

WESTERN CORN ROOTWORM (COLEOPTERA: CHRYSOMELIDAE; *DIABROTICA VIRGIFERA*  
*VIRGIFERA* LECONTE) EMERGENCE AND ABUNDANCE IN TRANSGENIC CORNFIELDS WITH  
STRUCTURED AND SEED BLEND REFUGES

BY

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THESIS

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## ABSTRACT

In the United States and Canada, western corn rootworm (Coleoptera: Chrysomelidae; *Diabrotica virgifera virgifera* LeConte) larvae have been managed using transgenic Bt (*Bacillus thuringiensis* Berliner) corn hybrids expressing toxic Cry proteins (i.e., Cry3Bb1, Cry34Ab1/35Ab1, mCry3a) for a decade. To delay western corn rootworm resistance to Bt toxins, growers must plant a non-Bt “refuge” in each Bt cornfield. This allows susceptible larvae to develop without exposure to the Bt toxin(s). Matings between susceptible adults and potentially resistant adults developing on Bt plants may delay the development of Bt resistance in their offspring. The refuge strategy relies on the movement of mate-seeking adults from refuge to Bt corn. The research summarized here focused on the distribution of western corn rootworm adults in Bt cornfields planted with structured refuges as blocks at the west end of each plot or as seed blend refuges. The spatial and temporal distribution of western corn rootworm emergence and live collected adults were analyzed in four refuge treatments (20% structured refuge, 5% structured refuge, 5% seed blend refuge and 0% refuge). Abundance was compared across plots and corn phenology stages (vegetative, pollination and post-pollination) from 2010 – 2012. Adult abundance in adjacent soybean fields was also analyzed. Males emerged 6.4 days before females and both males and females emerged from Bt corn 7.3 days after adults from refuge corn plants. In seed blends, emergence from refuge and Bt corn was more synchronous than that of structured refuge treatments. Adult emergence rates (adults/trap/day) from refuge corn in seed blends were significantly lower than those in structured refuge treatments. Adult emergence rates from Bt corn did not differ between

structured and seed blend refuge treatments. Adult collection rates in 5% and 20% structured refuge treatments were significantly greater in refuge rows during vegetative and pollination stages. Adults became more evenly distributed across plots during post-pollination. In contrast, adults in 5% seed blend and 0% refuges were evenly distributed across the plots throughout the season. Adults were most abundant in soybeans during the post-pollination phenology of nearby corn plots; few adults were collected in soybeans during vegetative or pollination stages. Most of the adults collected in soybeans each year were female; the percentages were 93%, 88% and 81% in 2010, 2011 and 2012, respectively. The concentration of adults in refuge rows suggest that structured refuge configurations do not generate a distribution of western corn rootworms that facilitate the mixing of adults between refuge and Bt corn. Deploying refuge as a seed blend would serve to delay resistance to Bt technology by producing uniform distributions of western corn rootworms that better promote the mixing of mate-seeking adults between refuge and Bt corn plants

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## TABLE OF CONTENTS

CHAPTER 1: ASPECTS OF WESTERN CORN ROOTWORM (COLEOPTERA: CHRYSOMELIDAE; <i>DIABROTICA VIRGIFERA VIRGIFERA</i> LECONTE) BIOLOGY AND DISPERSAL BEHAVIOR IN A TRANSGENIC LANDSCAPE.....	1
BIBLIOGRAPHY .....	17
CHAPTER 2: WESTERN CORN ROOTWORM (COLEOPTERA: CHRYSOMELIDAE; <i>DIABROTICA VIRGIFERA VIRGIFERA</i> LECONTE) EMERGENCE AND ABUNDANCE IN TRANSGENIC CORNFIELDS WITH STRUCTURED AND SEED BLEND REFUGES .....	24
FIGURES AND TABLES .....	52
BIBLIOGRAPHY .....	64

# CHAPTER 1: ASPECTS OF WESTERN CORN ROOTWORM (COLEOPTERA: CHRYSOMELIDAE; *DIABROTICA VIRGIFERA VIRGIFERA* LECONTE) BIOLOGY AND DISPERSAL BEHAVIOR IN A TRANSGENIC LANDSCAPE

## INTRODUCTION

The western corn rootworm, *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae), is the most economically important pest of corn in the United States (US). It has been estimated that annual losses and management costs related to the western corn rootworm and, the closely related, northern corn rootworm (*Diabrotica barberi* Smith and Lawrence) exceed USD \$1 billion (Metcalf 1986, Rice 2004). The 1992 discovery of western corn rootworm in Europe and its subsequent spread has only enhanced the status of the western corn rootworm as an important pest (Baca 1994, Kiss et al. 2005). Projected costs for the management of economic infestations of western corn rootworm across European (Wesseler and Fall 2010) add substantially to the worldwide economic impact of this pest. The Commonwealth Agricultural Bureaux International recently designated the western corn rootworm as the world's most expensive pest to control (Cock 2011).

In North America, the western corn rootworm has long been controlled by crop rotation and the use of soil and foliar insecticides (Levine and Oloumi-Sadeghi 1991). The first Bt (*Bacillus thuringiensis* Berliner) hybrids targeting corn rootworm species were commercialized in 2003. Bt hybrids have revolutionized rootworm management and grower adoption has increased dramatically since 2003. By 2012, 67% of US corn expressed one or more Bt toxins to manage coleopteran or lepidopteran pests (USDA-ERS 2012). Bt hybrids have been engineered to express insecticidal proteins derived from crystalline (Cry) toxins originating from the soil

microbe *Bacillus thuringiensis*, and have been an effective management tool. As with other management methods, Bt technology is vulnerable to insect resistance. In an effort to delay resistance to Bt toxins, an insect resistance management (IRM) plan was set forth by the US Environmental Protection Agency (EPA) regulating the use of this technology (EPA-OPP 2001, 2012). Aspects of the IRM plan developed for corn rootworm Bt hybrids were adapted from the IRM plan that was already in place for European corn borer (*Ostrinia nubilalis* Hübner) and other lepidopteran pests, a group with very different biology from corn rootworm beetles. Much is known about the biology of western corn rootworm, but how does that knowledge apply to current IRM strategies? The focus of this review is to better understand the role of western corn rootworm biology and dispersal behavior in a Bt transgenic landscape.

## GENERAL BIOLOGY

### Life cycle

The life history of the western corn rootworm is closely tied to corn. The first publication recognizing it as a pest suggested annual rotation of corn with another crop was a “simple remedy” (Gillette 1912). Historically, females deposited their eggs almost exclusively, in the soil of cornfields from July to August (Ball 1957). After overwintering as eggs (Ball 1957) in an obligatory diapause (Krysan and Branson 1977), the larvae begin to eclose in late May and early June (Levine et al. 2002). The neonates must feed on corn root tissue (Krysan and Branson 1983). The majority of control costs and yield losses related to western corn rootworm are a result of larval feeding which may disrupt water and nutrient absorption (Kahler et al. 1985), make plants more susceptible to disease (Palmer and Kommedahl 1969) and reduce

structural stability. This causes the plants to lodge, making them poor interceptors of light and difficult to harvest. After pupation, adults emerge from the soil beginning in late June (Murphy et al. 2010) though older sources recorded adult emergence from July to October (Ball 1957). Western corn rootworms are protandrous, adult males begin to emerge from the soil 5-7 days before females (Branson 1987, Murphy et al. 2010, Quiring and Timmins 1990) but 98% of male emergence overlaps with that of females (Quiring and Timmins 1990). Adults feed on foliage, tassels, silks, pollen and corn ears (Ball 1957). At high densities, silk feeding (i.e., silk clipping) may reduce yield by interfering with pollination and impeding the production of mature kernels (Levine and Oloumi-Sadeghi 1991).

### **Mating behavior**

Western corn rootworm males reach sexual maturity 5-7 days after emergence (Guss 1976, Quiring and Timmins 1990). Previous laboratory studies suggested that males mate eight or more times over a period of 42 days (Branson et al. 1977, Quiring and Timmins 1990). However, Kang and Krupke (2009b) have determined that males mate on average 2.5 times in the 10-day period after their initial mating. Beyond this 10-day period they mated on average 0.15 times (Kang and Krupke 2009b). This effective reproductive lifespan of 10 days varies considerably from the 42 days described by Branson et al. (1977). The more conservative estimate may be due to the larger arenas used by Kang and Krupke (2009a) that allowed a more natural behavior.

Females are sexually mature at emergence (Hammack 1995) and, shortly after, begin releasing sex attractant pheromone (Ball 1957, Hill 1975). The primary component of this



pheromone is 8*R*-methyl-2*R*-decenyl-propanoate (Guss et al. 1982) which attracts the males (Hammack 1995). When a male locates a calling female, there is a brief period of courtship before he is accepted (Lew and Ball 1979). Kang and Krupke (2009a) found that larger females were more attractive to males. Most females mate only once (Branson et al. 1977) within their natal cornfield shortly after emergence (Quiring and Timmins 1990). Quiring and Timmins (1990) report that 96.6% of females are mated while they are still teneral, that is, within the 12 – 24 hr period before their elytra are fully sclerotized (Cates 1968).

Adults mate for 3 – 4 hours (Lew and Ball 1979), during which the male passes a spermatophore to the female that provides enough sperm to fertilize all of her eggs making multiple mating unnecessary (Hill 1975). Spermatophores are composed of 7.40% protein and 0.66% carbohydrate (Murphy and Krupke 2011). Nitrogenous compounds derived from spermatophores were eventually transferred to the eggs (Murphy and Krupke 2011). After copulation, the spermatophore degrades over a 5-7 day period (Lew and Ball 1980, Murphy and Krupke 2011). Males make a significant investment in each spermatophore, comprising up to 10% of male body mass. In the lab, Quiring and Timmins (1990) determined that spermatophore mass declined with the quality of a male's food source. They also determined that the size of the male was not a factor in the size of the spermatophore, suggesting that small males transfer a proportionately larger fraction of their bodily resources to the female during copulation (Quiring and Timmins 1990). In the lab, females have been recorded mating more than once during their lifetime. Branson et al. (1977) used normal and irradiated sterile males to determine sperm precedence. When females mated twice, the eggs were fertilized by the second male's sperm.

After mating, females will feed for several days and begin to disperse from their natal field before oviposition (Isard et al. 2004). When western corn rootworm beetles were collected ascending from cornfields, 85% were female. Of these females, 84% contained spermatophores, indicating that they had recently mated (Isard et al. 2004). Oviposition occurs preferentially on moist soil substrates (Gustin 1979). Kirk (1979) recorded female western and northern corn rootworms entering drought cracks and ovipositing at a variety of depths. The depth of oviposition was dependent on the presence of moist soil (Kirk 1979). Peak ovipositional activity usually occurs between 8.00 hr and 12.00 hr (Ball 1971). Boetel and Fuller (1997) stated that females were capable of producing 440 viable eggs but Hill (1975) observed females depositing as many as 1000 eggs in the laboratory.

## **MOVEMENT BEHAVIOR**

### **Adult intrafield movement**

The movement of mate-seeking western corn rootworm males through cornfields has been a challenge to study. High population densities have made mark-release-recapture studies difficult. Spencer et al. (2003) developed a novel method for measuring adult movement within Bt cornfields, allowing beetles to mark themselves by consuming Bt corn tissue and later testing their gut content for the presence of different Bt Cry proteins. By recording the collection location for each beetle they could determine that the beetle had moved if it contained a Cry protein that differed from that expressed by the corn plants where it was collected. Because Bt Cry proteins are detectable in the insect gut for up to 32hr, they were able to estimate a daily movement rate for beetles containing a different Bt Cry protein

based on the distance between the collection location and the nearest source of the different Bt Cry protein. Using a variation of this method, Marquardt and Krupke (2009) found that females move little before mating and were less likely to move within the field than males.

### **Interfield movement**

When mated females and mate-seeking male western corn rootworms disperse from their natal field, they often move into adjacent fields that may contain corn or other crops like soybeans. Female bias is characteristic of insects engaging in interfield dispersal, especially when entering soybean fields (Godfrey and Turpin 1983). Local interfield movement of adults has largely focused on studies of movement between corn and soybeans (Spencer et al. 2005). When adults disperse from corn into soybeans, they are unable to remain in soybeans for long periods. Soybean foliage is a poor food source and beetles must return to corn to feed (Mabry and Spencer 2003). Beetles often leave cornfields when pollination is complete (O'Neal et al. 2004). Adults tend to move toward younger corn and are attracted by volatiles from pollen and silks (Naranjo 1994, O'Neal et al. 2004). After pollination, or when cornfields are maturing, they become less attractive sites for feeding and beetles will leave to exploit weeds as a pollen source (Moeser and Vidal 2005).

Estimates of western corn rootworm interfield movement vary. Adults have been recorded moving up to 300 m from a release location into nearby cornfields (Toepfer et al. 2006) but averaged 4.6 - 9.1 m/day between Bt cornfields and soybean fields (Spencer et al. 2003).

## **Long-distance flight**

Western corn rootworm females engage in long-distance flight after they have mated but before they become gravid (Coates et al. 1987, Isard et al. 2004). Post-mating levels of juvenile hormone are a factor in initiating long-distance flight (Coates et al. 1987). Analyses of flying beetles collected at a 10 m elevation revealed peaks in flight activity from 6:45 – 11:00 am and again from 5:00 – 8:30 pm when transitional atmospheric conditions (i.e., atmospheric instability) facilitate flight (Isard et al. 2004). As beetles ascend, they are carried in the direction of prevailing winds (Isard et al. 2004). Adult long-distance flight was studied to help understand and predict the spread of rotation resistant populations. Models using patterns of prevailing wind directions and storm data, estimated that rotation resistant populations spread 10 – 30 km per year (Onstad et al. 1999). The most extreme examples of movement by adults are associated with the passage of summer convective cells (Grant and Seevers 1989). Updrafts associated with approaching storms can draw in airborne beetles and carry them long distances.

## **DISTRIBUTION AND SPREAD**

### **North America**

The western corn rootworm was first described by LeConte who collected two specimens from wild gourd near Fort Wallace, Kansas in 1867 (LeConte 1868). The species was first recorded as a pest of cultivated corn in 1909 and 1910 in Colorado (Gillette 1912). Its range was limited to the US central Great Plains prior to the early 1900s after which it expanded eastward following increasing cultivation of corn that was made possible by the availability of

irrigation systems and fertilizers (Gray et al. 2009). Western corn rootworm had become a significant pest of corn by the 1940s (Branson and Krysan 1981). From Colorado it spread across the Corn Belt, was established throughout Nebraska by 1955, entered Illinois in 1964, and during the 1980s western corn rootworm reached U.S. east coast states (Gray et al. 2009). The range of this pest also spread southward to Texas and as far north as Montana, North Dakota and Ontario (Meinke et al. 2009). As corn cultivation increased so did corn rootworm population densities. In an effort to control corn rootworm species, corn growers employed a variety of management techniques including broad spectrum insecticides such as chlorinated hydrocarbons, cyclodienes (a sub-group of chlorinated hydrocarbons), organophosphates and carbamates (Spencer and Levine 2008). Over time, intensive use led to corn rootworm resistance to cyclodienes (e.g., aldrin) in the early 1960s, organophosphates (e.g., methyl parathion) and carbamates (e.g., carbaryl) in the 1990s (Bigger 1963, Metcalf 1983, Spencer and Levine 2008).

In addition to the increased cultivation of corn, the rapid eastward spread of western corn rootworm was facilitated by the direction of prevailing winds and storm fronts as beetles engaged in long-distance flight (Grant and Seevers 1989, Isard et al. 2004, Onstad et al. 1999). Long-distance flight is a major factor of western corn rootworm expansion, allowing small populations to establish themselves ahead of the majority and accelerate the species' spread (Onstad et al. 1999). The recent establishment of western corn rootworm in Europe and concern about the spread of resistant variants in the US has spurred interest in long-distance flight and interfield movement.

## Europe

The first established population of western corn rootworm in Europe was observed in a cornfield near the Belgrade airport, in Serbia, in 1992 (Baca 1994). It has since been identified in 16 European countries (Ciosi et al. 2008, Kiss et al. 2005). Through genetic testing of populations in North America and Europe, Miller et al. (2005) determined that there have been at least three introductions of western corn rootworm from North America that became established, in Serbia, Italy and France. Genetic testing by Ciosi et al. (2008) indicated that there have been five introduction events: one each in Serbia, Italy, the United Kingdom and two introductions in France. Models suggest that Europe could suffer losses averaging € 472 million Euro, annually, if no control measures are taken (Wesseler and Fall 2010).

### **CROP ROTATION AND ROTATION RESISTANCE**

The strong ovipositional fidelity of western corn rootworms to cornfields and the inability of larvae to feed on other economic crops (Branson and Ortman 1967) allowed annual rotation of corn with non-host crops to control this pest for over a century (Gillette 1912, Shaw et al. 1978, Levine and Oloumi-Sadeghi 1991). Annual rotation of corn with soybeans (*Glycine max* Merrill) is prevalent in the Corn Belt; a majority of farmers (75.2%) reported using it as part of a rootworm management program (Wilson et al. 2005). By planting soybeans in fields that had been planted to corn and had experienced heavy oviposition the previous year, growers create an environment inhospitable for larval development. However, there are circumstances when females will oviposit in non-host fields that may result in larval injury in first-year corn. When adult females and eggs were collected from soybean fields, there was no subsequent

larval injury to first-year corn except when volunteer corn had also been present in those soybean fields during the preceding year (Shaw et al 1978). It is likely that a small proportion of the western corn rootworm population had always expressed reduced fidelity to cornfields. When crop rotation had been broadly adopted, this behavior provided an advantage to their offspring and their representation in the population increased (Levine et al. 2002). This intense selective pressure led to the evolution of behavioral resistance to crop rotation (rotation resistance) in western corn rootworm (Levine and Oloumi-Sadeghi 1996, Onstad et al. 2001, Levine et al. 2002).

The first evidence of rotation resistance was documented in 1987 in a field of rotated corn in Ford County, Illinois (Levine and Oloumi-Sadeghi 1996). By 1995, rotation resistant beetles were causing economic damage in first-year corn throughout east central Illinois and northwestern Indiana (Levine et al. 2002). Since their discovery, the rotation resistant western corn rootworm has spread to parts of Indiana, Ohio, Michigan, Wisconsin, Missouri, Iowa and the Canadian province of Ontario (Levine and Oloumi-Sadeghi 1996, Levine et al. 2002, Gray et al. 2009).

Corn phenology plays a role in western corn rootworm movement into soybeans but this is not specific to rotation resistant populations (O'Neal et al. 2004). Through feeding assays it was determined that while both rotation resistant and rotation susceptible beetles were able to consume soybean foliage, it was a poor food source and the habit would require beetles to return to nearby cornfields to feed or they would die (Mabry and Spencer 2003). The consumption of soybean foliage for 1 – 3 days caused stress that increased rates of oviposition (Mabry et al. 2004). This effect of soybean herbivory may bolster rotation resistant

populations by increasing the number of eggs deposited in fields that are likely to be rotated the following year (Spencer et al. 2005). Western corn rootworms entering soybeans had a tendency to fly near the level of the corn canopy, distinguishing them from beetles engaging in long-distance flight which tend to ascend and fly  $\geq 10$  m above the corn canopy (Spencer et al. 2005). Adults collected in soybean fields were predominately mature, gravid females (Godfrey and Turpin 1983, O'Neal et al. 1999). During two years of a three-year study, Pierce and Gray (2006), collected significantly more eggs by soil sample volume in soybean fields than cornfields.

O'Neal et al. (2001) developed an economic threshold for monitoring western corn rootworm adults in soybean using Pherocon AM traps (Trécé Inc., Adair, OK). This method offered producers an integrated pest management (IPM) approach to improve the management of rotation resistant beetles. However, many growers were reluctant to monitor soybeans, opting for a prophylactic application of soil insecticides to first-year corn at planting (Gray 2011a, 2013). This response to rotation resistance resulted in a dramatic increase in soil insecticide use in the eastern Corn Belt (Rice 2004).

### **BT TRANSGENIC CORN HYBRIDS**

Insecticidal Bt corn hybrids were originally introduced in 1996 to control lepidopteran pests including European corn borer (Wilson et al. 2005); the first Bt corn hybrid expressing a toxin (Cry3Bb1) that specifically targeted neonate corn rootworm larvae, was commercialized in 2003 (EPA-OPP 2001, Vaughn et al. 2005). Bt hybrids offered a welcome alternative to an increasing reliance on insecticides. Adoption of Bt hybrids reduced use of broad-spectrum soil



insecticides, reduced grower exposure to chemicals, protected non-target species and provided efficacy equivalent to insecticides (Rice 2004). Additional corn hybrids expressing Bt toxins targeting corn rootworms have since been introduced (Cry34Ab1/35Ab1 and mCry3a; Tabashnik and Gould 2012). Beginning in 2005, stacked hybrids expressing both a lepidopteran and a coleopteran toxin were commercialized. In 2008, pyramided hybrids were commercialized, expressing multiple toxins targeting lepidopterans and multiple toxins targeting coleopterans. In the decade since corn rootworm Bt corn hybrids were first commercialized, hybrids expressing insecticidal Bt traits have been rapidly adopted throughout the US, reaching 82% adoption by Illinois corn growers in 2010 and 67% throughout the US in 2012 (James 2010, USDA-ERS 2012).

Any pest management tool that is widely adopted and has high efficacy carries the risk of selection for insect resistance. In an effort to preserve the longevity of pest susceptibility to Bt technologies targeting western corn rootworm, the EPA developed an IRM plan mandating that a non-transgenic refuge be planted within each Bt cornfield (EPA-OPP 2001). Refuges are intended to preserve modest populations of susceptible western corn rootworms which will enter the Bt portion of cornfields and mate with potentially resistant individuals that emerge from Bt hybrids. This pattern of intermating will reduce the likelihood of matings that might produce homozygous resistant offspring. Based on an existing IRM plan for European corn borer that used a 20% refuge (planted within 0.5 mi of a Bt cornfield), a 20% rootworm refuge was proposed for hybrids expressing a single rootworm toxin (EPA-OPP 2001). The reduced mobility of corn rootworms, compared to corn borers, led to the requirement that a structured refuge (one refuge block or multiple row strips) be planted within each Bt cornfield targeting

corn rootworm. With the introduction of stacked and pyramided Bt hybrids, the required refuge percentage was reduced to 10% and 5%. Depending on the particular hybrids and toxins used, refuges could be planted in a variety of configurations including structured block, structured row-strip refuges and as seed blends that contain the correct percentage of refuge seed blended into the bag of Bt seed so refuge will be randomly distributed across each Bt cornfield when planted (EPA-BPPD 2012).

The structured refuge strategy is based on a variety of assumptions. It is assumed that Bt hybrids express a high-dose of Bt toxins that will kill 98-99% of a target population (Tabashnik 2008). While true for the Cry toxins expressed in Bt corn hybrids that target lepidopteran corn pests, no current Bt hybrids express any rootworm specific toxins at high-dose (Meihls et al. 2008). The lack of a high-dose corn rootworm toxin in Bt corn hybrids raises some doubt as to the long-term durability of Bt technology for controlling corn rootworm species under current refuge requirements (Tabashnik and Gould 2012). Concern about toxin dosage in Bt cornfields is compounded by declining grower compliance with refuge requirements and a trend toward planting continuous corn (Jaffe 2009, Gray 2011a, 2011b; Onstad et al. 2011). In surveys conducted in 2010, Gray (2011a, 2011b) reported that 97% and 96.7% of Illinois corn growers planted Bt hybrids in 2008 and 2009, respectively. In the same group of growers 82% reported planting sufficient refuge in 2008 and 75.7% in 2009 (Gray 2011a, 2011b).

In 2009, the first cases of field evolved resistance to Bt toxin Cry3Bb1 were documented in three Iowa cornfields (Gassmann et al. 2011). All affected fields had been Cry3Bb1-expressing cornfields for at least three consecutive years (refuge compliance was not assessed)

(Gassmann et al. 2011). In this study, adults were collected from the fields with 'unexpected injury,' their offspring were reared in the laboratory and used in plant-based bioassays revealing reduced susceptibility to the Bt toxin Cry3Bb1 (Gassmann et al. 2011). In 2012, field resistance to Cry3Bb1 was also confirmed in Illinois using similar methods (Gray 2012b). In both studies, the Cry3Bb1 resistant populations remained susceptible to Bt toxin Cry34Ab1/35Ab1 (Gassmann et al. 2011, Gray 2012b)

Deploying refuge in a seed blend configuration may solve the problem of grower compliance with the refuge requirement. Because refuge seeds in seed blends are integrated into each bag of Bt corn seed in the appropriate proportion, there is no chance that an insufficient refuge will be planted. The convenience of seed blend refuges may lead to a further increase in adoption of Bt crops and reduction in IPM methods (Gray 2011a, 2011b; Onstad et al. 2001, 2011; Spencer et al. 2013). As grower adoption of Bt technologies has increased, their participation in traditional IPM methods like monitoring and the use of economic thresholds has declined (Gray 2011a, 2011b).

## **CONCLUSIONS**

Beyond assuring grower compliance with refuge requirements, the different spatial distribution of refuge in seed blends vs. structured block refuges will affect the distribution of susceptible adults by increasing their likelihood of encountering potential mates that developed on Bt hybrids. The refuge strategy relies on the assumption that early emerging males from the refuge will be able to mate many times in their adult life. When the limited male reproductive lifespan (Kang and Krupke 2009b) was incorporated into models, resistance developed more

quickly (Pan et al. 2011). Multiple mating by females could impact IRM similarly. Subsequent matings that occur after the early emerging refuge males have outlived their reproductive life span will result in more females mating with nearby Bt emerging males (Spencer et al. 2013). Multiple matings by females could also raise the question of sperm precedence if some females mate with both refuge and Bt emerging males. If such doubly-mated females did so in close succession, there may be opportunities for sperm competition to bias the proportion of progeny that may be heterozygous or homozygous for Bt resistance. Potentially Bt resistant males that are the second mates of females that emerged from Bt cornfield (and mated initially with a refuge male) may fertilize most of a female's eggs resulting in offspring that are homozygous for Bt resistance alleles and presumably phenotypically resistant to Bt toxins.

While adult western corn rootworms are capable of riding prevailing winds and dispersing great distances, they tend to move much shorter distances and less frequently when seeking mates within cornfields or dispersing between local fields. As a result, adults may not achieve the proportion of mixed matings between refuge and Bt emerging beetles needed to meet the assumptions of the refuge strategy for structured refuge configurations. The use of a seed blend more thoroughly mixes the distribution of susceptible and potentially resistant beetles, subsequently increasing the likelihood of mixed matings (Onstad et al. 2001, 2011; Spencer et al. 2013).

An important concern associated with seed blends is that larvae may move between individual Bt and refuge corn plants in a seed blend. Larvae may discontinue feeding on a Bt hybrid's roots before ingesting a lethal dosage of Bt toxin and then move onto a refuge plant's roots could lead to the development of resistance or tolerance to Bt toxins following exposure

(Hibbard et al. 2005). However, seed blends guarantee that a sufficient refuge will be planted in every Bt cornfield. The compliance benefits of seed blends may outweigh the risks of larval movement. Another concern is that pyramided seed blends encourage increased cultivation of Bt crops and create a Bt landscape with a smaller collective refuge leading to increased selective pressure (Onstad et al. 2011). Increasingly, growers of Bt crops are adopting an insurance-like approach to pest management that ignores the ecological principles of IPM. Bt cornfields are planted and pesticides are applied even when insect populations are predicted to be low (Gray 2011a, 2012a, 2013). A willingness to use Bt hybrids and insecticides on sub-economic populations of pests elevates the risk of pest resistance to both Bt toxins and insecticides. A return to traditional IPM including monitoring pest densities and use the of economic thresholds may alleviate some of the risks associated with both Bt and pesticide application (Gray 2011a).

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## **CHAPTER 2: WESTERN CORN ROOTWORM (COLEOPTERA: CHRYSOMELIDAE; *DIABROTICA VIRGIFERA VIRGIFERA* LECONTE) EMERGENCE AND ABUNDANCE IN TRANSGENIC CORNFIELDS WITH STRUCTURED AND SEED BLEND REFUGES**

### **INTRODUCTION**

The western corn rootworm (*Diabrotica virgifera virgifera* LeConte) is both the most important pest of corn and the world's most expensive pest to control (Cock 2011). Together, the western corn rootworm and northern corn rootworm (*Diabrotica barberi* Smith and Lawrence) cause over USD \$1 billion in control costs and losses every year in the US and Canada (Metcalf 1986).

The western corn rootworm is a univoltine pest with a life cycle closely tied to corn (Branson 1987). Larvae eclose from their eggs from May to June and immediately begin to feed on corn root tissue. Corn is the primary host of western corn rootworm; however, larvae may develop on some prairie grasses but nearly all of these alternate hosts have a reduced ability to support development (Branson and Ortman 1970). Larvae feed on corn roots through three instars (Levine and Oloumi-Sadeghi 1991). The majority of western corn rootworm damage is caused by the late instar larvae which bore into corn roots, disrupt water or nutrient flow (Kahler et al. 1985) and inflict feeding wounds that create points of entry for disease (Palmer and Kommedahl 1969). Larvae are capable of moving up to 0.46 m through the soil which allows them to move between plants in the same row or across one row, damaging multiple plants (Hibbard et al. 2003). Severe larval feeding can cause corn plants to lodge making them difficult to harvest and reducing photosynthetic efficiency, which ultimately reduces yield. Adults begin to emerge from the soil in June. Males emerge approximately 5 days before females but overlap female emergence for the remainder of the season (Branson 1987, Murphy

et al. 2010, Quiring and Timmins 1990). Adults feed on corn foliage, silks, tassels and kernels. Adult feeding on silks (termed silk clipping) can disrupt pollination causing kernels to remain unfertilized and reduce yield (Levine et al. 2002). Females predominately oviposit in cornfields from July to September. The diapausing eggs will overwinter in the soil to hatch the following spring (Krysan and Branson 1977, Shaw et al. 1978).

The ovipositional fidelity of western and northern corn rootworms to cornfields, and limited ability of larvae to develop on other plants (Branson and Ortman 1970), made them obvious targets for control using annual crop rotation (Gillette 1912), often with soybeans (Shaw et al. 1978, Levine and Oloumi-Sadeghi 1991). Annual crop rotation has been successful in controlling corn rootworms and was widely adopted across the US Corn Belt (James 2010). The resulting disruption of the rootworm lifecycle imposed heavy selective pressure that ultimately resulted in the development of physiological rotation resistance, by prolonged egg diapause, in northern corn rootworm (Chiang 1965) and behavioral resistance, by oviposition in fields of rotated non-host crops, in western corn rootworm (Levine and Oloumi-Sadeghi 1996, Levine et al. 2002). Prior to the evolution of rotation resistance, oviposition by female western corn rootworms in soybean fields was uncommon and there was little or no damage to first-year corn (Shaw et al. 1978). However, if volunteer corn was present in soybean fields, significant oviposition could occur around those plants leading to damage in first-year corn (Shaw et al. 1978). Since the development of rotation resistant populations in the late 1980s (Levine and Oloumi-Sadeghi 1996), females have become common in soybean fields (O'Neal et al. 1999), where they oviposit large numbers of eggs and feed on soybean foliage (Mabry et al. 2004, Mabry and Spencer 2003, Pierce and Gray 2006). Oviposition in soybean fields creates

the potential for economically significant larval damage in first-year corn (Levine and Oloumi-Sadeghi 1996, Levine et al. 2002).

The evolution of rotation resistance forced commercial corn growers in the eastern Corn Belt, who had long managed western corn rootworms with crop rotation, to return to soil insecticides to protect rotated cornfields (Rice 2004). While effective against corn rootworm larvae, insecticidal control is more costly than crop rotation and carries a risk of grower exposure to insecticides. Reducing this exposure risk was one of several factors that created demand for alternative methods of controlling corn rootworm larvae (Rice 2004). The need was met by the introduction of corn hybrids that expressed Bt (*Bacillus thuringiensis* Berliner) Cry proteins toxic to corn rootworms (in 2003). Bt corn hybrids are an effective method of controlling rotation susceptible and rotation resistant corn rootworm populations, offer economic benefits, reduce the need for soil insecticide application and reduce risks of pesticide exposure to growers and non-target insect species (Rice 2004, Tabashnik and Gould 2012).

To delay the development of rootworm resistance to the Bt toxins expressed by Bt hybrids, the United States Environmental Protection Agency (EPA) required that a certain percentage of each Bt cornfield be planted as a non-transgenic refuge (EPA-OPP 2001, Tabashnik 2008). The refuge is intended to support modest populations of Bt susceptible larvae that can develop without exposure to the Bt toxins expressed elsewhere in the field. Mate-seeking male beetles emerging from refuge corn are expected to move into the Bt portion of the cornfield and mate with the relatively sparse population of female beetles that survived their larval exposure to Bt toxins. The refuge strategy relies on matings between refuge and Bt

emerging beetles to dilute resistant alleles in the population and delay the evolution of resistance (EPA-OPP 2001, Tabashnik 2008, Tabashnik and Gould 2012).

The minimum refuge size for a Bt corn hybrid expressing one Bt Cry trait toxic to corn rootworms was initially set at 20% of the total area of Bt corn in the field (EPA-OPP 2001). These structured refuge areas were to be deployed as blocks or row strips located within or adjacent to the Bt cornfield. As the number of commercialized rootworm Bt toxins increased (i.e., Cry 3Bb1 was commercialized in 2003; Cry34Ab1/35Ab1 in 2005; and mCry3A in 2007) they were 'stacked,' combined with lepidopteran Bt toxins, or 'pyramided,' expressing toxins with different modes of action for both coleopteran and lepidopteran pests. The proliferation of hybrids with expressing combinations of traits complicated the process of deploying the proper percentage of refuge, which was reduced to 5% or 10% for certain trait combinations. Confusion about refuge requirements among the many commercial hybrids may have contributed to declining grower compliance with refuge requirements (Jaffe 2009).

Seed blend refuges ('refuge-in-a-bag') were introduced in response to declining grower compliance, offering a profitable and convenient alternative to structured refuges. Seed blends are currently sold with 10% or 5% refuge seeds integrated into a bag of Bt corn seed, simplifying the planting process. When planted, the appropriate proportion of refuge plants are randomly distributed across a Bt cornfield (EPA-BPPD 2012). By integrating the refuge in the bag, the seed industry has taken refuge compliance out of growers' hands and eliminated the possibility of planting an insufficient or nonexistent refuge.

The refuge strategy for corn rootworms was based on the successful application of the high-dose refuge strategy (Tabashnik 2008) for managing lepidopteran pests, like European



corn borer (*Ostrinia nubilalis* Hüber). However, replicating the lepidopteran refuge design poses problems because pests like European corn borer have life histories and dispersal patterns that are very different from those of corn rootworms. Also, unlike the Bt toxins in hybrids targeting lepidopterans, none of the Bt toxins targeting corn rootworms meet the high-dose criterion that is fundamental to the high-dose refuge strategy (Meihls et al. 2008, Tabashnik 2008, Tabashnik and Gould 2012). In addition, expectations of western corn rootworm behavior were based on data collected in laboratory and field conditions that are very different from current agricultural practices. Understanding western corn rootworm biology, particularly movement, is critical to establishing reasonable assumptions about beetle dispersal between refuge and Bt corn.

Studies of adults moving within cornfields have revealed that female beetles moved less often than males (Marquadt and Krupke 2009). In addition, little adult movement was recorded early in the season, after emergence, with increasing movement away from mature corn toward the end of the growing season (Cagan and Rosca 2012). It has been estimated that beetles engaging in interfield movement from corn into soybean fields traveled 4.6-9.1 m/day (Spencer et al. 2003). There are few published studies of western corn rootworm intrafield movement and even less information about the movement of mate-seeking beetles and mating relative to emergence in refuge as compared to Bt cornfields.

Detailed data about seasonal patterns of adult western corn rootworm emergence and abundance in different configurations of refuge and Bt corn is lacking. Documenting how specific refuge configurations affect the distribution of adults on a field level will provide the detail needed to understand movement and mating and make inferences about how Bt corn

affects western corn rootworm behavior in cornfields. Extensive adult movement between refuge and Bt corn are implicit in assumptions of the refuge strategy. If adult movement is more limited than is assumed, the shift toward integrated seed blend refuges may assure that mate-seeking adults from refuge corn plants are closer to potential mates in Bt corn than can be achieved in a structured refuge. Seed blend refuges may alter distributions of emergence and abundance (Onstad et al. 2001, 2011) and reduce distances between potential mates, ultimately increasing the likelihood that groups of mate-seeking beetles emerging from refuge and Bt corn rapidly become well mixed. The goal of this study was to measure the impact of refuge size and configuration on western corn rootworm ecology and to determine whether structured or seed blend refuge configurations generated beetle distributions that differed in their ability to facilitate mixing of mate-seeking beetles from refuge and Bt corn. This study specifically measures spatial and temporal patterns of adult western corn rootworm emergence and abundance in structured and seed blend refuge configurations.

## **MATERIALS AND METHODS**

### **Location and planting**

Field experiments were carried out at the University of Illinois' "Shaw Farm" located in northeast Urbana, IL (Champaign County; 40° 09' N, 88° 08' W) during 2010 – 2012. Four different configurations of refuge and Bt corn hybrids were planted in 0.76 m wide rows in four 1.66 hectare fields, subdivided into three 0.49 hectare subplots. There were three replicates of each refuge configuration planted in each year, with treatments assigned randomly to plots for a completely randomized design with three replications (Figure 1). Corn rows in each subplot

were on a north-south axis; subplots were configured with 110 – 116 rows of corn and were separated from each other by 8 m wide bare soil alleyways. Planting dates varied by year based on weather and field conditions (Table 1) and were similar to those of growers in the area.

## **Treatments**

Each year, a Herculex® XTRA CRW (Pioneer Hi-Bred) corn hybrid (expressing the Bt Cry1F and Cry34Ab1/35Ab1 proteins targeting lepidopteran caterpillars and corn rootworm larvae, respectively) was planted as the corn rootworm Bt corn hybrid (2010: Hybrid 34B41, RM109; 2011: Hybrid 33W84, RM111; 2012: Hybrid 33W84, RM111). The corn rootworm refuge hybrid, YieldGard® Corn Borer (Monsanto Co.), expressed the Bt Cry1Ab protein, targeting lepidopteran pests, (2010: Hybrid 34B94, RM113; 2011: Hybrid 33B54, RM113; 2012: Hybrid 33B54, RM113). All refuge and Bt corn hybrids expressed the Roundup Ready 2® herbicide tolerance trait and were factory-treated with a neonicotinoid seed treatment, 'Cruiser' or 'Cruiser Extreme' (thiamethoxam, Syngenta Crop Protection), at 0.25 ug a.i./kernel.

Refuge treatments were 1) 20% structured refuge (22 rows of non-Bt refuge corn and 88 rows of Bt corn), 2) 5.2% structured refuge (6 rows of non-Bt refuge corn and 109 rows of Bt corn), 3) 5.2% seed blend refuge (seed blend in all rows), and 4) 0% refuge treatment as a control (Bt corn in all rows). Hereafter, 5.2% refuge treatments will be referred to as 5% refuge treatments. The refuge rows in structured refuge treatments were always located on the west edge of each subplot.

The seed blend was prepared by weighing out appropriate portions of non-Bt and Bt corn seed (using seeds per Kg data available on the seed bag) and manually blending the seed

in multiple buckets with repeated stirring, tumbling and pouring between buckets to generate a blend that was distributed into seed bags for transport to the field. Major stages in corn phenology (Ritchie et al. 1993) were used to define three intervals during the growing season: vegetative, pollination, post-pollination (Table 1). Phenology was determined from observations of plants in Bt corn areas of fields.

### **Emergence trap collections**

Eight Illinois-style emergence traps (76.2 cm x 41.5 cm spanning the width of one row; Pierce and Gray 2007), were installed on 6 June 2010, 28 June 2011 and 7 June 2012. The traps were placed over single healthy corn plants at the V5 stage (Ritchie et al. 1993). The Cry protein expression status of each plant was verified using Quickstix™ (Envirologix, Portland ME 04103) which are qualitative test strips specific for detection of one Cry protein expressed in corn leaf or seed tissue. Each young plant was slipped through an opening in the top of the trap and a fabric skirt was tied loosely around the stalk of the plant to close the gap around the opening of the trap without hindering plant growth. A jar containing a paper cone was placed over a second opening in the top of each trap where climbing beetles would pass through an opening at the apex of the cone where they became trapped and could be easily collected.

The precise location and spatial arrangement of emergence traps were the same in all refuge treatments (n = 96 traps; Figure 2). In the 20% and 5% structured refuge treatments, four emergence traps were distributed within the refuge row that was second from the west side of each subplot (row W2). Two traps were placed ~ 3 m apart, ~ 7 m from the north edge of the plot and two others were placed ~ 3 m apart, ~ 7 m from the south edge. Traps were

positioned so they were not located where they might be damaged by insect collection activities. Four other emergence traps were installed over Bt corn plants in two additional rows. The selected rows were located 36 rows from the west and east sides of the subplots, respectively. In each row, one trap was located ~ 7 m from the north edge of the plot and the other was located ~ 7 m from the south edge. The emergence traps in the 5% seed blend and 0% structured refuge treatments were distributed in an identical configuration; thus the relative locations of emergence traps in all refuge treatments were identical.

Because the locations of refuge plants in the 5% seed blend refuge treatment were unpredictable, patches of refuge corn were planted in the locations where emergence traps would be installed. Immediately after machine planting, the seeds in four 1.5 m long patches in the designated emergence trap row were removed by excavating the furrow with a hand trowel. Refuge seed (7 – 8 seeds per patch) were replanted by hand with regular spacing and at the same 3 – 5 cm depth. The replanted refuge areas were marked with stakes and field flags. To avoid introducing refuge western corn rootworms into the 0% refuge subplots, no refuge patches were established in the 0% refuge treatment; in that treatment all emergence traps were placed over Bt corn plants.

Western corn rootworm adults were collected from emergence traps three times per week from June – August, 2010 - 2012. Abundance was recorded as the number of WCR/trap/day; beetle sex, collection location and corn type were recorded.

### **Adult live collections**

Western corn rootworm adults were collected from 11 designated sampling rows within each refuge treatment. In the 20% and 5% structured refuge treatments, the sampling rows were numbered based upon their distance (in rows) from the interface between the block of refuge corn and the Bt corn. A prefix letter was used to indicate whether a sample row was east (E) or west (W) of the refuge-Bt corn interface. In the 20% structured refuge treatment there were three sampling rows in refuge corn west of the interface: W22, W12 and W4, and eight sampling rows in Bt corn east of the interface: E3, E12, E24, E36, E48, E60, E72 and E82. In the 5% structured refuge treatment there were two sampling rows in refuge corn west of the interface: W4 and W2 and nine sampling rows in Bt corn east of the interface: E3, E12, E24, E36, E48, E60, E72, E84 and E 96. To facilitate comparisons, sampling rows in the 5% seed blend and 0% refuge treatments were labeled as though they were 5% structured refuge treatments. Sampling rows were clearly marked and labeled with a wooden marker at each end. The center of each sampling row was marked with a PVC pole (2.5 cm diameter) bearing brightly colored flag tape to indicate the location where each live collection was to be initiated.

Live collections were carried out during the morning peak of adult activity and mating. Any adults that had emerged very early in the day were still likely to be found in a teneral state (Cates 1968). Live collections were made by walking along one sampling row for a fixed time interval (typically 2 – 5 min) while searching amongst the foliage for single beetles and mating pairs. When encountered during a collection, a singleton or a mating pair were knocked from the corn foliage into collection jars consisting of a slippery plastic funnel secured to the top of a 1 quart glass jar containing an  $\sim 2 \text{ cm}^2$  piece of dry ice. Once inside the jar, beetles were rapidly killed by the intense cold and  $\text{CO}_2$  gas. Live collections were carried out three to five times per

week beginning after 3<sup>rd</sup> instar western corn rootworm larvae and pupae were first observed in the field. Completed samples were placed in a cooler of dry ice until they were transported to the lab. Beetle abundance, sex, location within the field and date were recorded for each collection row. Abundance was measured as the number of beetles collected per minute (beetles/min) in each collection row per refuge treatment. Each year, 6 – 12 persons assisted with live collections. To reduce sampling bias, each day collectors were randomly assigned to a treatment subplot (s) where they performed the full set of 11 live collections.

### **Abundance in soybeans**

The rotation resistant western corn rootworm is present in Champaign County. Monitoring seasonal changes in adult abundance in soybeans helps document the phenology of their behavior. Soybeans (variety P93Y40 in 2010 and 2011; variety P93Y70 in 2012, variety were Roundup Ready® to facilitate weed management) were planted in 76.2 cm rows in two 3.6 acre plots located between the cornfields described above. Stray weeds and volunteer corn were also removed manually throughout the season as necessary. Western corn rootworm adults were collected from soybean plots using 38 cm diameter sweep nets (BioQuip Products, Rancho Dominguez, CA 90220) modified with small removable collection bags. Sweep samples were made by passing the sweep net bag through the soybean canopy while walking down the designated soybean rows located 33 and 64 rows from the west side of the fields. When 100 sweeps were completed, the end of the sweep bag was removed, tied closed, labeled, placed on dry ice and returned to the lab where beetle abundance, sex and location were recorded.

## **Weather monitoring**

Weather data (e.g., insolation, temperature, wind, precipitation, and etc.) were recorded using a Watch Dog 900 Series Weather Station (Spectrum Technologies, Inc., Plainfield, IL 60585) that was placed in a soybean field near the center of the field site.

## **Analysis**

Counts of adult western corn rootworms from emergence traps were transformed using square root (+0.05) to stabilize variances and analyzed using SAS PROC MIXED and LSMEANS (SAS Institute 2011) to produce a Type 3 analysis of variance (ANOVA) of beetle emergence per day from 2010 through 2012. Mean male and female emergence per day from refuge and Bt corn in each refuge treatment and corn phenology were determined using SAS PROC MEANS procedure (SAS Institute 2011).

Live collections were transformed using square root ( $x + 0.05$ ) before PROC MIXED Type 3 ANOVA (SAS Institute 2011). The PROC MIXED LSMEANS procedure was used to perform comparison-wise analysis and error rates for live collected beetles/min by refuge treatments, host plant phenology and row number (SAS Institute 2011). Comparison-wise analysis and distribution of means were calculated in order to determine patterns of spatial and temporal abundance or male and female WCR. Distribution of means throughout the collection plots was analyzed using PROC MEANS (SAS Institute 2011).

Abundance data from male and female western corn rootworm adults collected in soybean sweeps were square root ( $x + 0.05$ ) transformed and analyzed using PROC MIXED



ANOVA and PROC MEANS (SAS Institute 2011). These data were analyzed by phenology of nearby cornfields to compare temporal patterns of beetle abundance in soybeans.

## RESULTS

### Emergence trap collections

Western corn rootworm adult emergence began in late June and early July and ended in late July and early August (Table 2). Overall cumulative emergence reached 50% in mid-to-late July with most male and female beetles from refuge and Bt corn reaching 50% cumulative emergence during pollination. Refuge males reached 50% emergence on average 7.9 days before refuge females. Males from Bt corn reached 50% emergence 5.3 days before females from Bt corn (Table 2). Cumulative male and female emergence from refuge corn reached 50% 7.3 days before Bt corn (Table 2). Emergence was more synchronous in treatments with smaller refuges and even more synchronous in the seed blend. The average delay in 50% cumulative emergence between refuge and Bt corn in 20% structured refuge treatments was 11.8 days (males: 14.3 days, females: 9.33 days). In the 5% structured refuge, the delay was 8.7 days (males: 12 days, females: 5.3 days) and in the 5% seed blend refuge, emergence was delayed 1.5 days (males: 0.7 days, females 2.3 days; Table 2). Total duration of emergence from first to last beetle collected each year ranged from 45 days in 2011 to 57 days in 2010. Beetles emerged from refuge corn for 43.1 days (males: 39.7 days, females: 46.4 days) and from Bt corn for 33.4 days (males: 29.3 days, females: 37.4 days; Table 2).

Male and female western corn rootworm emergence rates increased each year of the study with the greatest number of beetles emerging /trap/day in 2012 (males:  $F = 39.32$ ,  $df = 2$ ,

6821,  $P < 0.0001$ ; females:  $F = 62.95$ ,  $df = 2$ , 6821,  $P < 0.0001$ ). Mean female emergence rates (2010:  $0.0356 \pm 0.0037$  per trap/day ( $\pm SE$ ),  $n = 2336$ ; 2011:  $0.1196 \pm 0.0073$ ,  $n = 1919$ ; 2012:  $0.1779 \pm 0.1878$ ,  $n = 2569$ ) were greater than those of males each year ( $F = 19.68$ ,  $df = 1$ , 13646,  $P < 0.0001$ ; 2010:  $0.0214 \pm 0.0027$ ,  $n = 2336$ ; 2011:  $0.0510 \pm 0.0043$ ,  $n = 1919$ ; 2012:  $0.1203 \pm 0.0184$ ,  $n = 2569$ ; Table 3, Figures 3 – 5). Overall more females emerged than males, generating low sex ratios (2010: 0.56; 2011: 0.44; 2012: 0.67).

Corn phenology significantly affected daily beetle emergence each year (2010:  $F = 25.26$ ,  $df = 2$ , 2315,  $P < 0.0001$ ; 2011:  $F = 17.17$ ,  $df = 2$ , 1898  $P < 0.0001$ ; 2012:  $F = 156.35$ ,  $df = 2$ , 2548  $P < 0.0001$ ; Table 3). The significant effects of phenology and corn type in 2010 and 2012 revealed that emergence rates from refuge corn were greatest during the pollination (2010:  $F = 10.59$ ,  $df = 2$ , 2548,  $P < 0.0001$ ; 2011:  $F = 0.13$ ,  $df = 2$ , 2548,  $P = 0.8763$ ; 2012:  $F = 43.09$ ,  $df = 2$ , 2548,  $P < 0.0001$ ; Table 3). There was no difference in emergence rates from Bt corn by phenology in 2010 but emergence rates from Bt corn were greatest during pollination in 2012 (Table 3). The relationship between phenology and corn type had no effect on male and female emergence rates in 2011 (Table 3).

Overall emergence rates from refuge corn were greater than those from Bt corn in each refuge treatment during each year (2010:  $F = 15.20$ ,  $df = 2$ , 2315,  $P < 0.0001$ ; 2011:  $F = 12.31$ ,  $df = 2$ , 1898,  $P < 0.0001$ ; 2012:  $F = 13.41$ ,  $df = 2$ , 2548,  $P < 0.0001$ ). Male emergence rates from refuge corn were significantly affected by refuge treatment (2010:  $F = 18.36$ ,  $df = 2$ , 2315,  $P < 0.0001$ ; 2011:  $F = 4.50$ ,  $df = 2$ , 1898,  $P = 0.0113$ ; 2012:  $F = 19.62$ ,  $df = 2$ , 2548,  $P < 0.0001$ ). In 2010, the greatest emergence rate was in the 5% structured refuge, in 2011, in the 20% and 5% structured refuges (20% structured  $0.1236 \pm 0.0205$  adults/trap/day (mean  $\pm SE$ ); 5% structured

0.0826 ± 0.0165; 5% seed blend 0.0622 ± 0.0111) and in 2012, in 20% structured refuge (20% structured 0.2359 ± 0.0573 adults/trap/day; 5% structured 0.4108 ± 0.1283; 5% seed blend 0.0547 ± 0.0160). However, female emergence from refuge corn did not always differ by refuge treatment (2010:  $F = 5.98$ ,  $df = 2$ , 2315,  $P = 0.0026$ ; 2011:  $F = 10.98$ ,  $df = 2$ , 1898,  $P < 0.0001$ ; 2012:  $F = 13.41$ ,  $df = 2$ , 2548,  $P < 0.0001$ ). Female emergence did not differ between refuge treatments in 2010 (20% structured 0.0596 ± 0.0139 adults/trap/day (mean ± SE); 5% structured 0.1134 ± 0.01773 adults/trap/day; 5% seed blend 0.0551 ± 0.0147 adults/trap/day) or 2011 (20% structured 0.2625 ± 0.0312 adults/trap/day; 5% structured 0.1476 ± 0.0253 adults/trap/day; 5% seed blend 0.1889 ± 0.0255 adults/trap/day) but in 2012 emergence rates from structured refuge treatments were greater than those from the seed blend (20% structured 0.3224 ± 0.0727 adults/trap/day; 5% structured 0.4487 ± 0.1139 adults/trap/day; 5% seed blend 0.1474 ± 0.0406 adults/trap/day). Male and female emergence rates from Bt corn did not differ between refuge treatments (means not shown).

### **Adult live collections**

Male and female western corn rootworm live collection rates increased each year of this study (Male:  $F = 414.72$ ,  $df = 2$ , 11366,  $P < 0.0001$ ; Female:  $F = 364.42$ ,  $df = 2$ , 11366,  $P < 0.0001$ ). Mean female collection rates were greater than male collection rates in 2010 (male: 0.3548 ± 0.0116 adults/min (mean ± SE); female: 0.3709 ± 0.0109 adults/min) and 2012 (male: 0.8875 ± 0.0186 adults/min; female: 0.8877 ± 0.0179 adults/min); however, male and female collection rates did not differ in 2011 (male: 0.5098 ± 0.0145 adults/min; female: 0.9798 ± 0.0275 (adults/min ± SE); 2010:  $F = 5.95$ ,  $df = 1$ ,  $P = 0.0147$ ; 2011:  $F = 299.94$ ,  $df = 1$ ,  $P < 0.0001$ ;

2012:  $F = 0.00$ ,  $df = 1$ ,  $P = 0.9895$ ). The male:female sex ratio was nearly even in 2010 (0.99) and 2011 (1.01) but lower in 2012 (0.53).

Corn phenology significantly affected western corn rootworm collection rates during each year of this study; collection rates during pollination and post-pollination were significantly greater than those during vegetation (2010:  $F = 282.42$ ,  $df = 2$ , 3847,  $P < 0.0001$ ; 2011:  $F = 858.07$ ,  $df = 2$ , 2904,  $P < 0.001$ ; 2012:  $F = 115.17$ ,  $df = 2$ , 4222,  $P < 0.001$ ). In 2010 and 2012, collection rates differed during all three stages of phenology with the greatest male collection rates recorded during pollination (2010: Vegetative:  $0.2187 \pm 0.0195$  adults/min (mean  $\pm$  SE), Pollination  $0.4732 \pm 0.0261$  adults/min, Post-pollination  $0.3979 \pm 0.0168$  adults/min; 2011: Vegetative:  $0.3222 \pm 0.0255$  adults/min, Pollination:  $0.8276 \pm 0.0307$  adults/min, Post-pollination  $0.4826 \pm 0.0169$  adults/min) and the greatest female collection rates during post-pollination (2010: Vegetative:  $0.04612 \pm 0.0052$  adults/min, Pollination  $0.2845 \pm 0.0203$  adults/min, Post-pollination  $0.6417 \pm 0.0195$  adults/min; 2011: Vegetative  $0.1972 \pm 0.0205$  adults/min, Pollination  $1.2480 \pm 0.0494$  adults/min, Post-pollination  $1.6565 \pm 0.0565$  adults/min). In 2012, pollination and post-pollination did not differ; male collection rates were greatest in pollination (2012: Vegetative:  $0.4716 \pm 0.0320$  adults/min, Pollination:  $1.314 \pm 0.0433$  adults/min, Post-pollination  $1.0922 \pm 0.0233$  adults/min) but female collection rates did not differ during pollination and post-pollination (2012: Vegetative:  $0.1711 \pm 0.0130$  adults/min, Pollination:  $1.4060 \pm 0.0515$  adults/min, Post-pollination  $1.3314 \pm 0.0270$  adults/min).

The refuge treatment had a significant effect on beetle collection rates each year (2010:  $F = 23.65$ ,  $df = 3$ , 3847,  $P < 0.0001$ ; 2011:  $F = 23.35$ ,  $df = 3$ , 2904  $P < 0.0001$ ; 2012:  $F = 22.09$ ,  $df =$

3, 4222,  $P < 0.0001$ ). In 2010, collection rates in 20% and 5% structured refuges did not differ for male or females; numerically, beetles/min peaked in 5% structured refuge. Collection rates in the 5% seed blend did not differ from those in 20% or 0% structured refuge treatments though 20% and 0% refuge treatments differed from one another (Table 4). In 2011, collection rates were greatest in the 20% structured refuge. The 5% structured and seed blend refuge treatments did not differ and the 0% refuge treatment produced the fewest beetles/min (Table 5). In 2012, the 20% structured refuge treatment differed from all other treatments with the greatest beetles/min. The 5% structured and seed blend treatments did not differ and the 5% seed blend did not differ from the 0% refuge treatment (Table 6).

Western corn rootworm collection rates were also significantly influenced by an interaction among corn phenology, refuge treatment and row order in all three years of this study (2010:  $F = 1.93$ ,  $df = 60$ , 3847,  $P < 0.0001$ ; 2011:  $F = 2.85$ ,  $df = 60$ , 2904,  $P < 0.001$ ; 2012:  $F = 3.47$ ,  $df = 60$ , 4222,  $P < 0.001$ ). This pattern occurs because a specific corn type (refuge or Bt corn) was planted in only certain rows of the structured refuge treatments, Male and female beetles were collected at a greater rate in refuge rows and in Bt corn rows adjacent to refuge rows in 20% and 5% structured refuge treatments each year (Tables 4 – 6). Male and female collection rates from Bt corn rows in structured refuge treatments increased significantly during pollination in 2010 and during pollination and post-pollination in 2011 and 2012 (Tables 4 - 6). In 5% seed blend treatments in 2010 and 2012, male and female collection rates did not differ between rows during vegetative and pollination stages and were greatest during post-pollination; in 2011, collection rates among rows were greatest and did not differ during pollination and post-pollination (Tables 4 - 6). In 2010, the 0% refuge treatment showed no

difference in male and female collection rates between rows until post-pollination; in 2011 and 2012, there was no difference in male and female collection rates during pollination and post-pollination stages; however, the greatest adult collection rates were obtained during those stages of corn phenology (Tables 4 - 6). In 2011 and 2012, there were significantly greater rates of male and female collection in sampling rows on the west sides of 0% refuge treatments (i.e., rows corresponding to locations of refuges in structured refuge treatments that occupied these locations in previous years; Tables 4 - 6).

### **Abundance in soybeans**

Western corn rootworm adult abundance in soybean plots, measured as beetles/per 100 sweeps, increased significantly each year of the study ( $F = 114.82$ ,  $df = 2, 720$ ,  $P < 0.0001$ ). Overall mean female abundance (2010:  $1.30 \pm 0.16$  adults/100 sweeps (mean  $\pm$  SE); 2011:  $2.04 \pm 0.34$  adults/100 sweeps; 2012:  $11.60 \pm 1.36$  adults/100 sweeps) was significantly greater than male abundance (2010:  $0.10 \pm 0.03$  adults/100 sweeps; 2011:  $0.27 \pm 0.05$  adults/100 sweeps; 2012:  $2.74 \pm 0.37$  adults/100 sweeps) each year ( $F = 92.13$ ,  $df = 1, 720$   $P < 0.0001$ ). Male:female sex ratios were consistently low in soybeans (2010: 0.12; 2011: 0.32; 2012: 0.31).

Corn phenology in the adjacent cornfields had a significant influence on the total number of beetles collected in soybeans (2010:  $F = 42.27$ ,  $df = 2, 129$ ,  $P < 0.001$ ; 2011:  $F = 12.72$ ,  $df = 2, 89$ ,  $P < 0.0001$ ; 2012:  $F = 237.68$ ,  $df = 2, 142$ ,  $P < 0.001$ ). Male abundance in soybean did not differ by corn phenology in 2010 or 2011; however, significantly greater male abundance was observed during post-pollination 2012 (Table 7). Each year the female

abundance was greatest during post-pollination, there were no differences in female abundance between the vegetative and pollination periods (Table 7).

## DISCUSSION

### Emergence trap collections

Comparisons of 50% emergence between refuge and transgenic plants, in treatments containing both refuge and Bt corn types, revealed a delay in emergence from Bt corn compared with refuge corn. This is underscored by peaks in emergence rates from refuge corn during pollination and from Bt corn during post-pollination. Overall emergence rates were greatest during pollination which may reflect limited larval ability to develop on maturing corn roots during post-pollination. Hibbard et al. (2008) found that larval development was affected by plant phenology, with fewer adults emerging when larvae eclosed and fed on roots of reproductive age corn plants, a conclusion consistent with the findings of this study. Adult emergence rates from refuge and Bt corn were more synchronous in smaller refuges and seed blends. Murphy et al. (2010) reported that emergence between refuge and Bt corn was more synchronous in cornfields planted as seed blends than in cornfields with the same percentage of refuge corn planted in a structured refuge configuration. This was also true for male and female emergence in this study. In some cases (Table 2), females emerged before males. In contrast to the findings of Murphy et al. (2010), who reported that more beetles emerged from Bt corn in seed blends than in structured refuges, I found no difference in rates of emergence from Bt corn between refuge treatments. The lack of variation in the numbers of beetles

emerging from Bt corn may be related to the local suppression of western corn rootworm populations that has followed the wide adoption of Bt hybrids that target corn rootworms.

Average duration of the adult emergence interval was shorter for females than males, and shorter for beetles emerging from Bt compared with those emerging from refuge corn. These differences in duration of beetle emergence were likely due to protandry and the delay in emergence from Bt corn. The declining quality of Bt corn plants during emergence from those plants (Hibbard et al. 2008) also likely contributes to shortening the period of Bt female emergence. The vigor of beetles emerging from maturing corn plants during post-pollination and the lack of preferred foods late in the growing season arguably put these adults at a significant competitive disadvantage compared with beetles that emerged during or before pollination.

Early emergence of refuge males and the delayed emergence of females in Bt corn (Murphy et al. 2010) may limit the mixing of susceptible and potentially resistant males and females in structured refuges. Pan et al. (2011) produced models indicating that resistance to Bt evolved more quickly as the delay between emergence of male and female beetles from refuge and transgenic corn grew. The increased synchrony of emergence in seed blends compared with structured refuge configurations should generally benefit IRM based on Pan et al. (2011) model outcomes related to emergence delays. However, the mechanism that facilitates the greater synchrony of emergence must be considered. Synchrony may be a result of larval movement between refuge and Bt corn plants described by Hibbard et al. (2005). The random distribution of refuge plants amidst Bt corn plants within a seed blend means that refuge plants are likely to be surrounded by Bt corn plants. Seed blends make it possible for



larvae that are capable of retreating after feeding on a Bt plant to encounter a more hospitable refuge plant. The possibility of this larval movement increases the likelihood of survival among larvae that have been exposed to, but not killed by, Bt toxins (Hibbard et al. 2005). In a structured refuge configuration there is only a limited linear interface between refuge and Bt corn. While larval movement between Bt and refuge plants can occur across the interface, only a small fraction of larvae will be situated to take advantage of the nearby refuge. Because of limited larval movement capabilities, structured refuge configurations limit opportunities for Bt exposed larvae to retreat from Bt corn to find refuge corn where they may complete development. If movement away from a Bt corn plant after consumption of a sub-lethal dose of toxin is heritable, larval movement has the potential to become a mechanism of western corn rootworm resistance to Bt corn. The risk of behavioral resistance to Bt corn among western corn rootworm larvae in seed blends must be weighed against the benefit of assured refuge compliance that seed blends guarantee.

The homogenous distribution of refuge plants within seed blend fields provides another important benefit of an integrated refuge that likely affects adult mate-seeking behavior. The scattered spatial distribution of refuge plants throughout a seed blend field assures that beetles emerging from refuge plants are not concentrated in small areas as was observed in structured refuge treatments. Combined with the greater temporal synchrony between male and female emergence from refuge and Bt corn plants, the scattered distribution of refuge plants that produce Bt susceptible adults generates homogeneous patterns of male and female abundance. These uniform distributions will arguably facilitate a more rapid and thorough

mixing of mate-seeking western corn rootworm adults from refuge and Bt corn, an expectation that is fundamental to basic assumptions about how refuges function.

The five percent structured refuge treatment produced beetles at a greater rate than 5% seed blend configurations (which had emergence rates that were not different from those in cornfields with 0% refuge) suggesting that an aggregation rather than sparse placement of refuge plants may support a greater overall larval population. Another explanation for this pattern is that some larvae feeding on the roots of Bt corn plants may opt to move between plants (Hibbard et al. 2005). With a 90 - 95% probability that a moving larva will find a Bt corn plant in a seed blend, wandering refuge larvae are likely to settle on a Bt plant and be killed or exhaust themselves searching for a palatable root. Since only first instar larvae are highly susceptible to Bt toxin (Binning et al. 2010), the age at which larvae move between roots is an important factor in the survival of moving larvae. The consequences of failing to find a refuge plant may be quite different for vulnerable neonates compared to second instars in seed blends. In addition to the risk of failing to find a refuge plant, moving exposes larvae to risks like predation. While the consequences of larval movement may reduce the number of western corn rootworm larvae present in a field, if the tendency to move is heritable and a portion of movers are successful in finding refuge plants, the use of seed blend could select for evolution of behavioral resistance to Bt.

### **Adult live collections**

The greatest abundance of live collected western corn rootworm adults per minute was measured in refuge rows within structured refuge treatments. My data suggest that refuge

adults fail to move far from refuge rows for long periods after emergence, resulting in greater abundance around refuges and a sustained scarcity of beetles in Bt corn, especially in the rows farthest from structured refuges. These data suggest that western corn rootworm adults may not be as mobile or as likely to engage in intrafield movement as was once assumed. In Bt corn rows, adult abundance did eventually increase, but not until late in the season. This change in abundance may have been influenced more by late emergence of adults from Bt corn than movement of mate-seeking adults from refuge rows.

The seed blend refuge configuration generated an even distribution of western corn rootworm adults across the plot throughout the season. After the first year of the study, there was an increase in the row-to-row abundance variability in treatments with uniform distributions of plants (i.e., seed blend and 0% refuge treatments). This may be a consequence of the random assignment of treatments to subplots and aggregation of adults in and around refuge corn rows. Refuge corn in structured refuge plots was always located along the west side of the plots. During this study, the annual concentration of beetles in structured refuges likely contributed to increased oviposition in the west sides of these plots. When a 5% seed blend or 0% refuge treatment was planted in a subplot that previously hosted a structured refuge treatment more beetles were collected from rows on the west side of the subplots. This was likely because more egg laying occurred in the soil where beetle abundance was greater the previous year. Thus, the unexpected peaks of abundance in the 5% seed blend and 0% refuge treatments may be evidence of a growing population or aggregated oviposition in continuous refuge corn.

The combination of adults lingering in refuge rows, delayed adult emergence from Bt corn and the limited reproductive lifespan of males (Kang and Krupke 2009), suggest that few refuge males may be capable of mating when females emerge from Bt corn (Spencer et al. 2013). In simulation models, Pan et al. (2011) found that Bt resistance evolved faster when the duration of the male reproductive life span was shortened. Pan et al. (2011) also explored effects of delayed female emergence from Bt corn; they questioned whether late emerging females could complete their reproductive development and deposit eggs before corn plants become poor food sources. In the current study, females emerging from Bt corn sometimes did not reach 50% cumulative emergence until the post-pollination stage of corn phenology. Murphy et al. (2010) also reported peak cumulative emergence on collection dates when corn would have been during post-pollination. These Bt females would emerge into a field of maturing corn plants and have little or no access to the high quality corn tissues that were available to early emerging females. If Bt resistant individuals are among late emerging adults, resource constraints may limit their fecundity, dispersal and opportunity to contribute their resistance alleles to the next generation. Knowing whether Bt resistant individuals are present or absent among the late emerging beetles could affect IRM and monitoring plans in fields where resistant beetles may be present.

While the increase in late season adult emergence from Bt corn may focus attention on individuals surviving on Bt plants, perhaps the focus should be shifted to adults that emerge from Bt plants concurrent with those emerging from refuge plants. Unlike late emerging adults, if early emerging adults from Bt plants are Bt resistant, they may have a long period of reproduction, while host plant tissues are most nutritious, and have more opportunities to

move between fields or to move long distances. Determining when Bt resistant adults emerge would enable resistance monitoring and management to be focused on the most threatening portion of the population.

### **Abundance in soybeans**

The sex ratio of western corn rootworm adults collected in soybeans was strongly female biased, with 1 male for every 3 or more females. This is characteristic of rotation resistant populations (O'Neal et al. 1999), which have been documented throughout east central Illinois, and in surrounding states (Spencer et al. 2005, Gray et al. 2009). It also suggests that many females are leaving their natal cornfields after emergence and dispersing to nearby fields. The presence of numerous western corn rootworm adults moving between corn and soybean fields, while feeding and laying eggs in both crops, are also common in rotation resistant populations (Mabry et al. 2004, Mabry and Spencer 2003, Pierce and Gray 2006). The continued presence of rotation resistant western corn rootworm adults at the study site was documented during the course of this experiment. Female western corn rootworms were consistently more abundant in soybean sweep samples than males and abundance was always greatest during post-pollination. O'Neal et al. (1999), similarly, found a greater proportion of females in soybeans than corn using yellow sticky traps and vial traps in east central Illinois. Pierce and Gray's (2006) study of egg accumulation in soybeans also indicated that populations of female beetles in soybeans increase as the season progresses and the corn matures and enters the reproductive (i.e., pollination) phenology stage. It has been suggested that movement from corn late in the season could be related to the declining quality of corn as a

food source (O'Neal et al. 2004) or as a more general response to corn phenology, particularly at the R2 stage of corn development (Pierce and Gray 2006). The dramatic increases in western corn rootworm abundance in soybeans following the end of corn pollination in this study were consistent with the response to phenology documented by Pierce and Gray (2006).

The mean number of beetles collected per 100 sweeps has declined dramatically compared with mean collection rates recorded before the introduction of Bt hybrids targeting corn rootworm. Spencer et al. (1997) recorded 59 beetles per 100 sweeps in soybean at the same location. This comparison provides additional illustration of the overall suppression of western corn rootworm population in east central Illinois since the introduction of Bt hybrids. If Bt resistant western corn rootworms are found in the rotation resistance area, it is reasonable to expect that severe injury to first-year Bt cornfields will be observed. The link between long term continuous Bt corn cultivation and field selection for Bt resistance (Gassmann et al. 2011), may slow the spread of Bt resistance into areas where corn is still commonly rotated. However, once resistance allele frequencies rise among rotation resistant populations, Bt resistance may be observed in rotated corn. This would dramatically increase the potential for Bt resistance to affect corn production.

Over the course of this study, western corn rootworms were collected with increasing abundance each year. This increase may be a consequence of planting continuous corn, which allows rootworm populations to grow. However, planting Bt hybrids continuously will limit population growth in the absence of Bt resistance. The increasing beetle abundance in this study may be attributed to a very local buildup of western corn rootworm populations in the study fields which experienced focused oviposition in refuges as hypothesized above.

Weather was also likely a contributing factor in abundance pattern variation at times during the experiments. In 2010, high precipitation left portions of two 20% refuge replicates and corresponding emergence traps submerged for part of June. Subsequently, few beetles emerged from these locations until later in the season. In 2011, high rainfall early in the season led to muddy field conditions and delayed planting by one month. In 2012 and the latter half of 2011, the Midwest experienced an historic drought which resulted in severe plant stress, dry hardened soils and other conditions that likely affected beetle emergence and survival.

Data presented in this study clearly demonstrate how refuge configuration (i.e., structured or seed blend refuge) can affect patterns of western corn rootworm emergence and abundance. I have documented abundance patterns suggesting that beetles emerging within the refuge tend to remain there rather than dispersing to Bt corn. It is likely that emergence, rather than movement, caused increases in western corn rootworm abundance from Bt corn within structured refuge treatments. Structured refuge configurations have been favored for addressing concerns about potential negative consequences of larval movement. However, a lack of significant intrafield movement by mate-seeking adults from the refuge, will not yield the mixed matings central to expectations of the refuge strategy. The seed blend refuge configuration offers a distribution of Bt corn plants in the landscape that will produce an even distributions of emergence and adult abundance that will better facilitate the mixed matings of adults from refuge and Bt corn (Onstad et al. 2001, 2011; Spencer et al. 2013). Seed blends also ensure grower compliance with refuge requirements which declined after the introduction of stacked hybrids (Jaffe 2009).

While seed blends are expected to be used on a majority of corn acres in 2013, this mechanism of deploying refuge in Bt cornfields is still a relatively new option for corn rootworm management in the US and Canada. The presumed benefits of adopting integrated refuge should not overshadow a continued need to document their impact on western corn rootworm ecology. Broad adoption of seed blends will increase risks associated with larval movement and increased selection pressure caused by increasing adoption of Bt hybrids expressing just a few different Cry proteins. The protection of susceptibility through adult western corn rootworm movement, offered by guaranteeing that sufficient refuge is planted, may outweigh these risks.



FIGURES AND TABLES

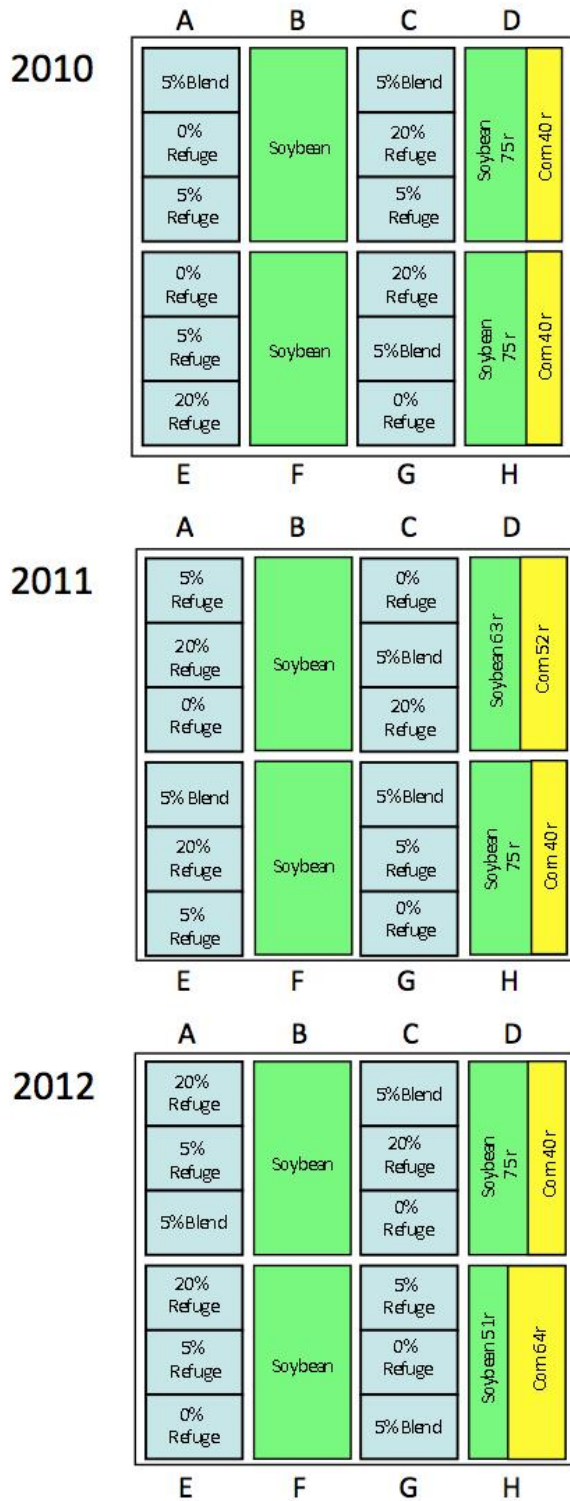


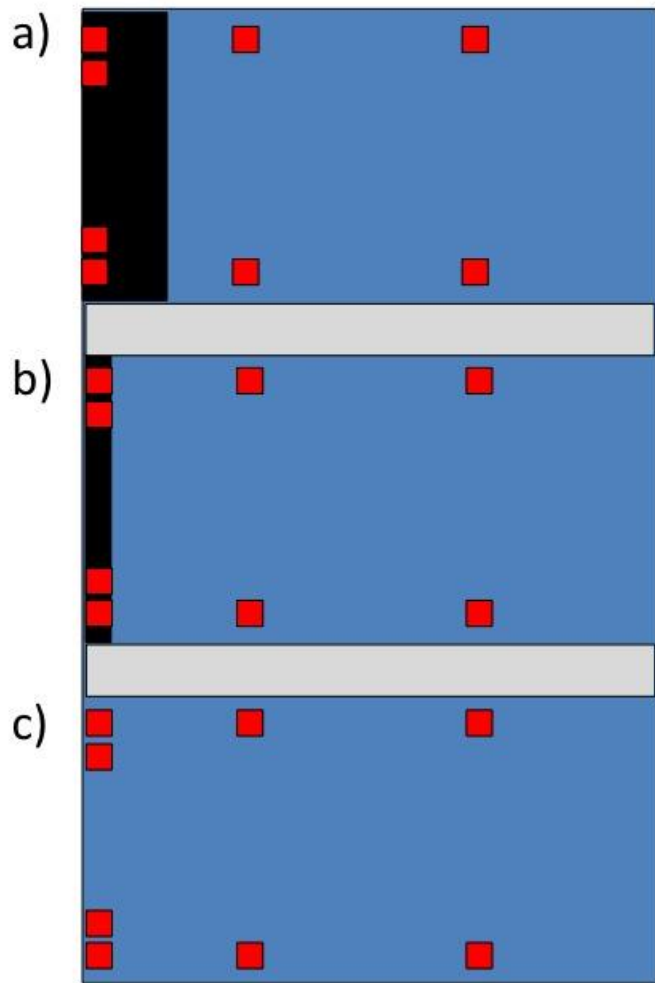
Figure 1. 2010-2012 treatment subplot layouts in lettered plots at the Lost 40 field location. Treatment locations were randomly assigned each year. See text for detailed descriptions.

**Table 1.** Dates of corn planting and duration of phenology intervals in 2010, 2011 and 2012.

	2010	2011	2012
Corn Planting Date	20 April	20 May	19 April
Phenology Interval <sup>a</sup>			
Vegetative	21 June - 5 July (JD 172 - 186 <sup>b</sup> )	30 June - 18 July (JD 181 - 199)	12 June - 1 July (JD 164 - 183)
Pollination	6 July - 16 July (JD 187 - 197)	19 July - 29 July (JD 200-210)	2 July - 12 July (JD 184 - 194)
Post-pollination	17 July - 26 August (JD 198 - 238)	30 July - 16 August (JD 211 - 228)	13 July - 14 August (JD 195 - 227)

<sup>a</sup> Phenology was determined based on Ritchie et al. 1993.

<sup>b</sup> JD indicates Julian date



**Figure 2.** Emergence trap distribution within plots of varying refuge treatments; (a) 20% structured refuge treatment, (b) 5% structured refuge treatment, (c) 5% seed blend refuge and 0% refuge treatment. Black strip along the left side of plots a and b represent refuge corn, blue represents Bt corn, red squares represent emergence traps and bare soil alleyways are gray.

**Table 2.** Timing and duration of male and female western corn rootworm emergence from refuge and Bt corn by treatment in 2010, 2011 and 2012.

Treatment	Refuge Males				Refuge Females				Bt Males				Bt Females			
	Start	50% <sup>a</sup>	End	Days <sup>b</sup>	Start	50%	End	Days	Start	50%	End	Days	Start	50%	End	Days
2010																
20% Structured	179	183	221	43	181	183	237	57	197	197	197	1	193	197	211	19
5% Structured	179	186	221	43	179	197	230	52	186	195	200	15	190	197	218	29
5% Seed Blend	179	188	204	26	179	200	232	54	179	195	204	26	193	204	237	45
0% Refuge	---	---	---	---	---	---	---	---	181	195	221	41	181	200	228	48
2011																
20% Structured	192	183	227	36	192	210	231	40	199	208	222	24	183	217	231	29
5% Structured	186	196	227	42	186	206	229	44	217	217	227	11	199	215	227	29
5% Seed Blend	186	213	231	46	196	217	231	36	192	208	227	36	192	213	229	38
0% Refuge	---	---	---	---	---	---	---	---	196	213	229	34	192	220	231	40
2012																
20% Structured	170	187	209	40	172	188	219	48	174	191	205	32	184	195	219	36
5% Structured	170	187	209	40	170	188	212	43	177	193	219	44	177	195	219	44
5% Seed Blend	174	188	214	41	177	193	219	44	177	188	214	38	174	200	214	41
0% Refuge	---	---	---	---	---	---	---	---	163	188	212	50	174	198	221	48

<sup>a</sup> 50% indicates the date at which 50% of total western corn rootworm adult males or females had emerged from the specified corn type and treatment.

<sup>b</sup> Days indicate the duration of western corn rootworm emergence from the specified corn type and treatment.

**Table 3.** Western corn rootworm emergence per trap per day (mean  $\pm$  SE) from refuge and Bt corn in 2010, 2011 and 2012.

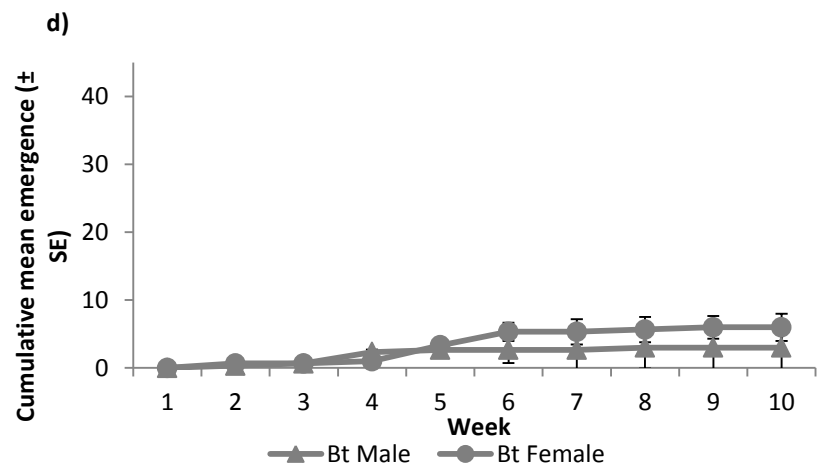
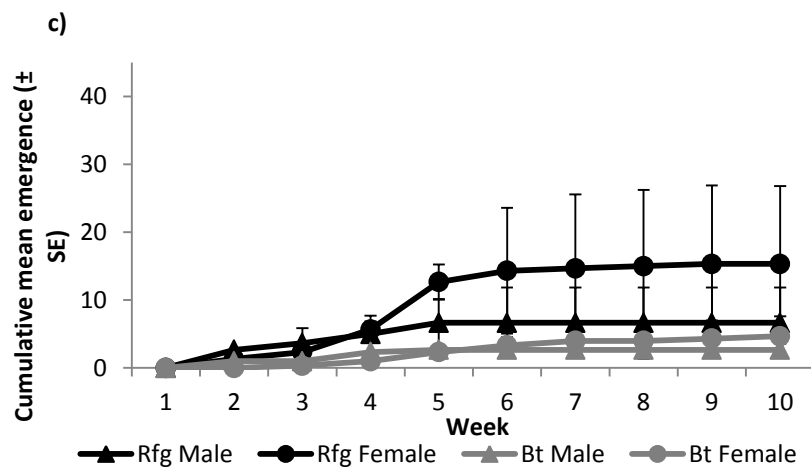
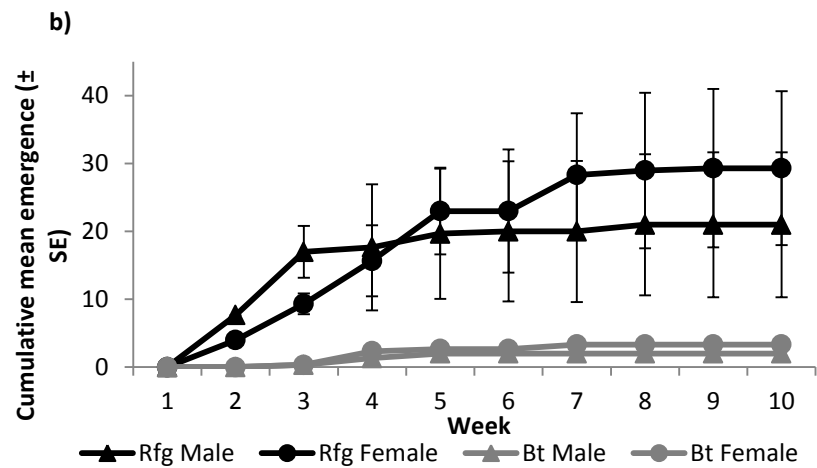
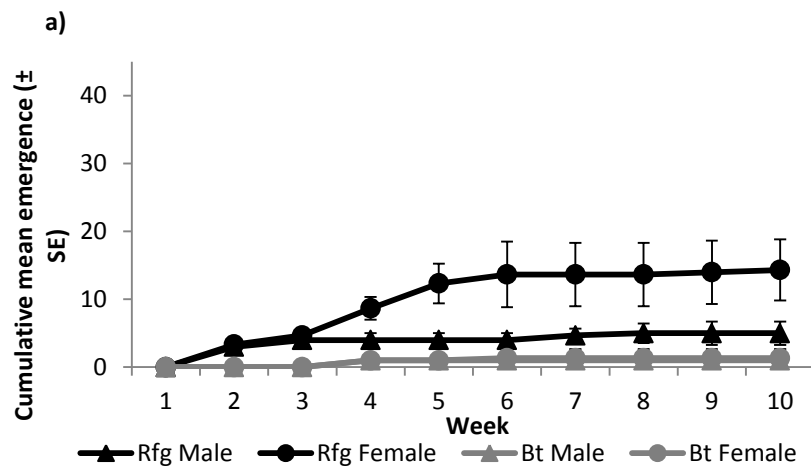
Year	Corn Type <sup>b</sup>	Corn Phenology <sup>a</sup>								
		Vegetative			Pollination			Post-pollination		
		n <sup>c</sup>	Male	Female	n	Male	Female	n	Male	Female
2010	All	576	0.0440 $\pm$ 0.0088 a	0.0284 $\pm$ 0.0060 a	480	0.0363 $\pm$ 0.0070 a	0.0662 $\pm$ 0.0110 a	1280	0.0056 $\pm$ 0.0014 a	0.0275 $\pm$ 0.0044 a
2011	All	576	0.0511 $\pm$ 0.0087 a	0.0560 $\pm$ 0.0107 b	384	0.0577 $\pm$ 0.0109 a	0.0998 $\pm$ 0.0150 b	959	0.0483 $\pm$ 0.0054 a	0.1658 $\pm$ 0.0115 a
2010	All	889	0.0896 $\pm$ 0.0146 b	0.0397 $\pm$ 0.0087 c	432	0.4491 $\pm$ 0.1030 a	0.6223 $\pm$ 0.1036 a	1248	0.0283 $\pm$ 0.0045 c	0.1225 $\pm$ 0.0102 b
2010	Rfg	216	0.1073 $\pm$ 0.0225 c	0.0710 $\pm$ 0.0150 b	180	0.0559 $\pm$ 0.0149 a	0.1404 $\pm$ 0.0265 a	480	0.0116 $\pm$ 0.0035 b	0.1070 $\pm$ 0.0002 b
	Bt	360	0.0006 $\pm$ 0.0027 a	0.0527 $\pm$ 0.0028 a	300	0.0246 $\pm$ 0.0065 a	0.0217 $\pm$ 0.0066 a	800	0.0020 $\pm$ 0.0009 a	0.0542 $\pm$ 0.0111 a
2011	Rfg	216	0.1169 $\pm$ 0.0215 a	0.1362 $\pm$ 0.0272 b	144	0.0856 $\pm$ 0.0227 a	0.2037 $\pm$ 0.0265 ab	360	0.0824 $\pm$ 0.0115 a	0.2602 $\pm$ 0.0248 a
	Bt	360	0.0116 $\pm$ 0.0037 a	0.0079 $\pm$ 0.0031 b	240	0.0410 $\pm$ 0.0107 a	0.0375 $\pm$ 0.0092 b	599	0.0278 $\pm$ 0.0048 a	0.1091 $\pm$ 0.0102 a
2012	Rfg	337	0.1988 $\pm$ 0.0367 c	0.0959 $\pm$ 0.0222 b	160	0.9104 $\pm$ 0.2704 a	1.2635 $\pm$ 0.2648 a	468	0.0296 $\pm$ 0.0067 b	0.1318 $\pm$ 0.0185 b
	Bt	552	0.0229 $\pm$ 0.0054 b	0.0054 $\pm$ 0.0024 c	272	0.1777 $\pm$ 0.0289 a	0.2451 $\pm$ 0.0390 a	780	0.0276 $\pm$ 0.0060 b	0.1169 $\pm$ 0.0119 b

Means followed by same letter(s) and sex in a row are not significantly different ( $P = 0.0009$ ) with Bonferroni correction. Analyses of variance performed using square root transformed ( $x + 0.05$ ) data, untransformed data are shown.

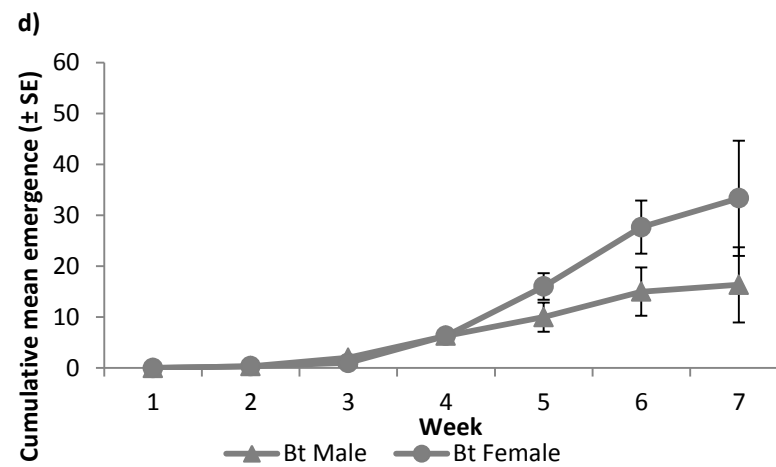
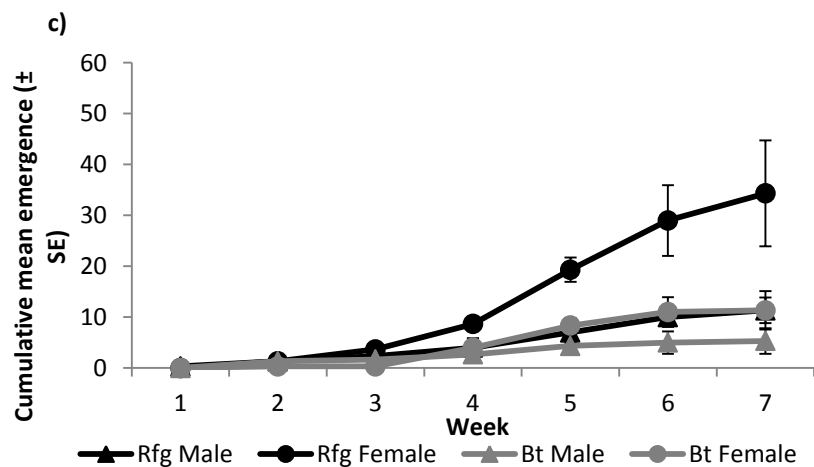
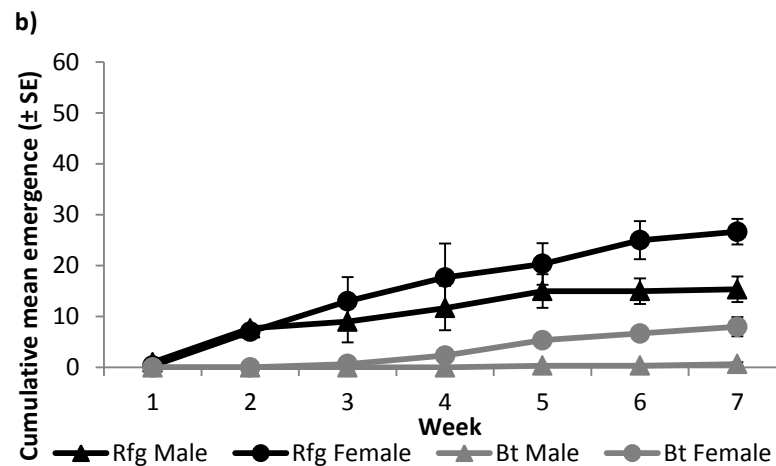
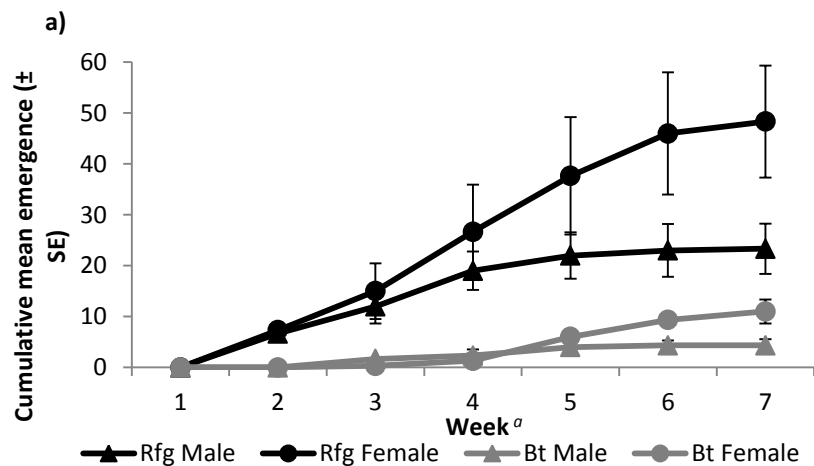
<sup>a</sup> Phenology was determined based on Ritchie et al. 1993.

<sup>b</sup> Abbreviations represent the corn type from which beetles emerged: refuge corn (Rfg), Bt corn (Bt) and emergence regardless of corn type (All). See text for further explanation.

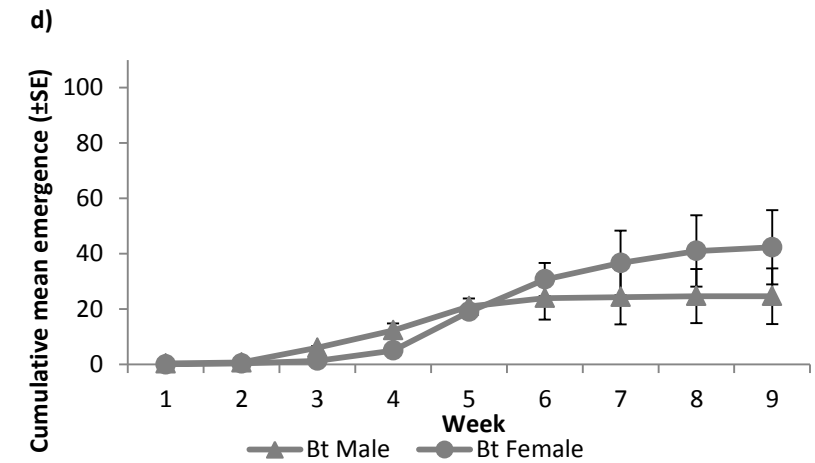
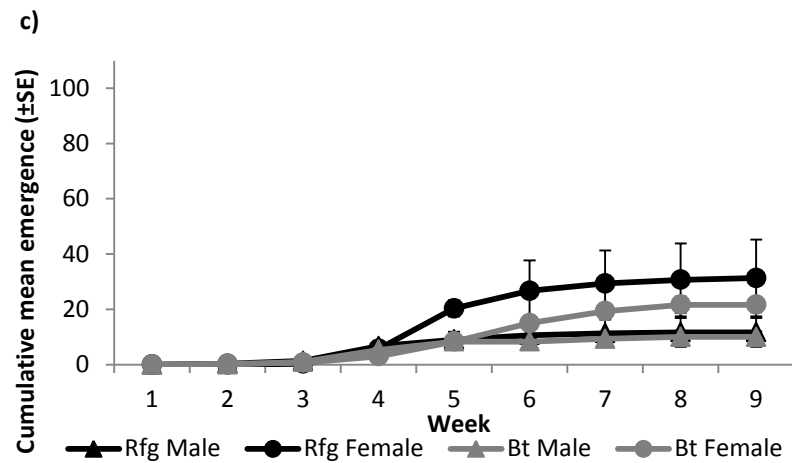
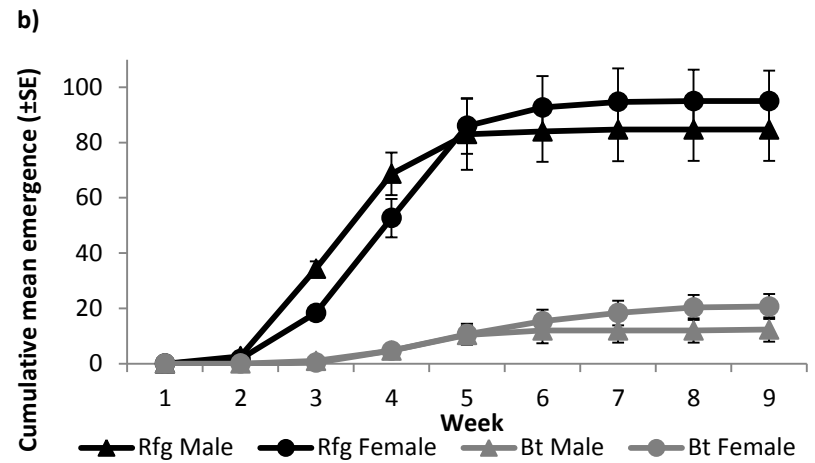
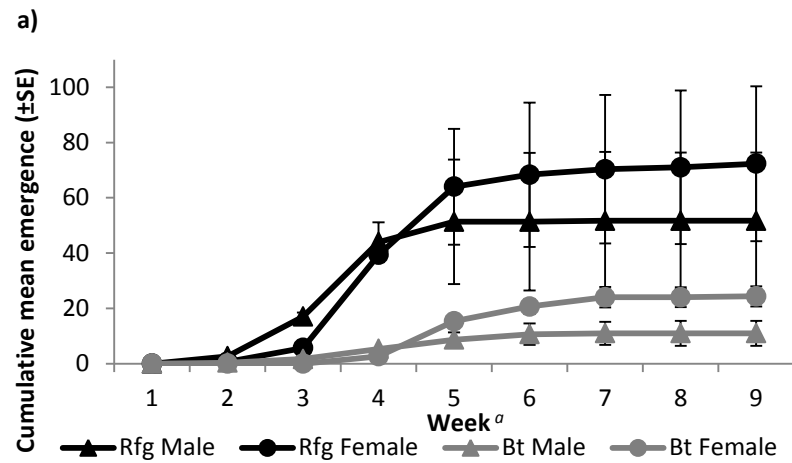
<sup>c</sup> n indicates the number of samples within each mean.



**Figure 3.** Cumulative mean emergence ( $\pm$  SE) of adult male and female western corn rootworm per week from refuge and Bt corn by treatments in 2010; (a) 20% structured refuge, (b) 5% structured refuge, (c) 5% seed blend refuge and (d) 0% refuge. Week 1 began 6/25/10 (Julian date 176).



**Figure 4.** Cumulative mean emergence ( $\pm$  SE) of adult male and female western corn rootworm per week from refuge and Bt corn by treatments in 2011; (a) 20% structured refuge, (b) 5% structured refuge, (c) 5% seed blend refuge and (d) 0% refuge. Week 1 began 6/30/11 (Julian date 181).



**Figure 5.** Cumulative mean emergence ( $\pm$  SE) of adult male and female western corn rootworm per week from refuge and Bt corn by treatments in 2012; (a) 20% structured refuge, (b) 5% structured refuge, (c) 5% seed blend refuge and (d) 0% refuge. Week 1 began 6/12/12 (Julian date 164).



**Table 4.** Spatial and temporal abundance (adults/min; mean  $\pm$  SE) of male and female western corn rootworm adults by corn phenology, refuge treatment and sampling row in 2010.

Treatment	Row <sup>b</sup>	n <sup>c</sup>	Corn Phenology							
			Vegetative <sup>a</sup>			Pollination			Post-pollination	
			Males/min ( $\pm$ SE)	Females/min ( $\pm$ SE)	n	Males/min ( $\pm$ SE)	Females/min ( $\pm$ SE)	n	Males/min ( $\pm$ SE)	Females/min ( $\pm$ SE)
20% Structured Refuge	W20-Rfg	30	0.92 $\pm$ 0.26 a	0.17 $\pm$ 0.05 a	19	1.46 $\pm$ 0.29 a	1.04 $\pm$ 0.14 a	40	0.42 $\pm$ 0.14 a	0.88 $\pm$ 0.17 a
	W12-Rfg	30	1.33 $\pm$ 0.32 a	0.31 $\pm$ 0.09 a	19	1.45 $\pm$ 0.29 a	0.96 $\pm$ 0.19 a	40	0.44 $\pm$ 0.13 a	0.77 $\pm$ 0.11 ab
	W3-Rfg	30	1.02 $\pm$ 0.30 a	0.21 $\pm$ 0.08 a	19	1.15 $\pm$ 0.29 ab	0.66 $\pm$ 0.12 ab	40	0.33 $\pm$ 0.10 a	0.69 $\pm$ 0.08 ab
	E3-Bt	30	0.22 $\pm$ 0.08 b	0.04 $\pm$ 0.02 a	19	0.38 $\pm$ 0.09 c	0.17 $\pm$ 0.07 bc	40	0.36 $\pm$ 0.08 a	0.68 $\pm$ 0.14 abc
	E12-Bt	30	0.16 $\pm$ 0.06 b	0.02 $\pm$ 0.01 a	19	0.26 $\pm$ 0.06 c	0.04 $\pm$ 0.02 c	40	0.27 $\pm$ 0.07 a	0.52 $\pm$ 0.11 abc
	E24-Bt	30	0.13 $\pm$ 0.04 b	0.03 $\pm$ 0.02 a	19	0.14 $\pm$ 0.05 c	0.03 $\pm$ 0.02 c	39	0.32 $\pm$ 0.08 a	0.59 $\pm$ 0.17 abc
	E36-Bt	30	0.12 $\pm$ 0.06 b	0.04 $\pm$ 0.03 a	19	0.18 $\pm$ 0.08 c	0.04 $\pm$ 0.03 c	39	0.21 $\pm$ 0.06 a	0.33 $\pm$ 0.08 c
	E48-Bt	30	0.09 $\pm$ 0.04 b	0.02 $\pm$ 0.01 a	19	0.41 $\pm$ 0.12 c	0.09 $\pm$ 0.05 c	39	0.28 $\pm$ 0.06 a	0.57 $\pm$ 0.11 abc
	E60-Bt	30	0.10 $\pm$ 0.03 b	0.03 $\pm$ 0.02 a	19	0.34 $\pm$ 0.10 c	0.11 $\pm$ 0.04 c	39	0.26 $\pm$ 0.08 a	0.54 $\pm$ 0.11 abc
	E72-Bt	30	0.07 $\pm$ 0.03 b	0.02 $\pm$ 0.02 a	19	0.44 $\pm$ 0.11 c	0.14 $\pm$ 0.07 c	39	0.27 $\pm$ 0.07 a	0.43 $\pm$ 0.09 bc
E84-Bt	29	0.08 $\pm$ 0.04 b	0.03 $\pm$ 0.01 a	19	0.22 $\pm$ 0.11 c	0.05 $\pm$ 0.03 c	39	0.23 $\pm$ 0.08 a	0.43 $\pm$ 0.10 bc	
5% Structured Refuge	W4-Rfg	30	1.52 $\pm$ 0.31 a	0.28 $\pm$ 0.08 a	19	2.17 $\pm$ 0.31 a	1.56 $\pm$ 0.25 a	43	0.71 $\pm$ 0.15 a	1.33 $\pm$ 0.21 a
	W2-Rfg	29	1.54 $\pm$ 0.32 a	0.41 $\pm$ 0.11 a	19	1.84 $\pm$ 0.27 a	1.67 $\pm$ 0.30 a	43	0.71 $\pm$ 0.17 a	1.19 $\pm$ 0.14 a
	E3-Bt	30	0.23 $\pm$ 0.09 b	0.04 $\pm$ 0.02 a	19	0.63 $\pm$ 0.13 b	0.36 $\pm$ 0.14 b	43	0.55 $\pm$ 0.11 ab	1.04 $\pm$ 0.19 ab
	E12-Bt	30	0.03 $\pm$ 0.01 b	0.00 $\pm$ 0.00 a	19	0.29 $\pm$ 0.08 b	0.35 $\pm$ 0.15 b	43	0.38 $\pm$ 0.09 ab	0.52 $\pm$ 0.09 c
	E24-Bt	30	0.03 $\pm$ 0.03 b	0.00 $\pm$ 0.00 a	19	0.40 $\pm$ 0.13 b	0.17 $\pm$ 0.07 b	42	0.23 $\pm$ 0.07 b	0.38 $\pm$ 0.07 c
	E36-Bt	30	0.03 $\pm$ 0.02 b	0.01 $\pm$ 0.01 a	19	0.29 $\pm$ 0.07 b	0.08 $\pm$ 0.04 b	43	0.30 $\pm$ 0.08 ab	0.55 $\pm$ 0.13 c
	E48-Bt	30	0.06 $\pm$ 0.02 b	0.03 $\pm$ 0.01 a	19	0.33 $\pm$ 0.08 b	0.11 $\pm$ 0.06 b	44	0.37 $\pm$ 0.11 ab	0.58 $\pm$ 0.13 c
	E60-Bt	30	0.02 $\pm$ 0.02 b	0.01 $\pm$ 0.01 a	19	0.36 $\pm$ 0.11 b	0.15 $\pm$ 0.06 b	44	0.43 $\pm$ 0.09 ab	0.64 $\pm$ 0.15 bc
	E72-Bt	30	0.03 $\pm$ 0.02 b	0.00 $\pm$ 0.00 a	19	0.46 $\pm$ 0.11 b	0.02 $\pm$ 0.08 b	44	0.42 $\pm$ 0.12 ab	0.49 $\pm$ 0.13 c
	E84-Bt	30	0.05 $\pm$ 0.03 b	0.01 $\pm$ 0.01 a	19	0.60 $\pm$ 0.20 b	0.28 $\pm$ 0.13 b	44	0.25 $\pm$ 0.08 b	0.34 $\pm$ 0.10 c
E96-Bt	30	0.08 $\pm$ 0.03 b	0.00 $\pm$ 0.00 a	19	0.51 $\pm$ 0.18 b	0.23 $\pm$ 0.07 b	44	0.24 $\pm$ 0.11 b	0.42 $\pm$ 0.11 c	
5% Seed Blend	W4-Sb	30	0.08 $\pm$ 0.04 a	0.00 $\pm$ 0.00 a	19	0.25 $\pm$ 0.10 a	0.19 $\pm$ 0.12 a	41	0.49 $\pm$ 0.13 a	1.04 $\pm$ 0.16 a
	W2-Sb	30	0.15 $\pm$ 0.05 a	0.03 $\pm$ 0.02 a	19	0.30 $\pm$ 0.10 a	0.25 $\pm$ 0.12 a	41	0.54 $\pm$ 0.20 a	0.95 $\pm$ 0.17 ab
	E3-Sb	30	0.15 $\pm$ 0.07 a	0.01 $\pm$ 0.01 a	19	0.33 $\pm$ 0.15 a	0.17 $\pm$ 0.06 a	41	0.48 $\pm$ 0.16 a	0.81 $\pm$ 0.15 abc
	E12-Sb	29	0.12 $\pm$ 0.05 a	0.02 $\pm$ 0.01 a	19	0.38 $\pm$ 0.15 a	0.16 $\pm$ 0.09 a	41	0.42 $\pm$ 0.12 a	0.62 $\pm$ 0.13 abc
	E24-Sb	29	0.09 $\pm$ 0.04 a	0.01 $\pm$ 0.01 a	19	0.19 $\pm$ 0.08 a	0.18 $\pm$ 0.11 a	41	0.39 $\pm$ 0.13 a	0.48 $\pm$ 0.11 abc
	E36-Sb	29	0.15 $\pm$ 0.05 a	0.03 $\pm$ 0.02 a	19	0.23 $\pm$ 0.11 a	0.14 $\pm$ 0.07 a	41	0.50 $\pm$ 0.13 a	0.53 $\pm$ 0.12 abc
	E48-Sb	29	0.06 $\pm$ 0.03 a	0.03 $\pm$ 0.01 a	19	0.27 $\pm$ 0.06 a	0.17 $\pm$ 0.05 a	41	0.42 $\pm$ 0.10 a	0.61 $\pm$ 0.10 abc
	E60-Sb	29	0.16 $\pm$ 0.05 a	0.00 $\pm$ 0.00 a	19	0.27 $\pm$ 0.09 a	0.16 $\pm$ 0.06 a	41	0.45 $\pm$ 0.10 a	0.72 $\pm$ 0.13 bc
	E72-Sb	29	0.22 $\pm$ 0.08 a	0.09 $\pm$ 0.04 a	19	0.38 $\pm$ 0.12 a	0.18 $\pm$ 0.07 a	41	0.34 $\pm$ 0.10 a	0.59 $\pm$ 0.10 bc
	E84-Sb	29	0.07 $\pm$ 0.05 a	0.03 $\pm$ 0.02 a	19	0.38 $\pm$ 0.12 a	0.30 $\pm$ 0.07 a	41	0.36 $\pm$ 0.11 a	0.62 $\pm$ 0.15 c
E96-Sb	29	0.14 $\pm$ 0.05 a	0.04 $\pm$ 0.03 a	19	0.38 $\pm$ 0.13 a	0.30 $\pm$ 0.14 a	41	0.45 $\pm$ 0.10 a	0.51 $\pm$ 0.12 c	
0% Refuge	W4-Bt	29	0.01 $\pm$ 0.01 a	0.01 $\pm$ 0.01 a	19	0.26 $\pm$ 0.12 a	0.33 $\pm$ 0.22 a	43	0.50 $\pm$ 0.12 a	0.94 $\pm$ 0.15 a
	W2-Bt	30	0.03 $\pm$ 0.02 a	0.03 $\pm$ 0.01 a	19	0.42 $\pm$ 0.19 a	0.23 $\pm$ 0.15 a	43	0.48 $\pm$ 0.11 a	0.99 $\pm$ 0.17 a
	E3-Bt	30	0.02 $\pm$ 0.01 a	0.01 $\pm$ 0.01 a	19	0.22 $\pm$ 0.07 a	0.10 $\pm$ 0.07 a	43	0.42 $\pm$ 0.10 a	0.56 $\pm$ 0.08 ab
	E12-Bt	30	0.04 $\pm$ 0.02 a	0.01 $\pm$ 0.01 a	19	0.23 $\pm$ 0.06 a	0.11 $\pm$ 0.05 a	43	0.42 $\pm$ 0.10 a	0.59 $\pm$ 0.10 ab
	E24-Bt	30	0.05 $\pm$ 0.02 a	0.00 $\pm$ 0.00 a	19	0.15 $\pm$ 0.05 a	0.07 $\pm$ 0.04 a	43	0.40 $\pm$ 0.10 a	0.62 $\pm$ 0.10 ab
	E36-Bt	30	0.04 $\pm$ 0.02 a	0.00 $\pm$ 0.00 a	19	0.27 $\pm$ 0.06 a	0.09 $\pm$ 0.08 a	43	0.47 $\pm$ 0.14 a	0.61 $\pm$ 0.12 ab
	E48-Bt	30	0.04 $\pm$ 0.03 a	0.00 $\pm$ 0.00 a	19	0.24 $\pm$ 0.12 a	0.08 $\pm$ 0.04 a	43	0.39 $\pm$ 0.10 a	0.57 $\pm$ 0.11 ab
	E60-Bt	30	0.04 $\pm$ 0.03 a	0.00 $\pm$ 0.00 a	19	0.42 $\pm$ 0.16 a	0.16 $\pm$ 0.08 a	43	0.45 $\pm$ 0.13 a	0.51 $\pm$ 0.10 b
	E72-Bt	30	0.05 $\pm$ 0.03 a	0.00 $\pm$ 0.00 a	19	0.39 $\pm$ 0.14 a	0.20 $\pm$ 0.08 a	43	0.32 $\pm$ 0.08 a	0.48 $\pm$ 0.07 b
	E84-Bt	30	0.02 $\pm$ 0.02 a	0.01 $\pm$ 0.01 a	19	0.35 $\pm$ 0.13 a	0.14 $\pm$ 0.07 a	43	0.51 $\pm$ 0.13 a	0.50 $\pm$ 0.09 b
E96-Bt	30	0.04 $\pm$ 0.03 a	0.01 $\pm$ 0.01 a	19	0.21 $\pm$ 0.08 a	0.21 $\pm$ 0.07 a	43	0.33 $\pm$ 0.09 a	0.46 $\pm$ 0.11 b	

Means followed by same letter(s) in a column are not significantly different ( $P = 0.0009$ ) with Bonferroni correction. Analyses of variance performed using square root transformed ( $x + 0.05$ ) data, untransformed data are shown.

<sup>a</sup> Phenology was determined based on Ritchie et al. 1993.

<sup>b</sup> Letter and number indicates the location of each sampling row relative to the interface between refuge (Rfg) and Bt corn types. See text for further explanation.

<sup>c</sup> n indicates the number of samples within each mean.

**Table 5.** Spatial and temporal abundance (adults/min; mean  $\pm$  SE) of male and female western corn rootworm adults by corn phenology, refuge treatment and sampling row in 2011.

Treatment	Row <sup>b</sup>	n <sup>c</sup>	Corn Phenology							
			Vegetative <sup>a</sup>			Pollination			Post-pollination	
			Males/min ( $\pm$ SE)	Females/min ( $\pm$ SE)	n	Males/min ( $\pm$ SE)	Females/min ( $\pm$ SE)	n	Males/min ( $\pm$ SE)	Females/min ( $\pm$ SE)
20% Structured Refuge	W20-Rfg	27	1.83 $\pm$ 0.40 a	1.48 $\pm$ 0.39 a	18	3.05 $\pm$ 0.33 a	4.91 $\pm$ 0.43 a	24	0.64 $\pm$ 0.10 a	3.05 $\pm$ 0.31 b
	W12-Rfg	27	1.73 $\pm$ 0.41 a	1.12 $\pm$ 0.36 a	18	1.64 $\pm$ 0.21 b	3.43 $\pm$ 0.46 b	24	0.45 $\pm$ 0.08 a	3.54 $\pm$ 0.42 b
	W3-Rfg	27	1.41 $\pm$ 0.35 a	0.98 $\pm$ 0.31 ab	18	0.98 $\pm$ 0.20 bc	2.53 $\pm$ 0.31 bc	24	0.56 $\pm$ 0.09 a	5.74 $\pm$ 0.56 a
	E3-Bt	27	0.40 $\pm$ 0.12 b	0.24 $\pm$ 0.09 bc	18	0.73 $\pm$ 0.15 c	1.62 $\pm$ 0.29 cd	24	0.48 $\pm$ 0.10 a	1.03 $\pm$ 0.16 c
	E12-Bt	27	0.17 $\pm$ 0.06 b	0.15 $\pm$ 0.07 c	18	0.73 $\pm$ 0.15 c	0.71 $\pm$ 0.19 de	24	0.42 $\pm$ 0.07 a	1.07 $\pm$ 0.17 c
	E24-Bt	27	0.14 $\pm$ 0.05 b	0.12 $\pm$ 0.06 c	18	0.65 $\pm$ 0.14 c	1.14 $\pm$ 0.28 de	24	0.34 $\pm$ 0.07 a	1.10 $\pm$ 0.23 c
	E36-Bt	27	0.13 $\pm$ 0.06 b	0.06 $\pm$ 0.03 c	18	0.48 $\pm$ 0.10 c	0.55 $\pm$ 0.12 e	24	0.37 $\pm$ 0.09 a	0.66 $\pm$ 0.12 c
	E48-Bt	27	0.07 $\pm$ 0.03 b	0.09 $\pm$ 0.04 c	18	0.77 $\pm$ 0.17 c	0.61 $\pm$ 0.13 e	24	0.33 $\pm$ 0.08 a	0.50 $\pm$ 0.08 c
	E60-Bt	27	0.06 $\pm$ 0.03 b	0.01 $\pm$ 0.01 c	18	0.49 $\pm$ 0.09 c	0.66 $\pm$ 0.10 de	24	0.25 $\pm$ 0.07 a	0.40 $\pm$ 0.08 c
	E72-Bt	27	0.06 $\pm$ 0.03 b	0.05 $\pm$ 0.03 c	18	0.56 $\pm$ 0.10 c	0.67 $\pm$ 0.14 de	24	0.26 $\pm$ 0.08 a	0.51 $\pm$ 0.10 c
E84-Bt	27	0.16 $\pm$ 0.07 b	0.10 $\pm$ 0.05 c	18	0.57 $\pm$ 0.16 c	0.81 $\pm$ 0.20 de	24	0.32 $\pm$ 0.08 a	0.39 $\pm$ 0.08 c	
5% Structured Refuge	W4-Rfg	27	1.89 $\pm$ 0.38 a	1.24 $\pm$ 0.35 a	18	1.58 $\pm$ 0.21 a	3.53 $\pm$ 0.38 a	24	0.60 $\pm$ 0.10 ab	5.18 $\pm$ 0.35 a
	W2-Rfg	27	1.59 $\pm$ 0.36 a	0.85 $\pm$ 0.26 ab	18	1.54 $\pm$ 0.23 ab	3.50 $\pm$ 0.49 a	24	0.70 $\pm$ 0.11 a	5.53 $\pm$ 0.61 a
	E3-Rfg	27	0.43 $\pm$ 0.10 b	0.19 $\pm$ 0.07 bc	18	1.44 $\pm$ 0.27 ab	1.75 $\pm$ 0.30 b	24	0.47 $\pm$ 0.07 abc	1.22 $\pm$ 0.16 bc
	E12-Rfg	27	0.20 $\pm$ 0.06 b	0.12 $\pm$ 0.05 bc	18	1.05 $\pm$ 0.18 abc	1.18 $\pm$ 0.29 bc	24	0.45 $\pm$ 0.09 abc	1.29 $\pm$ 0.18 bc
	E24-Rfg	27	0.12 $\pm$ 0.05 b	0.08 $\pm$ 0.05 c	18	0.84 $\pm$ 0.13 bcd	1.04 $\pm$ 0.16 bc	24	0.40 $\pm$ 0.08 abc	1.24 $\pm$ 0.19 bc
	E36-Rfg	27	0.13 $\pm$ 0.03 b	0.04 $\pm$ 0.02 c	18	0.48 $\pm$ 0.08 cd	0.58 $\pm$ 0.13 c	24	0.33 $\pm$ 0.07 abc	1.61 $\pm$ 0.44 b
	E48-Rfg	27	0.10 $\pm$ 0.05 b	0.05 $\pm$ 0.03 c	18	0.41 $\pm$ 0.09 d	0.66 $\pm$ 0.16 c	24	0.19 $\pm$ 0.04 bc	1.35 $\pm$ 0.26 bc
	E60-Rfg	27	0.17 $\pm$ 0.06 b	0.05 $\pm$ 0.02 c	18	0.41 $\pm$ 0.08 d	0.48 $\pm$ 0.11 c	24	0.25 $\pm$ 0.05 abc	1.28 $\pm$ 0.30 bc
	E72-Rfg	27	0.03 $\pm$ 0.02 b	0.05 $\pm$ 0.02 c	18	0.44 $\pm$ 0.08 d	0.88 $\pm$ 0.22 bc	24	0.26 $\pm$ 0.08 abc	0.66 $\pm$ 0.11 bc
	E84-Rfg	27	0.05 $\pm$ 0.03 b	0.00 $\pm$ 0.00 c	18	0.35 $\pm$ 0.04 d	0.53 $\pm$ 0.12 c	24	0.12 $\pm$ 0.04 c	0.55 $\pm$ 0.11 c
E96-Rfg	27	0.04 $\pm$ 0.02 b	0.00 $\pm$ 0.00 c	18	0.46 $\pm$ 0.09 cd	0.57 $\pm$ 0.11 c	24	0.22 $\pm$ 0.05 abc	0.68 $\pm$ 0.12 bc	
5% Seed Blend	W4-Sb	27	0.40 $\pm$ 0.21 a	0.23 $\pm$ 0.12 a	18	1.77 $\pm$ 0.25 a	1.50 $\pm$ 0.25 ab	24	1.28 $\pm$ 0.13 ab	3.19 $\pm$ 0.46 a
	W2-Sb	27	0.26 $\pm$ 0.09 a	0.10 $\pm$ 0.04 a	18	1.83 $\pm$ 0.23 a	1.65 $\pm$ 0.31 a	24	1.46 $\pm$ 0.16 a	2.68 $\pm$ 0.30 ab
	E3-Sb	27	0.15 $\pm$ 0.05 a	0.12 $\pm$ 0.04 a	18	1.47 $\pm$ 0.22 ab	1.54 $\pm$ 0.28 ab	24	1.04 $\pm$ 0.13 abc	2.26 $\pm$ 0.30 abc
	E12-Sb	27	0.10 $\pm$ 0.03 a	0.09 $\pm$ 0.04 a	18	1.29 $\pm$ 0.27 abc	1.67 $\pm$ 0.34 ab	24	0.69 $\pm$ 0.10 cde	2.21 $\pm$ 0.30 abc
	E24-Sb	27	0.17 $\pm$ 0.06 a	0.08 $\pm$ 0.03 a	18	0.88 $\pm$ 0.13 bcd	0.89 $\pm$ 0.21 abc	24	0.78 $\pm$ 0.11 bcd	1.91 $\pm$ 0.33 bc
	E36-Sb	27	0.18 $\pm$ 0.06 a	0.07 $\pm$ 0.03 a	18	0.72 $\pm$ 0.12 cd	0.89 $\pm$ 0.20 abc	24	0.45 $\pm$ 0.09 ef	1.50 $\pm$ 0.30 cd
	E48-Sb	27	0.11 $\pm$ 0.04 a	0.10 $\pm$ 0.04 a	18	0.29 $\pm$ 0.05 d	0.69 $\pm$ 0.19 bc	24	0.46 $\pm$ 0.08 de	1.33 $\pm$ 0.23 cd
	E60-Sb	27	0.11 $\pm$ 0.04 a	0.06 $\pm$ 0.03 a	18	0.51 $\pm$ 0.08 d	0.50 $\pm$ 0.12 c	24	0.30 $\pm$ 0.07 ef	0.91 $\pm$ 0.15 d
	E72-Sb	27	0.12 $\pm$ 0.03 a	0.07 $\pm$ 0.03 a	18	0.31 $\pm$ 0.07 d	0.47 $\pm$ 0.09 c	24	0.28 $\pm$ 0.06 ef	0.77 $\pm$ 0.11 d
	E84-Sb	27	0.15 $\pm$ 0.05 a	0.08 $\pm$ 0.03 a	18	0.44 $\pm$ 0.08 d	0.72 $\pm$ 0.14 abc	24	0.19 $\pm$ 0.06 f	0.96 $\pm$ 0.16 d
E96-Sb	27	0.21 $\pm$ 0.06 a	0.06 $\pm$ 0.03 a	18	0.47 $\pm$ 0.10 d	0.75 $\pm$ 0.14 abc	24	0.45 $\pm$ 0.07 de	1.13 $\pm$ 0.17 d	
0% Refuge	W4-Bt	27	0.14 $\pm$ 0.05 a	0.05 $\pm$ 0.03 a	18	1.25 $\pm$ 0.29 a	1.37 $\pm$ 0.26 a	24	1.11 $\pm$ 0.19 a	3.12 $\pm$ 0.48 a
	W2-Bt	27	0.15 $\pm$ 0.06 a	0.09 $\pm$ 0.04 a	18	1.16 $\pm$ 0.23 a	1.30 $\pm$ 0.29 a	24	1.14 $\pm$ 0.23 a	2.04 $\pm$ 0.33 ab
	E3-Bt	27	0.08 $\pm$ 0.04 a	0.04 $\pm$ 0.02 a	18	0.72 $\pm$ 0.18 ab	1.19 $\pm$ 0.26 a	24	0.71 $\pm$ 0.10 ab	1.51 $\pm$ 0.30 bcd
	E12-Bt	27	0.11 $\pm$ 0.04 a	0.05 $\pm$ 0.03 a	18	0.51 $\pm$ 0.10 b	0.93 $\pm$ 0.20 a	24	0.36 $\pm$ 0.07 b	1.27 $\pm$ 0.18 bcde
	E24-Bt	27	0.10 $\pm$ 0.04 a	0.04 $\pm$ 0.02 a	18	0.50 $\pm$ 0.10 b	0.95 $\pm$ 0.20 a	24	0.36 $\pm$ 0.07 b	0.84 $\pm$ 0.11 de
	E36-Bt	27	0.08 $\pm$ 0.04 a	0.01 $\pm$ 0.01 a	18	0.43 $\pm$ 0.10 b	0.85 $\pm$ 0.29 a	24	0.23 $\pm$ 0.05 b	1.00 $\pm$ 0.19 cde
	E48-Bt	27	0.21 $\pm$ 0.07 a	0.06 $\pm$ 0.03 a	18	0.67 $\pm$ 0.14 ab	0.99 $\pm$ 0.23 a	24	0.26 $\pm$ 0.06 b	0.93 $\pm$ 0.21 cde
	E60-Bt	27	0.13 $\pm$ 0.05 a	0.06 $\pm$ 0.04 a	18	0.25 $\pm$ 0.08 b	0.80 $\pm$ 0.25 a	24	0.26 $\pm$ 0.07 b	0.59 $\pm$ 0.12 e
	E72-Bt	27	0.10 $\pm$ 0.04 a	0.06 $\pm$ 0.03 a	18	0.38 $\pm$ 0.09 b	0.94 $\pm$ 0.27 a	24	0.30 $\pm$ 0.08 b	0.75 $\pm$ 0.11 de
	E84-Bt	27	0.11 $\pm$ 0.03 a	0.06 $\pm$ 0.03 a	18	0.35 $\pm$ 0.08 b	1.05 $\pm$ 0.21 a	24	0.35 $\pm$ 0.09 b	1.68 $\pm$ 0.27 bc
E96-Bt	27	0.10 $\pm$ 0.05 a	0.04 $\pm$ 0.02 a	18	0.54 $\pm$ 0.11 ab	0.94 $\pm$ 0.26 a	24	0.38 $\pm$ 0.12 b	1.76 $\pm$ 0.29 bc	

Means followed by same letter(s) in a column are not significantly different ( $P = 0.0009$ ) with Bonferroni correction. Analyses of variance performed using square root transformed ( $x + 0.05$ ) data, untransformed data are shown.

<sup>a</sup> Phenology was determined based on Ritchie et al. 1993.

<sup>b</sup> Letter and number indicates the location of each sampling row relative to the interface between refuge (Rfg) and Bt corn types. See text for further explanation.

<sup>c</sup> n indicates the number of samples within each mean.

**Table 6.** Spatial and temporal abundance (adults/min; mean  $\pm$  SE) of male and female western corn rootworm adults by corn phenology, refuge treatment and sampling row in 2012.

Treatment	Row <sup>b</sup>	n <sup>c</sup>	Corn Phenology							
			Vegetative <sup>a</sup>		n	Pollination		Post-pollination		
			Male/min ( $\pm$ SE)	Female/min ( $\pm$ SE)		Male/min ( $\pm$ SE)	Female/min ( $\pm$ SE)	Male/min ( $\pm$ SE)	Female/min ( $\pm$ SE)	
20% Structured Refuge	W20-Rfg	39	1.98 $\pm$ 0.40 a	0.76 $\pm$ 0.18 a	18	2.52 $\pm$ 0.36 a	2.84 $\pm$ 0.37 a	45	1.00 $\pm$ 0.14 b	2.42 $\pm$ 0.30 a
	W12-Rfg	39	2.30 $\pm$ 0.50 a	0.65 $\pm$ 0.16 abc	18	2.31 $\pm$ 0.19 a	2.54 $\pm$ 0.31 ab	45	1.46 $\pm$ 0.19 ab	2.89 $\pm$ 0.37 a
	W3-Rfg	39	2.03 $\pm$ 0.48 a	0.67 $\pm$ 0.16 ab	18	1.99 $\pm$ 0.28 ab	2.88 $\pm$ 0.31 a	45	1.27 $\pm$ 0.18 ab	2.24 $\pm$ 0.27 ab
	E3-Tg	39	0.52 $\pm$ 0.15 b	0.13 $\pm$ 0.05 bcd	18	1.69 $\pm$ 0.27 abc	1.56 $\pm$ 0.23 bcd	45	1.85 $\pm$ 0.20 a	2.25 $\pm$ 0.21 a
	E12-Tg	39	0.25 $\pm$ 0.10 b	0.11 $\pm$ 0.04 bcd	18	1.44 $\pm$ 0.24 bc	1.63 $\pm$ 0.28 bc	45	1.70 $\pm$ 0.20 ab	1.27 $\pm$ 0.14 c
	E24-Tg	39	0.22 $\pm$ 0.08 b	0.06 $\pm$ 0.03 d	18	1.51 $\pm$ 0.25 abc	1.43 $\pm$ 0.38 cd	45	1.60 $\pm$ 0.17 ab	1.31 $\pm$ 0.13 c
	E36-Tg	39	0.24 $\pm$ 0.16 b	0.09 $\pm$ 0.04 cd	18	1.00 $\pm$ 0.31 cd	1.24 $\pm$ 0.28 cd	45	1.39 $\pm$ 0.16 ab	1.49 $\pm$ 0.13 bc
	E48-Tg	39	0.15 $\pm$ 0.05 b	0.04 $\pm$ 0.02 d	18	1.10 $\pm$ 0.25 cd	0.84 $\pm$ 0.22 cd	45	1.20 $\pm$ 0.15 ab	1.11 $\pm$ 0.11 c
	E60-Tg	39	0.09 $\pm$ 0.03 b	0.05 $\pm$ 0.02 d	18	1.31 $\pm$ 0.54 bcd	0.79 $\pm$ 0.25 cd	45	1.29 $\pm$ 0.17 ab	1.15 $\pm$ 0.12 c
	E72-Tg	39	0.16 $\pm$ 0.05 b	0.04 $\pm$ 0.02 d	18	0.68 $\pm$ 0.18 cd	0.74 $\pm$ 0.21 cd	45	1.25 $\pm$ 0.14 ab	1.00 $\pm$ 0.12 c
E84-Tg	39	0.10 $\pm$ 0.05 b	0.03 $\pm$ 0.02 d	18	0.51 $\pm$ 0.24 d	0.70 $\pm$ 0.23 d	45	1.03 $\pm$ 0.13 b	1.05 $\pm$ 0.21 c	
5% Structured Refuge	W4-Rfg	39	2.75 $\pm$ 0.49 a	1.24 $\pm$ 0.27 a	18	2.62 $\pm$ 0.43 a	2.89 $\pm$ 0.46 a	42	0.79 $\pm$ 0.10 bc	1.69 $\pm$ 0.20 a
	W2-Rfg	39	2.54 $\pm$ 0.56 a	1.04 $\pm$ 0.21 a	18	2.00 $\pm$ 0.36 abc	2.71 $\pm$ 0.48 a	42	0.78 $\pm$ 0.08 bc	1.69 $\pm$ 0.22 a
	E3-Tg	39	0.77 $\pm$ 0.21 b	0.29 $\pm$ 0.10 b	18	1.97 $\pm$ 0.23 ab	1.81 $\pm$ 0.27 ab	42	1.41 $\pm$ 0.15 ab	1.63 $\pm$ 0.18 a
	E12-Tg	39	0.33 $\pm$ 0.08 bc	0.10 $\pm$ 0.04 b	18	1.68 $\pm$ 0.29 abcd	1.20 $\pm$ 0.23 bc	42	1.58 $\pm$ 0.20 a	1.53 $\pm$ 0.14 ab
	E24-Tg	39	0.17 $\pm$ 0.05 bc	0.06 $\pm$ 0.03 b	18	1.22 $\pm$ 0.17 bcd	1.26 $\pm$ 0.27 bc	42	1.26 $\pm$ 0.18 abc	1.22 $\pm$ 0.13 abc
	E36-Tg	39	0.16 $\pm$ 0.05 c	0.10 $\pm$ 0.03 b	18	0.92 $\pm$ 0.20 cde	1.12 $\pm$ 0.22 bcd	42	0.99 $\pm$ 0.14 abc	1.24 $\pm$ 0.11 abc
	E48-Tg	39	0.16 $\pm$ 0.05 bc	0.04 $\pm$ 0.02 b	18	0.74 $\pm$ 0.17 de	0.81 $\pm$ 0.20 cd	42	0.92 $\pm$ 0.11 abc	1.07 $\pm$ 0.15 abc
	E60-Tg	39	0.09 $\pm$ 0.03 c	0.03 $\pm$ 0.02 b	18	0.51 $\pm$ 0.09 e	0.81 $\pm$ 0.20 bcd	42	0.68 $\pm$ 0.07 c	0.83 $\pm$ 0.10 c
	E72-Tg	39	0.06 $\pm$ 0.04 c	0.01 $\pm$ 0.01 b	18	0.46 $\pm$ 0.14 e	0.56 $\pm$ 0.14 cd	42	0.64 $\pm$ 0.09 c	0.71 $\pm$ 0.08 c
	E84-Tg	39	0.08 $\pm$ 0.03 c	0.01 $\pm$ 0.01 b	18	0.38 $\pm$ 0.12 e	0.50 $\pm$ 0.12 cd	42	0.80 $\pm$ 0.09 bc	0.97 $\pm$ 0.12 bc
E96-Tg	39	0.04 $\pm$ 0.02 c	0.02 $\pm$ 0.01 b	18	0.48 $\pm$ 0.16 e	0.34 $\pm$ 0.11 d	42	0.81 $\pm$ 0.11 bc	1.06 $\pm$ 0.15 abc	
5% Seed Blend	W4-Sb	39	0.33 $\pm$ 0.12 a	0.05 $\pm$ 0.02 a	18	1.35 $\pm$ 0.30 a	1.76 $\pm$ 0.45 a	41	1.15 $\pm$ 0.15 a	1.62 $\pm$ 0.17 a
	W2-Sb	39	0.24 $\pm$ 0.07 a	0.12 $\pm$ 0.03 a	18	1.50 $\pm$ 0.22 a	1.54 $\pm$ 0.28 a	41	1.10 $\pm$ 0.13 a	1.28 $\pm$ 0.16 ab
	E3-Sb	39	0.31 $\pm$ 0.13 a	0.13 $\pm$ 0.04 a	18	1.32 $\pm$ 0.18 a	1.28 $\pm$ 0.20 a	41	1.02 $\pm$ 0.13 a	1.06 $\pm$ 0.11 ab
	E12-Sb	39	0.29 $\pm$ 0.10 a	0.10 $\pm$ 0.05 a	18	1.29 $\pm$ 0.24 a	1.19 $\pm$ 0.23 a	41	1.08 $\pm$ 0.15 a	1.09 $\pm$ 0.14 ab
	E24-Sb	39	0.17 $\pm$ 0.06 a	0.12 $\pm$ 0.04 a	18	1.31 $\pm$ 0.22 a	1.24 $\pm$ 0.25 a	41	0.90 $\pm$ 0.14 a	1.21 $\pm$ 0.18 ab
	E36-Sb	39	0.33 $\pm$ 0.10 a	0.11 $\pm$ 0.04 a	18	1.21 $\pm$ 0.22 a	1.07 $\pm$ 0.23 a	41	0.85 $\pm$ 0.16 a	1.03 $\pm$ 0.16 b
	E48-Sb	39	0.42 $\pm$ 0.12 a	0.15 $\pm$ 0.05 a	18	1.39 $\pm$ 0.22 a	1.17 $\pm$ 0.20 a	41	0.84 $\pm$ 0.14 a	1.25 $\pm$ 0.14 ab
	E60-Sb	39	0.19 $\pm$ 0.06 a	0.06 $\pm$ 0.03 a	18	1.43 $\pm$ 0.25 a	1.22 $\pm$ 0.19 a	41	0.84 $\pm$ 0.13 a	0.77 $\pm$ 0.10 b
	E72-Sb	39	0.36 $\pm$ 0.13 a	0.07 $\pm$ 0.03 a	18	1.35 $\pm$ 0.36 a	1.44 $\pm$ 0.34 a	41	1.10 $\pm$ 0.19 a	0.83 $\pm$ 0.12 b
	E84-Sb	39	0.46 $\pm$ 0.17 a	0.21 $\pm$ 0.10 a	18	1.44 $\pm$ 0.31 a	1.48 $\pm$ 0.46 a	41	1.03 $\pm$ 0.16 a	1.06 $\pm$ 0.13 ab
E96-Sb	39	0.26 $\pm$ 0.07 a	0.14 $\pm$ 0.07 a	18	1.14 $\pm$ 0.27 a	1.83 $\pm$ 0.64 a	41	0.95 $\pm$ 0.16 a	1.05 $\pm$ 0.16 ab	
0% Refuge	W4-Tg	39	0.30 $\pm$ 0.09 a	0.08 $\pm$ 0.02 a	17	1.50 $\pm$ 0.37 abcd	1.75 $\pm$ 0.29 ab	41	1.31 $\pm$ 0.18 a	2.02 $\pm$ 0.19 a
	W2-Tg	39	0.32 $\pm$ 0.08 a	0.08 $\pm$ 0.03 a	17	1.78 $\pm$ 0.34 ab	1.61 $\pm$ 0.28 abc	41	1.37 $\pm$ 0.18 a	1.90 $\pm$ 0.19 ab
	E3-Tg	39	0.26 $\pm$ 0.07 a	0.10 $\pm$ 0.03 a	17	2.43 $\pm$ 0.27 a	2.50 $\pm$ 0.46 a	41	1.37 $\pm$ 0.16 a	1.31 $\pm$ 0.14 bcd
	E12-Tg	39	0.29 $\pm$ 0.08 a	0.08 $\pm$ 0.03 a	17	1.83 $\pm$ 0.22 ab	2.34 $\pm$ 0.43 a	41	1.31 $\pm$ 0.16 a	1.49 $\pm$ 0.16 abc
	E24-Tg	39	0.21 $\pm$ 0.07 a	0.04 $\pm$ 0.02 a	17	1.83 $\pm$ 0.20 ab	2.06 $\pm$ 0.40 a	41	1.24 $\pm$ 0.18 ab	1.39 $\pm$ 0.18 abcd
	E36-Tg	39	0.19 $\pm$ 0.06 a	0.08 $\pm$ 0.03 a	17	1.46 $\pm$ 0.18 abc	1.52 $\pm$ 0.40 abc	41	1.07 $\pm$ 0.15 abc	1.35 $\pm$ 0.14 abc
	E48-Tg	39	0.16 $\pm$ 0.05 a	0.04 $\pm$ 0.02 a	17	1.02 $\pm$ 0.22 bcde	0.92 $\pm$ 0.21 bcd	41	0.97 $\pm$ 0.12 abc	1.13 $\pm$ 0.15 cd
	E60-Tg	39	0.15 $\pm$ 0.05 a	0.08 $\pm$ 0.03 a	17	0.65 $\pm$ 0.16 cde	0.77 $\pm$ 0.18 cd	41	0.80 $\pm$ 0.11 abc	1.00 $\pm$ 0.14 cd
	E72-Tg	39	0.13 $\pm$ 0.04 a	0.04 $\pm$ 0.01 a	17	0.66 $\pm$ 0.16 cde	0.75 $\pm$ 0.20 cd	41	0.62 $\pm$ 0.10 bc	0.82 $\pm$ 0.12 d
	E84-Tg	39	0.13 $\pm$ 0.04 a	0.05 $\pm$ 0.03 a	17	0.48 $\pm$ 0.14 de	0.53 $\pm$ 0.17 d	41	0.60 $\pm$ 0.08 bc	1.00 $\pm$ 0.13 cd
E96-Tg	39	0.10 $\pm$ 0.03 a	0.03 $\pm$ 0.01 a	17	0.42 $\pm$ 0.14 e	0.53 $\pm$ 0.16 d	41	0.55 $\pm$ 0.09 c	0.82 $\pm$ 0.11 d	

Means followed by same letter(s) in a column are not significantly different ( $P = 0.0009$ ) with Bonferroni correction. Analyses of variance performed using square root transformed ( $\sqrt{x + 0.05}$ ) data, untransformed data are shown.

<sup>a</sup> Phenology was determined based on Ritchie et al. 1993.

<sup>b</sup> Letter and number indicates the location of each sampling row relative to the interface between refuge (Rfg) and Bt corn types. See text for further explanation.

<sup>c</sup> n indicates the number of samples within each mean.

**Table 7.** Mean male and female western corn rootworm collection rates (adults/100 sweeps; mean  $\pm$  SE) in soybean fields during the vegetative, pollination and post-pollination periods of corn phenology in 2010, 2011 and 2012.

Corn Phenology <sup>a</sup>	2010				2011			2012	
	n <sup>b</sup>	Male	Female	n	Male	Female	n	Male	Female
Vegetative	20	0.00 $\pm$ 0.00 a	0.00 $\pm$ 0.00 b	36	0.17 $\pm$ 0.07 a	0.25 $\pm$ 0.13 b	48	0.06 $\pm$ 0.04 b	0.04 $\pm$ 0.03 b
Pollination	24	0.04 $\pm$ 0.04 a	0.13 $\pm$ 0.07 b	24	0.25 $\pm$ 0.09 a	0.58 $\pm$ 0.17 b	28	0.82 $\pm$ 0.15 b	1.79 $\pm$ 0.48 b
Post-pollination	88	0.14 $\pm$ 0.04 a	1.92 $\pm$ 0.21 a	32	0.41 $\pm$ 0.12 a	5.15 $\pm$ 0.69 a	69	5.38 $\pm$ 0.64 a	23.62 $\pm$ 2.02 a

Means followed by same letter(s) in a column are not significantly different ( $P = 0.0009$ ) with Bonferroni correction. Analyses of variance performed using square root transformed ( $x + 0.05$ ) data, untransformed data are shown.

<sup>a</sup> Phenology of corn in adjacent fields was determined based on Ritchie et al. 1993.

<sup>b</sup> n indicates the number of samples within each mean.

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