Impact of simulated corn earworm damage on field corn yield and the influence of chlorantraniliprole and flubendiamide on fall armyworm and agronomic characteristics of field corn

By

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The introduction of pyramided *Bacillus thuringiensis* (Bt) in field corn, *Zea mays* L. has helped reduce kernel and foliar damage from caterpillar pests including fall armyworm, *Spodoptera frugiperda* (J.E. Smith) and corn earworm, *Helicoverpa zea* (Boddie). These pests can also be controlled with diamide insecticides. No compensation for manual kernel damage occurred at any section of the ear for Bt or Non-Bt corn. No consistent impacts on corn growth or yield were observed following foliar applications of diamide insecticides at various growth stages. The diamides exhibit longer residual efficacy than other insecticides available for fall armyworm management in field corn. Based on the level of mortality observed on young tissue developed after treatment with chlorantraniliprole, chlorantraniliprole translocated in corn and could therefore be more beneficial than other insecticides when applied during vegetative growth stages.
DEDICATION

I would like to dedicate this thesis to my parents, Martha P. Olivi and Richard F. Olivi. For everything I have put both of you through, you have never lost faith in me and have always stuck by my side with unconditional love and support. I truly appreciate everything you have done for me and my brother Christopher.

I would also like to dedicate this thesis to my wonderful wife Tatum G. Olivi and my daughter Ava F. Olivi. I thank you both from the bottom of my heart for supporting me through all the good and bad times we have been through together.
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CHAPTER I
LITERATURE REVIEW

Introduction

Field corn, *Zea mays* (L.), also referred to as maize, is grown mainly for consumption as livestock feed, cereal products, ethanol, and processed human-food products including corn syrup, high fructose corn syrup, corn starch, and corn oil. Corn is one of the oldest cultivated grains. (Farnham et al. 2003). Field corn is the most commonly grown field crop in the United States (USDA NASS 2014). Corn production in the U.S. was estimated at 37,915,822 hectares (ha) during 2014 and 39,632,544 ha during 2015 (USDA NASS 2015). Production in Mississippi was estimated at 352,344 ha during 2014 and 350,459 ha during 2015 (USDA NASS 2015).

Several insect pests, such as the seedcorn maggot, *Delia platura* (Meigen), southern corn rootworm, *Diabrotica undecimpunctata howardi* Barber, and black cutworm, *Agrotis ipsilon* (Hufnagel) can infest seeds and seedlings reducing plant populations (Fischhoff 1996). Some insect pests, such as fall armyworm, *Spodoptera frugiperda* (J. E. Smith), can infest corn during the vegetative stage and reproductive stage, causing decreased leaf area. Southwestern corn borer, *Diatraea grandiosella* (Dyar), corn earworm, *Helicoverpa zea* (Boddie), fall armyworm, are targets for control with Bt transgenic corn hybrids and are considered annual pests of field corn in the southern U.S. (Fischhoff 1996).
The corn earworm, is considered one of the most damaging insect pests in North America because it is an economic pest of multiple crops. It causes millions of dollars in economic damage annually (Capinera 2000). This species is polyphagous, has high fecundity, and can diapause as a pupa to aid in over wintering (Fitt 1989, Bohenblust et al. 2013). The corn earworm’s host range includes many agricultural crops such as corn, cotton, *Gossypium hirsutum* (L.), soybean, *Glycine max* (L.) Merr., and many vegetable crops. Corn is one of the most preferred host plants compared to soybeans or cotton (Johnson et al. 1975).

Corn is most attractive to corn earworm adults during the silking stage (Johnson et al. 1975). Oviposition normally occurs on leaf hairs or silks (Lingren et al. 1982). To manage infestations in sweet corn, multiple insecticide applications (generally 7-10) are needed to reduce damage from Lepidoptera pest and make the crop marketable. Foliar insecticide applications are made when corn is in the reproductive stage and eggs and neonates are on leaf hairs or silks. If foliar insecticide applications are not timed well, the larvae enter the ear where they are protected from insecticides. Management of corn earworm infestations in field corn with foliar insecticides has not been considered economically viable and is generally not recommended.

The fall armyworm is another highly polyphagous insect pest. As early as 1797, Smith and Abbott reported that the fall armyworm was an agricultural pest in the U.S. (Sparks 1979). However, fall armyworm does not have a diapause mechanism and over winters only in South Texas and South Florida in the U.S. (Bohnenblust et al. 2012). Therefore, it migrates to the northern latitudes in the U.S. each year when temperatures become warm. Out of some 50 known host species, corn is the most preferred by fall
armyworm (Lynch et al. 1981) and is subject to attack during every growth stage (Wiseman et al. 1979).

Crystalline toxins, from the soil bacterium, *Bacillus thuringiensis* (Berliner), have specific activities against insect species of the orders: Lepidoptera (butterflies and moths), Diptera (mosquitoes and flies), and Coleoptera (beetles) (Dean 1984). Bt has been a valuable source of Cry toxins that have been used in the production of biological insecticides and insect-resistant genetically modified crops. Once insects ingest the toxins, the alkaline pH of their digestive tract begins to break down the crystals. The proteases activate the toxin, which then creates pores in the midgut of the insect (Dean 1984). Insect death often occurs within several hours after ingestion.

Corn, cotton, and soybean have been the main focus of genetic modification. These crops have been genetically engineered for resistance to insects, pathogens, and herbicides. The first generation transgenic Bt corn hybrids were developed primarily to manage the corn borer complex and expressed one Bt protein (Ostlie et al. 1997). Since the initial release of transgenic Bt crops in 1995, there has been several Bt proteins commercialized. These proteins provide protection against Lepidoptera or Coleoptera. The first generation Bt traits in field corn (YieldGard® Corn Borer, Cry1Ab, Monsanto, St. Louis, Mo and Herculex® I, Cry1F, Dow AgroSciences, Indianapolis, IN) have demonstrated minimal activity against corn earworm feeding in ears. However, the newer transgenic Bt corn hybrids [Genuity® VT Triple Pro®, Cry1Ac, Cry1Ab, and Cry1F (Monsanto, St. Louis, Mo), Genuity® SmartStax®, Cry1A, Cry1F, Cry2Ab2, CryBb1, Cry34Ab1, CryAb1 (Monsanto, St. Louis, MO; Dow AgroSciences, Indianapolis, IN) and Agrisure Viptera®, Vip3A (Syngenta, Greensboro, NC)] express multiple Bt toxins
with significant efficacy against corn earworm and fall armyworm. Transgenic corn hybrids expressing insecticidal Cry proteins from Bt have been available in the Southeast since 1998 and offer the potential to reduce losses from fall armyworm and corn earworm in field corn (Buntin et al. 2001, 2004). The proteins are expressed in the tissue of the plant, and offer better control than foliar insecticides against corn earworm. These technologies offer more economical insect protection to producers, because they also provide control of several other insect pests.

Two recently introduced insecticides have been registered in corn and several other crops for control of fall armyworm, corn earworm, and other lepidopteran pests. Flubendiamide (Belt® 4SC, Bayer CropScience, Research Triangle Park, North Carolina) and chlorantraniliprole (Prevathon® 0.43SC, DuPont Crop Protection, Wilmington, Delaware) represent the diamide class of insecticides that react with ryanodine receptors in the muscle cells of insects. When insects are exposed to these insecticides, the calcium channels open and continuously release calcium into the cytoplasm leading to muscle paralysis and eventual death (Cordova et al. 2006). There have been undocumented reports of foliar applications of chlorantraniliprole having a positive influence on corn growth and yield. While there have been several studies that evaluated the potential impact of several insecticides and fungicides on growth and yield on different crops in the absence of insect and disease infestations (Weichel & Nauen 2004, Spiers et al. 2008, Lehoczki-Krsjak et al. 2013), there is little no published data on the impact of diamide insecticides on plant growth and yield of any crop apart from insect protection.
Field Corn

Corn is a member of the grass family Poaceae. The origin of corn has been an issue of historical debate. The center of origin of corn is the Mesoamerican region, primarily in the Mexican highlands. It was in this area where corn spread rapidly. Phylogenetic analysis and archaeological records report that domestication started around 6,000 years ago (Piperno et al. 2001). Corn is one of the oldest cultivated grains and also one of the most productive crop species. It has a global average yield of more than 4 tons per hectare (Farnham et al. 2003).

Corn is a monocot and grows upward from the whorl. It has a determinate growth habit with distinct vegetative and reproductive stages (Hanway et al. 1997). Corn has several vegetative stages identified by the number of collars on the plant. For standardization of definitions, corn researchers developed a guide for determining the different growth stages of corn. Not all plants in a field will reach a particular stage at the same time. Researchers determine a crop stage when at least 50% of the plants have reached a vegetative stage (Kling et al. 1997). The reproductive stages are defined by the ear development stages and include silking (R1): when silks are present and the cob is beginning to form, blister (R2): when the developing kernels are “blisters” of clear fluid on the cob, milk (R3): the majority of the blisters have turned yellow and have a “milky” fluid, dough (R4): the milky fluid is changing into a “doughy” substance, dent (R5): the kernels are starting 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 completely disappeared and a black layer has formed at the end of the kernel stopping the nutrient exchange to the kernel; this stage is referred to as “black layer” (Hanway
1963 and Ritchie et al. 1993). Corn has a C4 photosynthetic pathway that allows a continued response to increasing solar radiation up to full sunlight coupled with low levels of photorespiration. The maximum level of leaf photosynthesis per unit area occurs between full leaf expansion and silking (Lee et al. 2007). Corn is a day neutral plant and growth and development depends upon temperature (Lauer 1997). Thermal time is expressed in units of growing degree-days (GDD) or heat unit (HU) accumulation and is used to categorize different corn cultivars into early or late maturing varieties (McMaster et al. 1997). Growing degree days are the measure of accumulated heat units that is required for a phenological process (such as tasseling) to take place. The critical temperature for corn is 10º C (50º F). Heat units are calculated by subtracting the critical temperature from the average daily temperature (Wang 1960). Different corn hybrids vary in heat unit requirements for development.

**Insect pests of corn**

Several insect pests can infest planted corn seed and seedlings. Insect pests in the Mid-South include southern corn rootworm, white grubs, *Phyllophaga* spp; wireworms, *Melanotus* spp.; seedcorn maggot, bean aphid, *Aphis fabae* (Scopoli); lesser cornstalk borer, *Elasmopalpus lignosellus* (Zeller); potato aphid, *Macrosiphum euphorbiae* (Thomas); brown stink bug, *Euschistus servus* (Say); green stink bug, *Acrosternum hilare* (Say); southern green stink bug, *Nezara viridula* (L.); greenbug, *Schizaphis graminum* (Rondani); corn root aphid, *Aphis middletoni* (Thomas); billbugs, *Sphenophorus* spp; bird cherry-oat aphid, *Rhopalosiphum padi* (L.); chinch bug, *Blissus leucopterus* (Say); black cutworm, and sugarcane beetle, *Eutheola humilis rugiceps* (Le Conte) (Steffey et al. 1999, Akin et al. 2012).
Insects that infest corn during the whorl stage include: fall armyworm, corn earworm, true armyworm, *Pseudoletia unipuncta*; Southwestern corn borer, Southern cornstalk borer, *Diatraea crambidoides* (Grote); corn leaf aphid, *Rhopalosiphum maidis* (F.), and European corn borer, *Ostrinia nubilalis* (Hübner); (Steffey et al. 1999).

Corn earworm and fall armyworm will infest ears, causing direct loss of grain. Insecticidal applications to control corn earworm during the ear stage in field corn is difficult due to crop height and the difficulty of reaching larvae once they enter the ear husk (Buntin 2008). Insecticidal control to prevent ear damage in field corn is generally not cost effective. The eggs must be targeted in order for foliar insecticide applications to be successful so that newly emerged larvae cannot enter the ear. This process is complicated by the rapid growth rate of corn silks exposing untreated silks for oviposition, and the limited time larvae are outside the ear (Storer et al. 2001). Planting dates for field corn are planned to meet agronomic potential, but optimum planting dates tend to be early, which helps avoid corn earworm and fall armyworm infestations (Buntin 2007). The feeding habits of corn earworm and fall armyworm as well as the lower market value of field corn typically attribute to foliar applications against both insects not being economical.

**Corn earworm**

Corn earworm, is in the order Lepidoptera and a member of the Noctuidae family. Corn earworm has a wide host range that includes many crop species throughout South and North America (Hardwick 1965, Covell 1984, Capinera 2005). The corn earworm feeds on the whorl, tassel, silks, and kernels of field corn (Cook et al. 2004). First generation larvae may feed in tightly rolled leaves of whorl-stage corn. A wet, tan to
brown waste droppings, commonly referred to as frass, is deposited between the whorl and the base of the leaves. The second-generation larvae cause damage that is more economically important due to the larvae feeding on the corn kernels around the tip of the ear (Boyd et al. 2001). Corn plant leaves will develop a ragged appearance due to severe feeding by corn earworm. Feeding on kernels near the ear tip creates entry for diseases as well as decreasing kernel weight and reducing yields (Cook et al. 2004). Annual corn yield losses can range from (5-7%) for field corn and (10-15%) for sweet corn (Boyd et al. 2001).

The corn earworm has a holometabolous life cycle that normally lasts 21-28 days (Drees et al. 1999). The life cycle consists of four stages: egg, larva, pupa, and adult (Miller et. al 2003). Eggs are light green in color, but over time turn yellowish and then grey (Neunzig 1964). In general, the head capsule varies from a dark yellow to orange color, but body color can range from green to yellow, pink, or brown. A pair of dark dorsal stripes runs along the back of the body and black microspines cover the cuticle of the body (Rector et al. 2002). Adult moths have yellowish brown forewings that have a dark spot located in the center of their body. Adult moths have yellowish brown forewings that have a dark spot located in the center. The moth’s wingspan ranges from 32 to 45mm (Kogan et. al. 1978). Corn earworm is distributed widely throughout North America, except for Alaska and northern Canada, but does not normally overwinter successfully in the northern United States (Capinera 2010). The number of generations per year is dependent upon different climatic regions (Miller et. al 2003).

Due to its polyphagous nature, corn earworm has several common names (Harding 1967). The common names reflect the major crops that it infests, i.e. cotton,
bollworm; corn, corn earworm; soybean, *Glycine max* (L.) Merr., podworm; tomato, *Solanum lycopersicum* (L.); tomato fruitworm; and sorghum, *Sorghum bicolor* (L.) Moench; headworm. Tomato and sweet corn are the most favored vegetable hosts, but corn earworm also infests asparagus, *Asparagus officinalis* (L.); cabbage, *Brassica oleracea* (L.); lima bean, *Phaseolus lunatus* (L.); spinach, *Spinacia oleracea* (L.); artichoke, *Cynara scolymus* (L.); cowpea, *Vigna unguiculata* (L.) Walp.; cucumber, *Cucumis sativus* (L.); lettuce, *Lactuca sativa* (L.); snap bean, *Phaseolus vulgaris* (L.); eggplant, *Solanum melongena* (L.); okra, *Abelmoschus esculentus* (L.) Moench; and squash, *Cucurbita pepo* (L.). Alfalfa, *Medicago sativa* (L.); field corn; cotton; oat, *Avena sativa* (L.); rice, *Oryza sativa* (L.); sorghum; soybeans; vetch, *Vicia orobus* (L.); wheat, *Triticum aestivum* (L.); and sugarcane, *Saccharum officinarum* (L.) are other crops injured by corn earworm. Ornamental plants and fruit may also be attacked. Ripening avocado, *Persea americana* Mill.; pear, *Pyrus spp.* (L.); grape, *Vitis labrusca* (L.); strawberry, *Fragaria × ananassa* (Duchesne); geranium, *Geranium spp.* (L.); raspberry, *Rubus spp.* (L.); plum, *Prunus spp.* (L.); peaches, *Prunus persica* (L.) Stokes; zinnia, *Zinnia peruviana* (L.); and rose, *Rosa spp.* (L.) are all subject to corn earworm injury (Capinera 2007). Martin et al. (1976) observed corn earworm infesting 17 vegetable and field crops in Florida, with sorghum and corn being the most preferred. Uncultivated plants have also been reported as larval hosts. Corn earworm is often found on common wild annual plants including crown vetch, *Securigera varia* (L.); common lambsquarters *Chenopodium album* (L.); sunflower, *Helianthus annuus* (L.); Spanish needles, *Bidens alba* (L.) DC.; fall panicum, *Panicum dichotomiflorum* (Michx.); prickly sida, *Sida spp.* (L.); lupine, *Lupinus albus* (L.); pigweed, *Amaranthus palmeri* (S. Wats.); common
ragweed, *Ambrosia artemisiifolia* (L.); and velvetleaf, *Abutilon theophrasti* (Medik.). However, in a study conducted in Texas, Harding (1976) observed that only sunflower served as a good wild host relative to 10 other species. In Mississippi, crimson clover, *Trifolium incarnatum* (L.); cranesbill species, *Geranium spp*. (L.); and winter vetch, *Vicia villosa* (Roth) were indicated by Stadelbacher (1981) as important early season hosts. Also, tree and shrub species are often host plants frequented by adult corn earworm. Host plants include *Salix spp*. (L.); *Quercus spp*. (L.); *Prunus spp*. (L.); *Betula spp*. (L.); *Pithecellobium spp*. (Mart.); and other trees (Capinera 2010).

The corn earworm has extensive flight capabilities and can migrate considerable distances along with favorable weather patterns which contribute to its overall distribution and widespread pest damage (Sandstrom et al. 2007). Depending on location and weather, overwintering generally starts in mid to late fall with emergence occurring in early May (Mayer et al. 2003). Dispersing adults may arrive as early as May in southern states to as late as August in northern states due to the variations associated with weather. Corn earworms can complete their life cycle in about a month in the southern states, and may have up to seven generations a year (Mayer et al. 2003). One generation is generally reported in northern areas, and most of Canada, two in northeastern states; three in the central Great Plains; and northern California (Capinera 2010).

Corn earworm infests field corn during the vegetative and reproductive stages (Fitt 1989). Female moths are strongly attracted to silking corn (Johnson et al. 1975), and this is how moths choose their oviposition sites (Miller et al. 1984). An individual moth may lay over 1000 eggs (Lingren et al. 1982). Eggs are individually deposited on leaf hairs and corn silks, and hatch in about 3 to 4 days (Adler et al. 1989). Larvae begin
feeding on young silks and move their way down the silk channel into the ear (Rector et al. 2002). Corn earworm larvae generally feed on the upper part of the ear near the tip (Buntin et al. 1986), and continue until they reach the 6th instar (Fitt 1989). The first three instars are not aggressive and will not cannibalize other larvae (Barber 1936). Older larvae become more aggressive and cannibalistic. This often leaves only one or two large larvae per ear (Boyd 2008). This helps reduce populations when larval density per ear is high as competition for food increases (Barber 1936). Prior to pupation, larvae will exit the ear, and move to the soil. Larvae will burrow into the soil and pupate 5 to 10 cm below the soil surface. Temperature, primarily soil temperature, influences pupal developmental rate, and soil temperatures below 0°C can result in increased pupal mortality (Barber 1937). Soil moisture can also impact pupal survival. Pupal mortality is often high when the soil moisture level is greater than 18 percent (Ditman et al. 1940). Pupal stage duration ranges from 10 to 25 days (Neunzig 1969). Once pupation is completed, moths will emerge from the soil. Moths are most active at night (Lingren et al. 1982). The adult life span typically ranges from 5 to 15 days, and they utilize vegetation to rest during the day (Kogan et. al. 1978). They can live over thirty days under optimum conditions (Kogan et. al. 1978).

**Fall armyworm**

The fall armyworm, is in the order Lepidoptera and a member of the Noctuidae family. In the U.S. it can colonize over 80 different plant species. The presence of the fall armyworm has been recorded in Georgia as early as 1797 (Sparks 1979). It is a strong flier that generally migrates to the northern part of Mississippi by late May or early June (Sparks 1979).
The fall armyworm has a holometabolous life cycle that consists of an egg, larva, pupa, and adult stage. Females lay their eggs in masses that will begin to darken as they age (Flanders et al. 2011). Eggs are dome-shaped and have a white to gray appearance (Bohnenblust et al. 2012). Larval appearance closely resembles corn earworm and true armyworm larvae. The main feature that helps identify the fall armyworm from the corn earworm is a darker head capsule with a white inverted “Y” on the head. The color of the body can vary from green, tan, or black, and three stripes run down the dorsal side (Bohnenblust et al. 2012). Pupation generally takes place in the soil, and pupa appear reddish brown in color. Moths have a wingspan that averages (32-40 mm). Male moths have a forewing that is shaded gray and brown. A triangular white spot is located at the tip of the forewings. Female forewings are less distinctly marked, and range from a uniform grayish brown to a mottling of gray and brown (Capinera 2005).

The fall armyworm is an aggressive polyphagous feeder (Ashley 1979). It is often an economic pest in vegetable crops and sweet corn (Bohnenblust et al. 2012). If available, fall armyworm will feed on several members of the grass family (Poaceae). The host range includes Bermuda grass, *Cynodon dactylon* (L.) Pers.; field corn, sweet corn, and sorghum (Luginbill 1928). Several field crops are frequently injured, including alfalfa, *Medicago sativa* (L.); buckwheat, *Fagopyrum esculentum* (Moench); cotton; clover, *Trifolium* spp. (L.); field corn; oat; peanut, *Arachis hypogaea* (L.); rice; ryegrass, *Lolium* spp. (L.); sorghum; soybean; sugarcane; and wheat. Uncultivated hosts include bentgrass, *Agrostis* spp.; crabgrass, *Digitaria* spp.; Johnson grass, *Sorghum halepense* (L.); morning glory, *Ipomoea* spp.; nutsedge, *Cyperus* spp.; pigweed, *Amaranthus* spp.; and sandspur, *Cenchrus tribuloides* (Capinera 2005).
The pre-oviposition period lasts from 3-4 days. Female moths attach their eggs in clusters of 50 or more in a single layer on foliage of plants, and eggs hatch between 2 to 4 days after oviposition (Flanders et al. 2011). Larvae consume foliage until they have completed 6 instars and pupated (Sparks 1979). The larvae will move to the soil surface and pupate 2-8 cm below the soil in a loosely constructed cocoon of debris. The duration of the pupal stage can range from 37-7 days when the mean soil temperatures are 15-29°C, respectively. The pupa measures 4.5 mm in width and 13-18 mm in length (Sparks 1979). Adults are nocturnal, and are most active during warm, humid evenings. The adult life span is estimated to average about (10 days), with a range of (7-21 days) (Capinera 2005).

The number of generations in an area varies. During warm summer months in the southeastern U.S. and through the Gulf Coast states, the fall armyworm life cycle is completed in about 30 days. Sometimes the life cycle can take up to 60 days during spring and autumn. During winter, life cycles can take between 80-90 days to complete. Fall armyworms will also complete more generations in the Gulf region of the United States than farther north (Capinera 2005). The fall armyworm has no true diapause unlike many other insects, and overwinter in the Gulf of Texas and South Florida where host plants are always available and temperatures rarely reach below 10°C (Luginbill 1928).

The fall armyworm generally feed on foliage, but larvae will also feed on corn ears during heavy infestations. Fall armyworm feeding causes reduced grain weight and yields due to foliar and direct ear feeding in field corn. At heavy infestations, densities of larvae will be reduced down to one or two per plant because of cannibalism (Sparks 1979). Characteristic foliar damage to corn usually includes ragged feeding and moist
sawdust-like frass near the upper leaves and whorl of the plant. Early feeding may appear similar to European corn borer damage, but European corn borer larvae bore into the stalk (Bessin 2004). Ear damage is similar to corn earworm damage. Corn earworm damage includes chewed kernels and visible frass, except fall armyworm burrows through the husk anywhere on the ear and does not feed consistently down through the silks like corn earworm (Bohnenblust et al. 2012).

**Bacillus thuringiensis**

*Bacillus thuringiensis* (Berliner) is a common soil inhabiting gram-positive bacterium which forms parasporal crystals (Shelton 2008). In 1901, Bt was discovered by a Japanese biologist, Shigetane Ishiwata, in a diseased silkworm colony. *Bacillus thuringiensis* was first characterized as an insect pathogen of flour moths in 1911 from the province of Thuringia, Germany (Beegle et al. 1992). The insecticidal activity it expresses, depending on the type of insect, was attributed to the parasporal crystals (Schneqf et al. 1998). In order for Bt proteins to work, they must first be ingested by an insect. Proteins are then activated by the alkaline conditions inside the insect’s gut. The toxin binds to receptors located on epithelium cells in the midgut, and pores begin to form. These pores allow the release of the contents of the midgut into the hemocoel and feeding will diminish. Within several hours, insect mortality will occur (Shelton 2008).

*B. thuringiensis* can be regarded as opportunistic insect pathogens with differing Cry proteins (Schneqf et al. 1998). More than 60 Cry proteins have been identified. Proteins have been discovered with insecticidal activity against the Colorado potato beetle, *Leptinotarsa decemlineata* (Say); (Cry3A, Cry3C); corn earworm (Cry1Ac, Cry1Ab); and European corn borer, *Ostrinia nubilalis* (Hübner) (Cry1Ab, Cry1Ac,
Cry9C). Most early Bt corn hybrids targeting European corn borer only produced Cry1Ab protein and a few produced the Cry1Ac or Cry9C protein (Ostlie et al. 1997). Two of the most widely used strains in foliar insecticides are *B. thuringiensis* subsp. *israelensis* and *B. thuringiensis* subsp. *kurstaki* (Thorne et al. 1986). *Bacillus thuringiensis israelensis* is primarily used to control mosquito and blackfly larvae (Aronson et al. 1986), while *Bacillus thuringiensis kurstaki* is used to control a wide range of lepidopteran species that are important pests in forestry and agriculture (Adang et al. 1985). *Bacillus thuringiensis* is the leading biorational pesticide, and transgenic corn expressing insecticidal proteins from the bacterium *Bacillus thuringiensis* (Bt) has become important in agriculture.

Genetically modified corn is one of the most abundant transgenic crops planted in the United States that is resistant to insect pests. The first generation transgenic Bt corn hybrids genetically engineered with the Mon810 event to express the Cry1Ab protein from *Bacillus thuringiensis* (Bt). It was mainly produced to combat damage by stalk-boring pests, in particular, European corn borer, and southwestern corn borer (Koziel et al. 1993). However other lepidopterous pests, including *H. zea*, are also affected to varying degrees by Bt toxins on field corn (Sims et al. 1996).

Genetically modified corn hybrids expressing insecticidal *Bacillus thuringiensis* (Bt) toxins were first planted commercially in the United States in 1996 (Burkness et al. 2011). Seed companies are beginning to market Bt hybrids (e.g. Viptera, Smartstax) that contain increased insecticidal activity against corn earworm, fall armyworm, and several other noctuid species (Dow Agrosciences 2010, Syngenta 2011). Before these newer hybrids were developed, Bt events (e.g., MON810) suppressed corn earworm populations
by 70-90% (Kennedy and Storer 2000). Currently, hybrids containing several Bt events can offer near complete control of corn earworm, partially those that produce the Vip3A protein (Burkness et al. 2010). However, this level of control is only needed if populations of corn earworm pose an economic risk to field corn, and little research appears to have characterized damage of corn earworm and fall armyworm in modern hybrids of field corn in the southern states.

**Impact of Bt corn on target pests**

Bt corn hybrids have provided protection against the European corn borer equal to, and usually far greater than, optimally timed foliar insecticides. Ostlie (et al. 1997) found that Bt corn hybrids (regardless of event) provided 99% control of first generation European corn borer larvae in whorl-stage corn. Events BT11 (Cry1Ab) and MON810 were observed to provide a higher level of control than event 176 (Cry1Ab) (Mycogen Indianapolis, IN). This is due to event 176 hybrids only producing Bt protein in green tissues and pollen. MON810 and BT11 events produce Bt protein throughout the plant (Ostlie et al. 1997).

Bt offers the potential for reducing damage by fall armyworm, and corn earworm, which are lepidopteran pests of corn in some areas. Field and laboratory studies have shown that hybrids containing the Bt11 event reduce fall armyworm and corn earworm growth and survival (Williams et al. 1997, 1998). Due to the natural geographic variability in corn earworm and fall armyworm susceptibility to Bt toxins (Siegfried et al. 2000) and the increased use of Bt transgenic corn throughout the United States, it is important to determine the effects of Bt corn hybrids in reducing corn earworm and fall armyworm damage in different corn growing regions (Chilcutt et al. 2006).
Since the release of Bt corn, research has been conducted to test the efficacy of technologies in Bt corn against fall armyworm and corn earworm. Corn hybrids vary in their susceptibility to feeding damage by corn earworm and fall armyworm, and in agronomic traits, making it important that comparisons of Bt transgenic and non-Bt corn be performed using similar hybrids Chilcutt et al. (2006). Hybrids expressing the Cry1Ab protein reduced larval growth of corn earworm, and kernel damage was reduced by an average of 80% in trials conducted by Storer et al. (2001). Storer et al. (2001), also observed a significant reduction in adult emergence with Cry1Ab expressing corn lines compared to non-Bt lines. Buntin et al. (2004) found that in fall armyworm whorl infestations, all Bt events in 1999, 2000, and 2001 studies significantly reduced mean whorl damage rating per plant compared with the non-transgenic varieties on all sample dates. Buntin et al. (2004) also concluded that despite manual infestations of ears with fall armyworms in 2000, virtually all lepidopteran larvae recovered from corn ears were corn earworm. All Bt treatments also had considerably less ear infestation and damage by corn earworm compared with the non-transgenic varieties. In studies conducted by Chilcutt et al. (2006), corn earworm densities were higher on Bt corn when compared to non-Bt plants, and in some instances more early instar larvae were observed on Bt plants than non-Bt plants, which may be a result of growth inhibition caused by Bt proteins (Storer et al. 2001, Chilcutt et al. 2006). Chilcutt et al. (2006) concluded that the higher densities could have resulted from a reduction in cannibalism. Horner et al. (2003) found that the percentage of Mon810 (Cry1Ab) ears damaged by corn earworm was reduced by 33%, and kernel damage was reduced by 60% compared with non-Bt hybrids. Buntin et al. (2004), found that MON84006 (Cry2Ab2) singly and pyramided with Cry1Ab
proteins had superior control of whorl-stage damage by fall armyworm and ear stage
damage by corn earworm compared with MON810 (Cry1Ab) alone.

Cry1Ab proteins and probably Cry2Ab2 proteins are not expressed a season-long at a high-dose for fall armyworm (Buntin et al. 2004). There is a high chance of repeated exposure within a season, but this probably doesn’t matter since fall armyworm does not overwinter in Mississippi (Sparks 1979) and any individuals that are resistant would not survive until the next season (Buntin et al. 2004).

Since the introduction of transgenic Bt corn technologies that exhibit substantial activity against corn earworm during the ear stage, there has been more interest in managing ear infestations of corn earworm in field corn. Positive yield responses have been observed in some studies following reductions in kernel damage (DeLamar et al. 1999a-e; Lauer & Wedberg 1999; Storer et al. 2001; Buntin et al. 2004), while no responses have been observed in other studies (Buntin 2008; Bowen et al. 2014; Reay-Jones and Reisig 2014; Steckel & Stewart 2015).

**Diamide Chemistry**

Several new insecticides that have novel modes of action have been developed for use in integrated pest management. The diamide insecticides are classified as reduced risk because of their low mammalian toxicity and narrow spectrum of activity (Villanueva et al. 2005). Diamides control insects by controlling the release of calcium from intracellular stores via Ca$_{2+}$ channels such as ryanodine channels (RyR). The RYR mediates several cellular and physiological activities such as hormone secretion, neurotransmitter release, muscle contraction and gene expression (Hirooka et al. 2007). The discovery of a new chemical class of insecticides based on insecticidal diamide that
binds to insect RyR at a site distinct from ryanodine and strongly interferes with the receptor's role in calcium homeostasis used RYR to achieve toxicity of some species (Cordova et al. 2006). Diamide insecticides have been recently introduced to the market, and are represented by two commercial compounds: flubendiamide and chlorantraniliprole (Hirooka et al. 2007). Lepidopteran larvae treated with diamide insecticides show unique symptoms of poisoning including feeding cessation, complete contraction paralysis, and ultimately death (Tohnishi et al. 2005, Hirooka et al. 2007). It was selected for development based on the combination of insecticidal potency and excellent mammalian safety (Lahm et al. 2007). This chemical has excellent larvicidal activity against many Lepidoptera and Coleoptera (Lahm et al. 2007). Toxicity has also been observed in some Diptera, Isoptera, and Hemiptera (DuPont 2007, Hannig et al. 2009). Chlorantraniliprole is more selective to ryanodine receptors of insects than mammals, which accounts for its low mammalian toxicity (Lahm et al. 2007, 2009).

Chlorantraniliprole is relatively harmless to beneficial arthropods, and has not been found to show cross-resistance with existing insecticides (Lahm et al. 2005; Lahm et al. 2009). Consequently, chlorantraniliprole provides rapid plant protection through feeding cessation in the target pest soon after consumption (Hirooka et al. 2007) and eventual death because of starvation within 1-3 d after exposure by ingestion or contact (Lahm et al. 2009). Both chlorantraniliprole and flubendiamide are now being used worldwide for a broad range of crops to control several pests belonging to Lepidoptera.

These diamide insecticides have been reported to have high residual efficacy against the target pests. The level of mortality observed indicates that chlorantraniliprole was translocated from treated tissue to tissue that developed after application (Cordova et
Sial & Brunner (2010) determined the residual toxicity of chlorantraniliprole to obliquebanded leafroller, *Choristoneura rosaceana* (Harris). A mortality of 100% was recorded at field rate applications of chlorantraniliprole, 10, 38, and 59 (DAT). Hardke et al. (2009) conducted leaf tissue bioassays in grain sorghum on fall armyworm mortality with chlorantraniliprole and flubendiamide, and observed that plant tissue treated with chlorantraniliprole or flubendiamide significantly impacted fall armyworm mortality compared to that of larvae on the non-treated tissue 7 DAT and 14DAT. Also, translocation within the plant has been observed with foliar applications of chlorantraniliprole but not flubendiamide (Chen et al. 2015).

The objectives of this project were to determine the critical level of kernel damage to field corn that would reduce yield using manual damage methods, determine the influence of chlorantraniliprole and flubendiamide on agronomic characteristics including yield of field corn, and evaluate the residual efficacy and potential translocation of chlorantraniliprole and flubendiamide in field corn.
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CHAPTER II

IMPACT OF SIMULATED CORN EARWORM (*Helicoverpa zea*)

DAMAGE ON FIELD CORN YIELD

Abstract

In recent years, Bt corn has been observed to efficiently control corn earworm feeding and help protect plants from indirect and direct damage. In this study Bt corn hybrids expressing the Agrisure Viptera trait were used to minimize impacts of natural infestations of corn earworm and fall armyworm. Manual ear damage was imposed at milk stage to mimic corn earworm feeding while avoiding interference with pollination. A significant relationship between the number of damaged kernels and total yield was observed. Each increase of one damaged kernel resulted in a reduction of 0.27g of yield per ear. A significant relationship between the number of damaged kernels and the total number of harvested kernels per ear was also observed. Each increase of one damaged kernel at milk stage resulted in reduction of 0.86 kernels at harvest. While the relationship is statistically significant, the small amount of yield lost to corn earworm seldom justifies incurring any additional expense to protect hybrids from this feeding damage.
Introduction

Field corn, *Zea mays* (L.), also referred to as maize, is grown mainly for consumption as livestock feed, cereal products, ethanol, and processed human-food products including corn syrup, high fructose corn syrup, corn starch, and corn oil. Field corn is the most commonly grown field crop in the United States (USDA NASS 2014). Corn production in the U.S. was estimated at 37,915,822 hectares (ha) during 2014 and 39,632,544 ha during 2015 (USDA NASS 2015). Production in Mississippi was estimated at 352,344 ha during 2014 and 350,459 ha during 2015 (USDA NASS 2015) with a value of $4.50/bu during 2014 and $4.10/bu during 2015, respectively.

In North America, corn earworm, *Helicoverpa zea* (Boddie), (Lepidoptera: Noctuidae), is often considered to be one of the most damaging insect crop pests across all crops (Hellmich and Hellmich 2012). Corn earworm feeds on many different plant families including, but not limited to, Asteraceae, Fabaceae, Leguminaceae, Malvaceae, Poaceae, and Solanaceae (Fitt 1989). Corn earworm is distributed widely throughout North America, except for Alaska and northern Canada, but does not normally overwinter successfully in the northern United States (Capinera 2010). The corn earworm life cycle normally begins in corn due to the insect emerging in May (Hardwick et al. 1965). However, corn earworm can emerge as earlier as March in clover before corn is infested (Mayer et al. 2003).

Corn earworm and fall armyworm infest field corn during the vegetative and reproductive stages (Fitt 1989). Female corn earworm moths are strongly attracted to silking corn (Johnson et al. 1975), and the attractiveness and robustness of the plant is how moths choose oviposition sites (Miller and Strickler 1984). Eggs are individually
deposited on leaf hairs and corn silks, and hatch in about 3 to 4 days (Adler and Charles 1989). Following eclosion, larvae typically feed on the reproductive structures of the plant when available. The larvae begin feeding on young silks and move down the silk channel into the ear. Corn earworm larvae generally feed on the upper part of the ear near the tip (Buntin 1986), and continue until they reach the 6th instar (Fitt 1989).

*Bacillus thuringiensis* (Berliner) is a common soil inhabiting gram-positive bacterium which forms parasporal crystals, and the insecticidal activity it expresses is attributed to the parasporal crystals (Schneqf et al. 1998). In 1901, *Bacillus thuringiensis* was discovered by a Japanese biologist, Shigetane Ishiwata, in a diseased silkworm colony (Beegle and Yamamoto 1992).

*Bacillus thuringiensis* produces proteins that express highly specific insecticidal activity during the stationary phase of its growth cycle. (Schnepf et al. 1998). Bt proteins must first be ingested by an insect for insecticidal activity to occur. Proteins are activated by the alkaline conditions inside the insect’s peritrophic matrix, which is an extracellular envelope that lines the digestive tract of most insects. The toxins bind to receptors located on epithelium cells in the midgut, and pores begin to form. These pores allow the release of the contents of the midgut into the hemocoel and feeding declines. Within several hours, insect mortality will occur (Shelton 2008).

Corn genetically modified with Bt toxins is the most widely planted transgenic crop in the United States with resistance to insect pests. The first generation transgenic Bt corn hybrids were commercialized beginning in 1995 and expressed only one Bt protein (Ostlie et al. 1997). They were genetically engineered to express the Cry1Ab or Cry1F proteins, and were mainly produced to control stalk-boring pests, in particular, European
corn borer, *Ostrinia nubilalis* (Hübner) and southwestern corn borer, *Diatraea grandiosella* Dyar (Koziel et al. 1993). Two of the most widely planted single gene technologies were Herculex® containing Cry1F and YieldGard®, containing Cry1Ab (Hellmich and Hellmich 2012).

As part of the resistance management strategy for Bt corn, newer products have been introduced that express multiple Bt traits targeting lepidopteran pests (Hellmich and Hellmich 2012). These include several products that exhibit substantial activity against corn earworm, (e.g. Viptera®, Smartstax®, VT3P®). This study was conducted to examine the relationship between kernel damage and yield and to determine the number of damaged kernels required to impact yield.

**Materials and Methods**

**Impact of simulated corn earworm damage on field corn yield**

Experiments were conducted to examine the impact of kernel damage on field corn yield using manual damage methods. Manual damage methods were used so that defined levels of damage and levels higher than those normally observed with natural infestations of corn earworm could be studied. Research was conducted during 2013 and 2014 at the Delta Research & Extension Center, Stoneville, MS. Bt corn hybrids expressing the Agrisure Viptera trait (NK77P-3111 during 2013 and Pioneer 1319VYHR during 2014) were used to minimize impacts of natural infestations of corn earworm and fall armyworm. Both hybrids were planted at 84,000 seeds per ha. Trials were planted from late March through late May during 2013 and 2014. A randomized complete block design was used with four to six replications. Plots were 1 row x 12.19 m with 1.016 m row spacing. Manually damaged treatments included 0, 10, 20, 40, 60, and 100 kernels
damaged per ear. Damage treatments were imposed on 10 ears per plot during R3 (milk stage) to avoid interference with pollination. The starting point for kernel damage treatments was the uppermost kernels on each ear that were pollinated and had reached R3. Additional kernels were damaged around the circumference toward the base of the ear until the requisite number of kernels for the particular treatment had been damaged. Kernels were damaged by pressing a sharp object into an individual kernel until the pericarp had ruptured and the contents of the kernel were expelled. After damage was imposed, each ear was surface sterilized with 95% ethanol by spraying approximately 8 ml of ethanol on each ear with a trigger spray bottle. The husk was then pulled back up around the ear and covered with a 14 x 35 cm water repellant pollination bag (Midco, Kirkwood, MO). The bags were placed individually on each ear, pulled tightly around the base of the ear and the bag was stapled to itself to secure the bag on the ear. Surface sterilization and covering with pollination bags were measures taken to minimize fungal contamination and growth. Ears were individually hand harvested when grain moisture content was no higher than 15%. Each ear was equally divided into 4 sections based upon total ear length, with section 1 representing the ear tip and section 4 representing the ear base. Mean grain weight, number of kernels, and weight of individual kernels was determined for each ear section. Grain weight was adjusted to 15% moisture.

**Data Analysis**

Kernel damage and yield were subjected to linear regression analysis using Proc GLIMMIX (SAS Institute 2013). For all analyses site year (trial within a year) and replication nested within site year were considered random effects. Degrees of freedom were calculated using the Kenwood-Roger method. Data for individual kernel weight for
each ear section of the control plots were subjected to analysis of variance using Proc GLIMMIX (SAS Institute 2013). Kernel weight means were separated using Fisher’s protected LSD test, and differences were considered significant for α=0.05.

Results

Total Yield

A significant relationship between the number of damaged kernels and total yield was observed \((F=42.22, \text{df}=1, 99; \ P<0.01)\) (Figure 2.1A). Each increase of one damaged kernel resulted in a reduction of 0.27g per ear of yield. Also, a significant relationship between the number of damage kernels and the total number of kernels per ear was observed \((F=113.78, \text{df}=1, 99; \ P<0.01)\) (Figure 2.1B). Each increase of one damaged kernel at milk stage resulted in reduction of 0.87 kernels at harvest. No significant relationship between the number of damaged kernels and individual kernel weight was observed \((F=1.74, \text{df}=1, 99; \ P=0.19)\) (Figure 2.1C).

Yield Components

A significant relationship between damaged kernels and yield was observed for ear section one (ear tip) \((F=124.9, \text{df}=1, 99; \ P<0.01)\) (Figure 2.2A). For each increase of 1 damaged kernel, grain yield was reduced by 0.18g per ear. This was expected since much of the kernel damage imposed occurred in this portion of the ear. Also, a significant relationship between damaged kernels and yield was also observed for ear section two \((F=23.99, \text{df}=1, 99; \ P<0.01)\) (Figure 2.2B). Yield reductions were lower than that observed in section 1, with each increase of 1 damaged kernel resulting in a 0.06g per ear yield loss. No significant relationship between damaged kernels and yield was observed
for ear section three \( (F=1.91, \text{df}=1, 99; P=0.17) \) (Figure 2.2C), or ear section 4 \( (F=0.01, \text{df}=1, 99; P=0.95) \) (Figure 2.2D).

A significant relationship between damaged kernels and number of kernels was observed for ear sections one \( (F=160.06, \text{df}=1, 99; P<0.01) \) (Figure 2.3A), two \( (F=29.13, \text{df}=1, 99; P<0.01) \) (Figure 2.3B), and three \( (F=4.72, \text{df}=1, 99; P<0.03) \) (Figure 2.3C). For each increase of 1 damaged kernel at milk stage the number of kernels present at harvest for ear sections 1, 2, and 3 was reduced by 0.6, 0.16, and 0.08 kernels per ear, respectively. No significant relationship between damage and number of kernels was observed for ear section four \( (F=0.25, \text{df}=1, 99; P=0.62) \) (Figure 2.3D).

Consistent with the finding that individual kernel weight did not change overall with increasing kernel damage, individual kernel weight was not impacted by the number of damaged kernels for any section (data not shown). Natural variation in individual kernel weight can occur among kernels on the same ear depending on location and is illustrated in Figure 2.4. In the absence of kernel damage (non-damaged ears), ear section 1 (ear tip) had significantly lower individual kernel weights compared to ear sections 3 and 4 (base of ear) \( (F=12.66; \text{df} = 3, 57; P<0.01) \).

**Discussion**

For many years, corn earworm infesting field corn during the ear stage has not been deemed an economic pest, primarily due to the number of foliar insecticide applications that would be required for control. Since the introduction of transgenic Bt corn technologies that exhibit substantial activity against corn earworm during the ear stage, there has been more interest in managing ear infestations of corn earworm in field corn. Results from studies with natural infestations of corn earworm have varied.
Positive yield responses have been observed in some studies following reductions in kernel damage (DeLamar et al. 1999a-e; Lauer & Wedberg 1999; Storer et al. 2001; Buntin et al. 2004), while no responses have been observed in other studies (Bibb 2015; Buntin 2008; Bowen et al. 2014; Reay-Jones and Reisig 2014; Steckel & Stewart 2015).

The current study utilized mechanical damage methods so that defined levels of damaged kernels and levels greater than those normally observed with natural infestations of corn earworm could be imposed. Minimal amount of any fungal infections were observed. Results of the current study indicate that no compensation for kernel damage occurred at any section of the ear. Steckel & Stewart (2015) observed compensation for kernel damage in some instances, but not others in simulated damage studies. Steckel and Stewart (2015) also reported varied responses among corn hybrids for individual kernel weight in studies with kernel damage from naturally occurring corn earworm infestations. Also, our results showed a significant negative relationship between the number of damaged kernels and the total number of kernels per ear. Steckel & Stewart (2015) also observed that as the number of damaged kernels per ear increased, total number of kernels per ear decreased significantly in simulated damage studies. In the current study a significant negative relationship was observed between the number of damaged kernels and total kernel weight per ear (yield). Steckel & Stewart (2015) observed varied responses in total kernel weight per ear (yield) to a range of levels of damaged kernels, with a significant negative response in some cases and no response in others.

The current study demonstrates that destruction of kernels can negatively impact yield, and that there are possibilities to improve field corn yield through reduction in
kernel damage from ear feeding lepidopteran insects. However, with a total yield reduction of only 0.27 g per damaged kernel and an average of 10 damaged kernels per ear (10 damaged kernels per ear is within the range of damage from natural corn earworm infestations reported by Steckel and Stewart (2015)), on an average ear weighing 184 g, yield loss would only be 2.7g. On a high yielding field, this could cost the grower $40/ha, but preventing ear feeding with transgenic hybrids or foliar insecticides will often exceed this cost and seldom provides absolute control, so the economic benefit of controlling ear-feeding insects in field corn is minimal and may be negative. Damage to ears from feeding by lepidopteran larvae can provide an entry point for fungal organisms that produce aflatoxins (Widstrom et al. 1975), but insect damage does not appear to be the 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). Therefore, the selection of corn hybrids and foliar insecticides in field corn should be based on agronomics and control of pests other than ear-feeding corn earworm.
Figure 2.1  Linear regressions between the number of damaged kernels per ear and total yield (A) total number of kernels per ear (B) and individual weight per kernel (C).
Figure 2.2  Linear regressions between the number of damaged kernels and yield for ear tip (A), mid-tip (B), mid-bone (C), and base (D).
Figure 2.3  Linear regression between the number of damaged kernels and number of total kernels for ear tip (A), mid-tip (B), mid-base (C), and base (D).
Figure 2.4  Mean kernel weight (±SEM) by ear section, for non-damaged ears, with section one representing the tip of the ear and section four representing the base section of the ear.

Bars containing the same letter are not significantly different (Fishers Protected LSD, \( \alpha=0.05 \)).
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CHAPTER III
THE INFLUENCE OF CHLORANTRANILIPROLE AND FLUBENDIAMIDE ON AGRONOMIC CHARACTERISTICS OF FIELD CORN

Abstract

There have been undocumented claims of foliar applications of chlorantraniliprole having a positive influence on corn growth and yield, so research studies were conducted in 2013 and 2014 to determine the influence of chlorantraniliprole and flubendiamide on agronomic characteristics of field corn. A non-Bt hybrid, Pioneer 1319R (Pioneer Hi-Bred International Johnston, IA) and a Bt hybrid, Pioneer 1319VYHR, expressing the (Vip 3A) trait were used to distinguish between insect control and agronomic improvement. Trials were planted during the normal planting window for corn in Mississippi, mid-March through mid-April, and observations for plant height, chlorophyll and yield were recorded. In general, few significant interactions between application timing and foliar treatment or main effects of the two factors were observed. There was a significant interaction between growth stage when applied and the foliar treatment for manually harvested yield in Bt corn trials, but not for machine harvested yield in the same trial. Overall, the data suggest that diamide insecticides do not provide any agronomic benefit to corn and therefore their application should be based only on a need for insect protection.
Introduction

Field corn, *Zea mays* (L.), also referred to as maize, is the most commonly grown field crop in the United States (USDA NASS 2014). Corn is a monocot and grows upward from the whorl (Hanway and Ritchie 1997). Corn is a member of the grass family Poaceae and is one of the oldest cultivated grains and also one of the most productive crop species. Field corn is grown mainly for consumption as livestock feed, cereal products, ethanol, corn starch, and corn oil. The advancements in field corn breeding programs in the United States have resulted in increased yields and tolerance of environmental stresses (Farnham et al. 2003).

Several new insecticidal compounds in the diamide class that have novel modes of action are being developed. These compounds are classified as reduced risk insecticides as they are said to reduce pesticide risks to human health and the environment (Villanueva and Walgenbach 2005). These compounds may result in less damage to corn from corn earworm and fall armyworm damage, which could increase overall plant yield.

Both chlorantraniliprole and flubendiamide are now being used worldwide to control several pests belonging to Lepidoptera (Hardke et al. 2010). These diamide insecticides have been reported to have high residual efficacy and cause feeding cessation in the target pest. The diamides act on the ryanodine receptors. Calcium is released from intracellular stores that is controlled by a ryanodine receptor (RyR). The RyR mediates several cellular and physiological activities such as hormone secretion, neurotransmitter release, muscle contraction and gene expression (Hirooka et al. 2007). Lepidopteran larvae treated with diamide insecticides show unique symptoms of poisoning including
feeding cessation, complete muscle paralysis, and ultimately death (Tohnishi et al. 2005, Hirooka et al. 2007, Lahm et al. 2005). Chlorantraniliprole is the first pesticide from this new class of chemistry. It was selected for development based on the combination of insecticidal potency and excellent mammalian safety. This chemical has larvicidal activity against many Lepidoptera and Coleoptera (Lahm et al. 2007). Toxicity has also been observed in some Diptera, Coleoptera, Isoptera, and Hemiptera (Hannig et al. 2009). Chlorantraniliprole is more selective to ryanodine receptors of insects than mammals, which accounts for its low mammalian toxicity (Lahm et al. 2007, 2009). Chlorantraniliprole and flubendiamide share this novel mode of action.

While not commonly observed, there have been several studies conducted to evaluate the impact of insecticides and fungicides on growth and yield on different crops in the absence of insect pressure (Weichel & Nauen 2004, Spiers et al. 2008, Lehoczki-Krsjak et al. 2013). Although claims of foliar applications of chlorantraniliprole having a positive influence on corn growth and yield have been made, no published data on diamides affecting plant growth factors in the absence of insect pressure could be found. This current study was conducted to evaluate impacts of both chemicals on field corn’s growth and yield.

**Materials and Methods**

Studies were conducted to determine the influence of chlorantraniliprole and flubendiamide on agronomic characteristics of field corn. The research was conducted in 2013 through 2014 at the Delta Research & Extension Center, Stoneville, MS; and Mississippi State University R. R. Foil Plant Science Farm, Starkville, MS. Two trials were conducted at each location, one using a non-Bt hybrid, Pioneer 1319R (Pioneer Hi-
Bred International Johnston, IA) and one using a Bt hybrid, Pioneer 1319VYHR, expressing the Vip 3A trait. Both hybrids were planted at 84,000 seeds per ha. A randomized complete block design with a split plot treatment arrangement with 4 replications was used. Trials were planted during the normal planting window for corn in Mississippi, mid-March through mid-April. Plot size was 4 rows x 12.2 m (13.3 ft x 40 ft.) with 101.6 cm (40 in) row spacing in Stoneville, MS and 4 rows x 12.2 m (12.7 ft x 40 ft.) with 96.5 cm (38 inch) row spacing in Starkville, MS. The main plot factor was growth stage at the time of diamide application and included V7, V10, tassel (VT), and silk (R4) stages. Foliar applications of 0.0105 kg Ai/ha of flubendiamide (4SC Belt®, Bayer CropScience, Research Triangle Park, NC) 0.075 kg Ai/ha of chlorantraniliprole (0.43SC Prevathon™, DuPont, Wilmington, DE), or a non-treated control. Diamides were applied once with a John Deere 6000 sprayer at designated growth stages for specific plots in Stoneville, MS and with a hand held spray boom in Starkville, MS with an application volume of 93.5 liters/ha for both the hand held spray boom and John Deere 6000 sprayer. Once a foliar application was made, height of 10 plants was determined 7 days after treatment, and every week after until plants reached R1 stage. Plant chlorophyll content of 10 plants was also measured 7 days after treatment and every week after until physiological maturity using a SPAD 502 Chlorophyll Meter (Spectrum Technologies, Aurora, IL). When grain reached 15% moisture, 10 ears were hand harvested and individually shelled into four sections with section 1 being the tip of the ear and section 4 being the base of the ear from each plot. The mean grain weight of 10 ears per plot was determined. Plots were machined harvested after the hand harvesting was completed, and yields were adjusted to 15% moisture.
Data Analysis

Data were subjected to analysis of variance using SAS 9.3 (SAS Institute 2013). Year, location, year by location and replication nested within year by location were designated as random effects to allow for inference to be made over a range of environments. Error degrees of freedom were calculated using the Kenward-Roger method. All means were separated using Fisher’s protected LSD test. Differences were considered significant for α=0.05.

Results

Non-Bt Corn

No significant interaction between foliar treatments applied at V7 and weeks after treatment and no main effect of foliar treatments applied at V7 was observed for plant height or chlorophyll content (Table 3.1). Because the plants were still in vegetative growth, there was a significant effect of weeks after treatment for plant height and chlorophyll content (Table 3.1, Table 3.2).

No significant interaction between foliar treatments applied at V10 and weeks after treatment was observed for plant height or chlorophyll content (Table 3.1). No significant effect of foliar treatment applied at V10 was observed for plant height, but foliar treatment at V10 did result in a change in chlorophyll content (Table 3.1). A significant effect of weeks after treatment was observed for plant height and chlorophyll content (Table 3.1, Table 3.2) as expected.

The only factor significant from an application during VT or R1 stages was weeks after application for plant height during VT stage (Table 3.1, Table 3.3).
There were no significant factors or interactions impacting machine or manually harvested yield of non-Bt corn (Table 3.1) (Table 3.4).

**Bt Corn**

Similar to the results of the non-Bt corn, the only significant factor of plant height and chlorophyll content for the V7 application was weeks after treatment (Table 3.6). The results from a V10 application were similar, with only weeks after treatment being significant for plant height and chlorophyll content (Table 3.6). From the VT application, weeks after treatment was significant for chlorophyll content, but not plant height, while no factors resulted in significant differences from an R1 application (Table 3.6).

There were no significant factors or interactions impacting machine harvested yield of Bt corn (Table 3.5) (Table 3.8). However, a significant interaction between growth stage and foliar treatment was observed for manual harvested yield ($F=2.71; df=6, 56; P<0.02$) (Table 3.8).

**Discussion**

There have been rumors from growers and consultants that foliar applications of chlorantraniliprole had a positive influence on corn growth and yield beyond what could be attributed to insect control. The current studies were conducted to evaluate these impacts. Few impacts on corn growth or yield were observed following foliar applications of chlorantraniliprole or flubendiamide at various growth stages, with no consistent trends. This is not the first time that reports of pesticides promoting plant growth has been rejected by scientific data. Spiers et al. (2008) observed that abamectin, spinosad, acephate, and bifenthrin did not impact photosynthesis or growth and
development of gerbera (*Gerbera ambigua*) plants. Also, Navi (2013) observed that foliar applications of triazole or strobilurin fungicides or mixtures of the two at the R3 growth stage did not impact chlorophyll content or yield of soybeans (*Glycine max*) in the absence of disease. Weichel & Nauen (2004) observed that imidacloprid did not affect the overall plant growth or yield of hops, *Humulus lupulus* L., in the absence of any insect infestation. Weichel & Nauen (2004) observed no impact on plant development or yield when prothioconazole and tebuconazole were applied to wheat, *Triticum aestivum* L., in the absence of any plant disease.

Although there have been rumors of foliar applications of chlorantraniliprole having a positive influence on corn growth and yield, our studies indicate that neither chlorantraniliprole nor flubendiamide impacted chlorophyll content, plant height, or yield. Several other studies also indicate that other insecticides and fungicides have no effect on plant growth and yield in the absence of pest infestations. Therefore, the application of these insecticides should be based on sound IPM principles including scouting and economic thresholds and not on any perceived agronomic benefit.
Table 3.1  Analysis of variance table for plant height, chlorophyll content, machine harvest yield, and manual harvest yield of non-Bt field corn receiving foliar applications of diamide insecticides at various plant growth stages.

<table>
<thead>
<tr>
<th></th>
<th>Plant Height</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Growth Stage</td>
<td>V7</td>
<td>V10</td>
<td>VT</td>
<td>R1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>df</td>
<td>P</td>
<td>F</td>
<td>df</td>
<td>P</td>
<td>F</td>
<td>df</td>
</tr>
<tr>
<td>Foliar Treatment</td>
<td>0.02</td>
<td>2, 79</td>
<td>1.0</td>
<td>0.07</td>
<td>2, 44.3</td>
<td>0.85</td>
<td>2.61</td>
<td>2, 40.2</td>
</tr>
<tr>
<td>Foliar Treatment x</td>
<td>0.05</td>
<td>12, 224</td>
<td>1.0</td>
<td>0.06</td>
<td>8, 124</td>
<td>1.00</td>
<td>0.31</td>
<td>4, 75.1</td>
</tr>
<tr>
<td>Weeks after Treatment</td>
<td>448.30</td>
<td>6, 215</td>
<td>&lt;0.01</td>
<td>61.8</td>
<td>4, 117</td>
<td>&lt;0.01</td>
<td>12.36</td>
<td>2, 71.5</td>
</tr>
<tr>
<td>Weeks after Treatment</td>
<td>0.76</td>
<td>12, 211</td>
<td>0.7</td>
<td>0.53</td>
<td>8, 134</td>
<td>0.83</td>
<td>1.36</td>
<td>2, 45.2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Chlorophyll Content</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Growth Stage</td>
<td>F</td>
<td>df</td>
<td>P</td>
<td>Growth Stage</td>
<td>F</td>
<td>df</td>
<td>P</td>
</tr>
<tr>
<td>Foliar Treatment</td>
<td>1.22</td>
<td>2, 86</td>
<td>0.3</td>
<td>5.0</td>
<td>2.48</td>
<td>&lt;0.01</td>
<td>0.22</td>
<td>2, 30</td>
</tr>
<tr>
<td>Foliar Treatment x</td>
<td>0.76</td>
<td>12, 211</td>
<td>0.7</td>
<td>0.53</td>
<td>8, 134</td>
<td>0.83</td>
<td>1.36</td>
<td>2, 45.2</td>
</tr>
<tr>
<td>Weeks after Treatment</td>
<td>106.6</td>
<td>6, 198</td>
<td>&lt;0.01</td>
<td>17.8</td>
<td>4, 128</td>
<td>&lt;0.01</td>
<td>2.88</td>
<td>1, 45.2</td>
</tr>
<tr>
<td>Weeks after Treatment</td>
<td>0.76</td>
<td>12, 211</td>
<td>0.7</td>
<td>0.53</td>
<td>8, 134</td>
<td>0.83</td>
<td>1.36</td>
<td>2, 45.2</td>
</tr>
</tbody>
</table>

|                        | Machine Harvest Yield |                      |                      |                      | Manual Harvest Yield |                      |                      |                      |
|------------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|                      |
|                        | Growth Stage          | F                     | df                    | P                     | Foliar Treatment      | F                     | df                    | P                     |
| Foliar Treatment       | 1.21                 | 2, 128                | 0.30                  | Foliar Treatment      | 0.74                 | 2, 56                | 0.48                  |
| Foliar Treatment x     | 1.97                 | 6, 128                | 0.07                  | Foliar Treatment x    | 0.81                 | 5, 56                | 0.57                  |
| Growth Stage           | 1.12                 | 3, 56.6               | 0.35                  | Growth Stage          | 2.26                 | 3, 21                | 0.11                  |
Table 3.2  Least squared mean plant height and chlorophyll content over time after treatment during V7 and V10 to non-Bt corn, Starkville and Stoneville MS, 2013-2014.

<table>
<thead>
<tr>
<th>Application Growth Stage</th>
<th>Treatment</th>
<th>Weeks After Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Plant Height (cm) (SEM)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V7</td>
<td>Non-Treated</td>
<td>86.4f (22.2)</td>
</tr>
<tr>
<td>V7</td>
<td>Chlorantraniliprole</td>
<td>84.5f (22.2)</td>
</tr>
<tr>
<td>V7</td>
<td>Flubendiamide</td>
<td>83.3f (22.2)</td>
</tr>
<tr>
<td>Chlorophyll Content</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V7</td>
<td>Non-Treated</td>
<td>51.4f (1.68)</td>
</tr>
<tr>
<td>V7</td>
<td>Chlorantraniliprole</td>
<td>51.7f (1.68)</td>
</tr>
<tr>
<td>V7</td>
<td>Flubendiamide</td>
<td>51.6f (1.68)</td>
</tr>
<tr>
<td>Plant Height (cm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V10</td>
<td>Non-Treated</td>
<td>222.8d (25.7)</td>
</tr>
<tr>
<td>V10</td>
<td>Chlorantraniliprole</td>
<td>225.0d (25.7)</td>
</tr>
<tr>
<td>V10</td>
<td>Flubendiamide</td>
<td>227.1d (25.7)</td>
</tr>
<tr>
<td>Chlorophyll Content (nm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V10</td>
<td>Non-Treated</td>
<td>57.8gh (1.84)</td>
</tr>
<tr>
<td>V10</td>
<td>Chlorantraniliprole</td>
<td>56.7h (1.83)</td>
</tr>
<tr>
<td>V10</td>
<td>Flubendiamide</td>
<td>58.3fgh (1.83)</td>
</tr>
</tbody>
</table>

Means within rows and columns for plant height within a growth stage and chlorophyll content within a growth stage followed by a common letter are not significantly different (FPLSD, α=0.05).
Table 3.3  Least squared mean plant height and chlorophyll content over time after treatment during VT and R1 to non-Bt corn, Starkville and Stoneville MS, 2013-2014.

<table>
<thead>
<tr>
<th>Application Growth Stage</th>
<th>Treatment</th>
<th>Weeks After Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td><strong>Plant Height (cm)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VT</td>
<td>Non-Treated</td>
<td>291.1a (10.34)</td>
</tr>
<tr>
<td>VT</td>
<td>Chlorantraniliprole</td>
<td>289.2ab (10.34)</td>
</tr>
<tr>
<td>VT</td>
<td>Flubendiamide</td>
<td>289.2ab (10.34)</td>
</tr>
<tr>
<td><strong>Chlorophyll Content (nm)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VT</td>
<td>Non-Treated</td>
<td>60.0ab (1.29)</td>
</tr>
<tr>
<td>VT</td>
<td>Chlorantraniliprole</td>
<td>60.8a (1.29)</td>
</tr>
<tr>
<td>VT</td>
<td>Flubendiamide</td>
<td>60.0ab (1.29)</td>
</tr>
<tr>
<td><strong>Plant Height (cm)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R1</td>
<td>Non-Treated</td>
<td>287.7a (8.85)</td>
</tr>
<tr>
<td>R1</td>
<td>Chlorantraniliprole</td>
<td>289.9 (8.85)</td>
</tr>
<tr>
<td>R1</td>
<td>Flubendiamide</td>
<td>290.2 (8.85)</td>
</tr>
<tr>
<td><strong>Chlorophyll Content (nm)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R1</td>
<td>Non-Treated</td>
<td>60.5ab (1.25)</td>
</tr>
<tr>
<td>R1</td>
<td>Chlorantraniliprole</td>
<td>60.4ab (1.25)</td>
</tr>
<tr>
<td>R1</td>
<td>Flubendiamide</td>
<td>60.7ab (1.25)</td>
</tr>
</tbody>
</table>

Means within rows and columns for plant height within a growth stage and chlorophyll content within a growth stage followed by a common letter are not significantly different (FPLSD, α=0.05).
Table 3.4  Impact of foliar treatments applied at different growth stages to non-Bt corn on machine harvested yield (kg/ha) and manual harvested yield (g/ha).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Growth Stage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>V7</td>
</tr>
<tr>
<td>Non-Treated</td>
<td>12,653a</td>
</tr>
<tr>
<td>Chlorantraniliprole</td>
<td>12,955a</td>
</tr>
<tr>
<td>Flubendiamide</td>
<td>12,555a</td>
</tr>
</tbody>
</table>

Means within columns for machine harvest yield and manual harvest yield followed by a common letter are not significantly different (FPLSD, α=0.05).
Table 3.5  Analysis of variance table for plant height, chlorophyll content, machine harvest yield, and manual harvest yield of Bt field corn receiving foliar applications of diamide insecticides at various plant growth stages.

<table>
<thead>
<tr>
<th></th>
<th>Growth Stage/Application Timing of Foliar Treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>V7</td>
</tr>
<tr>
<td><strong>Plant Height</strong></td>
<td></td>
</tr>
<tr>
<td>Foliar Treatment</td>
<td>0.08</td>
</tr>
<tr>
<td>Weeks after Treatment</td>
<td>2185.3</td>
</tr>
<tr>
<td>Foliar Treatment x</td>
<td>0.20</td>
</tr>
<tr>
<td>Weeks after Treatment</td>
<td></td>
</tr>
<tr>
<td><strong>Chlorophyll Content</strong></td>
<td></td>
</tr>
<tr>
<td>Foliar Treatment</td>
<td>0.34</td>
</tr>
<tr>
<td>Weeks after Treatment</td>
<td>96.08</td>
</tr>
<tr>
<td>Foliar Treatment x</td>
<td>1.18</td>
</tr>
<tr>
<td>Weeks after Treatment</td>
<td></td>
</tr>
<tr>
<td><strong>Machine Harvest Yield</strong></td>
<td></td>
</tr>
<tr>
<td>Growth Stage</td>
<td>0.38</td>
</tr>
<tr>
<td>Foliar Treatment</td>
<td>0.71</td>
</tr>
<tr>
<td>Foliar Treatment x</td>
<td>0.52</td>
</tr>
<tr>
<td><strong>Manual Harvest Yield</strong></td>
<td></td>
</tr>
<tr>
<td>Growth Stage</td>
<td>1.14</td>
</tr>
<tr>
<td>Foliar Treatment</td>
<td>0.05</td>
</tr>
<tr>
<td>Foliar Treatment x</td>
<td>2.71</td>
</tr>
<tr>
<td>Growth Stage</td>
<td></td>
</tr>
</tbody>
</table>
Table 3.6  Least squared mean plant height and chlorophyll content over time after treatment during V7 and V10 to Bt corn, Starkville and Stoneville MS, 2013-2014.

<table>
<thead>
<tr>
<th>Application Growth Stage</th>
<th>Treatment</th>
<th>Weeks After Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td><strong>Plant Height (cm)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V7</td>
<td>Non-Treated</td>
<td>81.9e</td>
</tr>
<tr>
<td>V7</td>
<td>Chlorantraniliprole</td>
<td>82.4e</td>
</tr>
<tr>
<td>V7</td>
<td>Flubendiamide</td>
<td>83.6e</td>
</tr>
<tr>
<td>V10</td>
<td>Non-Treated</td>
<td>215.8d</td>
</tr>
<tr>
<td>V10</td>
<td>Chlorantraniliprole</td>
<td>218.6d</td>
</tr>
<tr>
<td>V10</td>
<td>Flubendiamide</td>
<td>217.5d</td>
</tr>
<tr>
<td><strong>Chlorophyll Content (nm)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V7</td>
<td>Non-Treated</td>
<td>53.4g</td>
</tr>
<tr>
<td>V7</td>
<td>Chlorantraniliprole</td>
<td>53.0g</td>
</tr>
<tr>
<td>V7</td>
<td>Flubendiamide</td>
<td>52.4g</td>
</tr>
<tr>
<td>V10</td>
<td>Non-Treated</td>
<td>57.8a</td>
</tr>
<tr>
<td>V10</td>
<td>Chlorantraniliprole</td>
<td>56.9d</td>
</tr>
<tr>
<td>V10</td>
<td>Flubendiamide</td>
<td>57.7cd</td>
</tr>
</tbody>
</table>

Means within columns for machine harvest yield and manual harvest yield followed by a common letter are not significantly different (FPLSD, α=0.05).
Table 3.7  Least squared mean plant height and chlorophyll content over time after treatment during VT and R1 to Bt corn, Starkville and Stoneville MS, 2013-2014.

<table>
<thead>
<tr>
<th>Application Growth Stage</th>
<th>Treatment</th>
<th>Weeks After Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td><strong>Plant Height (cm)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VT</td>
<td>Non-Treated</td>
<td>289.4a (8.44)</td>
</tr>
<tr>
<td>VT</td>
<td>Chlorantraniliprole</td>
<td>287.7ab (8.44)</td>
</tr>
<tr>
<td>VT</td>
<td>Flubendiamide</td>
<td>287.1ab (8.44)</td>
</tr>
<tr>
<td><strong>Chlorophyll Content (nm)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VT</td>
<td>Non-Treated</td>
<td>58.9b (0.92)</td>
</tr>
<tr>
<td>VT</td>
<td>Chlorantraniliprole</td>
<td>59.0b (0.92)</td>
</tr>
<tr>
<td>VT</td>
<td>Flubendiamide</td>
<td>59.3ab (0.92)</td>
</tr>
<tr>
<td><strong>Plant Height (cm)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R1</td>
<td>Non-Treated</td>
<td>289.0ab (6.19)</td>
</tr>
<tr>
<td>R1</td>
<td>Chlorantraniliprole</td>
<td>289.7ab (6.19)</td>
</tr>
<tr>
<td>R1</td>
<td>Flubendiamide</td>
<td>288.3abc (6.19)</td>
</tr>
<tr>
<td><strong>Chlorophyll Content (nm)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R1</td>
<td>Non-Treated</td>
<td>59.6a (1.18)</td>
</tr>
<tr>
<td>R1</td>
<td>Chlorantraniliprole</td>
<td>59.6a (1.18)</td>
</tr>
<tr>
<td>R1</td>
<td>Flubendiamide</td>
<td>59.5a (1.18)</td>
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</table>

Means within rows and columns for plant height within a growth stage and chlorophyll content within a growth stage followed by a common letter are not significantly different (FPLSD, α=0.05).
<table>
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<tr>
<th>Treatment</th>
<th>Growth Stage</th>
<th>V7</th>
<th>V10</th>
<th>VT</th>
<th>R1</th>
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<tr>
<td><strong>Machine Harvested (kg/ha)</strong></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Non-Treated</td>
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<td>11,431a</td>
<td>11,777a</td>
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<td>12,039a</td>
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<td>11,873a</td>
<td>11,858a</td>
<td>12,009a</td>
</tr>
<tr>
<td><strong>Manual Harvested (g/ear)</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Non-Treated</td>
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<td>206.3abc</td>
<td>201.9bcd</td>
<td>193.6d</td>
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<td>200.8bcd</td>
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<td>197.0cd</td>
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<td>209.2ab</td>
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</tbody>
</table>

Means within columns for machine harvest yield and manual harvest yield followed by a common letter are not significantly different (FPLSD, α=0.05)
References


CHAPTER IV
RESIDUAL EFFICACY OF CHLORANTRANILIPROLE AND FLUBENDIAMIDE
ON FALL ARMYWORM (Spodoptera frugiperda) IN FIELD CORN

Abstract
Studies were conducted during 2013 and 2014 at the Delta Research & Extension Center, Stoneville, MS to evaluate the residual efficacy of chlorantraniliprole and flubendiamide against fall armyworm in field corn. The corn hybrid Pioneer 1319R (Pioneer Hi-Bred, Johnston, IA) was used in all trials. For the V7 application timing trials, leaf tissue was collected at 7 DAT, V10, VT, R1, and milk stage (R3). In the V10 application timing trials, leaf tissue was collected at 7DAT, silk stage (R1), and milk stage (R3). In V7 and V10 application timing trials, plants were marked to differentiate plant material that developed after application. V7 and V10 applications showed significant fall armyworm mortality on tissue present at the time of application treated with flubendiamide or chlorantraniliprole up to 21DAT. Fall armyworm mortality on tissue not present at the time of application for V7 and V10 was significant for chlorantraniliprole (40%) and declined significantly with each successive assay timing, but was not significant for flubendiamide (<20% mortality). Applications of chlorantraniliprole or flubendiamide applied at VT and R1 both had a significant impact on fall armyworm mortality 15DAT. These studies indicate that chlorantraniliprole could
potentially be more beneficial than other insecticides in early applications. As observed in this study, chlorantraniliprole translocated upward in the plant, however flubendiamide did not appear to be translocated in corn plants. Applications of chlorantraniliprole during the vegetative growth stages of corn for management of one pest, may provide residual efficacy in newly developed tissue to control future infestations of other pests.

Introduction

In the United States, the fall armyworm, *Spodoptera frugiperda* (J. E. Smith), can colonize over 80 different plant species. Fall armyworm has been recorded in Georgia since 1797. It can migrate to the northern part of Mississippi by late May and early June (Sparks 1979). The fall armyworm has a holometabolous life cycle which consists of egg, larva, pupa, and adult stages (Flanders et al. 2011). Larval appearance closely resembles corn earworm and true armyworm larvae. The main feature that helps identify the fall armyworm from the corn earworm is a darker head capsule with a white inverted “Y” on the head. Pupation generally takes place in the soil. (Capinera 2005).

It is an economic pest in numerous vegetable crops, including sweet corn (Bohnenblust et al. 2012). If available, fall armyworm predominately feeds on members of the grass family (Poaceae) (Capinera 2005).

Female moths attach their eggs in clusters of 50 or more in a single layer on foliage of plants, and eggs hatch in 2 to 4 days (Flanders et al. 2011). Larvae will feed on the foliage of the corn plant and also can be observed in the corn whorl. Larvae will devour foliage until they have completed 6 instars and pupated (Sparks 1979). When they are ready to pupate, larvae will move to the soil surface and pupate 2-8 cm below the soil in a loosely constructed cocoon of debris. The length of the pupal stage can range from 7-
37 days when the mean soil temperatures are 15-29°C, respectively. Once the adult moths emerge from the cases, they move to the soil surface and attach to plants or plant debris (Sparks 1979).

The number and duration of generations in an area varies. During warm summer months, in the southeastern US and through the Gulf Coast states, the fall armyworm life cycle is completed in about 30 days. The life cycle can take up to 60 days during the spring and autumn. During the winter, a life cycle can take between 80-90 days to complete. Fall armyworms will also complete more generations in the Gulf region of the United States than farther north (Capinera 2005). The fall armyworm has no true diapause unlike many other insects. They overwinter in the Gulf of Texas, South Florida, and further south where host plants are always available and temperatures rarely fall below 10°C (Luginbill 1928).

In corn, fall armyworm generally feed on foliage, but larvae will also feed on corn ears during heavy infestations. Fall armyworm feeding causes reduced grain weight and yields due to foliar and direct ear feeding in field corn (Sparks 1979). Larvae can consume all leaf tissue except the midrib. In heavy infestations, densities of larvae will be reduced to one or two per plant because larvae are cannibalistic (Sparks 1979). The most common damage is defoliation of late pre-tassel corn (Bessin 2004). Characteristic symptoms on corn usually include moist sawdust-like frass on the upper leaves and whorl of the plant. Early instar feeding may appear similar to European corn borer damage but late instar European corn borer larvae bore into the stalk, while fall armyworm late instars continue to feed on foliage. Early instar damage is also similar to corn earworm damage. Corn earworm damage includes chewed kernels and visible frass, at the ear tip.
Fall armyworm burrows through the husk and does not always feed down through the silks like corn earworm (Bohnenblust et al. 2012).

Both chlorantraniliprole and flubendiamide are now being used worldwide on a broad range of crops to control several pests belonging to Lepidoptera (Hardke et al. 2009). These diamide insecticides have been reported to provide long residual efficacy and cause feeding cessation in the target pest. Chlorantraniliprole and flubendiamide have a novel mode of action. As a selective agonist for ryanodine receptors in insects, they cause unregulated Ca\textsuperscript{2+} release from intracellular calcium stores, which impairs in the insect’s ability to regulate muscle function. Poisoning symptoms include rapid feeding cessation, lethargy, muscle paralysis, and ultimately insect death (Lahm et al. 2005). Chlorantraniliprole is relatively harmless to beneficial arthropods, and has not been found to show cross resistance with existing insecticides (Lahm et al. 2009). Consequently, chlorantraniliprole provides rapid plant protection through feeding cessation in the target pest soon after exposure (Hirooka et al. 2007) and eventual death because of starvation within 1-3 d after exposure by ingestion or contact (Lahm et al. 2009). The objective of these studies was to estimate the residual efficacy and of selected diamide insecticides applied at various growth stages against fall armyworm. By conducting bioassay procedures, these studies will help establish baseline data for future studies by determining mortality rate, and help consultants observe when applications would be best economically applied to insure protection against fall armyworm infestations and reduce the possibility of yield loss.
Materials and Methods

Studies were conducted during 2013 and 2014 at the Delta Research & Extension Center, Stoneville, MS to evaluate the residual efficacy of chlorantraniliprole and flubendiamide against fall armyworm in field corn. Planting dates and application dates for each trial are listed in Table 4.1. The corn hybrid Pioneer 1319R (Pioneer Hi-Bred, Johnston, IA) was used in all trials, and planted at 84,000 seeds per ha. Plot size measured 4 rows 4.1 x 12.2 m (13.3 x 40 ft.) with 1.0 m row spacing. Foliar treatments consisting of either 0.015 kg Ai/ha of flubendiamide (Belt®, Bayer CropScience, Research Triangle Park, NC) or 0.075 kg Ai/ha of chlorantraniliprole (Prevathon™, DuPont, Wilmington, DE) were applied with a John Deere 6000 sprayer. A non-treated control was also included. For the V7 application timing trials, leaf tissue was collected at 7 DAT, V10, VT, R1, and milk stage (R3). In the V10 application timing trials, leaf tissue was collected at 7DAT, silk stage (R1), and milk stage (R3). In V7 and V10 application timing trials, plants were marked with orange ribbon tied around the corn stalk to differentiate plant material that developed after application. Both plant material that was present at the time of application and plant material that developed after application were included in bioassays. For the VT application timing trials, leaf tissue was collected at 7DAT, and milk stage (R3). For the R1 application timing trials, leaf tissue was collected at 7DAT, and milk stage (R3). In this trial treatments were arranged in a randomized complete block design with repeated measures and 4 replications.

Leaf material collected from plots were utilized in laboratory bioassays. Filter paper was placed in petri dishes and tissue samples measuring 5.08 x 5.08cm were placed on the filter paper. Fall armyworm larvae (1st instar), were placed on diet for 24 hours,
and then placed on top of the tissue sample. A small amount of water was added to the filter paper to provide moisture and parafilm was used to seal the petri dishes. Larval mortality was determined 48 hours after infestation by turning larva on the dorsal side. If larvae did not move or turn over to ventral side within 10 seconds larvae were considered dead. Control mortality in all assays was <2%. Mortality was corrected using Abbott’s Formula (Abbott 1928).

**Data Analysis**

Data for trials with vegetative stage application timings (V7, V10) were analyzed as a split plot RCB with repeated measures, with foliar treatment as the main plot, tissue location as the subplot, and time after application expressed as subsequent growth stages following application as the repeated measure. Data were log transformed to meet assumptions of normality. For trials with reproductive growth stage application timings (VT, R1), data were analyzed as a RCB with repeated measures with time after application expressed at subsequent growth stages following application as the repeated measure. Data were subjected to analysis of variance using SAS 9.3 with an alpha equal to 0.05 (SAS Institute 2013). Year, location, year by location and replication nested within year by location were designated as random effects to allow for inference to be made over a range of environments. Error degrees of freedom were calculated using the Kenward-Roger method. All means were separated using Fisher’s protected LSD test. Differences were considered significant for $\alpha=0.05$. 

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Results

For trials with V7 application timing, a significant interaction between treatment, leaf position and growth stage ($F=6.12; \text{df}=4, 126; P<0.01$) was observed for fall armyworm mortality (Figure 4.1). Mortality of FAW larvae was greatest on tissue present at the time of application treated with chlorantraniliprole at 7DAT. Fall armyworm mortality on chlorantraniliprole treated tissue declined significantly at each successive assay timing. Fall armyworm mortality on tissue not present at the time of application from chlorantraniliprole treated plants was greatest (39%) at 7DAT and declined significantly with each successive assay timing until VT (tassel). Fall armyworm mortality on flubendiamide treated tissue (tissue present at application) was greatest at 7DAT, and declined significantly at each successive assay timing until R1. Fall armyworm mortality on tissue not present at the time of application from flubendiamide treated plants was greatest (8%).

For trials with V10 application timing, a significant interaction between treatment, leaf position and time after application was observed for fall armyworm mortality ($F=21.69; \text{df}=2, 70; P<0.01$) (Figure 4.2). On tissue treated tissue present at the time of application with chlorantraniliprole or flubendiamide fall armyworm mortality was greatest at 7DAT and declined significantly at each successive assay timing.

For trials with VT application timing, no significant interaction between foliar treatments and time after application ($F=0.86; \text{df}=1, 21; P=0.36$) was observed for fall armyworm mortality (Figure 4.3). Also, there was no difference between the 2 foliar treatments ($F=0.46; \text{df}=1, 21; P=0.50$). However, a significant effect of time after
application ($F=27.71; \text{df}=1, 21; P<0.01$) was observed. Mean mortality at 7DAT was 85.6% and declined to 70.9% by 15DAT (R3).

For trials with R1 application timing, no significant interaction between foliar treatments and time after application ($F=0.16; \text{df}=1, 21; P=0.70$) was observed for fall armyworm mortality (Figure 4.4). Also, no significant effect of foliar treatment was observed ($F=2.09; \text{df}=1, 21; P=0.16$) was observed. However, a significant effect of time after application ($F=82.42; \text{df}=1, 21; P<0.01$) was observed. Mean mortality at 7DAT was 93.1% and declined significantly to 71.6% by 14DAT (Figure 4.4).

**Discussion**

When applied at V7 or V10, chlorantraniliprole and flubendiamide resulted in >40% mortality of fall armyworm larvae for 26 and 28 DAT, respectively, on plant tissue present at the time of application. With plant tissue that developed after application, very low levels of mortality (<10%) were observed with flubendiamide indicating that little to no translocation occurred. For chlorantraniliprole, mortality of fall armyworm larvae on plant tissue that developed after application ranged from 38.8% to 7.6% at 7 DAT and 26 DAT, respectively with the V7 application timing and from 36.3% to 9.4% at 7 DAT and 28 DAT, respectively with the V10 application timing. Mortality of this magnitude on plant tissue that developed after application indicates that translocation within the corn plants did occur. When applied at the growth stage VT, chlorantraniliprole (81%) and flubendiamide (83%) resulted in significantly greater mortality at 7DAT compared to at 15DAT (73%). Similar results were observed when chlorantraniliprole and flubendiamide were applied at the R1 growth stage.
The length of residual efficacy for chlorantraniliprole and flubendiamide reported in this study was consistent with some insecticides, cyantraniliprole and methoxyfenozide, that have been reported to date (Hardke et al. 2011; Sial & Brunner 2010; Chen et al. 2015). Sial & Brunner (2010), conducted studies to determine the residual toxicity of chlorantraniliprole to obliquebanded leafroller, *Choristoneura rosaceana* (Harris). A mortality of 100% was recorded at field rate applications of chlorantraniliprole, 10, 38, and 59 (DAT), which resulted in significant reductions in defoliation compared to the untreated control. Hardke et al. (2011), conducted leaf tissue bioassays in grain sorghum on fall armyworm mortality with chlorantraniliprole, cyantraniliprole, flubendiamide, lambda-cyhalothrin, methoxyfenozide, and novaluron. Plant tissue treated with chlorantraniliprole, flubendiamide, cyantraniliprole, and methoxyfenozide significantly impacted fall armyworm mortality compared to that of larvae on the non-treated tissue 7 DAT and 14DAT. Chen et al. (2015), conducted studies on the uptake of chlorantraniliprole and flubendiamide in rice plants through foliar absorption. Translocation within the plant has been observed with foliar applications of chlorantraniliprole but not flubendiamide (Chen et al. 2015). This is consistent with our studies that also indicated chlorantraniliprole has uptake characteristics in corn. Adams et al. (2016), conducted studies to determine the residual and systemic efficacy of flubendiamide and chlorantraniliprole and against corn earworm in soybean at the V4 and R3 stage. *H. zea* mortality was significant with both insecticides on leaves that were present at the time of application for at least 31 d after application. Chlorantraniliprole resulted in greater mortality than flubendiamide at 24 and 31 d.
The current study demonstrates that diamides may exhibit longer residual efficacy than other insecticides available for fall armyworm management in field corn. Also based on the level of mortality observed with chlorantraniliprole that translocated from treated tissue to tissue that developed after application, these studies indicate that chlorantraniliprole could potentially be more beneficial than other insecticides in early applications. As observed in this study, chlorantraniliprole translocated upward in the plant, however flubendiamide did not appear to be translocated in corn plants. Applications of chlorantraniliprole during the vegetative growth stages of corn for management of one pest, may provide residual efficacy in newly developed tissue to control future infestations of other pests such as corn borer sp. including European corn borer *Ostrinia nubilalis* (Hübner), southwestern corn borer, *Diatraea grandiosella* (Dyer), and sugarcane borer *Diatraea saccharalis* (Fabricius). However, efficacy of chlorantraniliprole against fall armyworm on tissue that developed after application (tissue that chlorantraniliprole was translocated to) was not high, so control may not be satisfactory unless insects are very susceptible to the insecticide or infestations are light.
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<th>Application Date</th>
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</tr>
<tr>
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<td>15 Apr</td>
<td>15 May</td>
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<td>2013</td>
<td>15 Apr</td>
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<tr>
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<td>2013</td>
<td>15 Apr</td>
<td>6 Jun</td>
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<tr>
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Figure 4.1  Impact of foliar treatments applied at V7 growth stage, leaf tissue location (present at time of application or developed after application) and sample timing on fall armyworm mortality.

Statistical analysis performed on transformed data (arcsine square root), however actual means reported.
Figure 4.2  Impact of foliar treatments applied at V10 growth stage, leaf tissue location (present at time of application or developed after application) and sample timing on fall armyworm mortality.

Statistical analysis performed on transformed data (arcsine square root), however actual means reported.
Figure 4.3  Impact of foliar treatments applied at VT growth stage and sample timing on fall armyworm mortality.

Figure 4.4  Impact of foliar treatments applied at R1 growth stage and sample timing on fall armyworm mortality.
References


Sial, A., and Brunner J. 2010. Toxicity and residual efficacy of chlorantraniliprole, spinetoram, and emamectin benzoate to obliquebanded leafroller (Lepidoptera: Tortricidae. J. Econ. Entomol. 103: 1277-1285.