ABSTRACT

HOWELL, FORREST C. Evaluating Seed Applied Neonicotinoid use to Manage Pests of Corn and Wheat in the Southeastern U.S. (Under the direction of Dominic Reisig and Hannah Burrack).

Seed applied insecticides, specifically those in the neonicotinoid class, have been shown to offer protection in the presence of many soil insect pests. This class of insecticides has become one of the fastest growing in crop protection. Neonicotinoids have many advantages, including long lasting residual activity and efficacy at low rates. Insecticides in this class can provide both seed and foliar protection for several weeks or months after planting.

The first chapter of this thesis details field experiments conducted during the summer of 2014 and 2015, where insecticide seed treatments and in-furrow insecticides were evaluated in corn. The majority of the US corn fields are planted with a seed-applied neonicotinoid-class insecticide. Major objectives of these studies were to show the impacts of insecticidal seed treatments on several species of insects in corn and to document the interaction with plant growth characteristics. Field sites were selected based on known previous pest pressure and contained wireworms (*Melanotus communis* (Gyllenhal), *Conoderus vespertinus* (F.) and *C. lividus* (Degeer)) and annual white grubs (*Popillia japonica* (Newman), *Cotinis nitida* (Linnaeus), and *Cyclocephala spp*.). An additional site contained southern corn billbugs (*Sphenophorus callous* (Olivier)). Treatments included untreated corn seed, as well as in-furrow and seed-applied insecticides (terbufos 560.424g a.i./ha, clothianidin 3.088mg a.i./ha, clothianidin 1.235mg a.i./ha, terbufos 560.424g a.i./ha + clothianidin 3.088mg a.i./ha, terbufos 560.424g a.i./ha + clothianidin 1.235mg a.i./ha, bifenthrin 448.339g a.i./ha). Above-ground injury and root injury were assessed from the

non-billbug locations at two, three, and four weeks after planting. Root weights and concentrations of clothianidin from the stalk and roots were taken from the samples collected at these locations. Soil was sifted from each plot to document soil-dwelling insect pest density, and wireworms were counted from root samples. Only injury was assessed at the billbug locations in 2014, but billbug injury, billbug mortality and presence of billbugs in root crown and stalks were assessed in 2015. Seedlings with neonicotinoid seed treatments alone had fewer wireworms, greater root mass, and less billbug injury compared to seedlings that had no seed treatment. White grub and wireworm abundance was also lower in plots planted with neonicotinoid treated seed compared to in-furrow treatments and untreated seed. Neonicotinoid seed treatments also reduced southern corn billbug injury, increased mortality of feeding adults, and decreased stalk tunneling by larvae. In-furrow treatments of bifenthrin decreased stalk tunneling by southern corn billbug larvae, while terbufos caused mortality among feeding adults. This is the first study to examine concentrations of insecticides in corn plants where these particular insect pests feed (roots and crown) and to contrast this with the incidence and associated injury of this particular soil insect pest complex. Clothianidin concentrations in the corn plant tissue were associated with their degree of insect control, and were highest in the treatment with 3.088mg a.i./ha, intermediate in the treatment with 1.235mg a.i./ha, and lowest with no seed treatment. Concentrations of insecticide in the roots and crown where these insects feed increased at two WAP after planting, but decreased by four WAP.

The second chapter of this thesis addresses a neonicotinoid seed treatment in wheat used to control the Hessian fly, *Mayetiola destructor* (Say). The Hessian fly is a common economically important pest of wheat throughout the southeastern US. Hessian flies are

multi-voltine, with generation number dependent on temperature. Management approaches rely on resistant wheat varieties, crop rotation, timely plantings, and insecticide treatments. The objectives of this research were to show the impacts of a common insecticide seed treatment (imidacloprid) and a common foliar spray (lambda-cyhalothrin) on Hessian fly populations in wheat. An early planting date and an Extension-recommended planting date were compared at two different coastal North Carolina locations. Main plots with different planting dates were subdivided into treatments including untreated wheat seed, semi-monthly foliar lambda-cyhalothrin, imidacloprid seed treatment, and imidacloprid seed treatment + semi-monthly foliar lambda-cyhalothrin. Number of eggs present on leaves, number of larvae, and the number of pupae in the plant were counted for the fall Hessian fly generation or generations; the spring generation was eliminated through foliar sprays. There was no benefit from foliar sprays during the 2014 study when eggs, larvae, and pupae were assessed, except for tiller densities at one location in untreated plots that received semi-monthly foliar sprays. Timely applications of foliar sprays are crucial for the control of Hessian fly and sprays may not have been initiated soon enough during 2014. Imidacloprid treated seed provided the greatest amount of control from Hessian fly compared to untreated wheat and wheat treated with a foliar spray. Warm weather during fall 2015 extended the pest presence, and multiple generations of Hessian fly infested wheat during the fall. Foliar sprays were timed more appropriately and provided some protection from Hessian fly, by reducing adults that laid eggs and larvae that migrated to the crown. However wheat treated with imidacloprid still had fewer eggs, larvae, and pupae compared to all other treatments.

Evaluating Seed Applied Neonicotinoid use to Manage Pests of Corn and Wheat in the Southeastern U.S.

by
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BIOGRAPHY

Forrest Chandler Howell was born on September 25, 1992. He is the oldest of three children of Glenn and Fran Howell. Forrest was raised in Pinetown, North Carolina located in rural Beaufort County, where his love for the land was instilled in him at an early age through his work on his family's farm. His love for the land didn't stop with just agriculture, but included his cultivated passion for the great outdoors through countless hunting trips with his father and brothers. His passion for agriculture was emanate in high school where he joined the school's local FFA chapter and worked to obtain both his State Farmers Degree and his American Farmer Degree from the National FFA Organization. Forrest graduated from Pungo Christian Academy in 2010. He attended North Carolina State University where he earned a Bachelor of Science degree in Agricultural Science with minors in Agricultural Leadership, Agroecology, Agricultural Business Management and Crop Science in 2013. During his undergraduate work, Forrest had the opportunity of working for Dr. Tommy Carter, at the Soybean and Nitrogen Fixation Unit, where he learned about plant breeding, furthered his interest in graduate studies

Forrest was accepted into the graduate program of the Department of Entomology at North Carolina State University in 2014 where he began his Masters research under the direction of Dr. Dominic Reisig and Dr. Hannah Burrack. His research aimed to evaluate the value of neonicotinoid seed treatments in corn and wheat in coastal North Carolina agricultural systems.

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CHAPTER 1

Impact of seed applied clothianidin and in-furrow insecticides on the corn (*Zea mays* L.) seedling insect complex, plant injury and growth, and clothianidin concentration in the plant.

Introduction

Corn, *Zea mays*, L., is a crop of major economic importance in the United States, with over 35 million hectares planted. It was valued over $52 billion in 2014 (USDA 2015). Approximately two million hectares of corn were planted across the southeastern United States in 2014, making it one of the largest crops grown in the region, with over 200,000 hectares planted on any given year in North Carolina alone. Corn is widely used for livestock feed, and shortages of grain often require livestock producers in the southeast to import grain from the Midwest. More than thirty arthropod species or groups of species are known to cause economic injury to US corn (Pedigo 2014). Some of these insects are considered important seedling or soil pests, including black cutworms (Noctuidae), corn rootworms (Chrysomelidae), wireworms (Elateridae), and billbugs (Curculionidae). Soil dwelling insects remain a particularly serious problem, because of their ability to economically damage crops across many agricultural systems (Jackson et al. 2000) and because of their cryptic behavior, making sampling difficult. Wireworms are considered among the most significant soil pests across the US Corn Belt (Youngman et al. 1993).

The major corn production region in North Carolina, in terms of total hectares and yield, is located in the eastern portion of the state. Planting in this region generally occurs in early April through early May, when soil temperatures begin to warm. Corn is often used in a rotation system with wheat and soybean throughout the southeastern United States, but is also used as a rotational crop with some vegetable production throughout North Carolina. Previous work has showed that insecticidal seed treatments can provide protection from many of these soil dwelling pests (Jeschke et al. 2011). The insect pest complex affecting southeastern grown corn differs from that in other parts of the United States. The insect pests of major concern in the southeastern U.S. does not reflect the insect pests of concern such as *Diabrotica* spp. (Coleoptera: Chrysomelidae) in the mid-western U.S. My study focuses on common soil dwelling coleopteran pests that form a complex in the southeast in the orders Elateridae, Scarabaeidae, and Curculionidae.

Wireworms (Coleoptera: Elateridae) are the subterranean and highly polyphagous larval stage of click beetles, which are global pests (Traugott et al. 2015). Historically, they have been difficult to manage because of their subterranean habitat and sporadic occurrence in space and time (Finney 1946). Life history varies greatly between genera and species, including the duration of life cycles and portion of time spent below ground (Thomas 1940). Evidence of wireworm is commonly observed on field edges and in low lying areas of the field (Thomas 1940). Little is known about exact species composition and their life history for the wireworm complex in the southeastern US. The reasons for the lack of knowledge are the same in current times as in the past; many pestiferous species of wireworm require multiple years to complete their life cycle and spend a majority of that time as larvae in the soil (Hawkins 1936, Rabb 1963, Esser et al. 2015).

Corn is generally planted in the southeastern US during early April through early May into fields with existing wireworm populations. Because of warmer soil temperatures in the spring, wireworms are found near to the soil surface, where they feed on the recently planted corn crop (Thomas 1940, Fisher et al. 1970). Fourteen wireworm species are frequently encountered at detrimental levels in corn, of which three genera are commonly found in corn in the southeastern US: *Conoderus spp.*, *Glyphonyx spp.*, and *Melanotus spp* (Riley et al. 1974, Riley and Keaster 1979). In the coastal regions of North Carolina, *Melanotus* species are the most prevalent wireworm species, especially in crops that are planted following corn and soybeans (Willis et al. 2010). Wireworm larvae are able to locate germinating seeds in the soil because the developing seedlings emit CO_2 (Doane et al. 1975). Roots of seedlings are often fed on by these pests and some tunneling in the stalk occurs at this stage. Economic loss by wireworms occurs through crop stand reduction or direct plant injury leading to yield reduction (Smith et al. 1981). Management strategies for wireworms include delaying planting date, crop rotation, tillage, and application of seed applied insecticides (Hall and Cherry 1970).

Organic matter influences the soil's ability to absorb and retain water. Wireworms are most commonly found in soils with high moisture, compared to soils with lower soil moisture (Fulton 1928, Seal and Chalfant 1994, Lefko et al. 1998, Parker and Howard 2001, Kuhar et al. 2003, Kuhar and Alvarez 2008). A common hypothesis is that when soil conditions become dry, wireworms migrate in search of a more suitable and moister environment. As noted earlier, corn is used in rotation with other grain crops, but in North Carolina it is also used in rotation with some vegetable crops, including potatoes, *Solanum tuberosum*. Langdon et al. (2012) surmised that potatoes were a source of moisture for wireworms when soil moisture levels become inadequate, since crop injury was more prevalent in xeric conditions. Fields in coastal North Carolina are typically low in elevation (<2.4m) and, coupled with a high water table, are often saturated. Drainage ditches are a necessity in most production settings in this region to eliminate excess water. These ditches can harbor a variety of suitable grasses that can serve as hosts for wireworms when a non-preferred crop is planted into a field.

Root-feeding white grubs are another major global pest of diverse agricultural crops (Klein 2006). Grubs such as *Popillia japonica* (Newman), *Cotinis nitida* (Linnaeus), and

Cyclocephala spp. (Coleoptera: Scarabaeidae) belong to this group of root-feeding pests (Ritcher 1958). *Popilla japonica* is commonly known as the Japanese beetle. Native to Japan, as their name implies, they were first discovered in North America in 1916 and quickly spread throughout the US (Potter 2002). These beetles can injure corn in both the larval and adult stage, feeding on the roots as larvae and on the silks as adults (Gould 1963, Steckel et al. 2013). Typically, larvae (also known as grubs in Scarabaeidae) feed on roots of grasses; however when weedy grasses are not present, as in non-organic production settings, corn can serve as an alternative food source. Grubs move closer to the soil surface when soil temperatures rise above 10°C (Fleming 1972), which, in coastal North Carolina, occurs in the early spring. Corn is, therefore, often planted where grubs are already present and soil temperatures are suitable for larval conditions. Injury from the grubs can be characterized by stunted growth as a result of root pruning (Tiwari et al. 2009). As grubs, they reside in the soil for approximately one year before developing into adults in most areas of the US, except in northern New England where development takes 2 years to complete (Potter 2002). Adults emerge in the southeastern US during early May through June. Once emerged, adults mate and females deposit their eggs in to the soil over their 4-6 weeks before dying (Potter 2002). After hatching the root- feeding grubs remain in the soil until pupation. Grubs overwinter in the soil at a depth of 5-15cm (Dalthorp et al. 2000).

Cyclocephala spp., annual white grubs, and *Cotinis nitida* (Linnaeus), green June beetles, are both similar in appearance as larvae (grubs). Annual white grubs are an early season pest that feed on corn seeds and seedling roots directly following planting (McLeod et al. 1999 and Youngman et al. 1993). Feeding on root hairs can stunt plants and cause stand loss in severe cases (Youngman and Tiwari 2004). Injury can be identified above ground by purpling of the leaves, as below ground root pruning interferes with the plant's ability to uptake phosphorous. Fall sampling methods have been developed to predict spring infestations in Virginia, and clothianidin seed treatment (3.08mg/ha) is recommended when pest populations are above sampling thresholds, which is ≥ 1.04 white grubs in the spring using a compact sampling method, in which a known (20.3 x 20.3cm square x 15cm deep) area of soil is sampled (Jordan et al. 2012).

Sphenophorus spp. (Coleoptera: Curculionidae), billbugs, are common pests of turfgrass (Potter 1998). There are more than 60 native species that can be found throughout the United States and most are believed to be univoltine (Niemczyk and Shetlar 2000). *Sphenophorus callous* (Olivier), the southern corn billbug, (Coleoptera: Curculionidae), is a major pest of corn (DuRant 1975, Wright et al. 1982). It is distributed throughout the southeastern US, found mostly in the coastal plains of the Carolinas and Georgia (Wright et al. 1983). Adults overwinter around field edges in residues, hedgerows, and wooded areas, emerging when corn is planted (Morgan and Beckham 1960). From their overwintering sites, southern corn billbug adults will disperse into nearby corn fields where they feed at the base of corn plants (Wright et al. 1983). This feeding can result in injury to the foliage that becomes apparent as the plants age, leading to stunting that can result in tillering, with relatively light feeding, or plant death, with heavier feeding (Morgan and Beckham 1960, DuRant, 1975, All et al. 1984). Injury is not only synonymous with adult feeding; larvae develop in the root crown area and often tunnel the lower portions of corn stalks, causing corn plants to be more vulnerable to drought stress and die prematurely (Van Duyn 2004). There is a negative relationship between yield and the point at which billbugs begin to feed, with infestations in the early growth stages causing the most yield loss (DuRant 1982). Early planting, proper drainage, fertilization, liming, and crop rotation are recommended control tactics (Webster 1912 and Metcalf 1917). Despite research by DuRant (1979), regarding post-emergence applications of insecticides on corn to control southern billbug injury, little published research is available concerning insecticidal seed treatments, which are the ubiquitous management tactic for this insect across the southeastern US.

Seed applied insecticides have shown to offer some protection in the presence of wireworms, specifically neonicotinoid class of insecticides. With the exception of organic corn, nearly all corn seed planted in the US is treated with a neonicotinoid (Krupke et al. 2012). It has become one the fastest growing class of insecticides in crop protection in recent years (Jeschke 2008). Neonicotinoids have long residual activity and can often be used at low rates, making them attractive for use against many crop pests (Elbert et al. 2008). More importantly, neonicotinoids can provide both seed and foliar protection for several weeks or months after planting (Wilde et al. 2001, Nault et al. 2004, Koch et al. 2005, Jeschke et al. 2011), which has facilitated grower adoption in numerous agricultural systems worldwide. This class of insecticides targets the central nervous system of insects as an agonist of the post-synaptic nicotinic acetylcholine receptors. In corn, a neonicotinoid is applied to the seed coat before planting; the active compound is taken up by the plant roots after germination and distributed throughout the entire plant, allowing for systematic protection from insects (Girolami et al. 2009).

The main purpose of this study was to investigate the damage by insect pests of corn seedlings treated with a neonicotinoid, clothianidin, as compared to those treated at planting with bifenthrin, a liquid in-furrow planting insecticide, or terbufos, a granular in-furrow insecticide in southern US corn production. These treatments were selected as placeholders to represent classes of insecticides commonly used in a commercial field setting. A further objective was to measure the concentration of clothianidin in the zone where seedling insect pests feed. A final objective was to increase understanding the biology of the soil insect pest species complexes present in corn agricultural systems across the southeast US to improve management practices.

Materials and Methods

Wireworm studies. *Site selection.* Fields were chosen based on past wireworm incidence; these varied in size from 2 to 8ha. To determine the wireworm species present in each field, as well as their density, bait traps were placed at potential field sites. Baiting occurred in the month of February of both years during the study. Approximately 150g dry steam crimped oats, *Avena sativia* L. (Purina Mills, St. Louis, MO) was placed in a plastic bag and soaked in 600mL water for 48h at room temperature. After 48h, nine bait traps were placed 15m apart on two outer edges of each selected field. Generally traps were placed on field margins near drainage ditches or hedgerows. A post-hole digger was used to extract a uniform core of soil, 20-25cm in depth, with an approximate diameter of 15cm. About 25g of soaked oats was placed at the bottom of each hole and covered with soil. A surveyor’s flag was placed in the center of each trap to allow easy identification of the bait trap in the field. After 14 days, a garden shovel, with a 10cm spade, was used to excavate the soil surrounding the trap and the oats. The contents were placed in a large plastic bag labeled with date and location of the trap, which was then transported to the Tidewater Research Station in Plymouth, NC and stored at 4^{o}C until samples could be processed. At the Tidewater Research Station bags were emptied onto cafeteria trays, and examined for wireworms. Wireworms were placed in vials with 80% ethanol solutions labeled with collection date and location and later identified to species using the keys of Rabb (1963), Riley and Keaster (1979), and Seal (1990). Plots were established at the two locations that had highest wireworm populations determined from preseason bait traps, using a randomized complete block design with four replicates at all field locations.

Field experiments. Treatments included untreated corn seed (P1498YHR, DuPont Pioneer, Johnston, IA), as well as seed planted with in-furrow and seed-applied insecticides. Insecticide treatments included 1) terbufos in furrow at 560.424g a.i./ha (Counter, AMVAC Chemical Corporation, Newport Beach, CA), 2) seed-applied clothianidin at 3.088mg a.i./ha (Poncho, Bayer CropScience, Research Triangle Park, NC), 3) seed-applied clothianidin at 1.235mg a.i./ha, 4) in-furrow terbufos at 560.424g a.i./ha + seed-applied clothianidin at 3.088mg a.i./ha, 5) in-furrow terbufos at 560.424g a.i./ha + seed-applied clothianidin at 1.235mg a.i./ha, and 6) in-furrow bifenthrin at 448.339g a.i./ha (Capture LFR, FMC Corporation, Philadelphia, PA). Clothianidin seed treatments were applied with a laboratory-scale seed treater to untreated corn seed. Along with the clothianidin, a color dye slurry (2 ml PRO-IZED red seed colorant at 2.26kg seed + 25 ml of water, Bayer CropScience, Research Triangle Park, NC) was added to the insecticide solution, which provided a visual reference to ensure uniform application.

Corn was planted on 14 April 2014 (2014 Location 1 and 2014 Location 2) and 24 April 2015 (2015 Location 1 and 2015 Location 2) in fields near Elizabeth City, NC. An application of Bicep Magnum II (*S*-metolachlor and atrazine, Syngenta Crop Protection, Greensboro, NC) at 1.89L/ha for control of broadleaf weeds and grasses was made prior to planting. Plots were planted using a John Deere 5085M tractor (John Deere, Moline, IL), equipped with a Kinze Base (Kinze Manufacturing, North Liberty, IA), with Almaco Cone

Units (ALMACO, Nevada, IA). Individual plots were four rows by 12m with 91cm row centers. No start-up fertilizer was applied at the time of planting in an attempt to slow initial seedling growth and to maximize injury. All plots received an application of fertilizer at sidedress following Extension recommendations. Plots were planted along field edges where wireworms were most prevalent based on baiting traps. At two, three, and four weeks after planting (WAP) plots were rated for wireworm injury. Ratings were taken from the center two rows of the plot. The number of corn plants with wireworm injury and white grub injury were recorded, along with the total number of plants in the two center rows for stand counts. Wireworm injury was characterized by a whorl that did not completely unfurl (whipped whorl), visible leaf chlorosis, or stunted growth compared to the most vigorously growing plants in the trial. White grub injury was also identified by stunted growth, but included wilted plants and purpled stems. Because both insect species were present in the experimental systems and above ground injury symptoms were difficult to differentiate, any of above symptoms were classified as injury, and injured plants were grouped together for analysis.

Root samples were also collected from the plots at two, three, and four WAP. Plants were selected at random from the two outside rows of the plots, to preserve the center two rows for yield. Ten plants per plot were collected at two WAP, but only six plants were collected at three and four WAP because the plants were large enough to reduce the subsample size for adequate data precision. A garden shovel with a 10cm spade was used to carefully unearth the plants in order to ensure roots remained as intact as possible. Plants were then placed in labeled plastic bags and transported in ice chests to the laboratory, where the roots were washed to remove any excess soil. They were placed on cafeteria trays, with a paper-towel to help dry and absorb any moisture for 15 minutes. The stalks were cut from the plants leaving approximately 3cm of the coleoptile; these sections were then weighed.

Although species were identified from the baiting prior to planting, soil-insect sampling was also done on 23 May 2014 from border rows of all plots at both Location 1 and 2. An area of about 0.092m^2 was excavated around the plant. Soil-insects that could cause seedling injury (wireworms and white grubs) were sampled from all plots during 2014. During 2015, soil-insects were sampled two, three, and four WAP from untreated, clothianidin 1.235mg a.i./ha, and clothianidin 3.088mg a.i./ha plots. All plots at 2015 Location 1 were sampled for soil-insects five WAP. These insects were placed in vials labeled with plot number and date. The vials were then transported to the laboratory and an 80% ethanol solution was added to each vial to preserve specimens until they could be identified. Because insect numbers were low at 2014 Location 1, these data were not analyzed.

Billbug studies. *Field experiments*. The experimental design was a randomized complete block small plot study with four replicates. Treatments were applied to seed from the same hybrid and using the same process as for wireworm experiments. Seed was planted on 8 May 2014 (2014 Location 3) and 23 April 2015 at (2015 Location 3), on privately owned locations in Hyde County selected based on previous billbug history. Prior to site selection, field visits were made to view surrounding landscape and to investigate possible overwintering habitats for southern corn billbugs (ditchbanks, tree-lines, and fields adjacent to previous corn; Wright et al. 1982a). At two, three, and four WAP, plots were rated for billbug injury and stand counts were taken using the same methods as described for wireworm studies. One additional injury characteristic for these studies included counting all plants that had transverse holes in the leaves, which were presumed to result from billbug feeding though the base of the stalk while the unfurled leaves were developing. Root samples were also collected at two, three, and four WAP using the same procedures as listed in the wireworm study in the 2015 Location 3 study.

The number of dead adult billbugs visible on the soil surface was counted from the two center rows of all plots on 7 May 2015 from 2015 Location 3. The number of billbug larvae developing in stalks were quantified on 6 June 2015 from 2015 Location 3. Larvae and dead adults were not present in the 2014 trials and were not quantified. For larval counts, five plants were selected at random from the border rows of each plot and unearthed. Using a hacksaw, stalks were removed at approximately 25cm from the base and split. Billbug larvae within the stalks were recorded.

Neonicotinoid concentrations. *Plant tissue.* At two, three, and four WAP, plant samples were collected for neonicotinoid concentration testing from 2014 Location 1 and 2015 Location 3. Plants were collected at random from the border rows of plots planted with untreated seed and seed coated with clothianidin at both rates, but without terbufos in-furrow, following the same procedure detailed for root samples in the wireworm study. Bags containing the roots were then placed in an ice chest and transported to the laboratory where they were stored at -80^{o}C until they could be processed.

Plant samples were partitioned into stalk and root samples, the regions of the plant where the target insect pests feed. Tissue samples ranging 3-15g each, were cut, weighed, and then ground with solvents, acetonitrile and 1% glacial acetic acid, according to protocol procedures (WI-MET124-02) obtained from National Science Laboratories, Gastonia Lab (Gastonia, NC) to extract clothianidin from each sample. The extracted insecticide samples from 2014 were analyzed by the North Carolina State Department of Chemistry using LC/MS. Samples from 2015 were sent to the David H. Murdock Research Institute, Analytical Sciences Laboratory, Kannapolis, NC and analyzed using LC/MS, by a UPLC-MS system (ACQUITY UPLC-Quattro Premier XE MS, Waters Corp., Milford, MA); the system was operated, and data were collected, in electrospray ionization positive mode. A calibration curve was made using methanol into which clothianidin was spiked at 10 concentrations varying from 1.95 ng/mL to 1000 ng/mL to determine the dynamic range of the assay.

Soil testing. Soil samples were collected from 2015 Location 3, pre-plant, to test for clothianidin residues. In order to obtain a uniform collection to represent the plot area, soil samples were collected in the marked plot area approximately 21m apart. A soil probe was used to obtain four samples. The probe was pushed into the ground at a depth of six cm to eight cm deep. The extracted soil core was then placed in a plastic bag, labeled with date and location. Samples were analyzed by the USDA AMS Science and Technology Laboratory Approval and Testing Division of the National Science Laboratories Gastonia Lab, to determine the levels of clothianidin residues. The samples were extracted for analysis by the laboratory in Gastonia and analyzed by LC/MS for the presence of clothianidin as described before.

Data analysis. All data points from each trial were analyzed using individual analyses of variances, or repeated measures analyses, if data were collected from the same plot during subsequent periods of time (SAS PROC MIXED, SAS Institute 2012). A single dependent variable was used in each analysis of variance and these included: stand count, number of injured plants, root weights, concentrations of clothianidin in plant tissue, deceased southern corn billbug number, southern corn billbug larvae in the root crown and stalk, and yield. The independent fixed effect for the models was treatment, while replication was considered a random effect. Data were transformed when needed in order to satisfy assumptions of the model. Repeated measures analysis was performed using the REPEATED statement in SAS. The SLICE statement SAS was used to partition interactions. Repeated measures analysis was not performed on 2014 neonicotinoid data, because relatively small amounts of tissue were collected, requiring samples from each collection date to be pooled for analysis; however, it was used on 2015 data. The SLICE statement was also used on 2015 neonicotinoid data, when there were significant interactions between treatment and sampling date, to determine inter-week variation. Mean separations were based on Tukey's honesty significant differences test, where treatments differed significantly at $\alpha \leq 0.05$.

Results

Wireworm studies. *Site selection.* Baiting was done prior to planting during both years at all locations. *Melanotus communis* was the most abundant wireworm species collected from bait traps prior to planting in both years (Fig 1.1). A total of 37 wireworm larvae were collected from 18 traps at the 2014 wireworm locations, including 23 *M. communis* (62%), four *C. lividus* (11%), four *C. vespertinus* (11%), and six *G. bimarginatus.* (16%). During 2015, a total of 43 wireworm larvae, including 25 *M. communis* (58%), seven *C. lividus* (16%), seven *C. vespertinus* (16%), four *G. bimarginatus.* (10%) were collected from the 18 oat bait traps prior to planting at the wireworm field locations.

Field experiments. Post-planting insect sampling was performed once during 2014. Both wireworms and white grubs were collected from all plots on 23 May 2014 at 2014 Location 1. A total of eight wireworms were collected from Location 1 on this sampling date. Seven wireworms were collected from untreated plots, while a single wireworm was collected from a plot treated in-furrow with bifenthrin; no wireworms were collected from other plots. A total of 22 white grubs were collected from the plots on this date and there were no differences among treatments (*F* = 0.41; d.f. = 6,21; *P* = 0.8615).

At 2014 Location 2, insects were sampled from all plots on 23 May 2014. Although no white grubs were found, there was a total of 68 wireworms collected from this location. Insecticide treatment had a significant effect on the average number of wireworms collected per plant (*F* = 9.65; d.f. = 6,18; *P* < 0.0001; Figure 1.2). Wireworm densities were higher in untreated plots compared to plots with either rate of clothianidin seed-treatment or clothianidin with the addition of terbufos, while the remaining treatments did not differ from the untreated control plots.

During 2015, wireworms and white grubs were collected from untreated and clothianidin-alone seed treated plots two, three, and four WAP, while all plots were sampled for soil insect pests at five WAP. Hence, a repeated measures analysis was performed for samples at two, three, and four WAP, while a single analysis of variance was performed for samples from five WAP. There was no interaction of treatment with sampling date for wireworm densities (*F* = 1.97; d.f. = 4,18; *P* = 0.1421). Wireworm densities did differ over time (*F* = 4.5; d.f. = 2,22; *P* = 0.0229), and differed significantly among treatments (*F* = 17.43; d.f. = 2,9; *P* = 0.0008; Figure 1.3). The same number of wireworms, 52, were collected both at two and three WAP, but had decreased to 31 by four WAP across treatments. Wireworm densities were higher in untreated plots (82 wireworms) compared to clothianidin seed-treatment alone at either rate, clothianidin at 1.235mg a.i./ha (39 wireworms) and clothianidin at 3.088mg (14 wireworms). There was no interaction of treatment and date for white grub abundance (*F* = 1.23; d.f. = 4,18 ; *P* = 0.2758) and white grub abundance did not change across dates (*F* = 1.39; d.f. = 4,18; *P* = 0.2758). However, treatment had a significant effect on white grub abundance (*F* = 6.71; d.f. = 2,9; *P* = 0.0164; Figure 1.3), while date did not (*F* = 0.21; d.f. = 2,18; *P* = 0.8109). There were higher wireworm and white grub densities in untreated plots compared the plots treated with clothianidin alone.

At five WAP, more wireworms were collected from untreated plots (20 wireworms), terbufos treated plots (16 wireworms), and bifenthrin treated plots (16 wireworms) compared to plots with clothianidin at 3.088mg a.i./ha (3 wireworms), plots with clothianidin 1.235mg a.i./ha (5 wireworms), plots with clothianidin 3.088mg a.i./ha + terbufos (4 wireworms), and plots with clothianidin 1.235mg a.i./ha + terbufos (7 wireworms; *F* = 17.86; d.f. = 6,21; *P* < 0.0001; Figure 1.4). Insecticide treatment had no effect white grub densities at this date (*F* = 0.83; d.f. = 6,18; *P* = 0.5587; Figure 1.4).

Above-ground wireworm and white grub injury was reduced using clothianidin seed treatment at both rates and without the addition of terbufos compared to the untreated check. There was not an interaction of treatment and sampling date, but treatment (*F* = 3.49; d.f. = 6,18; *P* = 0.0182; Figure 1.5) and date (*F* =49.07; d.f. = 2,42; *P* < 0.0001) had significant effects on plant injury during 2014 at Location 1. More plant injury had occurred by four WAP, compared to two and three WAP. In the same trial, injury following in-furrow applications of bifenthrin and terbufos was not different from either rate of clothianidin seed treatment alone or clothianidin seed treatment with the addition of terbufos. Number of injured plants was the lowest in clothianidin 1.235mg a.i./ha plots while the highest amount of injury was recorded in untreated plots (Figure 1.5). At 2015 Location 1, there was an interaction of treatment and sampling date for plant injury from wireworms and white grubs, with more injury occurring during three and four WAP (*F* = 4.14; d.f. = 12,42; *P* = 0.0003; Table 1.1), compared to two WAP. There was also an interaction between treatment and sampling date for wireworm and white grub injury at 2015 Location 2 (*F* = 2.09; d.f. = 12,42; *P* = 0.0389; Table 1.2), with the most injury occurring four WAP. Wireworm and white grub injury was also lower at 2015 Location 2 in plots with either rate of clothianidin seed treatment alone or clothianidin seed treatment with the addition of terbufos.

Root weights were collected at a single sampling date. At 2014 Location 1, the highest mean root weights were collected from plots with either rate of clothianidin seed treatment alone or clothianidin seed treatment with the addition of terbufos (*F* = 6.15; d.f. = 6,18; *P* = 0.0012; Figure 1.6). Root weights were lowest in untreated, bifenthrin, and terbufos treated plots when compared to plots treated with either rate of clothianidin seed treatment alone of clothianidin seed treatment with the addition of terbufos. At 2015 Location 1 seed treatment also had a significant effect on mean root weight (*F* = 5.68; d.f. = 6,18; *P* = 0.0018; Figure 1.6). Plots treated with clothianidin 3.088mg/ha had higher mean root weights compared to other seed treatments. However, the remaining treatments did not have higher mean root weights compared to the untreated check plots. Treatment did not have a significant effect on root weight at 2015 Location 3 (*F* = 1.18; d.f. = 6,21; *P* = 0.3541).

Yield components. The 2014 Location 1 total average stand count was 14.1 plants per row meter and plant number did not differ between insecticide treatments (*F* = 0.56; d.f. = 6,18; *P* = 0.756). In 2015, Location 1 had an average stand count of 15.9 plants per row meter, and the Location 2 average was 16.8 plants per row meter. Stand did not differ among insecticide seed treatments in either location (2015 Location 1: *F* = 1.06; d.f. = 6,18; *P* = 0.4199; 2015 Location 2: *F* = 0.64; d.f. = 6,18; *P* = 0.7008). There were no significant differences in yield during 2014 (Location 1: *F* = 2.01; d.f. = 6,21; *P* = 0.1089) or 2015 (Location 1: *F* = 0.93; d.f. = 6,18; *P* = 0.4947; Location 2: *F* = 1.52; d.f. = 6,18; *P* = 0.2292).

Neonicotinoid concentrations. A single sample was taken from combined stalk and root tissue in 2014. The combined tissue collected from plants with clothianidin-treated seed at 3.088mg/ha had higher concentrations of clothianidin in the plant compared to the untreated check at 2014 Location 1 at 2 WAP (*F* = 9.03; d.f. = 2,6; *P* = 0.0155; Figure 1.7). Concentrations in combined tissue from plots planted with clothianidin at 1.23mg/ha were not different from the untreated check. At 2014 Location 1 at 3 WAP there were also significant differences (*F* = 6.88; d.f. = 2,6; *P* = 0.028; Figure 1.7). Again, plots with clothianidin-treated seed at 3.08mg/ha had higher concentrations of clothianidin in the plant compared to the untreated check, but the concentration in plots with clothianidin-treated seed at 3.088mg/ha was not different from concentrations in plots with clothianidin-treated seed at 1.235mg/ha. Finally, clothianidin concentration in plots with clothianidin-treated seed at 1.235mg/ha was also not different from the untreated check. Combined tissue collected from 2014 Location 2, at 4 WAP, did not have significant differences between treatments (*F* = 0.24; d.f. = 2,5.3; *P* = 0.7946; Figure 1.7).

Multiple samples were taken of the tissue during 2015. Concentrations of clothianidin found in both stalk and root tissue combined, collected from plots varied significantly in untreated plots and either rate of clothianidin plots from 2015 Location 3. There was a significant interaction between tissue collection date and treatment (*F* = 6.4; d.f. = 4,35.5; *P* = 0.0005; Figure 1.8). The first collection date had significant interactions among treatments, with higher concentrations found in either rate of clothianidin compared to the untreated tissue. When the variance was partitioned to hold time constant, clothianidin varied between plant parts (*F* = 4.21; d.f. = 2,33.4; *P* = 0.0234; Figure 1.9), with higher rates found in roots of clothianidin 1.23mg a.i./ha and clothianidin 3.088mg a.i./ha root tissue compared to roots or stalk of plants from untreated plots.

Soil testing. Clothianidin was found prior to planting in the soil collected from 2015 Location 3. Soil sampling was not performed during 2014. All four 2015 samples contained clothianidin. The average concentration of clothianidin in soil for all four samples was 37.5 ng/g and ranged from 28.7 ng/g to 47.5 ng/g

Billbug studies. *Field experiments.* A repeated measures analysis was used on 2014 and 2015 southern corn billbug data collected at two, three, and four WAP. During 2014, both rates of clothianidin seed treatment alone or clothianidin seed treatments with the addition of terbufos provided a reduction in southern corn billbug injury incidence (*F* = 3.99; d.f. = 6,18; *P* = 0.0103; Figure 1.10) compared to the untreated check, and there was no interaction of treatment with date (*F* = 1.9; d.f. = 12,42; *P* = 0.0621). Injury increased over time and was higher at four WAP, compared to two and three WAP (*F* = 33.3; d.f. = 2,54; *P* < 0.0001). Injury in plots treated with bifenthrin and terbufos was not different from the clothianidin seed treatments or the untreated check plots. At the 2015 southern billbug location there was a significant interaction of treatment with sampling date (F = 3.96; d.f. = 12,42; P = 0.0004; Table 1.3). The most injury occurred during the three and four WAP, compared to two WAP.

Number of dead southern corn billbugs and billbugs in root crowns and stalks were only assessed at a single time point during 2015. Number of dead southern corn billbugs differed across treatments (*F* = 8.89; d.f. = 7,21; *P* < 0.0001; Figure 1.11). Clothianidin seed treatment alone, at both rates, or clothianidin seed treatment with the addition of terbufos had higher numbers of dead southern corn billbugs compared to the untreated check. Furthermore, the number of southern corn billbug larvae found in the stalks of corn plants was different across treatments (*F* = 6.67; d.f. = 7,20.1; *P* = 0.0004; Figure 1.12). Bifenthrin and either rate of clothianidin seed treatment alone or clothianidin seed treatment, with the addition of terbufos, reduced the number of plants infested with larvae compared to the untreated check.

Yield Components. Stand count during 2014 did not differ among treatments and averaged 13.8 plants per row meter (*F* = 1.13, d.f. = 6,18; *P* = 0.3865); there were no differences in yield among treatments (*F* = 1.35; d.f. = 6,18; *P* = 0.2869). The average stand count for 2015 was 17 plants per row meter. There were no differences in stand (*F* = 1.47; d.f. = 7,21; *P* = 0.2331) or yield (*F* = 1.8; d.f. = 7,21; *P* = 0.1409) among insecticide treatments during the 2015 season.

Discussion

Either rate of clothianidin seed treatment alone or clothianidin seed treatment with the addition of terbufos consistently reduced wireworm, white grub, and southern corn billbug injury, pest incidence, and increased root weight when compared to the untreated check and other in-furrow insecticides tested. Moreover, clothianidin was found in the plant tissue at higher concentrations from plots that received the highest seed applied rate of clothianidin compared to the lower rate and untreated check. While other studies have examined concentrations of neonicotinoids in leaf tissue (Wallingford et al. 2012) and the movement of these insecticides into root zones (Juraske et al. 2009), this is the first published study to associate the concentration of clothianidin in the root and lower portion of the stalk (coleoptile) with the incidence and associated injury of the soil insect pest complex.

Moreover, although previous studies have examined the interaction of above ground and below ground pest with response to plant fitness (Poveda et al. 2003), none have considered the effect of below-ground pests such as wireworms and white grubs on root mass. Additionally, this is the first published study to document the association of clothianidin with billbug mortality, both as adults on the soil surface, and the reduction of billbug larvae in the stalk. Finally, this is the first study to note the reduction of billbug larvae in corn stalks when bifenthrin has been used in-furrow.

The translocation of systematic insecticides into plant tissue has been widely studied (Stein-Dönecke et al. 1992 and Westwood et al. 1998), but analyzing partitioned tissue into zones specifically where pests specifically feed is relatively new (Westwood et al. 1998). Determining the concentration of insecticides in zones where target insect pest feed allows researchers to understand the concentration at which the insecticide is effectively preventing feeding from target insect pest. These studies demonstrate a consistent pattern with increasing rates of clothianidin on the seed and increasing clothianidin concentrations in the plant. Furthermore, by partitioning the plant tissue into zones where our target insect pest feed, there is likely a correlation between efficacy between the billbugs, wireworms, and white grub control observed and the concentration of neonicotinoid observed. These results also demonstrate that, when insect pressure was relatively low, a lower rate of clothianidin was just as effective as the high rate. It is worth noting that clothianidin was detected in plant samples from untreated plots in both years, at concentrations that always significantly differed from treated plots. Although, all measures were taken in the laboratory to ensure contamination did not occur, there is still a possibility. Untreated corn seed also passed through the same seed tubes on the planter that clothianidin treated seed passed through, so that may have been route for exposure to clothianidin. As a result, before planting corn, during the 2015 season, I collected soil samples from all of my field locations and tested the soil for clothianidin concentrations. However, soil tests prior to planting during 2015 indicated clothianidin was already present in the soil. As other studies have discovered, clothianidin and other neonicotinoids can accumulate in the soil (Goulson 2013), and it is likely that this was responsible for the detection in plant tissue collected from the untreated plots. Clothianidin present as residue in soil likely moved into plants from seed not treated with clothianidin.

Understanding the period of time during which clothianidin concentrations are present in plant tissue is an important part of understanding efficacy across different pest species. In terms of insecticide concentrations in the partitioned tissue, root tissue had higher concentrations of clothianidin across all treatments compared to the stalk tissue, which may have implications for pest control. Wireworms and white grubs are exposed to clothianidin by feeding on the roots, likely consuming small amounts of root tissue. Southern corn billbugs feed on the meristematic tissue of corn by inserting their proboscis into corn stalks; which houses xylem tissues (Williams 1947), through which clothianidin is translocated throughout the plant (Bonmatin et al. 2015), thus exposing them to the insecticide. Furthermore, the roots and stalks serve as transient locations for clothianidin. Concentrations of clothianidin were examined over a three week period, during which time there was a decline in the amount insecticide in the specific tissue where billbugs feed; significantly, there was no difference of clothianidin concentration among treatments at four WAP in the coleoptile. However during the 2015 season, there were still significant differences of clothianidin concentration among treatments in root tissue at three WAP. Wireworms were present near the soil surface during the early season, presumably before environmental factors such as temperature forced them to go deeper in the soil profile or find alternate hosts, while white grubs were more apparent later in the season. Hence, the higher rates of clothianidin likely provided extended protection when pests such as white grubs were apparent later in the season. Other studies have noted neonicotinoid seed treatments provide protection for both seed and foliage for weeks to months in some cases (Elbert et al. 2008). These experiments revealed that clothianidin as seed treatment provided protection from seedling pest until three weeks after planting and, after this point, clothianidin concentrations in the plant began to decline. Injury from wireworms, white grubs, and adult billbugs also declined by the third week, indicating that the majority of injury occurred early in the season, immediately after the seed was planted.

There are many species of wireworms, and species composition likely varies greatly by geographical region. Understanding wireworm species that are present in North Carolina agricultural systems will allow for management strategies that target the most abundant species to result in reductions in injury. From bait traps I determined there were four species present at the field locations before plots were planted (*M. communis, C. lividus, C. vespertinus, and G. bimarginatus*). The most abundant species from both the 2014 and 2015 seasons was *M. communis,* which composed 60% of the wireworm population collected from bait traps during those seasons. *Melanotus communis* is a well-known pest of corn and can be found in agricultural systems throughout much of the mid-eastern United States (Fenton 1926, Riley et al. 1979, Riley and Keaster 1979). Wireworms, however, were not the only soil insect pest present during in many of these studies, likely reflective of the term “soil insect complex” referred to in pest management. White grubs of at least three species (*P.*

japonica, C. nitida, Cyclocephala spp.) were also present during soil insect sampling and are also known to feed on the roots of crops such as corn (Klein 2006 and Richter 1958). White grub densities surpassed thresholds previously set by Jordan et al. (2012) in some plots during both years of the study. The protection provided by clothianidin in plant tissue is evident in root masses collected from both 2014 and 2015 trials. Moreover, clothianidin seed treatments with and without the addition of terbufos protected plant roots in the presence of feeding wireworms and white grubs. In contrast, root weights, wireworm number and number of injured plants planted with bifenthrin and terbufos alone were not always significantly different from the control.

Although threshold levels of pests and differences in injury among treatments were detected and were not coupled with significant differences in yield among treatments, these experiments were not appropriate for detecting yield differences. There were numerous issues that were encountered that prevented yield. For example, nutrient deficiencies were artificially imposed on the plots (i.e., no starter fertilizer used at planting). Perhaps most importantly, the planting equipment used for these trials was not appropriate for detecting differences in yield. Spacing between plants was not even in all of the plots, which were planted using a cone planter. Corn plants are highly competitive, and variations in plant density have been known affect corn yield considerably (Luque et al. 2006, Abuzar et al. 2011). Competition between plants affected the plants ability to compensate for injury; thus when plants were grouped together competition was high between plants, however when injury was high and caused reductions in stand, competition between plants was reduced. To further this point, the coefficient of variation (CV) for all yield data was greater than 10%, meaning there was substantial variation, making it difficult to measure differences in yield

(Cochran and Cox 1957). These experiments were designed to specifically test for insecticide efficacy, not yield. In order to test yield, changes such as proper plant spacing and fertilizer use would have needed to have been made.

Periods of high temperatures resulted in a relatively shortened pest presence at locations with wireworms. The 2014 wireworm trials were planted on 14 April, when soil temperatures were still relatively cool (14^{o}C); the 2015 wireworm locations were planted on 24 April. Growers typically plant corn earlier (late March – early April) in the coastal regions of North Carolina in order to avoid severe, coastal storms and hurricanes that can pose a major threat throughout southeastern coastal states. Wireworm and white grub injury was most severe by the third week of injury ratings from the 2014 studies By the third week, day temperatures increased, likely warming the soil and providing for a suitable environment for these pests to move towards the soil surface to feed on young seedlings and roots of plants. Once soil temperatures became too warm, wireworms likely migrated to more suitable environments such as locations deeper in the soil profile away from corn roots or to nearby ditch banks that harbor plants that can serve as alternate hosts (Belcher 1989 and Nash and Rawlins 1941).

Corn roots stabilize the plant, take up water, and distribute essential nutrients (McCully 1999). Root weights in plots treated with clothianidin, regardless of whether or not terbufos was applied, were, on average, 20%-32% heavier compared to the untreated check. Furthermore, plots that were treated with clothianidin had fewer wireworms present in the soil that were found feeding on plants. Wireworms exposed to clothianidin have been documented as entering a moribund stage, from which they later recover (Vernon et al. 2009). Hence, in my study, it is possible that insecticide exposure in clothianidin treated plots deterred wireworms from migrating into plots treated with clothianidin and preventing them from feeding on roots of clothianidin treated plants.

Clothianidin seed treatment and terbufos, alone, in combination, and independent of rate caused mortality among southern corn billbug adults, with clothianidin being the most effective. In terms of preventing injury from feeding southern corn billbugs, clothianidin was the most effective compared to other treatments. Furthermore, clothianidin also reduced the number of larvae that moved into the tissue of the plant. Corn and yellow nutsedge (*Cyperus esculentus* (L.)), are known billbug reproductive hosts in commercial corn production (Wright et al. 1982a). Moreover, corn is only susceptible to southern corn billbug feeding for a short period of time, which should encourage growers to plant early in order to minimize the period of time in which corn is susceptible to billbug injury and to counteract billbug feeding times (Wright et al. 1982b). While that management strategy can been effective, these studies reveal the importance of preventative clothianidin seed treatment to prevent both adult and larval feeding even during early plantings. Moreover, once adults have infested corn, managing them with foliar insecticides is difficult, because once the damage is apparent little can be done to help the crop recover. In addition, there are no current methods to treat infested plants once larvae have entered the plant tissue, which could potentially create opportunities for plant diseases and other pathogens to enter the plant.

Previous studies have noted wireworms are found more often in high moisture soils (Fulton, 1928, Seal and Chalfant 1994, Lefko et al. 1998, Parker and Howard 2001, Kuhar et al. 2003, Kuhar and Alvarez 2008). Soils with higher percentages of organic matter generally retain water compared to other soils with low organic matter content (Hudson 1994). Soils in these studies contained a relatively high organic matter (15-30%). The soil at the wireworm studies consisted of Hyde series and Pasquotank series of soil, while the billbug studies were conducted on the Ponzer series of soil. Rainfall during April 2014 was 15.3cm and was 10.5cm during 2015, which is above the average of 9.2cm during April for the region (CRONOS 2016). The combination of high soil moisture, amplified by water retention in the organic soils likely contributed to wireworm and white grub presence, feeding, and resulting injury on the plants. Excess rainfall also likely resulted in the movement of southern corn billbug larvae into the stalks of corn plants, up from the crown where they normally reside (Van Duyn 2004). Clothianidin seed treatment, with or without terbufos, and independent of rate consistently protected corn seedlings from southern corn billbug injury. Terbufos alone and bifenthrin alone in-furrow did not always reduce southern corn billbug injury relative to the control. However, in-furrow treatments of bifenthrin and clothianidin seed treatments reduced the number of southern corn billbug larvae moving into the tissue of corn. Furthermore, this study documents the first recorded activity of bifenthrin on southern corn billbug larvae in corn. Bifenthrin is commonly used in the turf-grass industry to control larva of other billbug species (Buss 2002), but little to no work as evaluated the efficacy on the southern corn billbug larvae in corn. This pyrethroid has also been noted to have longer lasting residual activity compared to other insecticides in the pyrethroid class (Baumler and Potter 2007). Future studies should further investigate to determine the residual activity of bifenthrin on southern corn billbug larvae.

Clothianidin seed treatments alone consistently provided protection from wireworms, white grubs, and southern corn billbug incidence in 2014 and 2015. Other soil treatments, bifenthrin and terbufos, provided moderate levels of control against southern corn billbugs, defined as a numerical reduction injury relative to the control, but were not as effective as clothianidin. The neonicotinoid class of insecticides is a critical tool used by growers to manage wireworm, white grub, and southern corn billbug in corn. These insects comprise a unique pest complex in the southeastern US and vary greatly in terms of biology, which highlights the need to conduct insecticide efficacy trials in a diversity of corn production regions. As mentioned previously, soil insect pests in the southeastern U.S. differ from those in the midwestern U.S., where *Diabrotica virgifera virgifera* (LeConte) (Coleoptera: Chrysomelidae), western corn rootworm, causes the most injury to corn. Insecticide resistance is one of the major concerns growers in the midwestern U.S. have regarding this pest, because of its documented ability to adapt to management strategies (Van Rozen and Ester 2010). While southeastern U.S. corn producers are aware of these issues, the pest complex differs, and insecticide resistance in this region is not a known issue for the corn seedling insect pest complex. Due to the wide commercial use of neonicotinoid seed treatments in corn, growers have enjoyed the benefits of a likely suppression of the soil insect pest complex, because this class of insecticides is systemic and provides prolonged protection. However, with recent discoveries linking neonicotinoids to pollinator health decline, there are concerns of the restricted use of neonicotinoids in production agriculture throughout the United States (Blacquiere et al. 2012). As of January 2016, the U.S. National Wildlife Refuge System banned the use of neonicotinoids as tool for managing pest on U.S. National Wildlife Refuge land (Kurth 2014). This decision may cause corn producers in these areas to rely on other alternatives, such as the organophosphate or pyrethroid-class insecticides (represented by terbufos and bifenthrin in this study) that are likely less effective. Furthermore, chemicals in these classes are known to be environmentally harmful (Satoh and Hosokawa 1999, Solomon et al. 2001). The southern corn billbug studies were located near

Pocosin Lakes National Wildlife Refuge. The 2015 studies during revealed that the use of clothianidin could potentially reduce southern corn billbug population in the landscape over time. Without the use of neonicotinoids in this region southern corn billbug populations could potentially rise, making it harder for growers to produce corn.

In conclusion, clothianidin seed treatments were shown to manage wireworm, white grub, and southern corn billbug injury in corn and also reduce the need for other insecticides often used in combination with seed treatments for these pests. These results serve as a foundation for future research that will develop better pest management strategies with future effort focused on yield assessment in order to further quantify the value of neonicotinoid seed treatments.

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Table 1.1. Mean number of wireworm and white grub injured corn plants per 24.3 row-meters at 2015 Location 1, during two, three, and four weeks after planting (WAP). Mean separation was carried out using Tukey-Kramer adjustment for multiple comparisons, α = 0.05. Means sharing the same letter are not statistically different and separations are unique to each WAP column.

Insecticide treatment	**Mean number of injured plants per 24 row-meters**		
	2 WAP	**3 WAP**	**4 WAP**
untreated	5.75 A	6.50 A	9.00 A
bifenthrin	6.75 A	2.75 AB	8.75 A
terbufos	4.00 A	1.00 B	6.25 AB
clothianidin 1.25mg a.i./ha	5.00 A	0.50 B	1.50 BC
clothianidin 1.25mg a.i./ha + terbufos	3.50 A	0.25 B	2.00 BC
clothianidin 3.088mg a.i./ha	2.25 A	1.75 AB	0.25 C
clothianidin 3.088mg a.i./ha + terbufos	4.25 A	0.50 B	0.75 C

Table 1.2. Mean number of wireworm and white grub injured corn plants per 24.3 row-meters at 2015 Location 2, during two, three, and four WAP. Mean separation was carried out using Tukey- Kramer adjustment for multiple comparisons, α = 0.05. Means sharing the same letter are not statistically different and separations are unique to each WAP column.

Insecticide treatment	**Mean number of injured plants per 24 row-meters**		
	2 WAP	**3 WAP**	**4 WAP**
untreated	6.50 A	5.50 A	16.25 A
bifenthrin	9.00 A	6.50 A	6.50 AB
terbufos	7.25 A	3.00 A	4.75 B
clothianidin 1.25mg a.i./ha	5.00 A	7.75 A	2.25 B
clothianidin 1.25mg a.i./ha + terbufos	3.75 A	3.25 A	2.50 B
clothianidin 3.088mg a.i./ha	3.50 A	3.75 A	1.00 B
clothianidin 3.088mg a.i./ha + terbufos	1.75 A	2.25 A	1.00 B

Table 1.3. Mean number of southern corn billbug injured corn plants per 24.3 row-meters at 2015 Location 3, during two, three, and four WAP. Mean separation was carried out using Tukey- Kramer adjustment for multiple comparisons, α = 0.05. Means sharing the same letter are not statistically different and separations are unique to each WAP column.

Insecticide treatment	**Mean number of injured plants per 24 row-meters**		
	2 WAP	**3 WAP**	**4 WAP**
untreated	35.25 A	69.25 A	106.25 A
bifenthrin	36.25 A	40.00 AB	59.50 B
terbufos	23.50 A	28.75 AB	40.75 B
clothianidin 1.25mg a.i./ha	18.25 A	22.25 B	36.00 B
clothianidin 1.25mg a.i./ha + terbufos	19.75 A	18.50 B	29.75 B
clothianidin 3.088mg a.i./ha	19.25 A	22.00 B	25.75 B
clothianidin 3.088mg a.i./ha + terbufos	30.25 A	17.50 B	19.75 B

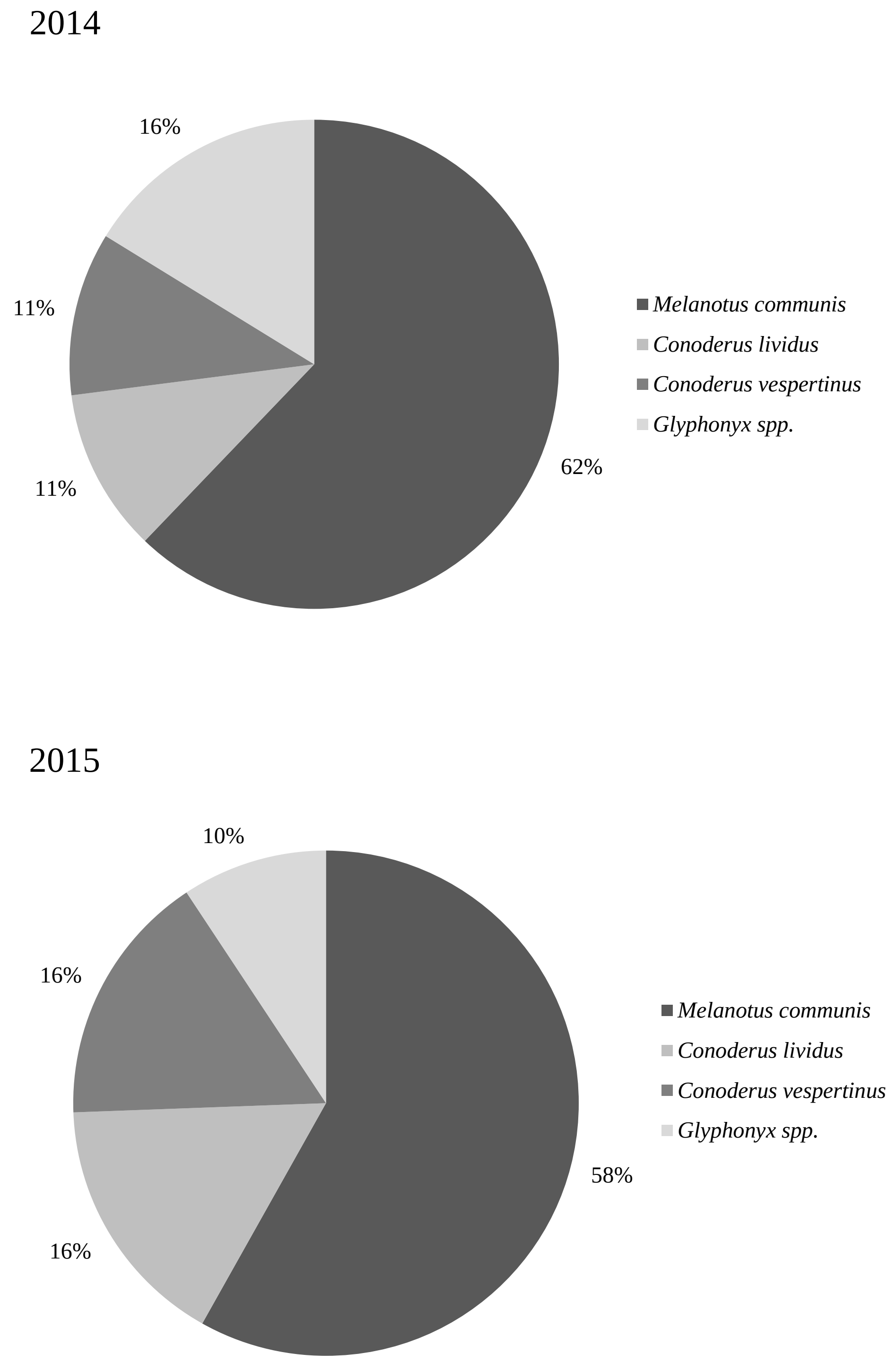

Figure 1.1. Species composition for wireworms collected in oat baits from wireworm locations prior to planting during the 2014 and 2015 growing seasons.

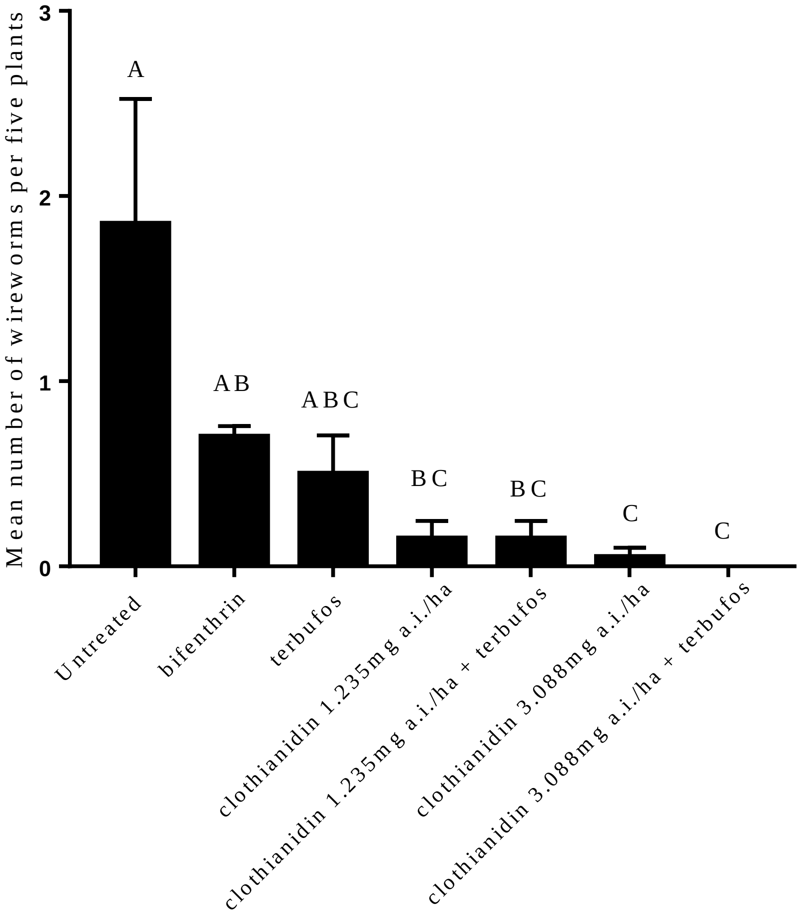

Figure 1.2. Abundance of total wireworms collected from soil surrounding plant roots at 2014 Location 2. Bars sharing the same letter are not significantly different at $\alpha<0.05$. Error bars represent standard error.

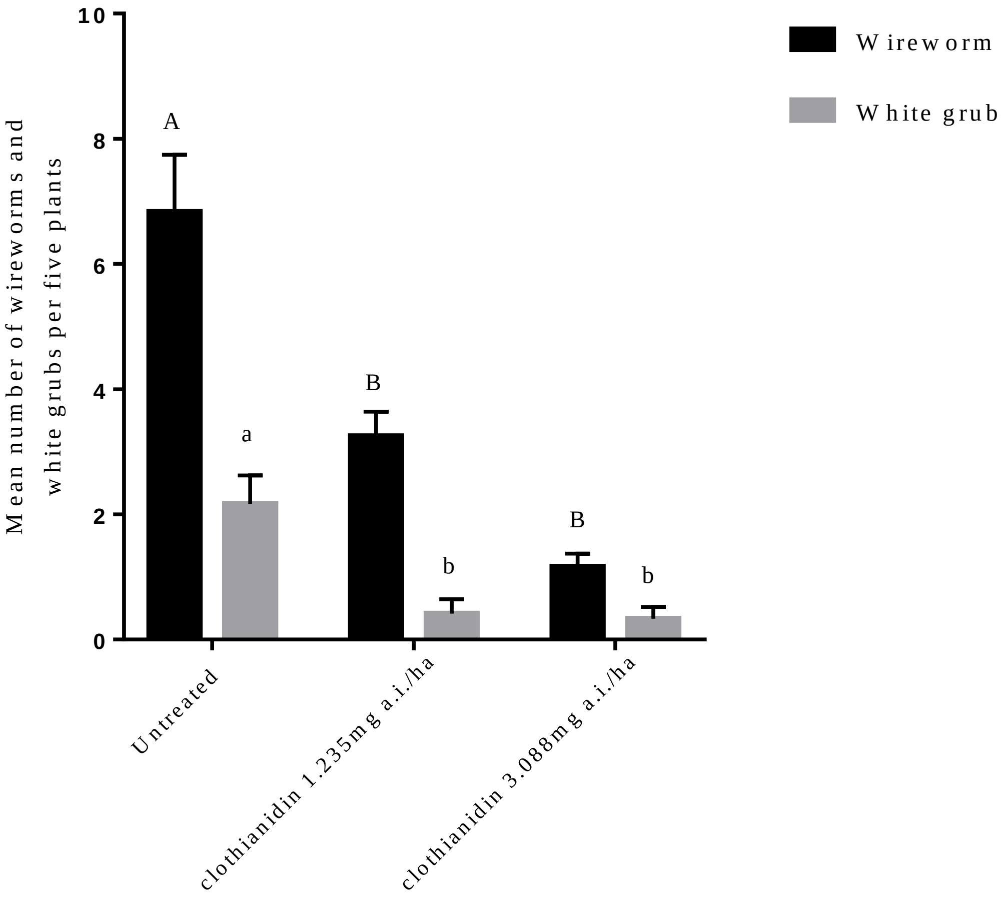

Figure 1.3. Abundance of total wireworms and white grubs, collected from untreated, clothianidin 1.25mg a.i./ha, and clothianidin 3.088mg a.i./ha plots at 2015 Location 1. Bars sharing the same letter are not significantly different at α<0.05 and comparisons are unique to insect pest species. Error bars represent standard error.

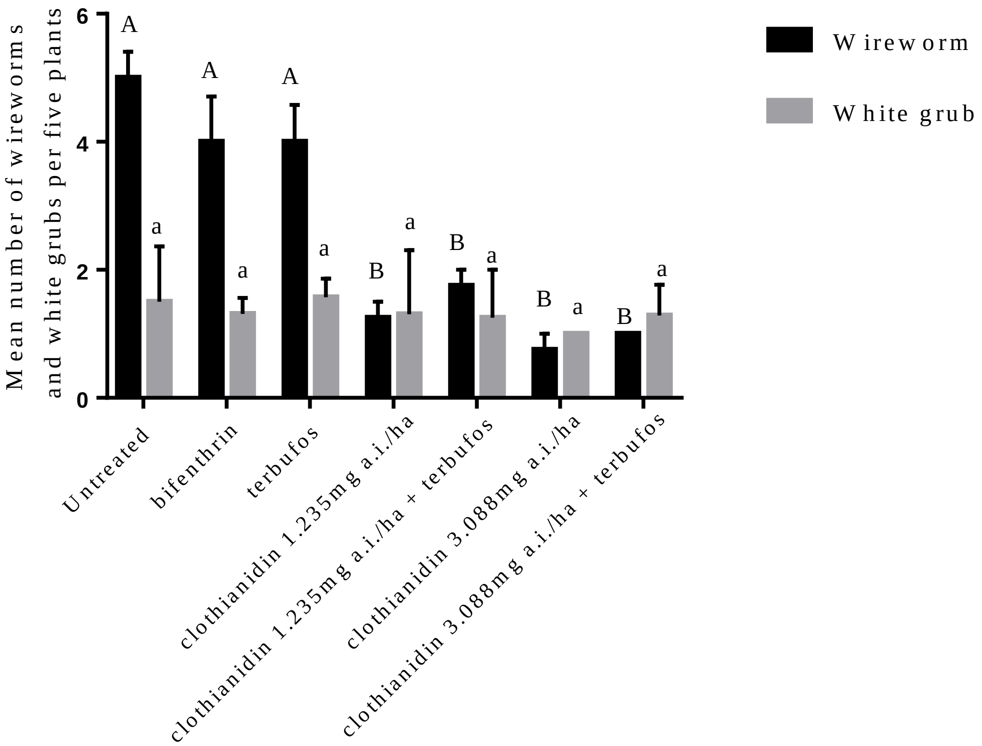

Figure 1.4. Abundance of total wireworms and white grubs collected from all treatments at five WAP, from 2015 Location 1. Bars sharing the same letter are not significantly different at $\alpha<0.05$ and comparisons are unique to insect species. Error bars represent standard error.

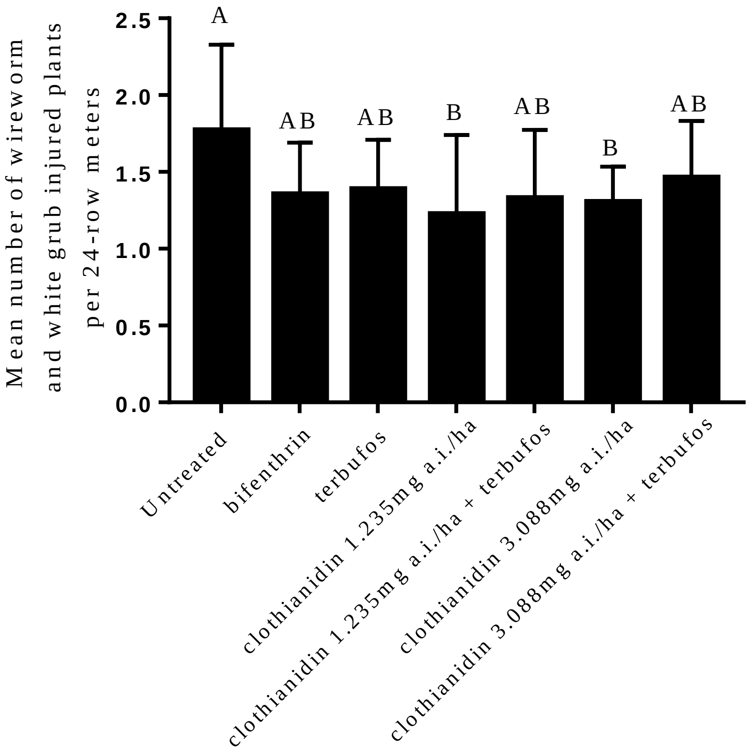

Figure 1.5. The mean number of wireworm and white grub injured plants noted during injury ratings at 2014 Location 1. Bars sharing the same letter are not significantly different at α<0.05. Error bars represent standard error.

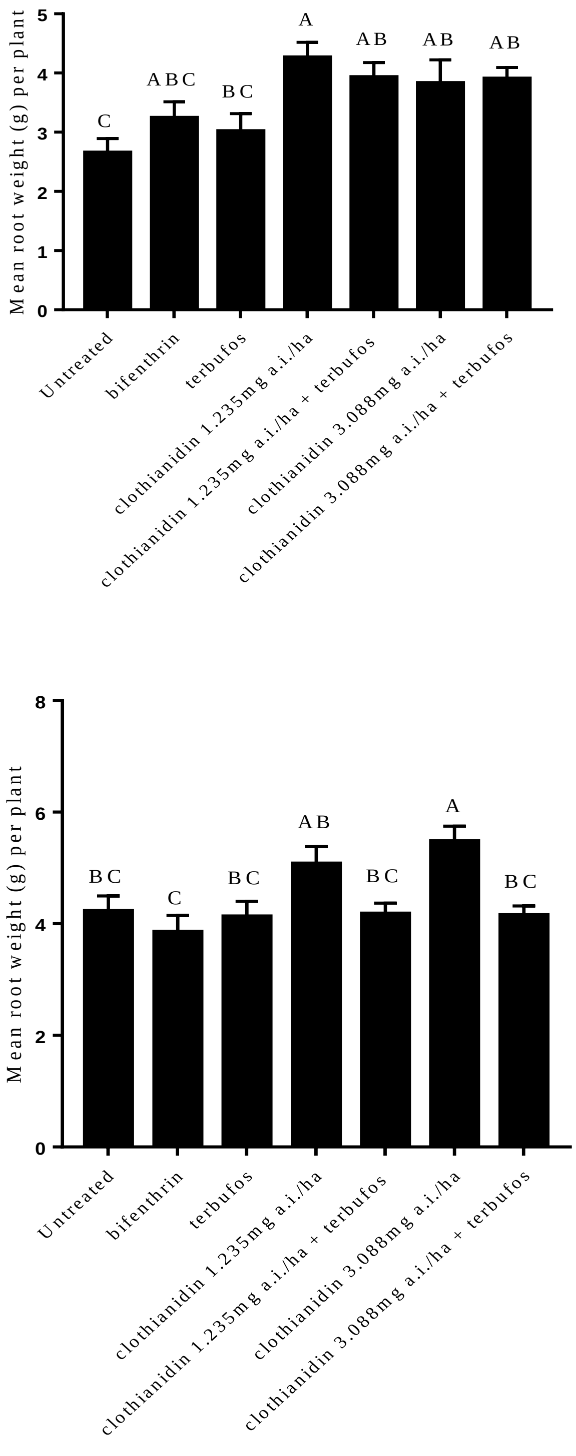

Figure 1.6. The mean root weight of samples collected during 2014 (top) and 2015 (bottom) field studies. Bars sharing the same letter are not significantly different at α<0.05. Error bars represent standard error.

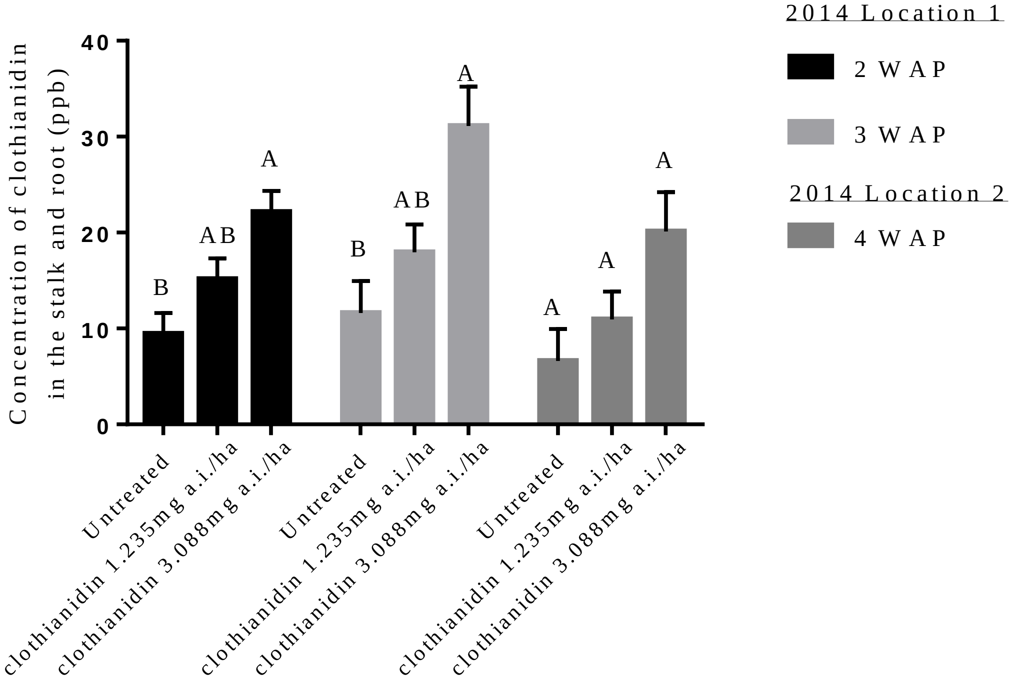

Figure 1.7 Concentration of clothianidin in combined corn (stalk and root) tissue collected at two, three, and four WAP during 2014 at Locations 1 and 2. Repeated measures analysis was not performed. Bars within a given sample date sharing the same letter are not significantly different at α<0.05. Error bars represent standard error.

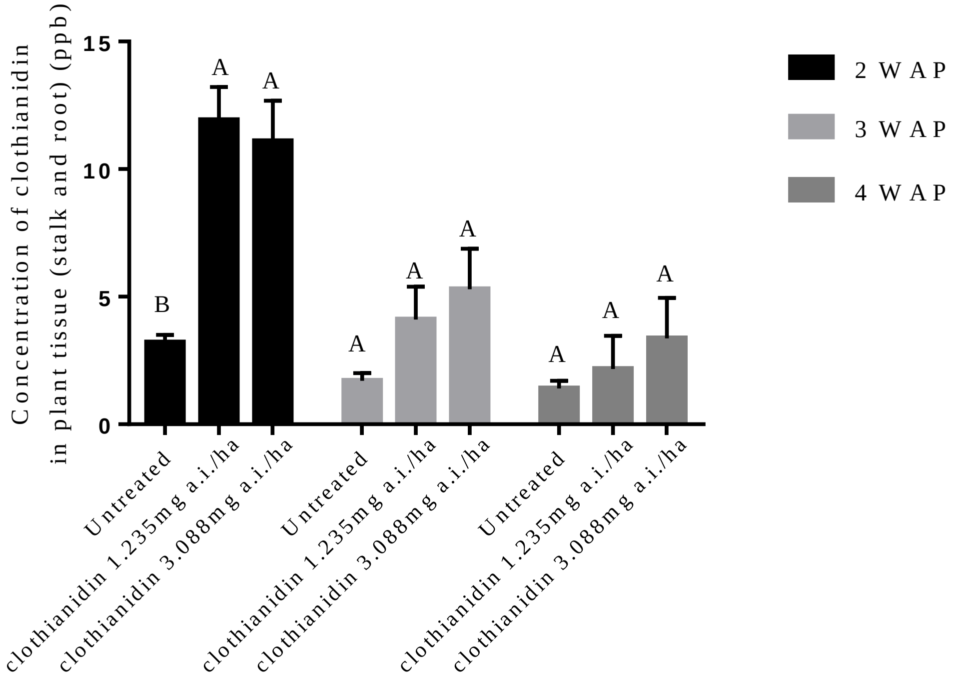

Figure 1.8. Concentration of clothianidin in combined (stalk and root) corn tissue collected at two, three, and four WAP during 2015 field studies. Repeated measures analysis was performed and data were sliced to partition variation by date. Therefore, letter groupings for mean separation are unique to each date. Bars within a given sample date sharing the same letter are not significantly different at $\alpha<0.05$. Error bars represent standard error.

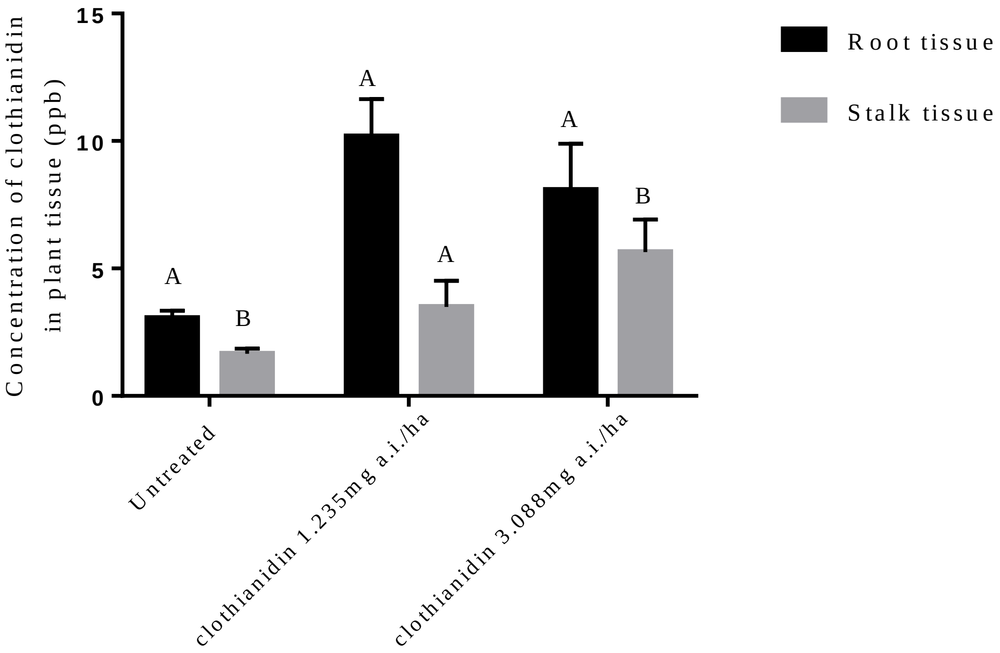

Figure 1.9. Concentration of clothianidin in root and stalk tissue collected from untreated, clothianidin 1.25mg a.i./ha, and clothianidin 3.088mg a.i./ha plots from 2015 Location 3. Repeated measures analysis was performed and data were sliced to partition variation by treatment. Therefore, letter groupings for mean separation are unique to each treatment. Bars within a given treatment sharing the same letter are not significantly different at α<0.05. Error bars represent standard error.

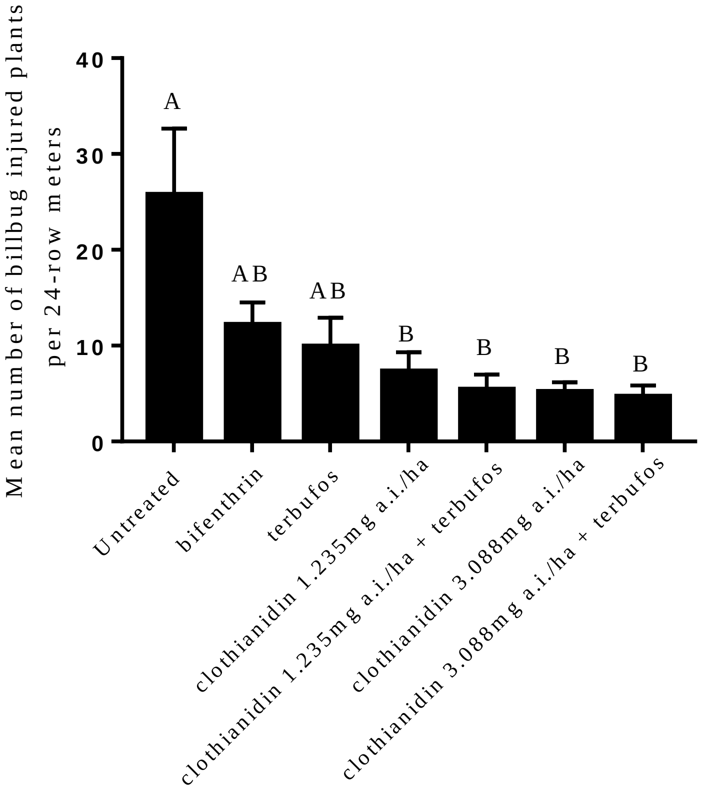

Figure 1.10. Mean number of southern corn billbug injured plants observed during 2014 injury ratings at 2014 Location 3. Bars sharing the same letter are not significantly different at α<0.05. Error bars represent standard error.

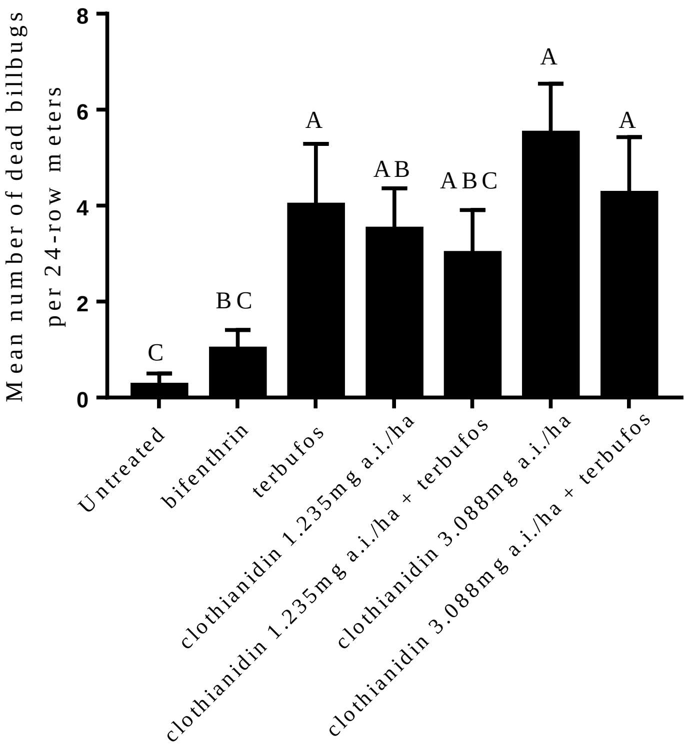

Figure 1.11. Mean number of dead southern corn billbugs recorded on soil surface in each plot at two WAP at 2015 Location 3. Bars sharing the same letter are not significantly different at α<0.05. Error bars represent standard error.

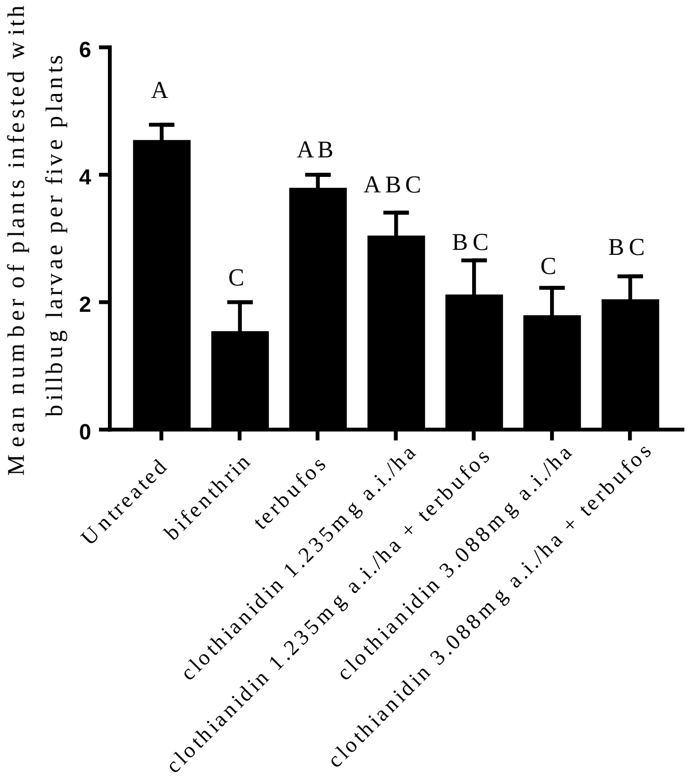

Figure 1.12. Mean number of plants infested with southern corn billbug larvae from five randomly selected plants in all treatments collected from 2015 Location 3. Bars sharing the same letter are not significantly different at α<0.05. Error bars represent standard error.

Chapter II

Impact of imidacloprid treated seed and foliar insecticides on Hessian fly in wheat (*Triticum aestivum* L.)

Introduction

Wheat, *Triticum aestivum* L., is a major crop of economic importance throughout the United States, ranking third in area planted behind corn and soybean and valued over 11 billion dollars in 2014 (USDA 2016). Wheat is also grown on all continents and is considered the most important cereal crop in the Northern hemisphere, as well as in Australia and New Zealand (Oerke 2006). Globally, it is estimated that two-thirds of the world population is dependent on wheat as a food source (WHO 2012). There are six classes of wheat grown in the United States: hard red winter, hard red spring, hard white, soft white, soft red winter, and durum. Soft red winter wheat is a major crop in the southeastern U.S.

Growers in the southeastern U.S. typically plant wheat in the fall, and it is often harvested in late May through June. Wheat is normally used in a double-cropping system in this region in combination with soybean, *Glycine max* L. A number of insect pests threaten wheat productivity, including aphids (Aphididae), armyworms (Noctuidae), cereal leaf beetle (Chrysomelidae, *Oulema melanopus* (L.)), and Hessian fly (Cecidomyiidae, *Mayetiola destructor* (Say)).

Of these insect pests of wheat, Hessian fly is among the most serious throughout the southeastern U.S. (Buntin and Chapin 1990). Hessian fly prefers wheat, but can also feed on grasses in the genera Triticeae (Zeiss et al. 1993, Chen et al. 2009). This pest damages both winter and spring wheat, resulting in worldwide yield losses averaging 32-42% (Amri et al. 1992 and Lhaloui et al. 1992). Hessian flies are believed to have originated in Southwest Asia, where wheat also originated (Barnes 1956), and it is speculated that it was transported to the United States in straw bedding used by Hessian soldiers during the American Revolution (Gallun 1977, Pauly 2002). Wheat production in the Southeast increased during the late 1970's, which also contributed to the increase in Hessian fly incidence and damage (Buntin and Chapin 1990). Injury from Hessian fly varies from year-to-year, but they are more prevalent in high-residue and annual cropping systems (Pike and Antonelli 1981).

Hessian fly adults are small, dark colored, gall-making flies often mistaken for other insects. The adults are present during a relatively short time (three to five days) and do not feed. Females can oviposit 100-400 eggs during a one to two day period (McColloch 1923). Hessian fly eggs are small, reddish in color, oblong in shape, and are laid end-to-end on the adaxial side of wheat leaves and between the grooves of the longitudinal veins (Dean and McCulloch 1915). Females typically only mate with one male; however, males mate with numerous females which they locate using sex-pheromones released by the female (Harris and Foster 1999). Males may fertilize as many as 3,445 eggs in their lifetime (Foster et al. 1992, Bergh et al. 1992). Temperature and humidity regulate the three to 10 day period in which larvae (maggots) emerge and migrate to the base of the wheat plants, where they feed between leaf sheaths at the crown (Dean and McColloch 1915, McCulloch 1923, Ratcliff and Hatchett 1997). Larvae have three instars and the first and the second instars cause the most damage to wheat (McCulloch 1923, Ratcliffe and Hatchett 1997). Hessian fly populations appear to be more significant in wet years (Criddle 1917), but there is little information regarding how moisture impacts adult emergence; it is unclear whether this is reflective of abundance, injury to the plants, or a combination of these factors. The Hessian fly pupae will remain enclosed in the case, or puparia, that is synonymous with dipterous insects, until environmental conditions permit emergence (McColloch 1923). Hessian fly infestations in wheat are often confused with other ailments such as nutrient deficiencies, which makes identification and management of these pests problematic. Hessian fly injury can increase tillering, as the plant attempts compensate for damage (Anderson et al. 2011), and can result in stunted growth, dead wheat seedlings, and increased susceptibility to winter kill (Buntin 1999). Stunting is caused by unknown toxic substances injected into the plant from larvae while they are feeding (Cartwright et al. 1959, Byers & Gallun 1972a,b, Shukle et al. 1985). Larvae that feed during the spring prevent small tillers from heading, cause lodging, and reduce the weight of the grain (Hill and Smith 1925).

Traditionally Hessian fly has been managed in wheat by delayed plantings (McColloch 1923 and Gallun 1965) and the use of resistant wheat varieties (Painter 1951, Gallun and Reitz 1971), but Hessian fly populations can also be affected by tillage and burning practices (Chapin et al. 1992). Unlike in the Midwestern United States, there is no “fly free” date for Hessian fly, or fall date after which flies are no longer active, in the southeast. Plowing infested wheat stubble can bury larvae to prevent adults from emerging, and is a widely used management tactic. For example, post-harvest disking of wheat stubble has the potential to lower Hessian fly emergence by 70% the following season compared to un-disked wheat (Zeiss et al. 1993), although this is not always effective. Previous work has also suggested pest that population sizes may increase in reduced tillage systems, because reduced tillage increases surface residues which serve as overwintering habitats for the fly (Pike et al. 1993, Smiley et al. 2004, Castle del Conte et al., 2005). With adoption of no-till and conservation tillage practices, wheat growers have turned to other alternatives for managing this serious pest when a resistant variety is not planted. One of those alternatives is the use of insecticides. Foliar insecticides have not been widely adopted, because control is inconsistent (Buntin and Hudson 1991). The short adult life span, the brief period larva is exposed to leaf surface, and the inability to reach larvae once in the leaf sheaths of wheat plants contribute to the inconsistency of foliar insecticides. For example, multiple foliar sprays are needed to control spring generations of Hessian fly and those sprays must be applied before peak egg laying occurs (Buntin and Hudson 1991). Moreover, timing of insecticide applications is crucial in order to achieve control, but is often times very difficult. At planting insecticides have been demonstrated have some efficacy. For example, at planting in-furrow applications of disulfoton and phorate increased wheat yields under high Hessian fly infestations (Buntin et al. 1992). Furthermore, carbofuran is effective as either a seed treatment or soil treatment and was shown to reduce fall Hessian fly generations during the late 1970's (Nelson and Morrill 1978). However, these broad-spectrum organophosphate and carbamate insecticides are no longer registered for use on wheat.

Neonicotinoids have lasting residual activity that can often be used at low rates, making them widely used for many crop pests (Elbert et al. 2008). Neonicotinoids can provide both seed and foliar protection for several weeks or months after planting (Wilde et al. 2001, Nault et al. 2004, Koch et al. 2005, Jeschke et al. 2011), which has facilitated grower adoption in numerous agricultural systems worldwide. This class of insecticides targets the central nervous system of insects as an agonist of the post-synaptic nicotinic acetylcholine receptors. Before planting, a neonicotinoid is applied to the seed coat; the active compound is taken up by the plant roots after germination and distributed throughout the entire plant, allowing for systematic protection from insects (Girolami et al. 2009). There is some information on the efficacy of neonicotinoid seed treatments in wheat against wireworms (Elateridae) and aphids, but little efficacy information is available for Hessian fly control in the peer-reviewed literature.

The main purpose of this study was to investigate the efficacy of insecticides as management tools for Hessian fly in southeastern U.S. wheat, including a neonicotinoid seed treatment, multiple foliar insecticide sprays, and a combination of both foliar sprays and a seed treatment. These treatments represent the common insecticide classes of insecticides used in commercial field settings.

Materials and Methods

Site Selection. Field studies were conducted over two years in coastal North Carolina, where most Hessian fly injury occurs in the state. Sites during 2014 were located in Washington County at the Tidewater Research Station and Lenoir County at the Cunningham Research Station. During 2015 all study locations were in Lenoir County at the Cunningham Research Station. Field locations were chosen based on previous reported Hessian fly occurrences.

Field Experiment. Small plots were established in field locations with previous Hessian fly occurrence, and treatments were arranged in a split-plot design. The whole plot effect of seed-applied insecticidal treatment was split with a foliar insecticide treatment in an attempt to create a range of Hessian fly abundance. There were four replications of each treatment, which included: 1) untreated wheat seed, 2) untreated wheat seed + semi-monthly (twice a month) foliar applications of lambda-cyhalothrin 56.7ml a.i./ha (Warrior II, Syngenta Crop Protection, Greensboro, NC), 3) seed-applied imidacloprid 68.03g a.i./ha (Gaucho 600 FS, Bayer CropScience, Research Triangle Park, NC), and 4) seed-applied imidacloprid 68.03g a.i./ha + semi-monthly foliar application of lambda-cyhalothrin 56.7ml a.i./ha. Imidacloprid seed treatments were applied with a laboratory-scale seed treater to untreated wheat seed. Along with the clothianidin, a color dye slurry (2 ml PRO-IZED red seed colorant at 2.26kg seed + 25 ml of water, Bayer CropScience, Research Triangle Park, NC) was added to the insecticide solution, which provided a visual reference to ensure uniform application. Plots at all locations were sprayed using a CO_2 pressurized backpack sprayer calibrated to apply 71.9 liters per hectare applied at a speed of 4.8 kilometers per hour with 13.6 kilograms of pressure. The insecticide was applied using four, XR 8001 spray nozzles (TeeJet, Dillsburg, VA), on a 1.2m boom.

Wheat, DG Shirley (Dyna-Gro Seed, Geneseo, IL), was planted on 9 October 2014 (2014 Location 1 and 2). The same variety was planted on 4 November 2015 (2015 Location 1) and 2 December 2015 (2015 Location 2). Shirley is highly susceptible to Hessian fly and was planted using a John Deere 5085M tractor (John Deere, Moline, IL), equipped with a John Deere 8300 grain drill (John Deere, Moline, IL), with 19.05cm spacing. Wheat was seeded at a rate of 61.23 kg/ha. Plots in 2014 were 9.1m long by 1.8m wide; during 2015, plots were 9.1m long by 3.6m wide. All plots received applications of fertilizer according to state Extension recommendations. Moreover, all plots also received applications of Harmony Extra SG 22.1ml/ha (thifensulfuron-methyl and tribenuron-methyl, DuPont, Wilmington, DE) and Osprey 140.4ml/ha (mesosulfuron-methyl, Bayer CropScience, Research Triangle Park, NC) for control of broadleaf weeds and grasses after planting.

Data Collection. Hessian fly presence was monitored weekly after wheat seed had germinated. A destructive sampling method was used to collect data from each plot. Wheat plants were excavated from the soil, and ten random tillers from each plot were examined for Hessian fly eggs, larvae, and pupae, and the total number of each per plot was recorded. In order to count the number of larvae and pupae, leaves from each tiller were peeled back to expose the larvae and pupae hidden in the leaf sheath. Tiller densities were also recorded by calculating the number of tillers per square meter in each plot. The number of sampling dates varied by year and planting date. Samples were collected weekly until Hessian fly presence (eggs, larvae, and pupae) had diminished to near undetectable populations.

Data Analysis. Egg, larvae, and pupae numbers were analyzed using separate individual general linear mixed repeated measures analysis of variance models (PROC MIXED, SAS Institute 2012). In each analysis, sampling date and treatment were dependent fixed variables along with the interaction between sampling date and treatment, while the replication was a random variable. The REPEATED statement was used and, because sampling events were evenly spaced, the covariance was modeled as compound symmetry. If independent variable effects were significant, means were separated using Tukey's honestly significant differences test, where treatments differed significantly at $\alpha \leq 0.05$. Transformations were used, as needed, to comply with the assumptions of analysis of variance.

Results

Data Collection. At 2014 Location 1, the number of eggs in each plot differed between treatments ($F = 4.49$; d.f. = 3,12; P = 0.0247; Figure 2.1) and over time ($F = 56.42$; d.f. = 6,72; $P < 0.0001$; Figure 2.2), but effects of seed treatments did not differ among sample dates ($F = 1.71$; d.f. = 18,72; $P = 0.0572$). More eggs were counted in plots without imidacloprid treated seed compared to those with imidacloprid. There was, however, a significant interaction of seed treatment with sampling date for larvae ($F = 5.29$; d.f. = 18,72; $P < 0.0001$; Figure 2.3). More larvae were counted in plots without seed treatment during the third and fourth week of sampling compared to other weeks. There was no interaction of treatment with sampling date for pupae. Treatment ($F = 5.21$; d.f. = 3,9; $P = 0.0233$; Figure 2.4) and sampling date ($F =$

2.51; d.f. = 6,72; *P* = 0.0292) had significant effects on the number of pupae counted. However, mean separations for pupae by treatment and date using the conservative Tukey's procedure were not significant.

There was no interaction of treatment with sampling date at 2014 Location 2 for number of eggs counted, but treatment (*F* = 16.76; d.f. = 3,12; *P* = 0.0001; Figure 2.5) and sampling date (*F* = 28.39; d.f. = 6,72; *P* < 0.0001; Figure 2.6) each significantly impacted egg numbers. Fewer eggs were counted in plots with seed treatments, regardless of semi-monthly foliar sprays, compared to plots with seed treatments, regardless of semi-monthly foliar sprays. Egg sampling was consistent throughout much of the seven week sampling period, until the last sampling date, when egg numbers decreased. Larval and pupal counts at 2014 Location 2 were unaffected by treatment (Larvae: *F* = 2.69; d.f. = 3,9; *P* = 0.1091; Pupae: *F* = 0.67; d.f. = 3,12; *P* = 0.5885), sample date (Larvae: *F* = 1.48; d.f. = 6,72; *P* = 0.1958; Pupae: *F* = 0.83; d.f. = 6,72; *P* = 0.5481), or their interaction. At 2015 Location 1, there was a significant interaction between treatment and sample date (*F* = 4.71; d.f. = 24,96; *P* < 0.0001; Figure 2.7) for number of eggs counted. More eggs were counted in weeks seven and eight of sampling compared to other weeks, but there was no interaction of treatment with sampling date while sampling for larvae. Larval counts did differ between treatments (*F* = 17.62; d.f. = 3,12; *P* = 0.0001; Figure 2.8) and sample dates (*F* = 15.35; d.f = 8,96; *P* < 0.0001; Figure 2.9). More larvae were count in plots without a seed treatment and without semi-monthly foliar sprays compared to all other treatments. Pupae differed significantly between treatments over time (*F* = 3.74; d.f. = 24, 96; *P* < 0.0001; Figure 2.10), where weeks three, four, and five had higher pupal counts compared to other weeks.

At 2015 Location 2, there was a significant interaction between treatment and sample dates (*F* = 3.04; d.f. = 6,24; *P* = 0.0234; Figure 2.11) for number of eggs counted. More eggs were counted in weeks two and three in untreated plots, regardless of foliar spray, compared to treated plots. Differences between insecticide treatments occurred during weeks two and three during the sampling period. There was also a significant interaction of treatment with sampling date for larvae (*F* = 6.08; d.f. = 6,24; *P* = 0.0006; Figure 2.12); Larvae counts were higher in untreated plots compared to treated plots during the first and second sampling dates. Finally, there was a significant interaction of treatment with sampling date for pupal counts (*F* = 5.65; d.f. = 6,24; *P* = 0.0009; Figure 2.13), with higher pupal numbers during weeks two and three in plots without imidacloprid treated seed, compared to other treatments and sample dates.

Treatment had a significant effect on tiller density at both 2014 Location 1 (*F* = 17.47; d.f. = 3,9; *P* = 0.0004; Figure 2.14) and 2014 Location 2 (*F* = 12.31; d.f. = 3,9; *P* = 0.0015; Figure 2.15). Tiller densities were highest in plots with imidacloprid treated seed, compared to untreated plots. Again in 2015 at both locations, treatment had a significant effect on tiller densities (2015 Location 1 (*F* = 11.24; d.f. = 3,9; *P* = 0.0021; Figure 2.16; 2015 Location 2 (*F* = 22.92; d.f. = 3,9; *P* = 0.0002; Figure 2.17)). Plots with imidacloprid treated seed had higher tiller densities compared to untreated plots. In lambda-cyhalothrin treated plots, tiller density was not effected when combined with a seed treatment, but when no seed treatment was applied, tiller density was higher at 2014 Location 2.

Discussion

Imidacloprid treated seed generally reduced Hessian infestations during both the fall and spring in all experiments. Moreover, tiller densities were the highest in plots planted with imidacloprid treated seed in all experimental locations. Repeated foliar applications of lambda-cyhalothrin, alone, were only effective in a single experimental location, increasing tiller number in one case, and decreasing larval abundance in another. Hence, the insecticidal seed treatment was the most consistent method of insecticide protection against Hessian fly in these experiments.

In order for foliar insecticides to be an effective method of control Hessian fly, they need to be applied before peak egg laying occurs for maximal efficacy (Buntin and Hudson 1990). Moreover, multiple applications are likely needed to manage spring infestations, since the titer of insecticide in the plant from a seed treatment from a fall planting is likely low. My study was designed to eliminate the need to properly synchronize foliar insecticide spraying with Hessian fly emergence, since I sprayed every other week when Hessian flies were active. Despite this effort, the foliar sprays did not decrease the number of eggs, larva, or pupae when compared to other treatments during 2014; however, during 2015 foliar spays were beneficial since egg, larva, and pupa numbers were reduced in plots that received semi-monthly foliar applications of lambda-cyhalothrin without an insecticidal seed-treatment. One possible explanation for this phenomenon could be initiation of the first foliar spray during 2014. It is possible that the initial application of lambda-cyhalothrin during 2014 was late and wheat was already infested with Hessian fly. Foliar applications in 2014 were likely made after eggs had hatched and larvae had migrated to the crown and concealed themselves in the leaf sheath, but in 2015 applications were synchronized with adult emergence and applied before peak egg laying occurred. However, on the foliar spray date during 2015, average egg number (two to three per tiller), exceeded that of 2014 (one to two per tiller). Therefore, another possible explanation could be due to differences in Hessian fly pressure between the two years. For example, more eggs in 2015 developed into larvae than in 2014. Bi-monthly applications of lambda-cyhalothrin only appeared to benefit plots when pest populations were high during 2015. Even when foliar insecticide sprays were paired with imidacloprid treated seed there were minimal benefits, measured in reductions of egg, larva, and pupal counts and increases in tiller number. More importantly, because there was no significant difference in egg counts at early sampling dates, but there were at later sampling dates, my studies demonstrate an effect of seed treatment for up to two months after planting. Wheat growth likely contributed to this finding. Winter wheat is in vegetative growth stages during fall months and then vernalizes over the winter, which induces reproductive growth in the spring (Sacks et al. 2010). Because of these growth processes, the overall size of the wheat plant remains relatively small during the fall and winter; thus, the dilution effect of the systemic insecticide, such as imidacloprid, is not very high during this period, because once it is taken up, it remains in the plant (Elbert et al. 1998).

Hessian fly completes one to six generations per year, dependent on climate and latitude (Osborn 1898, Barnes 1956). Mild winter weather may allow for Hessian flies to complete three to six generations per year in the southern U.S. (Buntin et al. 1990). Optimal conditions for growth, development, and adult emergence are at 21.1°C (Foster and Taylor 1975). While most offspring emerge soon after entering the pupal stage, some puparia may not emerge for an extended period of time (McColloch 1923). Like other states in the southeast U.S., North Carolina does not have a “fly-free” planting date, but growers that plant later tend to have reduced incidence of fall Hessian fly infestations. However, there is an optimal planting window to balance maximum yield potential and minimize Hessian fly pressure. For example, in Georgia, if plantings occur after 1 December, wheat yield potential declines, and there is minimal benefit gained for fly suppression (Buntin and Chapin 1990).

During 2014, my trials were planted before the state Extension service recommended planting date of 30 October to 4 November in these regions of coastal North Carolina (Weisz 2013); however during 2015 wheat plantings were both timely (4 November 2015) and late (2 December 2015), due to rainfall that precluded earlier planting. The purpose of planting wheat early was to foster as much Hessian fly pressure as possible. The early plantings increased the severity of Hessian fly injury during the fall, but the timely plantings did not avoid damage as measured by egg, larvae, pupae, and tiller counts. The temperatures during our the 2014 studies were one to four degrees lower than monthly averages for the region (CRONOS 2016) and Hessian fly activity (egg, larvae, and pupae counts) decreased dramatically by December. However, during the 2015 studies, temperatures from November through February were unseasonably warm. Temperatures during these months were, on average, five to ten degrees above normal (CRONOS 2016). A peak in egg counts was measured during the month of December at 2015 Location 1 that was not measured in other trials. Based on number of eggs counted, flies were emerging from puparia through much of the fall and into parts of winter during 2015 in North Carolina. With temperatures remaining above average through late fall and into the winter months the number of Hessian fly generations likely increased compared to the 2014 studies.

Precipitation is a known factor that is positively correlated Hessian fly adult emergence (Morgan et al. 2005). My study did not specifically examine the role of precipitation on Hessian fly emergence. However, precipitation recorded at Cunningham Research Station (2015 Location 1 and 2), was 12-25cm above average for the region, during

2015-16 (CRONOS 2016). Moisture is positively correlated with pupal mortality (Yokoyama et al. 1994), but the effect of moisture on pupal survival and adult emergence in not known. Ample amounts of moisture and moderate temperatures during 2015 likely created a suitable environment for pupae to emerge and lay eggs that developed into larvae that damaged wheat in my studies into late December at both locations.

The results of this study demonstrate the value of neonicotinoid seed treatment throughout the southeast U.S. on susceptible cultivars of wheat. These findings are not only important for the southeastern U.S., but globally where Hessian flies are pest. For example, in parts of North Africa, it has been documented that Hessian fly can cause complete crop loss to susceptible cultivars of wheat (Amri et al. 1992). My studies have documented that a highly susceptible Hessian fly wheat cultivar, treated with imidacloprid, was able to avoid economically significant infestations in the southeastern U.S. Managing Hessian fly with the use of neonicotinoid seed treatments is a preventative strategy. Hence, growers should use information about farm history, long-range weather forecasts of temperature and moisture, and knowledge of planting date to make decisions of whether or not to include a seed treatment to prevent Hessian fly infestations. Resistant cultivars remain one of the best management tools for Hessian fly, because they are preventative and the seeds are similar in cost to non-resistant cultivars. However, when a resistant cultivar is not selected, the cost of applying a neonicotinoid seed treatment to a susceptible cultivar of wheat is economically justified when there is a high risk of economic infestations by Hessian fly (Buntin et al. 1992).

Future studies should determine the neonicotinoid seed treatment rates that are the most effective, as well as differences among specific neonicotinoid insecticide active ingredients. Furthermore, additional research should focus on the timing of foliar sprays as method of control for Hessian fly and, perhaps, the use of pheromone trapping, or other sampling methods, to monitor Hessian fly presence and to better time foliar sprays to control this pest.

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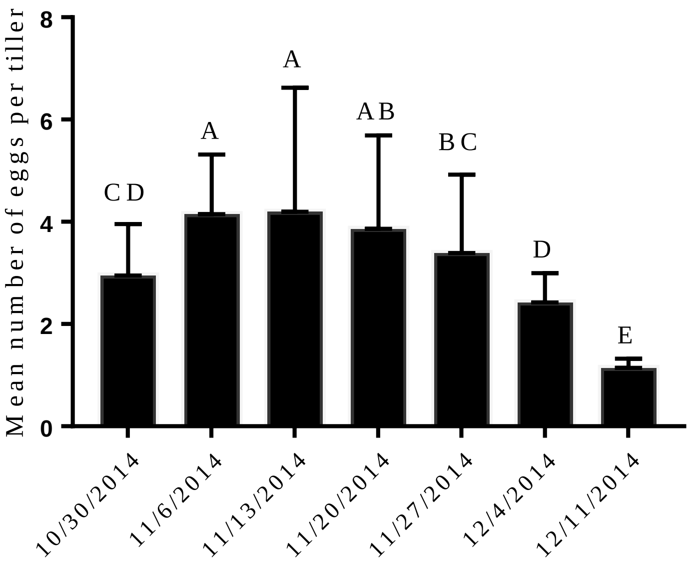

Figure 2.1. Mean number of Hessian fly eggs across all treatments at 2014 Location 1. Egg counts were significantly lower at the last sampling dates. Bars sharing the same letter are not significantly different at $\alpha<0.05$. Error bars represent standard error.

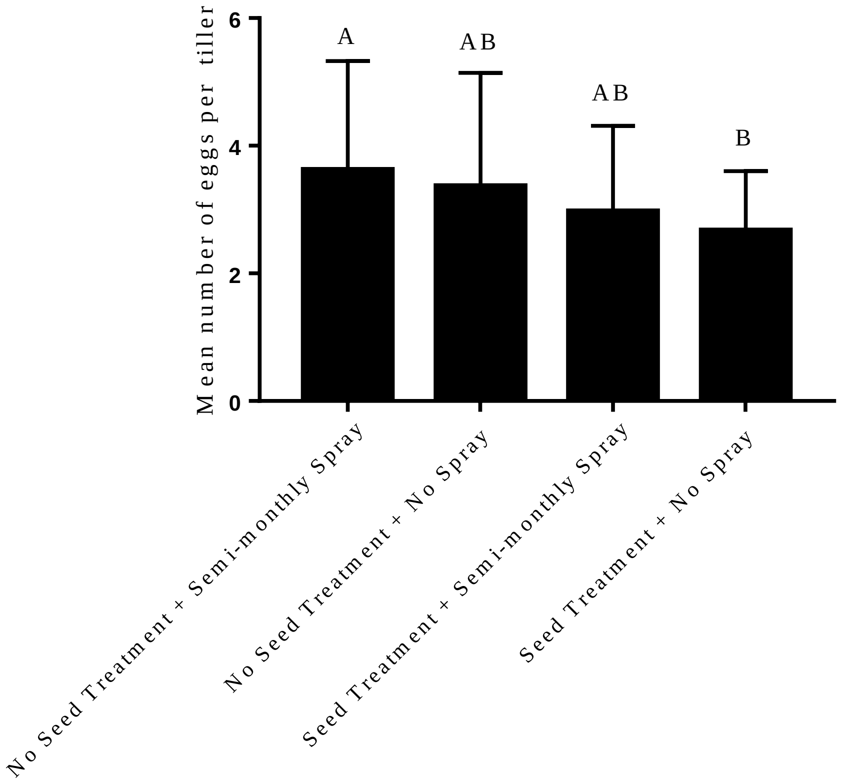

Figure 2.2 Mean number of Hessian fly eggs counted at 2014 Location 1. Egg counts varied among treatments. Higher densities were found in plots without seed treatment, semi-monthly sprays provided no benefit at 2014 Location 1. Bars sharing the same letter are not significantly different at α<0.05. Error bars represent standard error.

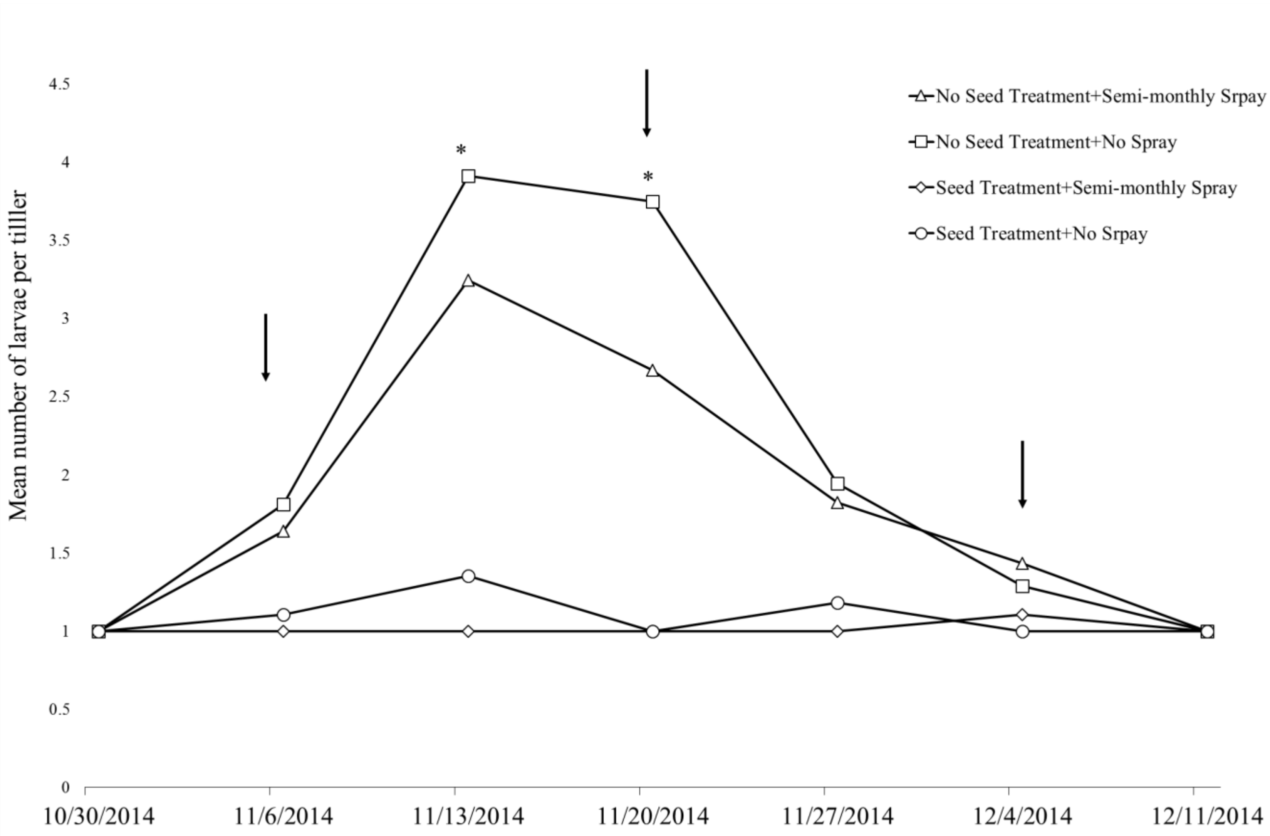

Figure 2.3 Mean number of Hessian fly larvae collected over the seven week sampling period from 2014 Location 1. Significant differences are denoted by *, and occurred on 13 November and 20 November, where plots without a seed treatment differed from plots with treated seed. Arrows represent the dates that foliar spray applications were made. Means were significantly different at α<0.05.

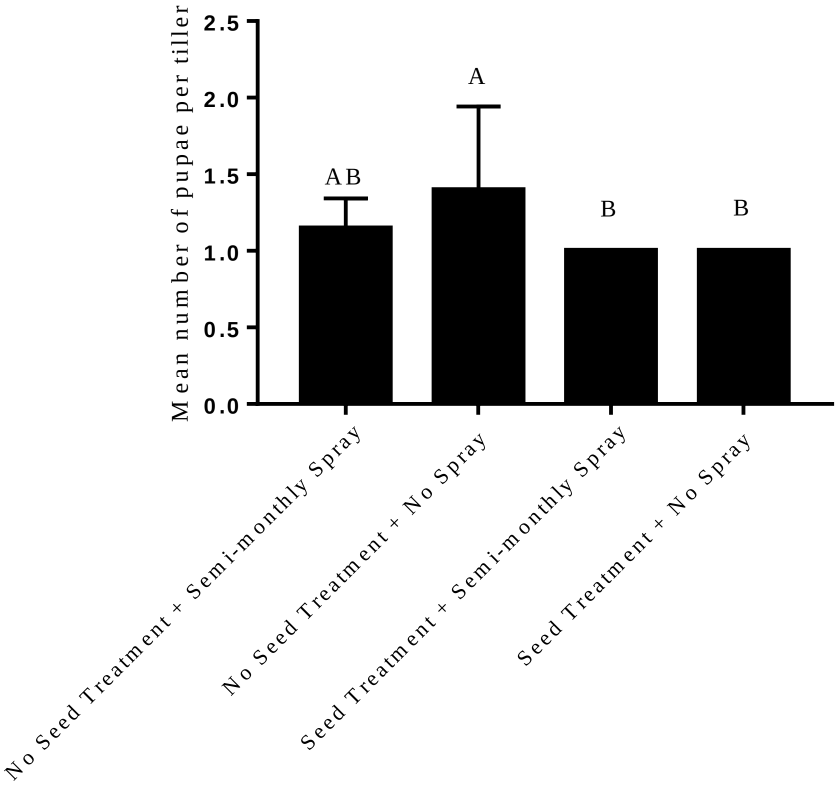

Figure 2.4. Mean number of Hessian fly pupae counted at 2014 Location 2. Pupae counts varied among treatments. Higher densities were found in plots without seed treatment, semi-monthly sprays provided no benefit at 2014 Location 2. Bars sharing the same letter are not significantly different at α<0.05. Error bars represent standard error.

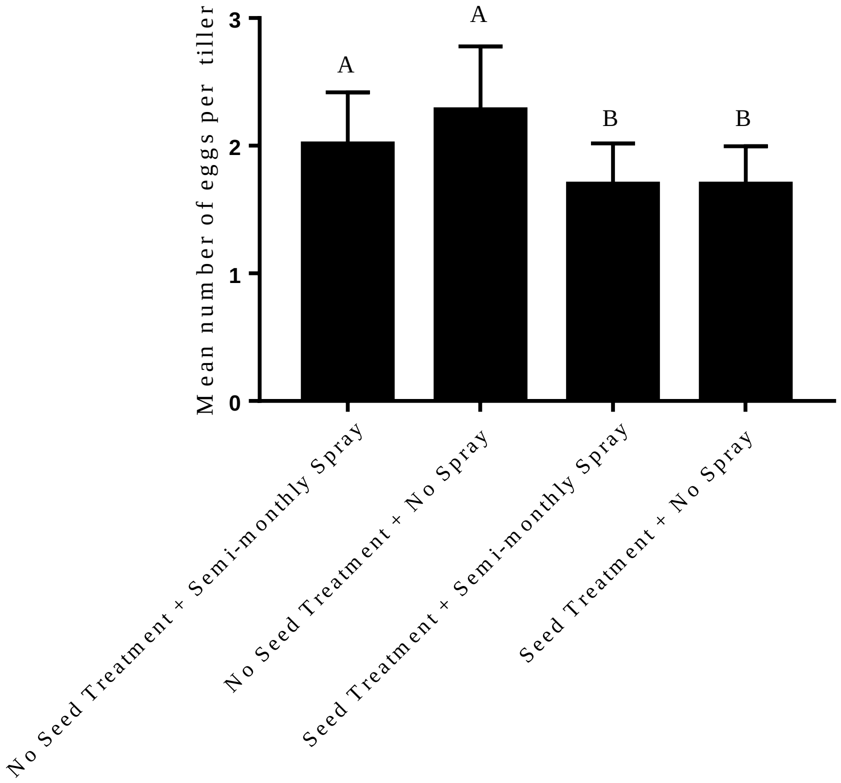

Figure 2.5. Mean number of Hessian fly eggs counted at 2014 Location 2. Egg counts varied among treatments. Higher densities were found in plots without seed treatment, semi-monthly sprays provided no benefit at 2014 Location 2. Bars sharing the same letter are not significantly different at $\alpha<0.05$. Error bars represent standard error.

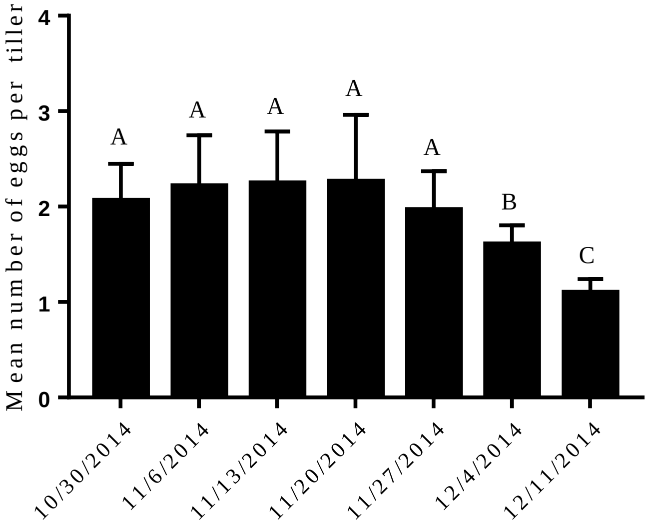

Figure 2.6. Mean number of Hessian fly eggs per tiller across treatments during the seven week sampling period at 2014 Location 2. Differences occurred during the last two sampling dates. Bars sharing the same letter are not significantly different at α<0.05. Error bars represent standard error.

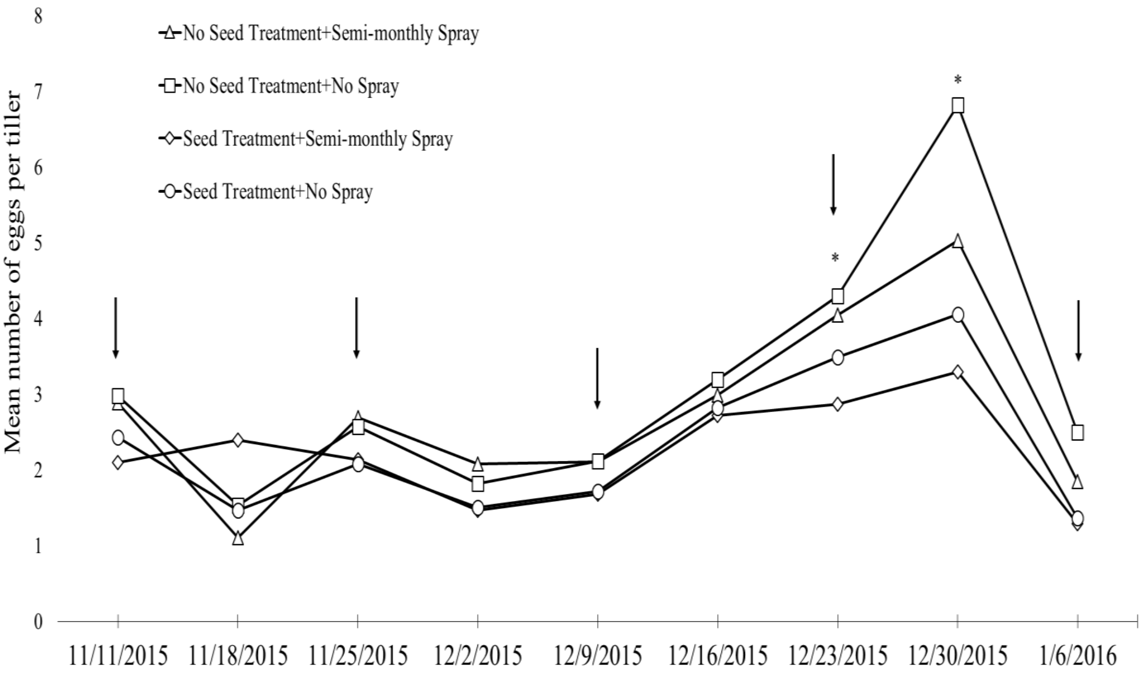

Figure 2.7. Mean number of Hessian fly eggs collected per tiller over the nine week sampling period from 2015 Location 1. Significant differences are denoted by *, and occurred on 23 December and 30 December, where plots without a seed treatment differed from plots with treated seed. Arrows represent the dates that foliar spray applications were made. Means were significantly different at $\alpha<0.05$.

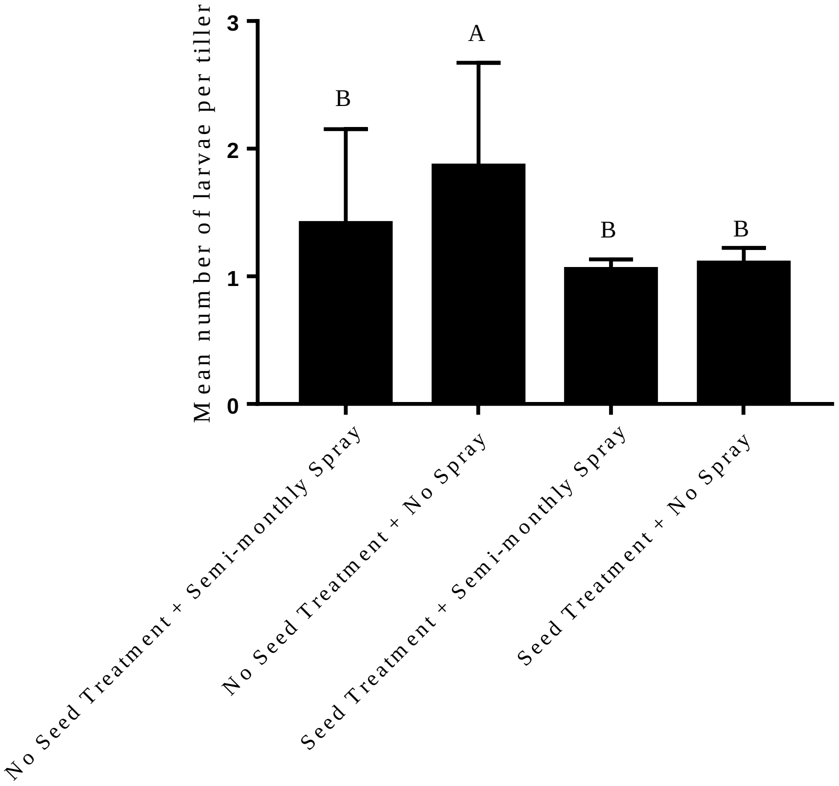

Figure 2.8. Mean number of Hessian fly larvae collected from each treatment at 2015 Location 1. Insecticide treatment affected the number of larvae counted in plots and more were recorded in plots without seed treatment and foliar sprays. Foliar sprays benefited plots without seed treatment. Bars sharing the same letter are not significantly different at α<0.05. Error bars represent standard error.

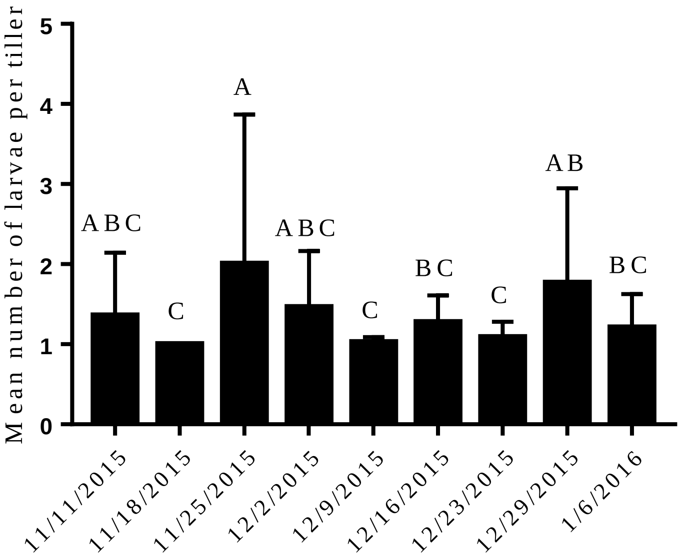

Figure 2.9. Mean number of Hessian fly larvae across treatments during the over the nine week sampling period. More larvae occurred on 25 November and 29 December compared to other dates. Bars sharing the same letter are not significantly different at $\alpha<0.05$. Error bars represent standard error.

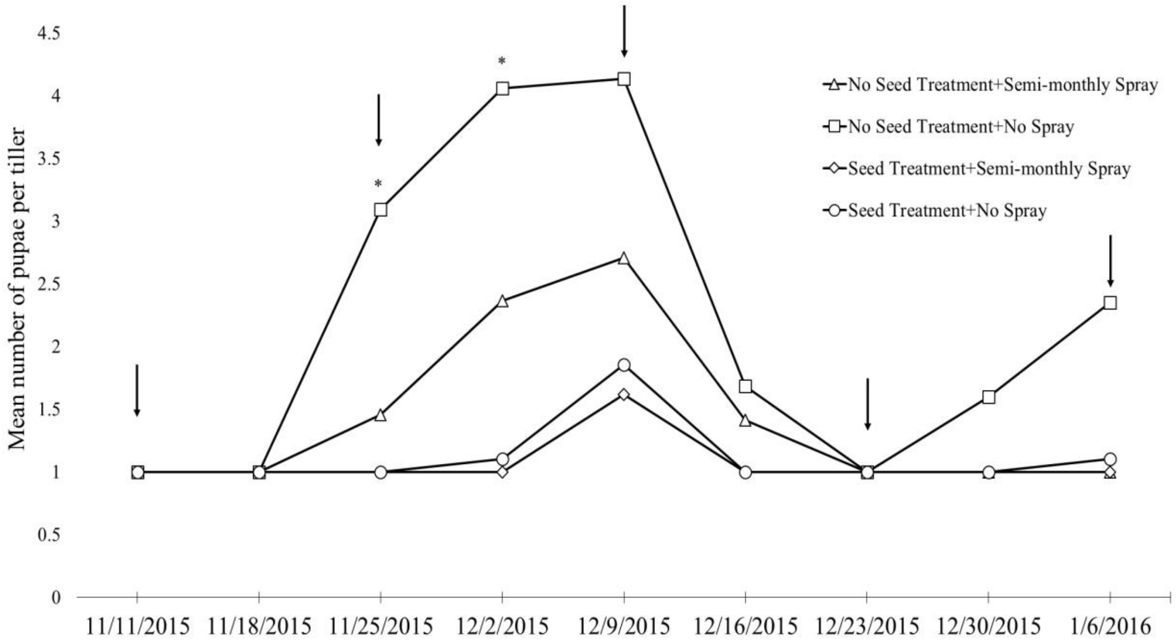

Figure 2.10. Mean number of Hessian fly pupae collected per tiller over the 9 week sampling period from 2015 Location 1. Significant differences are denoted by *, and occurred on 25 November, 2 December, and 9 December, where plots without a seed treatment differed from plots with treated seed. Arrows represent the dates that foliar spray applications were made. Means were significantly different at $\alpha<0.05$.

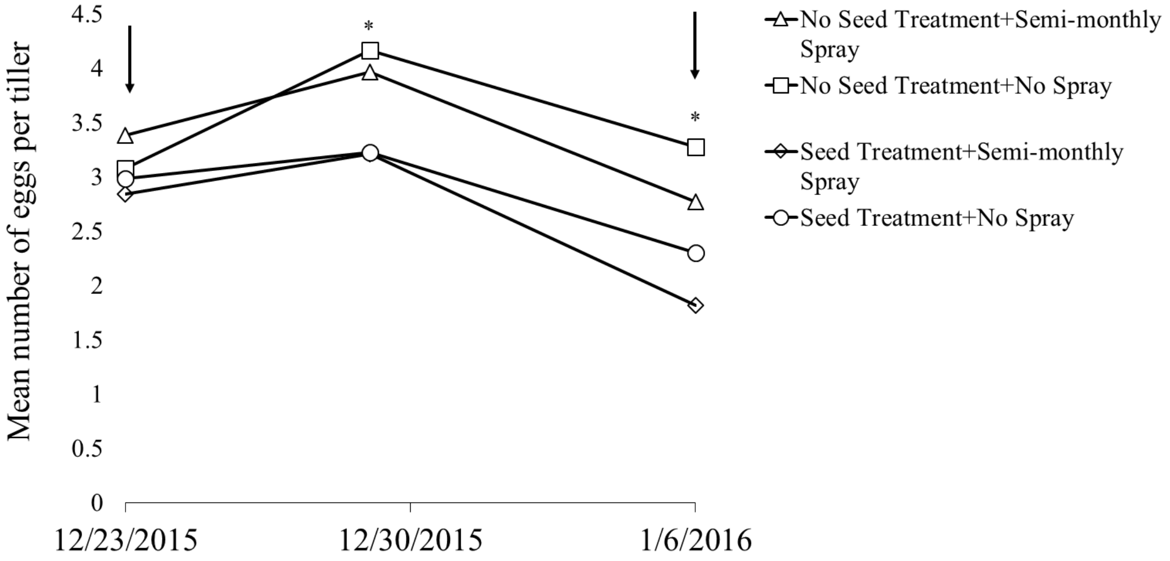

Figure 2.11. Mean number of Hessian fly eggs collected per tiller over the 3 week sampling period from 2015 Location 2. Significant differences are denoted by *, and occurred on 30 December and 6 January, where plots without a seed treatment differed from plots with treated seed. Arrows represent the dates that foliar spray applications were made. Means were significantly different at $\alpha<0.05$.

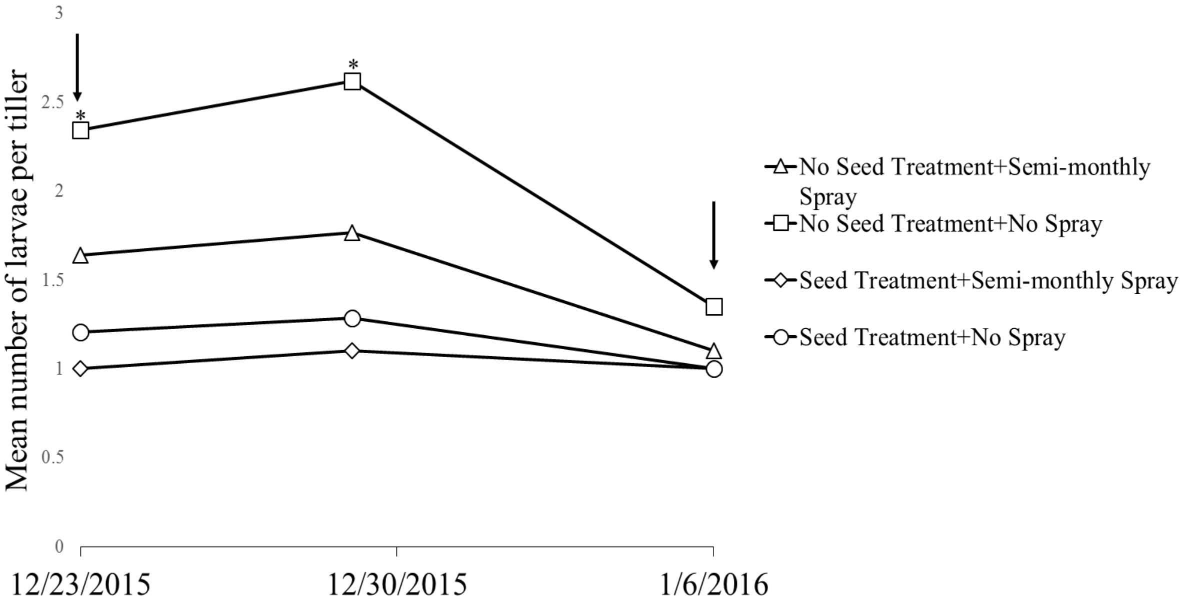

Figure 2.12. Mean number of Hessian fly larvae collected per tiller over the 3 week sampling period from 2015 Location 2. Significant differences are denoted by *, and occurred on 23 December and 30 December, where plots without a seed treatment differed from plots with treated seed. Arrows represent the dates that foliar spray applications were made. Means were significantly different at α<0.05.

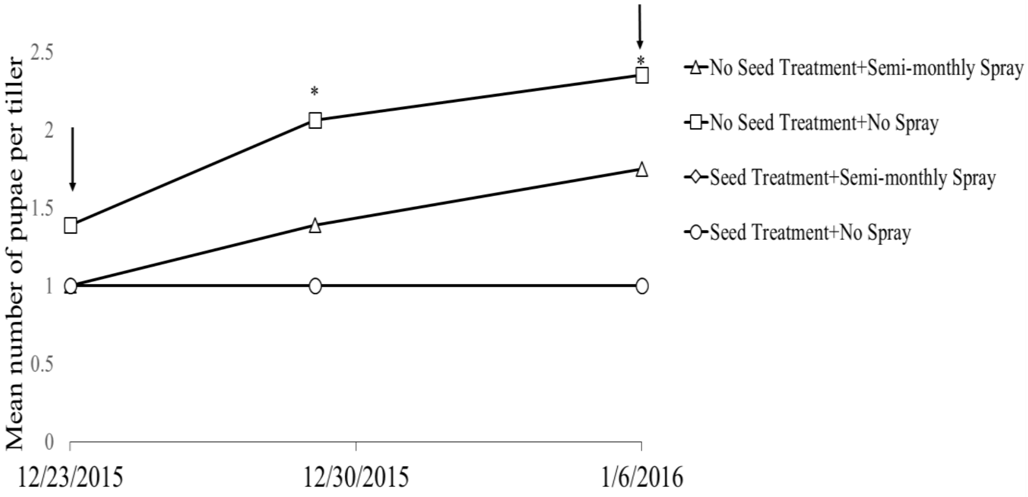

Figure 2.13. Mean number of Hessian fly pupae collected per tiller over the 3 week sampling period from 2015 Location 2. Significant differences are denoted by *, and occurred on 30 December and 6 January, where plots without a seed treatment differed from plots with treated seed. Arrows represent the dates that foliar spray applications were made. Means were significantly different at $\alpha<0.05$.

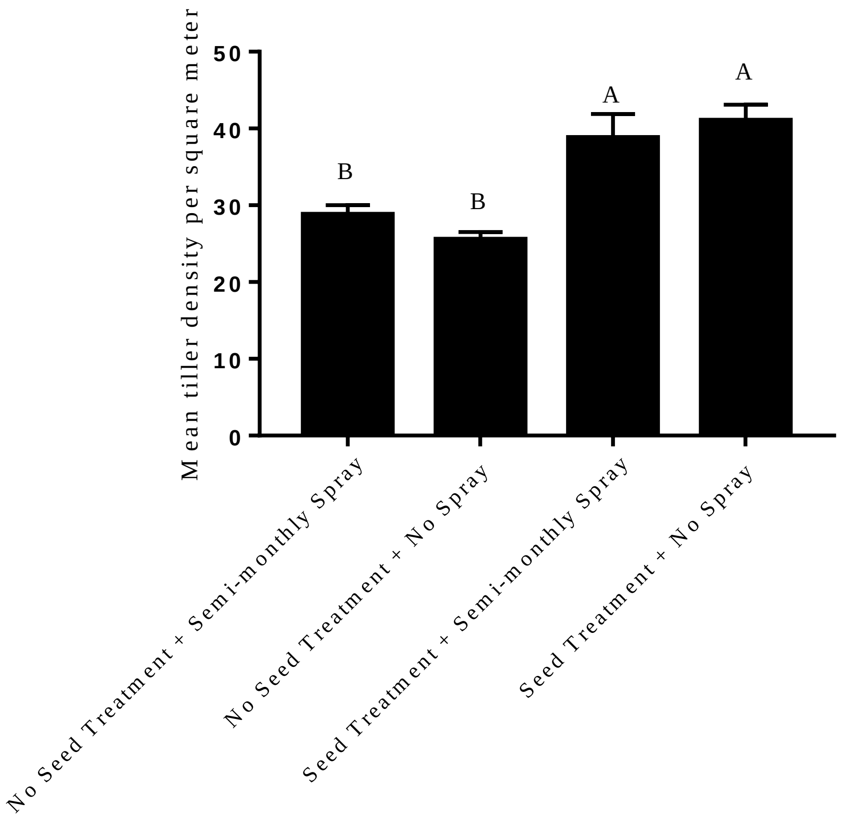

Figure 2.14. Mean tiller density in each treatment at 2014 Location 1. Tiller density was highest in plots with seed treatment, semi-monthly sprays provided no benefit. Bars sharing the same letter are not significantly different at $\alpha<0.05$. Error bars represent standard error.

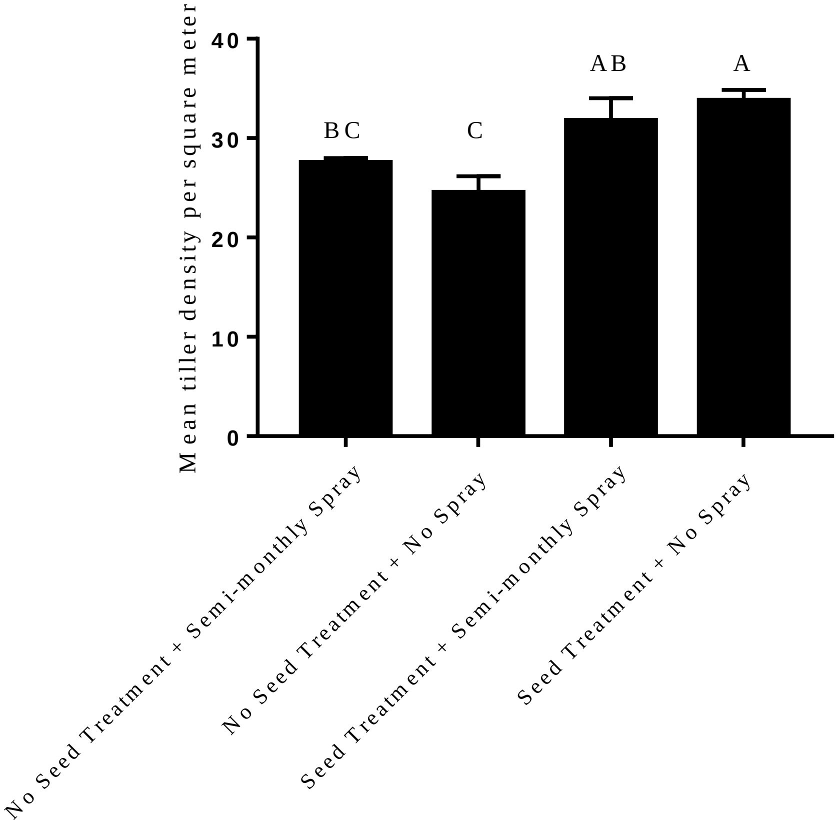

Figure 2.15. Mean tiller density calculated per square meter in each treatment at 2014 Location 2. Tiller density was highest in plots with seed treatment. Bars sharing the same letter are not significantly different at α<0.05. Error bars represent standard error.

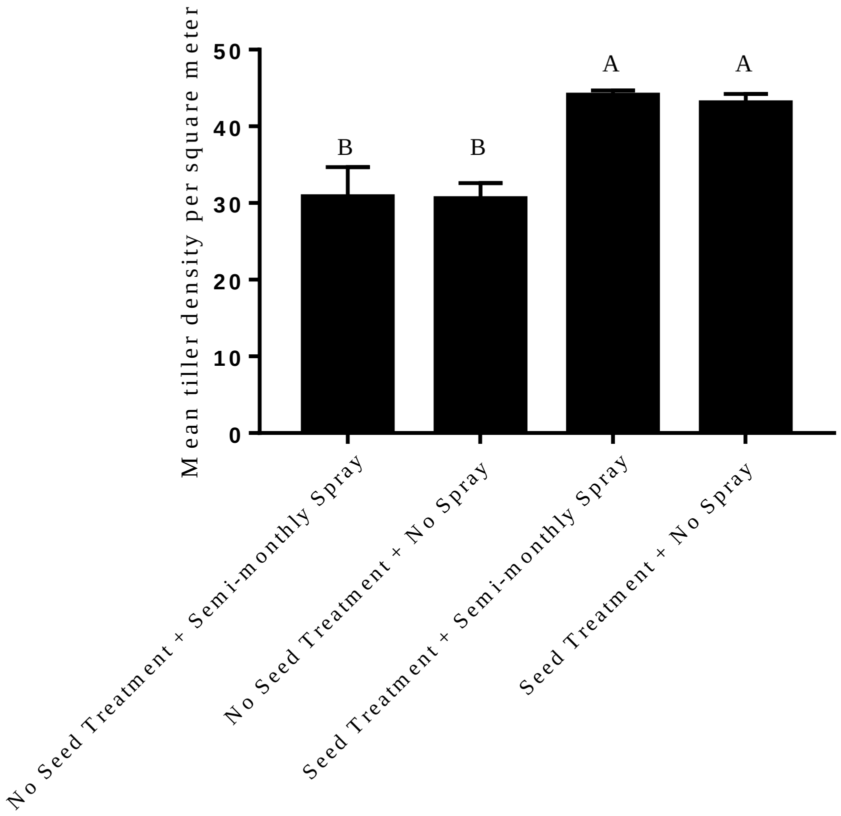

Figure 2.16. Mean tiller density calculate per square meter at 2015 Location 1. Tiller density was highest in plots with seed treatment, semi-monthly sprays provided no benefit. Bars sharing the same letter are not significantly different at $\alpha<0.05$. Error bars represent standard error.

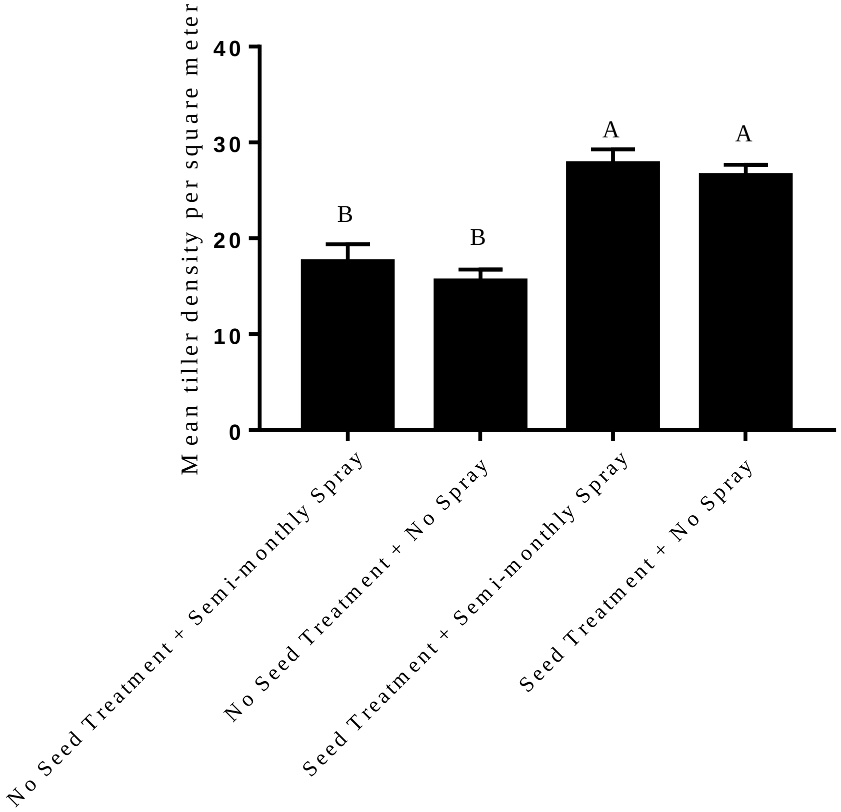

Figure 2.17. Mean tiller density calculated per square meter at 2015 Location 2. Tiller density was highest in plots with seed treatment, semi-monthly sprays provided no benefit. Bars sharing the same letter are not significantly different at α<0.05. Error bars represent standard error.