12-11-2015

Impact of Corn Earworm on Field Corn Yield and Grain Quality

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By

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A Thesis
Submitted to the Faculty of
Mississippi State University
in Partial Fulfillment of the Requirements
for the Degree of Master of Science
in Agricultural Life Sciences
in the Department of Biochemistry, Molecular Biology,
Entomology, and Plant Pathology

Mississippi State, Mississippi

December 2015

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Pages in Study: 83

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Field corn, *Zea mays* L., has been introduced to the market with pyramided *Bacillus thuringiensis* (Bt) corn technologies. These technologies reduce kernel damage from ear feeding caterpillar pests, including corn earworm, *Helicoverpa zea* (Boddie). The first generation Bt traits in field corn demonstrated limited activity on corn earworm feeding on grain in ears. The pyramided corn technologies have greater cumulative protein concentrations and improved expression throughout the plant, so these corn traits should provide an effective management tool against these pests. In addition, reduced kernel injury may also have a direct effect on physical grain quality. The results for this trial showed no relationship between number of damaged kernels and yield was observed for corn hybrid expressing the Herculex®, YieldGard®, or Genuity VT Triple Pro® technologies. A significant relationship between both damaged kernels and test weight and damaged kernels and aflatoxin concentrations was observed in two technologies.

DEDICATION

I dedicate this thesis to my late father, Ronnie Bibb. If it was not for you I would have never discovered my passion for agriculture and everything it involves, nor would I have discovered my love for entomology. Thank you for your never ending support of every endeavor I was ever involved in. From softball to cattle to insects you were always there rooting me on. I would not be where I am or the person I am today if it was not for you. I love you dad!

ACKNOWLEDGEMENTS

I would like to thank my advisors, Drs. Angus Catchot, and Don Cook, for taking a chance on an animal agriculture student and showing me a whole new world of entomology and row crops. Your guidance and support has been never ending and always available whenever I needed it and for that I am greatly appreciative. I would also like to thank the rest of my graduate committee: Dr. Fred Musser, Dr. Scott Stewart, Dr. B.R. Leonard, and Dr. Tom Allen for all their guidance and help on this research project and thesis. I would also like to thank Dr. David Buntin for his support of this project and allowing me to have research plots at his location.

The Mississippi Corn Promotion Board and Monsanto for providing financial support of this study. The Mississippi Agricultural and Forestry Experiment Station (Blake Garrard), Mississippi State Extension Service, Mississippi State University Delta Research and Extension Center (Neil Wright), University of Tennessee West Tennessee Experiment Station (Sandy Steckel), LSU AgCenter's Macon Ridge Research Station (Shelby Williams and Karla Emfinger), and University of Georgia Griffin Experiment Station (W.R. Slaughter and J.T. Strickland) personnel for all their support and supplement of space and resources while conducting this study. Thanks to all the faculty, staff, and students at Mississippi State University for making this a positive work and learning experience for me.

I would like to thank Kevin Lanford, Chris Hood, Will Scott, Jonathan Sykes, Lucas Owen, John Smith, Blake Goldman, John Randle Wells, Scott Graham, Thomas Shipp, Walt Grant, Joel Moore, Angus Catchot III, Brian Adams, Ben VonKanel, Wes McPherson, Joshua Jones, Ryan Mann, John North, Adam Whalen, Andrew Adams, Wes Humphreys, Michael Temple, Cody Smith, Teresa Ziegelmann, Kathy Knighten, Dung Bao and everyone else for all their endless hours sampling my research and for all their support and teachings in everything that is entomology and row crop, from rearing insects to planting corn plots.

I would like to thank my mother for her never ending support and encouragement through this entire process and for always picking up the phone no matter how many times I had already called that day/hour. I would like to thank my boyfriend Lloyd, not just for his never ending love and support of me and my goals, but for as all his help and assistance in my research. Without his help and support I would not be where I am today. I would also like to thank both my family and friends and Lloyd's family and friends for all their support and words of encouragement. Finally, and most of all I would like to thank our Lord and Savior Jesus Christ, with whom this entire journey was made possible.

"I Can Do All Things through Christ Who Strengthens Me"

Philippians 4:13

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CHAPTER I

LITERATURE REVIEW

Corn

Field corn, *Zea mays* (L.), is the most commonly grown field crop in the United States (USDA NASS 2012). Approximately 37,231,000 and 38,850,000 hectares (ha) of corn were grown in the United States during 2001 and 2012, respectively (USDA NASS 2012). In Mississippi, 327,795 and 339,936 ha of corn were grown during 2011 and 2012, respectively (USDA NASS 2012), with a value of $0.24/kg ($6.01/bu) during 2011 and $0.26/kg ($6.67/bu) during 2012.

Corn, also known as maize, is a C4 grass that is capable of using water, nutrients and CO_2 to produce grain starch (Mabberly 1997, Subedi and Ma 2009, Mangelsdorf 1974). Corn originated from Mexico and Central America as a wild grass that over the years has been improved upon to become one of our most productive crops (Galinat 1988). A corn plant is a vigorous, monoecious, annual plant capable of both self and cross pollination (Galinat 1988, Morris 2002). It has distinct vegetative and reproductive stages (Hanway and Ritchie 1997, Lauer 1997, Abendroth 2011). The vegetative stages are defined by the number of collars on the plant. Vegetative stages range from emergence (VE) to ca. 15-16 leaf collars (V15-V16) or tassel emergence (VT) (Hanway 1963). The reproductive stages are defined by the stages of ear development, the reproductive stages include silking (R1): silks are present and cob is beginning to form, blister (R2): when the developing kernels are "blisters" of clear fluid on the cob, milk (R3): the kernels have mostly turned yellow and contain a "milky" fluid, dough (R4): the milky fluid is changing into a "doughy" substance, dent (R5): the kernels are beginning to dent in the top center and the "milk line" has formed showing the difference in solid and liquid endosperm, and reproductive maturity (R6): the milk line has disappeared completely and a black layer has formed at the base of the kernel ceasing nutrient exchange to the kernel; this stage is also known as "black layer" or physiological maturity (Hanway 1963, Ritchie et al. 1993). The reproductive stage lasts ca. 30 days (Denmean and Shaw 1960). Corn is a day neutral plant with the growth and development dependent upon temperature (Lauer 1997, Brown 1993, Nielsen 2010). Heat unit (HU) accumulation or growing degree-days (GDD) can be used to describe the timing of biological processes, such as plant development (McMaster and Wilhelm 1997). Heat units are calculated using the average daily temperature (adding the high and low temp then dividing by 2) subtracted from the critical temperature for the crop of interest (Wang 1960, SuPak 1984, Brown 1993, Nielsen 2010). The critical temperature for corn is 10ºC (50ºF). Corn hybrids vary in heat unit requirements to reach maturity. Optimum planting times in Mississippi range from as early as February 25th to as late as April 25th (Larson 2012a). Recommended relative maturities for corn hybrids in Mississippi range from 111 to 119 days for dryland production and from 113 to 120 days for irrigated production (Larson 2012b).

In the Mid-South, numerous insect pests can infest newly planted corn seed and emerged seedlings, and include seedcorn maggot, *Delia platura* (Meigen); white grubs, *Phyllophaga* spp; Japanese beetle, *Popillia japonica* (Newman); southern corn rootworm, *Diabrotica undecimpunctata howardi* (Barber); wireworms, *Melanotus* spp. and other spp. also; lesser cornstalk borer, *Elasmopalpus lignosellus* (Zeller); bean aphid, *Aphis fabae* (Scopoli); corn root aphid, *Aphis middletoni* (Thomas); potato aphid, *Macrosiphum euphorbiae* (Thomas); greenbug, *Schizaphis graminum* (Rondani); corn leaf aphid, *Rhopalosiphum maidis* (F.); bird cherry-oat aphid, *Rhopalosiphum padi* (L.); billbugs, *Sphenophorus* spp; chinch bug, *Blissus leucopterus leucopterus* (Say); black cutworm, *Agrotis ipsilon* (Hufnagel); sugarcane beetle, *Euetheola humilis rugiceps* (LeConte); green stink bug, *Acrosternum hilare* (Say); brown stink bug, *Euschistus servus* (Say); and southern green stink bug, *Nezara viridula* (L.) (Steffey et al. 1999, Akin et al. 2012, Catchot et al. 2013). During the whorl stage: corn earworm, *Helicoverpa zea* (Boddie); fall armyworm, *Spodoptera frugiperda* (J.E. Smith); true armyworm, *Pseudaletia unipuncta*; European corn borer, *Ostrinia nubilalis* (Hübner); Southwestern corn borer, *Diatraea grandiosella* (Dyar); Southern cornstalk borer, *Diatraea crambidoides* (Grote); grasshoppers, and stink bugs can inflict damage (Steffey et al. 1999, Akin et al. 2012, Catchot et al. 2013). Stink bugs, corn borers, corn earworm, fall armyworm, Japanese beetle, and *Moodna bisinuella* (Hampson), can injure corn plants at different stages during the pre-tassel to the black layer stage (Steffey et al. 1999, Akin et al. 2012, Catchot et al. 2013).

Corn Earworm

The corn earworm, *Helicoverpa zea*, (Boddie) is a member of the family Noctuidae and the order Lepidoptera. The corn earworm is a polyphagous pest attacking multiple agronomic crops including cotton, *Gossypium hirsutum* (L.), corn, *Zea mays* (L.), soybean, *Glycine max* (L.), sorghum, *Sorghum bicolor* (L.), among others (Hayes

1988, Fitt 1989, Kennedy and Storer 2000). Depending on the crop that the earworm is found in, its common name can vary, i.e. corn: corn earworm, cotton: bollworm, soybean: podworm, sorghum: headworm, and tomato: tomato fruitworm.

The corn earworm has a holometabolous life cycle usually lasting 21-28 days during normal summertime temperatures (Drees and Jackman 1999). The number of generations can range from 1 per year in northern areas (northern U.S. and Canada) to 8 generation per year in Florida and some parts of Texas (Fitt 1989, Capinera 2000). Adults are most active at night (Lingren et al. 1982). Corn is most attractive to corn earworm adults (females) during the silking stage because of a specific kairomone produced by corn (Johnson et al. 1975). Oviposition sites are chosen on the attractiveness and robustness of the plant (Miller and Strickler 1984, Schneider et al. 1986). Oviposition generally occurs on the leaf hairs and the silks and the oviposition period can range from several days to several weeks. An individual moth can oviposit over 1,000 eggs during her lifespan (Lingren et al. 1982). Eggs are 0.5 - 0.6 mm in diameter (Fitt 1989) and will change colors from clear white to cream to brown as the embryo develops. Eggs hatch in 2-10 days depending on temperature. Upon eclosion the larvae begin feeding on young silks and move down the silk channel to the ear. Little time is spent outside the ear (Storer et al. 2001, Rector et al. 2002, Akin et al. 2012, Catchot et al. 2013). After reaching the ear, larvae begin feeding on the small, developing kernels near the tip of the ear (Buntin 1986). Many larvae can be present at the time of eclosion, but due to cannibalism, usually only one or two will survive to fully complete larval development (Mally 1892, Dial and Adler 1990). Cannibalism in corn earworm is not due to the lack of food, but is thought to be a genetic ancestral trait (Stinner et al. 1977, Chilcutt 2006).

Surviving larvae will remain on the ear and develop through 5 to 6 instars before moving to the soil to pupate (Fitt 1989). The pupal stage lasts from 10 to 25 days (Neunzig 1969, Mayer et al. 2003), unless pupae are in an overwintering state. Corn earworm overwinters as a pupa, but cannot successfully overwinter north of the 40th parallel (Hardwick 1965). Overwintering generally begins in mid-to-late fall with emergence usually in May.

Corn earworm control in field corn is difficult (Storer et al. 2001, Rector et al. 2002, Akin et al. 2012, Catchot et al. 2013). Management of ear feeding lepidopteran insect pests, particularly corn earworm, with insecticides has not been considered economical because numerous applications would be required to obtain control (Akin et al. 2012, Catchot et al. 2013). For foliar insecticide applications to be successful, they must be targeted at eggs and newly emerged larvae before they enter the ear. This is complicated by the rapid growth rate of corn silks and the short period of time larvae are outside the ear (Storer et al. 2001). Newly emerged unprotected silk material can be present within one day of an insecticide application due to rapid silk growth. Very low infestation densities can render sweet corn unmarketable (DePew 1966). Due to the high value of fresh market sweet corn, multiple insecticide applications (12 to 40 insecticide applications) for corn earworm and fall armyworm are economically viable (Janes and Green 1969, Foster 1989). The value of field corn is considerably lower and corn earworm feeding does not destroy the whole ear as with fresh market sweet corn (USDA NASS 2012). The combination of the biology of the corn plant, biology of the corn earworm, and the lower value of field corn renders control of corn earworm with foliar insecticides unprofitable; therefore corn earworm has not been considered an economic pest of field corn. Host plant resistance has the potential to control corn earworm larvae that have entered the ear (Rector et al. 2002). Also, *Bacillus thuringiensis* (Bt) corn hybrids have been introduced into the market that have significant activity against corn earworm (Akin et al. 2012). However, the impact of corn earworm infestations on field corn yield has not been fully investigated. Therefore, the value of enhanced corn earworm control provided by these new technologies is unknown.

Bacillus thuringiensis

Bacillus thuringiensis is a gram-positive soil bacterium which produces proteins that exhibit highly specific insecticidal activity (Schnepf et al. 1998, Crecchio and Stotzky 2001, Ibrahim et al. 2010). *Bacillus thuringiensis* (Bt) was discovered in a diseased silkworm colony by Japanese biologist, Shigetane Ishiwata in 1901 (Beegle and Yamamoto 1992, Reardon et al. 1994, Roh et al. 2007, Ibrahim et al. 2010). German bacteriologist, Ernst Berliner also isolated Bt from Mediterranean flour moth in 1911 (Beegle and Yamamoto 1992, Ibrahim et al. 2010). There are numerous Bt proteins and each has activity against a specific group of insects generally confined within one of the following insect orders: Lepidoptera, Diptera, or Coleoptera. The proteins produced by Bt can vary in their spectrum of activity. For example, tobacco budworm, *Heliothis virescens* (F.) is extremely sensitive to the Cry 1Ac protein, while black cutworm, *Agrotis ipsilon* (Hufnagel), is very tolerant. The two most widely used strains in commercial insecticides are *B. thuringiensis subsp. kurstaki* and *B. thuringiensis subsp. israelensis* (Thorne et al. 1986). *Bacillus thuringiensis kurstaki* has activity against a wide range of lepidopteran species that are important pests in agriculture and forestry (Adang et al. 1985, Aronson et al. 1986). *Bacillus thuringiensis israelensis* is used primarily for the control of mosquito and blackfly larvae (Aronson et al. 1986, Nester et al. 2002). All Bt proteins must be ingested for activity to occur. Most if not all Bt proteins are converted to their active form by the alkaline conditions in the insects gut. In susceptible insect species, the activated Bt toxin binds to receptors in the midgut epithelium cells. This results in pore formation in the midgut and the release of the midgut contents into the hemocoel and a cessation of feeding within several hours (Shelton 2008). Death of the insect occurs within several hours to several days.

The U.S.-EPA first approved transgenic Bt plants were Bt potatoes which were introduced in 1995. In 1996, Ciba (now part of Syngenta Seeds) and Mycogen Seeds (now part of Dow) introduced the first Bt corn hybrids. Transgenic Bt corn, potatoes, and cotton are currently grown in the United States. During 2001 transgenic Bt varieties/hybrids accounted for 69% of the cotton and 26% of the corn planted in the U. S. (Nester et al. 2002). During 2011, the percentage of U. S. corn planted to stacked Bt varieties/hybrids was 49%. In 2012, the percentage of stacked Bt varieties/hybrids had risen to 52% of the corn planted in the U. S. (USDA NASS 2012).

The first generation transgenic Bt corn hybrids expressed one Bt protein and were developed primarily to manage the corn borer complex (Ostlie et al. 1997). YieldGard® and Herculex® were two of the most widely planted single gene technologies, containing the proteins Cry1Ab (YieldGard®) and Cry1F (Herculex®). These were followed by single trait technologies for control of corn rootworm and hybrids that expressed a single trait for lepidopteran insects and one for corn rootworm. The newest Bt technology trait packages now express multiple toxins for lepidopteran pests, corn rootworm, or both. Expression of multiple traits for a target pest is referred to as pyramided traits (Hellmich and Hellmich 2012). Based on current evidence, the gene pyramiding strategy has been advantageous in preventing insect resistance to Bt technologies (Huang et al. 2002, Zhao et al. 2003).

To delay development of insect resistance to Bt crops, the EPA requires an insect resistance management plan. The insect resistance management plan is based on the 'high-dose/refuge' strategy (Livingston et al. 2000, Ostlie et al. 1997). The high-dose/refuge strategy was initially developed for single gene Bt technologies, but also applies to pyramided gene Bt technologies (Gryspeirt and Grégoire 2012).The high-dose/refuge strategy is based on several major assumptions, namely that, resistance in the insect is a recessive trait, the plant expresses a high dose of the toxin, and random mating occurs between resistant individuals from the Bt crop and susceptible individuals from the refuge crop. A high-dose is defined as expression of the toxin at a great enough concentration to kill all susceptible and heterozygous resistant individuals (Roush 1997, Gould 1998, Roush 1998, Caprio et al. 2000, Huang et al. 2002, Vacher et al. 2004, Huang et al. 2011). This results in a very low frequency of homozygous resistant individuals in the population.

The refuge component of the 'high dose/refuge' strategy for field corn involves planting part of the crop to non-Bt hybrids. The purpose of the refuge is to produce Bt susceptible individuals to mate with Bt resistant individuals that may emerge from the Bt portion of the crop. Mating between the two genotypes would produce the heterozygous individuals that would be killed by the high dose produced by the Bt crop. Single trait corn technologies with lepidopteran active traits have a refuge requirement of 50% in cotton growing regions and 20% in non-cotton growing regions. Single trait corn technologies with rootworm active traits have a refuge requirement of 20% in all areas.

Multiple or pyramided trait technologies for management of lepidopteran pests have a refuge requirement of 20% in cotton growing regions and 5% in non-cotton growing regions. Multiple or pyramided trait technologies for management of rootworms have a refuge requirement of 5% in all areas. Also, refuge deployment options vary depending on which pests the technology targets. Refuge plantings for rootworm traits are required to be in the same field as the Bt crop or directly adjacent, while refuge plantings for lepidopteran traits may be in the same field, adjacent to the field or within 0.8km.

There are several advantages to pyramiding traits including: increased efficacy, activity against a wider range of insect pests, and resistance management. Many of the pyramided trait corn technologies demonstrate much higher efficacy against certain insect pests, such as corn earworm, than the single trait technologies (Siebert et al. 2012). Another advantage to growers of the pyramided Bt corn technologies is a reduced refuge requirement (Siebert et al. 2012). Trait pyramiding should delay resistance based on an expected lack of cross resistance between two Bt toxins, so the likelihood of one individual being resistant to more than one toxin is remote (Gryspeirt and Grégoire 2012). So far, no evidence of cross resistance has been observed in pyramided technologies (Tabashnik et al. 2003).

Effect of Bt Corn Transgenic Hybrids on Corn Earworm

Many studies have been conducted to investigate the efficacy of Bt corn technologies against corn earworm (Buntin et al. 2001, Abel and Pollan 2004, Buntin et al. 2004a, Buntin et al. 2004b, Buntin 2008, Reay-Jones and Wiatrak 2011, Bohnenblust et al. 2013, Reay-Jones and Reisig 2014, Bowen et al. 2014). Storer et al. (2001) observed a significant reduction in ear damage from corn earworm, reduced larval growth, and reduced adult emergence with Cry1Ab expressing corn lines compared to non-Bt lines. Buntin et al. (2004a) observed significant reduction of grain damage from corn earworm and fall armyworm on Bt11 (Cry1Ab) and MON810 (Cry1Ab) expressing corn hybrids compared to non-Bt corn isolines. However, no differences in yield or test weight were observed between the Bt and non-Bt hybrids, which was attributed to genetic differences between the hybrids (Buntin et al. 2004a). In a different study, Buntin et al. (2004b) reported that fall armyworm injury during the whorl stage, corn earworm densities and ear damage were lower on corn expressing Cry1Ab, Cry2Ab, or Cry1Ab x Cry2Ab compared to non-Bt corn isolines. Also, the pyramided traits (Cry1Ab x Cry2Ab) resulted in lower corn earworm numbers and reduced ear injury compared to single traits. Sachs et al. (1996) proposed that stacking or pyramiding Cry1Ab with other Bt proteins could be used to delay resistance development to other Cry1A proteins (i.e. Cry1Ac used to control tobacco budworm *Heliothis virescens* (F.)).

The ability of transgenic Bt corn technologies to reduce corn earworm injury has been demonstrated; however, yield responses to reductions in insect damage have been inconsistent in field corn (Buntin et al. 2001, Buntin et al. 2004a) and in sweet corn (Burkness et al. 2001, Burkness et al. 2002). Chilcutt et al. (2007) reported that corn earworm numbers were higher on Bt corn when compared to non-Bt plants. In some instances, earlier instars were observed on Bt plants rather than non-Bt plants, which may be a result of growth inhibition caused by Bt proteins (Storer et al. 2001, Horner et al. 2003, Chilcutt et al. 2007). Chilcutt (2006) concluded that the higher densities could be a result of reduced cannibalism. Buntin (2008) did not observe significant yield differences between Bt and non-Bt corn planted early in the optimum recommended dates when corn earworm infestations were low, but did observe significant yield differences between when Bt and non-Bt corn was planted later when corn earworm infestations were higher.

There are many different factors that can offer insight into why yield increases are not commonly observed on Bt corn traits, even in the presence of significant corn earworm populations. Coop et al. (1992) indicated that higher numbers of early instar larvae on Bt corn had little to no effect on yield. Cannibalism may play a role in reducing densities of corn earworm that could have caused potential yield losses. Nishikawa and Kudo (1973) stated that many of the initial kernels found on the top part of the ear will not develop into mature kernels. Most corn earworm feeding can be found on these initial kernels which could offer another explanation to why a yield response was not observed. A masked yield response could also be the result of harvest inefficiency. Typically, smaller kernels near the ear tip, the area where corn earworm typically feeds, are expelled from the combine at harvest potentially masking any yield response that could be present. Finally, planting early in the recommended planting window provides some ability to avoid corn earworm and fall armyworm infestations making it possible to avoid yield losses caused by these pests.

Grain Quality

Both yield and grain quality are important to producers. The quality of harvested grain can influence or limit the end use of grain, which influences the value. Grain quality can be impacted by hybrid/variety, growing conditions, harvest practices, and drying operations (Pioneer 2009). In corn, quality can be defined as grain that consists of 50 percent or more of whole kernels and may contain no more than 10.0 percent of other grains for which standards have been established under the United States Grain Standards Act (Pitchford 1995). There are many components of grain quality; these include test weight, moisture content, damaged kernels, contaminated kernels (insect or fungi), odor, and foreign materials. Test weight is defined as bulk density measured under specific conditions (Bern and Brumm 2009). Test weight is a general indicator of grain quality and higher test weight normally means higher quality grain (Bern and Brumm 2009). High test weight indicates that kernels can resist breakage during threshing and handling (Pioneer 2009). Damaged kernels are kernels that have been damaged due to insects, harvest, or handling. Grain odor can be an indication of fungal contamination and/or deterioration (Pitchford 1995). Foreign material contamination (weeds, plant parts, etc.), as well as the ability of the combine to exclude or eject these things, is often associated with harvest machinery efficiency. Many grain elevators have their own standard for accepting or rejecting grain, but they are generally similar. The following are typical parameters of grain inspection that are checked at the granary: level of aflatoxin and other mycotoxins, test weight, foreign material content, and percent damaged grain. Mycotoxin levels can determine and or impact the end use of grain. Reduction of insect damage with Bt crops can contribute to fewer pathways for fungi to develop into mycotoxins and may result in higher grain quality (Zhao et al. 2003).

Mycotoxins

Agronomic crops, including corn, cotton, peanut (*Arachis hypogea* L.); rice (*Oryza sativa* L.); and wheat (*Triticum aestivum* L.) are frequently infected by fungi in the field that can result in the production of toxic metabolites referred to as mycotoxins (Peraica et al. 1999, Varga et al. 2003). More importantly, research has suggested that

25% of the world's crops are, in some form, contaminated with mycotoxins as either a result of infection in the field or post-harvest contamination, especially in poorly maintained grain storage facilities (Fink-Gremmels 1999). However, not all fungi produce mycotoxins. In fact, the production of mycotoxins that affect animals and humans are typically caused by specific members of the genera *Aspergillus, Fusarium* and *Penicillium* (Bennett and Klich 2003). Mycotoxins were first extensively studied following reports that the turkey X disease was caused by a toxin produced by *Aspergillus flavus* Link: Fr. growing on harvested peanuts (Davis et al. 1966). Biologically, the production of the toxin results when the fungus infects the plant and produces secondary metabolites (Rao et al. 1997, Wright et al. 2000, Wu 2006). The mycotoxins can be poisonous to humans and animals that consume the contaminated grain, a situation that can produce an immediate reaction to the toxin, termed mycotoxicosis (Agrios 1997, Bennett and Klich 2003). Generally, the fungi that produce mycotoxins can be classified as primary fungi that are aggressive and infect healthy hosts or opportunistic fungi that infect stressed or weakened hosts (Bennett and Klich 2003). Most fungi that cause human mycoses are opportunistic and require the host to be stressed (Bennett and Klich 2003). The severity of mycoses can vary from minimal impact to life threatening. Depending on the specific situation and the level of exposure, mycotoxins can be allergens, carcinogens, mutagens, and teratogens; however, in most cases a frequent, prolonged exposure to the toxin is necessary for cancer or genetic mutations to occur (Bennett and Klich 2003).

Two of the most important mycotoxins that affect corn are aflatoxin and fumonisin (Abbas et al. 2002). Corn mycotoxins are generally produced as a result of environmental stress causing factors during significant growth stages such as pollination and grain development that include high-temperatures, poor irrigation management, nutrient-deficiency, insect damage, and poor soil fertility management (Jones et al. 1981, Dorner et al. 1999). In addition to the factors that can impact the production of aflatoxins in the field, injury to the corn kernel during harvest can result in the production of aflatoxins post-harvest (Anderson et al. 1975, Jones et al. 1981, Lillehoj 1983, Lisker and Lillehoj 1991, Bruns 2003). Aflatoxins are considered to be more of a problem in the South Eastern and Mid-Southern U.S. due to the ideal growing temperatures and low average rainfall that can occur from silking (R1) to late dough (R4) stage of the corn crop (Lillehoj et al. 1975, Jones et al. 1981, Goldman and Osmani 2007). More specifically, in Mississippi, high-temperatures are thought to contribute to greater corn seed contamination by both aflatoxins and fumonisins (Abbas et al. 2002). The optimal growing temperature for aflatoxin is 30°C, but the fungus, *A. flavus*, can grow at a temperature range of 12 to 48°C (Hedayati et al. 2007, Goldman and Osmani 2007). Aflatoxin production ceases when temperatures exceed 36°C due to the destabilization of the aflatoxin regulatory gene (Goldman and Osmani 2007). Therefore, it is possible for the fungi to be present without the accompaniment of the toxin.

Aflatoxins are a group of mycotoxins produced by several fungal species within the genus *Aspergillus*. The following members of the genus *Aspergillus* have been reported to produce aflatoxins: *A. arachidicola* Pildain, Frisvad and Samson, *A. bombycis* S.W. Peterson, Yoko Ito, B.W. Horn and T. Goto, *A. flavus*, *A. minisclerotigenes* Vaamonde, Frisvad and Samson, *A. nomius* Kurtzman, B.W. Horn and Hesselt., *A. parasiticus* Speare, *A. parvisclerotigenus* (Mich. Saito and Tsuruta) Frisvad and Samson, *A. pseudocaelatus* Varga, Samson and Frisvad, and *A. rambellii* Frisvad and Samson (Hesseltine et al. 1966, Dorner et al. 1999, Hedayati et al. 2007). However, in corn, the two most notable species of *Aspergillus* that result in aflatoxin production are *A. flavus* and *A. parasiticus* (Dorner et al. 1999).

As previously stated, mycotoxin production can be greatly influenced by abiotic factors (e.g., temperature, humidity, drought) (Zuber and Lillehoj 1979). However, even though the fungus can be observed on corn kernels this does not necessarily result in toxin contamination. This makes it difficult to consistently correlate insect infestations and mycotoxin concentrations, specifically higher concentrations. Several conditions, including those that cause stress, as well as yield reduction must be met for the production of aflatoxin by *A. flavus* (Jones et al. 1981). In some instances reductions of multiple stress factors such as drought and insect damage have to occur for a reduction in aflatoxin to be observed (Smith and Riley 1992). An example would be if insect damage occurs while plants are drought stressed, then a corresponding increase in aflatoxin contamination will most likely occur (Fortnum and Manwiller 1985).

Aflatoxins are considered to be the most toxic and most potent carcinogenic compounds known to man (Goldman and Osmani 2007). In 1971, aflatoxins were reviewed by the International Agency for Research on Cancer (IARC) and, due to the limited data available, were labeled as a potential carcinogen; twenty years later these compounds were reclassified as group 1 carcinogens (Eaton and Gallagher 1994). In addition, aflatoxins are considered to be powerful hepatotoxins, teratogens, and mutagens resulting in liver damage, embryo malformation, and genetic mutation of both animals and humans (Dunn et al. 1982, Kihara et al. 2000, Butler and Neal 1973, Abbas et al. 2002).

Even though aflatoxins are generally stated as a group, there are several different specific compounds that are referred to as analogs. The four most common aflatoxin analogs are B1, B2, G1, and G2 (Raper and Fennell 1965) and different species of *Aspergillus* produce specific aflatoxin analogs. Generally, *A.flavus* produces analogs B1 and B2, while *A. parasiticus* can produce analogs B1, B2, G1, and G2 (Goto et al. 1996, Goldman and Osmani 2007). Of the four common aflatoxin analogs, B1 is the most important. Specifically, analog B1 is the most toxic and potent hepatocarcinogenic natural compound characterized (Bennett and Klich 2003, Hedayati et al. 2007). In addition to the four mentioned aflatoxin analogs, there are an additional 16 related aflatoxin analogs that have not been identified in corn grain (Wogan 1969). Because of their toxicity and potential carcinogenicity, aflatoxins pose serious health risks to both humans and animals, which can impact the value of effected crops (Dorner et al. 1999). The greatest concern regarding aflatoxin contamination is the concentration in harvested corn grain. The FDA established acceptable concentration of aflatoxins in grain for human consumption is 20 parts per billion (ppb). Additional guidelines for other uses of grain with concentrations up to 300 ppb have been established (FDA 1989, Stoloff et al. 1991).

Aspergillus flavus is not generally considered to be an aggressive organism, its growth and development can be influenced by environmental stress factors that include temperature, drought, and the presence of available moisture (Hesseltine et al, 1966, Goldman and Osmani 2007). In general, *A. flavus* can be characterized as a weak, opportunistic plant pathogen that can infect both above and below-ground plant parts of monocots and dicots (Goldman and Osmani 2007). *A. flavus* is also responsible for producing an ear rot when corn ears are infected and conducive environmental conditions prevail (Woloshuk, et al. 1997). When grown in Czapek's solution agar, *A. flavus* can be morphologically characterized by its yellow to olive green color and profuse sporulation (Thom and Church 1921). *A. flavus* has a worldwide distribution (Hedayati et al. 2007). The widespread distribution of this particular fungus could be due to the ease of the conidia to be transported through the air. Even though *A. flavus* is more often observed on aboveground plant parts, most of its life is spent in the soil where it has been reported to aid in decomposition (Scheidegger and Payne 2003). Spores of *Aspergillus* spp. have the ability to overwinter in plant debris or on stored grain and can then be disseminated by air currents or by insects (Robertson et al. 2008). Normally, *A. flavus* enters the developing corn ear by way of the silks; however, the spores can also enter the ear through areas damaged by insects. Aflatoxin contamination can occur under specific environmental conditions within the developing corn kernels in response to the fungal infection. Fungal infection and disease symptoms (ear rot) do not always result in aflatoxin contamination.

Relationship Between Insect Damage and Mycotoxin Contamination

Insect injury to plant reproductive parts can increase the risk of mycotoxin contamination which can occur in undamaged grain, but is more likely to occur in damaged grain (Diener et al. 1987, Niu et al. 2009). The type of injury an insect causes can be an important factor to consider (Setamou et al. 1998). For example, Widstrom et al. (1975) reported a difference in aflatoxin contamination between corn infested with

European corn borer and fall armyworm in the same field. Both caused damage, but the type of damage affected aflatoxin production. Widstrom et al. (1975) suggested that lepidopteran insects do not directly transport fungus into the ear, but provide a pathway of fungal entry. European corn borer and fall armyworm both cause damage that would allow for entrance of the fungus into the ear. Fall armyworm damage is directly in the ear by feeding on kernels, while European corn borer cause damage by boring in the stalk and ear shank. Several studies have investigated the relationship between insect damage with *A. flavus* presence and aflatoxin production with responses being variable between studies (Barry et al. 1992). Many studies reported a significant relationship between fungal infection and ears damaged by corn borers when compared to non-damaged ears (Fennell et al. 1975, Widstrom et al. 1975, Lillehoj et al. 1976). Fennell et al. (1975) and Taubenhaus (1920) reported that *A. flavus* was usually present on areas of corn ears that sustained corn earworm injury and that as the level of corn earworm damage decreased, the aflatoxin concentrations also decreased. Ni et al. (2011) observed no significant correlation between corn earworm damage and aflatoxin concentration during 2006, but maize weevils, *Sitophilus zeamais* (Motschulsky), and the number and the percentage of stink bug damaged kernels were significantly correlated with aflatoxin concentrations. A positive correlation between corn earworm and aflatoxin concentrations was observed during 2007 (Ni et al. 2011).

The efficacy of Bt corn technologies against target pests has been demonstrated by numerous researchers (Dowd 2000, Buntin et al. 2001, Odvody et al. 2000, Williams et al. 2002, Wu et al. 2004, Wu 2007). Also, research suggests that impacts on aflatoxin concentrations are through reduced insect injury which reduced avenues for fungal infection and not from direct action of the Bt traits on the fungus (Dowd 2000, Williams et al. 2002). However, reductions in insect injury do not consistently translate into reduced aflatoxin concentrations (Buntin et al. 2001, Odvody et al. 2000, Wu et al. 2004, Wu 2007). These studies indicate that insect injury is not the primary factor that influences aflatoxin production.

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CHAPTER II

IMPACT OF CORN EARWORM (*Helicoverpa zea*) ON YIELD AND GRAIN QUALITY IN FIELD CORN

Abstract

Corn earworm, *Helicoverpa zea* (Boddie) is a common insect that infests field corn, *Zea mays* (L.) in Mississippi. Due to the combination of the biology of the corn plant, biology of the corn earworm, and the low value of field corn, it is not economical to control corn earworm with insecticide applications. Recently, transgenic Bt corn technologies have been introduced that exhibit much greater efficacy against corn earworm and may also potentially reduce mycotoxin contamination. Experiments were conducted during 2011 and 2012 to investigate the impact of corn earworm on yield and grain quality of field corn. Treatments included field corn hybrids expressing the Herculex®, YieldGard®, and Genuity VT Triple Pro® technologies. Supplemental insecticide applications were made every 1-2 days from silk emergence to silk senescence to create a range of damaged kernel levels for each technology. No relationship between number of damaged kernels and yield was observed for corn hybrid expressing the Herculex®, YieldGard®, or Genuity VT Triple Pro® technologies. A significant relationship between damaged kernels and test weight was observed in two technologies. Also, a significant relationship between damaged kernels and aflatoxin concentrations was observed in two technologies.

Introduction

The corn earworm, *Helicoverpa zea* (Boddie), is a polyphagous pest that infests corn, *Zea mays* (L.), cotton, *Gossypium hirsutum* (L.), soybean, *Glycine max* (L.), sorghum, *Sorghum bicolor* (L.), among other crops (Hayes 1988, Fitt 1989, Kennedy and Storer 2000). Corn earworm in the mid-south initially emerges from overwintering in April and May, with Mississippi local emergence occurring around mid-April to mid-May (Lincoln et al. 1967, Rummel et al. 1986, Schneider 2003). Depending on when emergence occurs other host plants including: crimson clover, *Trifolium incarnatum* L. (mid-April to 1st week of May), Persian clover, *Trifolium resupinatum* L. (third week of April to late May), *Geranium dissectum* L. (third week of April-Late May), winter vetch, *Vicia villosa* Roth. (mid-April onwards), and caley pea, *Lathyrus hirsutus* L., may be preferred to cultivated crops (Snow and Brazzel 1965, Parker 2000).

Corn is highly attractive to corn earworm adults during the silk stage. Female moths are attracted to corn during the silk stage because of kairomones produced by corn (Fitt 1989, Johnson et al. 1975). Oviposition usually occurs on leaf hairs and silks at night when adults are most active (Lingren et al. 1982). The eggs hatch within 2-10 days depending on temperature (Fitt 1989). Upon eclosion larvae begin feeding on the silks and make their way down the silk channel to feed on the immature kernels forming on the ear (Rector et al. 2002). Larvae will feed on kernels and remain in the ear until they reach the 5th to 6th instars, then move to the soil to pupate (Fitt 1989). Adult emergence usually occurs 2-3 weeks later, unless pupae are in an overwintering state (Neunzig 1969, Mayer et al. 2003). At this point, depending on the attractiveness, robustness and availability of corn, female moths may choose to oviposit on later corn, cotton, soybean, or other host plants (Miller and Strickler 1984, Schneider et al. 1986). Climate and region play a huge role in the number of generations per year for corn earworm. The number of generations can range from 1 per year in northern areas (northern U.S. and Canada) to 8 generations per year in Florida and some parts of Texas (Fitt 1989, Capinera 2000).

Corn earworm infests both field corn and sweet corn. The tolerances for larvae in fresh market sweet corn are very low (DePew 1966). With the high value of fresh market sweet corn, corn earworm management with foliar insecticides is economically viable. Foliar insecticide applications must be targeted at eggs and newly emerged larvae before they enter the ear. Residual activity of foliar applications can be compromised by the rapid growth rate of silks (Storer et al. 2001). This can result in the presence of newly emerged unprotected silk material within one day of an insecticide application. Because of these factors, multiple insecticide applications (12 to 40 insecticide applications) are required to manage corn earworm and fall armyworm in sweet corn (Janes and Green 1969, Foster 1989). With the lower value of field corn, this strategy is not economical and corn earworm has not been considered an economic pest of field corn (USDA NASS 2012).

Bacillus thuringiensis (Bt) was discovered in a diseased silkworm colony by Japanese biologist, Shigetane Ishiwataf in 1901 (Beegle and Yamamoto 1992, Reardon et al. 1994, Roh et al. 2007, Ibrahim et al. 2010). Bt is a gram positive soil bacterium that when ingested by susceptible insects, cause cessation of feeding and death within several hours to several days (Shelton 2008).

The insertion of genes that encode for certain Bt proteins into field corn has allowed for the control of corn earworm in field corn during the ear stage. In 1996, Ciba (now part of Syngenta Seeds) and Mycogen Seeds (now part of Dow) introduced the first Bt corn hybrids. Transgenic Bt corn and cotton are currently grown in the United States. During 2001 transgenic Bt varieties/hybrids accounted for 26% of the corn planted in the U. S. (Nester et al. 2002). In 2015, the percentage of stacked Bt varieties/hybrids has risen to 79% of the corn planted in the U. S. (USDA NASS 2012).

The first generation transgenic Bt corn hybrids expressed one Bt protein and were developed primarily to manage the corn borer complex (Ostlie et al. 1997). YieldGard® and Herculex® are two of the most widely planted single gene technologies, containing the proteins Cry1Ab (YieldGard®) and Cry1F (Herculex®). These were followed by single trait technologies for control of corn rootworm and hybrids that expressed a single trait for lepidopteran insects and one for corn rootworm. The newest Bt technology trait packages for corn now include multiple toxins for lepidopteran pests, corn rootworm, or both. Expression of multiple traits for a target pest is referred to as pyramiding traits (Hellmich and Hellmich 2012).

The value of enhanced corn earworm control provided by these new technologies is unknown because the impact of corn earworm infestations on field corn yield has not been fully investigated. The ability of transgenic Bt corn technologies to reduce corn earworm injury has been demonstrated; however, yield responses to reductions in insect damage have been inconsistent in field corn (Buntin et al. 2001, Buntin et al. 2004a, Buntin 2008, Reay-Jones and Wiatrak 2011, Reay-Jones and Reisig 2014, Bowen et al. 2014). In addition to corn earworm control, these new Bt technologies may potentially reduce mycotoxin contamination (Odvody et al. 2000, Buntin et al. 2001, Williams et al. 2002, Zhao et al. 2003).

Mycotoxins are the toxic secondary by-products of certain fungi under specific conditions (Koenning and Payne 1999, Bennett and Klich 2003). One of the most notable mycotoxins, with regard to corn production, is aflatoxin. Aflatoxin is a mycotoxin produced by the fungus *Aspergillus flavus*, and several other species of fungi in the genus *Aspergillus* (Dorner et al. 1999, Hesseltine et al. 1966, Hedayati et al. 2007). Plant stresses including: hot, dry weather, poor irrigation management, nutrient-deficiency, and insect damage can be conducive to aflatoxin production (Jones et al. 1981, Dorner et al. 1999, Koenning and Payne 1999). By reducing insect injury (i.e. corn earworm), the new Bt technologies have the potential to reduce mycotoxin levels.

These new technologies could be extremely beneficial to corn growers in the South. Therefore, determining the impact and value of these technologies to mid-southern field corn production is needed.

Materials and Methods

Impact of corn earworm on field corn yield

Experiments were conducted at 5 locations which include the Mississippi State University R. R. Foil Plant Science Farm, Starkville, MS; Delta Research and Extension Center, Stoneville, MS; University of Tennessee West Tennessee Experiment Station, Jackson, TN; LSU AgCenter Macon Ridge Research Station, Winnsboro, LA; and University of Georgia Bledsoe Research Station, Griffin, GA. Corn was planted during the recommended planting window and after the recommended planting window during 2011 and 2012 at each location to increase the probability of encountering corn earworm infestations (McClure 2010, Anonymous 2012, Larson 2012, Lee 2013). Planting and harvest dates are detailed in Table 2.1. Three transgenic Bt corn technologies, each expressing proteins active against lepidopteran pests, were used, namely Herculex® 1 (Cry1F protein, Dow AgroSciences, Indianapolis, IN; "P1615HR", Pioneer Hi-Bred, Johnston, IA), YieldGard® (Cry1Ab protein, "DKC 69-40", Monsanto Company, St. Louis, MO), and Genuity VT Triple Pro® (Cry1A.105 + Cry2Ab2, "DKC 67-88", Monsanto Company, St. Louis, MO). Hybrids (technologies) were arranged in a randomized complete block design with four replications. Plot size was 8 rows and row spacing ranged from 76.2 to 101.6 cm (30 to 40 inch) with a plot length of 9.1 to 15.2 m (30-50 ft). Individual rows of each plot were designated for different purposes to include border, harvest (treated and non-treated), and in-season destructive sampling (Figure 2.1). In 2011, two rows in each plot were treated with either 0.105 kg ai/ha (0.094 lb ai/acre) of flubendiamide (Belt®, Bayer CropScience, Research Triangle Park, NC) or 0.075 kg ai/ha (0.067 lb ai/acre of chlorantraniliprole (Prevathon™, DuPont, Wilmington, DE) beginning at silk emergence (R1) and continuing until silk senescence (R4) (ca. 21 days). Foliar applications applied by CO2 backpack sprayer with the "spraying to wet" (40GPA) method targeted the ear only and were made every 1-2 days. In 2012, foliar insecticide applications were made to the center 2 rows and consisted of flubendiamide at 0.105kg ai/ha (0.094 lb ai/acre) (Belt®, Bayer CropScience, Research Triangle Park, NC) every 1-2 days beginning at silk emergence (R1) until 5 days after silk emergence. On the 5th day of silking, 0.5 ml of a 0.2% solution of formulated flubendiamide plus 0.05% solution of methylated seed oil was injected into the area inside of the husk surrounding the silks and tip of each ear. Injections were made using a cattle injection syringe (Allflex® 25MR2 Repeater Syringe, 25ml, Allflex USA, Inc., DFW Airport, TX) with the end of the needle (16 gauge) ground blunt. These treated rows along with the corresponding non-treated rows in each plot were utilized to provide a range of corn earworm damage within each plot. Twenty ears from the destructive sampling rows were examined to determine larval density, larval stage, and species present at the R3 growth stage (milk). A second sample was collected at R4 growth stage (dough). Within 1-2 days of harvest, 50 ears each from the treated rows and the non-treated rows of each plot were examined for kernel damage from corn earworm. The remaining portions of the treated and non-treated rows of each plot were mechanically harvested and yields were corrected to 15% moisture content. Fertilizer application, irrigation and other agronomic practices, except insecticide sprays, were applied as per the recommendations of each State's Cooperative Extension Services (McClure 2010, Anonymous 2011, Anonymous 2012, Lee 2013).

Impact of corn earworm on grain quality

Grain samples were collected from each plot of the experiments outlined above to determine aflatoxin presence and concentration. Kernel samples of approximately 308 grams were ground using a Wiley Mill (No. 4 Grinder, Thomas Wiley Mills, Thomas Scientific, Swedesboro, NJ) fitted with a 0.05 mm sieve. Using a validated Mississippi State Chemical Laboratory (MSCL) method, the mill was cleaned between samples using compressed air to prevent cross contamination. A mixture of 5 grams of ground corn kernels and 25 ml of a 70% methanol:water solution was incorporated in a beaker by hand-swirling for 3 minutes. The solution was then filtered using grade 1 Whatman filter paper and the liquid extraction portion collected for analysis in 15 ml screw top centrifuge tubes. The liquid extraction was refrigerated (4°C) until analysis no longer than 72 hours. Quantitative analysis for aflatoxin concentrations was conducted using ELISA test kits (Neogen Corp., Lexington, KY) by the Department of Biochemistry and

Molecular Biology, Mississippi State, MS. Aflatoxin concentration results are expressed in parts per billion (PPB).

Data Analysis

Data for number of damaged kernels between the treated and non-treated portions of plots within each technology and number of damaged kernels for the non-treated portions of plots only within each technology were subjected to analysis of variance procedures using Proc Glimmix (SAS Institute 2013). Site year and replicate nested within siteyear were considered random effects. Degrees of freedom were calculated using Kenwood-Roger method. Means were separated according to Fisher's Protected Least Significant Difference (α=0.05).

Due to variation in yield potential among site years (combination of year and trial), data for yield, test weight, and aflatoxin were separated into higher and lower yield environments based on the median of the yield within each of the three technologies of the trial. This procedure was conducted independently for each technology.

Data for each technology within each yield potential category were analyzed independently using regression analysis with PROC GLIMMIX (SAS Institute 2013) to determine the relationships between yield and number of damaged kernels, test weight and number of damaged kernels, and aflatoxin concentration and number of damaged kernels. Data for the treated and non-treated portions of each plot within each technology were combined to represent a range of damaged kernels. Data for locations at which no damaged kernels were observed for both the treated and non-treated portions of each plot were excluded from analysis. Observations greater than two standard deviations from the mean were considered outliers and excluded from analysis. Site year and replicate nested within siteyear were considered random effects. Degrees of freedom were calculated using Kenwood-Roger method.

Results

Corn earworm infestations occurred in all trials (infestation data not presented for LA location) (Table 2.2). Insect densities were on average 75% greater during the R3 growth stage compared to the R4 growth stage. A decline in insect densities from R3 to R4 was expected as cannibalism can occur leaving only one or two older larvae to successfully complete development and the maturation of larvae and their movement to the soil to pupate (Mally 1892, Dial and Adler 1990). Differences in kernel damage for each technology were achieved with foliar insecticide applications directed at the ears: YieldGard® (F=73.97; df=1, 131; *P*<0.0001), Herculex® (F=97.50; df=1, 130; *P*<0.0001), and VT Triple Pro® (F=67.15; df=1, 122.8; *P*<0.01) (Figure 2.2).

When foliar insecticide applications were not applied, VT Triple Pro® resulted in significantly lower numbers of damaged kernels than the Herculex® and YieldGard® technologies (F=18.12; df=2, 205; *P*<0.01) (Figure 2.3). These technologies were chosen for this study due to their varying levels of activity against of corn earworm.

No significant relationship between yield and damaged kernels was observed for the Herculex® (F=1.73; df=1, 51.64; *P*=0.19), YieldGard® (F=2.44; df=1, 59.72; *P*=0.12), or VT Triple Pro® (F=2.39; df=1, 39.35; *P*=0.13) technologies in higher yield environments (Figures 2.4, 2.5, 2.6). In lower yield environments, there was also no relationship between yield and damaged kernels for Herculex® (F=3.44; df=1, 47.74; *P*=0.07), YieldGard® (F=0.01; df=1, 46.16; *P*=0.91), or VT Triple Pro® (F=0.11; df=1, 60.92; *P*=0.75) (Figures 2.7, 2.8, and 2.9).

No relationship between test weight and damaged kernels was observed for the Herculex® (F=0.75; df=1, 42.96; *P*=0.39), YieldGard® (F=0.42; df=1, 41.49; *P*=0.52), or VT Triple Pro®® (F=0.00; df=1, 29.76; *P*=0.99) technologies in higher yield environments (Figures 2.10, 2.11, and 2.12). In the lower yield environments, a significant relationship between test weight and damaged kernels was observed for the Herculex® (F=15.37; df=1, 30.9; *P*=0.0005) and the YieldGard® (F=11.70; df=1, 42.66; *P*=0.0014) technologies. No significant relationship between test weight and damaged kernels was observed for the VT Triple Pro® (F=.84; df=1, 41.32; *P*=0.37) technology (Figures 2.13, 2.14, and 2.15).

In the higher yield environments a significant linear relationship between number of damaged kernels and aflatoxin levels was observed for the Herculex® (F=6.75; df=1, 42.36; *P*=0.01) and YieldGard® (F=5.70; df=1, 50.61; *P*=0.02) technologies, while no significant relationship was observed for the VT Triple Pro®® (F=0.52; df=1, 13.64; *P*=0.48) technology (Figures 2.16, 2.17, and 2.18). In the lower yields environments, no significant relationship between aflatoxin and damaged kernels was observed for the Herculex® (F=3.01; df=1, 49.29; *P*=0.09), YieldGard® (F=0.15; df=1, 44.39; *P*=0.69), or VT Triple Pro® (F=1.31; df=1, 60.06; *P*=0.26) technologies (Figures 2.19, 2.20, and 2.21).

Discussion

The efficacy of Bt corn technologies against corn earworm resulting in a reduction of kernel damage is well documented and ranges from moderate to very good (Storer et al. 2001, Buntin et al. 2004a, Buntin 2008). Corn earworm infestations levels were generally high in my trials, and I was successful in establishing substantial differences in kernel injury by using different Bt technologies and insecticides. However, even with the technologies that have more efficacy, yield responses have been variable (Buntin et al. 2004a, Buntin 2008, Reay-Jones and Wiatrak 2011, Reay-Jones and Reisig 2014, Bowen et al. 2014, Steckel and Stewart 2015). In some studies a yield increase was observed with kernel damage reduction from the use of Bt corn (Buntin et al. 2004a, DeLamar et al. 1999a-e), while in others no impact on yield was observed (Buntin et al. 2004a, Buntin 2008, Reay-Jones and Wiatrak 2011, Bowen et al. 2014, Reay-Jones and Reisig 2014, Steckel and Stewart 2015). There were also no significant differences in test weight between Bt and non-Bt hybrids where corn earworm kernel damage was reduced (Buntin et al. 2004b, Buntin 2008). In the current study, no significant relationship between kernels damage and yield or test weight was observed for any of the included technologies, with one exception for test weight. There are several possibilities as to why reductions in corn earworm feeding on kernels have not resulted in significant yield increases. Corn earworm normally feeds on kernels near the tip of the ear (Klostermeyer and Rasmussen 1953, Steffey et al. 1999, Bohnenblust et al. 2013), and many of the kernels near the ear tip will not develop into mature kernels that contribute to yield (Nishikawa and Kudo 1973). The kernels near the ear tip generally have a lower weight than those further down the ear (Tollenaar and Daynard 1978) which would increase the chance of those kernels being expelled from the combine at harvest with cobs and stalk material. It is also possible that kernels further down the ear may compensate for damage occurring near the ear tip (Dyer 1975, White and Scott 1983, Woronecki et al. 1980, Steckel and Stewart 2015). Another possibility could be related to harvest efficiency. Many of the published studies utilized mechanical harvest methods (Buntin et al. 2004a, Buntin 2008, Bowen et al. 2014, Reay-Jones and Reisig 2014). Surveys of harvested commercial corn fields in Mississippi reported up to 143,000 volunteer corn plants/ha (Babu et al. 2014). These densities are greater than 1.5 times the recommended seeding rate for corn (Larson 2009, McClure 2010, Lee 2011, Anonymous 2012). Many of the factors mentioned above may contribute to the lack of yield responses to kernel damage reductions. Improved harvest efficiency may or may not result in positive responses to kernel damage reduction, but would result in higher harvest yields for growers.

Lepidopteran larvae do not appear to directly transport fungus into the ear, however, larval feeding can provide a pathway of fungal entry (Widstrom et al. 1975). It appears that any impacts on aflatoxin concentrations associated with Bt corn hybrids are through reductions in insect injury which reduces avenues for fungal infection and not from direct action of the Bt traits on the fungus (Dowd 2000, Williams et al. 2002). The efficacy of transgenic Bt corn technologies against lepidopteran larvae is well documented. However, reductions in kernel damage have not consistently translated into reduced aflatoxin concentrations (Odvody et al. 2000, Buntin et al. 2001, Wu et al. 2004, Wu 2007). Injury to plant reproductive parts caused by insects can increase the risk of mycotoxin contamination which can generally occur in undamaged grain, but is more likely to occur in damaged grain (Diener et al. 1987, Niu et al. 2009). In the current study, a significant relationship between kernel damage and aflatoxin concentration was observed with two technologies. Published research indicates that insect injury, such as that caused by lepidopteran and coleopteran insects, is not a primary factor that influences aflatoxin production (Widstrom et al. 1975, Buntin et al. 2001, Odvody et al. 2000, Wu et al. 2004, Wu 2007, Ni et al. 2011). Other factors including plant stress factors such as drought and nutrient deficiencies appear to have a much greater influence on the incidence and overall aflatoxin production (Jones et al. 1981, Dorner et al. 1999).

Table 2.1 Planting and Harvest Dates.

Year	Location	Planting Date	Harvest Date
2011	Starkville, MS	April 12	August 30
2011	Starkville, MS	June 1	October 14
2011	Starkville, MS	June 21	November 2
2012	Starkville, MS	March 29	August 30
2012	Starkville, MS	May 22	October 9
2011	Stoneville, MS	April 8	August 23
2011	Stoneville, MS	May 10	September 9
2012	Stoneville, MS	April 5	August 28
2012	Stoneville, MS	June 7	September 24
2011	Jackson, TN	May 9	September 6
2011	Jackson, TN	May 31	October 4
2012	Jackson, TN	April 25	September 11
2012	Jackson, TN	May 10	September 23
2011	Winnsboro, LA	April 11	August 12
2011	Winnsboro, LA	May 23	September 21
2012	Winnsboro, LA	April 10	August 24
2012	Winnsboro, LA	May 2	September 10
2011	Griffin, GA	April 15	August 25
2011	Griffin, GA	May 31	September 27
2012	Griffin, GA	April 4	September 12
2012	Griffin, GA	June 4	October 10

Table 2.2 Average number of corn earworm larva per 20 ears present at milk (R3) and dough (R4) growth stage at each location over all plant dates.

Year	Location	Technology (Hybrid)	R3 Small	R3 Med	R3 Large	R4 Small	R4 Med	R4 Large
2011	Starkville, MS	Herculex®	2.1	6.4	10.1	0.3	1.1	2.1
2011	Starkville, MS	YieldGard®	0.8	4.8	8.9	0.1	0.5	1.1
2011	Starkville, MS	VT Triple Pro®	3.1	6.4	8.6	0.3	1.4	3.3
2012	Starkville, MS	Herculex®	14.9	12.0	7.9	2.4	4.4	4.5
2012	Starkville, MS	YieldGard®	16.8	11.8	5.3	3.4	4.1	5.9
2012	Starkville, MS	VT Triple Pro®	12.9	9.3	9.0	3.3	4.5	5.6
2011	Stoneville, MS	Herculex®	26.5	12.4	5.5	7.6	6.3	6.6
2011	Stoneville, MS	YieldGard®	16.1	13.1	7.3	6.6	4.8	3.9
2011	Stoneville, MS	VT Triple Pro®	21.5	5.1	1.1	8.8	5.8	2.8
2012	Stoneville, MS	Herculex®	9.9	26.3	6.4	0.6	1.9	1.6
2012	Stoneville, MS	YieldGard®	3.4	14.9	6.1	0.0	0.4	0.4
2012	Stoneville, MS	VT Triple Pro®	12.6	9.4	1.1	1.5	1.5	1.3
2011	Jackson, TN	Herculex®	18.8	24.4	20.9	1.5	3.0	8.8
2011	Jackson, TN	YieldGard®	19.9	22.4	17.0	0.3	3.4	6.4
2011	Jackson, TN	VT Triple Pro®	23.0	6.4	1.8	2.0	4.8	6.5
2011	Griffin, GA	Herculex®	22.6	11.5	2.5	8.6	4.4	1.8
2011	Griffin, GA	YieldGard®	35.1	12.6	3.0	4.6	6.3	2.4
2011	Griffin, GA	VT Triple Pro®	17.0	3.9	0.4	9.8	4.3	2.3
2012	Griffin, GA	Herculex®	22.8	7.3	1.4	2.8	2.9	7.9
2012	Griffin, GA	YieldGard®	31.1	9.3	1.5	2.4	5.9	6.4
2012	Griffin, GA	VT Triple Pro®	10.4	1.0	0.4	6.6	3.0	1.1

(Small: <0.64cm (0.25 inch); Medium: 0.64 to1.27cm (0.25 to 0.5 inch) cm); Large: >1.27 cm (0.5inch)).

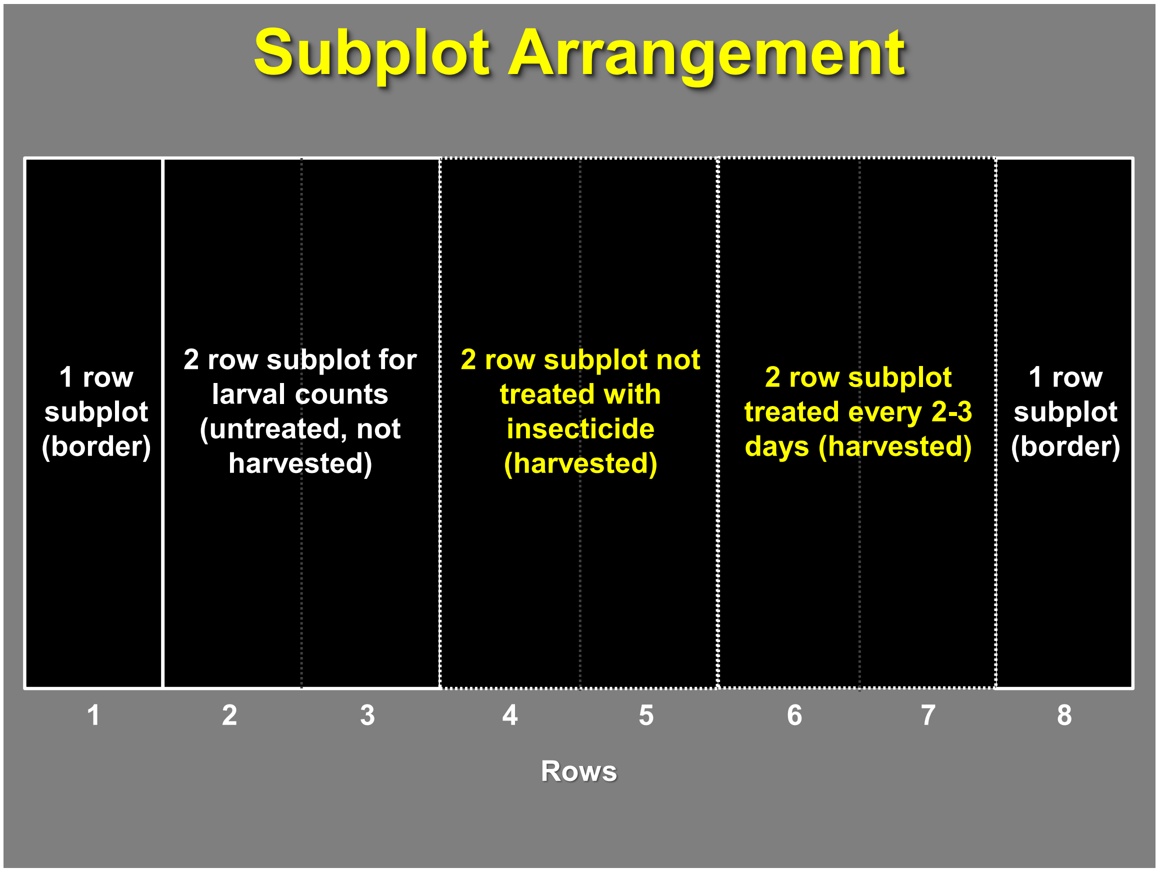

Figure 2.1 Example Plot Plan.

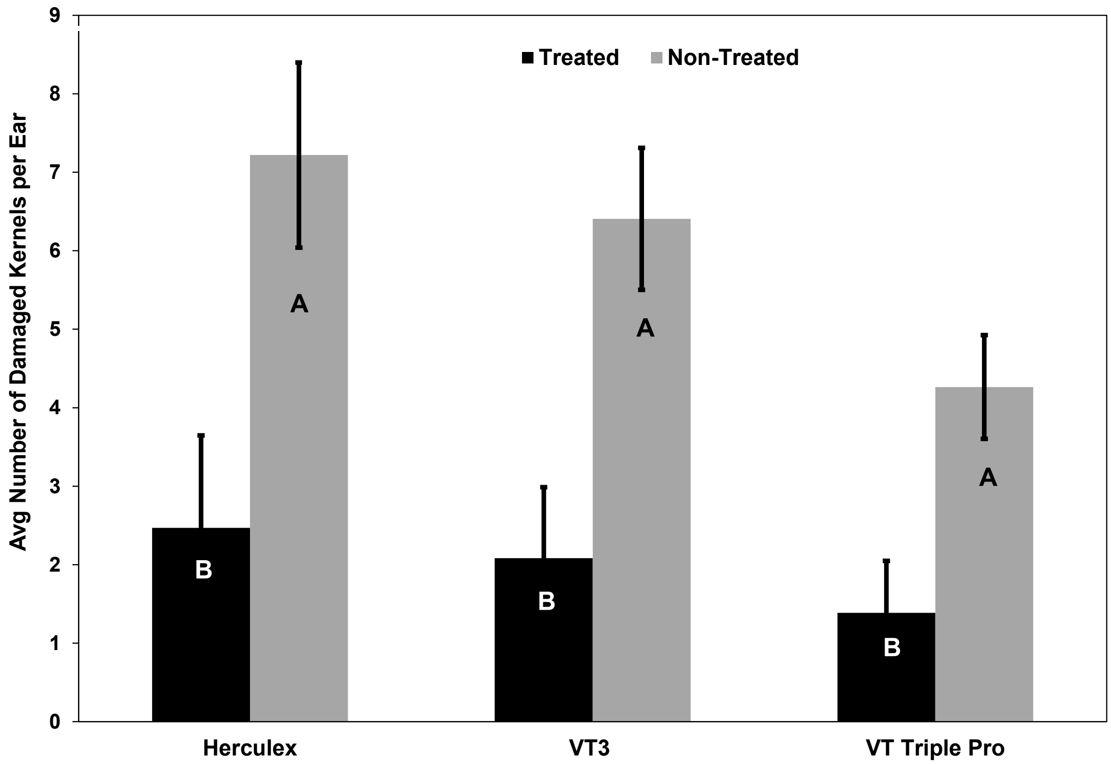

Figure 2.2 Mean number of corn earworm damaged kernels (Mean ± SEM) for corn hybrids expressing the Herculex®, YieldGard®, or Genuity VT Triple Pro® technologies.

Bars within a technology with a common letter are not significantly different ($P \geq 0.05$, Fisher's PLSD).

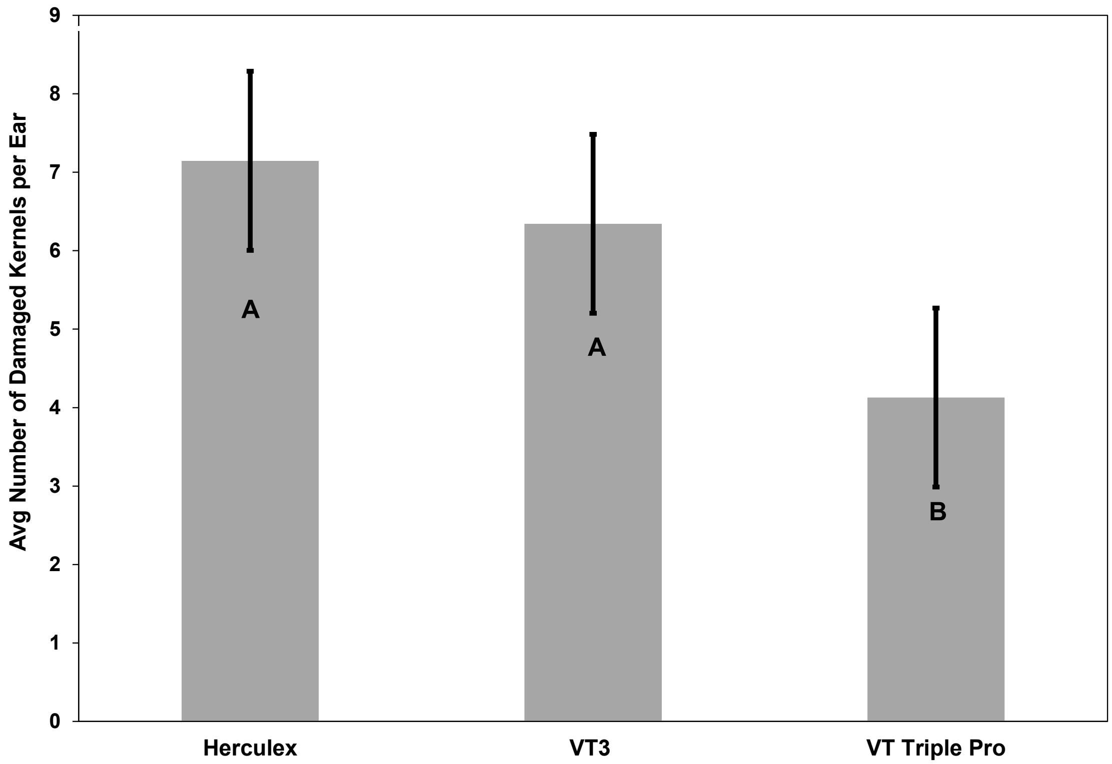

Figure 2.3 Mean number of damaged kernels (Mean ± SEM) for corn hybrids expressing the Herculex®, YieldGard®, or Genuity VT Triple Pro® technologies not treated with a foliar insecticide application.

Bars with a common letter are not significantly different (*P*≥0.05, Fisher's PLSD).

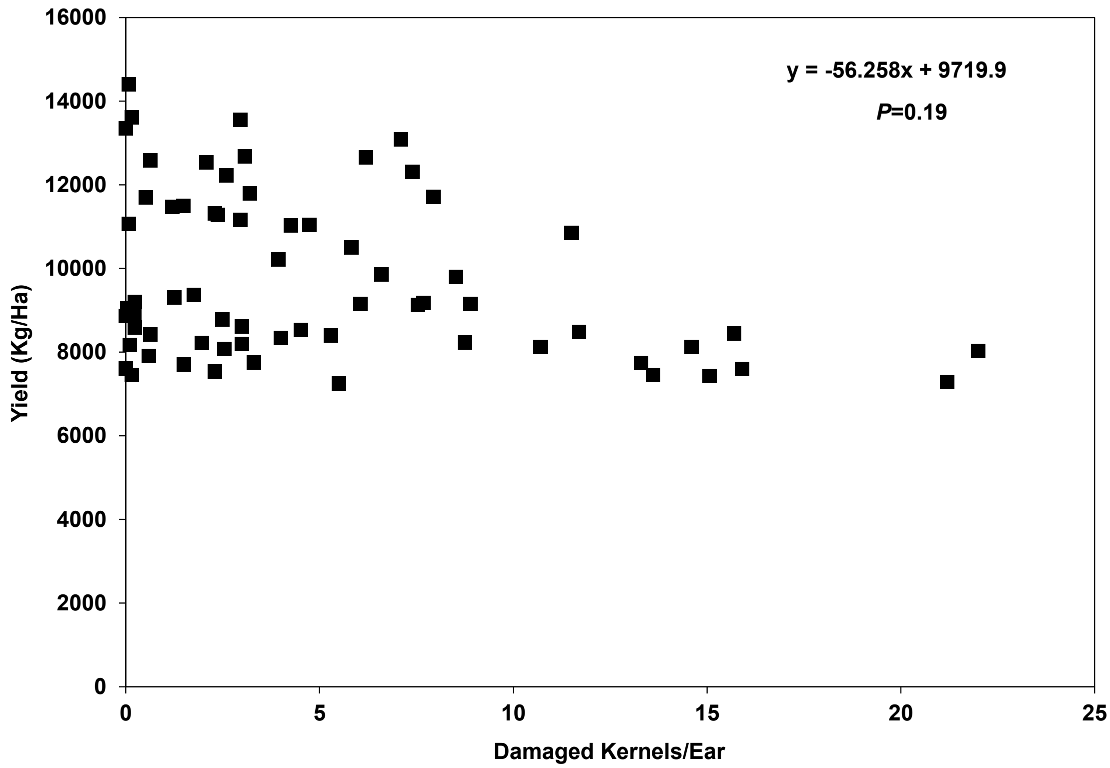

Figure 2.4 Relationship between numbers of damaged kernels per ear and yield for a corn hybrid expressing the Herculex® technology in higher yield environments.

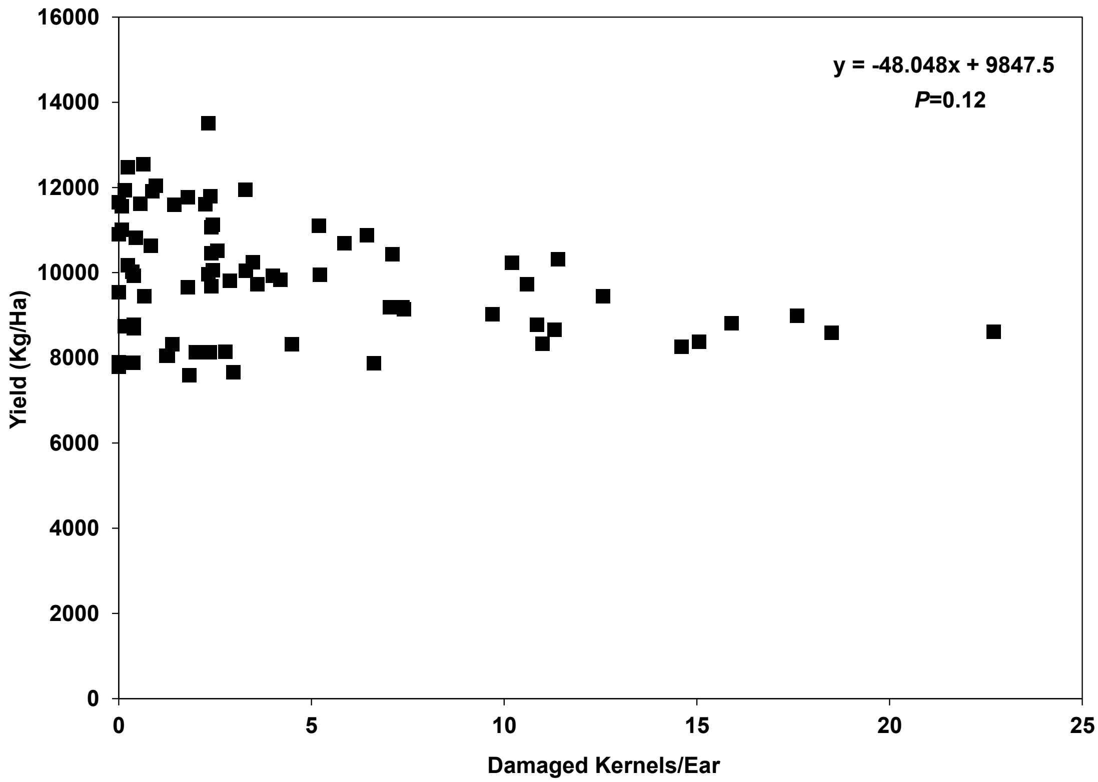

Figure 2.5 Relationship between numbers of damaged kernels per ear and yield for a corn hybrid expressing the YieldGard® technology in higher yield environments.

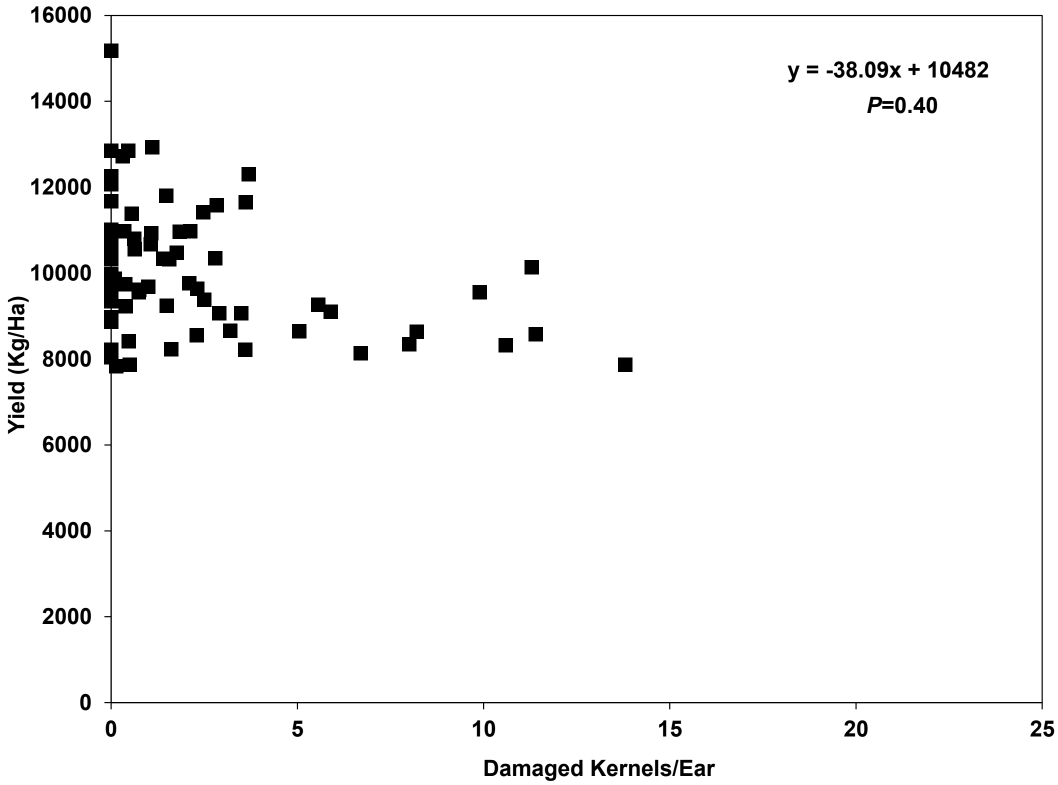

Figure 2.6 Relationship between numbers of damaged kernels per ear and yield for a corn hybrid expressing the VT Triple Pro® technology in higher yield environments.

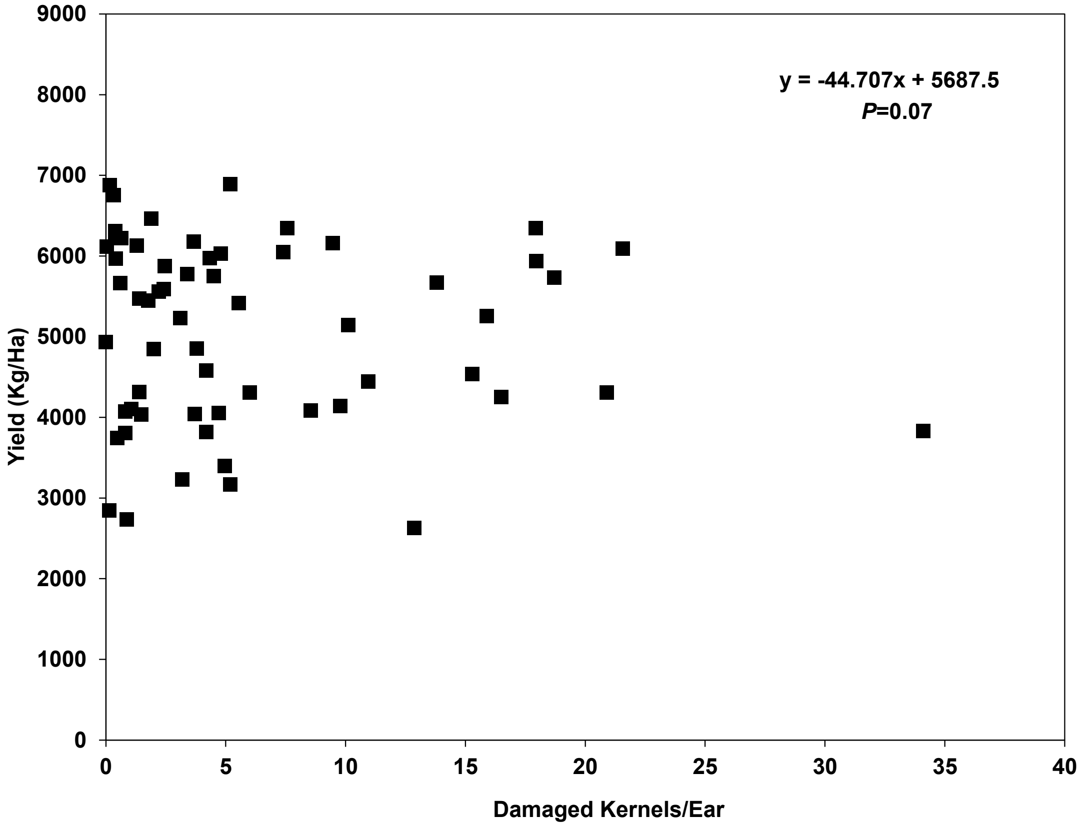

Figure 2.7 Relationship between numbers of damaged kernels per ear and yield for a corn hybrid expressing the Herculex® technology in lower yield environments.

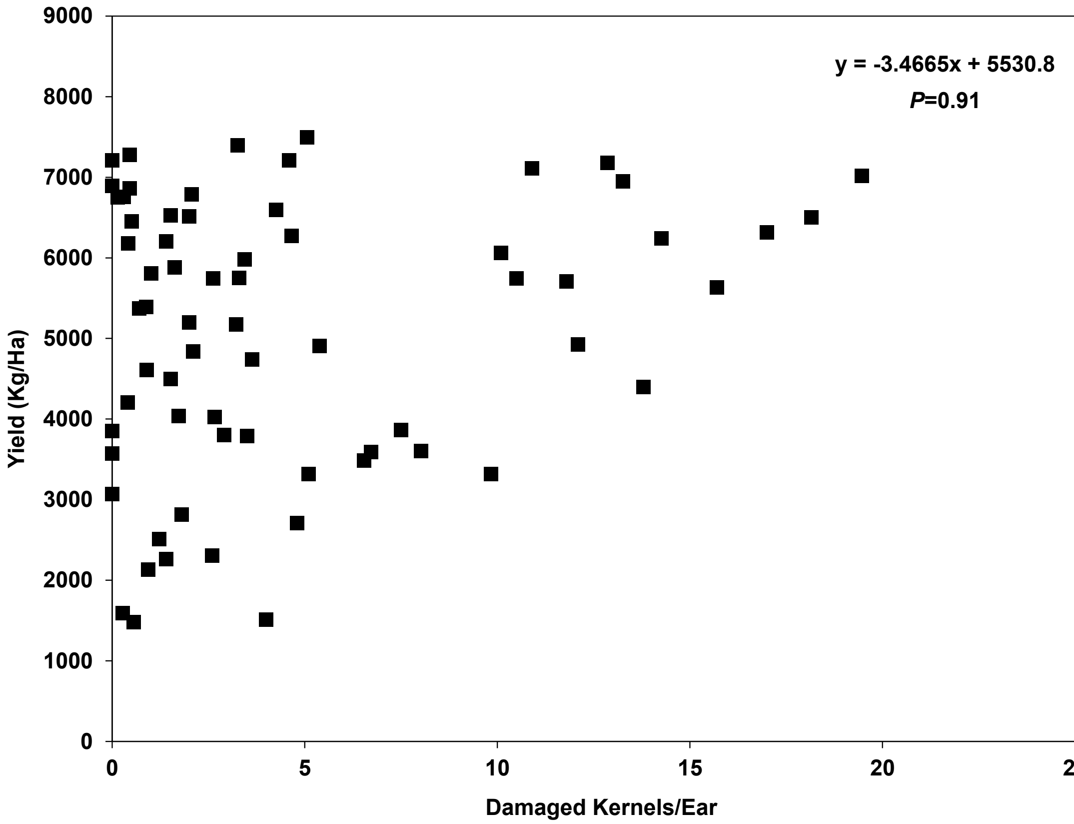

Figure 2.8 Relationship between numbers of damaged kernels per ear and yield for a corn hybrid expressing the YieldGard® technology in lower yield environments.

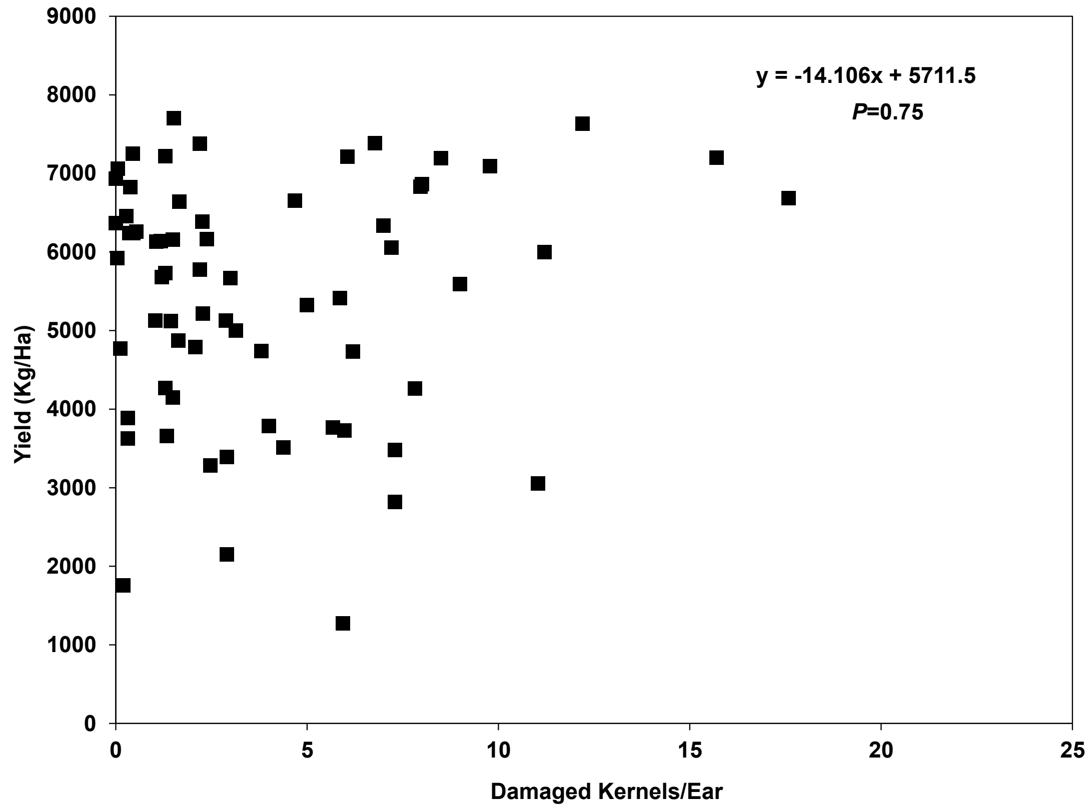

Figure 2.9 Relationship between numbers of damaged kernels per ear and yield for a corn hybrid expressing the VT Triple Pro® technology in lower yield environments.

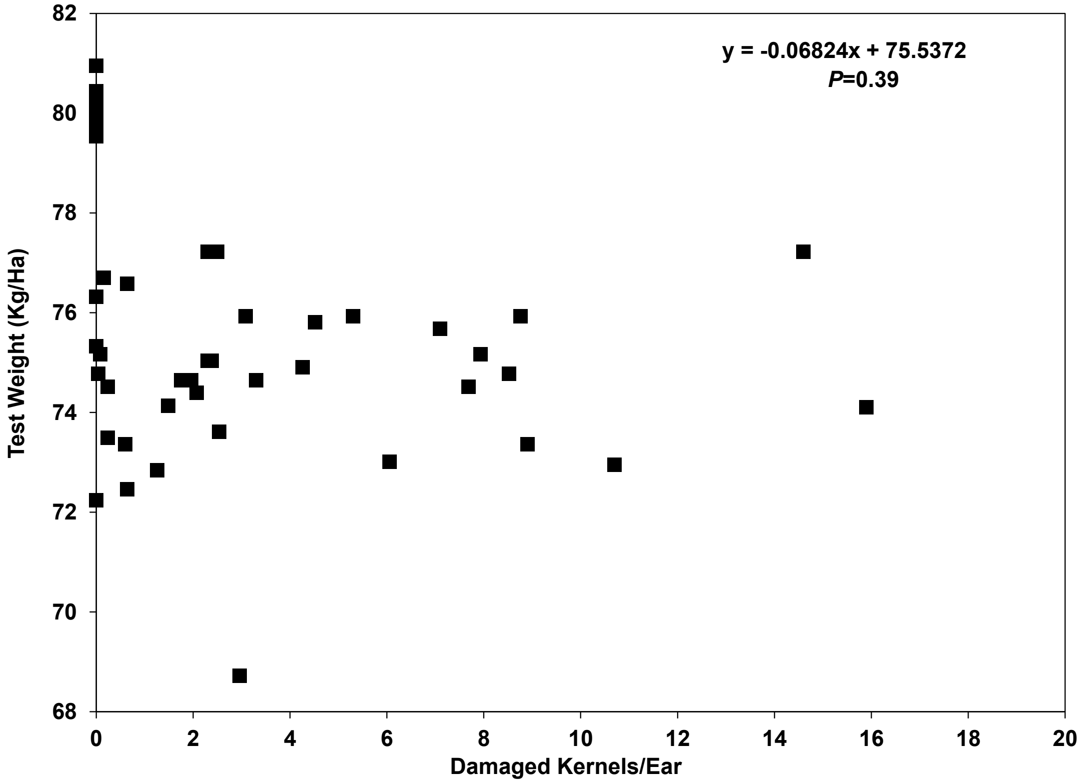

Figure 2.10 Relationship between numbers of damaged kernels per ear and test weight for a corn hybrid expressing the Herculex® technology in higher yield environments.

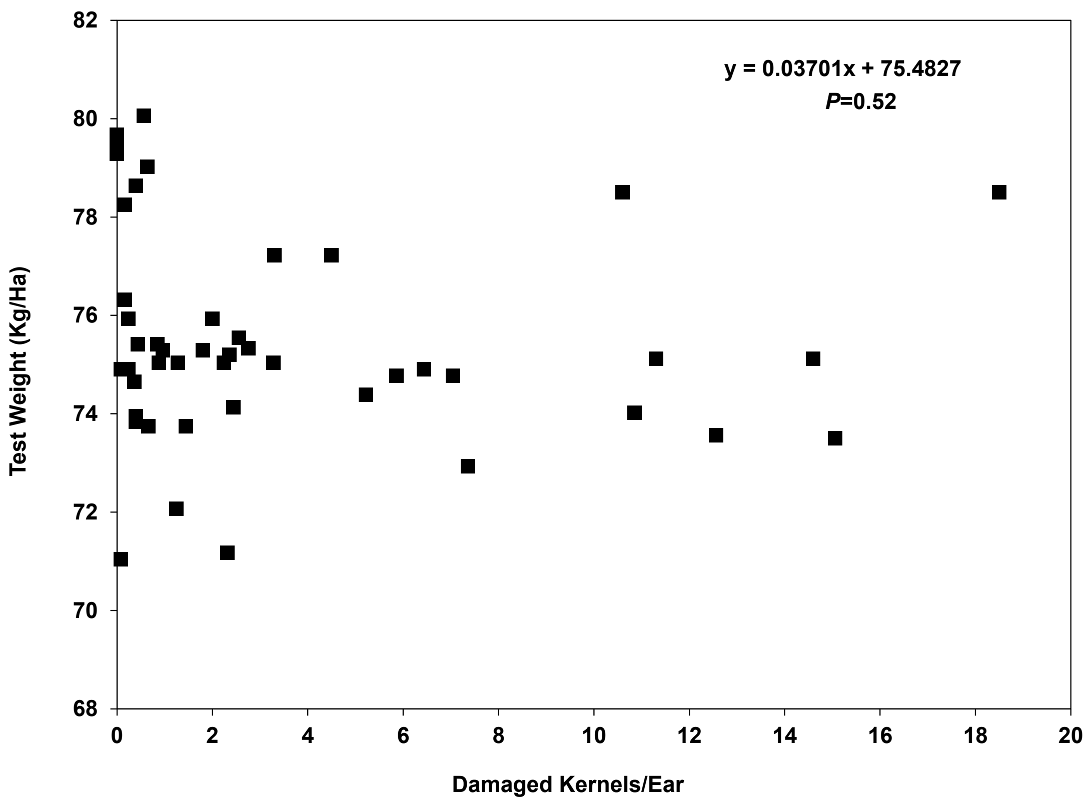

Figure 2.11 Relationship between numbers of damaged kernels per ear and test weight for a corn hybrid expressing the YieldGard® technology in higher yield environments.

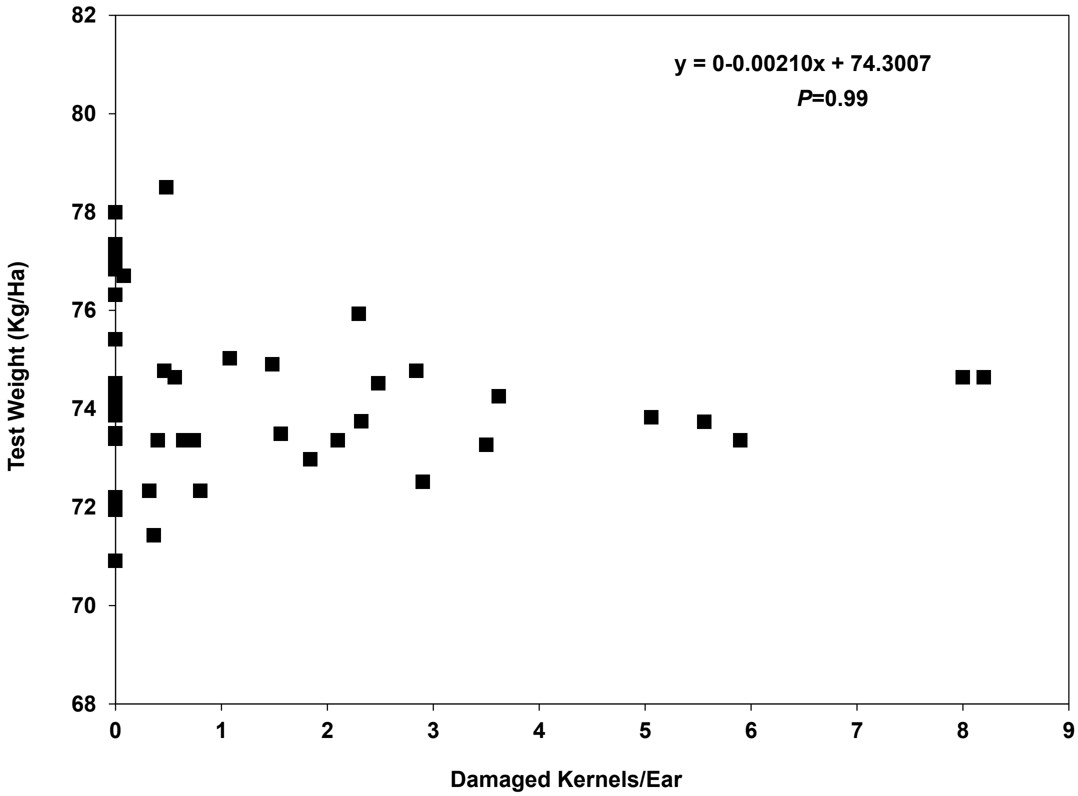

Figure 2.12 Relationship between numbers of damaged kernels per ear and test weight for a corn hybrid expressing the VT Triple Pro® technology in higher yield environments.

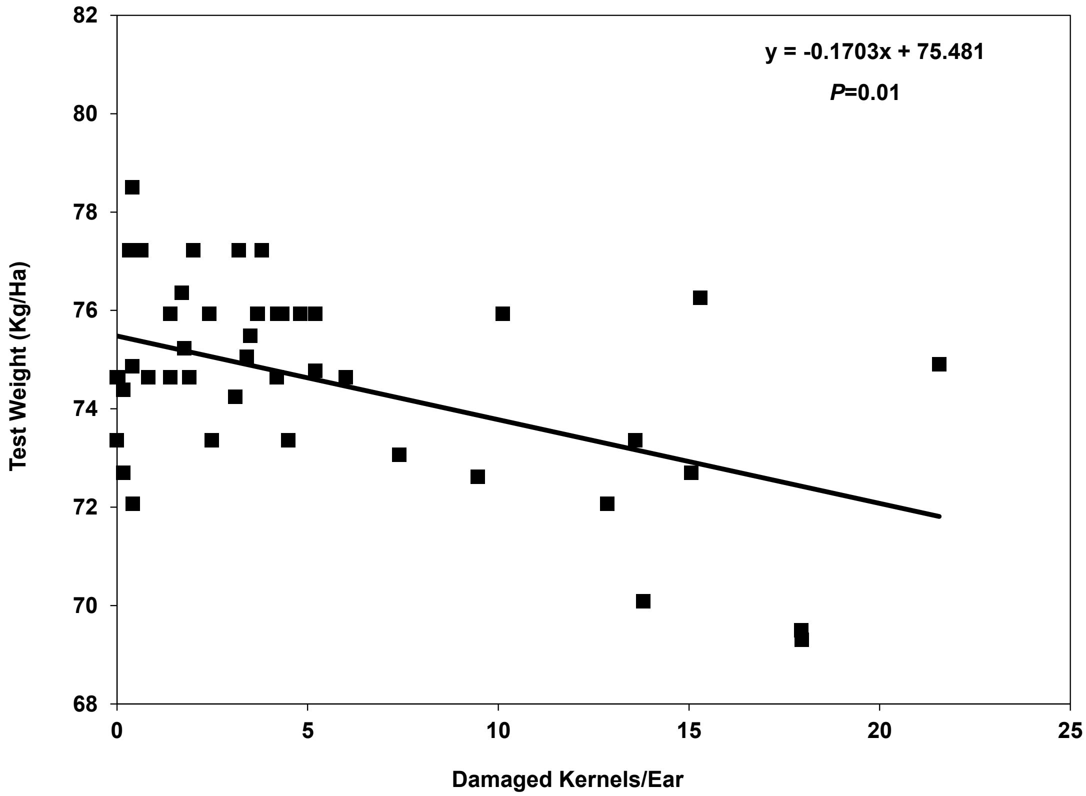

Figure 2.13 Relationship between numbers of damaged kernels per ear and test weight for a corn hybrid expressing the Herculex® technology in lower yield environments.

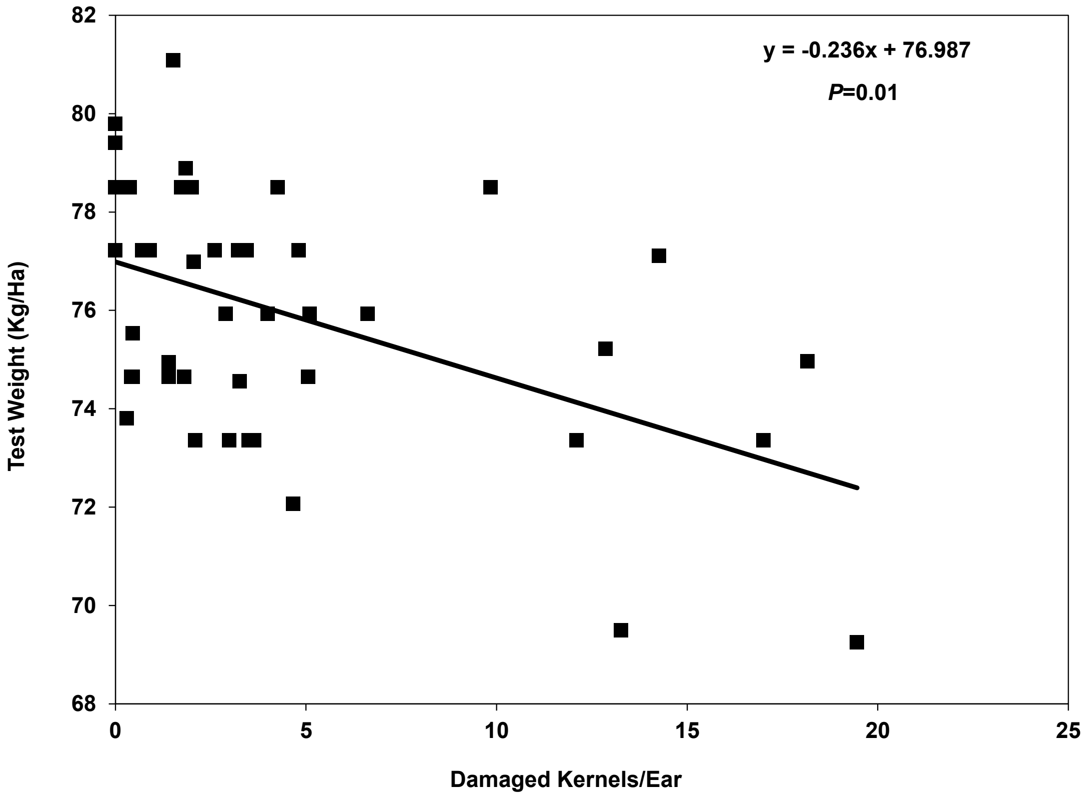

Figure 2.14 Relationship between numbers of damaged kernels per ear and test weight for a corn hybrid expressing the YieldGard® technology in lower yield environments.

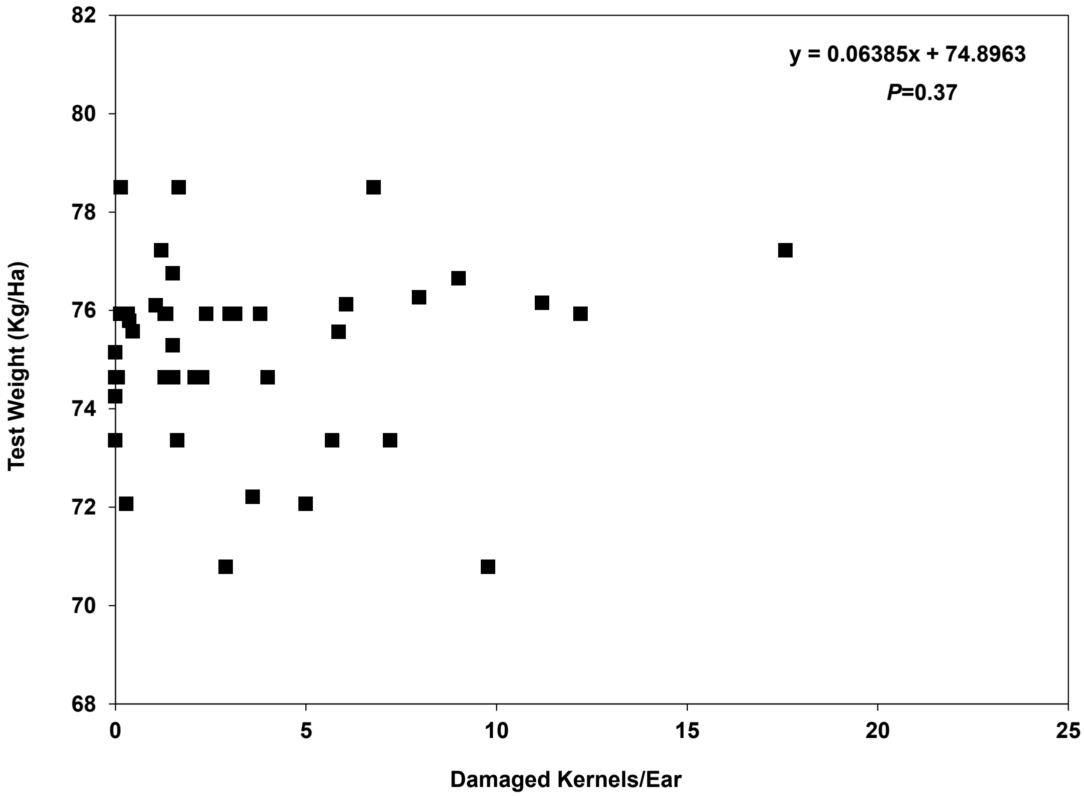

Figure 2.15 Relationship between numbers of damaged kernels per ear and test weight for a corn hybrid expressing the VT Triple Pro® technology in lower yield environments.

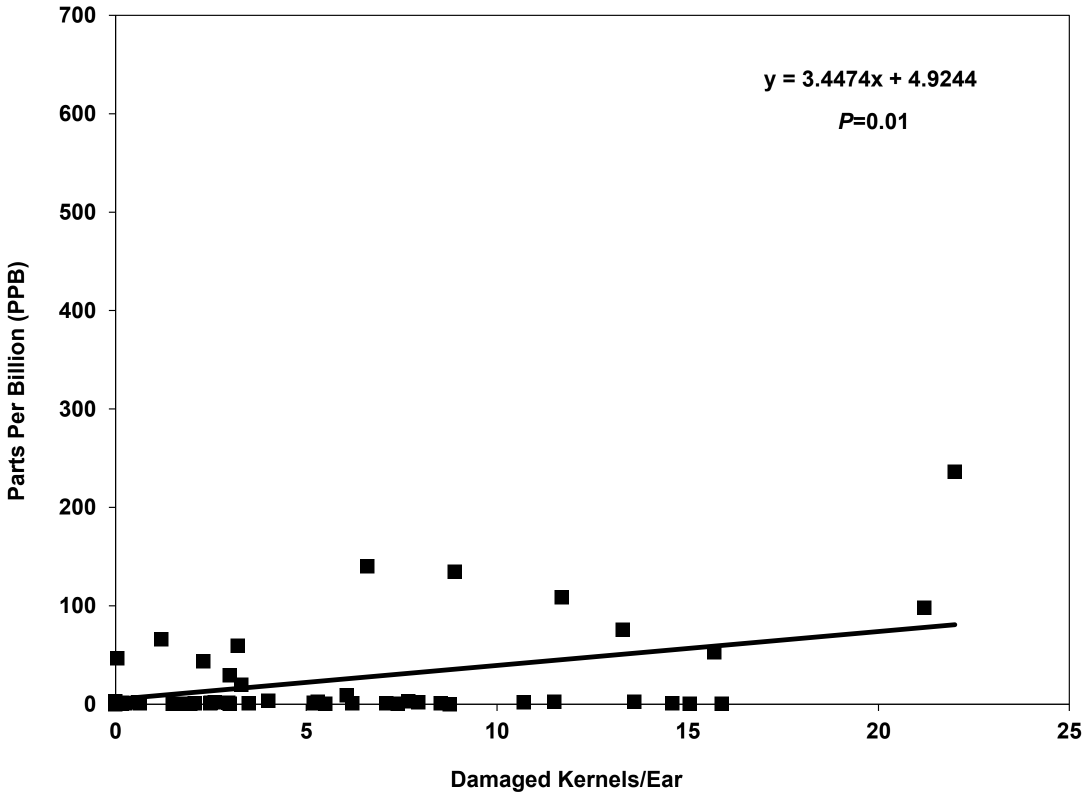

Figure 2.16 Relationship between numbers of damaged kernels per ear and aflatoxin concentration for a corn hybrid expressing the Herculex® technology in higher yield environments.

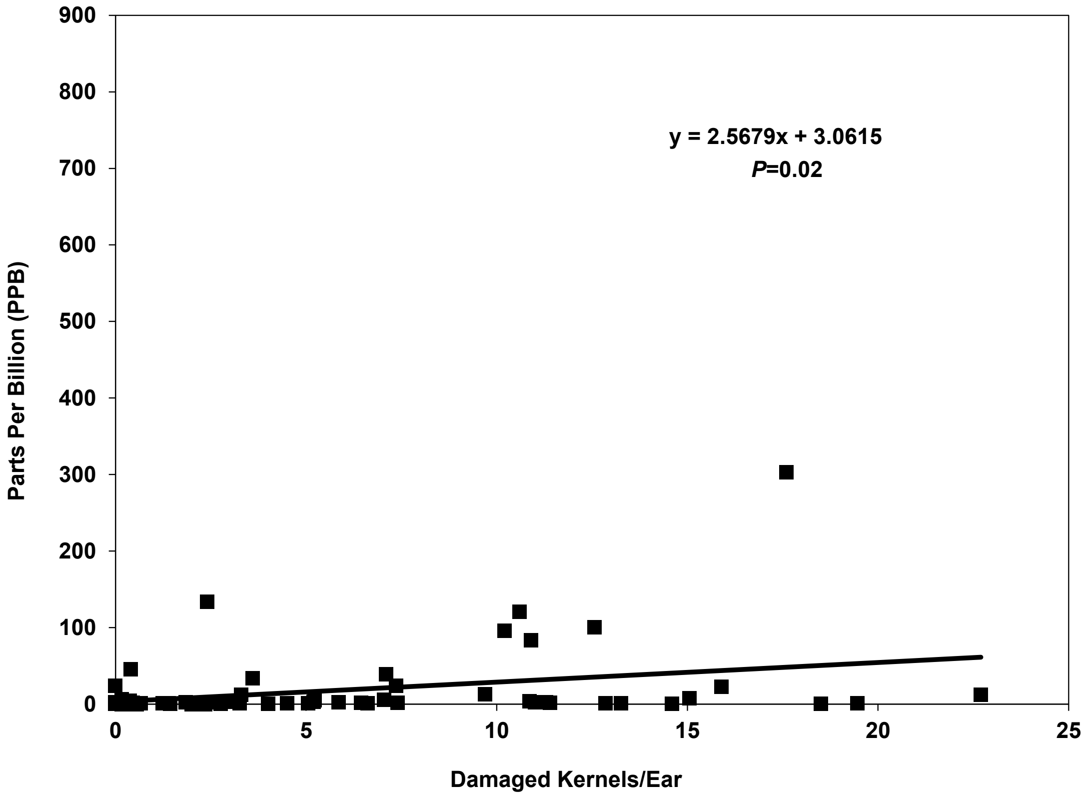

Figure 2.17 Relationship between numbers of damaged kernels per ear and aflatoxin concentration for a corn hybrid expressing the YieldGard® technology in higher yield environments.

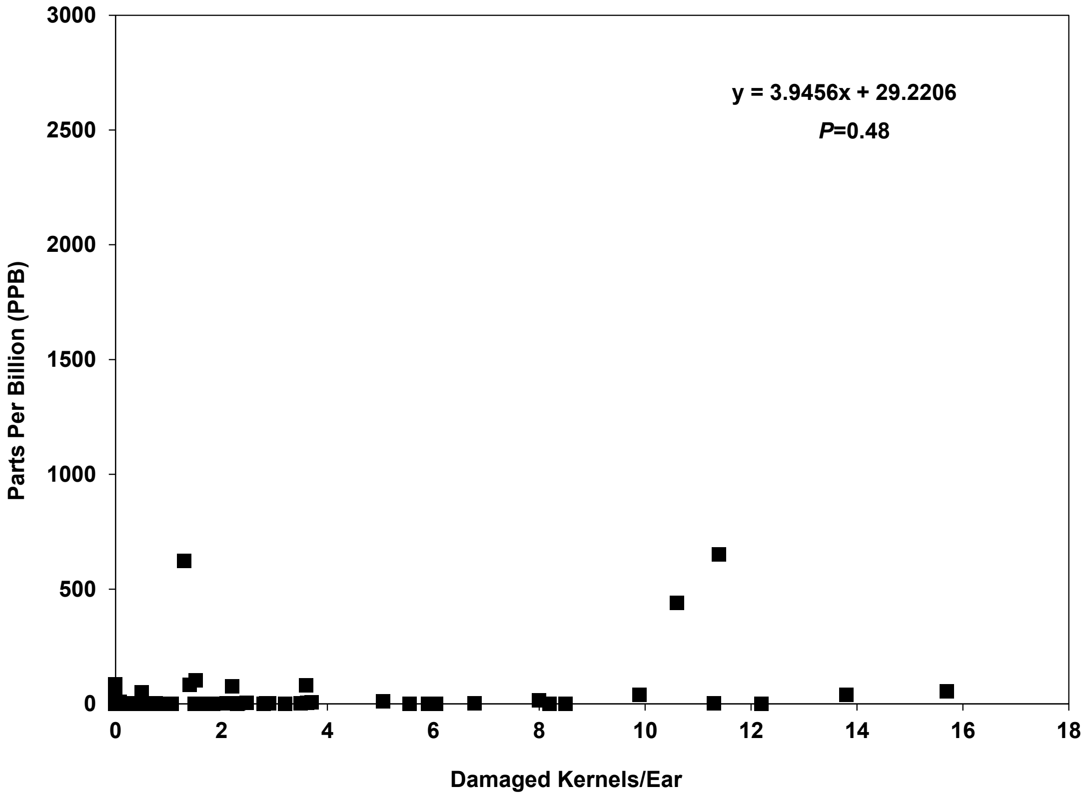

Figure 2.18 Relationship between numbers of damaged kernels per ear and aflatoxin concentration for a corn hybrid expressing the VT Triple Pro® technology in higher yield environments.

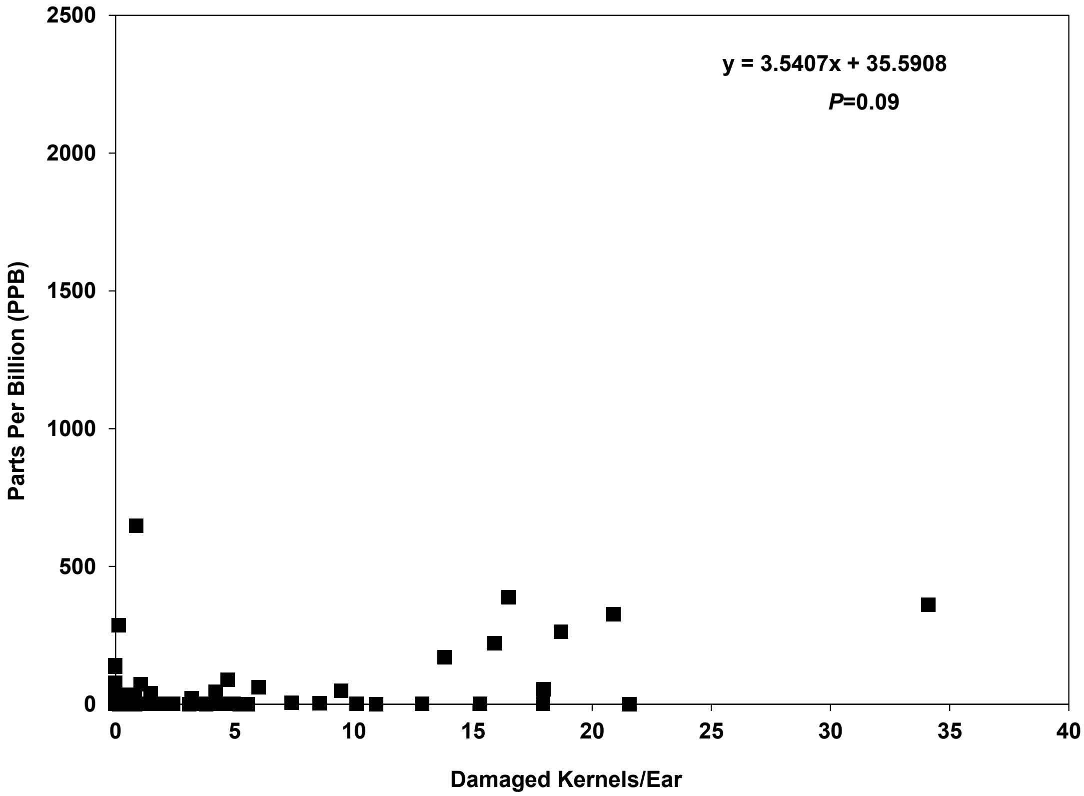

Figure 2.19 Relationship between numbers of damaged kernels per ear and aflatoxin concentration for a corn hybrid expressing the Herculex® technology in lower yield environments.

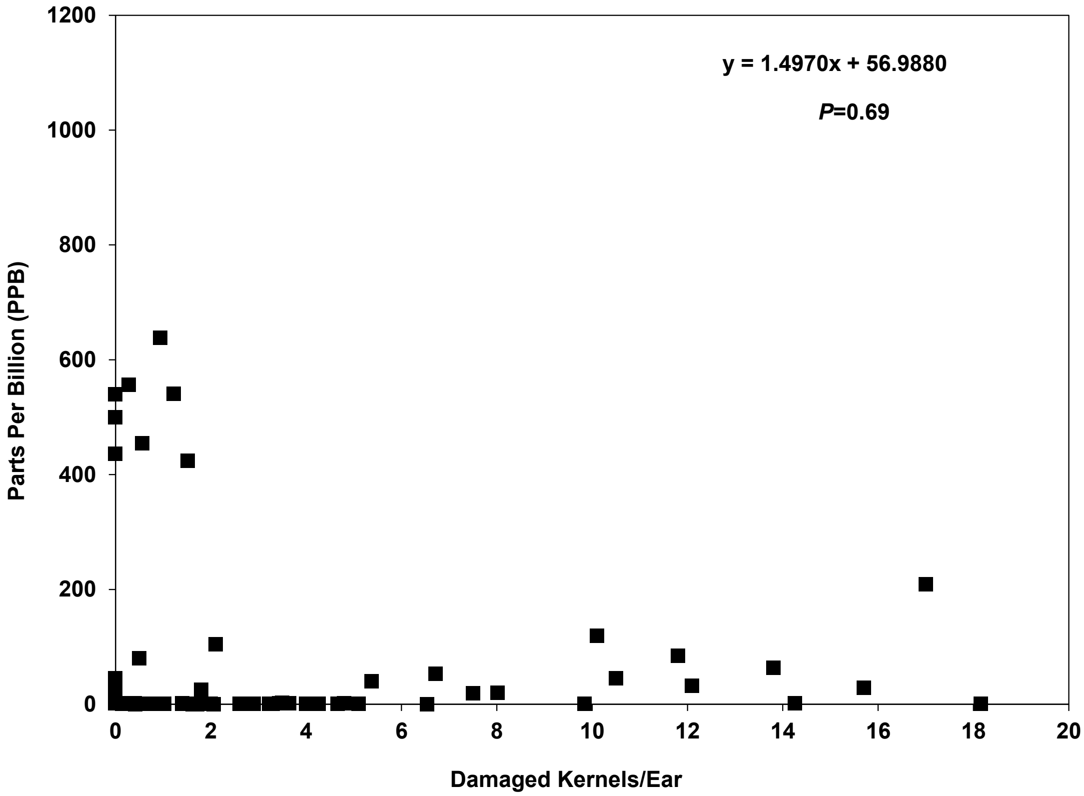

Figure 2.20 Relationship between numbers of damaged kernels per ear and aflatoxin concentration for a corn hybrid expressing the YieldGard® technology in lower yield environments.

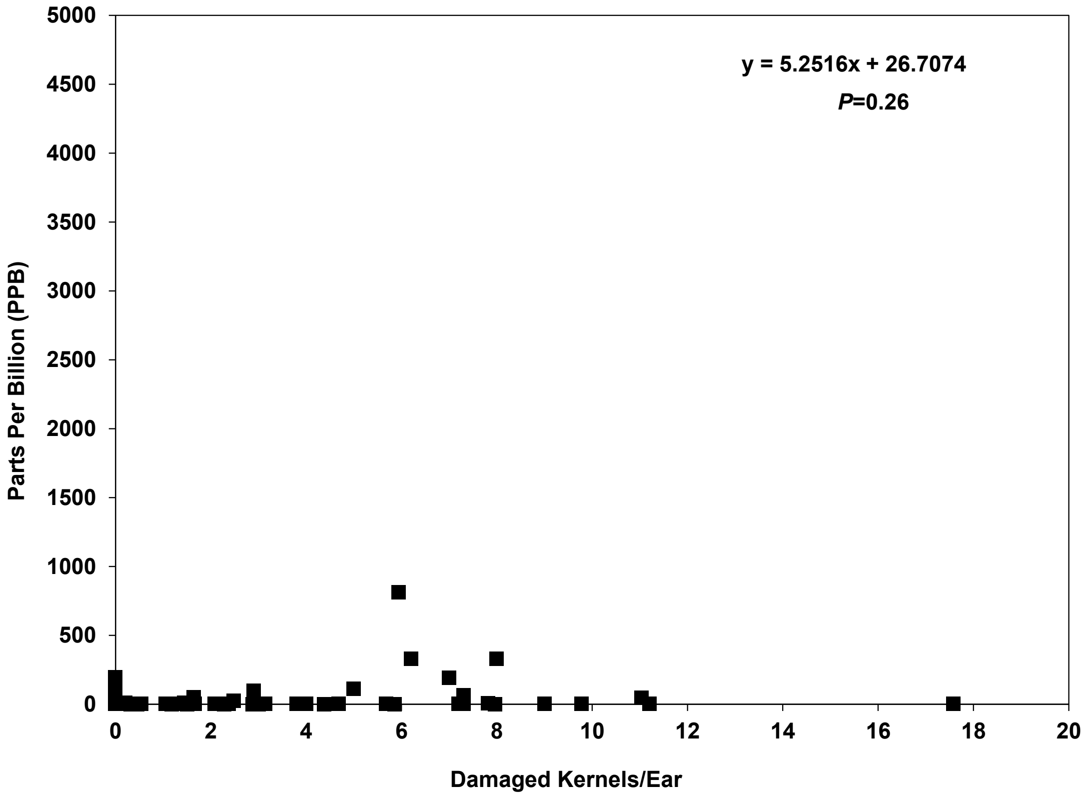

Figure 2.21 Relationship between numbers of damaged kernels per ear and aflatoxin concentration for a corn hybrid expressing the VT Triple Pro® technology in lower yield environments.

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CHAPTER III

IMPACT OF HARVEST METHODS ON YIELD IN FIELD CORN INFESTED BY CORN EARWORM (*Helicoverpa zea*)

Abstract

Historically corn earworm has not been considered an economic pest of field corn due to available management strategies being cost prohibitive. Recently, transgenic Bt corn technologies have been introduced that exhibit a much greater efficacy against corn earworm; however, yield responses have been variable. Experiments were conducted during 2012 and 2013 to evaluate the impact of harvest method on detecting a relationship between the number of corn earworm damaged kernels per ear and yield. No relationship between the number of damaged kernels per ear and yield was observed regardless of harvest method. Also, no differences in yield among harvest methods was observed. However, many factors can influence harvest efficiency including grain moisture, harvest speed, and type and condition of machinery. Additional research is needed to determine if harvest efficiency influences yield response to corn earworm management in field corn.

Introduction

Corn, *Zea mays* (L.), also known as maize, is a C4 grass that is capable of using water, nutrients and CO_2 to produce grain starch (Mabberly 1997, Subedi and Ma 2009, Mangelsdorf 1974). Corn originated from Mexico and Central America as a wild grass that over the years has been improved upon to become one of our most productive crops (Galinat 1988). Approximately 37,231,000 and 38,850,000 hectares (ha) of corn were grown in the United States during 2001 and 2012, respectively (USDA NASS 2012). In Mississippi, 327,795 and 339,936 ha of corn were grown during 2011 and 2012, respectively (USDA NASS 2012), with a value of \$0.24/kg (\$6.01/bu) during 2011 and \$0.26/kg (\$6.67/bu) during 2012.

During kernel development corn earworm, *Helicoverpa zea* (Boddie), infests corn ears. Larvae typically feed on kernels near the tip of the ear. Historically corn earworm has not been considered an economic pest of field corn during the ear development stage because of the high cost of control measures. This feeding has been speculated to cause a reduction in grain yield. Recently, transgenic Bt corn technologies have been introduced that exhibit much greater efficacy against corn earworm and the ability of these transgenic Bt corn technologies to reduce corn earworm injury has been demonstrated. However, yield responses to reductions in insect damage have been inconsistent (Buntin et al. 2001, Buntin et al. 2004, Buntin 2008, Reay-Jones and Wiatrak 2011, Bohnenblust et al. 2013, Reay-Jones and Reisig 2014, Bowen et al. 2014). In the previous chapter and other studies, no significant impact of corn earworm feeding on field corn yield was observed (Buntin et al. 2004, Buntin 2008, Reay-Jones and Wiatrak 2011, Bowen et al. 2014, Reay-Jones and Reisig 2014). This could be the result of smaller kernels near the ear tip, the area where corn earworm typically feeds, being expelled from the combine at harvest. The objective of this study was to evaluate the impact of harvest method (machine vs hand) on the relationship between kernel damage and yield.

Materials and Methods

Experiments were conducted at the Mississippi State University R. R. Foil Plant Science Farm, Starkville, MS and the Delta Research and Extension Center, Stoneville, MS. Plots were planted on 22 May, 2012 at the R.R. Foil Plant Science Farm and 21 May, 2013 at the Delta Research and Extension Center. A two factor experiment with four replications was conducted. The two factors included foliar insecticide applications (treated and a non-treated control) and harvest method (mechanical and hand). These treatments were placed in a factorial arrangement within a split plot design. Trials were conducted using "P1615HR", Pioneer Hi-Bred, Johnston, IA at Starkville and "P1745HR", Pioneer Hi-Bred, Johnston, IA at Stoneville. Both varieties expressed Cry1F (Herculex® 1, Dow AgroSciences, Indianapolis, IN). Plot size for each location was 4 rows and the dimensions of the Stoneville plots were 101.6 cm (40 inch) row spacing with a plot length of 6.09 m (20 ft). The dimensions of the Starkville plots were 96.5 cm (38 inch) row spacing with a plot length of 7.62 m (25 ft). Insecticide applications were made to the center 2 rows and consisted of flubendiamide at 0.105kg ai/ha (0.094 lb ai/acre) (Belt®, Bayer CropScience, Research Triangle Park, NC) every 1-2 days beginning at silk emergence (R1) until 5 days after silk emergence. On the 5th day of silking, 0.5 ml of a 0.2% solution of formulated flubendiamide plus 0.05% solution of methylated seed oil was injected into the area inside of the husk surrounding the silks and tip of each ear. Injections were made using a cattle injection syringe (Allflex® 25MR2 Repeater Syringe, 25ml, Allflex USA, Inc., DFW Airport, TX) with the end of the needle (16 gauge) ground blunt. Immediately prior to harvest, 50 ears per plot were examined for number of corn earworm damaged kernels. The remainder of each plot was harvested according to the designated harvest method. Plots designated for machine harvest were harvested with a Kincaid 8XP (Kincaid Equipment Manufacturing, Haven, KS) small plot combine at both the Starkville and Stoneville locations. Plots designated for hand harvest were harvested by hand and carried back to the lab for shelling using a Seedburo (Seedburo Equipment Comapany, Des Plaines, IL) aluminum hand corn sheller (Starkville) and a Maximizer™ (Pleasant Hill Grain, Hampton, NE) hand crank corn sheller (Stoneville). Plots were harvested on 9 October, 2012 at the R.R. Foil Plant Science Farm; 5 November, 2013 at the Delta Research and Extension Center and yields adjusted to 15% moisture content. Fertilizer application, irrigation and other agronomic practices except insecticide sprays were applied as per the recommendations of the Mississippi State University Extension Service (Anonymous 2011).

Data were subjected to regression analysis using PROC GLIMMIX (SAS Institute, 2013) to determine the relationship between the number of damaged kernels per ear and hand harvest yield and number of damaged kernels per ear and machine harvest yield. The supplemental insecticide applications were utilized to create a range of damaged kernels. For regression analyses, data for plots that received the supplemental insecticide applications were combined with data for the non-treated plots within each harvest method. Data for locations at which no damaged kernels were observed for both the treated and non-treated plots were excluded from analysis. Observations greater than two standard deviations from the mean were considered outliers and excluded from analysis. Site year and replicate nested within site year were considered random effects. Degrees of freedom were calculated using Kenwood-Roger method. Proc GLIMMIX was used to test for equal slopes of the two regression lines. Treated and non-treated plot yields were combined and the overall yield data for harvest methods were subjected to analysis of variance procedures using Proc Glimmix (SAS Institute 2013). Site year and replicate nested within siteyear were considered random effects. Degrees of freedom were calculated using Kenwood-Roger method. Means were separated according to Fisher's Protected Least Significant Difference (α=0.05).

Results and Discussion

No significant relationship between number of damaged kernels and hand harvest yields were observed (F=0.08; df=1, 12.97; *P*=0.78) (Figure 3.1). Also, no significant relationship between number of damaged kernels and machine harvest yields were observed (F=0.05; df=1, 9.39; *P*=0.82). Additionally, the slopes of the regression lines were not significantly different (F=1.67; df=1, 20; *P*=0.21).

Harvest efficiency can substantially influence yield. In the current study there were no significant differences in yield among harvest methods (F=1.09; df=1, 27; *P*=0.31) (Figure 3.2), but no relationship between number of damaged kernels and hand harvest yield was observed either.

However, many factors can influence harvest efficiency including grain moisture, harvest speed, and type and condition of machinery (Johnson and Lamp 1966, Hanna 2008). Harvest losses of 1,255.4 kilograms of corn per hectare have been observed (Hanna 2008). The number of volunteer plants in a field following harvest is also a measure of harvest efficiency, and up to 143,000 volunteer corn plants/ha have been observed in commercial corn fields in Mississippi following harvest (Babu et al. 2014). The current study is limited in scope and additional research is needed to determine if harvest efficiency influences yield response to corn earworm management in field corn.

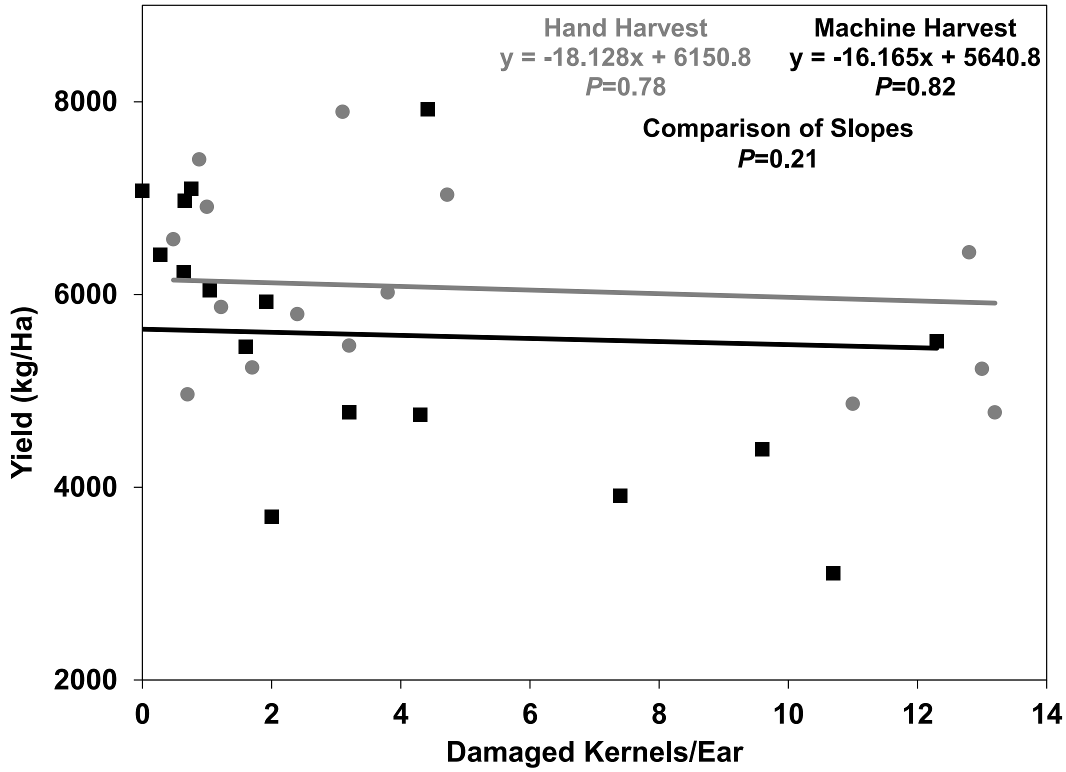

Figure 3.1 Relationship between the total number of damaged kernels per ear and hand harvest yield and the relationship between the total number of damaged kernels per ear and machine harvest yield.

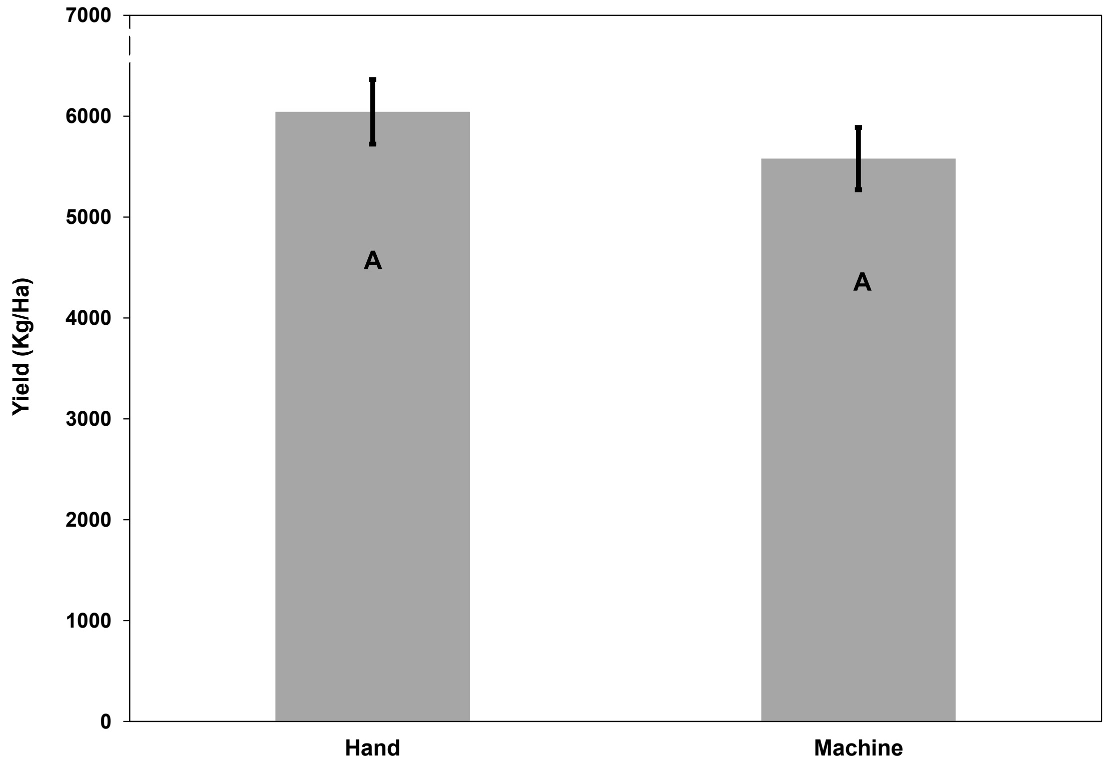

Figure 3.2 Mean yield (Mean ± SEM) differences for hand harvesting compared to machine (combine) harvesting.

Bars with a common letter are not significantly different (P≥0.05, Fisher's PLSD).

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