EVALUATING THE EFFECT OF FOLIAR INSECTICIDES ON SUSPECTED BT-RESISTANT WESTERN CORN ROOTWORMS IN ROTATED SOYBEAN

BY

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ABSTRACT

The western corn rootworm, *Diabrotica virgifera virgifera* LeConte, is the most economically significant insect pest of U.S. corn production and can inflict substantial yield loss. Historically, crop rotation was a successful management strategy because oviposition occurred predominantly in cornfields. However, beginning in 1995, crop rotation failed to adequately manage larval injury to first-year corn in east central Illinois and northwestern Indiana. Adoption of crop rotation had selected for female western corn rootworm beetles that laid eggs in both corn and soybean fields. Commercial Bt corn hybrids targeting this pest have been widely adopted and were an effective tool to manage the rotation-resistant western corn rootworm. Until recently, documentation of field-evolved resistance by western corn rootworm to Bt traits was associated with the cultivation of continuous corn expressing the same Bt toxin. In 2013, severe injury to rotated Bt corn was documented in Illinois, adding urgency to existing concerns about Bt resistance. Unexpected western corn rootworm injury to Bt corn hybrids in rotated cornfields and high beetle densities in corn and soybean have increased grower interest in adult management.

In 2014, an experiment was initiated to determine how applying foliar insecticides to soybean affects patterns of western corn rootworm beetle abundance at trial sites where resistance to Cry3Bb1 was suspected and subsequently confirmed. Treatments were soybean foliar-applied insecticide applications and included: (1) an early application of Warrior II (lambda-cyhalothrin) applied during the tasseling stage of nearby corn, (2) a late application of Warrior II applied when nearby corn silks were brown, and (3) an untreated check. Adult western corn rootworm abundance in soybean was evaluated using unbaited yellow sticky traps and sweep samples.

No significant differences were observed in beetle abundance across all treatments as measured with sticky traps and sweep samples for 2014 and 2015 with the exception of sweep samples from after the late spray. This exception did not correlate with sticky trap data for that time period. These results suggest that a single application of an insecticide in soybean timed to coincide with phenological events in adjacent cornfields does not have an effect on western corn rootworm beetle abundance throughout the growing season in soybean.

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CHAPTER 1: LITERATURE REVIEW

INTRODUCTION

The western corn rootworm, *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae), is a major insect pest of corn, *Zea mays* L., in the United States and was first identified as a pest of corn in 1909 in Colorado (Gillette 1912). Metcalf (1986) estimated that the combined value of control costs and yield losses associated with the western corn rootworm and related species is approximately $1 billion annually in the United States. However, Dun et al. (2010) estimated that this number may be much larger. The western corn rootworm is an incredibly adaptive pest, and has evolved resistance to a variety of pest management tactics, including insecticides (Ball 1968, Call et al. 1977, Meinke et al. 1998), crop rotation (Levine et al. 2002), and genetically engineered corn hybrids (Gassmann et al. 2011, 2014; Wangila et al. 2015).

The western corn rootworm is thought to have originated in Central America and is likely to have evolved alongside corn in Mexico (Chiang 1973, Krysan and Smith 1987, Ciosi et al. 2008). Approximately 3,000 years ago, the western corn rootworm made its way to what is the present-day southwestern United States (Ciosi et al. 2008). Expansion of the western corn rootworm occurred quickly across the United States. In the 1950s, it was restricted to the southwestern Corn Belt, but by the 1980s, it had spread to the U.S. Atlantic coast (Spencer et al. 2005).

Globalization of trade and travel has led to increases in non-native species in many countries (Peck et al. 1998). The western corn rootworm is no longer only a pest in North America; it can now be found internationally. Western corn rootworms were first detected in

Europe in 1992, near the international airport in Belgrade, Serbia (Baca 1993, Berger 2001). Since then, there have been numerous outbreaks in Europe, including Croatia and Hungary in 1995; Romania in 1996; Bosnia and Herzegovina in 1997; Bulgaria, Italy and Venice in 1998; Switzerland in 2000; Ukraine in 2001; Austria, the Czech Republic and France in 2002; and finally in the Netherlands, Belgium, and England in 2003 (Kiss et al. 2005, Ciosi et al. 2008). Western corn rootworm movement throughout Europe is likely due to transportation and packaging of materials throughout the continent (Kiss et al. 2005) as well as long distance flights by dispersing adults (Coats et al. 1986). The beetle has now spread to more than 15 European countries, and the economic costs of managing this pest in Europe are approximately €472 ($532) million annually (Wesseler and Fall 2010).

BIOLOGY

Eggs

The western corn rootworm is a univoltine pest; only one generation is produced each year (Gray et al. 2009). Eggs overwinter in the soil until the following spring, and hatch begins in late May through early June (Shaw et al. 1978, Levine and Oloumi-Sadeghi 1991). Overwintering is not required for egg development, and therefore, some eggs can hatch in the same season as oviposition; however, long-term survival is unlikely due to the onset of cold temperatures and food scarcity (Chiang 1974). The survival rate of eggs is noticeably reduced with severe winter temperatures (Chiang 1974). Egg diapause ensures survival in cold temperatures and synchronization of development with corn (Coats et al. 1986). Gustin (1981) found that hatching success significantly decreased when temperatures fell below –7.5°C. Egg survival can also be affected by the amount of surface residue, which serves as an insulator and provides additional spatial separation between the soil surface and the eggs (Godfrey et al. 1995).

Because snow also acts as a soil insulator, mortality will increase due to lack of snowfall during the winter months (Ellsbury and Lee 2004). Extended diapause (diapause lasting more than one winter) has been documented in populations of northern corn rootworms, (*Diabrotica barberi* Smith and Lawrence) (Chiang 1965), it occurs in 0.14 to 0.21% of western corn rootworm eggs (Levine et al. 1992).

Larvae

The most susceptible life stage of the western corn rootworm is the neonate larva (Pike et al. 1995); Branson (1989) reported that survival rates decrease if larvae are unable to locate a suitable host plant within 24 h. In the laboratory, Oloumi-Sadeghi and Levine (1989) found no significant effects on survival if corn roots were located within 72 h. Strnad et al. (1986) revealed that larvae use carbon dioxide to locate corn roots. Clark et al. (2006) noted that larvae begin to feed immediately once the roots have been located. The larval feeding period extends from the middle of June through the latter part of July and is greatly influenced by climate, soil conditions, and other environmental factors (Bryson et al. 1953). Western corn rootworm larvae were once thought to only survive on the roots of corn; however, larvae can also survive and mature on multiple grass species (Branson and Ortman 1967). More recently, Wilson and Hibbard (2004) studied 22 grass species; larvae were recovered from 21 grass species after 14 days, 18 species after 26 days, and third instars were recovered from 16 species. Western corn rootworm larvae will feed on roots throughout all three instars; the majority of the injury to roots is caused by second and third instars (Levine and Oloumi-Sadeghi 1991, Tuska et al. 2002). The properties of the soil in which larvae hatch can affect their development (Turpin and Peters 1971). If soil is too wet or dry, a decrease in larval movement and increased mortality can be observed (MacDonald and Ellis 1990). Movement of larvae is greatest in silty clay or loam and less in loamy sand (Macdonald and Ellis 1990).

Larvae that hatch in corn initially feed on fine root hairs and later burrow into root tips of seedlings (Devos et al. 2013). As larvae feed and tunnel throughout the root system into younger nodes of adventitious roots, nutrient and water uptake by the plant is disrupted (Kahler et al. 1985, Meinke et al. 2009). Larval root injury can also lead to yield loss, lodging, and increased susceptibility to diseases (Chiang 1973). A yield loss of approximately 15% can occur from the loss of each node of corn roots (Tinsley et al. 2013). Rootworm larvae pupate after completion of three larval instars; adult emergence occurs about 5–10 days after pupation (Fisher 1986).

Adults

The initial emergence of western corn rootworm beetles in Illinois occurs in late June through early July, and adults survive until the first killing frost in the fall (Onstad et al. 1999, Spencer et al. 2009, Steffey and Gray 2009). There is protandry in the western corn rootworm, male beetles emerge approximately 5 days prior to females because larval development occurs faster for males than females (Branson 1987). Western corn rootworm beetles will eat any aboveground part of a corn plant, but most economic injury caused by adults occurs when excessive silk clipping reduces the effectiveness of pollination (Ball 1957). If corn silks extend at least 2.54 cm beyond the tip of the husk, the corn kernels can still be fertilized (Calvin 2003). If pollination has yet to occur, silks that have been clipped can regrow and pollination can occur normally (Capinera et al. 1986). Poor pollination due to silk clipping generally will occur when drought stress is also an issue (Calvin 2003). Extensive feeding on the leaves can also occur but generally will only cause cosmetic issues when the plant is fully developed (Burkhardt 1954). Once the corn silks have matured, adults will move to younger volunteer corn due to the appeal of fresh plant tissue; ear tips are fed upon as silks and tassels dry, often resulting in hollowed kernels (Ball 1957). However, this feeding is not often economically significant, unless there are high densities of adults (Chiang 1976).

Male beetles reach sexual maturity approximately 5–7 days post-emergence, as determined by their response to female sex pheromones (Guss 1976). Conversely, females are sexually mature immediately following emergence (Hammack 1995). Mating generally occurs in July and throughout August, but can sometimes extend through October (Ball 1957). Females often mate within just a few hours of adult emergence (Ball 1957, Lew and Ball 1979). Female beetles usually only mate once, but males may mate with multiple females (Hill 1975, Branson et al. 1977, Kang and Krupke 2009). Adults are responsible for field to field dispersal; females lay eggs as they move within and between fields (Derr et al. 1964). Adults live an average of 52 days in the field (Elliott et al. 1990); however, in the laboratory, adults may live up to 95 days under controlled conditions (Branson and Johnson 1973).

Historically, oviposition (egg-laying) primarily occurred in cornfields starting in late July and continued through the first killing frost (Ball 1957, Gray et al. 1992). Optimal temperatures for oviposition are between 15.3° and 18.6°C (Levine and Oloumi-Sadeghi 1991, Ball 1957). Pierce and Gray (2006) hypothesized that intense selection pressure resulting from decades of crop rotation resulted in the variant western corn rootworm expanding oviposition into other crops such as soybean, *Glycine max* (L.) Merr. Mabry et al. (2004) concluded that oviposition in soybean fields is influenced by dietary stress that occurs when females feed on soybean foliage. Curzi et al. (2012) suggest that higher levels of cathepsin L enable western corn rootworms to overcome soybean defenses and therefore ultimately circumvent crop rotation. Generally, females lay eggs deeper in dryer soil conditions due to the presence of drought cracks (Weiss et al. 1983), with the majority of eggs found in the top 15 cm of soil (Chiang 1973). Weiss et al. (1983) also concluded that the upper 10 cm of soil included 80% of the eggs in irrigated fields and only 45% in the non-irrigated fields. Within the non-irrigated fields, eggs were found at depths up to 35 cm.

MANAGEMENT TACTICS

Chemical Control

Soil-Applied Insecticides

Soil-applied insecticides are used to manage western corn rootworm larval injury and are generally applied 4–8 wk before larvae begin to emerge in the soil (Mayo 1980). Planting date is a crucial consideration, it influences the efficacy of the soil-applied insecticide (Mayo 1980). When selecting a soil insecticide, persistence is an important factor to take into consideration; the ideal insecticide should persist for 6–10 wk in the soil to ensure protection against larvae for the majority of the hatching period (Levine and Oloumi-Sadeghi 1991).

It was not until the late 1940s that control of subterranean insects became feasible with the development of DDT; this was followed by cyclodiene insecticides years later (Harris 1972). However, in the 1970s, the use of DDT and cyclodiene decreased substantially because they were found to be pollutants (Metcalf 2002). Organophosphates react in light, air, and moisture and although they are highly toxic to humans, there is very little accumulation in the soil (Metcalf 2002). Pyrethroids are very effective against pests at low dosages; however, they are also extremely toxic to beneficial insects and fish (Metcalf 2002). Today, the majority of soil insecticides consist of organophosphates, neonicotinoids, and pyrethroids.

Soil-applied insecticides have historically been used to manage larval injury in continuous corn (Meinke et al. 1998) and remain a successful management tactic to prevent injury caused by rotation-resistant western corn rootworm larvae (Gray et al. 2006). However, occasional performance problems are possible due to early planting, poor equipment calibration, high larval densities, dry soil, and poor of incorporation of the insecticide (Gray and Steffey 1998). Gray et al. (1992) found that planting time soil insecticides are intended to prevent excessive root feeding, pruning and lodging of corn plants; they are not intended to manage established populations of western corn rootworms.

Foliar Insecticides

Foliar insecticides are used to prevent injury to aboveground portions of the corn plant caused by adult rootworm beetles. Foliar insecticides have been used to manage adults since the 1940s in the Corn Belt (Ball and Weekman 1962). These insecticides can be applied by aircraft or high-clearance ground sprayers. Levine and Oloumi-Sadeghi (1991) suggested that the level of protection against larval injury with foliar insecticides is no greater than that achieved with soil insecticides. Foliar insecticide applications may also reduce egg-laying if they are timed to coincide with the presence of gravid females; this practice suppresses adult densities and decreases oviposition (Zhu et al. 2005). When successful, adult management can decrease oviposition sufficiently to prevent economic levels of root damage the following season (Zhu et al. 2005). Post-application rainfall can decrease the efficacy of foliar applied insecticides; therefore, timing the applications to coincide with favorable weather patterns is important (Mayo 1984).

Insecticide Resistance

Western corn rootworm resistance has been documented for several classes of insecticides. Ball and Weekman (1962) reported ineffective control of rootworms after use of chlorinated hydrocarbon insecticides beginning in 1959. It was determined that successive years of corn planted following corn and continuous use of chlorinated hydrocarbon insecticides were to blame (Ball and Weekman 1962). Cyclodiene resistance coincided with the eastward expansion of the western corn rootworm; resistance has persisted for years after the use of cyclodiene soil insecticides was discontinued (Metcalf 1986, Siegfried and Mullin 1989). After the development of cyclodiene resistance, management tactics for rootworms changed to the use of organophosphates and carbamates either at planting or the first cultivation (Mayo and Peters, 1978, Ball 1981).

Resistance to organochlorine, organophosphate, carbamate and pyrethroid insecticides has also been confirmed (Meinke et al. 1998, Call et al. 1977, Pereira et al. 2015). Hamilton (1965) found that rootworm beetles had developed resistance to aldrin; however, the rootworms were still susceptible to diazinon. The following year, Hamilton (1966) found that both rootworm larvae and adults were resistant to aldrin. LC_{50} values for organophosphates diazinon and phorate, nearly doubled between 1963 and 1967 (Ball 1968). Reduced susceptibility to carbaryl (a carbamate) was documented in 2004 (Siegfried et al. 2004). Resistance to carbofuran (a carbamate) is worsened by microbes degrading the insecticide (Felsot et al. 1982).

Biological Control

Fungi and nematodes have been studied as potential biological control agents for western corn rootworm management. In 2003, an estimated 58% of corn acreage in Illinois was treated with an insecticide (USDA NASS 2015). This percentage is not further broken down into target pests, but the primary threat would have been the western corn rootworm. Addition of another management tactic based on biological control could help to greatly reduce the usage of chemical insecticides (Jackson 1996). A number of biological control agents have recently been studied for efficacy against corn rootworms including fungi, nematodes, predatory insects, and parasitoids (Mulock and Chandler 2001, Kurtz et al. 2007, Lundgren et al. 2010, Zhang et al. 2004).

Mulock and Chandler (2001) found that applying an entomopathogenic fungus, *Beauveria bassiana* (Balsamo) Vuillemin, could potentially reduce adult populations and oviposition. A properly timed application of the entomopathogenic fungus could result in 75% mortality of beetles and reduce oviposition by approximately 70%. Early applications of *B. bassiana* resulted in greater reduction of eggs than did late applications. However, Hoffman et al. (2014) showed that *B. bassiana* had little to no impact on the mortality rates of western corn rootworm larvae. Pilz et al. (2007) concluded that the fungus *Metarhizium anisopliae* (Metschnikoff) Sorokin caused higher mortality than *B. bassiana* in some instances.

Nematodes are another agent of biological control that has been studied. For nematodes to be successful, both establishment and persistence must occur (Kurtz et al. 2007). *Steinernema carpocapsae* Weiser is a nematode that has been tested and proven effective as a biological control agent for western corn rootworm larvae (Journey and Ostlie 2000). Nematode applications can reduce root damage and adult emergence; however, application timing has a significant effect on efficacy. Later applications targeting second- and third-instar larvae were more effective than earlier applications targeting eggs and first instars. Species of *Heterorhabditis* have also been proven capable of suppressing western corn rootworm populations (Georgis et al. 1991). Root injury and adult emergence can be considerably reduced with *Heterorhabditis* (Jackson 1996, Toepfer et al. 2005). Nematodes of the *Heterorhabditis* and *Steinernema* are unlikely to negatively affect nontarget species (Georgis et al. 1991).

Predatory insects have limited use for biological control of the western corn rootworm. This is due to a larval hemolymph defense, which makes the larvae sticky, toxic, or unpleasant to consume (Lundgren et al. 2010). Stoewen and Ellis (1991) found that many egg predators exist; however, none significantly reduced numbers of western corn rootworm eggs. In contrast, Kirk (1981) determined that larval populations were greatly reduced by an ant, *Lasius neoniger* Emery, when compared to populations without ants. Corn plants with a colony of ants nearby had 74–80% fewer larvae in areas beneath the plants. Beetles in the Carabidae family are known to be predators of other insects (Kirk 1982). The two most successful carabids for rootworm management are active during the same time that rootworms are typically ovipositing in drought cracks (Kirk 1982). However, Best and Beegle (1977) found that five species of carabids preferred to feed on dead insects.

Cultural Control

Agronomic Practices

The United States Environmental Protection Agency (USEPA (2002)) reported that the highest rates of mortality for larvae feeding on Bt transgenic corn roots occur during the first instar. This could be problematic if some neonate larvae are able to feed on grassy weeds when they are most vulnerable to Bt corn. Branson and Ortman (1970) showed that rootworms could complete larval development on 13 of 44 grass species that they evaluated. Therefore, larvae could hatch, begin eating the roots of grassy weeds, move on to corn roots in the second and third instar, and complete growth on a transgenic corn plant (Wilson and Hibbard 2004). Furthermore, Oyediran et al. (2004) reported that adults were produced from 14 grass species other than corn. Three of these species did not yield significantly fewer larvae or adults when compared with corn. The presence of grassy weeds in cornfields presented rootworm management challenges before commercialization of Bt corn. Kirk et al. (1968) found that beetles prefer weedy grass clumps, as opposed to corn stalks, for oviposition sites. Shaw et al. (1978) noted that oviposition in soybean fields was insignificant in fields that were nearly free of weeds and volunteer corn, but could result in damage where weeds and volunteer corn were abundant. However, during the late 1980s, some populations of the western corn rootworm began laying eggs in soybean fields regardless of the presence of volunteer corn or grass weeds (Levine and Oloumi-Sadeghi 1996).

Larval densities and root injury were less than predicted for no-till treatments when compared with other tillage treatments (Gray and Tollefson 1987). The no-till treatment decreases the soil temperature leading to smaller root systems and less root tissue for larvae to feed upon (Gray and Tollefson 1987). Initial beetle emergence is delayed in conservation tillage, however, the rate of emergence increases and is comparable to tillage treatments (Gray and Tollefson 1988a). Tillage does not significantly affect western corn rootworm survival throughout the growing season (Gray and Tollefson 1988b).

Delaying planting time by 10 days decreases the root damage rating by 0.48 to 1.08 (on the Hills and Peters (1971) 1-6 scale) depending on the location (Musick et al. 1980). Branson (1989) found that western corn rootworm larvae survival rates were significantly lower if they were starved one day before being placed on corn roots. In this same study, after 3 days of starvation, only 3.4 out of 74 larvae survived to the adult stage. The effects of delayed planting on the populations of western corn rootworms is probably related to reduced availability of corn roots for early hatching larvae (Bergman and Turpin 1984).

Crop Rotation

Annual crop rotation was first recommended as a western corn rootworm management strategy by Gillette (1912). Rotation to a non-host crop, such as soybean, has historically been a successful management tactic due to the ovipositional affinity of western corn rootworm females for cornfields (Levine et al. 2002). Crop rotation was also successful because larvae are only able to survive on corn roots (and roots of a select number grass species), but not soybean roots or roots of other small grains, which can disrupt the lifecycle of this pest (O'Neal et al. 1999). Crop rotation was used effectively for decades; however, in 1987, severe injury to rotated corn was observed in seed production cornfields near Piper City, Illinois (Levine and Oloumi-Sadeghi 1996). By the mid-1990s, crop rotation had become ineffective as a western corn rootworm management tactic in nine counties across east-central Illinois, and fifteen counties in northwestern Indiana (Levine et al. 2002). The rotation-resistant western corn rootworm has a current range that includes the northern two-thirds of Illinois and Indiana, southern Wisconsin and Michigan, western Ohio, and parts of eastern Iowa and Missouri (Gray et al. 2009). Recent monitoring in eastern Iowa suggests that while rotation resistant WCR are present, their densities are below economic thresholds (Dunbar and Gassmann 2013). Knight et al. (2005) also reported rotation resistance in southeastern parts of Ontario.

Many studies have been conducted to determine the cause of rotation resistance in the western corn rootworm. Levine and Oloumi-Sadeghi (1996) eliminated extended diapause as a possible cause. It is hypothesized that rotation resistance evolved in response to many years of strict annual rotation of corn and soybean (Spencer et al. 1999, Levine et al. 2002). According to

Onstad et al. (2001), 98% of soybean fields in northwest Indiana and east-central Illinois are rotated to corn the following season. Therefore, larvae emerging from eggs that were oviposited in soybean are able to survive the following spring on the roots of rotated corn. Multiple studies have shown that in areas with rotation resistance, beetle densities are greater in soybean fields than in cornfields, especially female populations (O'Neal et al. 1999, Levine et al. 2002, Rondon and Gray 2003). Ultimately, it was a change in the behavior of egg-laying females that allowed western corn rootworms populations to overcome crop rotation leading to evolution of rotation resistance (Levine et al. 2002). Rotation-resistant females are more likely to move outside of cornfields, which leads to oviposition in other crops (Levine and Oloumi-Sadeghi 1996) prompting many farmers to use soil-applied insecticides (and later to plant Bt hybrids) in first-year corn (Spencer et al. 2003).

Oviposition by rotation-resistant females is not limited to soybean fields, but may also occur in alfalfa, *Medicago sativa* L.; wheat, *Triticum aestivum* L.; and oat, *Avena sativa* L (Rondon and Gray 2004). Spencer et al. (1997) found that 71–91% of beetles collected from sweep samples in soybean were female. The percentage of female western corn rootworm beetles collected from yellow sticky traps was greater in soybean than in cornfields, though not many of these females were gravid (O'Neal et al. 1999). Feeding on soybean foliage causes stress in western corn rootworms because it is a poor quality diet and the resulting dietary stress increases the oviposition rate (Mabry et al. 2004). Rondon and Gray (2003) state that producers that rotate to crops such as alfalfa, soybean, or wheat may still be at risk to economic larval injury to corn roots the following year. Initial male and female beetle counts were greater in maize than other crops; shortly after emergence, densities of females began to decrease in maize and increase in the other crop (alfalfa, soybean, and oat stubble). O'Neal et al. (2002, 2004) hypothesized that earlier planted corn might play a role in rotation resistance as well. Late in the growing season, early planted corn may be senescing while nearby soybean is still green, which can influence late season western corn rootworm movement.

The study of western corn rootworm adult dispersal has become more important through the years (Marquardt and Krupke 2009); interest in dispersal spiked when rotation-resistant populations of western corn rootworm were found in Illinois and Indiana in the late 1980s (Levine and Oloumi-Sadeghi 1996). A four-year study conducted from 1996 to 1999 confirmed that western corn rootworm beetles are most abundant in corn for approximately 1 wk (18–25 July), and then densities begin to increase rapidly in nearby soybean fields and often exceed densities in cornfields (O'Neal et al. 1999). This study also concluded that western corn rootworm movement occurred throughout the growing season regardless of whether fresh corn silks were still available, suggesting that movement is not due solely to overcrowding or lack of food (O'Neal et al. 1999).

When rotation-resistant western corn rootworms are present, interfield movement can lead to widely distributed western corn rootworm populations (Spencer et al. 2009). Dispersal by western corn rootworm adults is a primary activity that affects the local population abundance and spreading infestations of this insect pest (Grant and Seevers 1989). Naranjo (1989) found that western corn rootworm beetles had more flight time in a 23 h period compared with northern corn rootworm beetles. Furthermore, young mated females participate in more long-distance dispersal, which can increase the geographic spread of resistance (Coats et al.1986, Isard et al. 2004, Spencer et al. 2009). Naranjo (1991) found that a majority of beetles collected in late planted fields are immigrants from earlier planted fields.

Bt Hybrids

In 2003, the (USEPA) approved the use of genetically modified corn designed to limit losses caused by western corn rootworm larvae. These Bt hybrids produced insecticidal toxins derived from *Bacillus thuringiensis* Berliner (Bt), which is a naturally occurring, rod-shaped, soil-borne bacterium (Al-Deeb and Wilde 2005, Head and Ward 2009). These toxins include Cry3Bb1, Cry34/35Ab1, mCry3A, and eCry3.1Ab (Head and Ward 2009, Frank et al. 2015). Today, Bt corn is the primary method of protection used in continuous corn to prevent western corn rootworm larval injury, and there has been a rapid increase in the use of Bt corn in the past 16 years (Gassmann et al. 2014). In 2014, a record 181.5 million ha were planted with Bt corn globally and 73.1 million in the United States (ISAA 2014).

Bt toxins are harmless to humans, other vertebrates and plants and are also biodegradable (Mendelsohn et al. 2003, NRC 2010, Bravo et al. 2007). During sporulation, this gram-positive bacterium produces a proteinaceous parasporal crystalline inclusion (Gill et al. 1992). When ingested by susceptible insects, this inclusion is solubilized in the midgut and releases proteins known as δ–endotoxins (Gill et al. 1992). The proteins are then activated by the midgut and interact with the epithelium to cause disruption which leads to death of the insect (Gill et al. 1992). With respect to the western corn rootworm, Bt toxins only affect larvae feeding on corn roots; emerging beetles that feed on aboveground tissues have no significant reaction to the toxins (Al-Deeb and Wilde 2005).

Selection of Bt-resistant biotypes is a major concern associated with the repeated annual use of Bt corn. Field-evolved resistance is the decrease in susceptibility of a population due to repeated exposure to the toxin in the field (Tabashnik 1994, Gould 1998, Carrière et al. 2010). Field-evolved resistance to rootworm-Bt toxins has been confirmed in Iowa (Gassmann et al.

2011, Gassmann et al. 2012, Gassmann et al. 2014, Tabashnik et al. 2014), Nebraska (Wangila et al. 2015), and Illinois (Gray 2014, Schrader et al. In Press). Gassmann et al. (2011) found an increase in western corn rootworm survival on corn expressing Cry3Bb1 after as few as three generations. Gassmann et al. (2014) suggested that Bt resistance may be the result of independent evolution within each field or the dispersal of individuals from fields where resistance was already present.

Resistance to Bt corn may also be accelerated if farmers do not comply with refuge requirements. The refuge (an area of non-Bt corn plants) is used to produce individuals that have not been exposed to the Bt toxin during larval development and will thus increase the number of susceptible beetles emerging from a field (Tabashnik et al. 2003). In theory, these susceptible individuals will mate with any rare resistant individuals emerging from Bt corn plants. Refuge requirements have changed over the years. Initially, a separate 20% in-field refuge was required for corn rootworm Bt corn hybrids (Difonzo 2016). As new rootworm Bt traits entered the market and were combined into multi-trait (multiple different modes of action against corn rootworm) ‘pyramided’ hybrids, the refuge percentage has been cut to as low as 5% (DiFonzo 2016). To assure compliance with refuge requirements, refuge seed is commonly premixed in the bag with Bt seed in what is referred to as a ‘refuge in a bag’ (RIB) format. Proper use of refuge seed is an important tactic that growers are required to follow; however, refuge compliance may suffer when guidelines are confusing (Andow et al. 2010). Tabashnik and Gould (2012) recommend a 50% refuge when using a Bt hybrid with only one toxin active against western corn rootworm larvae and a 20% refuge for Bt hybrids that have two toxins.

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CHAPTER 2: EVALUATING THE EFFECT OF FOLIAR INSECTICIDES ON SUSPECTED BT-RESISTANT WESTERN CORN ROOTWORMS IN ROTATED SOYBEAN

INTRODUCTION

Diabrotica virgifera virgifera LeConte, the western corn rootworm, has become one of the most economically important insect pests of corn, *Zea mays* (L.), in the United States (Gray et al. 2009). Corn is the most widely planted field crop in the United States, with 35.98 million ha planted in 2015 (USDA 2015). Metcalf (1986) estimated that between management expenditures and yield losses, corn rootworms, *Diabrotica* spp., cost US corn producers over $1 billion annually.

The larval stage of the western corn rootworm is the most damaging stage to corn, causing physiological yield loss, harvest loss, and reduced nutrient and water uptake by feeding on corn roots (Godfrey et al. 1995). Lodging may occur if root injury is severe, making corn plants difficult to harvest and causing yield loss from 11 to 34% (Spike and Tollefson 1991). After progressing through three instars during the late spring and early summer, larvae pupate in the soil and adults emerge in late June through early July; adults survive until the first frost in the fall (Onstad et al. 1999). Western corn rootworm adults will feed on any of the aboveground plant parts, the most critical being silk tissue during pollination (Burkhardt 1954). Adults start laying eggs in the soil beginning in late July, and oviposition occurs through the first frost (Ball 1957).

Historically, a widely adopted tactic for managing western corn rootworm larval injury was annual rotation to a non-host crop such as soybean, *Glycine max* (L.) Merr. Crop rotation was successful because corn rootworm larvae survive only on corn and other closely related grass species (Oyediran et al. 2004). However, damage was reported to first-year corn in areas of east-central Illinois in the late 1980s, which was subsequently attributed to oviposition in crops other than corn. It is hypothesized that strict adherence to annual rotation of corn and soybean imposed strong selection against females that laid eggs only in cornfields (Spencer et al. 1999, Levine et al. 2002, Pierce and Gray 2006). Across much of east-central Illinois, eggs deposited in cornfields are destined to hatch in fields that have been rotated to soybean, a crop that is not a host for western corn rootworm larvae. Meanwhile, eggs deposited in soybean fields (or in fields of other rotated crops) during the previous year will hatch in cornfields where the emerging larvae can survive. Thus, where annual crop rotation is widely practiced, females that leave cornfields to lay some of their eggs in other crop fields will have more surviving offspring than females with strict ovipositional fidelity to cornfields. Spencer et al. (1997) reported adult movement to soybean fields prior to depletion of food sources in corn. According to Mabry et al. (2004), oviposition will not only occur in cornfields but also in soybean fields, where dietary stress experienced while feeding on soybean foliage can stimulate oviposition.

The threat posed by the rotation-resistant western corn rootworm prompted approximately 33 to 39% of Illinois growers to use soil insecticides in order to protect first-year corn (Rice 2004). In 2003 the United States Environmental Protection Agency (USEPA) approved the use of genetically modified corn that expressed the Cry3Bb1 toxin to control western corn rootworm. The insecticidal Cry3Bb1 protein is derived from the naturally occurring, soil-borne, and rod-shaped bacterium, *Bacillus thuringiensis* Berliner (Bt) (Ostlie et al. 1997, Al-Deeb and Wilde 2005, Head and Ward 2009). Reduction of insecticide usage due to increased planting of Bt corn hybrids has been documented (Rice 2004). To delay the development of western corn rootworm resistance to Bt toxins, the USEPA initially made a requirement that 20% of a cornfield be planted as a refuge (non-Bt) (USEPA 2001). Since 2003, three additional Bt toxins, Cry34/34Ab1, mCry3A, and eCry3.1Ab, with activity against corn rootworms have been engineered into commercial corn hybrids (Head and Ward 2009, Walters et al. 2010). The required refuge percentage in Bt cornfields has dropped to as low as 5% for hybrids expressing multiple rootworm-active Bt toxins (i.e. pyramided hybrids) (DiFonzo 2016).

Gassmann et al. (2011) reported the first instance of field-evolved resistance to a Bt toxin by the western corn rootworm. This resistance to the Cry3Bb1 toxin was likely caused by insufficient use of refuge seed and corn-on-corn planting with repeated use of Bt hybrids expressing the same toxin (Gassmann et al. 2011). In 2013 Bt performance problems were observed in east-central Illinois (Gray 2013). Rotation resistance is also prevalent in this area of Illinois (Gray et al. 2009). Co-occurrence of rotation resistance and Bt resistance in the same population presents significant challenges to growers who must manage western corn rootworms that are resistant to both management tactics. Is it possible to control adult populations in soybean thereby reducing oviposition and subsequent root injury to corn the following season? The objective of this on-farm research was to determine how the timing of a foliar insecticide application to soybean affects the abundance of rotation-resistant adult western corn rootworm adult abundance.

MATERIALS AND METHODS

Three on-farm locations were used for this study and were located in Ford (Site 1) and Livingston (Sites 2 and 3) Counties in east-central Illinois. Trial sites were selected based on severe root injury and lodging in 2013 to first-year corn planted with Genuity VT Triple Pro Bt-corn hybrids that express the Cry3Bb1 protein. Based on the observed 2013 injury, western corn rootworm populations at these locations were suspected to be resistant to the Cry3Bb1 toxin prior to beginning this experiment. Subsequent plant-based bioassays following the methods of Gassmann et al. (2011) confirmed resistance to Cry3Bb1 along with cross resistance to mCry3A in the offspring of local western corn rootworm adults collected from ear tips and the corn canopy of these fields in August of 2013 (J.L. Spencer, unpublished data).

This experiment was conducted using a randomized complete block design with three replications. Each of the three locations used for the experiment served as a single replication with three plots. The soybean field trial area measured 165 m by 152 m and was divided into thirds to accommodate three soybean foliar insecticide treatments. Plots were separated with an 18 m wide buffer zone to prevent insecticide drift across plots. The 2014 growing season was the first year of the experiment; a soybean trial was established at each location on 3 July. The 2015 soybean field trials were established on 1 July.

During 2015, corn trials were planted using a variety of Bt hybrids with and without soil insecticide where foliar applications to soybean were applied the year before. The Bt hybrid and soil insecticide combinations included, Genuity SmartStax RIB Complete (Cry3Bb1 and Cry34/35Ab1 rootworm toxins, 5% seed blend), Genuity SmartStax RIB Complete + Force 3G (4 oz./1000 ft.), Genuity VT Triple Pro RIB Complete (Cry3Bb1 rootworm toxin, 10% seed blend), Genuity VT Triple Pro RIB Complete + Force 3G (4 oz./1000 ft., and an untreated check. Root injury evaluations were conducted using the 0–3 node injury scale (Oleson et al. 2005). Root injury data will be used to evaluate the effectiveness of the soybean foliar insecticide on corn hybrid/soil insecticide treatments.

Treatments

Three soybean foliar insecticide treatments were applied to measure their effects on subsequent adult western corn rootworm abundance in soybean. Treatments were randomly assigned and applied to three adjacent 55 m wide by 152 m long soybean plots. At each location, there was an early treated plot, a late treated plot, and an untreated (check) control plot. Treated soybean plots received a broadcast application of Warrior II (lambda-cyhalothrin, Syngenta Crop Protection LLC, Greensboro, NC), a foliar insecticide applied at a rate of 29 g a.i/ha. The phenology of nearby corn plants was used to determine the timing of the insecticide application. The early treatment was applied to soybean when the adjacent cornfield was at the VT stage (tasseling, Abendroth et al. 2011). The late treatment was applied when the adjacent cornfield reached R3 (brown silk, Abendroth et al. 2011) and was no longer appealing to the western corn rootworm as a viable food source.

During the 2014 season, the early insecticide applications were applied on 7 July at Site 1 and on 24 July at Sites 2 and 3. The late insecticide applications were applied on 14 August for Site 1 and on 8 September for Sites 2 and 3, respectively. For the 2015 season, the early application was applied on 22 July and the late application was on 13 August at all three locations. Detailed agronomic information for all sites can be found in the appendix (Table 5–Table 7).

Data Collection

Western corn rootworm adult abundance data were collected once per week in the soybean fields. The abundance of western corn rootworm beetles in soybean was evaluated using unbaited yellow sticky traps (Pherocon AM, Great Lakes IPM, Inc., Vestaburg, MI) as well as sweep net samples. Twelve sticky traps were used per soybean plot. The 2014 sticky traps were deployed on 3 July and the 2015 sticky traps were deployed on 1 July for all three sites. The traps were changed weekly throughout adult emergence during the growing season until the soybean plants had senesced.

Three 100-sweep samples were taken from each plot once per week using a 38 cm sweep net (Catalog No. IPM-315MS, Great Lakes IPM, Inc.). Sweep sampling also continued until the onset of soybean senescence. The number of beetles/trap/day and beetles/100 sweeps were used to measure the effect of soybean foliar insecticide applications on adult abundance populations at three different time intervals. The intervals were before the VT spray, between the VT and R3 spray, and after the R3 spray.

Statistical Analysis

Sweep sample, sticky trap data, and root injury were analyzed using SAS 9.3 (SAS Institute, Inc., Cary, NC). Data were transformed using a square root transformation to stabilize the variances and to meet all assumptions of analysis of variance. Statistical tests for the fixed effect of foliar insecticide application were performed using the mixed model procedure (PROC MIXED). Fixed effects were declared significant at $P \leq 0.05$. Means were compared using the least square means (LSMEANS) option of PROC MIXED with a Tukey adjustment for multiple pairwise comparisons (Tukey 1949). Means were declared significantly different at $P \leq 0.05$. Response variables included beetles/trap/day for sticky traps and beetles/100 sweeps for sweep sampling. Each variable was analyzed independently for three discrete time periods; pre-VT spray, between the VT and R3 spray, and post-R3 spray.

RESULTS

Sweep Sample Data

In 2014, there was no significant effect of treatment on the mean number of beetles per 100 sweeps for any sampling period (Table 1). Prior to the early spray treatment, mean densities were low and never exceeded 0.45 per 100 sweeps across all treatments (Table 2, Figure 1). Between the early (VT) and the late (R3) insecticide application, mean densities never exceeded 29.87 per 100 sweeps across all treatments (Table 2, Figure 1). After the late spray, mean beetle densities never exceeded 4.47 beetles per 100 sweeps (Table 2, Figure 1).

In 2015, a similar trend was observed. There was no significant effect of treatment on the mean number of beetles per 100 sweeps for any sampling period (Table 1). Before the early treatment, no beetles were recovered in any of the sticky traps deployed (Table 2, Figure 2). For the period between the late and early spray, mean densities never exceeded 0.19 beetles per trap per day (Table 2, Figure 2). After the late spray, the mean number of beetles never surpassed 0.48 beetles per 100 sweeps (Table 2, Figure 2).

Sticky Trap Data

In 2014, there was a significant effect of treatment on the mean number of beetles per trap per day during the post-late spray period, but not during the two preceding periods (Table 1). Prior to the early treatment, mean densities never exceeded 0.12 beetles per trap per day (Table 2, Figure 3). During the period between the early and late spray applications, mean densities never exceeded 2.9 beetles per trap per day (Table 2, Figure 3). During the period after the late spray, the late spray treatment had a significantly different mean number of beetles per trap per day than the early spray or untreated control treatments, which did not differ significantly (Table 2, Figure 3).

No significant effect of treatment on the average number of beetles per trap per day was observed for any sampling period during 2015 (Table 1). Prior to the early treatment, mean beetle densities never exceeded 0.01 beetles per trap per day (Table 2, Figure 4). Between the early and the late spray, mean densities were lower than 0.06 beetles per trap per day (Table 2, Figure 4). Finally, after the late treatment, the mean beetle densities did not exceed 0.04 beetles per trap per day (Table 2, Figure 4).

Root Injury

Excessive rainfall from 1 May through 30 June (Table 3, Figure 5) resulted in extremely low western corn rootworm larval (and adult) populations. There was very little root injury. There were no significant effects of foliar insecticide treatment on larval injury to Bt hybrids. The node injury scores for 2015 are reported in Table 4.

DISCUSSION

Very low beetle densities were observed prior to the early application of foliar insecticide. Between the early and late sprays, the untreated check and the late treatment were expected to have similar densities and the early treatment to have fewer beetles. Spraying early had no effect on the western corn rootworm beetle population buildup in soybean. After the late spray, it was expected that population density in the late treatment would be lower than that in the early treatment and the untreated check. However, no significant differences were observed. Soybean foliar insecticide treatments did not significantly reduce adult western corn rootworm abundance in soybean. These results suggest that attempting to control western corn rootworm populations in soybean may have little effect on adult abundance and therefore, very little effect on the abundance of egg-laying females. To have any impact on adult abundance in soybean, multiple spray applications may be necessary throughout the growing season to counteract the continuous movement of beetles into fields. This lack of significant differences in beetle density in the early and late spray treatments is consistent with evidence for significant interfield movement by rotation-resistant western corn rootworm (Isard et al. 2000). The daily movement of western corn rootworm adults leads to the eventual recolonization of insecticide-treated soybean fields after the residual effects of the insecticide diminish. This evidence of the western corn rootworm capacity to rapidly recolonize treated soybean fields underscores the importance of considering the role of movement in management of resistant rootworms. Interfield movement in this area is also facilitating the spread of Bt resistance on a local scale.

Due to excessive rainfall in May and June of 2015, beetle densities remained very low throughout the 2015 growing season. All three locations received an excess of rainfall ranging from 16.8 to 22.4 cm during May and June when compared with the historical average rainfall (1981-2010) for the area (Table 3, Figure 5). Western corn rootworm larvae typically emerge from their eggs during late May to early June (Bryson et al. 1953), the sustained period of heavy rainfall likely resulted in saturated soil conditions coinciding with egg hatch. Such conditions reduce successful larval establishment on corn roots and increase mortality (Sutter et al. 1989). Saturated soil during larval emergence is believed to be a cause of the extremely low beetle densities observed during the 2015 growing season. Low western corn rootworm densities during 2015 resulted in collection of little useful data.

Another planned aspect of this experiment was evaluation of node injury scores for first-year corn planted where foliar insecticide treatments were applied the previous year. In 2015, a selection of various Bt hybrids were planted both with and without soil-applied insecticide in the location of the soybean plots from 2014. Use of sticky traps in the soybean plots allowed adult beetles density data to be compared to established economic thresholds for western corn rootworm in soybean. Previously, an economic threshold for western corn rootworm beetle density in soybean was set at 4.7 beetles/trap/day (O'Neal et al. 2001). In 2013, Dunbar and Gassmann (2013) revised the threshold downward to 1.5 beetles/trap/day. Based on these thresholds, the relatively high beetle densities on the soybean sticky traps in the 2014 (Figure 3) suggested that there was a potential for significant root injury in the first-year corn planted in those fields during 2015. In fact, during the period after the early spray and before the late spray in 2014, all plots exceeded the economic threshold set by Dunbar and Gassmann (2013). Unfortunately, due to the extensive rainfall, little to no damage was seen across all treatments. If abundant rainfall had not inundated the study sites in 2015, higher node injury scores would have been anticipated.

It is expected that western corn rootworm populations will remain low for the 2016 growing season. The extremely low number of adults in 2015 makes it likely that very few eggs were available to overwinter into the 2016 season in our monitored fields. However, given that rainfall was not uniformly excessive everywhere (Figure 5), there may be a risk of economic injury in rotated cornfields in portions of Illinois. Going forward, the best advice for growers facing Bt- and rotation-resistant western corn rootworms is to adopt an IPM-based approach to pest management that includes routine scouting of fields and rotation of management technologies. Based upon the results observed, it is concluded that a single application of insecticide in soybean fields timed to coincide with a particular growth stage of nearby corn offers little value for managing western corn rootworm beetle populations in soybean.

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TABLES

Table 1. Statistical tests[1] of the fixed effect of treatment for sticky traps and sweep samples (2014–2015)

Response variable	Year	Period[2]	*F* value	*P* value
Beetles/trap/day	2014	A	1.36	0.35
		B	4.09	0.11
		C	7.94	0.04
	2015	A	3.75	0.12
		B	1.16	0.40
		C	0.64	0.57
Beetles/100 sweeps	2014	A	0.20	0.53
		B	0.58	0.60
		C	5.43	0.07
	2015	A	0.00	1.00
		B	0.36	0.72
		C	3.27	0.14

[1] Statistical tests were performed using PROC MIXED of SAS 9.3; means within a column for a given time period are significant if $P \leq 0.05$. Square-root transformed data were analyzed. [2] Period A refers to the time period before the early (VT) spray. Period B refers to the time period between the early (VT) spray and the late (R3) spray. Period C refers to the time period after the late (R3) spray.

Table 2. Summary of western corn rootworm adult abundance in soybean for the effect of treatment (2014–2015)

			Sampling method	
Year	Period[1]	Treatment[2]	Sticky traps[3] (mean no. beetles/trap/day)	Sweep sampling[4] (mean no. beetles/100 sweeps)
2014	A	Early	0.10 a	0.37 a
		Late	0.11 a	0.39 a
		Check	0.09 a	0.44 a
	B	Early	2.67 a	29.03 a
		Late	2.46 a	17.06 a
		Check	2.88 a	29.08 a
	C	Early	1.10 a	4.83 a
		Late	0.64 b	0.72 a
		Check	1.23 a	4.71 a
2015	A	Early	0.09 a	0.00 a
		Late	0.09 a	0.00 a
		Check	0.12 a	0.00 a
	B	Early	2.53 a	0.07 a
		Late	2.84 a	0.15 a
		Check	2.64 a	0.19 a
	C	Early	0.85 a	0.25 a
		Late	1.19 a	0.00 a
		Check	0.92 a	0.47 a

Mean comparisons were performed using PROC MIXED of SAS 9.3; means within a column for a given time period that share a similar letter do not differ significantly ($P \leq 0.05$). Square root transformed data were analyzed; actual means are reported. [1]Period A refers to the time period before the early (VT) spray. Period B refers to the time period between the early (VT) spray and the late (R3) spray. Period C refers to the time period after the late (R3) spray. [2]Insecticide applications to soybean were timed to coincide with the phenology of nearby corn (early = VT or tasseling, late = R3 or brown silk); the insecticide used was Warrior II (lambda-cyhalothrin) applied at a rate of 116.9 ml/ha. [3]Twelve Pherocon AM sticky traps (Great Lakes IPM, Vestaburg, MI) were deployed per plot and changed at seven-day intervals. [4]Three 100-sweep samples were performed per each plot on each sampling date.

Table 3. 2015 corn planting and May-June precipitation (radar estimated) for cooperators. Normal May + June rainfall is 21.1 cm

Location	Planting date	Total rain	Rainfall excess
Site 1	2 May	43.4 cm	+22.4 cm
Site 2	1 May	41.1 cm	+20.1 cm
Site 3	14 May	37.8 cm	+16.8 cm

Source: FieldView, The Climate Corporation San Francisco, CA.

Table 4. Summary of 2015 node-injury scores (NIS) for rotated corn planted in the 2014 soybean foliar insecticide application plots

Treatment[1]	Range of NIS (0–3)[2, 3]
Early (VT)	0.00 – 0.02 a
Late (R3)	0.00 – 0.02 a
Unsprayed	0.00 – 0.04 a

Mean comparisons were performed using PROC MIXED of SAS 9.3; means that share a similar letter do not differ significantly ($P \leq 0.05$). Square root transformed data were analyzed; actual means are reported. [1]Treatment refers to the timing of insecticide applications to soybean which were timed to coincide with the phenology of nearby corn (Early = VT or tasseling, late = R3 or brown silk); the insecticide used was Warrior II (lambda-cyhalothrin) applied at a rate of 116.9 ml/ha. [2]Five corn hybrids were evaluated: Genuity SmartStax RIB Complete (Cry3Bb1 and Cry34/35Ab1 rootworm toxins, 5% seed blend), Genuity SmartStax RIB Complete + Force 3G (4 oz./1000 ft.), Genuity VT Triple Pro RIB Complete (Cry3Bb1 rootworm toxin, 10% seed blend), Genuity VT Triple Pro RIB Complete + Force 3G (4 oz./1000 ft), and an untreated check. [3]Mean Node Injury Scores (NIS) for each corn hybrid based on evaluation of n=75 roots per treatment; total roots =1125.

FIGURES

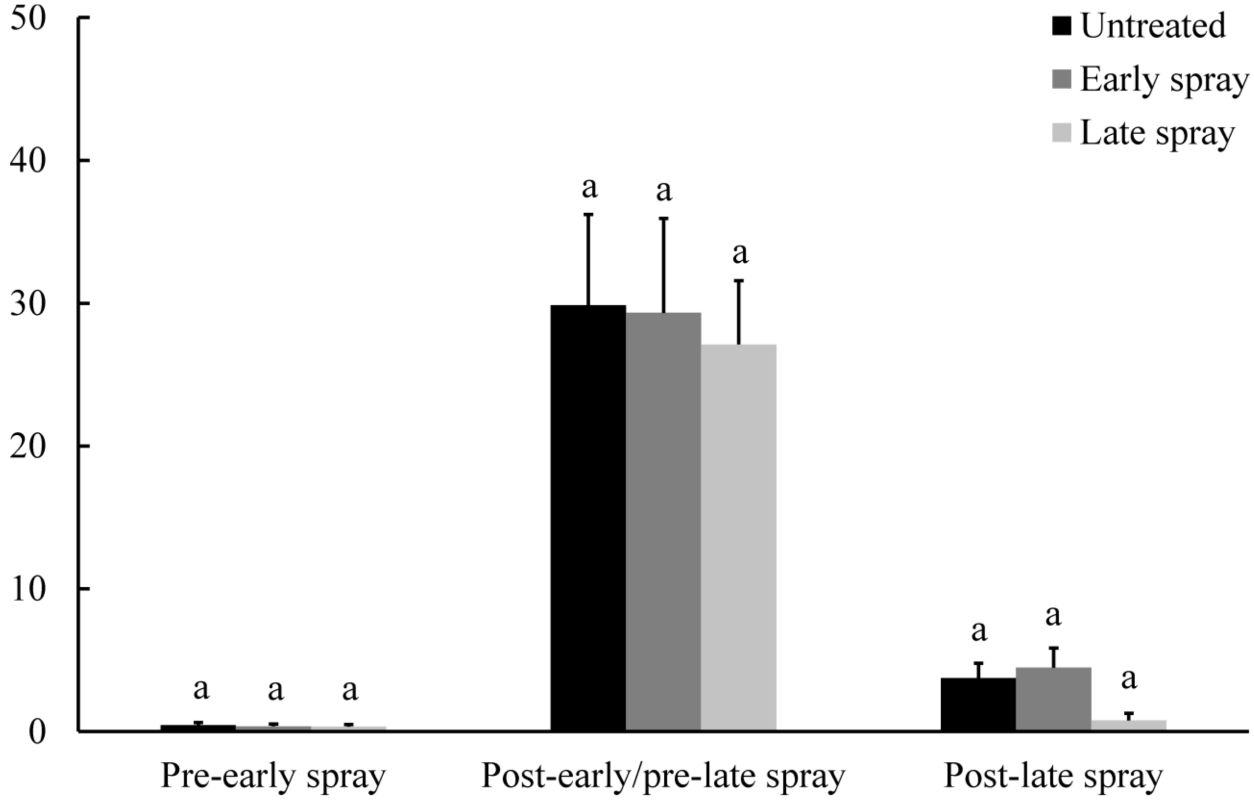

Figure 1. Mean number of beetles per 100 sweeps for 2014. Means sharing a similar letter during a given period are not significantly different ($P < 0.05$). Square-root transformed data were analyzed; actual means and standard errors are reported.

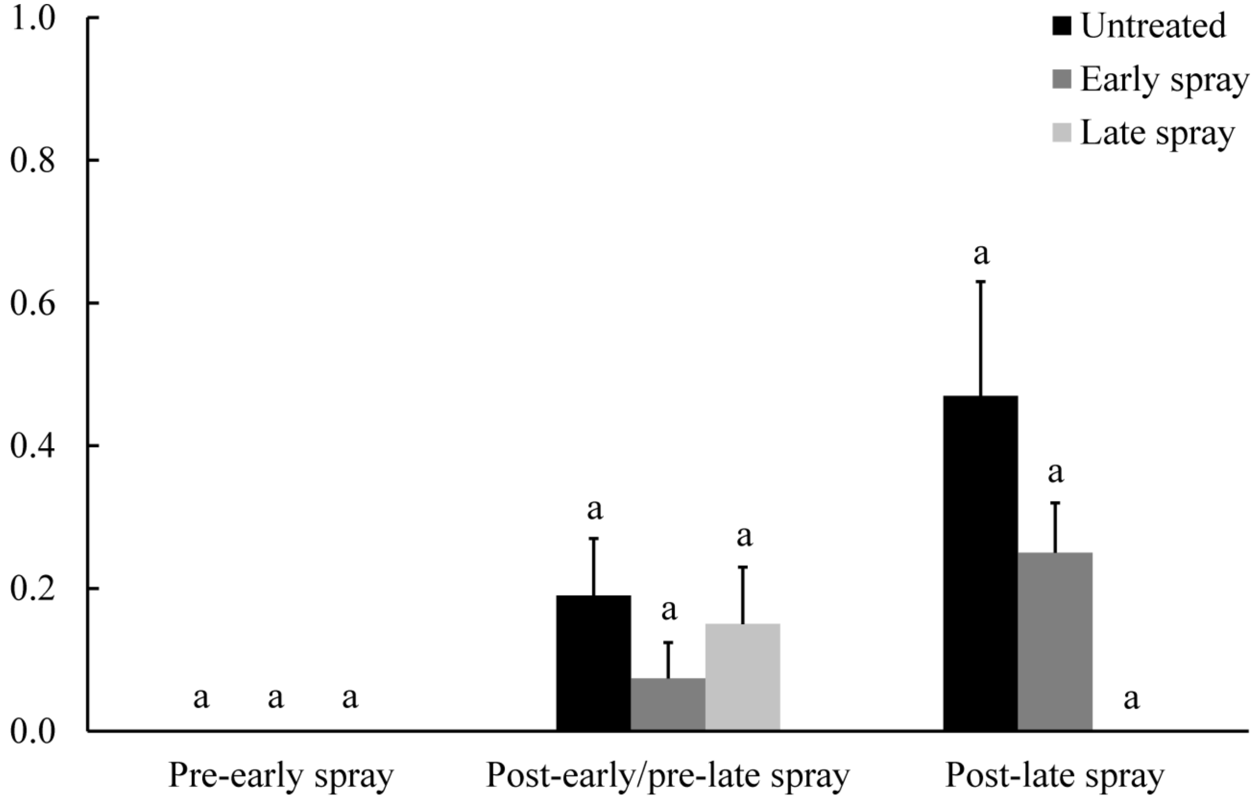

Figure 2. Mean number of beetles per 100 sweeps for 2015. Means sharing a similar letter during a given period are not significantly different ($P < 0.05$). Square-root transformed data were analyzed; actual means and standard errors are reported.

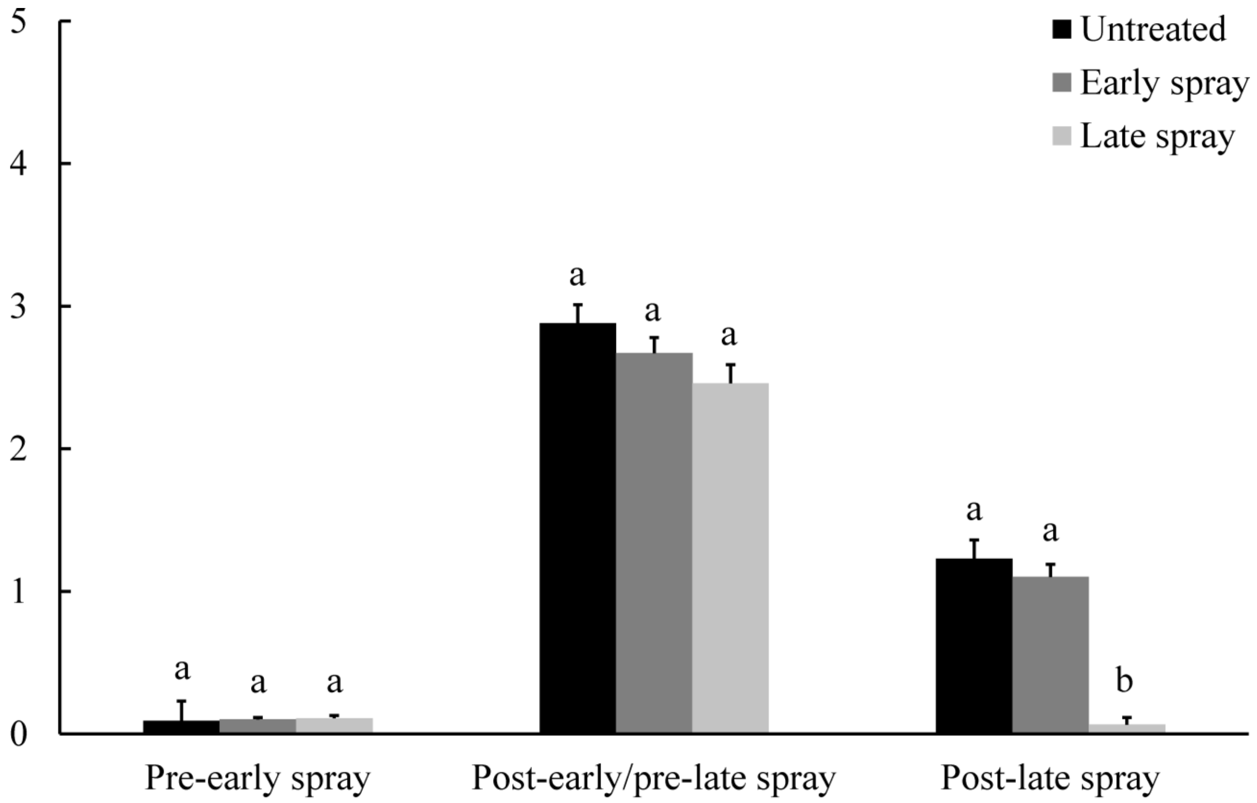

Figure 3. Mean number of beetles per trap per day for 2014. Means sharing a similar letter during a given period are not significantly different ($P < 0.05$). Square-root transformed data were analyzed; actual means and standard errors are reported.

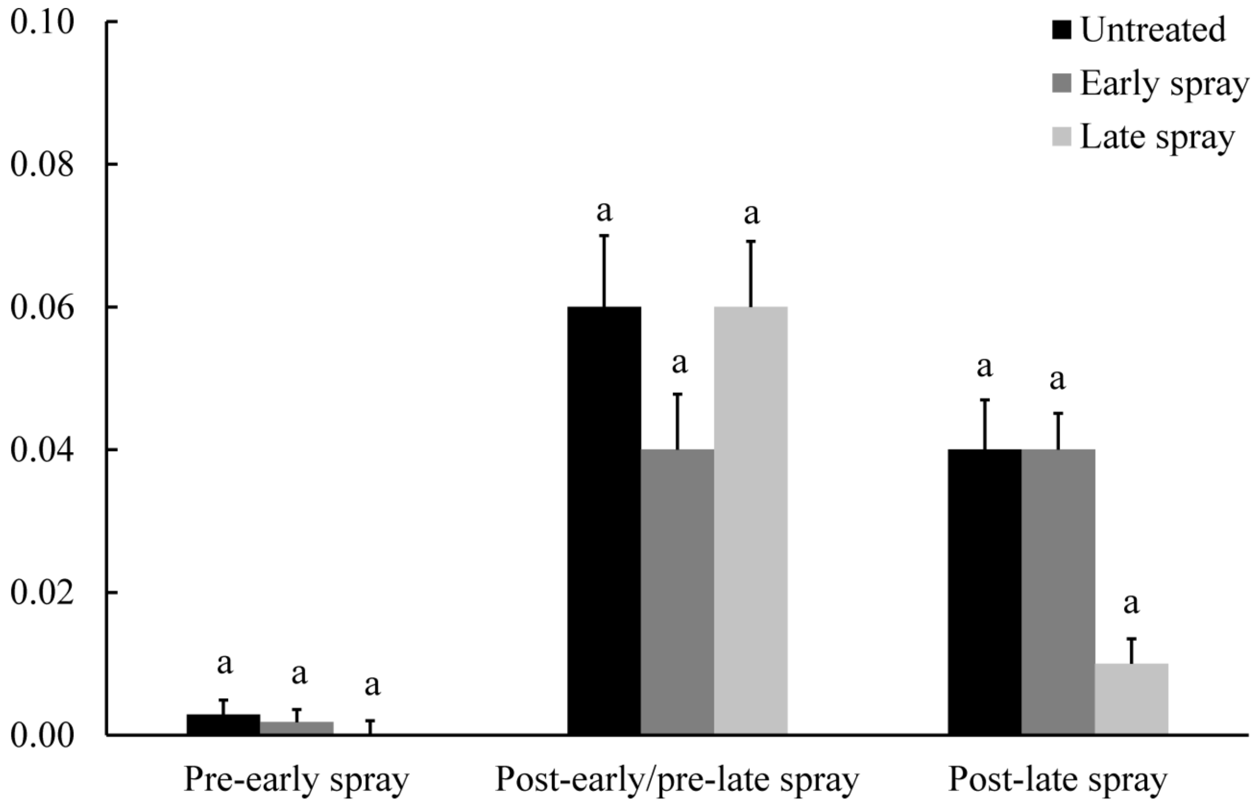

Figure 4. Mean number of beetles per trap per day for 2015. Means sharing a similar letter during a given period are not significantly different ($P < 0.05$). Square-root transformed data were analyzed; actual means and standard errors are reported.

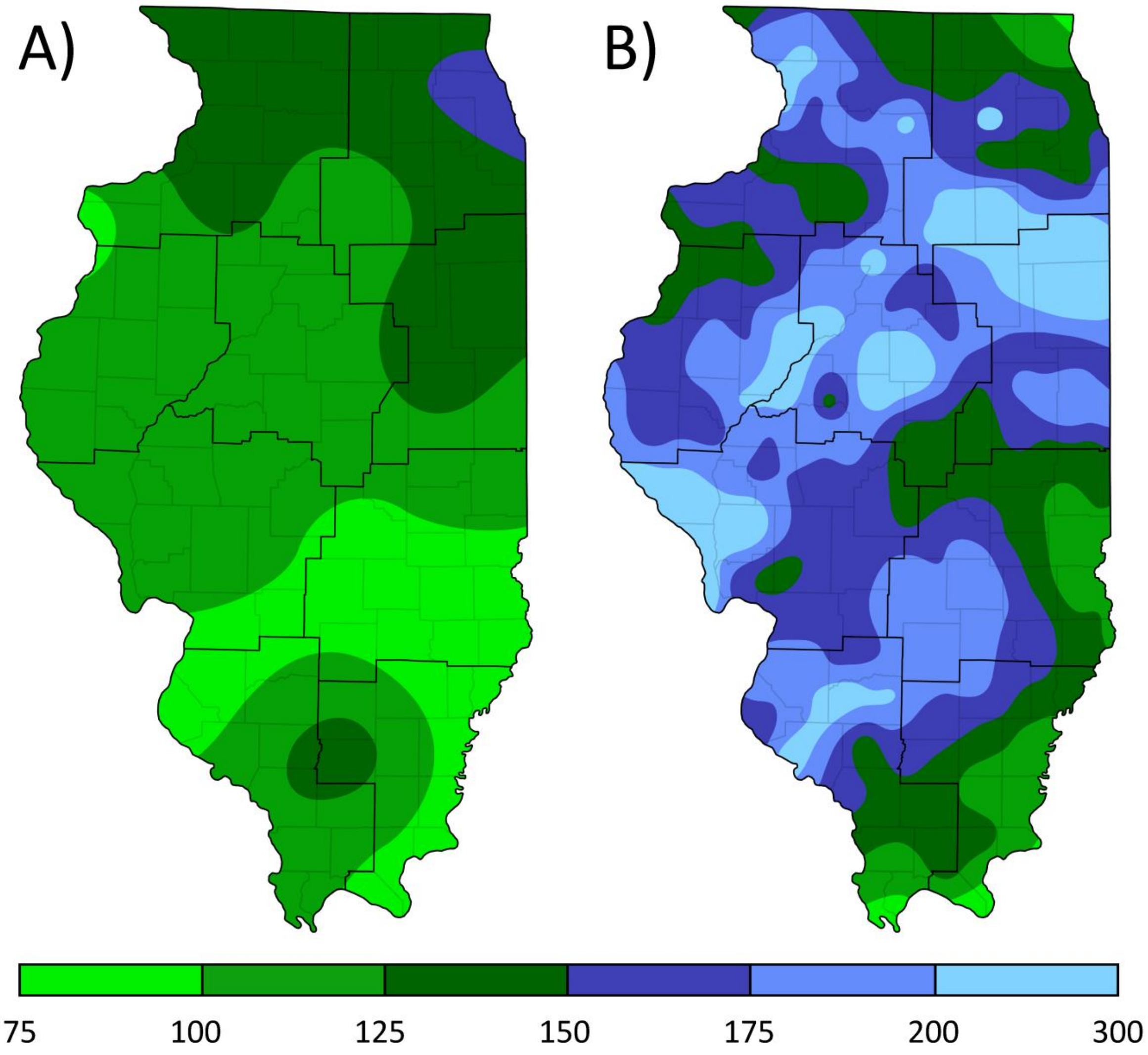

Figure 5. Precipitation accumulated from A) 1 May through 30 June during 2014 and B) 1 May through 30 June during 2015 reported as a percentage of the average amount of precipitation accumulated during the same time period from 1981 through 2010 (Source: Midwestern Regional Climate Center, Champaign, IL).

APPENDIX

Table 5. Agronomic Information for Site 1

2014	Corn	Hybrid	Pioneer P1352 AMXT (113 RM)
		Trait	Optimum AcreMax XTreme (mCry3A + Cry34/35Ab1)
		Planting date	24 April
		Seeding rate	33,600 seeds/acre (83,027 seeds/ha)
		Herbicide(s) used	Valor XLT
	Soybean	Variety	Pioneer 28T33R (MG 2.8)
		Planting date	29 May
		Seeding rate	167,000 seeds/acre (412,666 seeds/ha)
		Herbicide(s) used	Roundup
2015	Corn	Hybrid	Pioneer P0987AMX (109 RM)
		Trait	Optimum AcreMax Xtra (Cry34/35Ab1)
		Planting date	24 April
		Seeding rate	35,077 seeds/acre (86,677 seeds/ha)
		Herbicide(s) used	Valor XLT
	Soybean	Variety	Pioneer 28T33R (MG 2.8)
		Planting date	20 May
		Seeding rate	167,000 seeds/acre (412,666 seeds/ha)
		Herbicide(s) used	Roundup

Table 6. Agronomic information for Site 2

2014	Corn	Hybrid	Great Lakes 5939VT3PRIB
		Trait	Genuity VT Triple Pro RIB Complete (Cry3Bb1)
		Planting date	8 May
		Seeding rate	33,500 seeds/acre (82,780 seeds/ha)
		Herbicide(s) used	Glyphosate, Volley ATZ
	Soybean	Variety	Stine 30LC28LL
		Planting date	18 May
		Seeding rate	160,000 seeds/acre (395,368 seeds/ha)
		Herbicide(s) used	Liberty, Sonic
2015	Corn	Hybrid	Stone 6058RIB
		Trait	Genuity SmartStax RIB Complete (Cry3Bb1 + Cry34/35Ab1)
		Planting date	23 April
		Seeding rate	34,000 seeds/acre (84,016 seeds/ha)
		Herbicide(s) used	Glyphosate, Volley ATZ
	Soybean	Variety	Stine 30LC28LL
		Planting date	15 May
		Seeding rate	155,000 seeds/acre (383,013 seeds/ha)
		Herbicide(s) used	Liberty, Sonic

Table 7. Agronomic information for Site 3

2014	Corn	Hybrid	AgriGold A6442 (109 RM)
		Trait	Genuity SmartStax RIB Complete (Cry3Bb1 + Cry34/35Ab1)
		Planting date	24 May
		Seeding rate	33,000 seeds/acre (81,545 seeds/ha)
		Herbicide(s) used	Stalwart C, Atrazine, Roundup
	Soybean	Variety	ProHarvest 3135CR2Y (MG 3.1)
		Planting date	25 May
		Seeding rate	175,000 seeds/acre (432,434 seeds/ha)
		Herbicide(s) used	Authority, Roundup
2015	Corn	Hybrid	AgriGold A6442 (109 RM)
		Trait	Genuity SmartStax RIB Complete (Cry3Bb1 + Cry34/35Ab1)
		Planting date	3 May
		Seeding rate	33,000 seeds/acre (81,545 seeds/ha)
		Herbicide(s) used	Stalwart C, Atrazine
	Soybean	Variety	ProHarvest 2871CR2Y (MG 2.8)
		Planting date	27 May
		Seeding rate	175,000 seeds/acre (432,434 seeds/ha)
		Herbicide(s) used	Blanket, Clethodim, Durango, Resist