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Intra- and Inter-ear Compensation for Insect Injury to Field Corn, Zea mays L.

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To the Graduate Council:

I am submitting herewith a thesis written by Sandra Jean Steckel entitled "Intra- and Inter-ear Compensation for Insect Injury to Field Corn, Zea mays L.." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Entomology and Plant Pathology.

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Intra- and Inter-ear Compensation for Insect Injury to Field Corn, *Zea mays* L.

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Abstract

Research was conducted in 2010 and 2011 at the West Tennessee Research and Education Center in Jackson, TN, to investigate how southwestern corn borer, *Diatraea grandiosella* Dyar (Lepidoptera: Crambidae), when infested at different densities and growth stages, affected the yield of infested, non-Bt corn plants and neighboring Bt plants. Infesting non-Bt corn plants with southwestern corn borer larvae caused significant injury. The number of larvae infested on plants and the timing of these infestations were factors that affected the amount of yield loss. There was little compensation by Bt plants that were adjacent to infested plants.

Other studies were conducted in 2010 through 2012 to evaluate how silk clipping in corn affects pollination and yield. Manually clipping silks once daily had little effect on yield. Sustained clipping by either manually clipping silks three times per day or by caging Japanese beetles, *Popillia japonica* Newman (Coleoptera: Scarabaeidae), on ears affected yield if it occurred during early silking. Manually clipping silks three times per day for the first five days of silking reduced the numbers of kernels per ear and total grain weight. Caged beetles reduced the number of kernels per ear and also reduced yield at one location. Some compensation for this injury was observed where other kernels within the ear grew larger where clipping reduced the total number of kernels per ear.

Following either simulated or naturally-occurring corn earworm, *Helicoverpa zea* Boddie (Lepidoptera: Noctuidae), injury to ear tips, corn ears were evaluated to determine how yield was affected by different levels of kernel injury. In 2010 and 2011, simulated corn earworm injury reduced yield when kernels were injured at the blister and milk. In 2010, there was little or no

indication that other kernels within the ear compensated for this injury by getting larger. In 2011, simulated injury inflicted at both the blister and milk stage resulted in increased kernel size within the same ear. For naturally-occurring injury observed on multiple corn hybrids during 2011 and 2012, the analyses showed either no or a very weak relationship between number of kernels injured by corn earworm and yield.

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Introduction

Corn, *Zea mays* L., known as maize throughout most of the world, is a member of the Poacea, or grass, family (Mabberly 1997). Corn is native to Mesoamerica (Galinat 1988). The domestication of corn most likely began more than 8000 years ago with indigenous farmers of New World agriculture (Galinat1988). Modern corn has probably descended from a wild grass called teosinte, though there is some academic disagreement as to whether nature was the prominent selective agent or if it was human intervention (Galinat 1988, Mabberly 1997). Corn has become so highly domesticated that it cannot exist in the wild (Steffey et al. 1999).

Next to wheat and rice, corn is the third most important cereal crop in the world (Mabberly 1997). Corn ranks first in production and third in acreage worldwide. In 2009, over 817 million metric tons (MMT) of corn was harvested in the world, which was more than rice (678 MMT) or wheat (682 MMT). The top ten corn-producing countries in the world are the United States, China, Brazil, Mexico, Indonesia, India, France, Argentina, South Africa, and Ukraine (Edgerton 2009). Many cultivars are used throughout the world as human and animal food, cooking oil, beer and spirits, biofuel, industrial alcohol, and many other diverse uses (Mabberly 1997). The majority of the corn produced in the U.S. is used as livestock feed.

The United States Department of Agriculture (USDA) reported that 39,317,230 ha (97,155,000 acres) were planted to corn in the U.S. in 2012, giving a five-year average of 36,418,500 ha (89,992,000 acres) (USDA 2012a). The five-year average for U.S. corn production is over 308 million metric tons and this includes the 2012 season of record drought across much of the Midwestern Corn Belt (USDA 2012b). Tennessee planted 420,870 ha (1,040,000 acres) in 2012 and has a five-year average of 314,850 ha (778,000 acres) of corn (USDA 2012b).

The corn plant has a determinate growth habit, meaning that vegetative and reproductive growth does not occur at the same time. The vegetative growth occurs from germination and seedling emergence until the tassel is fully emerged. Reproductive growth takes place in six stages and begins with silk emergence and continues until the kernels are mature. The many divergent types of corn are grown over a wide range of climatic conditions throughout the world (Shaw 1988). Some cultivars grow short and some may grow to be up to 8 meters in height. Some cultivars require only 60 to 70 days to mature while others require up to 48 weeks (Shaw 1988). Though corn can grow in a wide range of climates, the bulk of world production is grown between the longitudes of 30 and 55° (Shaw 1988). Corn is grown in tropical, sub-tropical and temperate climates with the majority of production found in the latter two categories. It can be grown in latitudes from near sea level to several thousand meters above sea level. It can be grown in woodland and grassland climates, though its production is limited in drier areas. Corn does, however, have a cold limit which involves both a temperature and frost-free season limit. Hardly any corn is grown where the midsummer average temperature is less than 19°C or where average nighttime temperatures during summer are less than 13°C.

Corn is grown in climates where annual precipitation ranges from 25 to more than 500 cm (Shaw 1988). Summer rainfall of 15 cm is considered to be the lower limit for corn production without irrigation (Shaw 1988). In fact, shortage of water is the most important yield-limiting factor in corn production (Steffey et al. 1999). Corn yields can dramatically fluctuate with extreme variations in rainfall. In some drier steppe environments where the moisture demands of corn may exceed the available rainfall, other more drought-tolerant crops become more important.

Numerous insect pests attack corn (Steffey et al. 1999). Many of the most devastating agricultural pests are noctuids (Lepidoptera: Noctuidea), including the corn earworm, *Helicoverpa zea* Boddie, and fall armyworm, *Spodoptera frugiperda* J. E. Smith (Pedigo 2002). These are important insect pests of corn throughout the southern United States. The corn earworm is indigenous to North America and, due to its migratory ability, can be found anywhere on the continent where corn, *Zea mays* L., is grown (Pedigo 2002; Westbrook and Lopez 2010; Molina-Ochoa et al. 2010). Corn earworm successfully overwinters at latitudes below the 40^{th} parallel, which includes the southern U. S. where severe infestations from multiple generations may occur annually (Blanchard et al. 1942, Wiseman 1999, Pedigo 2002). Fall armyworm is native to South America, Central America and the southeastern U.S. (Quisenberry 1999, Pedigo 2002). Moths are capable of migrating as far north as Canada during the growing season (Pedigo 2002). Larvae feed on silks and kernels of developing ears. Fall armyworm damages corn ears similarly to corn earworm. Small larvae feed on silks and developing kernels in the ears (Ni et al. 2007). Heavy infestations of fall armyworm at the silking stage can destroy the tassel, ear, and leaves of the uppermost part of the plant (Quisenberry 1999). Feeding by corn earworm or fall armyworm may also provide entry wounds for secondary pests like sap beetles, *Carpophilis* spp. (Coleoptera: Nitidulidae), and pathogens such as *Aspergillus flavus* (Smeltzer 1958, McMillian et al. 1985, Rodriguez-del-Bosque et al. 1998, Pedigo 2002). *A. flavus* infection can result in aflatoxin and drastically reduce the market value of the corn grain.

The southwestern corn borer, *Diatraea grandiosella* Dyar, (Lepidoptera: Crambidae) is one of the most important insect pests of non-Bt corn, *Zea mays* L., in the southern region of the U.S. Southwestern corn borer is primarily distributed throughout the southern U.S. and Mexico

(Chippendale 1979, Davis and Williams 1986, Williams et al. 1997, Knutson and Davis 1999). Corn is its primary host, and it can damage the plant at all stages of its growth and development by tunneling into stalks, ear shanks and feeding in ears (Chippendale 1979).

Growers who plant Bt corn, *Zea mays* L., hybrids are required to plant non-Bt corn for resistance management. Refuge in a bag (RIB) is an emerging approach for resistance management where, for some hybrids having multiple Bt traits for a target species, the refuge is planted as a blend of Bt and non-Bt corn. Little is known about the effects of southwestern corn borer infestations in this type of scenario. Therefore, studies were conducted to simulate the refuge in a bag scenario to document the direct effects of southwestern corn borer infestation on non-Bt plants in this system; to determine if transgenic plants would compensate for injury caused by southwestern corn borers to a neighboring non-Bt plant; and to evaluate how the timing and intensity of injury to non-Bt plants affects any compensation by the transgenic neighbors.

Japanese beetles, *Popillia japonica* Newman (Coleoptera: Scarabaeidae), are an invasive pest that also feed in corn, especially preferring the silks of ears. Their range has recently expanded to include all of Tennessee where they are commonly reported feeding on silks. Tennessee corn growers have questions about whether treatment is needed for these pests clipping silks during pollination. There is little literature about treatment thresholds for this pest. Some Midwestern entomologists recommend treating for Japanese beetle when three Japanese beetles per ear are found, silks are clipped to less than 13 mm, and pollination is less than 50% complete. Studies were performed to evaluate the impact of continuous Japanese beetle feeding on pollination and yield in field corn. Another study was conducted to determine if it was possible to simulate Japanese beetle feeding by manually clipping silks multiple times daily.

Bt corn hybrids are genetically modified organisms that have been developed to control important insect pests. These transgenic corns are modified by inserting one or more genes from *Bacillus thuringiensis* Berliner (Bt) into the corn genome. Bt is a naturally occurring soil-borne bacterium found worldwide. This bacterium produces proteins which are toxic to lepidopteran and coleopteran pests. The primary targets of Bt corn in the southern U. S. are European corn borer *Ostrinia nubilalis* Hübner (Lepidoptera: Crambidae) and southwestern corn borer. Bt corn hybrids were introduced into the U.S. in 1996 (Ostlie et al. 1997). These hybrids provide excellent control of tunneling caterpillar pests. Some Bt corn technologies have moderate to good activity on ear-feeding pests, such as corn earworm and fall armyworm.

In recent corn trials conducted by the University of Tennessee and other universities, researchers have noticed that though these hybrids with newer Bt traits do indeed protect corn kernels, there has often been no yield advantage detected (unpublished). Studies were conducted to determine if undamaged kernels in an ear could compensate for corn earworm injury and how this compensation might be affected by ear maturity at the time of injury. This information would help predict potential yield increases, or lack thereof, as control of ear-feeding larvae such as corn earworm and fall armyworm is improved with these new Bt traits. Additionally, comparisons of different hybrids with various Bt traits were evaluated at harvest to determine if any correlation could be found between damage by ear-feeding larvae to kernels and yield parameters.

Research is needed to better understand how corn tolerates and potentially compensates for injury caused by ear-, silk- and stalk-feeding insects, such as that caused by corn earworm, Japanese beetles, and southwestern corn borer. These data would help provide a clearer picture of the economic benefits of Bt corn technologies or the need for foliar insecticide applications.

These answers will directly impact Tennessee corn producers and the integrated pest management systems they employ.

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Part I

Injury and Interplant Compensation for Southwestern Corn Borer (Lepidoptera: Crambidae) Infestations in Field Corn

Abstract

Growers who plant Bt corn, *Zea mays* L., hybrids are required to plant non-Bt corn for resistance management. Refuge in a bag (RIB) is an emerging approach for resistance management where, for some hybrids having multiple Bt traits for a target species, the refuge is planted as a blend of Bt and non-Bt corn. Studies were conducted to evaluate how southwestern corn borer, *Diatraea grandiosella* Dyar, when infested at different densities and growth stages, affected the yield of infested, non-Bt plants and neighboring Bt plants. Infesting non-Bt corn plants with SWCB larvae caused significant injury. Both the number of larvae infested on plants and the timing of these infestations affected the number of kernels per ear, total kernel weight and the weight of individual kernels. Infestation timing was more important than the number of larvae inoculated onto plants, with pretassel infestations causing more yield loss. There was little compensation by Bt plants that were adjacent to infested plants. Thus, the risk of yield loss from stalk tunneling larvae in a RIB scenario should be directly proportional to the percentage of non-Bt plants and the level of yield loss observed in these non-Bt plants. Because current RIB systems have 5 or 10% non-Bt corn plants within the seed unit, the likelihood of substantial yield losses from infestations of corn-boring larvae is remote given our results, especially for infestations that occur after silking has begun.

Key Words: corn, southwestern corn borer, *Diatraea grandiosella*, refuge, compensation.

Introduction

The southwestern corn borer, *Diatraea grandiosella* Dyar (Lepidoptera: Crambidae), is one of the most important insect pests of non-Bt corn , *Zea mays* L., in the southern region of the U.S. SWCB is primarily distributed throughout the southern U.S. and Mexico (Chippendale 1979, Davis and Williams 1986, Williams et al. 1997, Knutson and Davis 1999). Corn is its primary host, and it can damage the plant at all stages of its growth and development (Chippendale 1979).

Southwestern corn borer moths from the overwintering generation typically emerge in May in most regions of the southern U.S. and oviposit on whorl stage corn. Small larvae infest whorl stage plants causing leaf injury. More importantly, third instar and larger larvae tunnel into the stalks of corn plants (Hensley and Arbuthnot 1957). In young plants, this may cause stunting or deadheart, which is the destruction of meristematic tissue of the terminal (Davis et al. 1933, Chippendale 1979, Knutson and Davis 1999). Deadheart plants often die and survivors essentially become weeds as they do not produce an ear but compete for water and nutrients (Davis et al. 1933). During anthesis, small second-generation larvae feed between ear husks, on ear shoots, behind leaf collars, and on developing kernels, cob and ear shanks (Davis et al. 1972). Older larvae tunnel into the stalks, in ear shanks, or feed on ears. Shank tunneling may cause the ears to drop to the ground. Stalk tunneling greatly affects the plant’s ability to transport water, nutrients and minerals within the plant and to the ears thereby reducing plant height and yield and is the most serious damage inflicted by this pest (Davis et al. 1933, Chippendale 1979, Knutson and Davis 1999). Also, yield can be significantly decreased and harvesting slowed due to stalk lodging caused by southwestern corn borer (Rolston 1955).

Hybrids expressing the insecticidal crystal (Cry) proteins from *Bacillus thuringiensis* Berliner (Bt) were primarily developed to control stalk-tunneling lepidopteran pests such as the southwestern and European corn borer, *Ostrinia nubilalis* Hübner (Lepidoptera: Crambidae), (Abel et al. 2000, Buntin et al. 2004). Infestations of southwestern corn borer, the sugarcane borer, *Diatraea saccharalis* F. (Lepidoptera: Crambidae), and European corn borer may occur in the mid-south on non-Bt corn, especially when fields are planted after the optimum seeding dates. Bt corn hybrids were introduced into the southern U.S. in 1998 and have been widely adopted because they provide excellent control of these species (Ostlie et al. 1997, Williams et al. 1997, Buntin et al. 2001, Castro et al. 2004, Huang et al. 2012).

Insect resistance management guidelines mandate the planting of non-Bt corn refuges. Refuges are intended to mitigate insect resistance to specific Bt proteins produced in corn (Ostlie et al. 1997, EPA 2012a). When the hybrids conferring single gene resistance were introduced, the U. S. Environmental Protection Agency (EPA) mandated a 50% non-Bt corn refuge be planted in cotton-producing counties including much of the South where corn earworm, *Helicoverpa zea* Boddie (Lepidoptera: Noctuidae), is a significant problem. Recently, hybrids with multiple Bt genes that have activity on corn borers have been introduced. Refuge requirements for these hybrids have been reduced to 5 - 20% depending on the combination of Bt traits involved and whether the field is in a designated cotton growing county (EPA 2012b).

More recently, the commercial seed industry has expressed interest to incorporate the refuge requirement into the unit of seed. This practice is sometimes referred to as 'refuge in a bag' or RIB. For example, in areas where cotton is not grown, a producer may choose to plant a bag of seed containing as little as 5% non-Bt corn for some Bt technologies. This refuge planting strategy has not been approved for cotton-growing areas of the South. The objectives of this study were to simulate a refuge in a bag scenario to 1) document the direct effects of southwestern corn borer infestation on non-Bt plants, 2) determine if transgenic plants would compensate for injury caused by southwestern corn borer to a neighboring non-Bt plant, and 3) evaluate how the timing and intensity of injury to non-Bt plants affects any compensation by neighbors that might occur.

Materials and Methods

Field experiments were conducted in 2010 and 2011 at the West Tennessee Research and Education Center in Jackson, TN. On 31 March 2010 and 8 April 2011, a genetically modified corn hybrid, DeKalb DKC64-83 VT Triple Pro containing Cry1A.105, Cry2Ab2, and Cry3Bb1 (Monsanto Co., St. Louis, MO) was planted on a Lexington silt loam soil. This transgenic Bt hybrid has resistance to infestations of tunneling caterpillars such as southwestern corn borer and European corn borer (Ostlie et al. 1997, Williams et al. 1997, Abel et al. 2000, Buntin et al. 2004, Castro et al. 2004). This hybrid also has good activity on corn earworm and fall armyworm, *Spodoptera frugiperda* J. E. Smith (Lepidoptera: Noctuidae), both in the whorl and ear stage (Buntin et al. 2004, Huang et al. 2006, Hardke et al. 2010). These planting dates were at the beginning of the normally recommended planting season to help avoid confounding damage from naturally-occurring insect pests (McClure 2010). Corn was planted with a John Deere (Deere and Co., Moline, IL) 7200 Max Emerge Plus vacuum planter at a seeding rate of 75600 seeds/ha. All seed was treated with a commercial fungicide and insecticide. Row spacing was 76 cm, and plots were five rows by 13.7 m in length with one border row between treatment rows.

On the same day as planting, selected DKC64-83 seed were uncovered and replaced with a seed of the non-Bt isoline hybrid, DKC64-82 RR2 (Monsanto Co., St. Louis, MO). Each non-Bt seed was planted and flagged at a minimum distance of every 81 cm to insure an average of six seeds of DKC64-83 between every non-Bt DKC64-82 seed. This allowed a buffer between neighboring plants intended for harvest. Treatments were arranged in a randomized complete block design with six replications of five non-Bt plants for each treatment. Agronomic practices such as fertilization, seeding rates, and weed control followed University of Tennessee recommendations (McClure 2010). At the V4 growth stage, a QuikStix Field ELISA Kit (Envirologix, Portland, ME) was used that tests for the presence of Bt proteins expressed in Bt hybrids to confirm that the non-Bt plants were correctly identified.

Non-Bt plants were infested with either 2, 4 or 6 SWCB larvae per plant at three corn development stages including mid whorl (V6 in 2010, V8 in 2011), late whorl (V14) and blister stage (R2) as defined by Ritchie et al. (1986). A non-infested treatment was also included. Each treatment row included a non-Bt plant for each of the ten treatments (Table 1). Prior to infestation, color-coded Tyvek (DuPont, Wilmington, DE) tags were loosely attached to plants with a 22 gauge wire and labeled with the level of infestation. Plants were infested with second- or third-instar SWCB larvae from an insectary (Monsanto Co., Union City, TN) to improve the chances of establishment. Larvae were transferred to flagged, non-Bt corn plants using a camel's-hair brush. Plants were infested in the whorls and the base of the top one to two leaves at the V6 growth stage on 13 May 2010. Plants were similarly infested at the V8 growth stage on 6 June 2011. At the V14 growth stage, plants were infested in the whorls and top two to three leaf bases on 4 June 2010 and 19 June 2011. A third infestation was made at R2 on 24 June 2010 and

1 July 2011 by placing larvae at the base of the ear leaf or one to two leaves (nodes) above or below the ear leaf.

Ears were hand-harvested on 25 and 26 August 2010, and 25 and 26 August 2011. Stalks were cut at the brace root level of the infested plant and each adjacent neighboring plant in the same row. The ear of the infested plant was shucked and placed in a 10# paper sack along with its corresponding Tyvek tag. The ears from both neighbor plants were also shucked and placed in a 25# Shorty paper sack. The corresponding, bagged infested ear was then placed inside this larger sack. Samples were placed in a forced air dryer set to 65.6C (150F) and dried until grain moisture was 13%. Forced ambient air was applied for cooling purposes for 4 h afterward. Ears were stored in an air conditioned environment and moisture allowed to come to an equilibrium over one week. All stalks were split lengthwise at the time of harvest in 2010, and the total length of stalk tunneling caused by southwestern corn borer or other corn borer species that may have been present was recorded. Only stalks of non-Bt plants were split in 2011.

For infested and neighboring plants, total ear weight was taken and each ear was shelled individually using a hand-operated corn sheller (Seedburo Equip. Co., Des Plaines, IL). Grain was then screened through three 30.5 cm x 30.5 cm hand testing screens (5 mm x 19 mm slots, 4 mm x 19 mm slots, and a #18 sieve with round holes 7 mm in diameter) to remove small kernels and debris. Some hand removal of remaining debris was also necessary. Total kernel weight was measured for each non-Bt ear and both of its Bt neighboring ears collectively, and the number of kernels was counted using an automated seed counter (Old Mill Counter Model 850-3, Int'l Marketing and Design Corp., San Antonio, TX).

In 2010, one replicate was omitted because of severe damage caused by charcoal rot, *Macrophomina phaseolina* (Tassi) Goid. Data were analyzed as a randomized complete block using SAS statistical software (SAS 2008). Because the design was unbalanced, Dunnett's tests (Proc GLM, $\alpha = 0.05$) were done to make treatment comparisons between the non-infested treatment and each infested treatment. Proc MIXED and LS means for mean separation were used to test for the main effects and interactions of infestation density and infestation timing on the amount of stalk tunneling, kernel numbers per ear, total kernel weight per ear and the weight of individual kernels in each ear ($\alpha = 0.05$). Each year and southwestern corn borer infestation level were considered fixed effects in the model and blocks and all interaction with blocks were considered random effects.

Results

For both years of the study, there were no statistically significant interactions between the main effects of infestation level (0, 2, 4 and 6 larvae per plant) and the timing of infestation for the average number of kernels per ear, total kernel weight per ear and the weight of individual kernels. Thus, the main effects of treatment are presented separately below.

Effects of Larval Infestation Level on Non-Bt Plants

In both years, the average length of stalk tunnels was significantly higher for infested plants than for plants that were not infested (Table 2). Some tunneling was observed in non-infested plants during both years as a result of natural infestations that mostly occurred after the blister stage (R2). There was generally more stalk tunneling when more southwestern corn borer larvae were infested onto plants. About one-half as much tunneling was observed in 2010 when

2 larvae were infested per plant versus when 6 larvae were infested. In 2011, tunneling significantly increased as the number of larvae infested per plant was increased.

In 2010, there were 35, 44, and 49% fewer kernels per ear when 2, 4, or 6 larvae were infested per non-Bt plant, respectively, compared with non-Bt plants that were not infested with SWCB. Results were similar in 2011 as the numbers of kernels was reduced by 18, 24 and 31% for plants infested with 2, 4 or 6 larvae, respectively. However, the difference between kernel numbers for plants infested with 2 and 4 larvae was not statistically significant in either year.

In both years, the total kernel weight per ear was significantly less for all levels of infestation compared with non-infested plants (Table 2). Kernel weight was reduced by 36 - 51% in 2010 and 22 - 36% in 2011 for plants that were infested with 2, 4, or 6 larvae. Kernel weight numerically decreased in a stepwise fashion as the number of larvae infested per plant increased. In both years, infesting 6 larvae per plant caused significantly more loss in kernel weight than infesting 2 larvae per plant. The difference between 2 and 4 larvae per plant was also significant during 2010.

Similarly, the average weight of individual kernels was heavier for non-infested plants compared with all infestation levels in both years. The weight reduction of individual kernels ranged from 23 - 36% in 2010 and 9 - 12% in 2011 compared with non-infested plants. Differences in individual kernel weight among treatments that were infested with larvae were not significantly different in 2011. In 2010, individual kernel weight was higher for plants infested with 2 larvae versus those infested with 4 or 6 larvae per plant.

Effects of Larval Infestation Level on Neighboring Bt Plants

Bt plants that were neighbors of non-infested plants had no tunneling in 2010 (Table 3). The average length of stalk tunnels for neighbors of infested plants was small, ranging from 0.45 - 0.88 cm. Thus, stalk tunneling was not measured in neighbor plants during 2011. The number of larvae infested on a neighboring non-Bt plant did not significantly affect the number of kernels per ear, total kernel weight, or average weight of individual kernels in either year. On average, total kernel weight only ranged 3% in either year, regardless of the number of larvae that were infested on the adjacent non-Bt plant.

Effects of Infestation Timing on Non-Bt Plants

In both years of this study, infested plants had significantly more tunneling than non-infested plants with one exception. Although numerically higher, plants infested at the V6 stage in 2010 did not have significantly more tunneling than uninfested plants (Table 4). In 2010, 72% of plants infested at the V6 stage were severely stunted or killed (deadheart) by the larvae, and tunnel lengths could not be measured given the deteriorated condition of these plants. Thus, data for the amount of tunneling are somewhat misleading. This did not occur in 2011 when the mid-whorl infestation was delayed until the V8 growth stage. However, for the amount of stalk tunneling, there was an interaction between the number of larvae infested per plant and the timing of infestation in 2011 ($F = 3.22$; $df = 4, 334$; $P = 0.0130$). There was a greater increase in tunneling for plants infested with 2, 4 or 6 larvae at the R2 stage than for other timings, especially those infested at V8. Regardless, in both years, the length of stalk tunneling was highest for infestations initiated at R2 and statistically less for infestations initiated at the mid whorl stage in both years.

Kernel numbers per ear were reduced 88, 33 and 7% for the V6, V14, and R2 timing, respectively, compared with non-infested plants in 2010 (Table 4). Kernel numbers were reduced by 40, 30 and 3% for the V8, V14 and R2 timing, respectively, in 2011. Plants that were not infested with SWCB larvae had more kernels compared with those infested at the mid whorl (V6 or V8) and late whorl timings (V14) in both years. Although numerically less, plants infested at the R2 stage did not have statistically fewer kernels than non-infested plants in either year. Kernel numbers among treatments that were infested with larvae were significantly different from each other, and kernel numbers increased as infestation timing was delayed in both years.

Results for total kernel weight per ear followed a similar pattern with significantly lower weights for plants infested at mid whorl (V6 or V8) and late whorl (V14) than for plants not infested with larvae. Total kernel weight for plants infested at the blister stage (R2) was less than uninfested plants but not statistically significant. Infesting plants at the V6, V14 or R2 growth stages reduced total kernel weight by 88, 34 and 10% in 2010 and by 40, 33 and 15% in 2011 compared with non-infested plants. Except for infestations at V8 or V14 in 2011, these differences among infested treatments were statistically significant.

The average weight of individual kernels in the V6 treatment was 79% lower than non-infested plants in 2010. The average weight of individual kernels was also higher in the V14 and R2 infestation treatments compared with the V6 timing. In 2011, there were no differences among treatments that were infested with SWCB, but individual kernel weight was significantly less in plants infested at the V8 stage compared with non-infested plants.

Effects of Infestation Timing on Neighboring Bt Plants

Neighboring plants had little tunneling in 2010, and there were no differences compared with neighbors of non-infested plants (Table 5). In both years, the number of kernels, total kernel weight and individual kernel weight of plants that neighbored infested plants did not significantly differ from plants adjacent to non-infested plants.

In both years of the study, neighbors of infested plants had similar kernel numbers, kernel weights, and weights of individual kernels with one exception. In 2010, the plants infested at V6 had more kernels, heavier kernel weight and higher average weight of individual kernels compared with the V14 and R2 infestation timings. For example, the average kernel weight of plants neighboring a non-Bt plant that was infested at the V6 stage was 5-8% higher than neighbors of plants that were infested later.

Regression of Yield Components

Linear regression analyses were performed on some yield components across all treatments. The weight of entire ears was highly correlated with the total weight of kernels in 2010 (F = 233850; df = 1, 498; P <0.0001; R^2 = 0.99) and 2011 (F = 82732; df = 1, 698; P <0.0001; R^2 = 0.99). Total kernel weight and the number of kernels per ear were also highly correlated in 2010 (F = 15442; df = 1, 498; P <0.0001; R^2 = 0.97) and 2011 (F = 8766; df = 1, 698; P <0.0001; R^2 = 0.93).

Discussion

Most southwestern corn borer larvae begin tunneling into the plant during the third instar (Hensley and Arbuthnot 1957) and the vast majority of stalk tunneling is done by later-instar southwestern corn borer larvae (Whitworth et al. 1984). Plants were infested with second instar larvae to improve survival, which likely reduced mortality caused by natural enemies (Moulton et al. 1992). Success was achieved in the objective to establish different levels of injury at different growth stages to determine the relative susceptibility of non-Bt plants and evaluate whether compensation by neighboring plants would occur. Good establishment of larvae occurred with approximately 95% of infested plants showing signs of tunneling at the time of harvest. There were naturally-occurring infestations of southwestern corn borer in our non-infested treatments (30% and 40% of plants in 2010 and 2011, respectively) and presumably in our infested treatments as well. These infestations occurred late in the season and probably had little impact on our results.

A small but elevated level of tunneling observed in the stalks of Bt plants that were adjacent to infested plants indicated some interplant movement of larvae (Table 3). Evidence was observed of southwestern corn borer injury to plants that neighbored infested plants in the days following infestation. However, no evidence of prolonged survival was witnessed when larvae moved from non-Bt plants to Bt plants even though L2 stage larvae were used for infestation.

In 2010, 72% of non-Bt plants that were infested at the mid-whorl (V6) stage were severely stunted or killed (i.e., deadheart). We delayed infestation until the V8 growth stage in the following year. Deadheart was not observed when southwestern corn borer larvae were infested at V8 or later growth stages. Consequently, the effect of mid-whorl infestations on the yield parameters measured was greater in 2010. Similar to these results, Arbuthnot et al. (1958) found 36% deadhearted plants in corn infested with southwestern corn borer at 36 days after planting and no deadheart in corn infested at 47 days after planting.

Total kernel weight per ear (i.e. yield) of non-Bt corn plants that were infested with southwestern corn borer was reduced by 10 - 88% compared with non-infested plants depending upon the timing or level of larval infestation (Tables 2 and 4). Similar to these results, Whitworth et al. (1984) found that infestations when the twelfth leaf was fully emerged caused more yield loss than did later infestations at the R1 (beginning silk) or R3 (milk) stage. Unlike their study, significant yield reductions did not occur at all timings of infestation compared with non-infested plants in this experiment (Whitworth et al. 1984). A positive relationship was found between the numbers of southwestern corn borer larvae infested, the amount of stalk tunneling, and how much kernel weight per ear was reduced (Table 2). This differs from previous research (Whitworth et al.1984) where no greater yield loss occurred as tunneling damage increased.

In my experiments, the timing of infestation had a greater effect on total kernel weight than did the number of larvae that were inoculated onto non-Bt plants. Scott and Davis (1974) also found greater impact on yield from late whorl infestations of southwestern corn borer versus infestations that occurred during pollination. The yield losses observed in this study were primarily reflected by reduction in kernel numbers, but individual kernel weights were also reduced by infestations of southwestern corn borer. Scott and Davis (1974) also attributed yield losses caused by infestation of southwestern corn borer to a reduction of kernels per plant. I found little evidence of compensation for yield loss by plants that neighbored infested plants. Indeed, the only significant indication of compensation by neighboring plants occurred when the non-Bt plant was functionally killed, which was commonly observed at theV6 infestation timing in 2010. Even then, the kernel weight of neighboring ears only increased an average of 5 - 8% compared with plants neighboring other plants that were infested at V14 or R2.

Not surprisingly, a strong correlation was evident between the weight of an unshelled ear and the total kernel weight of that ear ($R^2 = 0.99$ in both years). I would have drawn the same conclusions about treatment effects on yield had I used either the weight of entire ears (data not shown) or the total kernel weight of shelled ears. It would require much less effort to use whole ear weights if similar experiments are done where the primary interest is treatment effects on yield.

This study simulated the refuge in a bag scenario where a relatively low percentage of non-Bt corn plants would be interspersed with Bt corn. Southwestern corn borer was used to model how well adjacent plants would compensate for injury to their neighbors by stalk tunneling larvae, but our results should generally apply to other stalk tunneling species that infest corn. Little or no compensation was observed under the conditions of my study. Growing conditions were good for both years of this test. The remaining portions of the fields used in these tests yielded 12870 and 10420 kg grain/ha in 2010 and 2011, respectively. It is unknown how much these results would differ under other environmental conditions or if different hybrids were used, but these results suggest that yield losses caused by infestations of stalk tunneling larvae would be directly proportional to the percentage of non-Bt plants within the refuge in a bag system and the level of yield loss observed in these non-Bt plants. Because current refuge in a bag systems have 5 or 10% non-Bt corn plants within the seed unit, the likelihood of substantial yield losses from infestations of corn boring larvae is remote given our results, especially for infestations that occur after silking has begun.

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Part II

Effects of Japanese Beetle (Coleoptera: Scarabaeidae) and Silk Clipping in Field Corn

Abstract

Japanese beetle, *Popillia japonica* Newman (Coleoptera: Scarabaeidae) is an emerging silk-feeding insect found in fields in the lower Corn Belt and midsouthern U.S. Studies were conducted in 2010 and 2011 to evaluate how silk clipping in corn affects pollination and yield parameters. Manually clipping silks once daily had modest effects on yield parameters. Sustained clipping by either manually clipping silks three times per day or by caging Japanese beetles onto ears affected total kernel weight if it occurred during early silking (R1 growth stage). Manually clipping silks three times per day for the first five days of silking affected the number of kernels per ear, total kernel weight and the weight of individual kernels. Caged beetles fed on silks and, depending upon the number of beetles caged per ear, reduced the number of kernels per ear. Caging eight beetles per ear significantly reduced total kernel weight compared with non-infested ears. Drought stress prior to anthesis appeared to magnify the impact of silk clipping by Japanese beetles. There was evidence of some compensation for reduced pollination by increasing the size of pollinated kernels within the ear. These results showed that it requires sustained silk clipping during the first week of silking to have substantial impacts on pollination and yield parameters, at least under good growing conditions. Some states recommend treating for Japanese beetle when three Japanese beetles per ear are found, silks are clipped to less than 13 mm, and pollination is less than 50% complete and that recommendation appears to be adequate.

Key words: corn, Japanese beetle, *Popillia japonica*, silk clipping

Introduction

Pollination of field corn, *Zea mays* L., is influenced by many factors including silk area, silk growth and health (Sadras et al. 1985). Silk receptivity is a key component of the fertilization process (Strachan and Kaplan 2001, Anderson et al. 2004). Many factors that interfere with the ability of silks to receive pollen, including environmental stress or silk clipping by insects, may reduce pollination and seed set (Westgate and Boyer 1986, Capinera et al. 1986, Culy et al. 1992b, Bassetti and Westgate 1993a, Strachan and Kaplan 2001, Anderson et al. 2004). In addition to seed losses from poor pollination, feeding from insects such as sap beetle (*Carpophilis* spp., Coleoptera: Nitidulidae) may expose the developing ear to secondary pest damage, infection by pathogens and damage by birds (Woodside 1954, Gould 1963, Woronecki et al. 1980, McMillian et al. 1985, Rodriguez-del-Bosque et al. 1998, Pedigo 2002).

Many insect species clip silks in field corn. The primary silk-feeding insect found in Tennessee and some other surrounding states is the corn earworm, *Helicoverpa zea* Boddie (Lepidoptera: Noctuidae) (Steffey et al. 1999). Other insects which may feed on fresh corn silks include grape colaspis *Colaspis brunnea* F. (Coleoptera: Chrysomelidae), corn rootworm beetles, *Diabrotica* spp. (Coleoptera: Chrysomelidae), fall armyworm, *Spodoptera frugiperda* J. E. Smith (Lepidoptera: Noctuidae), European corn borer, *Ostrinia nubilalis* HÜbner (Lepidoptera: Crambidae), and grasshoppers (Orthoptera: Acrididae) (Steffey et al. 1999). An emerging silk-feeding insect in Tennessee is the Japanese beetle, *Popillia japonica* Newman (Coleoptera: Scarabaeidae) (Steffey et al. 1999).

Japanese beetles are an invasive species introduced from Japan into the northeastern United States sometime before 1916 (Ladd 1987). Because they have few natural enemies and many hosts, numbers have increased greatly and their range of habitat continues to expand.

Fleming (1972) compiled a list of almost 300 species of host plants to Japanese beetles. Corn silks were identified as a preferred food source for adults (Ladd 1987). Due to their invasive nature and high reproductive capability (Yesudas et al. 2010), Japanese beetles have become well established in eastern Tennessee and their range has recently expanded to include the western parts of the state. Producers in Tennessee and other states are concerned with silk clipping in field corn by Japanese beetles.

Little has been written about the impact of Japanese beetle silk feeding on field corn. Japanese beetles are considered pests in the Midwestern U. S. where treatment is recommended if three or more Japanese beetles are found per ear, silks are clipped to less than 13 mm (0.5 inch), and pollination is less than 50% complete (Cook and Gray 2003, Hodgson 2009).

The objectives of this study were to 1) determine the critical duration and amount of silk clipping that will significantly affect pollination, 2) assess the impact of adult Japanese beetle feeding on corn silks, and 3) evaluate the level of compensation by other kernels on the ear when a reduction in seed set occurs due to silk clipping.

Materials and Methods

Daily, Manual Silk Clipping, 2010 – 2011

Field experiments were conducted in 2010 and 2011 at the West Tennessee Research and Education Center in Jackson, TN. On 31 March 2010, and 8 April 2011, a genetically modified corn hybrid, DeKalb DKC 64-83 VT Triple Pro containing Cry1A.105, Cry2Ab2, and Cry3Bb1 (Monsanto, St. Louis, MO), was planted on a Lexington silt loam soil. This Bt transgenic hybrid provides excellent control of tunneling caterpillars, such as southwestern corn borer, *Diatraea grandiosella* Dyar (Lepidoptera: Crambidae), and European corn borer (Abel et al. 2000, Buntin et al. 2004, Castro et al. 2004). It also has good activity on corn earworm and fall armyworm, both in the whorl and ear stage (Buntin et al. 2004, Huang et al. 2006, Buntin 2008, Hardke et al. 2010). Thus, confounding effects were avoided by other pests by using a Bt hybrid that expressed multiple toxins for lepidopteran and coleopteran pests. Plots were planted with a John Deere (Deere and Co., Moline, IL) 7200 Max Emerge Plus vacuum planter with a seeding rate of 76,000 seeds/ha at a planting depth of 3.8 cm. Row spacing was 76 cm and plots were five rows by 13.7 m with one border row between treatment rows. All seed was treated with a commercial fungicide and insecticide. Corn was planted no-till into soybean stubble from the previous year. Agronomic practices such as fertilization, seeding rates, and weed control, followed University of Tennessee recommendations (McClure 2010).

Plots were arranged in a randomized complete block design with six replications of five ears per treatment. Prior to silk emergence, plants were selected that were uniform in maturity and had developing primary ears. Each plant designated for treatment was separated by at least four plants from the next selected plant in the row. Color-coded Tyvek® (DuPont, Wilmington, DE) tags were loosely attached to plants at the ear ± one node with a 22 gauge wire and labeled with the treatment. On the first day of silking, clipping was initiated according to treatment level. Treatments were initiated when silks were first visible (day of silking 1) beyond the tip of the ear for less than 24 h. Silks were clipped at the tip of the husks using scissors. For each treatment, clipping occurred each day before 0700 hours to limit the likelihood of pollen transfer to silks due to this activity (Anderson et al. 2004).

The nine treatments in this study consisted of untreated plants with no silks clipped and plants with all silks being clipped once daily for the days of silking 1 - 5, 1 - 10, 1 - 15, 6 - 10, 6 - 15, and 11 - 15. Also, a treatment was included where approximately 50% of the silks, those nearest the stalk, were clipped daily for the 15 days of silking. For another treatment, all silks were clipped daily to a length of 38 mm for the 15 days of silking.

Ears were hand-harvested on 25 August 2010 and 25 August 2011. The treatment ear was shucked and placed in a 10# paper sack along with its corresponding Tyvek® tag. Sacks were placed in a forced air dryer set to 65.6°C and dried until grain moisture was 13%. Forced ambient air was applied for cooling purposes for 8 h afterward. Ears were stored in an air conditioned environment and moisture allowed to come to an equilibrium over one wk. Total ear weight was taken and each ear was shelled individually using a hand operated corn sheller (Seedburo Equip. Co., Des Plaines, IL). Grain was then screened through three 30.5 cm x 30.5 cm hand testing screens (5 mm x 19 mm slots, 4 mm x 19 mm slots, and a #18 sieve with round holes 7 mm in diameter) to remove small kernels and debris. Total kernel counts were done using an automated seed counter (Old Mill Counter Model 850-3, Int'l Marketing and Design Corp., San Antonio, TX).

Data were analyzed as a randomized complete block design with five observations of each treatment in each replication using SAS statistical software (SAS 2008). Data were analyzed using the MIXED procedure in SAS. Main effects and all possible interactions were analyzed using the appropriate expected mean square values as recommended by McIntosh (1983). In the model years and locations (environments) as well as blocks, nested within environments, were considered random effects while treatments were considered fixed effects. Any interaction between fixed and random variables was also considered a random effect. Designating environment as a random effect broadens the possible inference space the experimental results are applicable to (Carmer et al. 1989). The LS means statement was used to provide the standard error of the differences between means. Then Fisher's Protected LSD was used for mean separation of clipping on the number of kernels per ear, total kernel weight per ear and weight of individual kernels on each ear ($\alpha < 0.05$). Single degree of freedom contrast statements were conducted to compare combined data from treatments where 100% of silks were clipped early (days of silking 1 – 5, 1 – 10, and 1 - 15) with treatments that were clipped later (days of silking 6 - 10, 6 - 15, 11 – 15).

Three-Times Daily, Manual Silk Clipping, 2011 and 2012

An experiment was performed where silks were clipped three times per day. For 2011, the corn hybrid and planting date were the same as described in the once daily silk clipping experiment for 2011. In 2012, DeKalb hybrid DKC 67-88 VT Triple Pro containing Cry1A.105, Cry2Ab2, and Cry3Bb1 (Monsanto Co., St. Louis, MO), was planted on 15 May at the same population and depth and maintained as described for the previous experiment. Treatments were assigned to plants in a completely randomized design. Each treatment had 15 replications (ears). The three treatments in this study consisted of untreated plants with no silks clipped and plants with silks clipped three times daily for two timings (days of silking 1 - 5 and days of silking 6 - 10). Prior to silk emergence, uniform plants were selected that had a developing primary ear. Each plant designated for treatment was separated by at least four plants from the next selected plant in the row. Color-coded Tyvek® tags were labeled and loosely attached to plants as previously described. Silks were manually clipped with scissors at the tip of the ear husk leaves between 0700 and 0800 hours; again between 1130 and 1300 hours; and between 1600 and 1700 hours each day for the timing level. Ears were hand-harvested on 25 August 2011 and 9 September 2012 using a method previously described. All ears were dried, shelled and yield parameters measured at the West Tennessee Research and Education Center as previously described.

Data were analyzed as a completely randomized design using individual ears as replicates. Proc MIXED (SAS 2008) and LS means (Fisher's Protected LSD) for mean separation were used to test for effects of clipping on number of kernels per ear, total kernel weight per ear and weight of an individual kernel in each ear ($\alpha < 0.05$).

Silk Clipping by Japanese Beetles, 2011.

The impact of adult Japanese beetle feeding on corn silks was evaluated in 2011 by caging beetles on developing corn ears at the West Tennessee Research and Education Center in Jackson, TN, and at the T. E. Fisher Delta Research Center Lee Farm near Portageville, MO. DeKalb DKC 64-83 VT Triple Pro containing Cry1A.105, Cry2Ab2, and Cry3Bb1 (Monsanto, St. Louis, MO) hybrid field corn was planted in Tennessee on 8 April 2011 and maintained as previously described. Mycogen 2T784 SmartStax field corn containing Cry1A.105, Cry2Ab2, Cry1F, Cry3Bb1 and Cry 34/35Ab1 (Dow AgroSciences, Indianapolis, IN) was planted at the Delta Research Center Lee Farm 18 April 2011 with a row spacing of 76 cm. All agronomic practices at the Missouri location followed University of Missouri recommendations (Anonymous 2009).

Randomly selected plants were assigned a treatment, with 12 replications (ears) per treatment at Missouri and 15 replications at Tennessee. Treatments were zero, two, four, and eight beetles per ear, and plants were tagged accordingly as previously described. The beetles were confined for five days beginning the first day of silking (TN June 21 – 26; MO July 4 – 9) as this appeared to be the critical pollination window based on the author's previous experiences. Prior to silk emergence, uniform plants were selected that had a developing primary ear. Each treatment plant was separated by at least four plants from the next selected plant in the row.

Beetles were collected using Trece Catch Can traps with yellow top assembly and a double lure (one floral and one sex pheromone) system (Great Lakes IPM Inc., Vestaburg, MI)(Ladd 1986). Beetles were caged on ears beginning the first day of silking. Cages (12 x 30 cm) were made from 28 x 28 (thread count) synthetic filament screen closed at the bottom with a bread tie and folded over and closed at the top with a medium binder clip. Screening was amber color to allow good light penetration. Small gauge wire was used to support the cage by tying the binder clip at the top of the bag to the stalk. Japanese beetle adult survival in cages was monitored daily and dead beetles were replaced. I attempted to put at least one female in each cage. The percentage of clipped silks was visually estimated and average silk length was measured for each ear when the cages were removed. Hand-harvesting of ears was done 25 August 2011 in Tennessee and 7 September 2011 in Missouri as previously described. All ears were dried, shelled and yield parameters measured at the West Tennessee Research and Education Center as previously described.

Data were analyzed as a completely randomized design using SAS statistical software (SAS 2008). Proc MIXED and LS means (Fisher's Protected LSD) for mean separation were used to test for effects of infestation density on silk length, percentage of clipped silks, number of kernels per ear, total kernel weight per ear and weight of individual kernels in each ear ($\alpha < 0.05$).

Results

Daily, Manual Silk Clipping, 2010 – 2011

In both years of this study, the number of kernels per ear and total kernel weight per ear were statistically similar across all nine treatments when silks were only clipped once daily

(Table 6). The number of kernels per ear varied among treatments by 7.6% in 2010 and 3.0% in 2011. Total kernel weight varied by only 2.3% and 5.4% across treatments in 2010 and 2011, respectively.

In 2010, number of kernels per ear was reduced by 3.6% for the earlier clipping treatments compared with the late (Table 7). There was no difference in number of kernels per ear in 2011. Total kernel weight per ear was similar both years (Table 7). In 2010, individual kernel weight was 3.5% heavier for treatments where clipping was initiated on the first day of silking (early) but it was 3.1% less in 2011 for this treatment.

Three-Times Daily, Manual Silk Clipping, 2011 and 2012

No interaction between treatment and year was found for any response variables measured ($P > 0.25$ for all), so data were analyzed across years. There were 16% fewer kernels on ears where clipping was done three times daily during the first five days of silking compared with ears where no silks were clipped or clipping was delayed until the sixth day (Table 8). Similarly, total kernel weight was reduced 10.7% for ears with silks clipped during the first five days of silking versus those not clipped or those where clipping began the sixth day. Individual kernels from ears where silks were clipped during the first five days of silking weighed 5.4% and 6.7% more than kernels from ears where no silks were clipped or where clipping was delayed until the sixth day, respectively (Table 8).

Silk Clipping by Japanese Beetles, 2011

No interaction were found between locations and the percentage of silks clipped and average silk length at time of cage removal ($F = 2.03$; $df = 3, 100$; $P = 0.1141$ and $F = 2.11$; $df = 3, 100$; $P = 0.1040$, respectively), so these data were pooled across locations. The percentage of silks clipped by caged beetles ranged from 39.7 - 98.0% (Table 9). By the end of the 5-d caging period, Japanese beetle feeding reduced average silk length 45.7, 67.9, and 97.6% for cages with two, four, and eight beetles per ear, respectively, relative to the control. In this study, beetles were observed to feed only on the corn silks.

The total number of kernels and total kernel weight per ear was strongly affected by the number of beetles caged at the Missouri location (Table 9). Ears with eight beetles per cage had 34.6% fewer kernels per ear than non-infested ears. Ears infested with four beetles had 19.3% fewer kernels than non-infested ears and was different from the eight beetle treatment. Ears infested with two beetles had a similar number of kernels as non-infested ears. Eight beetles per ear reduced total kernel weight by 32.4% compared with non-infested ears. Total kernel weight was similar for non-infested ears and those with two or four beetles per cage. Individual kernel weight did not differ across treatments in Missouri, but kernels were numerically slightly heavier in ears infested with beetles (Table 9).

In Tennessee, the total number of kernels per ear did not differ significantly among treatments (Table 9), but trended toward an inverse relationship between kernels per ear and the number of beetles per cage. Total kernel weight was not affected by the number of beetles caged on ears at the Tennessee location. Individual kernel weight was 8% heavier for ears where eight beetles were caged compared with ears with two beetles, but neither treatment statistically differed from the non-infested ears.

Discussion

Overall, once daily silk clipping had modest effects on yield parameters; these effects were only notable in one year of our study and for treatments where 100% of silks were clipped beginning early in the silking process. Manually clipping silks three times per day reduced the number of kernels per ear and kernel weight per ear only if clipping occurred during early silking. Seed set was not affected if clipping was delayed until the sixth day of silking. Sustained Japanese beetle feeding on silks also had an effect on total kernel weight when it occurred early in the silking process.

Silks often emerge rapidly during the first two days of silking and all silks have emerged by the fourth day (Sadras et al. 1985). Maximum total silk area was exposed on the fifth day of silking (Sadras et al. 1985). Under optimum growing conditions, silks can grow 2.5 – 3.8 cm per day and will continue to elongate until fertilized (Ritchie et al. 1986). Silks grow rapidly during silking, especially during the first day, and typically cease nine to eleven days after initiation (Bassetti and Westgate 1993b).

Manually clipping silks multiple times daily appeared to simulate the effects of sustained Japanese beetle feeding in my studies. This observation may have implications for future research. For example, the number of kernels and kernel weight per ear were reduced in the test where silks were clipped three times per day the first five days of silking. The number of kernels was also reduced when four or eight Japanese beetles were caged on ears at the Missouri location, and kernel weight per ear was reduced when eight beetles were caged per ear. This finding agrees with Flynn and Reagan (1984) who found a reduction in the number of kernels on seed corn ears artificially infested with 10 or 15 *Colaspis louisianae* Blake beetles for the first week of silking. They also found similar results when they mechanically clipped silks every two days for the same duration. Strachan and Kaplan (2001) found that grain yield was significantly reduced when average silk length was < 25 mm for hybrid corn. However, another study found that densities of up to 20 adult western corn rootworms, *Diabrotica virgifera virgifera* LeConte, per ear did not significantly reduce total kernel weight, but beetles were infested at a later growth stage in this trial (Capinera et al. 1986). In my tests, evidence of an increase in individual kernel weight was found where there was a reduction in the number of kernels per ear.

The percentage of silks clipped by Japanese beetles and silk length at the time cages were removed was statistically the same for both Missouri and Tennessee (Table 4) and showed that beetles behaved comparably in both environments. Silk clipping by Japanese beetles may reduce the number of kernels per ear, reduce the total kernel weight, and increase the weight of an individual kernel. Though the number of kernels were reduced at the Missouri location by caging four beetles per ear compared with zero or two beetles, kernel weight per ear was not different among these treatments.

Total kernel weight was reduced by 32.4% at the Missouri location for the ears where eight beetles were caged on them, but there was not a significant reduction in total kernel weight for the same treatment at the Tennessee location. The dramatic reduction in total kernel weight at the Missouri location may have resulted from other environmental stressors. The Tennessee location had a good pollination and yield environment, whereas the Missouri location had a stressful one. Weather data collected from these locations for the two weeks prior to silking showed that maximum daily temperature averaged 0.32^{o}C lower in Missouri compared with Tennessee (data not shown). However, minimum temperatures were 2.28^{o}C higher in Missouri. Tennessee received 10.2 cm of precipitation compared with 0.51 cm in Missouri during this critical period. This suggests that a combination of drought stress and silk clipping by Japanese beetles reduced pollination and yield in the Missouri field corn. This agrees with Culy et al. (1992a) who found that moisture or heat stress during pollination magnified the effects of silk feeding by western corn rootworm beetles.

Drought stress during the period of two weeks prior to silking through two weeks after may cause substantial yield loss (Ritchie et al. 1986), at least in part by reducing number of kernels (Hall et al. 1981, 1982). Because silks have the highest water content compared with any other corn plant tissue, they are very sensitive to moisture levels in the plant (Neilsen 2010). Drought conditions may impact silk health and pollination in many ways. Water stress can delay silking, cause fertilization to take longer, and inhibit silk growth resulting in a decrease in kernel set (Westgate and Boyer 1986, Bassetti and Westgate 1993b, Carcova and Otegui 2001). Thus, the stress observed at our Missouri location likely explains why it was more affected by silk clipping by Japanese beetles.

My research indicates that protecting silks from clipping during the first five days of silking is critical for realizing optimum yield potential. However, the sensitive window may be longer in stressful environments. Moreover, my studies would suggest that silk clipping after drought stress will magnify yield loss from beetle damage. It is possible that some corn hybrids or other corn production practices may be more sensitive to silk clipping than the ones used in my studies. However, the threshold used by some states of three or more Japanese beetles per ear, silks are clipped to less than 13 mm (0.5 inch), and pollination is less than 50% complete (Cook and Gray 2003, Hodgson 2009) appears appropriate, especially in stressful pollination environments, given the results of my studies. My personal observation is that Japanese beetles tend to be more numerous along field margins, so scouting the field interior would be necessary to determine the need for a foliar insecticide application. One application, if needed, would likely be satisfactory given the relatively short duration of sensitivity to silk clipping observed in most studies.

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Part III

Intra-ear Compensation of Field Corn, *Zea mays* L., from Simulated and Naturally-Occurring Injury by Ear-Feeding Larvae

Abstract

Ear-feeding larvae, such as corn earworm, *Helicoverpa zea* Boddie, and fall armyworm, *Spodoptera frugiperda* J. E. Smith, (Lepidoptera: Noctuidae), can be important insect pests of field corn, *Zea mays* L., in the midsouthern U.S. Recently introduced, stacked Bt traits provide additional protection from ear-feeding larvae. Following either simulated or naturally occurring corn earworm injury to ear tips, corn ears were evaluated to determine how yield parameters including total kernel number, total kernel weight and individual kernel size were affected by different levels of kernel injury. Simulated corn earworm injury reduced total kernel weight when kernels were injured at the blister and milk stage in 2010 and when 60 or 120 kernels per ear were injured at the milk stage in 2011. In 2010, little or no indication was found that other kernels within the ear compensated for this injury. In 2011, simulated injury inflicted at both the blister and milk stage resulted in an increase in the size of kernels within the same ear. For naturally occurring injury observed on multiple corn hybrids during 2011 and 2012, the analyses showed either no or a very weak relationship between number of kernels injured by corn earworm and the total numbers of kernels per ear, total kernel weight or the size of individual kernels. The results indicate that intra-ear compensation for kernel injury to ear tips can occur under at least some conditions, and these data may explain why new Bt corn traits that provide better protection from ear feeding pests have not consistently improved yields in field corn.

Key words: corn, corn earworm, *Helicoverpa zea*, simulated injury, compensation

Introduction

Many of the most devastating agricultural pests are noctuids (Lepidoptera: Noctuidae), including the corn earworm, *Helicoverpa zea* Boddie, and fall armyworm, *Spodoptera frugiperda* J. E. Smith (Pedigo 2002). Both are important insect pests of corn throughout the southern U.S. The corn earworm is indigenous to North America and, due to its migratory ability, can be found anywhere on the continent where corn , *Zea mays* L., is grown (Pedigo 2002; Westbrook and Lopez 2010; Molina-Ochoa et al. 2010). Corn earworm successfully overwinters at latitudes below the 40th parallel, which includes the southern U. S. where severe infestations from multiple generations may occur annually (Blanchard et al. 1942, Wiseman 1999, Pedigo 2002).

In much of the South, the corn earworm is the most common caterpillar found infesting field corn. The early summer generation is often concentrated in corn where newly-hatched larvae feed on silks while making their way into the ear where they feed on developing kernels, potentially causing direct loss of grain (Buntin et al. 2001, Pedigo 2002). Corn earworms often feed on kernels at the ear tip and they may also feed on kernels down the sides of ears (Steffey et al., 1999). Corn earworm larvae spend little time outside the ear which makes them difficult to control with foliar insecticide applications (Rector et al. 2002). A young corn earworm larva can feed in the developing ear from early blister (R2) stage until it leaves to pupate, typically in the dough stage (R4) (White and Scott 1983, Ritchie et al. 1986). Often, only one large corn earworm is found per ear in non-Bt corn, most likely due to larval cannibalism (White and Scott 1983). White and Scott (1983) found no differences in level of damage incurred regardless of whether one, two, or four larvae were artificially infested. Field corn is commonly left untreated due to the relatively low cash value of the crop and the difficulty in controlling corn earworm even though its damage to corn ears can be severe (Buntin et al. 2001, Rector et al. 2002).

Fall armyworm is native to South America, Central America and the southeastern U.S. (Quisenberry 1999, Pedigo 2002). Moths are capable of migrating as far north as Canada during the growing season (Pedigo 2002). Fall armyworm damages corn ears similarly to corn earworm. Small larvae feed on silks and developing kernels in the ears (Ni et al. 2007). Heavy infestations of fall armyworm at the silking stage can destroy the tassel, ear, and leaves of the uppermost part of the plant (Quisenberry 1999). Feeding by corn earworm or fall armyworm may also provide entry wounds for secondary pests like sap beetles, *Carpophilis* spp. (Coleoptera: Nitidulidae), and pathogens such as *Aspergillus flavus* (Smeltzer 1958, McMillian et al. 1985, Rodriguez-del-Bosque et al. 1998, Pedigo 2002). *A. flavus* infection can result in aflatoxin and drastically reduce the market value of the corn grain.

Corn hybrids that express multiple Bt toxins with activity on ear-feeding larvae have been recently introduced. These hybrids may reduce kernel injury caused by corn earworm and fall armyworm (Reay-Jones and Wiatrak 2011, Siebert et al. 2012) although it is not clear if grain yield is increased with less ear-feeding. The objectives of this study were to determine how kernel injury at different ear stages of development affected yield and to evaluate how much intra-ear compensation might occur in response to injury to kernels in ear tips.

Materials and Methods

Simulated Corn Earworm Injury.

Experiments were conducted in 2010 and 2011 at the West Tennessee Research and Education Center in Jackson, TN. On 31 March 2010 and 8 April 2011, a genetically modified corn hybrid, DeKalb DKC 64-83 VT Triple Pro (Monsanto Co., St. Louis, MO) containing Cry1A.105, Cry2Ab2, and Cry3Bb1, was planted on a Lexington silt loam soil. This Bt hybrid provides exceptional control of southwestern corn borer, *Diatraea grandiosella* Dyar, and European corn borer, *Ostrinia nubilalis* Hübner (Lepidoptera: Crambidae) (Abel et al. 2000, Buntin et al. 2004, Castro et al. 2004, Siebert et al. 2012). It also has good activity on corn earworm and fall armyworm both in the whorl and ear stage (Buntin et al. 2004, Huang et al. 2006, Hardke et al. 2010, Siebert et al. 2012). Thus, any confounding effects of natural infestations of caterpillar pests were partly avoided by using a hybrid that expressed multiple Bt toxins for caterpillar pests.

Corn was planted with a John Deere (Deere and Co., Moline, IL) 7200 Max Emerge Plus vacuum planter with a seeding rate of 75,600 seeds/ha at a planting depth of 3.8 cm. All seed was treated with a commercial fungicide and insecticide. Row spacing was 76 cm, and plots were five rows by 13.7 m with one border row between each treatment row. Corn was planted no-till into soybean stubble from the previous year. Agronomic practices such as fertilization, seeding rates, and weed control followed University of Tennessee recommendations (McClure 2010).

Plots were arranged as a 4 x 2 factorial in a randomized complete block design with six replications of five ears per treatment. Main effects were four levels of kernel injury at two different growth stages, R2, blister, and R3, milk. Plants were selected that were uniform in maturity and had developing primary ears. Each plant designated for treatment was separated by four or more plants from the next selected plant in the row. Color-coded Tyvek® (DuPont, Wilmington, DE) tags were loosely attached to plants at the ear ± one node with a 22 gauge wire and labeled with the treatment. Simulated larval injury was done by husking back all leaf material on the ear to approximately 16 kernels deep from the ear apex. Only kernels that were visually assessed as pollinated were counted when implementing treatment levels. In 2010, larval injury was simulated by scraping a single blade of scissors around the entire circumference of the ear with the number of kernels targeted for injury being 0, 60, 120 and 240 kernels per ear, respectively.

The maximum level of kernel injury inflicted in 2011 was reduced. Also, instead of injuring kernels around the entire circumference of the ear tip, three consecutive rows by ten kernels deep from the ear apex were injured. This was done at zero, one, two or four places on an ear tip, resulting in treatments of 0, 30, 60 or 120 damaged kernels per ear. For the 120 kernel treatment, six adjacent rows of kernels to a depth of ten kernels at two places on the ear tip were injured; otherwise, injured areas were not adjacent. Kernels were injured using a serrated plastic table knife. All other materials and methods were consistent with those from 2010. Blister stage treatments were done 25 June 2010 and 30 June 2011. Milk stage treatments were done 1 July 2010 and 11 July 2011. Some natural infestations of small corn earworm larvae were observed at the milk stage timing during 2011, but little kernel injury resulted.

After kernel injury was inflicted, husks and silks were replaced back to their original state as much as possible, and a #33 rubber band was placed approximately 2.5 cm down from the ear (husk) tip to hold the husks in place. The scissors (2010) or knife (2011) was immersed into a small jar containing 99.5% ethanol between each ear to reduce possible contamination from disease pathogens between ears. To evaluate if husking back and replacing husks had an effect on kernel number and weight, a treatment was included each year where 15 plants were tagged but the ear left undisturbed until harvest.

Ears were hand-harvested 24 August 2010 and 25 August 2011. Each ear was shucked and placed in a 10# paper sack along with its corresponding Tyvek® tag. Sacks were placed in a forced air dryer set to 65.6°C and dried until grain moisture was 13%. Forced ambient air was applied for cooling purposes for 8 h afterward. Ears were stored in an air conditioned environment and allowed to come to equilibrium with ambient moisture. Total ear weight was taken, and each ear was shelled individually using a hand operated corn sheller (Seedburo Equip. Co., Des Plaines, IL). Grain was then screened through three 30.5 cm x 30.5 cm hand testing screens (5 mm x 19 mm slots, 4 mm x 19 mm slots, and a #18 sieve with round holes 7 mm in diameter) to remove small kernels and debris. Total number of kernels was counted using an automated seed counter (Old Mill Counter Model 850-3, Int'l. Marketing and Design Corp., San Antonio, TX).

Data were analyzed using SAS statistical software (SAS 2008). Proc MIXED and LS means (Fisher's Protected LSD) for mean separation were used to test for the main effects and interactions that the timing and level of kernel injury had on kernel numbers per ear, total kernel weight per ear, and the weight of an individual kernel on each ear ($\alpha < 0.05$). Ears that were not husked back were separately compared with husked ears that had no injury (Proc MIXED, $P < 0.05$).

Naturally-Occurring Corn Earworm Injury.

In a separate experiment conducted in 2011 and 2012, corn ears from a non-Bt corn (Monsanto Co., St. Louis, MO) and three hybrids with different Bt traits were evaluated at harvest for damage from ear-feeding insects (Table 10). The Bt hybrids included a Herculex corn (Pioneer Hi-Bred Int'l., Johnston, IA) containing Cry1F, a VT Triple hybrid (Monsanto Co., St. Louis, MO) containing Cry1Ab and Cry3Bb1, and a VT Triple Pro hybrid (Monsanto Co., St. Louis, MO) containing Cry1A.105, Cry2Ab2, and Cry3Bb1. All hybrids were planted in a randomized complete block design at a seeding rate of approximately 75,600 seeds/ha, planting depth of 3.8 cm, and a row spacing of 76 cm. All seed were treated with a commercial fungicide and insecticide. Corn was managed with standard agronomic practices such as fertilization, seeding rates, and weed control as recommended by The University of Tennessee (McClure 2010). No foliar applications of fungicide or insecticide were made to any hybrid. For each year and hybrid, 30 consecutive ears per plot were hand harvested. At the time of harvest, estimated kernel damage was evaluated by husking each ear and counting the number of harvestable kernels that were damaged by caterpillar feeding. The ears were then individually placed in labeled 10# paper sacks, and drying, storing and yield measuring were done as previously described.

Data were standardized by calculating the observed damaged kernels, total number of kernels, total kernel weight, and average weight of individual kernels for each ear as a percent of the mean observed for that hybrid and year. Actual mean estimates of these parameters are presented in Table 11. For each hybrid and year combination, linear regressions were done on standardized data for the number of damaged kernels versus total kernel weight and the number of damaged kernels versus individual kernel weight (Proc REG, SAS 2008). Linear regression of standardized data for number of damaged kernels versus total kernel weight, number of damaged kernels versus individual kernel weight, and total number of kernels versus total kernel weight was also done across all hybrids and years.

Results

Simulated Corn Earworm Injury.

The method and amount of kernel injury that was inflicted changed between years; therefore data for each year were analyzed separately. There were no differences in total kernel weight for ears that were not husked back compared with husked ears that had no injury at either timing in 2010 or 2011 ($P = 0.9339$ and $P = 0.9298$, respectively). Husking back ears but inflicting no injury also did not affect the number of kernels per ear ($P = 0.8290$ in 2010 and $P = 0.5318$ in 2011) or the average weight of individual kernels ($P = 0.9482$ in 2010 and $P = 0.7405$ in 2011) compared with ears that were not husked back.

In 2010, there was no interaction between timing of injury (either blister or milk) and the level of kernel damage on the number of kernels per ear ($P = 0.1223$) or total kernel weight per ear ($P = 0.4356$). There was a significant interaction between timing of injury and the level of kernel damage on the weight of an individual kernel ($P = 0.0275$). Therefore, these data are presented separately.

On average in 2010, the total numbers of harvested kernels per ear were reduced 42.4, 103.6 and 192.3 when the targeted treatment levels were 60, 120, and 240 kernels, respectively, at the blister stage (Table 12). All blister stage treatments differed from each other in the number of kernels and total kernel weight per ear. The total number of harvested kernels and total kernel weight decreased as the number of injured kernels was increased. The average weight of individual kernels was not affected by the amount of kernel injury inflicted on the ear.

For injury inflicted at the milk stage in 2010, the total numbers of kernels per ear were reduced by 56.8, 129.9, and 235.2 when the targeted treatment levels were 60, 120, and 240 kernels, respectively (Table 13). Similar to the blister stage treatments, all milk stage treatments differed from each other in the number of kernels and total kernel weight per ear. The total number of harvested kernels and total kernel weight decreased as more kernels were injured at the milk stage. There were no differences between the average weight of individual kernels from ears where kernels were injured and those where no kernel injury was inflicted.

In 2011, there was a significant interaction between the main effects of kernel stage at time of injury (blister or milk) and the level of kernel injury on the total number of kernels per ear ($P = 0.0151$) and total kernel weight per ear ($P = 0.0407$). Therefore, these data are presented separately. There was no interaction between kernel stage at time of injury and the level of kernel injury on individual kernel weight ($P = 0.9596$).

On average in 2011, the total numbers of harvested kernels per ear at the blister stage were reduced 11, 40 and 50 kernels when the targeted treatment levels were 30, 60 or 120 kernels, respectively. There were no differences ($P = 0.7400$) in total kernel weight regardless of the number of kernels that were injured at the blister stage (Table 14). The average weight of individual kernels increased as the number of injured kernels also increased. The average weight of individual kernels was heavier for ears where 60 or 120 kernels were injured compared with ears where no injury was inflicted. Individual kernel weight was also significantly increased for ears where 120 kernels were injured compared with those where 30 kernels were injured.

For injury inflicted during the milk stage in 2011, the total numbers of kernels per ear were reduced by 31.9, 85.1 and 115.1 for treatments where the targeted injury levels were 30, 60 or 120 kernels per ear, respectively (Table 15). Unlike injury inflicted during the blister stage, total kernel weight per ear was significantly reduced by inflicting injury at the milk stage. The treatments where 60 or 120 kernels were injured at the milk stage had significantly lower total kernel weight as harvest than ears where 0 or 30 kernels were injured. As observed at the blister stage, the average size of harvested kernels increased for treatments where more kernels were injured. Individual kernels were heavier for ear treatments where 60 or 120 kernels were targeted for damage compared with those where no injury was inflicted. Individual kernel weight also increased for ears where 120 kernels were injured compared with those where 30 kernels were injured.

Naturally Occurring Corn Earworm Injury.

The average amount of kernel damage caused by caterpillar feeding varied from approximately 1.8 to 12.3 kernels per ear, depending upon the year and hybrid (data not shown). The VT3Pro hybrid had less kernel damage and less variation in kernel damage than the other hybrids. Corn earworm was the only ear-feeding larvae observed in these experiments, and presumably all injury observed was caused by this species.

For individual regressions done by hybrid and year, there were weak and mostly non-significant relationships between of the numbers of damaged kernels per ear and total kernel weight (Table 16) or the average weight of individual kernels in that ear (Table 17). For VT3, there were significantly positive relationships between damaged kernels and total kernel weight (R^2 = 0.28) and individual kernel weight (R^2 = 0.17) in 2011. In contrast, there were negative relationships between damaged kernels and total kernel weight (R^2 = 0.14) and individual kernel weight (R^2 = 0.14) for the non-Bt hybrid in 2012. When the standardized data were regressed across years and hybrids (Figs. 1 and 2), there was no correlation between total kernel weight and the number of damaged kernels per ear (R^2 = 0.004; F = 0.99; df = 1, 237; P = 0.3215); nor was there a relationship between the average weight of individual kernels and the number of damaged kernels ($R^2 = 0.010$; $F = 2.37$; $df = 1, 237$; $P = 0.1250$). As might be expected, the total number of kernels per ear was positively and strongly correlated with total kernel weight for that ear ($R^2 = 0.80$; $F = 962.7$; $df = 1, 237$; $P < 0.0001$).

Discussion

In 2010, simulating corn earworm injury to ear tips resulted in significant reductions in the harvested number and total weight of kernels. There was little indication that the remaining, undamaged kernels compensated for injury by getting larger. The methods and amount of injury inflicted were changed in 2011, in part because the author did not feel the previous year's methods were representative of actual corn earworm injury. In 2011, injury inflicted at the blister stage did not result in a reduction in total kernel weight; whereas, higher levels of simulated corn earworm injury at the milk stages did cause yield loss. Unlike 2010, there was strong evidence that undamaged kernels compensated for injury by getting larger, and this occurred for injury inflicted at both the blister and milk stage. Why treatment responses were not consistent across years is unclear but may be due to different environmental conditions. However, final average ear weight varied only about 5% between the years. It is possible that compensation results from an increase in kernel size for those kernels adjacent and between injured kernels (i.e., those developed at the same time or after). Thus, no compensation may have been observed in 2010 because injury was inflicted around the entire circumference of the ear tips, leaving only older kernels to compensate for injury to less developed kernels. Data were not collected in a manner that allowed the testing of this hypothesis. Steckel et al. (2013) found that reduced pollination due to silk clipping resulted in other kernels within the same ear getting larger. However, previous research found that although kernels above and below partially developed or damaged kernels often appeared to be abnormally large, there was no significant weight difference (Duncan and Hatfield 1964, Woronecki et al 1980).

When compensation via increased kernel size was observed in 2011, the maximum increase in average kernel weight was 9.0% and 7.8% higher during the blister and milk stage, respectively, than that in uninjured ears. Statistically, complete compensation occurred for the reduction in kernel numbers from the most extreme treatment for injury inflicted at the blister stage (≈ 50 kernels/ear, Table 4). Thus, it is possible that I would have observed even greater compensation in the size of the remaining, uninjured kernels had I inflicted more injury. I presumably found the upper limit of compensation at the milk stage because significant yield losses were observed for the highest levels of kernel injury.

In a study of natural bird damage to maturing corn, Dyer (1975) found that damaged corn ears weighed more than undamaged ones, thus supporting a plant compensation process. It was also reported that overcompensation in ear weight could occur. Though some level of plant compensation occurred in my simulated injury test, no overcompensation was observed. Woronecki et al. (1980), when simulating bird damage, found slight compensation of kernel weight when corn was damaged at the milk stage but none at the dough (R4) growth stage. My results agree with theirs that corn compensated for a reduction in kernel number by significantly increasing individual kernel weight. White and Scott (1983) reported that kernel compensation for corn earworm damage was greater than that of blackbird damage, perhaps because corn earworm attacks ears at earlier development stages (Dyer 1975, Woronecki et al. 1980).

Another potential mechanism of compensation was indicated in my data. In these treatments, I carefully injured a targeted number of kernels that were judged to be pollinated.

However, I under-achieved the targeted goals of kernel injury. This under-achievement was especially true of injury inflicted to kernels near the ear tip that were at the blister stage in 2011 (e.g., Table 4). This suggests that some of these ear tip kernels were ultimately aborted, perhaps due to the ear not being able to adequately support all the kernels that were initially pollinated. This would indicate that these kernels at the ear tip may be more expendable and thereby able to sustain injury without impacting yield.

In a previous study, the ability of the corn plant to compensate for corn earworm injury to kernels was determined by timing of injury, duration and extent of damage to the developing ear (White and Scott 1983). Woronecki et al. (1980) found that visual assessment of low levels of damage occurring at the milk or dough stage was difficult to distinguish at harvest. Indeed, 38% and 17% of the ears that had six kernels removed at the milk and dough stages, respectively, showed no visual evidence of damage at harvest. It was hypothesized that low levels of damage could be obscured by the growth of adjacent kernels. Similar to my study, it is possible that kernels assessed as damaged were aborted before harvest. However, this seems less likely for injury inflicted at the milk or dough stages.

When evaluating non-Bt and Bt traits, the VT3Pro hybrid had the least kernel damage from a naturally-occurring infestation of corn earworm in both years of my study (Table 6). This agrees with research that found hybrids containing these multiple Bt traits reduce injury caused by lepidopteron larvae that feed in the ear (Siebert et al. 2012). Based on regression analyses, there was little evidence that natural infestations of corn earworm caused significant yield loss or that compensation occurred in our tests. The response by the VT3 hybrid in 2011 indicated some overcompensation due to an increase in average kernel size. However, the opposite effect was observed in the non-Bt corn in 2012. Thus, the only significant relationships observed were weak and contradictory. Based on visual assessments at the time of harvest, the maximum level of injury I observed from natural infestation of corn earworm averaged no more than 12.3 kernels per ear. This is only about one-third of that inflicted in my lowest simulated injury treatment, and based on my simulated injury studies would not have resulted in a statistically significant reduction in total kernel weight (i.e., yield) or a measureable impact on individual kernel size.

My study has shown that compensation for injury caused by ear-feeding larvae to ear tip kernels can occur in some cases. The level of compensation is likely determined by the timing and intensity of kernel damage as well as the environment.   These results would suggest that, although new Bt hybrids may reduce kernel injury by lepidopteran larvae compared with old Bt technologies, this may not translate into increased yield. This is consistent with observations by the author when comparing yields among various Bt corn technologies with near-isogenic genetic backgrounds (unpublished data).

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Conclusions

The overall objective of this research was to determine the compensation ability of corn to certain types of injury including stalk tunneling, silk clipping, and kernel feeding. The first component of this research emphasized that the effects southwestern corn borer infestations on non-Bt corn plants within a “refuge-in-a-bag” scenario where the non-Bt plants were surrounded by Bt corn plants. The main objective was to determine how infestations of southwestern corn borer impacted the yield of non-Bt plants and whether neighboring, Bt plants would compensate for yields losses. This information helps to predict what potential risk of yield loss there would be from southwestern corn borer infestations in a refuge in a bag scenario. Results from this study found that for non-Bt plants, there was a negative relationship between the number of infested larvae and the total kernel weight per ear (yield). However, the timing of the infestation had a greater impact on yield than did the number of larvae infested with earlier infestations causing the most yield loss. Little or no compensation occurred by neighboring Bt plants in this system. Thus, yield losses from southwestern corn borer infestations would be directly proportional to the percentage of non-Bt plants in the system and yield loss observed in these plants. These results would likely apply to other stalk-tunneling pests.

The second component of my research emphasized the effect of silk clipping on yield parameters using manually clipped silks and by caging Japanese beetle adults on ears. These results will help producers make decisions about the needs for insecticide applications to control silk clipping insects in pollinating field corn. Results indicated that manually clipping silks once daily had modest effects on yield parameters, but clipping more frequently or when done by caged beetles on ears may reduce the number of kernels per ear and reduce the total kernel weight per ear (yield). Increases in the weight of individual kernels on an ear occurred when pollination was reduced by silk clipping, suggesting some compensation occurred. In my tests, no yield losses were observed when clipping occurred after the first five days of silking. However, drought or heat stress may increase the sensitivity of corn to the effects of silk clipping. As suggested in some Midwestern states, a threshold of three or more Japanese beetles per ear, silks are clipped to less than 13 mm (0.5 inch), and pollination is less than 50% complete appears to be adequate. Manually clipping silks multiple times per day appeared to effectively simulate feeding by Japanese beetle clipping, and this should have implications for future research with silk clipping insects.

The third component of my research examined the potential effects of ear-feeding larvae on yield. The intent of this study was to elucidate how much yield might be lost by kernel feeding by insects such as the corn earworm, and thus, better understand the potential value of Bt technologies that reduce injury by ear-feeding larvae. Results from these studies found that simulated corn earworm injury could reduce total kernel weight (yield) when kernels were injured at the blister and milk stage. In the second year of my tests, other kernels in the ear compensated for injury by growing larger. For naturally-occurring injury observed on multiple corn hybrids, there was no or a weak relationship between number of kernels injured by corn earworm and the total numbers of kernels per ear, total kernel weight or the size of individual kernels. My results indicate that intra-ear compensation for kernel injury to ear tips can occur under at least some conditions, and these data may explain why new Bt corn traits that provide better protection from ear feeding pests have not consistently improved the yields of field corn grown in Tennessee.

Tennessee corn producers have a substantial investment made in their corn crop each year. Currently, Tennessee farmers have approximately $110 US in seed costs per acre of corn planted, representing a considerable "up front" investment. Hybrid selection is perhaps the most important decision a corn producer can make to influence the profitability or his/her crop. Stacked traits and newer traits cost a premium to these farmers. Also, the market price for corn continues to rise. This influences the economic threshold for insect pests in field corn as the threshold for damaging pests should decline as the value of the crop rises. Knowing how yield may be impacted by silk clipping insects is important to determining if and when an insecticide application is warranted. Knowing what yield protection newer Bt technologies will bring to the table for producers is imperative to helping them make sound judgments on the value of these traits.

Overall, my studies help corn growers determine 1) the potential risks and rewards of using a "refuge in a bag" system on their farms; 2) if and when a foliar insecticide application is warranted to prevent silk clipping by Japanese beetles during pollination; and 3) the potential value of new Bt corn technologies that provide more protection against ear-feeding pests, particularly the corn earworm.

Appendices

Appendix A

Tables

Table 1. Treatments used to assess the influence of density of southwestern corn borer larvae per plant at different growth stages of corn at time of infestation.

Treatment number	Number of larvae	Corn growth stage, 2010 and 2011
1	0	----
2	2	Mid whorl - V6 (2010) or V8 (2011)
3	2	Late whorl - V14
4	2	Blister - R2
5	4	Mid whorl - V6 (2010) or V8 (2011)
6	4	Late whorl - V14
7	4	Blister - R2
8	6	Mid whorl - V6 (2010) or V8 (2011)
9	6	Late whorl - V14
10	6	Blister - R2

Table 2. Effects of infesting different densities of southwestern corn borer larvae on non-Bt plants, 2010 and 2011.

Larvae infested per plant	Tunnel length per plant (cm)		Kernels per ear		Kernel wt per ear (g)		Individual kernel wt (g)	
	2010	2011	2010	2011	2010	2011	2010	2011
Not infested	7.4***	6.8***	585.4***	492.3***	194.0***	145.8***	0.3305***	0.2852***
2	17.7 a	14.4 a	378.0 a	401.5 a	124.3 a	113.4 a	0.2557 a	0.2602 a
4	31.2 b	19.4 b	328.9 ab	373.5 ab	106.6 b	102.4 ab	0.2247 b	0.2484 a
6	34.4 b	22.8 c	299.2 c	339.8 c	95.3 b	93.9 b	0.2114 b	0.2521 a
F-value	*6.36*	*12.26*	*5.6*	*4.0*	*5.5*	*4.2*	*4.6*	*0.49*
DF	*2, 159*	*2, 300*	*2, 212*	*2, 300*	*2, 212*	*2, 300*	*2, 212*	*2, 300*
P-value	*0.0021*	*<0.0001*	*0.0043*	*0.0198*	*0.0049*	*0.0162*	*0.0110*	*0.6108*

*, **, *** Indicates whether the non-infested treatment was significantly different from one, two or all three infested treatments, respectively (Dunnett's test, $P < 0.05$).

Means not followed by a common letter are significantly different (Proc MIXED, LS means, $P < 0.05$).

Table 3. Effects on Bt plants when a neighboring, non-Bt plant was infested with different densities of southwestern corn borer larvae, 2010 and 2011.

Larvae infested per plant	Tunnel length per plant (cm)		Kernels per ear		Kernel wt per ear (g)		Individual kernel wt (g)	
	2010	2011	2010	2011	2010	2011	2010	2011
Not infested	0	---	577.6	568.1	193.6	163.6	0.3334	0.2870
2	0.55	---	575.3	559.0	190.6	161.0	0.3299	0.2875
4	0.88	---	585.3	553.6	196.3	156.7	0.3349	0.2827
6	0.45	---	576.6	555.0	194.2	158.4	0.3363	0.2849
F-value	*0.39*		*0.6*	*1.02*	*0.89*	*1.15*	*1.66*	*1.94*
df	*2 ,212*		*2, 212*	*2, 300*	*2, 212*	*2, 300*	*2, 212*	*2, 300*
P-value	*0.6751*		*0.55*	*0.3632*	*0.4108*	*0.3181*	*0.1922*	*0.1455*

Means not followed by a common letter are significantly different (Proc MIXED, LS means, $P < 0.05$).

Table 4. Effects of infesting southwestern corn borer larvae on non-Bt corn plants at different growth stages, 2010 and 2011.

Growth stage at time of infestation	Tunnel length per plant (cm)		Kernels per ear		Kernel wt per ear (g)		Individual kernel wt (g)	
	2010	2011	2010	2011	2010	2011	2010	2011
Not Infested	7.4**	6.8***	585.4**	492.3**	194.0**	145.8**	0.3305*	0.2852*
V6 (2010) V8 (2011)	8.2 b	16.4 b	69.0 c	294.6 c	22.9 c	87.6 b	0.0694 b	0.2438
V14	29.0 a	18.4 b	391.1 b	343.9 b	128.7 b	97.5 b	0.3030 a	0.2573
R2	31.7 a	21.8 a	546.1 a	476.4 a	174.6 a	124.5 a	0.3193 a	0.2596
F-value	*12.26*	*5.13*	*163.9*	*36.84*	*154.5*	*15.88*	*173.5*	*0.99*
df	*2, 159*	*2, 300*	*2, 212*	*2, 300*	*2, 212*	*2, 300*	*2, 212*	*2, 300*
P-value	*<0.0001*	*0.0064*	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*	*0.3709*

*, **, *** Indicates whether the non-infested treatment was significantly different from one, two or all three infested treatments, respectively (Dunnett's test, $P < 0.05$).

Means not followed by a common letter are significantly different (Proc MIXED, LS means, $P < 0.05$).

Table 5. Effects on Bt plants when a neighboring, non-Bt plant was infested at different growth stages with southwestern corn borer larvae, 2010 and 2011.

Growth stage at time of infestation	Tunnel length per plant (cm)		Kernels per ear		Kernel wt per ear (g)		Individual kernel wt (g)	
	2010	2011	2010	2011	2010	2011	2010	2011
Not Infested	0	---	577.6	568.1	193.6	163.6	0.3334	0.2871
V6 (2010) V8 (2011)	0.39	---	595 b	558.5	202.6 b	158.8	0.3403 b	0.2840
V14	0.97	---	577.4 ab	549.4	192.4 a	156.5	0.3321 a	0.2843
R2	0.52	---	564.8 a	559.8	186.1 a	160.9	0.3287 a	0.2868
F-value	*0.74*		*4.69*	*1.01*	*7.49*	*1.14*	*5.27*	*0.76*
df	*2, 212*		*2, 212*	*2, 300*	*2, 212*	*2, 300*	*2, 212*	*2, 300*
P-value	*0.4783*		*0.0102*	*0.3657*	*0.0007*	*0.321*	*0.0059*	*0.4663*

Means not followed by a common letter are significantly different (Proc MIXED, LS means, $P < 0.05$).

Table 6. The number of kernels per ear, total kernel weight and individual kernel weight for ears where silks were manually clipped once daily beginning on different days and for different durations after first silk, 2010 and 2011.

Clipping, days of silking	Kernels per ear		Kernel wt per ear (g)		Individual kernel wt (g)	
	2010	2011	2010	2011	2010	2011
No clipping	588.2a	597.7a	199.6a	171.8a	0.3395cd	0.2867a
1 - 5	556.2a	580.0a	196.0a	164.8a	0.3526ab	0.2843a
1 - 10	568.3a	590.3a	199.8a	168.4a	0.3525ab	0.2860a
1 - 15	558.0a	598.0a	200.0a	167.4a	0.3596a	0.2801a
6 - 10	586.0a	577.8a	197.5a	168.4a	0.3369d	0.2923a
6 - 15	575.7a	590.5a	200.6a	173.9a	0.3482bc	0.2948a
11 - 15	583.7a	597.5a	199.8a	174.2a	0.3425bcd	0.2911a
50% of silks (1 - 15)	583.9a	595.6a	200.6a	174.2a	0.3442bcd	0.2927a
50% of length (1 - 15)	601.7a	581.2a	203.9a	172.6a	0.3389cd	0.2966a
F-value	*1.7*	*0.6*	*0.3*	*0.8*	*4.0*	*1.8*
df	*8, 256*	*8, 256*	*8, 256*	*8, 256*	*8, 256*	*8, 256*
P > F	*0.0933*	*0.8205*	*0.9750*	*0.6053*	*0.0002*	*0.0762*

Means in a column not followed by a common letter are significantly different (Proc MIXED, Fisher's Protected LSD, $P < 0.05$).

Table 7. The number of kernels per ear, total kernel weight and individual kernel weight for treatments where silks were manually clipped once daily during the first five days of silking versus treatments where clipping began on the sixth day or later, 2010 and 2011.

Initiation of clipping	Kernels per ear		Kernel wt per ear (g)		Individual kernel wt (g)	
	2010	2011	2010	2011	2010	2011
Early[a]	560.8b	589.4a	198.6a	166.8a	0.3549a	0.2835b
Late[b]	581.8a	588.6a	199.3a	172.2a	0.3425b	0.2927a
F-value	*4.8*	*0.01*	*0.04*	*3.2*	*14.6*	*8.8*
df	*1, 173*	*1, 173*	*1, 173*	*1, 173*	*1, 173*	*1, 173*
P > F	*0.0307*	*0.9257*	*0.8374*	*0.0760*	*0.0002*	*0.0035*

[a] Treatments where clipping occurred on days of silking 1 – 5, 1 – 10, and 1 – 15.

[b] Treatments where clipping occurred on days of silking 6 - 10, 6 - 15, and 11 - 15.

Means in a column not followed by a common letter are significantly different (Proc MIXED, Fisher's Protected LSD, $P < 0.05$).

Table 8. The number of kernels per ear, total kernel weight and individual kernel weight for ears where silks were manually clipped three times daily during the first five days of silking versus ears where clipping began on the sixth day and continued to the tenth day of silking, 2011 and 2012.

Clipping, days of silking	Kernels per ear	Kernel wt per ear (g)	Individual kernel wt (g)
No clipping	556.1a	161.5a	0.2913b
1 - 5	470.9b	144.2b	0.3078a
6 - 10	562.4a	161.3a	0.2872b
F-value	*20.7*	*7.8*	*5.8*
df	*2, 84*	*2, 84*	*2, 84*
P > F	*< 0.0001*	*0.0008*	*0.0045*

Means in a column not followed by a common letter are significantly different (Proc MIXED, Fisher's Protected LSD, $P < 0.05$).

Table 9. The number of kernels per ear, total kernel weight and individual kernel weight when different numbers of Japanese beetle adults were caged on ears during the first five days of silking, Missouri and Tennessee, 2011. The percent of silks clipped and average silk length when cages were removed is also shown.

Beetles infested per plant	Percent silks clipped	Average silk length (cm)	Kernels per ear		Kernel wt per ear (g)		Individual kernel wt (g)	
	Both states	Both states	Missouri	Tennessee	Missouri	Tennessee	Missouri	Tennessee
0	0.0d	8.1a	411.2a	601.7a	124.2a	181.3a	0.3021a	0.3014ab
2	39.7c	4.4b	399.8a	581.2a	123.2a	169.4a	0.3083a	0.2911b
4	79.7b	2.6c	331.9b	569.0a	107.1a	173.9a	0.3203a	0.3058ab
8	98.0a	0.2d	268.9c	555.2a	83.9b	175.1a	0.3110a	0.3164a
F-value	*305.8*	*173.7*	*12.6*	*1.5*	*7.1*	*0.7*	*0.7*	*2.8*
df	*3, 100*	*3, 100*	*3, 42*	*3, 56*	*3, 42*	*3, 56*	*3, 42*	*3, 56*
P > F	*< 0.0001*	*< 0.0001*	*< 0.0001*	*0.2195*	*0.0006*	*0.5405*	*0.5790*	*0.0500*

Means in a column not followed by a common letter are significantly different (Proc MIXED, Fisher's Protected LSD, $P < 0.05$).

Table 10. List of hybrids, planting date and harvest date, 2011 and 2012.

Bt trait[a]	Hybrid	Planting date	Harvest date
Non-Bt	DKC 63-80	8 April 2011	25 August 2011
HX	Pioneer 1615	31 May 2011	13 September 2011
VT3	DKC 67-40	31 May 2011	13 September 2011
VT3P	DKC 67-82	31 May 2011	13 September 2011
Non-Bt	DKC 66-94	18 May 2012	15 September 2012
HX	Pioneer 1615	10 May 2012	15 September 2012
VT3	DKC 69-40	10 May 2012	15 September 2012
VT3P	DKC 67-88	10 May 2012	15 September 2012

[a] Non-Bt = no Bt traits, HX = Herculex, VT3 = VT Triple, VT3P = VT Triple Pro.

Table 11. Naturally occurring corn earworm feeding. Means for damaged kernels, total number of kernels and total kernel weight per ear for various hybrids, 2010 and 2011.

Year	Hybrid[a]	Damaged kernels	Total kernels	Total kernel wt (g)
2010	Non-Bt	8.7 ± 2.2	495.2 ± 17.1	128.9 ± 4.8
	HX	6.9 ± 1.8	444.7 ± 18.2	85.0 ± 4.4
	VT3	9.3 ± 1.4	441.0 ± 19.4	116.4 ± 7.5
	VT3P	3.3 ± 0.9	493.8 ± 13.6	149.6 ± 5.7
2011	Non-Bt	6.2 ± 1.2	364.1 ± 12.7	117.3 ± 5.1
	HX	12.3 ± 2.5	578.9 ± 20.44	188.9 ± 7.8
	VT3	7.2 ± 1.5	521.7 ± 9.10	167.1 ± 3.4
	VT3P	1.8 ± 0.5	564.0 ± 13.2	196.7 ± 6.2

[a] Non-Bt = no Bt traits, HX = Herculex, VT3 = VT Triple, VT3P = VT Triple Pro.

Table 12. Effects of simulating injury by ear-feeding larvae at the blister stage of corn on the number of kernels per ear, kernel weight per ear and individual kernel weight, 2010.

No. of kernels targeted for injury	No. kernels per ear	Total kernel wt (g)	Individual kernel wt (g)
0	581.3a	193.9a	0.3335a
60	538.9b	182.1b	0.3378a
120	477.7c	161.8c	0.3389a
240	389.0d	127.3d	0.3263a
F-value	91.95	71.62	1.86
df	3, 96	3, 96	3, 96
$P > F$	<0.0001	<0.0001	0.1412

Means not followed by a common letter are significantly different (Proc MIXED, LS means, $P < 0.05$)

Table 13. Effects of simulating injury by ear-feeding larvae at the milk stage of corn on the number of kernels per ear, kernel weight per ear and individual kernel weight, 2010.

No. of kernels targeted for injury	No. kernels per ear	Total kernel wt (g)	Individual kernel wt (g)
0	597.2a	198.9a	0.3327a
60	540.4b	179.1b	0.3309a
120	467.3c	155.1c	0.3324a
240	362.0d	123.6d	0.3426a
F-value	107.35	64.92	1.40
df	3, 96	3, 96	3, 96
$P > F$	< 0.0001	< 0.0001	0.2481

Means not followed by a common letter are significantly different (Proc MIXED, LS means, $P <$ 0.05)

Table 14. Effects of simulating injury by ear-feeding larvae at the blister stage of corn on the number of kernels per ear, kernel weight per ear and individual kernel weight, 2011.

No. of kernels targeted for injury	No. kernels per ear	Total kernel wt (g)	Individual kernel wt (g)
0	564.9a	155.2a	0.2744c
30	553.3ab	157.2a	0.2841bc
60	524.8bc	151.2a	0.2880ab
120	514.3c	153.9a	0.2992a
F-value	4.94	0.42	6.14
df	3, 96	3, 96	3, 96
$P > F$	0.0031	0.7400	0.0007

Means not followed by a common letter are significantly different (Proc MIXED, LS means, $P < 0.05$)

Table 15. Effects of simulating injury by ear-feeding larvae at the milk stage of corn on the number of kernels per ear, kernel weight per ear and individual kernel weight, 2011.

No. of kernels targeted for injury	No. kernels per ear	Total kernel wt (g)	Individual kernel wt (g)
0	592.3a	165.2a	0.2791c
30	560.4b	162.0a	0.2888bc
60	507.2c	149.3b	0.2939ab
120	477.2d	143.5b	0.3009a
F-value	4.21	2.53	1.69
df	3, 96	3, 96	3, 96
$P > F$	< 0.0001	< 0.0001	0.0417

Means not followed by a common letter are significantly different (Proc MIXED, LS means, $P < 0.05$)

Table 16. Naturally-occurring corn earworm feeding: Regression of damaged kernels versus total kernel weight for various hybrids, 2011 and 2012.

Year	Hybrid[a]	Slope	R^2	F	df	$P > F$
2011	Non-Bt	-0.025 ± 0.028	0.0273	0.76	1, 27	0.3921
	HX	0.002 ± 0.038	0.0001	0.00	1, 28	0.9606
	VT3	0.224 ± 0.067	0.2848	11.2	1, 28	0.0024
	VT3P	0.040 ± 0.025	0.0809	2.47	1, 28	0.1276
2012	Non-Bt	-0.081 ± 0.038	0.1378	4.48	1, 28	0.0434
	HX	0.016 ± 0.038	0.0060	0.17	1, 28	0.6847
	VT3	0.014 ± 0.018	0.0198	0.57	1, 28	0.4579
	VT3P	0.002 ± 0.020	0.0003	0.01	1, 28	0.9229

[a] Non-Bt = no Bt traits, HX = Herculex, VT3 = VT Triple, VT3P = VT Triple Pro.

Table 17. Naturally-occurring corn earworm feeding: Regression of damaged kernels versus individual kernel weight for various hybrids, 2011 and 2012.

Year	Hybrid[a]	Slope	R^2	F	*df*	P > F
2011	Non-Bt	0.008 ± 0.015	0.0105	0.29	1, 27	0.5970
	HX	0.024 ± 0.013	0.1102	3.47	1, 28	0.0730
	VT3	0.082 ± 0.035	0.1650	5.53	1, 28	0.0259
	VT3P	0.011 ± 0.014	0.0215	0.62	1, 28	0.4390
2012	Non-Bt	-0.045 ± 0.021	0.1440	4.71	1, 28	0.0386
	HX	0.005 ± 0.014	0.0043	0.12	1, 28	0.7305
	VT3	0.004 ± 0.008	0.0099	0.28	1, 28	0.6001
	VT3P	0.003 ± 0.009	0.0034	0.10	1, 28	0.7581

[a] Non-Bt = no Bt traits, HX = Herculex, VT3 = VT Triple, VT3P = VT Triple Pro.

Appendix B

Figures

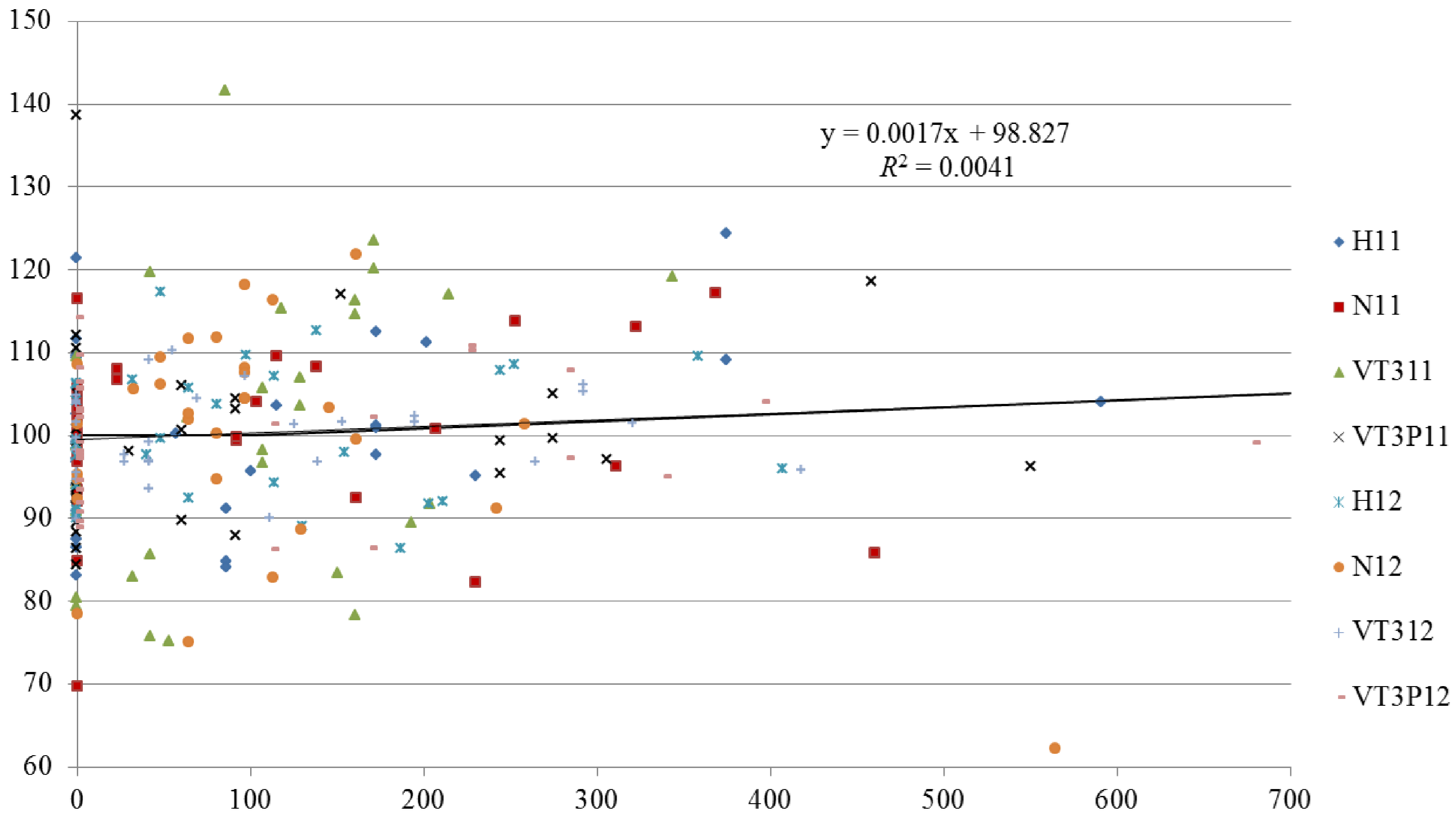

Figure 1. Regression of number of damaged kernels on total kernel weight across hybrids and years. H11 = Herculex 2011, N11 = Non-Bt 2011, VT3 11 = VT Triple 2011, VT3P = VT Triple Pro 2011, H12 = Herculex 2012, N12 = Non-Bt 2012, VT312 = VT Triple 2012, and VT3P12 = VT Triple Pro 2012.

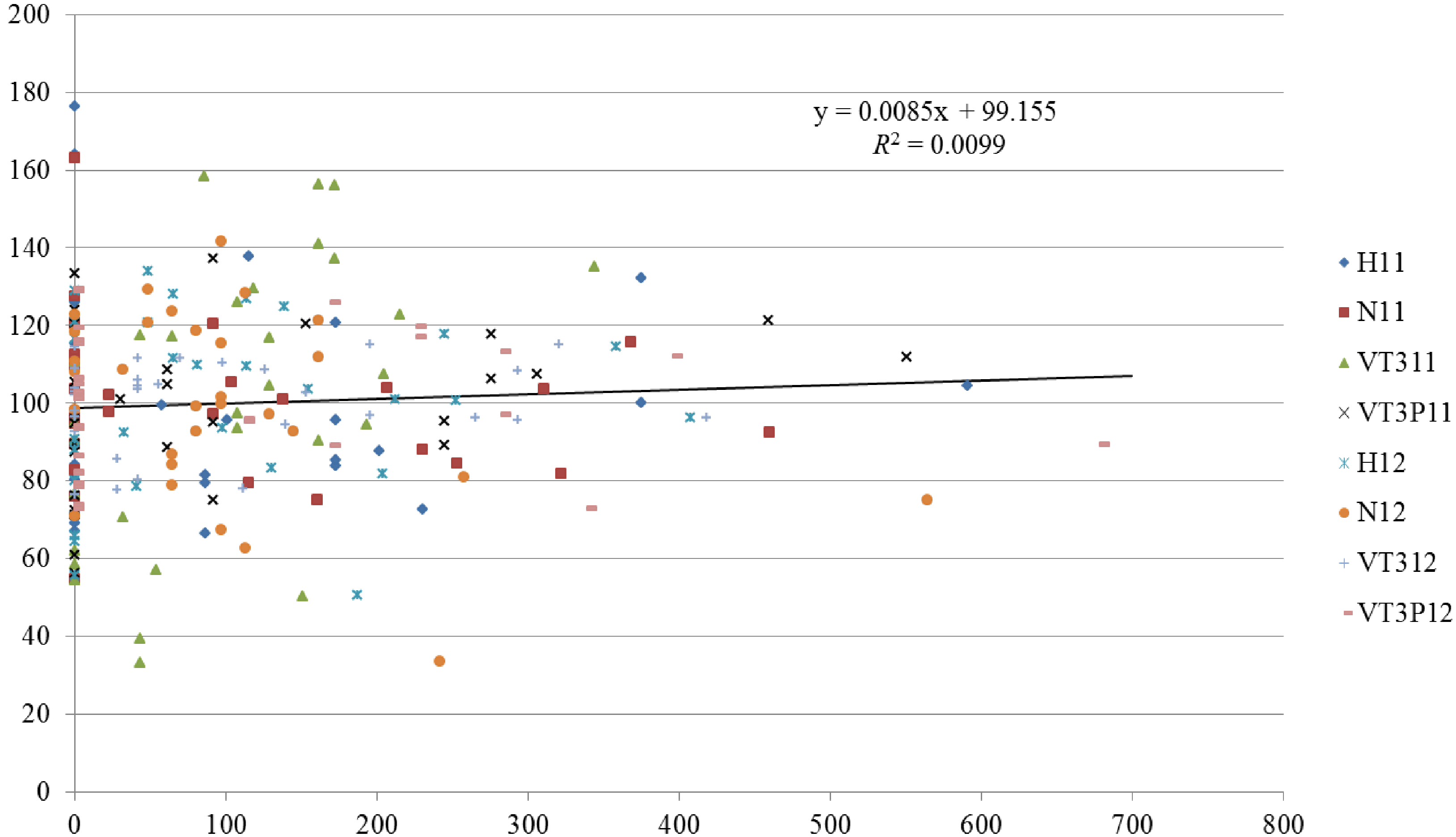

Figure 2. Regression of number of damaged kernels on individual kernel weight across hybrids and years. H11 = Herculex 2011, N11 = Non-Bt 2011, VT3 11 = VT Triple 2011, VT3P = VT Triple Pro 2011, H12 = Herculex 2012, N12 = Non-Bt 2012, VT312 = VT Triple 2012, and VT3P12= VT Triple Pro 2012.

Vita

Sandy is a native of Richview, IL where she grew up on a small livestock and grain farm. She worked on the family farm for 17 years and also farmed some land on her own for 12 of those years before returning to school. She received her B.S. in Agronomy from the University of Illinois at Urbana-Champaign in 2002. She has been a research technician in the Department of Entomology and Plant Pathology at the University of Tennessee since 2005.