduate school for education, she taught at an inner-city school in Seattle, WA for four years. She enjoyed teaching but realized she was missing entomology and wanted to find less traditional ways to teach and spread knowledge.

From Seattle, she moved to the south central region of Oregon where she worked as a field technician in noxious weed removal for the USDA Forest Service and taught at the local school. This experience was in anticipation that she was going to attend school at North Carolina State University for her Master's in Entomology. Upon graduation she will continue to pursue her dreams of studying entomology and teaching her community in non-traditional settings.

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CHAPTER 1:

DOES FLOWER FEEDING BY *HELICOVERPA ZEA* (BODDIE) CAUSE YIELD LOSS IN SOYBEANS?

Helicoverpa zea (Boddie), commonly known as the bollworm, corn earworm, and tomato fruitworm, is a significant insect pest of many crops, including soybeans [*Glycine max* (L.) Merr.]. *Helicoverpa zea* has the potential to cause extensive damage to plant tissue and has been documented as a pest of southeastern U.S.-grown soybeans for at least forty years (Smith and Bass 1972). *Helicoverpa* species are global pests because they are polyphagous, highly mobile, relatively fecund, and have short generation times with multiple generations per year (Fitt 1989; Sharma 2005). Losses to soybean from *H. zea* have been increasing in the southern U.S. For example, in 2010, higher than normal *H. zea* population densities resulted in severe injury to soybean plants throughout the southern U.S. (Musser et al. 2011), and the crop value lost to this pest has increased from an average of $0.05 to $34.03 per hectare from 2004 to 2010 (Musser et al. 2011; Musser et al. 2012). In 2011, this resulted in over 16 million bushels lost to *H. zea* in the southeastern U.S. region (Musser et al. 2012).

Approximately 31 million hectares of soybeans were planted in the Midwest and Southeast regions in 2012, making it the second largest crop grown by area in the United States, behind corn (USDA NASS 2012). Soybeans are an important livestock feed grain because of their high protein content in the seed. Cultivars are grouped based on the time needed to reach maturity. Indeterminate maturity group cultivars continue vegetative growth even after flowering, but determinate maturity group cultivars cease vegetative growth once flowering begins (Ritchie et al. 1997). Within each maturity group, cultivars have been selected for environmental differences, insect tolerance and herbicide resistance. In North Carolina, determinate soybeans from maturity groups IV-VI are the predominant cultivar.

Soybean plants naturally abort reproductive tissue (flowers and pods), both under ideal and stressful environmental conditions, and the causes of abortion are not well understood (Egli 2005). More flowers are generally aborted and shed during times of stress (Dybing et al. 1986). However, soybean plants have the ability to compensate if reproductive tissue is injured. Compensation is usually defined in terms of production of reproductive tissue after initial loss. After abortion and loss of flowers, for example, soybeans can produce more pods or create more and/or larger seeds (McPherson and Moss 1989). Compensatory ability decreases as a soybean plant matures (Terry et al. 1987b). For example, when injury occurs during the R4-R5 (seed-filling) growth stage (Ritchie et al. 1997) of a determinate soybean, plant photosynthate can be transported into remaining pods and seeds, resulting in larger seed size, but the plant cannot increase the number of pods (McAlister and Krober 1958). Compensation in the numbers of pods and seed weight will determine yield.

Helicoverpa zea larvae feed and reproduce on both cultivated and wild hosts (Sharma 2005). For their first two generations in eastern North Carolina, *H. zea* feed on wild hosts and corn following overwintering, although corn is a preferred host. After corn silks dry, *H. zea* move to other crops, including soybeans (Hardwick 1965; Terry et al. 1987b; Terry et al. 1987c; Head et al. 2010). Although *H. zea* larvae feed on all soybean host plant tissue types, leaf feeding and pod feeding have been studied the most and are known to contribute to yield loss. Flower feeding by *H. zea* has also been observed (Eckel et al. 1992a) and it is possible flower feeding may directly impact the yield of soybean crops by reducing the number of potential pods and seeds, however the relationship between flower feeding and economic loss is poorly understood.

Feeding from *H. zea* on soybeans has been demonstrated to delay both flower and seed growth, which can result in an overall decreased number of pods per plant (Eckel et al. 1992a). However, this observation was made with densities ten times higher than the current podding-stage threshold, the only threshold for *H. zea* in soybeans, and such densities are not normally encountered in the field. Furthermore, feeding during the R1 and R2 (flowering) growth stages were confounded with the effects of feeding later in the season when soybean plants were in the R3 and R5 (pod and seed development, respectively) growth stages. When oviposition happens during the R2 growth stage, significant yield loss can happen from the resulting larvae feeding on later soybean growth stages (Eckel et al. 1992b; Tipping et al. 2005). Neonates and early instar larvae eclosed from eggs laid on R2 plants will feed on leaves and flowers, but cause relatively little injury due to their small size. However, as soybeans mature, later instars feeding on pod and seed plant tissues may cause significant injury (Funderburk et al. 1999). The impact of a mixed age *H. zea* population feeding on flowering stage soybeans has not yet been explored, and the impact of *H. zea* feeding during flowering stages under lower pest densities, such as those commonly found in the field, has not been researched.

Currently, an economic threshold exists for *H. zea* in pre-bloom vegetative stages and reproductive stage soybeans, which include pod-filling plants (Reisig and Roberson 2011; Roberts and McPherson 2012; Catchot 2013; Greene 2013; Herbert 2013; Stewart and McClure 2013). The current threshold for soybeans during reproductive growth stages is 2.4 *H. zea* larvae per row meter (one per row foot) (Reisig and Roberson 2011; Herbert et al. 2003). One study has shown that population densities reaching elevated levels of 27.6 *H. zea*

per row meter on determinate soybeans during R3-R6 growth stages can decrease yield reducing the number of seeds per plant and seeds per pod. However, lower population densities of 3.0 to 4.5 *H. zea* per row meter were not shown to affect yield (McPherson and Moss 1989). This lower density reflects the current threshold during pod and seed developing stages (Eckel et al. 1992a). Accurate thresholds allow farmers to manage insects responsively rather than preventively. However, the current *H. zea* reproductive threshold does not include a distinction between *H. zea* damage to flowers and pods. Because soybeans are capable of compensating for flower or pod loss, they may continue to put energy into remaining tissues during the remaining growing season (Turnipseed and Kogan 1976). Therefore, it is important to assess potential differences in the impacts of *H. zea* on these different phenological stages of soybean.

It is difficult to predict the occurrence of *H. zea* infestations on a farm and landscape scale during a growing season (Mueller and Engroff 1980). However, *H. zea* population dynamics can be predicted to some degree on a regional scale based on plant phenology, climactic conditions and seasonality (Herbert et al. 1988). During hot and dry conditions, *H. zea* development rates are faster, generation times are shorter, and pupae tend to stay in the soil longer (Slosser 1980). This also shortens the time corn is an attractive host because pollination time is reduced, resulting in an earlier corn harvest (Hu 2003). This is important because corn is thought to be a source for *H. zea* infestations in soybeans (Herbert et al. 1991). Hot dry conditions also slow soybean growth and increase potential time for damage by *H. zea* (Tipping et al. 2005).

An effective *H. zea* cultural management tactic is reducing row width, which allows canopies to close sooner, possibly reducing oviposition rates (Terry et al. 1987a). The tactic of chemical management is also common and insecticidal treatments have increased in the southeastern United States (Musser et al. 2011). While economic thresholds may reduce the number of insecticide applications, they can still be costly. Pyrethroids are commonly used for *H. zea* management and tolerance to this class has been observed in the field (Abd-Elghafar 1993). Furthermore, even though soybeans self-pollinate, pollinators can increase yields, in varying degrees, by increasing fruit set on the plant (Erickson et al. 1978; Delaplane and Mayer 2000). The reduction of pesticide use, specifically during flowering, may increase insect pollinator abundances, perhaps increasing yields.

This study sought to determine the impact of flower feeding by *H. zea* larvae on soybeans in the field during the R1-R2 growth stages and on yield components, in order to develop phenology based economic thresholds. I predicted that *H. zea* feeding at natural densities would not cause significant injury to soybeans during R1-R2 growth stages, and that the reproductive threshold is too conservative to be applied when when soybeans are flowering.

Methods and Materials

Field studies were conducted over two years at sites in eastern North Carolina, where the majority of soybeans are grown in the state (NCDA&CS 2011). Sites were located in Edgecombe County at the Upper Coastal Plain Research Station (35.895N, -77.674W) during 2011 and 2012, in Tyrrell County (35.824N, -76.205W) during 2011, and in Washington County (35.817N, -76.598W) during 2012. Both years, soybeans (var. AG6130, Monsanto,

St. Louis, MO) were planted at an average of 25.6 plants per row meter with a two-row White vacuum planter (White, Burgaw, NC) at a depth of 1.9 cm. Plots consisted of eight 10.5 meter rows spaced 91-96 cm apart, depending on location. Treatments were arranged in a randomized split plot design and were developed to manipulate *H. zea* populations with disruptive and selective insecticides (Table 1.1). Natural infestation rates were also measured. The main factor was planting date (14 and 16 April, 12 and 15 May, and 15 June, in 2011 and 2012 respectively) and was designed to increase the period in which flowering soybeans (R1 and R2) were exposed to *H. zea*. In 2012, four rows of sweet corn were planted on both sides of the experiment to provide a source of *H. zea* for the plots.

Plots were split with insecticide treatments designed to create a range of *H. zea* abundances, with untreated plots serving as controls. Plots in Edgecombe County were sprayed using a CO_2 pressurized backpack sprayer calibrated to deliver 94.6 liters per hectare applied at a speed of at a 4.8 kilometers per hour with 3.5 kilograms of pressure. The insecticide was applied using a single TX-10 spray nozzle (TeeJet®, Dillsburg, VA). Tyrrell and Washington County sites were sprayed with at TX-6 spray nozzle (TeeJet®, Dillsburg, VA) in 117.5 liters per hectare applied at a speed of 4.8 kilometers per hour with 3.8 kilograms of pressure. Once all plots reached the pod-forming growth stages (R3-R6), they were treated with chlorantraniliprole (Coragen®, DuPont™, Wilmington, DE, 0.102 kg (a.i.)/ha) once a week using a John Deere 6000 sprayer (4 row boom, 2 nozzles per row, John Deere, Moline, IL) to prevent injury outside of the flowering period. Insects other than *H. zea* that may have affected yield and confound results were monitored. Treatment for other insect pests was only necessary at the Tyrrell County site on 10 August 2012 when methyl

parathion (Cheminova Methyl 4EC, Cheminova, Inc., Research Triangle Park, NC, 1.13 kg (a.i.)/ha) was applied with the intent of eliminating pentatomids, while having little impact of lepidopterans.

Insect Monitoring

Insects were sampled weekly using two sampling methods consisting of 25 sweep samples (37.5 cm diameter net; in rows two and three) and two beat sheet samples (1 meter length; in rows six and seven) for both sampling rows per plot. The two outside two rows served as border rows. Beat sheet data were used for analyses because this sampling method is considered more absolute compared to the sweep net and is commonly used to monitor *H. zea* in the southeast (Studebaker et al. 1991). Sweep net samples were useful to monitor other potential pest insects such as key Pentatomidae and Plataspidae pests. Adult *H. zea* moth populations were monitored weekly with a metal pheromone trap (Lopez et al. 1994). Every seven days, both live and dead adult males were counted and the pheromone lure (Pherocon® cap, Trécé® Inc., Adair, OK) was refreshed. The time of peak moth flights were determined by monitoring relative catches over time. A peak was designated as the highest counts of both dead and live moths found in the traps relative to the previous and following sampling dates. To confirm correct species identification between *Heliothis virescens* (F.) and *H. zea*, mandibular structure on L4-L6 instars was visually inspected using a hand lens. In the analysis, the insect counts that coordinated with the two weeks chosen for flower counts were used. Although sampling was performed across the season, *H. Zea* abundance for these two

weeks was used in figures to show the relationship of insect abundance in relationship to the current podding-stage threshold of 2.4 *H. zea* per row meter.

Plant Phenology

The total number of flowers and total number of injured flowers (in 2012) were counted on ten plants in the middle two rows per plot once per week. Each flower was counted using the Peterson et al. (1992) flower description. Flowers were counted from the time the bud was visible and fully extended until the time when the banner petal was beginning to collapse. Injured flowers were only counted during the summer of 2012. Any flower that exhibited feeding injury (e.g., frass, shot-hole, etc.) on any part of the flower during the flowering stages was considered injured. Flower injury was assumed to be a direct result from *H. zea* larval feeding, as this was the main chewing arthropod present in all experiments. The location of the first flower on the top-most raceme of the plant was recorded to measure plant development during R1-R2 growth stages.

Cumulative flowers and injured flower numbers were used in the analysis. The counts were taken during a two week period after peak moth flights when larval abundances were the highest (Fig. 1.1). Cumulative flower counts were determined by summing the total number of flowers per plant during two consecutive weeks (the week of the peak flight and the week following). This accounted for moths that may have been present and laying eggs during the week of a peak moth flight because flights generally occurred over a two-week period. Two week cumulative counts were also used to avoid arbitrarily weighting the analysis toward one week that may have had minimal insect density and resulting flower

injury, which takes time to develop. Hence, cumulative counts over two weeks allowed analysis during a wider duration when adults were laying eggs, larvae were feeding and plants were flowering. By design, larval peaks did not necessarily coordinate with peak flower time for all planting dates, so cumulative flower counts for plants in R1-R2 growth stages were calculated when larval abundance was highest (Table 1.2).

Yield Components

Stand counts were taken after all plants had emerged and consisted of the total plants from the two middle rows of each plot. Two sets of harvests were performed for mechanical and hand harvest yield. Mechanical yield was determined using a Gleaner K2 combine (Allis-Chalmber, Milwaukee, WI) equipped with a Harvestmaster™ weigh system (Juniper Systems, Inc., Logan, UT). Combine harvest dates in 2011 were 1 November and 9 November at Tyrrell County and Edgecombe County respectively. In 2012, harvest dates were 12 November and 1 November at the Washington County and Edgecombe County respectively. At the time of harvest, a Dickey-john GAC2100b (Dickey-john® Corporation, Minneapolis, MN) moisture meter was used to measure the moisture of each plot. After harvest, yields were adjusted to kg/hectare at standardized 13% seed moisture.

The hand harvest consisted of 20 plants per plot cut near the soil surface and bagged from the field prior to mechanical harvest. Hand harvest plants were cut when they were fully mature (R8) growth stage and averaged ca. 13% seed moisture. Hand harvest dates were on 5 November and 1 November in Tyrrell and Edgecombe Counties in 2011 and 6 November and 2 November in Washington and Edgecombe Counties in 2012, respectively. Plants were

stored in a refrigerator and later assessed in the lab to determine the number of pods per plant, number of seeds per pod and average seed weight per plot. Pods were stripped from the stems and stem circumference was measured. Once the pods were stripped from each plant, the seeds were counted and removed from each pod. When all seeds were separated from their pods, the average weight of 100 random seeds was recorded.

Data Analysis

Insect abundances, cumulative flowers, injured flower numbers, cumulative counts, and all yield components were each analyzed using a separate general linear mixed analysis of variance model (PROC MIXED, SAS Institute 2009). Cumulative counts were determined by summing two observations taken over two weeks and each site/year. In each analysis, the planting date, treatment and their interaction were fixed variables while the replication was a random variable. Although planting date was included in the analysis, it was not reported for single factor significance in the results because the objective was to focus on the impact of *H. zea* on flowering soybeans. However, interactions of insect densities with planting date were reported. In order to satisfy the assumptions of the model, insect abundances were transformed using a square root transformation. If tests were significant, Tukey's honestly significant differences tests were used to determine differences among groups.

Results

Insect Abundances

During both the 2011 and 2012 field seasons, *H. zea* population densities during the R1-R2 growth stages were at or above the current podding-stage economic threshold of 2.4 *H. zea* per row meter in Tyrrell ($F = 4.95$; df = 3, 33; $P = 0.006$) and Washington County ($F = 30.46$; df = 3, 12.6; $P < 0.0001$) (Figs. 1.2 and 1.3, respectively). There were significant interactions between planting date and insecticide treatment in both Tyrrell County ($F = 15.24$; df = 3, 33; $P < 0.0001$) and Washington County ($F = 6.52$; df = 6, 11.4; $P = 0.0035$) and the general trend was that insect abundances were higher in untreated and acephate-treated plots compared with chlorantraniliprole plots. During the two weeks analyzed, peak insect larval population densities reached 3.6 to 5.8 *H. zea* per row meter in some treatments in both Tyrrell County and Washington County.

Insect abundances in Edgecombe County in 2011 were not above the current podding-stage threshold and larval densities were not different among treatments ($F = 3.19$; df = 3, 12; $P = 0.0626$). In 2012, larval densities were different among treatments in Edgecombe County ($F = 12.23$; df = 3, 9; $P = 0.0016$), but were not above the current podding-stage threshold. There was a significant interaction between planting date and insecticide treatment in this analysis ($F = 8.58$; df = 6, 9; $P = 0.0002$), and the general trend was that insect abundances were higher in acephate-treated and untreated plots compared with chlorantraniliprole plots in 2012.

Flower Numbers

There were no significant differences in cumulative numbers of flowers during 2011 peak flowering periods in either Edgecombe County ($F = 0.40$; df = 3, 9; $P = 0.7556$) or Tyrrell County ($F = 1.83$; df = 3, 10.2; $P = 0.2042$). Because injured flowers were not counted during the summer of 2011, the relationship between *H. zea* and flower injury is unknown. During 2012, in Edgecombe County, cumulative flowers ($F = 15.29$; df = 3, 27; $P < 0.0001$) were higher in chlorantraniliprole-treated plots compared with both the untreated control and acephate-treated plots. There were no differences in cumulative injured flower counts ($F = 1.71$; df = 3, 27; $P = 0.1881$) or cumulative flowers ($F = 1.45$; df = 3, 27; $P = 0.2503$) in Washington County in 2012. Also in Washington County, flower injury was not correlated with varying larval *H. zea* densities even in plots where larval population densities were at or above the current podding-stage economic threshold. However, injured flowers at the Edgecombe County site were higher in untreated plots compared to chlorantraniliprole-treated plots (Fig. 1.4; $F = 6.04$; df = 3, 6.39; $P = 0.0274$).

Yield Components

The total average stand count was 26.7 plants per row meter and the number of plants in each plot was not significantly different among insecticide treatments ($F = 0.31$; df = 3, 188; $P = 0.816$). The stands for each year represented typical planting densities for soybeans grown in the southeast.

There were no significant differences in yield for the combine harvest. In 2011, Edgecombe County ($F = 1.06$; df = 3, 33; $P = 0.38$) and Tyrrell County ($F = 0.30$; df = 3, 33;

P = 0.83) averaged 2.28 and 2.29 tons per hectare, respectively. In 2012, yields were higher than 2011, averaging 3.95 and 3.02 tons per hectare, respectively, but were not different among treatments in Edgecombe County (*F* = 1.71; df = 3, 33; *P* = 0.21) or Washington County (*F* = 0.12; df = 3, 33; *P* = 0.95).

In 2011, there were no differences in pods per plant (*F* = 0.61; df = 3, 16.8; *P* = 0.6153), average seeds per pod (*F* = 1.14; df = 3, 16.8; *P* = 0.3629) and average seed weight (*F* = 1.49; df = 3, 18.2; *P* = 0.2518) in Edgecombe County. The same pattern was seen in Tyrrell County for pods per plant (*F* = 0.66; df = 3, 33; *P* = 0.5820), average seeds per pod (*F* = 1.23; df = 3, 33; *P* = 0.3161) and average seed weight (*F* = 1.59; df = 3, 33; *P* = 0.2099). Similarly, in 2012, there were significant differences in pods per plant (*F* = 1.92; df = 3, 16; *P* = 0.1672), average seeds per pod (*F* = 2.38; df = 3, 16.7; *P* = 0.1105), and average seed weight (*F* = 0.88; df = 3, 18.5; *P* = 0.4702) in Edgecombe County. Washington County hand harvest also did not show any significant differences in pods per plant (*F* = 2.52; df = 3, 12.1; *P* = 0.1074), average seeds per pod (*F* = 2.47; df = 3, 12.9; *P* = 0.1090), or average seed weight (*F* = 0.28; df = 3, 12.3; *P* = 0.8362).

Discussion

Helicoverpa zea densities were at or above current podding-stage economic threshold levels (2.4 larvae per row meter) in at least one experiment during both 2011 and 2012 growing seasons. However, there were no differences in flower numbers (on a whole-plant basis), yield or yield components (number of pods per plant, average number of seeds per pod and seed weight). In a single location during 2012, insect density, created by differential

and selective insecticide sprays, was positively correlated to flower injury and cumulative flowers. However, this location had relatively low *H. zea* abundance and there were no differences in yield or the components contributing to yield. This suggests that larval densities are positively correlated to flower injury, but are not related to yield loss at the densities encountered in these experiments. Thus, the current economic threshold for podding-stage soybeans is too conservative for determinate soybeans during R1-R2 growth stages.

Because flowers contribute to yield by forming the pods and seeds, flowers should eventually result in yield loss in determinate soybeans. However, yield loss was not measured in these experiments. It is possible that injury to soybean tissue in the early reproductive growth stages (R1 and R2) allows enough time for soybean plants to put energy into remaining reproductive structures (McPherson and Moss 1989). In this study feeding was confined to the flowering stages, and it was assumed that injury during R3-R7 growth stages was negligible, since weekly insecticide applications of lepidopteran-specific material were made. If this hypothetical explanation is true, the abundance of *H. zea* larvae was too low in these experiments to cause an observable yield loss.

During the summer of 2012, *H. zea* abundance in Edgecombe County did not exceed the podding-stage economic threshold, but there were significant differences in larval abundances, cumulative flowers, and injured flower counts in the plots. *Helicoverpa zea* abundances and injured flower numbers were higher in the untreated and acephate-treated plots compared to chlorantraniliprole plots; furthermore, there were higher cumulative flowers in chlorantraniliprole plots than untreated plots. This suggests that flower feeding

resulted from larval *H. zea* population densities. Ultimately, plots with increased *H. zea* abundances and more injured flowers had lower cumulative flower counts. However, populations were not large enough to cause significant yield loss; it is unknown if injured flowers affect the soybean plant. Because there were no differences in the yield components, following the compensation hypothesis above, it is possible injured flowers were aborted and energy was put into the remaining uninjured reproductive parts (number of seeds per pods and individual seed weight). If *H. zea* populations were higher at the Edgecombe County location, it may have shown a clearer trend of how injury to floral tissue impacts pods and seeds in later reproductive stages.

In contrast, during 2012, *H. zea* densities varied across treatments at the Washington County location, but there were no differences in injured flower numbers. This may have occurred because of environmental conditions at the site location. The Washington County location was in a no-till field that was not uniform. This increased variance among plots, ultimately affecting the yield of this site. Additionally, this may have impacted the attractiveness for adult moths to lay eggs and larval feeding because the plants were less vigorous than alternative portions of the soybean field surrounding the experiment.

Across experiments, *H.* zea abundances ranged from zero to 5.8 per row meter, above the 2.4 per row meter podding-stage threshold. These insect numbers occurred during the R1-R2 growth stages and did not result in yield differences or components contributing to yield. There were differences in flower injury with more injury occurring in untreated plots compared to "protected" plots (those treated with chlorantraniliprole). However, *H. zea* densities during the flowering stages and its impacts on soybean yields remain unanswered.

Clearer trends on the impacts of feeding during the flowering stages may emerge with higher densities of *H. zea* larvae, especially if adult moth flight and oviposition periods overlap when soybeans are flowering. Further research needs to be done investigating larval flower injury at higher *H. zea* densities in order to determine if flower feeding during the R1-R2 growth stages causes significant yield loss to determinate soybeans.

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Tables

Table 1.1: Insecticide treatments used to manipulate *H. zea* densities.

Desired effect on *H. zea* abundance	Insecticide	Rate	Timing of treatment
Eliminate	Chlorantraniliprole (Coragen®, DuPont™, Wilmington, DE)	0.102 kg (a.i.)/ha	Sprayed on plots every seven days from the time plants entered R growth stages until they had reached maturity or the R7 growth stage
Reduce	Esfenvalerate (Asana XL®, DuPont™)	7.5 kg (a.i.)/ha	Sprayed in coordination with peak insect flight before flowering stages
Increase	Acephate (Orthene® 97, AMVAC, Los Angeles, CA)	1.16 kg (a.i.)/ha	Sprayed before peak *H. zea* flights and flowering stages
Natural	None	NA	NA

Table 1.2: Two week flowering (R1-R2) period that coordinated with highest larval abundances for each planting date at all sites during years 2011-2012.

Planting Date	Year	Site	Peak Two-Week Flowering Period
April	2011	Edgecombe County	6 and 13 July
May			28 July and 4 August
June			18 and 23 August
April	2011	Tyrrell County	1 and 8 August
May			8 and 16 August
June			16 and 22 August
April	2012	Edgecombe County	1 and 8 August
May			1 and 8 August
June			8 and 15 August
April	2012	Washington County	31 July and 6 August
May			6 and 13 August
June			13 and 20 August

Figures

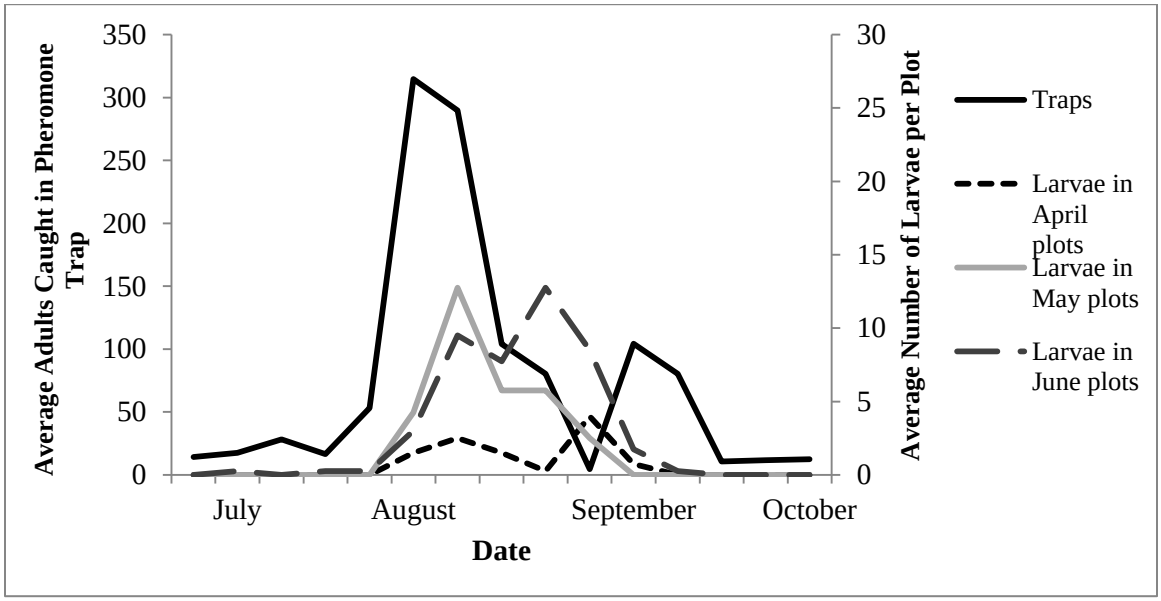

Figure 1.1: The average number of adults caught in pheromone traps for all planting dates with the average number of larvae found per plot for each planting date across all experiments.

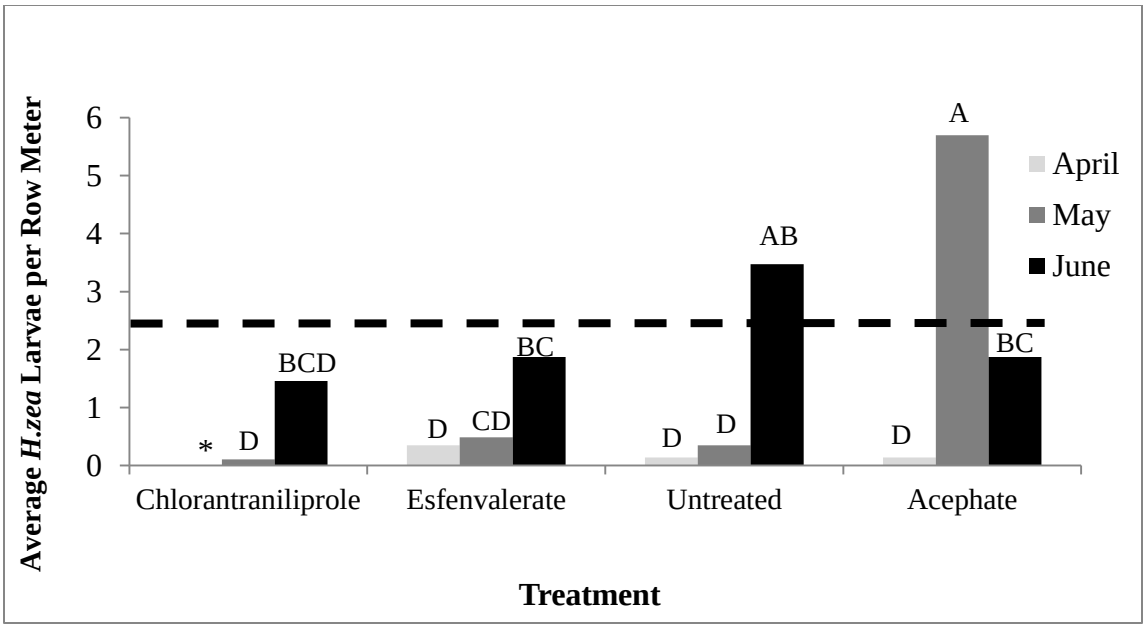

Figure 1.2: The average number of *H. zea* larvae sampled during the two peak flowering weeks in Tyrrell County during the summer of 2011 for each planting date (April, May, June). The dotted line represents the current economic threshold of 2.4 *H. zea* larvae per row meter when soybean plants are in their podding stages. The * indicates that the data were omitted from the analysis because no larvae were detected. Treatments sharing the same letter grouping are significantly the same by Tukey's means separation procedure.

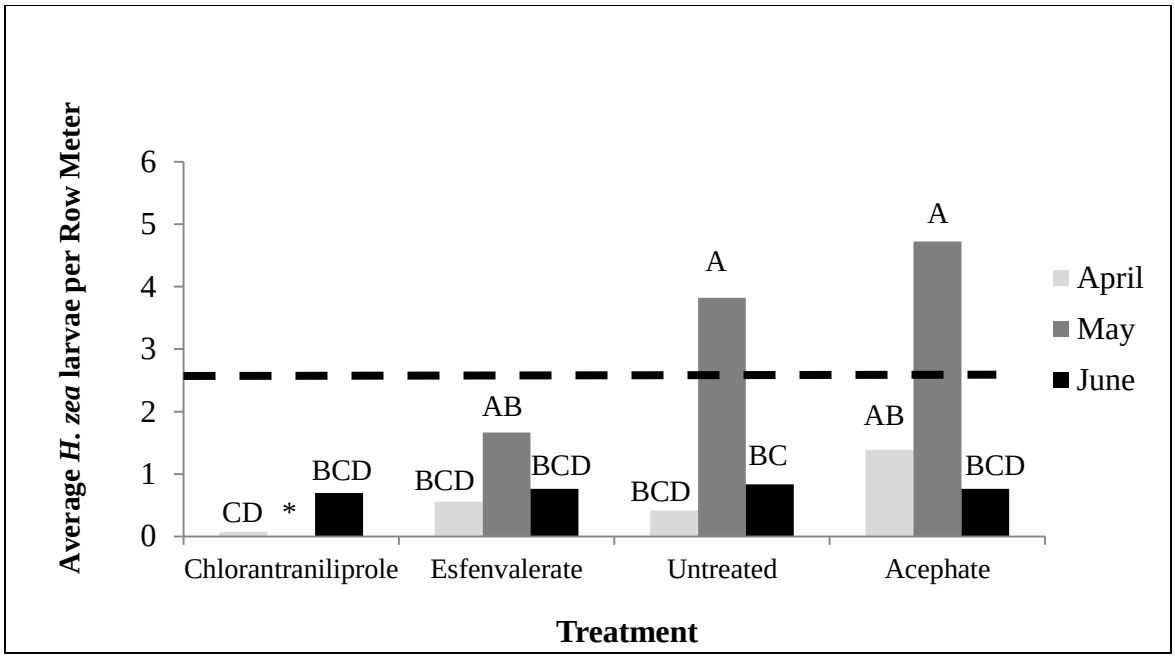

Figure 1.3: The average number of *H. zea* larvae sampled during the two peak flowering weeks in Washington County during the summer of 2012 for each planting date (April, May, June). The dotted line represents the current economic threshold of 2.4 *H. zea* larvae per row meter when soybean plants are in their podding stages. The * indicates that the data were omitted from the analysis because no larvae were detected. Treatments sharing the same letter grouping are significantly the same by Tukey's means separation procedure.

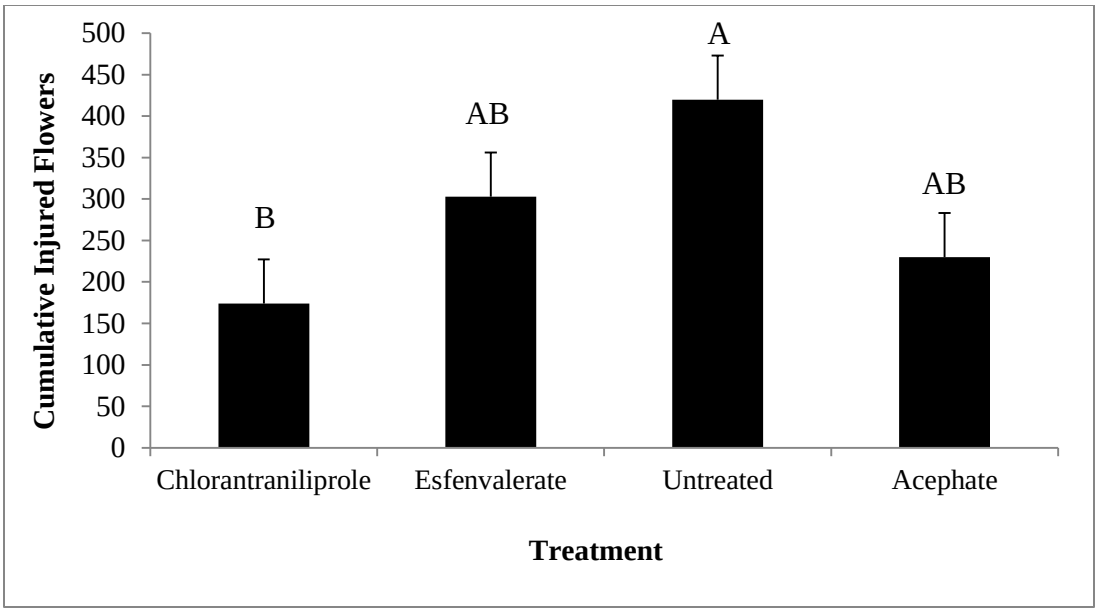

Figure 1.4: Cumulative number of injured flowers in Edgecombe County during 2012.

CHAPTER 2:

FEEDING BEHAVIOR AND PERFORMANCE OF *HELICOVERPA* ZEA (BODDIE) LARVAE ON SOYBEAN TISSUE TYPES

Helicoverpa zea (Boddie), commonly referred to as the bollworm, corn earworm and tomato fruitworm, has been recorded as a pest on soybeans [*Glycine max* (L.) Merr.] since the early 1900s (Hardwick 1965) and can cause widespread damage to this crop, especially late in the growing season. *Helicoverpa zea* may feed on wild and cultivated leaves, seeds, and fruits and are often difficult to control if they feed internally on reproductive structures (Nuenzig 1963; Hardwick 1965; Sharma 2005). Corn is the preferred *H. zea* host in eastern North Carolina (Hardwick 1965; Martin et al. 1965; Sharma 2005), but the first two annual generations may also feed on wild hosts. After corn matures, *H. zea* disperse to other crops including soybeans (Hardwick 1965; Terry et al. 1987a; Terry et al. 1987b; Head et al. 2010). Infestations from *H. zea* can affect yields of soybean crops (Eckel et al. 1992a), but the extent of this impact varies with infestation timing and density. Soybean development is divided into vegetative (pre-bloom) and reproductive growth stages. Reproductive growth stages are further divided into flowering (R1-R2), pod development (R3-R4), seed development (R5-R6) and seed maturation (R7-R8) growth stages (Ritchie et al. 1997). In the upper southeast, later-planted full season and double-cropped soybeans (planted in late May and June after wheat harvest) typically enter reproductive growth stages when corn matures. If moth oviposition coincides with soybean plant flowering, then larval development will correspond with the pod and seed-producing reproductive growth stages. Hence, *H. zea* feeding may cause significant damage to a crop (Hillhouse and Pitre 1976; McPherson and Moss 1989).

In the upper southeast, the most serious infestations of *H. zea* in soybeans happen in late July and early August, typically after corn silks dry, rendering the plant a less preferred host.

Helicoverpa zea infestations concurrent with soybean flowering can result in flower and pod feeding and dense infestations can cause severe crop loss (Eckel et al. 1992b), but flowering soybeans support more late-instar *H. zea* than podding soybeans (Terry et al. 1989). Soybean plants are able to compensate for loss by putting energy into remaining reproductive structures when flowers are damaged. However, it is harder for soybean plants to compensate when they are in later maturity stages, especially if injury occurs late in the season or plants are compromised by other stress factors. It is possible that higher densities of late-instar larvae on flowering soybean plants allow compensation to occur once that cohort pupates in the soil. A better understanding of the feeding preferences by *H. zea* may further clarify the relationship of feeding to soybean yield.

The preference-performance hypothesis (Thompson 1988) states that female insects are expected to oviposit where feeding allows higher survival rates and better performance, but evidence in support of this mechanism of adult host selection for *H. zea* is mixed. Female *H. zea* prefer to oviposit on flowering plants (Hardwick 1965; Johnson et al. 1975; Eckel et al. 1992a) and, in soybeans, lay more eggs on flowering (R2) plants compared to other growth stages (Hillhouse and Pitre 1976). Furthermore, *H. zea* prefer to oviposit on developing trifoliates in the top two-thirds of soybean plants (Terry et al. 1987b). This may suggest that oviposition on later-planted soybeans coordinates with larval feeding preferences, assuming that the optimal foraging hypothesis (Macarthur and Pianka 1966) is true. In this case, *H. zea* minimizes foraging costs when females oviposit in areas of preferred feeding. The resulting larvae can minimize energy expended by foraging and maximize fecundity. However, in cotton (*Gossypium hirsutum* L.), *H. zea* mainly oviposit on leaves or terminals and larvae are

found mainly on flowers and small bolls (Farrar and Bradley 1985; Gore et al. 2002; Torres and Ruberson 2006) meaning that female moths are not necessarily selecting the best location for their offspring, contradicting the preference-performance hypothesis.

Helicoverpa zea larvae have been observed feeding on soybean leaves, stems, flowers and pods (Eckel et al. 1992a) and neonates and young larvae may feed on soybean trichomes and blooms if present (Mueller and Engroff 1980). They survive on soybean plants during all growth stages, but establishment success decreases as plants mature to pod filling growth stages (Terry et al. 1989). In the field, *H. zea* larvae have been found in high numbers during the R1-R2 growth stages and can reduce flower number, especially if they are early instars (Eckel et al. 1992a). Small larvae injure the calyx and ovaries on the flowers, while large larvae typically injure the entire flower (Terry et al. 1987a; Terry et al. 1989). Injured flowers may abort or remain, and result in reduced number of seeds per pods, both of which can reduce yield (Eckel et al. 1992b; Bi et al. 1994). Once larvae reach later instars, they feed on pods and seeds (Terry et al. 1987a; Herbert 2003). Larvae perform best, as measured by pupal weight, when feeding on pod-filling plants, which suggests post-bloom plants may be a more suitable host for later instars (Terry et al. 1987a; Terry et al. 1987b).

Because flower feeding is observed in the field, while other tissues such as stems and leaves are present, but generally not fed upon, I hypothesized that flower tissue is preferred. However, because plant phenology and larval development change throughout the season, I also hypothesized that one host tissue type might not support the nutritional needs of a larva, resulting in a larva feeding on multiple tissue types throughout its development. The objectives of this experiment were to determine the performance of larvae when feeding on

different soybean tissues and the feeding preferences when given a choice of soybean tissues. I further expected that larvae would prefer to feed on different tissues as early and later instars because of host tissue availability and nutritional qualities of plant tissue types. Ultimately, if plant phenology and larval instars are timed to the availability of a preferred plant tissue type, feeding, especially during reproductive growth stages, could lead to significant yield reduction.

Materials and Methods

I compared the performance of second (early) and fourth (late) instar *H. zea* when fed a single soybean tissue type, and the preference of these same larval instars when given a choice of a range of soybean tissue types. A *H. zea* colony was initiated with insects obtained from a laboratory colony maintained for 10 years by the Corn Insect Host Plant Resistance Laboratory at USDA at Mississippi State University. The colony was reared using parameters adapted from Waldbauer et al. (1984) and held at 27 °C at a 16:8 light:dark cycle. Larvae used in experiments were chosen from the colony at random and returned upon reaching pupation.

Soybean tissues were collected twice each week and stored in a sealed container with a moist paper towel at 10° C until use or within five days of collection. Flowers senesced quickly after being removed from the plant and were collected more frequently and closer to the date they were used in the bioassays. Bioassay arenas were lined with Apex *Drosophila* agar (100 mesh; Genesee Scientific, San Diego, CA) at a 3% concentration. Soybean plant tissues used in the experiments included expanding and fully expanded trifoliates from the

top two-thirds of the plant, flowers, maturing pods (a mixture of R3-R4), developed pods with developing seeds (R5), pods with developed seeds (R6), stems, and petioles from the top two-thirds of the plant. The availability of each tissue type varied throughout the growing season; therefore replications were conducted based on host tissue type availability. A combination of greenhouse and field-grown soybean plants were used as sources of tissues. Source soybean plants were not treated with insecticides during the duration of their development and all soybean plants were variety AG6130 (Monsanto Company, St. Louis, MO).

Control tissue weight loss experiments without insects were conducted in order to determine if tissues lost or gained mass naturally once removed from the plant. Controlled experiments showed natural differences in weight loss for each tissue type. However, average tissue weight change during two days was not included in the analysis. Instead relative consumption rates measuring body weight over time were assessed. Because this study looked at survival, pupal weights, and observed feeding, it was not necessary to take natural tissue weight loss into consideration.

No-Choice Assays

Tissues used in the no-choice assays included fully expanded and expanding trifoliates, flowers (R1-R2), stems, petioles, maturing pods (R3-R4), developed pods with developing seeds (R5) and developed pods with developed seeds (R6). No-choice assays of each single tissue type were conducted either in petri dish (100 x 15 mm, Fisherbrand Polystyrene, Fisher Scientific, Hampton, NH) or plastic container (3 inch height, 4.5 inch

diameter, Berry Plastics, Evansville, IN) arenas with a single tissue type and a single insect larva (Fig. 2.1). Disposable petri dishes were used for the first 10 replications per tissue, but plastic containers were used for the remaining replications to reduce waste. Plastic containers were cleaned with dish soap and hot water and dried fully before each use. Plant parts and arenas were changed every two days. Insects were carefully moved from one arena to another using wide, round tip, featherweight forceps (BioQuip, Rancho Dominquez, CA) to minimize impact to the insect. Plant parts, frass, and insects were weighed at each replacement. Insect measurements continued from the time the insect was placed in the assay until pupation or until the insect died.

Choice Assays

Tissues included in each choice assay were emerging trifoliates, fully emerged trifoliates, flowers (R1-R2), developing pods (R3 to R4) and seed-filling pods (R5). Insects fed exclusively on stems and petioles in no-choice assays did not survive, and therefore, these tissues were excluded from choice assays. Each assay was set up in a large (150 x 15 mm, Fisherbrand Polystyrene) petri dish arena with a 3% agar (Apex *Drosophila* agar, 100 mesh) solution on the bottom of the petri dish and a mixture of host soybean tissue types (Fig. 2.2). Tissues were changed and weighed every two days, and consumption was calculated as the difference in mass between start and end of exposure. Tissue was assumed to have been fed on if it had visible feeding injury and frass around tissue. Feeding observations until pupation or death were included in the analysis.

Data Analysis

Consumption and growth indices were calculated for each assay by determining the amount of tissue consumed and the average weight over the entire life of each insect (Waldbauer 1968; Farrar et al. 1989). The amount of food consumed was calculated as the difference between the original weight of the tissue and the weight after each two day period. Insect development was recorded through different larval instars and the larval instars were considered complete when the individual reached pupation. The proportion of *H. zea* reaching pupation was calculated for each soybean host tissue type. Tissue types with insufficient numbers of individuals to pupation were omitted in analysis.

Consumption indices, growth rates, pupal weights and days to pupation were each analyzed using a separate general linear mixed analysis of variance model (ANOVA, PROC MIXED, SAS Institute 2009). Pupal weights were square root transformed to satisfy the assumptions of ANOVA, but the raw means are presented. Survival to pupation and observed feeding in choice assays were binomial variables (insects either survived or fed or they did not) and were each analyzed using a generalized linear mixed ANOVA model for binomial data (PROC GLIMMIX, SAS Institute 2009). For each analysis, insect, plant tissue source (field vs. greenhouse grown) and date were modeled as random variables and tissue type was modeled as a fixed effect. If tests were significant, Tukey's honestly significant differences tests were used to determine differences among groups.

Results

No-Choice Assays

Early-instars only survived on leaves and floral tissue. Survival rates were higher when early-instars fed on newly emerging trifoliates and fully emerged trifoliates compared to flowers (Fig. 2.3; $F = 7.05$; df = 2, 71; $P = 0.0016$). There were no significant differences between the total days required for early instars to pupate ($F = 1.09$; df = 2, 10; $P = 0.3732$), arithmetic body weight means ($F = 3.25$; df = 7, 3.91; $P = 0.1393$), consumption indices ($F = 0.28$; df = 3, 12.3; $P = 0.8362$), or relative growth rates ($F = 3.93$; df = 7, 1; $P = 0.3707$) among any of these tissues.

Late-instars survived better (Fig. 2.4; $F = 11.27$; df = 4, 19.1; $P < 0.0001$) and had higher pupal mass (Fig. 2.5; $F = 19.97$; df = 3, 47.5; $P < 0.0001$) when fed only fully developed pods with developed seeds. Late-instar no-choice assays for all tissue types showed no significant differences in arithmetic body weight means ($F = 1.7$; df = 5, 3.95; $P = 0.3145$), consumption indices ($F = 2.25$; df = 5, 9.34; $P = 0.1342$), or relative growth rates ($F = 1.82$; df = 5, 6.35; $P = 0.2375$). Control tissue weight loss experiments showed that leaves and flowers lost mass at the same rate and pod tissues lost mass at a different rate ($F = 63$; df = 4, 28.7; $P < 0.0001$).

Choice Assays

If given a choice of host soybean tissue types, early-instars fed more on newly emerging trifoliates and developing pods (Fig. 2.6; $F = 7.94$; df = 4, 83.6; $P < 0.0001$). Although early instars did not survive when fed only on developing pods (R4), developing

pods were preferred when they were given a choice of tissues. None of the early-instars survived until pupation during choice assays. Twenty-five percent of the individuals survived until the second instar, 55% survived until third instar and 20% until fourth instar. Fifteen percent of the individuals appeared killed by fungus, likely introduced from field collected tissues. Late-instars showed no significant difference in feeding activity ($F = 0.10$; df = 4, 60; $P = 0.9831$) when given a choice of tissues. Late-instar survival rate was much higher than early-instars, with 90% survival to pupation. Feeding preference patterns changed with stadia. Second instars preferred to feed on newly emerging trifoliates, third instars fed most on developing pods (R4), and fourth instars fed most on seed-filling pods (R5) (Fig. 2.7; $F = 42.69$; df = 8, 540; $P < 0.0001$).

Discussion

Early-instar survivorship on emerging and fully emerged trifoliates suggests that insects of this age survive on leaf tissue better than any other tissue. It is possible that leaf tissue provides all the nutrition needed for larval development. Late-instars survived best on fully developed pods with developing seeds, based on no-choice assay results. This suggests that feeding behaviors may change as larvae mature. However, although early instars did not perform well on R4 tissue, they did feed on these tissues when given a choice over their larval lifespan. This could suggest that soybean tissues, such as developing pods, are a good food source, but one that cannot be exploited by young larvae. Larvae may need a variety of tissue sources in order to obtain enough nutrients for survival to pupation.

Increased survival of early-instars on leaf tissues implies that larvae are able to infest soybean plants both pre-bloom and post-bloom, as they can survive solely on leaf tissue for their entire larval lifespan. Early-instar survival on leaf tissue could be one explanation why later-planted soybeans foster higher *H. zea* densities. Because soybean leaves are younger during larval infestations in later planted crops, larvae are able to feed on, presumably, higher-quality leaf tissue later in the season. While foliar feeding does not typically result in yield loss (Turnipseed and Kogan 1976), larval populations may develop on leaves until other tissue types are available. The increase of *H. zea* densities later in the season could also be a result of the population contribution from each successive generation. However, the contribution of soybeans to *H. zea* populations is unknown. Regardless, if plant and *H. zea* phenology are in sync (from the perspective of the insect) and if feeding preferences in laboratory assays mirror similar behaviors in the field, then early instars will feed on leaf tissue in the field. This could lead to populations composed of either later instars, or infestations from consecutive generations, that feed on soybean reproductive tissue.

In these experiments, *H. zea* fed on floral tissue at the same rate as other tissue types, but did not survive as well on flowers compared to leaf tissue. Early instars, in particular, had a high mortality rate when fed exclusively on flowers. This could suggest that floral tissue lacks nutrients necessary to complete larval development (McCall and Murphy 2013; Damle et al. 2005). These assays are not a perfect measurement of survival. It is possible that excised flowers in the assays were not an appropriate food source for early instars. For example, flowers were presented to the larvae soon after collection, but they quickly senesced and browned (Smith et al. 1992). Similar to flowers, early-instars did not survive

when given only R4 soybean tissue type, but did feed on it in choice assays. Therefore, it is possible that R4 tissue can be used by larvae at some stage, but does not contain all the nutrients necessary for complete larval development.

Early instars fed more on leaf tissue while later instars fed more on pods and seeds. This suggests that adult moth flights overlapping with pre-bloom and flowering growth stages could provide preferred host tissues for their later-instars progeny to feed on pod and seed tissues. Assuming that *H. zea* is a major limiting factor in production, this also justifies promoting early planting dates, allowing soybean crops to develop before *H. zea* emigrates.

Late-instars performed best on developed pods with developing seeds (R6), as shown by survival rates and pupal weights in no-choice assays. Because pupal weights correlate with fecundity (Honěk 1993), feeding on pod and seed tissues during later stadium may produce more fecund adults. This also implies that late-instars have the greatest potential to damage soybean crops if infestation rates are high. Furthermore, if larvae feeding on soybeans at R6 produce more fecund adults and if soybeans are an important source of *H. zea*, this may increase infestation rates for nearby later planted crops that may be in earlier growth stages. However, late-instars did not choose to feed on a specific tissue type. This could suggest they are able to feed on a wider range of tissue types than early-instars, and that they perform better when they are able to feed on soybeans in the later reproductive stages. With this information, plant phenology in the field can be manipulated by changing the planting date and/or cultivar to avoid periods of increased adult moth flight activity resulting in high larval densities.

These findings have direct implications for traditional breeding as well as the development of transgenic varieties with insect resistance. *Helicoverpa zea* larvae have different survival rates on different tissue types and preferentially feed on various tissue types during different larval developmental stages. Expression of insecticidal toxins or plant morphological characteristics (i.e., leaf shape or trichome density) could potentially be spatially modulated based on feeding preferences. Both transgenic cotton and corn differentially express of toxins in between different tissue types (Nguyen and Jehle 2007; Olsen et al. 2005). In order to target early-instar larvae, it will be more beneficial to have a higher expression in leaf tissues especially if soybeans are planted later in the season or double-cropped after wheat.

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Figures

Figure 2.1: No-choice assay arena with a single larva presented with a soybean host tissue type.

Figure 2.2: Choice assay arena with a single larva presented with five soybean host tissue types.

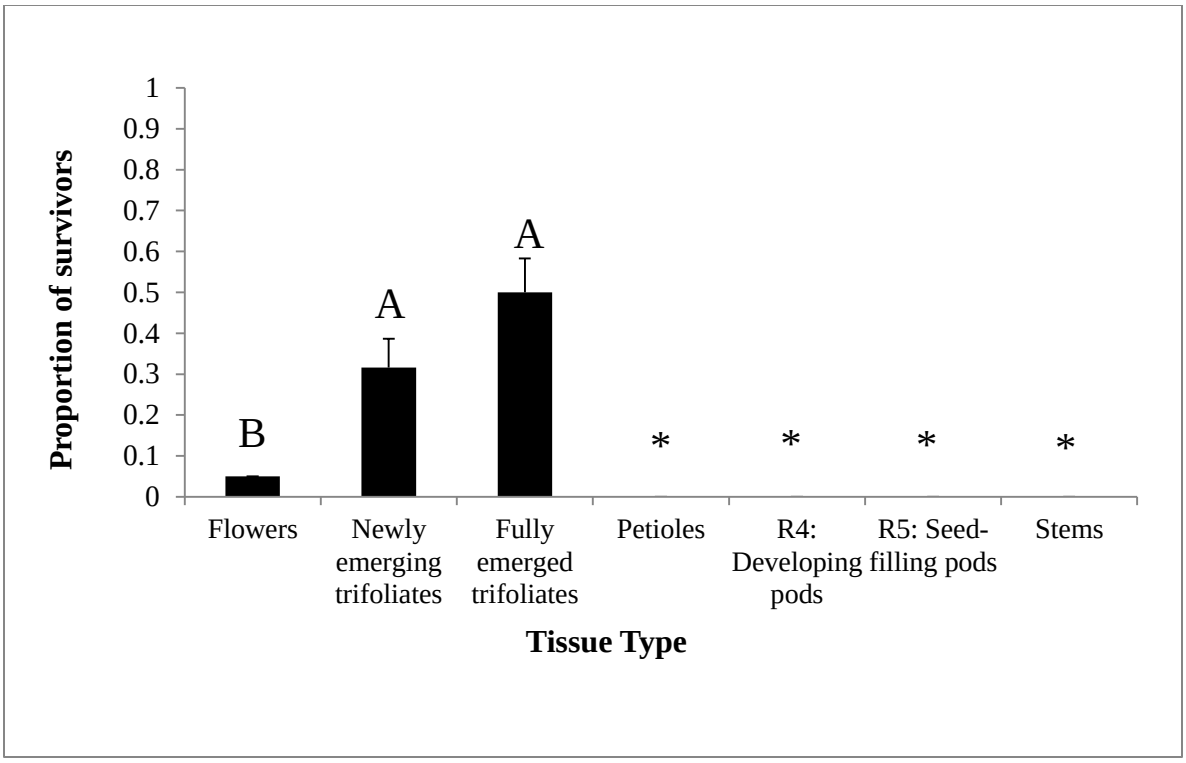

Figure 2.3: Early-instar no-choice proportion of survivors (defined as individuals who reached the pupal stage) when fed a single soybean host tissue type. Letters represent means separation by Tukey's HSD and error bars represent SE. The * data were omitted from analysis where there were no surviving individuals to pupation.

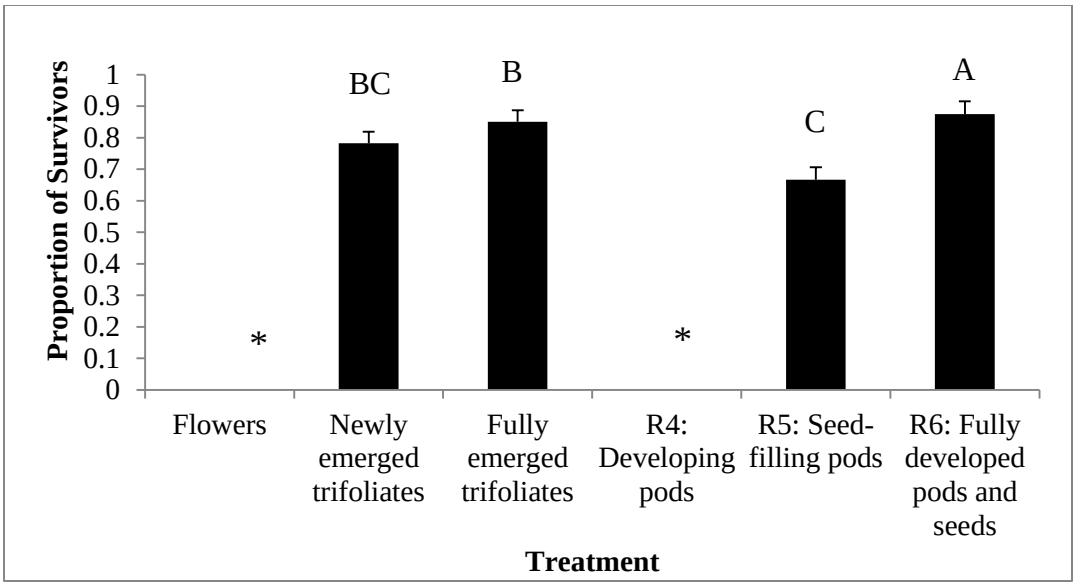

Figure 2.4: Late-instar no-choice proportion of survivors (defined as individuals who reached the pupal stage) when fed a single soybean host tissue type. Letters represent means separation by Tukey's HSD and error bars represent SE. The * data were omitted from analysis where there were no surviving individuals to pupation.

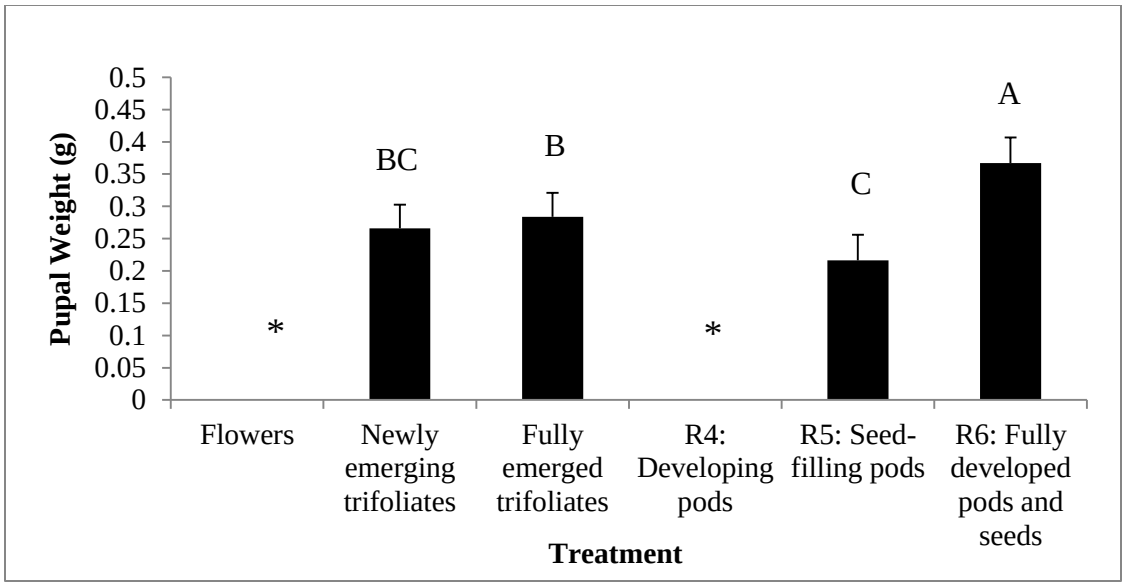

Figure 2.5: Late-instar no-choice pupal weights. Data were transformed for analysis but the raw means are represented. Letters represent means separation by Tukey's HSD and error bars represent SE. The * data were omitted from analysis where there were no surviving individuals to pupation.

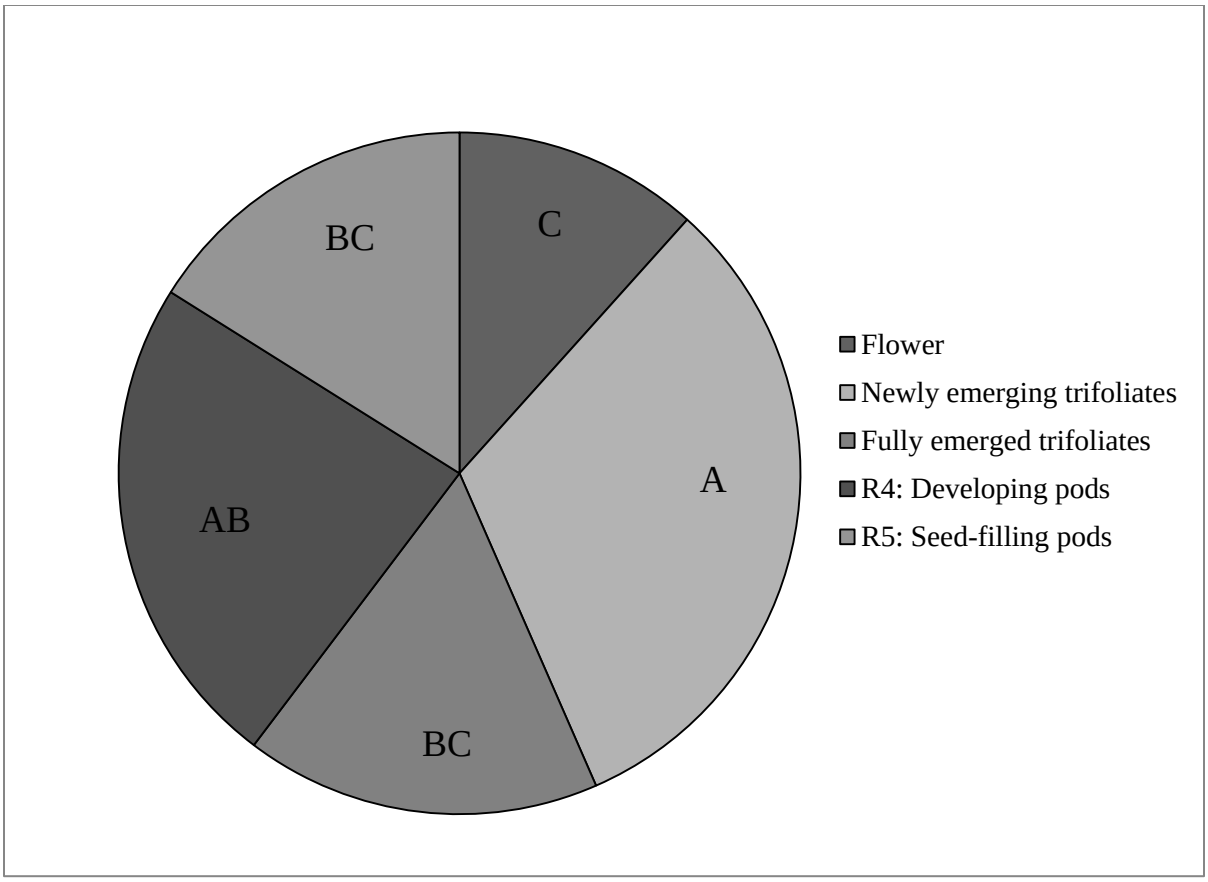

Figure 2.6: The proportion of larval feeding on different tissue types through second instar to pupation. Values indicated by the same letter are not significantly different via Tukey's HSD.

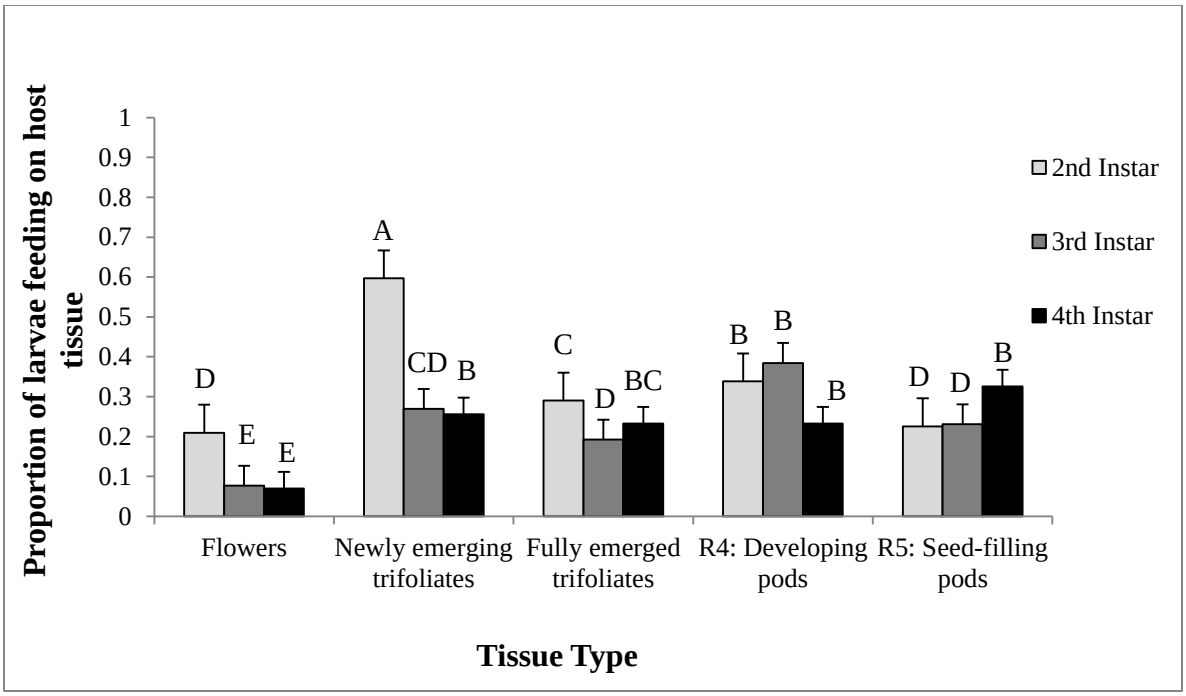

Figure 2.7: Proportion of larvae, by instar, feeding on each tissue type in choice assay.

Letters represent means separation by Tukey's HSD and error bars represent SE.