Utility of Scouting with Sticky Traps for Integrated Pest Management of Corn Rootworm

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Dedication

This thesis is dedicated to my mom, Jane. Her unwavering support and encouragement helped me find the motivation to continue through the long process of earning this degree.

Abstract

Corn rootworms (*Diabrotica virgifera virgifera* LeConte and *Diabrotica barberi* Smith and Lawrence) have been pests of economic importance on corn for over a century due to the injury they inflict on corn roots. Injury primarily takes place following larval feeding and occurs in the form of root pruning, stalk lodging, reduced nutrient up-take, and secondary attack by pathogens, but may also occur from adults feeding on silks and pollen.

For the past decade, growers have been relying primarily upon corn rootworm-active *Bacillus thuringiensis* Berliner (*Bt*) transgenic hybrids to control corn rootworms, and thereby disregarding classic IPM practices due to the reliability of these hybrids alone. In 2009, however, field-evolved resistance to corn rootworm- active *Bt* hybrids was found in Iowa. Since then, cases of problem fields (i.e., fields where excessive lodging and root pruning occur despite the use of transgenic hybrids) are becoming more prevalent across the Corn Belt where corn-on-corn is common, causing IPM to become a renewed focus in the effort to control corn rootworms. The frequency of problem fields and high cost of corn rootworm-resistant traits is leading growers to use outdated integrated pest management (IPM) decisions to control corn rootworms and hopefully reduce the injury in inflict on the corn plant.

Integrated pest management plans with corn rootworm-active *Bt* hybrids are a cornerstone for successful use of refuge-based Insect Resistance Management (IRM) plans as well. IRM plans combine corn rootworm biology with agronomic practices to

delay population resistance to current *Bt* hybrids containing corn rootworm-active events. While corn rootworm phenology has been extensively reviewed in the past, the influence of *Bt* hybrids on current phenology is not fully understood although developmental have been observed. A delay in adult emergence has been observed in beetles emerging from *Bt* hybrids in comparison to non-*Bt* (refuge) hybrids. The delay in emergence may result in reduced efficacy of refuge corn, which is essential to the IRM plan by increasing mating between the beetles from the refuge and *Bt* corn. Emergence differences may cause *Bt* tolerance to develop more quickly.

Planting date is known to play an important role in controlling the amount of damage that may occur on the corn plants as well but often coming at the expense of yield. Late planting results in a lack of food availability for corn rootworm larvae, which results in increased mortality, reduced injury, and delayed adult beetle emergence. The combination of planting date and *Bt* hybrid on corn rootworm adult emergence is largely unknown. This has implications for determining adult scouting windows in order to determine insect pressure the following season.

Current *Bt* hybrids exhibit superior genetics when compared to past inbred lines; in fact, no current data exists for adult beetle thresholds in relation to injury on *Bt* hybrids. With the new threat of *Bt*-resistant corn rootworm populations becoming more prevalent, recommendations on how and when to scout for resistant populations are needed. Updated beetle thresholds and scouting windows will also help prevent the overuse of pesticides and control beetle populations the following year. Studies which try to establish economic injury levels with adult beetles as well as economic thresholds in order to aid grower’s management decisions are important for extending the shelf life of corn rootworm-active *Bt* hybrids as well.

This study aimed to evaluate sticky traps as a scouting tool on current corn hybrids, establish precision and required sample sizes, define scouting windows based on planting date and transgenic hybrid-induced emergence delays, and determine the relationship to root injury the following season.

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Chapter I.

Review of the Literature

a. Corn Rootworm (*Diabrotica spp.*)

Corn rootworms (*Diabrotica spp.*), belonging to the family Chrysomelidae (Order: Coleoptera), have been pests of economic importance on corn (*Zea mays* L.) for over a century due to damage they inflict on corn in the Midwestern United States. *Diabrotica barberi* Smith and Lawrence (northern corn rootworm) and *Diabrotica virgifera virgifera* LeConte (western corn rootworm) are the two species most commonly found, but other species such as *Diabrotica undecimpuntata howardi* Barber (Southern corn rootworm) and *Diabrotica virgifera zeae* Krysan and Smith (Mexican corn rootworm) are also found in the United States on corn. *Diabrotica virgifera virgifera*, found throughout the Unites states, is believed to have originated from the same location in Central America as corn (Krysan et al. 1980). Conversely, *D. barberi*, is native to prairies of the United States, which may account for its ability to survive on more than one grass host (Branson and Krysan 1981). Both species have a high fidelity to corn and prefer to lay their eggs in fields that are planted to corn or near volunteer corn in fields currently planted to soybeans (Hill and Mayo 1980, Branson and Krysan 1981), which are likely be rotated to corn the following year.

Eggs

The corn rootworm life-cycle begins in soil as an egg that is laid during a two-month period beginning in late-July and ending in early-fall. When temperatures begin to drop, egg development ceases and the egg enters a diapause period until temperatures rise above 10.9°C and 10.5°C for *D. barberi* and *Diabrotica virgifera virgifera,* respectively (Schaafsma et al. 1991, Woodson et al. 1996). Conditions required to break diapause include temperatures above approximately 11°C (Krysan 1982) and moisture at appropriate levels (Krysan 1978). Upper developmental thresholds exist for egg development in both species as well with *D. virgifera virgifera* having an upper limit of 34.7 °C and *D. barberi* having an upper limit of 35.3 °C (Schaafsma et al. 1991, Woodson et al. 1996).

Corn rootworms spend the majority of their lifecycle in a subterranean habitat where they are subjected to a variety of edaphic, agronomic, and biological variables, which begin the moment they are oviposited into the soil. As they overwinter in the soil, *Diabrotica* spp. eggs are susceptible to damage and mortality from weather and biological sources. Soil moisture and temperature interact to cause mortality in *D. barberi* below -10°C (Gustin 1983, Ellsbury and Lee 2004). While *D. barberi* is unable to handle moisture in combination with low temperatures, *D. barberi* eggs are able to withstand cool temperature alone better than *D. virgifera virgifera* eggs. *Diabrotica virgifera virgifera* eggs are less affected by moisture, but mortality increases when soil temperatures fall below -10°C. While the supercooling points for *D. virgifera virgifera* reach as low as -27°C and as low as -21.5°C for *D. barberi*, mortality is believed to occur before the supercooling point due to pre-diapause chill resulting in increased susceptibility to cold temperatures (Ellsbury and Lee 2004).

Winter survival, studied on *D. virgifera virgifera* eggs, can also be impacted by egg depth and surface residue (Godfrey et al. 1995). In a study by Godfrey et al. (1995), without surface residue only 23 percent survived. Ground cover and reduced tillage have also been found to decrease egg mortality in cold winters (Gray and Tollefson 1988). In a study on *D. undecimpunctata howardi*, soil-drying time and soil texture also impacted egg survival as well as first-instar larval survival (Brust and House 1990). Once enough cumulative degree-days have been reached, eggs hatch and prepare to migrate to the corn root system.

Larvae

Upon egg hatch, larvae migrate through the soil towards corn roots. Larvae are attracted to CO_2 and other semiochemicals, produced by the corn roots (Branson 1982). Carbon dioxide may serve as the main signal for larvae searching for roots (Strnad et al. 1986, Bernklau et al. 2009). Upon reaching the plant, first-instar larvae burrow into seminal roots where they feed in the cortex and remain until molting (Strnad and Bergman 1987). Following molting, the second and third instar larvae redistribute on the root system to younger roots where they feed within and outside of the corn roots, depending on the larval size (Strnad and Bergman 1987).

Once larvae reach the corn roots, the possibility of mortality remains. *Diabrotica spp.* larvae have been found to suffer from density-dependent mortality due to high numbers of larvae competing for food and space on a single root system (Hibbard et al. 2010). Hibbard et al. (2010) determined that density-dependent mortality is likely to occur when a nodal injury rating of 1 (on a 0-3 node injury scale (Oleson et al. 2005)) occurs. Larvae are also prone to mortality depending on the phenology and growth stage of the corn plant. Larvae that hatch during late plant stages (VT to R1) are less likely to emerge as adults than larvae that hatch and feed on roots during earlier growth stages due to the lack of young roots as a food source (Hibbard et al. 2008).

While larvae suffer from mortality in multiple forms while feeding on the plant, most mortality occurs before larvae even reach a host plant. During this quest, larvae are subject to predation from beetles, nematodes, pathogen infection, and mortality due to drowning as well. Soil density can decrease larval movement while they attempt to migrate to corn roots (Gustin and Schumacher 1989). High bulk density soils restrict first instar larval movement to less than 5 cm when available pore size isn’t large enough for larval head-capsules (Gustin and Schumacher 1989). Soil moisture also affects larval survival and movement; very wet or very dry soils inhibit movement and prevent larvae from reaching a food source . Very wet soils can also result in mortality due to drowning (Macdonald and Ellis 1990). Additional soil properties such as drainage, percent clay, and amount of potassium in the soil have also been strongly correlated with the amount of damage a corn plant might incur due to larval feeding (Turpin et al. 1972). Conversely to mortality induced by high bulk- density soils restricting movement, clay content and its ability to retain moisture is positively correlated with larval survival due to a lowered risk of desiccation (Turpin and Peters 1971) .

Once corn rootworms reach a corn host, they injure the corn plant at two different life stages with the most injury occurring during the larval stage. Larval injury to roots results in plant stress in the form of water loss, reduced nutrient uptake, lodging, and yield loss (Kahler et al. 1985, Spike and Tollefson 1991). The injury sustained due to corn rootworm feeding has been quantified in multiple ways over the years with the current root rating system using the 0-3 Nodal Injury Scale (Oleson et al. 2005). The Nodal Injury Scale focuses on root nodes four through seven with timing of egg hatch determining available root number and size in the developing root system. When larvae complete their feeding, they move into s

Emergence

Following a 14-day pupal phase in this cell, emergence begins (Chiang 1973). Measurements of soil temperature (max. and min.), which are then used in degree-day models, are an accurate way to predict corn rootworm development and emergence. Degree-day accumulations depend on multiple factors, such as substrate availability, enzyme availability, accurate developmental minimum and maximum temperatures, and micro-habitat of the organism, to name a few (Higley et al. 1986). Peak adult emergence generally coincides with the silking and pollen-shed period in corn, which is adaptive considering food availability (Spencer et al. 2009).

Emergence of males begins before females (also known as protandry) in corn rootworm populations because males need to finish sexual development after they have emerged (Branson 1987). With non-transgenic hybrids, early emerging adults live longer and have greater fecundity than adults emerging later in the season (Boetel and Fuller 1997). The difference in egg-laying also has implications for scouting, which will be discussed at greater length in the scouting section. Species composition can also affect survival and emergence in populations. In populations composed primarily of one corn rootworm species, the species with fewer individuals tends to suffer greater mortality (Woodson 1993).

In addition to factors affecting larvae, there are multiple ways in which adult beetle emergence can be influenced. Soil compaction from wheel traffic, planting date or fertilizer application, can reduce beetle emergence as well as root injury (Ellsbury et al. 1994). If a transgenic hybrid producing *Bacillus thuringiensis* Berliner (*Bt*) crystalline proteins was chosen, influences on beetle emergence in the form of control efficacy and sublethal effects have been observed. Sublethal effects are demonstrated through delayed emergence (Frank et al. 2015a, Hitchon et al. 2015). Hybrids containing traits for producing Cry3Bb1 or Cry34/35Ab1 (active against corn rootworm) result in a delay (12.3 days and 3-7 days, respectively) when compared to emergence from a refuge hybrid (Hitchon et al. 2015). Emergence from a pyramided hybrid (Cry3Bb1 + Cry34/35Ab1) results in an even further delay (15.8 days) than the single-traited hybrids (Hitchon et al. 2015). Hybrids containing mCry3A + eCry3.1Ab resulted in a substantial delay (13.7 days) to 50% beetle emergence as well (Frank et al. 2015a). These delays can have large effects on mating between *Bt*-susceptible adults emerging from non-*Bt* corn and *Bt*-resistant adults emerging from traited hybrids.

Adults

Adult beetle population levels are a product of the challenges faced in their subterranean life stage. High larval populations can have detrimental effects to the beetle population overall (Branson and Sutter 1985). High larval densities result in increased competition for food, stressed larvae, and fewer larvae reaching adulthood. These factors ultimately result in fewer adult beetles with smaller head capsule widths, smaller egg clutch sizes and shorter life spans (Branson and Sutter 1985). Adult size also has a significant impact on mating. Female weight impacts the likelihood of mating with n mate during the vegetative stage (due to early emergence) may have longer periods between matings due to feeding on less nutritious food sources (i.e. leaf tissue instead of silks and pollen) (Branson and Krysan 1981). In order to mate, locate suitable food, or oviposit, corn rootworm adults are capable of traveling long distances. Most flight movement occurs during early morning or in the evening due to high temperatures during mid-day (Isard et al. 2000).

Adult corn rootworm beetles are not significant pests to corn, but they can cause damage to the ears. In a study by Capinera et al. (1986), where ears were artificially infested with varying adult beetle densities to obtain ear injury data, there was no significant yield loss to the plant even with moderate injury ratings. While unfilled kernels were found near the tip of the corn ear, this was not correlated with beetle herbivory. Severe silk clipping was found to have a significant effect on yield despite regular pollination and ear fill (Capinera et al. 1986)**.**

Mating and Egg Laying

Diabrotica virgifera virgifera beetles that emerge before optimal food is available (green silks and shedding pollen) feed longer before laying eggs and lay fewer eggs over the course of three months (Elliott et al. 1990). Beetles that are able to feed when silks are fresh and pollen is shedding not only live longer, but also lay more eggs and start laying those eggs earlier in the three-month period (Elliott et al. 1990). The initial host for larvae has been shown to influence the fecundity of adult corn rootworms, with an initial host of *Setaria faberi* Herrm (giant foxtail) resulting in the greatest amount of egg laying (Chege et al. 2009). Strong ovipositional preference is placed on soil that is moist and has soil cracks and debris covering the soil, which the beetles can crawl under or into (Mulock et al. 1995)

In *D. barberi*, the spermatophore mass has been found to account for 4.4 percent of total male weight (French and Hammack 2012). Male *Diabrotica* spp. size (head capsule width, pronotum width and elytral length) was found to directly correlate with spermatophore volume, further indicating that size does matter (Murphy and Krupke 2011). Females copulate only once while males may copulate multiple times over their long life span (Hill 1975). Females lay an average of 13.5 clutches (in ideal conditions) with each clutch laid between 3-8 days apart, for a total of approximately 1000 eggs (Hill 1975). Other studies, however, found field-collected *D. virgifera virgifera* females to lay 356-735 eggs per female and *D. barberi* only 132- 312 eggs per female depending on the timing of emergence (early season vs late) (Boetel and Fuller 1997). While high beetle numbers can be found over the course of their life span in corn and various weedy areas, research has shown that beetles preferentially return to corn fields or volunteer corn to lay their eggs; which in corn fields to provide optimal food source for hatching larvae the following year (Boetel et al. 1992). Due to this, corn rootworm beetles, especially females, will migrate to fresh food sources in order to continue feeding over the course of their ovipositional period (Naranjo 1991, Darnell et al. 2000, Pierce and Gray 2006).

Control History

Diabrotica spp. are highly adaptive insects, particularly to control measures the insect must overcome. The primary control tactic used for many years, and still today, is crop rotation. By rotating the next growing season to a non-host crop, such as soybeans, any corn rootworms will hatch the following year without a food source and die (Levine and Oloumisadeghi 1991). Some populations of *D. barberi* adapted to this control tactic by developing extended diapause, allowing the egg stage of the beetle to remain quiescent throughout one or more growing season(s) when a food source is not available (Krysan et al. 1986). Some populations of *D. virgifera virgifera* have responded to crop rotation by losing their egg-laying fidelity to corn. Laying eggs in soybean, wheat and even alfalfa fields ensures that corn is more likely to be the crop when the eggs hatch the following season. These western corn rootworm populations, exhibiting resistance to crop rotation, are known as the western corn rootworm variant or soybean variant (Levine and Oloumisadeghi 1996).

Delayed corn planting (occurring the end of May or later) has been used as an agronomic way to control *Diabrotica* spp.: reducing root system size at hatch reduces larval colonization success and beetle emergence as well as delay peak emergence (Bergman and Turpin 1984). Planting date does not affect density of first instar larvae, but does affect second and third instars due to limited availability of large roots suitable for burrowing and feeding of older larvae (Bergman and Turpin 1984). Planting date also significantly delays timing for second and third instars by ten to 11 days while peak emergence in adults is delayed 13-14 days between the first and last planting dates (Bergman and Turpin 1984). Conversely, early plantings, which result in corn being at a later growth stage when corn rootworms hatch, suffer less root injury than corn that is at an early growth stage (Hibbard et al. 2008).

Insecticides have also been frequently used as a reliable source of control strategy for corn rootworms. Foster et al. (1986) suggested the optimal strategy for controlling adult corn rootworms was to apply a planting-time soil insecticide instead of using adult sampling to predict following season damage, when populations are generally higher. Resistance has become a significant issue in Nebraska, however, with corn rootworms adapting to multiple foliar insecticides (Meinke et al. 1998, Wright et al. 2000, (Meinke, 1998 #550). In addition to foliar control, granular soil insecticides have been used historically and are still relied upon today. However, granular insecticides do not cause as much mortality to the corn rootworm populations and instead primarily protect the root zone enough to reduce lodging and / or yield loss (Levine and Oloumisadeghi 1991).

Corn producing *Bacillus thuringiensis* Berliner (*Bt*) crystalline proteins active against corn rootworm were developed as an efficient, safer way to control corn rootworms (Moellenbeck et al. 2001, Ellis et al. 2002, Vaughn et al. 2005). Hybrids have been developed to contain one trait (Cry3Bb1, Cry34/35Ab1, mCry3A), two or more traits (Cry3Bb1+Cry34/35Ab1, mCry3A+eCry3.1, Cry34/35Ab1+mCry3A, Cry1A.105 + Cry3Bb1), known as pyramided traits, for the control of corn rootworms. While larvae that survive on *Bt* hybrids have been shown to have the same egg hatch rates, life spans, and reproductive rates as larvae that develop on non-*Bt* hybrids, sub-lethal affects have been observed in the form of delays in adult emergence as stated earlier (Mohammad et al. 2005, Siegfried et al. 2005). Western corn rootworm has been shown to evolve resistance to Cry3Bb1 within three generations of greenhouse selection (Meihls et al. 2012). The resistance, however, comes with reduced fecundity in females and an eight-day shorter lifespan in males selected in greenhouse colonies, suggesting a fitness cost (Meihls et al. 2012).

Cornfields showing greater-than-expected injury to *Bt* hybrids, known as "problem fields", were first observed in 2009 (Ostlie, 2009). These fields were confirmed to have corn rootworm populations with resistance to Cry3Bb1 (Gassmann et al. 2011). In-field assessment tools to categorize the beetles in problem fields as resistant, or not, are not available yet. The only tools appear to be evaluating root injury and scouting adult emergence. Based upon increasing incidence and geographical distribution of 'problem fields', and growing knowledge that corn rootworm- active toxins are low-dose and resistance genes appear dominant, recommendations have been made for increased refuge (no-*Bt* corn) requirements (Tabashnik and Gould 2012).

Chapter I.

Review of the Literature

b. Scouting

Sampling with sticky traps is an effective way to estimate adult activity levels and has been shown to be easier to implement than other forms of sampling (Binns and Nyrop 1992). The use of economic thresholds, developed through scouting, has been shown to slow the evolutions of resistance in transgenic crops by (Crowder et al. 2006), and prevent unwanted damage to the crop. Sampling programs can occur in many different forms. Stratified random sampling is a technique often used when sampling insects within a large universe (Binns and Nyrop 1992). A study by Losey et al. (2003), however, found that transect sampling had a <1% decrease in precision when compared with sequential and systematic sampling plans for corn rootworm. In addition, categorizations of the fields as above or below threshold did not differ, but significant time was saved. Sampling can occur during multiple life stages (egg or adult), but it much easier and more cost efficient when viewing the above-ground portion of the life cycle.

Egg Sampling

Sampling eggs at the end of the season can serve as a good approximation method for the amount of injury the following season. Hein et al. (1985) determined a higher numbers of subsamples in a field that has been split up into quadrants can reduce the amount of variability found in egg samples but increases the overall cost for sampling. Park and Tollefson (2006) observed no spatial variability in egg samples on a whole-field scale, but did find some within small plots. Due to spatial variability, Park and Tollefson (2006) determined at least 25 m were necessary between eggs samples on a field scale and at least 0.4 m were needed between samples within plots. The sampling method used, however, a golf-hole cutter, was previously determined not to be an absolute method but a less efficient method of sampling eggs for both species.

A number of egg sampling methods have been developed (none of which were significantly different in efficacy), but the frame method in the only one that yields absolute densities and satisfies the need for both plant base and between row sampling. Sampling performed with metal frames accounts for differences in egg-laying habits between *D. barberi* and *D. virgifera virgifera* (Foster et al. 1979). *Diabrotica barberi* lay more eggs around the base of the plant while *D. virgifera virgifera* lay more eggs between rows and deeper in the soil profile (Foster et al. 1979). Foster et al. (1979) found 90% of the eggs (sampled in the top 20 cm of the soil) in the top 10 cm of the soil profile, with the base of corn stalks and cracks being concentrated egg laying sites.

One issue with the sampling of eggs, however, is the changes in specific gravity of eggs that occur with development or damage and mortality. Damage to the eggs results in higher specific gravity, which results in the eggs sinking during the separation process during cleaning (Palmer et al. 1976). This difference can lead to underestimates of the egg population in a field when trying to determine whether the threshold for eggs has been reached, especially for samples taken in the spring vs. fall. The economic threshold for *D. barberi* eggs is approx. 24.7 million eggs per hectare. The method provides a relatively reliable sampling method since eggs are stationary, present for an extended period for sampling and don't vary with time of day or weather. However, egg deposition is quite variable so sampling necessitates relatively large sample number and time investment in processing that becomes cost-prohibitive (Krysan and Miller 1986).

Adult Sampling

Adult beetle variability across a field necessitates counts using a systematic sampling plan, which involves sampling each quadrant of a field (Steffey et al. 1982) . Peak population estimates take the form of either whole plant counts or sticky traps to provide the most accurate predictions of root injury the following season (Hein and Tollefson 1984, Foster et al. 1986, Park and Tollefson 2005). Estimating adult corn rootworm population densities in order to predict injury is commonly performed utilizing sticky traps, but may also be done with whole-plant counts (Hein and Tollefson 1984).

Whole-plant beetle counts have been proposed as the most accurate to estimate beetle populations at the lowest cost. Subsequent yield loss, however, could not be predicted by beetle counts or root injury ratings, and damage levels below economic thresholds were inadequately estimated by adult *Diabrotica spp.* (Foster et al. 1986). In addition, there is a limited scouting window each day due to variable beetle activity depending on the weather, population age, and corn phenology. Foster et al. (1986) attempted to estimate the probability of damage levels by using Bayes' theorem and prior pest density data. Since rainfall also has a significant effect on *Diabrotica spp.* larval populations, season / location specific weather data must also be factored into economic thresholds (Chiang et al. 1980).

Only two sticky traps have been evaluated for their predictive abilities of root injury the following season based on trap beetle capture. Pherocon AM sticky traps have been shown to effectively capture *D. virgifera virgifera* and *D. barberi* when left out extended periods (6-21 days) (Karr and Tollefson 1987). Trap age and temperature have been shown to affect beetle capture, but the variability due to these factors are reduced when traps are left out for at least three days (Karr and Tollefson). In previous research, performed on non-transgenic hybrids, sampling proved highly correlated with injury when corn was in the blister through dent stages (Hein and Tollefson 1985), though only approximately 25 percent of the variability in subsequent root damage was explained by trap captures. The un-baited Pherecon AM sticky trap provides consistent corn rootworm population estimates. Based on the data from Hein and Tollefson (1985) an economic threshold of 40 beetles per trap per week (or six beetles per day) was recommended with twelve traps per field providing an accurate estimate for predicting subsequent injury (Hein and Tollefson).

Another option for scouting for corn rootworms is the Olson sticky trap. The Olson sticky trap provides accurate predictions of root injury the following year and accounts for more of the variability in root injury when additional edaphic and agronomic actors are included (Kuhar and Youngman 1998). In a study performed on Olson sticky traps in Virginia (U.S.), an economic threshold of 20 western corn rootworms per trap per week with an emphasis on trap capture in mid-August predicted injury the best (Kuhar and Youngman). Trap capture thresholds only cexist for *D. virgifera virgifera* with Olson sticky traps. When using this scouting method in continuous cornfields, 12 traps per week are recommended to obtain 95% certainty about corn rootworm concentrations. Sex composition has been quantified for Pherocon AM and Olson sticky traps in order to determine whether differences occurred between the traps, but no differences were found (Kuhar and Youngman 1995). While a higher proportion of the beetles captured were males, the two traps yielded similar results in sex composition.

Chapter I.

Review of the Literature

c. Corn

The exclusive host species for *D. virgifera virgifera* and primary host for *D. barberi* is corn or maize (*Zea mays* L.) (Smith 1966, Branson 1981). Corn is a monocotelydonous plant with development that mirrors that of *Diabrotica* spp. Corn originated in Central America alongside *D. virgifera virgifera* and spread northwards with the corn – cucurbit agriculture of native Americans. Today, corn is grown throughout much of North America, with the Corn Belt (a portion of the country located in the Midwest) producing the most field corn for the country. As the primary food source for *Diabrotica spp.* at all life stages, corn has the ability to influence emergence, migration, and oviposition (Darnell et al. 2000).

In terms of developmental temperature thresholds, the lower limit for corn is 10°C is 35°C) (Schaafsma et al. 1991, Woodson et al. 1996, Nafziger 2012). This similarity in developmental temperature profiles results in excellent synchrony between host and pest and makes it easier to predict life stages for the pest depending on the phenology of the corn plant. For instance, corn that is planted late tends to yield less than corn planted during the optimal time frame of April 20th – May 10th (resulting from less canopy intercepting solar radiation in the weeks around the summer solstice, fewer degree-days preceding frost in Minnesota) (Nafziger 2012). Delayed corn planting also reduces injury cause by *Diabrotica spp.* due to a lack of food availability (Hibbard et al. 2008).

Corn has gone through a series of genetic improvements over the years in order to increase benefits due to physical, morphological and phenological characteristics, disease and insect tolerance and / or resistance, and yield. Corn rootworm typically starts feeding on and causing injury to corn when it is at growth stage V6 (which stands for the vegetative growth stage when six leaves are fully emerged with the collar visible) (Abendroth et al. 2011). While this stage precedes the visible appearance of any reproductive tissue development, the plant is rapidly taking up nitrogen as this stage and beginning ear formation, which directly affects ear development and yield (Ciampitti and Vyn 2012).

Corn hybrids are typically chosen based on yield potential, relative maturity, and disease resistance (Nafziger 2012, Illinois Agronomy Handbook) related to local performance. Since corn does not possess strong host plant resistance aginst *Diabrotica* spp., this is not one of the deciding factors that goes into hybrid decisions. Instead, corn hybrids producing transgeic proteins, isolated from *Bacillus thuringiensis* Berliner (*Bt*), are used to combat corn rootworm insect pressure (Moellenbeck et al. 2001, Vaughn et al. 2005). *Bt* corn hybrids contain multiple forms of the *Bt* traits, which may be inserted singly or pyramided (multiple traits active on the same target insect) as described earlier in the control history section. As long as field corn remains a main crop in the Midwestern United States, corn rootworm will continue to be a pest of concern.

Chapter II.

Optimal Sticky Trap, Required Sample Size and Resulting Precision for Northern and Western Corn Rootworm Scouting

Introduction

Control of corn rootworms (*Diabrotica virgifera virgifera* LeConte [WCR] and *Diabrotica barberi* Smith and Lawrence [NCR]) has been a focal point of concern for growers in the Midwest U.S. for decades due to the injury the larval stage inflicts on corn (*Zea mays* L.) roots. While scouting methods, based on sticky traps, were developed and verified over 30 years ago for corn rootworm (*Diabrotica* spp.), the release of highly effective, corn-rootworm-active *Bt* transgenic hybrids beginning in 2003, largely eliminated the need for corn rootworm scouting. With the emergence of *Bt*-resistant rootworm populations, scouting for corn rootworm populations to verify trait performance, detect emerging resistance and predict root injury in the following year's corn crop is receiving renewed attention. Sticky traps, a common scouting tool, have been used in the past to assess risk of injury the following season and thereby assist in the use of IPM tactics, such as insecticide use, hybrid selection, and crop rotation. However, the criteria for management decisions have not been updated in more than 30 years.

For more than ten years, growers have relied primarily upon transgenic corn hybrids expressing Cry proteins from *Bt* corn to control corn rootworms (Moellenbeck et al. 2001, Vaughn et al. 2005). Rapid adoption of this highly efficacious technology eliminated the need for field-specific scouting information in corn rootworm management decisions, resulting in scouting for corn rootworm becoming an unnecessary expense that generated irrelevant data. However, in 2009, field-evolved resistance to corn-rootworm-active *Bt* hybrids (*Bt*-RW) was discovered in Iowa and Minnesota (Ostlie 2009, Gassmann et al. 2011). Since then, the incidence of problem fields (i.e., fields where excessive lodging and root pruning occur despite the use of transgenic hybrids) are becoming more prevalent in the Corn Belt, leading to a renewed focus on scouting in an effort to control corn rootworms. In addition to the increase in *Bt*-RW resistance, some growers are opting to return to planting non-*Bt*-RW hybrids due to the high costs associated with transgenic seed, resulting in a need for field-specific scouting information to monitor corn rootworm populations. Field-specific insect management relies on scouting to provide data integral to integrated pest management (IPM) decisions.

Despite changes in economics, shifting production practices, evolving corn hybrid genetics and root architecture, the introduction of corn rootworm-active *Bt* events, scouting thresholds for western corn rootworm have not been updated in over 20 years (Hein and Tollefson 1985). Thresholds for northern corn rootworms were developed with emergence cage data, but were never used commercially and data from sticky traps does not exist (Fisher 1985). In 2013, economic thresholds were estimated for northern corn rootworm to be 3.5 beetles per cage, however that study aimed to update thresholds based on economics (Dunbar and Gassman 2013). The study by Dunbar and Gassmann (2013) was performed on extended diapause beetles in Iowa and also used emergence cages instead of sticky traps. Multiple kinds of stick traps exist today (Olson^{TM} sticky trpa(Olson Products Inc., Medina, OH), Multigard ^{TM} sticky trap (Scentry, Billings, MT), Pherocon AM No-Bait^{TM} sticky trap (Trece, Adair, OK), and IPM Yellow^{TM} sticky trap (Great Lakes IPM, Vestaburg, MI)) and are marketed for corn rootworm: however, data (or updated data in the case of Pherocon AM WCR trap captures) is lacking for all of them.

While the re-adoption of scouting in some areas is encouraging, growers are using outdated thresholds to make management decisions to control corn rootworms and may be inaccurate decisions based on transfer of Pherocon-AM thesholds to captured levels with other traps. Outdated information provides challenges to growers when trying to determine what adult beetle captures mean in terms of assessing production risks, evaluating performance of current control tactics, detecting *Bt*-RW resistance, and in deciding when to switch control methods. By using yellow sticky traps to estimate corn rootworm population levels, time spent in the field can be minimized, the traps and the results can be shown directly to the grower. Updated beetle thresholds are needed especially for management decisions, in order to help reduce insecticide and trait costs against sub-economic populations and to determine when to switch *Bt* events as *Bt* trait resistance and density of corn rootworm survivors increase. This study explored the required sample size, precision, and utility of yellow sticky traps for effectively monitoring northern and western corn rootworm populations with four different yellow, non-baited, sticky traps and resulting egg populations the following spring within each site.

Materials and Methods

Estimation sampling using repeated measures design with factorial treatment arrangement was used to quantify trap capture rates of four different yellow sticky traps: Olson^{TM} (Olson Products Inc., Medina, OH), Multigard ^{TM} (Scentry, Billings, MT), Pherocon AM No-Bait^{TM} (Trece, Adair, OK), IPM Yellow^{TM} (Great Lakes IPM, Vestaburg, MI) (Table 1). The surface area of the current Olson trap is larger than the one used in the original study by Kuhar and Youngman (1998). The area of the Olson, Multiguard and IPM traps used in this study were standardized to the area (414 cm^2) Pherecon AM trap area for comparison purposes (Table 1).

Traps were evaluated at seven sites in Minnesota from 2013-2015 (Table 2). Sites differed in corn rootworm species composition, population density, and apparent *Bt*-trait resistance. Sticky traps were arranged in a RCB with four transects and three locations per transect for a total of 12 traps of each trap type at each site. Traps were placed with sufficient distance between each sticky trap to avoid local beetle depletion or interference (Losey et al. 2003). Transects were approximately 15-20 m apart with locations within transects approximately 8-10 m apart. Traps were changed once weekly for four to five weeks (depending on emergence status when we were notified of the site, set up traps and evaluated activity of corn rootworm beetles as sampling progressed) (Table 2) (Karr and Tollefson 1987).

Trap capture totals were reported on a capture-week basis with all 12 traps pooled for the week. A natural log transformation was used for all of the capture data in order to meet the necessary statistical assumptions for the analyses. Trap captures were analyzed as a repeated measures design using an ANOVA in R using package Agricole (Mendiburu 2014, R Core Team 2013). Trap type and week served as fixed effects while sites and reps (each of the 12 locations within a site) served as the random effects. Threshold equivalents were determined for Multiguard, Olson, and IPM sticky traps by regressing the trap captures for each of these traps against Pherocon AM trap captures. The threshold of six beetles per trap per day (Hein and Tollefson 1985) was then used in the regression equation to determine trap capture threshold equivalents.

Egg sampling occurred before planting the subsequent spring after sticky trap scouting. Samples were gathered from 10 locations in each of four different transects, which corresponded with sticky trap scouting transects used to monitor beetle populations the previous season. Eggs were sampled using the frame method (Foster et al. 1979). Soil was removed to a depth of 20.3 cm in order to obtain as many eggs as possible (Hein et al. 1985). Two subsamples, approx. 0.47L, were reserved from each frame for egg processing using the method described by Atyeo et al. (1964). Eggs were quantified in total, but not by species, due to difficulties with many of the samples in obtaining enough clarity to properly observe chorion differences for species identification. Peak beetle capture per week for each sticky trap was modeled against eggs at each locations, calculated as number of eggs per hectare, in order to determine the predictive ability of each trap for resulting egg populations in the soil (Park and Tollefson 2005).

The log mean-variance for each trap by species was compared to determine whether required sample sizes would differ for each separate trap and species. Linear mixed-effects models were used to compare these relationships in R. Taylor's power law (Taylor 1961) was used to determine the relationship between the mean and variance.

$$S^2 = a + b*\log(x) \qquad [1]$$

The coefficients for a and b were determined by regressing the log mean and log variance for each trap and each week on pooled sites. Southwood's equation for required sample size was used to calculate the number of traps needed to accurately predict a population level (Southwood 1978):

$$RSS = (t^2*S^2)/(D*\bar{x})^2 \qquad [2]$$

Where t is the student's t value, S^2 is sample variance (calculated using Taylor's power function), D is desired precision, and x is trap capture per day. Precision was set at 0.25.

Results

Sticky trap captures of WCR and NCR varied greatly between species and trap type. For WCR, population levels differed between sites (site: F=145.64; df=6, 96; p<0.0001). Traps also revealed large difference in captures across weeks (week: F=10.21; df=1, 96; p=0.0019), reflecting differences between the weeks leading up to and following peak emergence. Trap capture differed significantly between trap types (Trap: F=5.85; df=3, 96; p=0.0010) with the Multiguard sticky trap capturing the most WCR beetles (Fig. 3). Traps exhibited different capture capabilities between sites (site x Trap:

F=1.91; df=18, 96; p=0.0236) with Multiguard obtaining significantly higher capture rates than all other traps at Rosemount in 2014. Traps did not interact with weeks (Week x Trap: F=0.1924; df=2, 96; P=0.9014).

Northern corn rootworm captures were dependent on site (Site: F=59.08; df=6, 96; P<0.0001) and week (Week: F=41.31; df=1, 1320; P<0.0001). Traps differed significantly (Trap: F=2.32; df=3, 96; P<0.0001) with the Multiguard trap again capturing the most beetles. Trap captures were dependent on the site (Site x Trap: F=2.64; df=18, 96; P=0.0012), but not week (Week x Trap: F=1.64; df=3, 96; P=0.1855).

The number of traps required to precisely estimate population levels were determined for WCR and NCR on each trap type. After examining the relationship between the log mean and log variance across trap types for each species, it was determined that one required sample size would suffice across all trap types. For WCR, the traps did not differ in their log mean x log variance relationships (Traps: F=0.29; df=2, 67; P=0.2858). The relationships also did not significantly vary by week in the field (Week: F=0.62; df=1, 67; P=0.4355), nor was there a Trap x Site interaction (F=1.21; df=12, 67; P=0.2981). The required sample size for WCR, across all traps used in this study was found to be eight traps / location / week, which is significantly less than the 12 traps / week requirement reported by Hein and Tollefson (1985) for the Pherocon AM no-bait trap.

Northern corn rootworm trap captures followed a similar pattern to WCR; the relationship between the log mean and log variance did not differ among trap types (Trap: F=0.68; df=2, 67; P=0.5082). Note: The IPM Yellow Sticky trap had to be left out of this analysis, however, because of its inconsistent results from Site to Site. The relationship among remaining trap types was also consistent across weeks (Week: F=1.56; df=1, 67; P=0.215) with no interaction across locations (Site x Trap: F=1.16; df=12, 67; P=0.3276). The number of traps determined to provide a precise population estimate for NCR was 6 traps / Site / week, 25% lower than the estimate for western corn rootworm.

Analysis for WCR was taken a step further to determine threshold equivalents (assuming similar trap capture capabilities), based on the Pherocon AM trap (threshold = 6 beetles / trap / day (Hein and Tollefson 1985)), for the other three traps evaluated in this study. The equivalent values were determined from the regression relationship between Pherocon AM trap captures with trap capture for each respective trap type. While previous research suggested a threshold of approx. 3 beetles / trap / day for the Olson stick trap (Kuhar and Youngman 1998) in the Eastern U.S., our results indicated 10 beetles / trap / day provided a better fit. Multiguard was also found to have a threshold of 10 beetles / trap / day as well, while the IPM trap was found to need a threshold of only 7 beetles / trap / day (Table 3).

The linear regressions between trap capture and egg numbers the following year (as sampled in the spring) revealed some differences between trap types. The Olson sticky trap capture revealed a weak relationship with egg densities the following season ($p=0.0915$, $R^2=0.70$) for the limited number of sampling sites. While Multiguard caught the most beetles, its trap captures were not related to sampled egg numbers the following year with that trap, or any of the other traps.

Discussion

Sticky traps can be an effective and time-efficient way to monitor pest populations and gain information for use in decision-making on corn rootworm management. The traps are only useful, however, if information is known about their trapping abilities, precision, and relationship to future pest abundance (i.e. corn rootworm eggs in this case) or root injury. This study aimed to evaluate these parameters and determine what recommendations could be made for future use of this scouting tool under current production systems and in view of emerging *Bt* resistance.

Previous research determined the economic threshold for Pherocon AM trap capture per day to be six beetles per trap per day (Hein and Tollefson 1985) under a 1985 production and economic context. By examining Fig. 3, it can be surmised that all seven sites used in this study were above economic thresholds for WCR throughout the scouting period. The high WCR pressure at these sites allowed sufficient confidence regarding the trap capture results and conclusions.

Northern corn rootworm, however, does not have established thresholds for sticky traps. This lack of prior threshold, combined with the low population levels at five of the seven sites used in this study, only allows us to make preliminary interpretations about NCR, which will need to be evaluated further under a wide range of NCR populations and production situations.

All of the traps examined throughout this study exhibited differences across sites, weeks and traps in capture rates. Site differences were largely due to population levels at the location, as well as when the scouting period occurred and how that aligned with the phenology of corn rootworms at each location. Multiguard sticky traps provided the highest capture rates for WCR across all seven sites, followed by Olson sticky traps. Multiguard appears to provide the highest capture rates for NCR as well for sites with greater NCR populations. However, results were highly variable with Multiguard, which caught numbers similar to that of IPM and Pherocon,

Differences in trap captures rates between the two species, specifically for Olson sticky traps, raises the question of whether spectral reflectance plays a part in the capture capabilities for these traps. With all traps placed at the same height during the scouting period, spectral reflectance is one possible variable accounting for the difference in capture between the species. Adhesive differences do occur between the traps, but may not result in differences in trap capture for the two species. The finding that the IPM sticky trap requires a higher threshold (more captured WCR beetles per trap per day) reinforces the spectral hypothesis, considering the IPM trap uses the same adhesive (TangleTrap) as Pherocon AM, yet has a different threshold. This finding suggests spectral reflectance of the sticky trap may contribute to the trap's ability to capture corn rootworms.

Olson yellow sticky traps have been previously determined to have an economic threshold of 20 beetles per trap per week (which would be approximately three beetles per trap per day (Kuhar and Youngman 1998). While our study contained an Olson sticky trap, the trap in our study was a different size than the one used in the original study, which may explain the reason for the 10 beetles / trap / day threshold determined from this study, calculated by using Pherocon AM threshold equivalents. The range in thresholds calculated in this study (six to ten beetles per day), as well the number of traps required per scouted field differed among sticky trap types. All of the traps evaluated in this study demonstrated similar precision in predicting population levels, allowing growers and agricultural professionals to choose any of these traps when scouting. The ability to use a variety of traps could encourage increased adoption of scouting for people with limited access to a particular trap type. However, growers and ag advisors need to be aware that minimum trap number and thresholds are specific to each trap type. Compared to Foster and Tollefson (1988), however, fewer yellow sticky traps were needed to obtain desired precision under current corn production and beetle densities; a finding that's beneficial for those who would like to be more efficient in their scouting.

The poor relationships obtained from egg sampling in the spring results in additional questions about the relationship between trap capture and root injury and which explanatory parameters may be necessary. While the frame method only requires 15.8 samples to be within 20% standard error of the mean according to Foster et al. (1979), In contrast, Hein et al. (1985) determined that in order to obtain a desired precision of 0.25, 64 samples from 32 locations would be necessary. In this study, we sampled in 40 different locations within the study area, which is smaller than the whole-field sampling universe used by Hein et al. (1985). Egg survival can be impacted by temperature, moisture, surface residue, and egg depth: all of these factors impact the ability to recover eggs via sampling the following season (Gustin 1983, Brust and House 1990, Godfrey et al. 1995, Ellsbury and Lee 2004). Eggs that suffered from damage or mortality have higher specific gravity (Palmer et al. 1976). This results in the eggs sinking during separation procedures performed during the egg cleaning process. Spring egg populations may have been underestimated as a result of the cleaning process versus the sampling process.

Beetle captures by these traps differed weekly across the scouting window; these results indicate the need to run traps over a multi-week window to ensure estimates from peak week of activity. A glimpse into the differences that may occur between the species also revealed disparities between the traps used in this study. This finding raises additional questions of how *Bt* traits, which are known to cause shifts in beetle phenology, may extend this scouting window (Frank et al. 2015, Hitchon et al. 2015). Previous research indicates that delayed planting also shifts beetle phenology and reduces beetle emergence (Musick et al. 1980, Bergman and Turpin 1984, Hibbard et al. 2008). However, it is unknown whether delayed planting, when coupled with *Bt* traits, results in a further delay. This information would be crucial in designing a contemporary, field-specific scouting program based on yellow sticky traps

The utility of sticky traps as a scouting tool cannot be underestimated at a time when corn production costs are high and profits are low. With fewer traps being required for precise population estimates than previously reported, a higher adoption rate for this cornerstone for IPM may be achieved. While additional studies on the use of sticky traps for NCR population estimates are needed, this study provided a starting point for using a variety of sticky traps to assess WCR populations.

Table 1. Sticky trap types and properties of each trap used for scouting corn rootworm in the sticky trap comparison and scouting study from 2013-2015 at multiple locations in Minnesota.

Sticky Trap*	**Adhesive**	**color**	**Grid/No Grid**	**Sticky area measurements****
Olson	Hot Melt	yellow	No	28.5 cm long x 15.4 cm wide
Multiguard	Stickem Special	Neon Green	Yes	23.4 cm long x 15.5 cm wide
Pherocon AM	Tangle Trap	Yellow	Yes	23 cm long x 18 cm wide
IPM Yellow	Tangle Trap	Yellow	Yes	23 cm long x 18 cm wide

* Olson^{TM} (Olson Products Inc., Medina, OH), Multigard ^{TM} (Scentry, Billings, MT), Pherocon AM No-Bait^{TM} (Trece, Adair, OK), IPM Yellow^{TM} (Great Lakes IPM, Vestaburg, MI

**Sticky area measurements refers to the area of the trap where the adhesive is applied and functional in capturing corn rootworm.

Table 2. Years, locations, apparent *Bt*-RW susceptibility at time of scouting, cropping system and hybrid planted for sticky trap comparison and scouting study from 2013-2015 at multiple locations in Minnesota.

Year	**Location**	**Species Composition [Western: Northern]***	**Apparent *Bt* Susceptibility****	**Cropping System**	**Hybrid Planted**
2013	Rosemount, MN	51:1	Susceptible	Continuous corn >10 yrs.	Refuge
	Litchfield, MN	60:1	Resistant	Continuous corn >10 yrs.	2 pyramided hybrids
2014	Rosemount, MN	7:1	Susceptible	Continuous corn >10 yrs.	Refuge
	Lanesboro, MN	4:1	Resistant	Corn-soybean rotation	Refuge
	Rushford, MN	12:1	Resistant	Corn on corn	Refuge
	St. Joseph, MN	1:1	Susceptible	Conventional Corn	Conventional Corn
2015	St. Joseph, MN	1:6	Susceptible	Conventional corn	Conventional Corn

* Species composition presented at ratio of western corn rootworms to northern corn rootworms

*Apparent susceptibility determined by number of adult beetles during scouting and presence of excessive root injury on *Bt*-RW corn.

Table 3. Threshold Equivalents for western corn rootworms between Pherocon AM ™ No-Bait (6 beetles/trap/day) and three other sticky traps examined in this study: Olson ™, Multiguard ™, and IPM Yellow ™ sticky traps. Threshold Equivalents derived from linear regressions with sticky trap capture data from seven sites in MN (2013-2015).

Threshold Equivalents to Pherocon AM No-Bait (6 beetles/trap/day)*			
Trap	Slope	Intercept	Equivalent (beetles/trap/day)
Olson	3.8 (0.68)	0.98 (0.04)	10
Multiguard	2.92 (0.66)	1.17 (0.04)	10
IPM	3.12 (0.55)	0.72 (0.03)	7

* Un-baited Pherocon-AM sticky trap threshold as determine by Hein and Tollefson (1991).

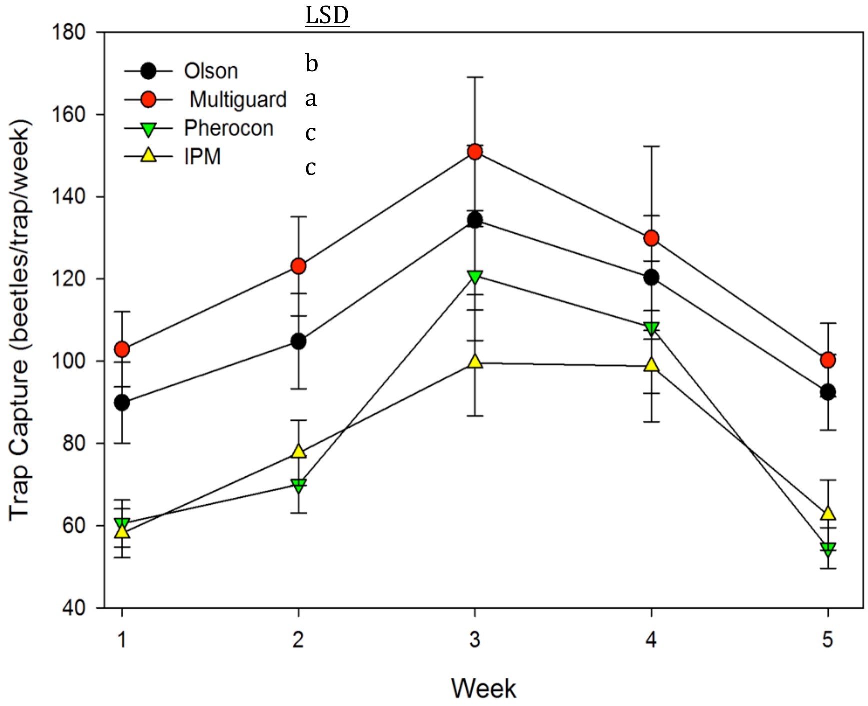


Figure 1. Mean trap capture (beetles/trap/week) and standard error of the western corn rootworm (*Diabrotica* virgifera virgifera) for four different sticky traps across a five-week sampling period (n=7 sites) (Week: F=10.21; df=1, 96; P=0.0019; Trap: F=5.85; df=3, 96; P=0.0010). Trap surface area standardized on the Pherocon AM No-bait trap area (414 cm^2). Letters represent Fisher's LSD results for significantly different traps.

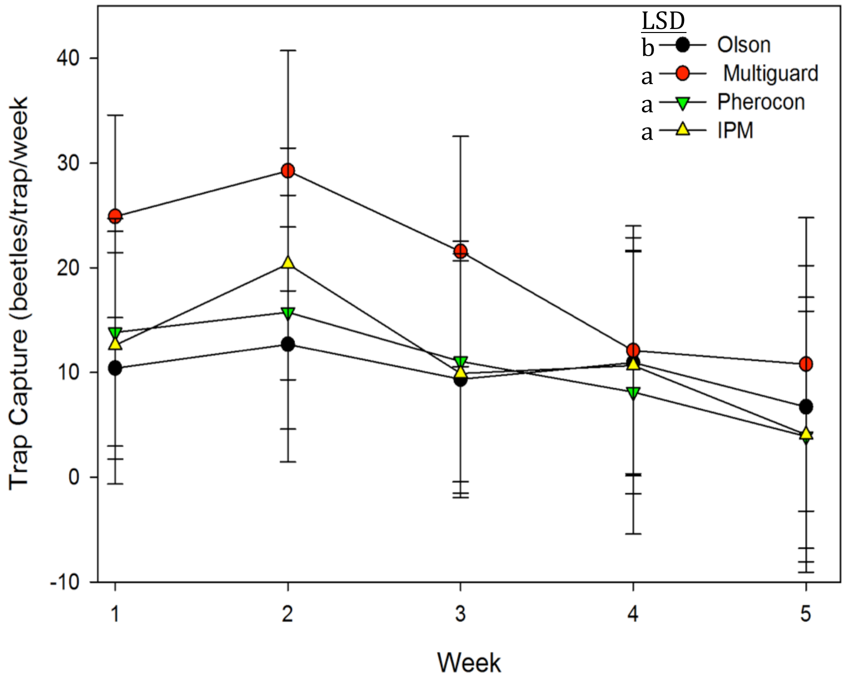


Figure 2. Mean trap captures (beetles/trap/week) and standard error of the northern corn rootworm (*Diabrotica barberi*) for four different sticky traps across a five-week scouting window (n=7 sites) (Week: F=41.31; df=1, 1320; P<0.0001; Trap: F=2.32; df=3, 96; P<0.0001). Trap surface area standardized on the Pherocon AM No-bait trap area (414 cm^2). Letters represent Fisher's LSD results for significantly different traps.

Chapter III.

The effect of planting date and transgenic hybrids on beetle emergence patterns

Introduction

Corn rootworm (CRW, *Diabrotica* spp.) beetles are highly adaptable pests that have overcome multiple control tactics throughout decades of corn (*Zea mays* L.) production. In the absence of control, corn rootworms primarily injure corn plants through larval feeding on the roots. Root injury reduces nutrient uptake, increases lodging, aggravates drought stress, and facilitates secondary attack by pathogens (Kahler et al. 1985). For every root node injured, a yield loss of 15% can be expected (Tinsley et al. 2012). At high populations, larvae compete for feeding sites and limited corn root tissue, the primary and nearly exclusive food source for larvae. The results of this competition: larvae die, body size of survivors is reduced and with it, mating success and fitness (Hibbard et al. 2008).

Delayed corn planting shifts developmental synchrony between corn rootworm phenology and corn root development with progressively smaller root systems available at egg hatch. Late-planted (with respect to the normal planting period) corn with its smaller root systems reduces larval colonization success (Hibbard et al. 2008), restricts food availability, and potentially enhances competitive mortality. Corn rootworm eggs that hatch when corn is at growth stages V4 to V11 (typical growth stages for corn planted during normal planting dates) produce more adults than eggs hatching at later corn growth stages (Hibbard et al. 2008). Corn rootworm beetle emergence is also delayed in late-planted corn, and occurs over a longer period (Musick et al. 1980, Bergman and Turpin 1984, Obopile et al. 2012). Beetles that emerge early (from non-*Bt*-RW corn) have been found to live longer than beetles that emerge late (Boetel and Fuller 1997). While the control tactic of delayed planting can result in less injury, yields can be significantly reduced so it's not employed as a control method as much as an artifact of weather-related constraints on planting.

Transgenic corn producing *Bacilus thuringiensis* (Berliner) (*Bt*-RW) crystalline (Cry) insecticidal protein(s) is the newest tactic for managing corn rootworm. The *Bt*-RW proteins, however, are low-dose toxins and do not provide complete control of corn rootworms (Siegfried et al. 2005). Larval exposure to Bt-RW proteins shifts phenology later, presumably due to sub-lethal effects of the toxin (Frank et al. 2015, Hitchon et al. 2015). The phenology shift, appears as delayed adult emergence, varies among Bt-RW traits and increases in magnitude with added Bt-RW traits in pyramided trait hybrids (Frank et al. 2015, Hitchon et al. 2015). For example, Hybrids containing Cry3Bb1 or Cry34/35Ab1 alone (single-trait for corn rootworm) result in a delay (12.3 days and 3-7 days, respectively) when compared to emergence from a refuge hybrid (non-Bt-RW) and emergence from hybrids containing Cry3Bb1 + Cry34/35Ab1 pyramided, results in an even further delay (15.8 days) than the single-traited hybrid (Hitchon et al. 2015). Similarly, hybrids expressing mCry3A + eCry3.1Ab pyramided also substantially delay (13.7 days) 50% beetle emergence (Frank et al. 2015).

Western corn rootworm resistance to *Bt-RW* hybrids has renewed effort to utilize scouting-based information in management decisions (Gassmann et al. 2011). Scouting corn rootworm beetles with sticky traps can be a useful tool for predicting risk, the number of eggs laid in a field, which directly relates to root injury expected the following season (Hein and Tollefson 1985). However, corn production practices, yield expectations and economics have changing markedly from 1980s (Hein and Tollefson 1985) and updated thresholds reflecting contemporary situations are needed. Dunbar and Gassmann (2013) revised existing thresholds for sticky trap scouting in rotated corn to reflect updated economic situation and yield-loss relationships. But this revision only focused on updating calculation; it did not consider how changing production practices in continuous corn affect risk, beetle populations, or their captures on yellow sticky traps. Management practices, such as *Bt*-RW hybrids or planting date, which affect corn rootworm populations, their synchrony with corn and fitness, can alter the underlying risk relationships between scouting data and subsequent impacts on corn production.

Bt-RW hybrids and planting date affect corn rootworm: corn synchrony, corn rootworm development and adult emergence. In order to update scouting and thresholds, management practices that may alter corn rootworm development need to be considered. How do *Bt*-RW transgenic traits and delayed-planting affect the scouting windows for accurate corn rootworm population estimates? Peak beetle activity in a field has been shown to provide the best predictions of root injury (Foster et al. 1986). Beetle phenology shifts due to these two different management tactics could lead to “misses” in trap deployment, underestimate ovipositional activity, and errors in corn rootworm management decisions.

This study examined how planting delays, when coupled with corn rootworm-active *Bt* hybrids, affect corn rootworm emergence phenology and how this may change optimal scouting windows for assessing corn rootworm populations. Our null hypothesis is that no further shifts in beetle emergence occur when delayed planting is coupled with corn rootworm-active *Bt* traits.

Materials and Methods

The effects of planting date coupled with *Bt*-RW traits on corn rootworm phenology were explored through four studies conducted at the University of Minnesota - Rosemount Research and Outreach Center, Rosemount, MN. These four studies spanned three field seasons: (2012: 1 locations, 2013: 2 locations, and 2014: 1 location). Fields used in this study were chosen based on moderate, natural corn rootworm pressure (estimated from yellow sticky trap data collected the previous year) and continuous corn cropping history without the use of *Bt*-RW hybrids for at least two years prior to the study. In the year prior to each study, locations were planted with long-maturity corn hybrids in order to increase oviposition in the field (trap crop effect) and amplify corn rootworm pressure the following year. Corn rootworm populations at the site were predominantly western corn rootworm with no significant issues with either extended diapause of NCRs or loss of egg-laying fidelity to corn by WCRs.

Studies were arranged in a split-plot design, consisting of two single-trait *Bt*-RW hybrids (one expressing Cry3Bb1 [VT Triple®[VT3]], one expressing Cry34/35Ab1 [Herculex RW® [HXX]), a pyramided hybrid (expressing both Cry3Bb1 and

Cry34/35Ab1 [Smart Stax® [SSX]]) (Table 4) and a non-*Bt*-RW hybrid (refuge) as main plot treatments, and four different planting dates (early May, mid-May, late May, and early June) as the subplots with four total replications of each treatment. Each planting date was approximately ten days after the prior planting date. Plots contained eight rows (15.2 m length, 0.76 m row-spacing) seeded at a rate of 85,000 seeds per ha^{-1} (35,500 seeds/A). No soil insecticide was applied: however, seed received 250 mg / kg seed of clothianidin (Poncho®) or thiomethoxam (Cruiser®).

Growing degree-days were determined by using the rectangle method with the program DEGDAY (Higley et al. 1986). Air temperatures, starting on 1 April each year, were used to calculate cumulative growing degree-days. Cumulative growing degree-days were substituted for planting date in order to allow for comparison across years and developmental rates. Cooling degree-days were calculated utilizing the sine-wave method (as described in DEGDAY by Higley et al. 1986) with an upper threshold of $-1^{o}C$ to determine the amount of mortality expected from winter temperatures the preceding winter.

Adult beetle emergence, used as an indicator of corn rootworm survival and phenology, was obtained by using cut-plant emergence cages (30 cm by 76 cm), modified from Chaddha et al. (1993). Six emergence cages were installed in rows two and seven (three cages per row) in order to minimize effects from adjacent plots. Emergence cage placement was chosen based on plant spacing (approximately 15 cm apart) with sufficient distance from the end of the plot (at least 60 cm) to ensure reproducibility of sampling units and minimize plot edge effects. Emerged beetles were collected twice per week (every three to four days) from July through mid-September. While the locations used for these studies contained mixed populations of northern corn rootworms (*Diabrotica barberi* Smith and Lawrence) and western corn rootworms (*Diabrotica virgifera virgifera* LeConte), during the three-year study period, the populations were dominated by western corn rootworm beetles. Emerged beetle totals reflect the mixed populations.

Data was analyzed with a mixed-model ANOVA using package Agricole (Mendibruru 2014) in R (R Core Team 2013). Cumulative beetle emergence was calculated based on the biweekly collections. Using the cumulative beetle emergence data, the day of year (DOY) when 50% beetle emergence was reached indicated peak beetle emergence (Bergman and Turpin 1984). Beetle emergence was transformed on the natural log scale in order to meet the necessary statistical assumptions for the mixed-model ANOVA. Years were not combined due to inconsistent treatments across years (Table 4) and weather-related location X season confounding. Growing degree-days, calculated with the rectangular method to the time of seed planting, were used to represent planting dates on a physiological basis, in place of DOY. Please note: root injury data were not included in this paper due to a high degree of variability across years and abnormally low injury levels in some years reflecting winter egg and spring larval mortality.

Results

Since planting date (Hibbard et al. 2008, Musick et al. 1980) and Bt-RW traits (Hitchon et al. 2015) are known to affect both the magnitude and phenology of corn rootworm adult emergence, this research focused on beetle emergence. Statistical analyses explored the individual and combined effects of these factors on adult emergence and its implications for corn rootworm scouting. Planting date (presented as growing degree-days) is known to change beetle phenology and predicted damage to plants based on previous research.

2012

Transgenic *Bt-RW* hybrids significantly delayed peak beetle emergence in comparison to the non- *Bt*-RW hybrid (F=30.58; df=2, 21; p<0.0001) (Fig. 3a). However, planting date (degree days at the time of planting) did not significantly affect peak beetle emergence (Fig. 3a) when evaluated across all hybrids (including non *Bt*-RW) (F=5.64; df=3, 9; p=0.0188). When the SSX hybrid was analyzed alone for the effect of planting date on 50% beetle emergence, there was a significant delay due to time of planting (F=10.72; df=1, 6; p=0.0169). There was also no interaction between planting date (degree days at planting) and the hybrids used in this study (F=0.83; df=6, 21; p=0.5598).

Total beetle emergence in response to cumulative degree-days at time of planting and *Bt*-RW hybrid was evaluated. Total beetles to emerge (per ha) were greatly affected by the hybrid treatment (F=19.02; df=1, 35; p<0.0001) (Fig. 4a). As expected, the most beetles emerged from the non-*Bt*-RW hybrid, followed by the VT3 hybrid and then the SSX hybrid. Planting date also had a significant effect on the number of emerged beetles (F=17.68; df=1, 3; p=0.0246) (Fig. 4a.). The first planting date resulted in the greatest number of beetles emerging from all hybrids, while the last planting date (most cumulative degree-days at time of planting) resulted in the fewest beetles. The VT3 hybrid (producing Cry3Bb1) produced more beetles in planting date three than planting date two, resulting in a nearly significant interaction between hybrids and planting dates in this study (F=2.99; df=2, 35; p=0.063).

2013

Hybrids (F=2.21; df=2, 21; p=0.1282) and planting date (F=0.82; df=1, 3; p=0.4328) failed to affect peak beetle emergence at site 1 (Fig. 3b). Also, no interaction between hybrids and time of planting was present (F=0.60; df=2, 27; p=0.5538). When only the *Bt* hybrids were analyzed, however, the *Bt* hybrids differed in the DOY at peak emergence at site 1 (F=12.98; df=1, 20; p=0.0018). Adult emergence was delayed longer with the pyramid Cry3Bb1+ Cry34/35Ab1 than with Cry34/35Ab1 alone. Planting date impact on DOY at peak beetle emergence bordered on significant (F=8.24; df=1, 3; p=0.0640), with DOY at peak emergence increasing with later planting dates.

At site 2, however, hybrids significantly delayed peak corn rootworm emergence (F=42.28; df=2, 34; p<0.0001) (Fig. 3c). Planting date changed phenology of corn rootworm (F=10.57; df=1, 2; p=0.474) (Fig. 3c), but in ways inconsistent with the other studies. In the SSX hybrid, the first planting date saw the greatest delay in peak emergence out of the four planting dates. In the non-*Bt*-RW hybrid, the last planting date produced beetles delayed more than either of the *Bt*-RW hybrids (Fig. 3c). An expected response would be for the earliest planting date to cause the least delay in emergence across all three hybrids and the last planting date to cause the greatest delay, with the SSX showing a greater delay than the other two hybrids. No interaction was present between hybrids and planting date for site 2 (F=2.28; df=2, 33; p=0.1173).

Total beetle emergence from site 1 in 2013 was not significantly impacted by either hybrid (F=2.79; df=2, 36; p=0.0745) or cumulative degree-days at time of planting (F=0.08; df=2, 36; p=0.7924) (Fig. 4b). A similar response was found at site 2 with neither hybrid (F=1.01; df=2, 36; p=0.3747) or cumulative degree-days at time of planting (F=0.01; df=1, 3; p=0.9106) (Fig. 4c.) having a significant impact on total beetle emergence. A large amount of variation in the abnormally low total beetle emergence was observed, especially at site 1, and extended across all hybrids and planting dates. Hybrids and planting dates did not interact in their effect on beetle emergence at site 1 (F=0.83; df=1, 2; p=0.4453) or site 2 (F=0.55; df=1, 2; p=0.5835).

2014

Corn rootworm-active *Bt* hybrids delayed emergence in comparison to the non-*Bt*-RW hybrid, but to varying degrees. For this study, both single trait hybrids (VT Triple [VT3] and Herculex RW [HXX]) were used in addition to the pyramided trait hybrid (Smart Stax [SSX]). The SSX hybrid produced the greatest delay, which is consistent with previous results, followed by the HXX hybrid, and lastly, the VT3 hybrid (F=31.89; df=3, 49; p<0.0001) (Fig. 3d.). In contrast to the previous studies just discussed, only a slight delay in western corn rootworm emergence when compared to the non-*Bt*-RW hybrid, was detected in the VT3 hybrid (3 days in 2014 vs. 13 days for site 1 and 11 days for site 2 in 2013 and 9 days in 2012). Loss of an emergence delay has been associated with developing *Bt*-RW resistance in western corn rootworm (Wangila and Meinke 2016). By inference, this dramatic reduction in VT3 delay in 2014 suggests *Bt*-RW resistance may be developing in this population. While previous bioassays results for *Bt*-

RW susceptibility conducted on western corn rootworms collected from Rosemount, MN in 2012 indicate a susceptible population (Zukoff et al. 2016); performance problems with VT3 in this field were detected in 2016 (Ostlie 2016).

There was no delay in beetle emergence due to planting date in this 2014 study (F=2.89; df=1, 3; p=0.1878) (Fig. 3d.). No interaction between hybrids and planting date and their effect on beetle emergence was detected (F=0.48; df=2, 49; p=0.6967).

As with DOY for peak emergence, hybrids differed markedly in total beetle emergence (F=29.75; df=3, 50; p<0.0001) (Fig. 4d.). In contrast to expected efficacy, VT3 and the non-*Bt*-RW hybrid produced similar numbers of beetles across the four planting dates. Both the HXX hybrid and the SSX hybrid performed as expected, significantly reducing adult emergence with the pyramided SSX hybrid producing the fewest number of beetles. Loss of efficacy with the VT3 hybrid, i.e., beetle production nearly equivalent to the non-*Bt* hybrid, provides a second indicator of developing resistance at this site.

Planting date, as represented by cumulative degree-days at time of planting, did not significantly impact total beetle emergence (F=0.01; df=1, 3; p=0.9237). Similarly, hybrids did not appear to interact with planting date to affect beetle emergence (F=0.28; df=1, 3; p=0.8396).

Discussion

Shifts in beetle emergence phenology, caused by delays in planting date (Bergman and Turpin 1984) or *Bt*-RW exposure (Frank et al. 2015, Hitchon et al. 2015), have been studied independently, but their combined effects on the magnitude of this delay and its implications for scouting have not been studied previously. In these studies, results varied across all four site-years at Rosemount, MN. Planting date effects varied in three of four site-years; these delays generally followed previously reported patterns by Hitchon et al. (2015) (pyramided *Bt*-RW traits > single *Bt*-RW traits > no *Bt*-RW traits) where single *Bt*-RW trait hybrids typically caused delays ranging from seven to twelve days (i.e. HHX and VT3 respectively) and 15.8 days for pyramided *Bt*-RW-traited hybrids (i.e. SSX). No interactive effects of planting date and *Bt*-RW hybrid on peak emergence were detected at any of the four sites.

The emergence delays caused by *Bt*-RW hybrids, on susceptible populations, were severe enough to hide any emergence delays due to cumulative degree-days at time of planting. Previous research indicated delays in adult emergence of approximately 13-14 days could be expected between the earliest and latest plantings (Bergman and Turpin 1984) under higher corn rootworm population densities but prospective delays were inconclusive under low population densities (Musick et al. 1980). Low population densities were evident in this trial in 2013 (see Fig. 4. a-d.).

While the winter of 2013/14 accumulated the most cooling degree-days, cold projected further into the winter of 2012/2013 with cumulative cooling degree days peaking at the end of March (Fig. 6), i.e., growing degree-days began accumulating around April 29th in 2013 compared to April 1st for 2012 and 2014 (Fig. 5). Extended cooling degree days in 2013, which presumably increased egg mortality, also delayed the accumulation of degree days needed for 50 percent corn rootworm egg hatch (380-426 degree days; Wilde 1971, Levine et al. 1992). The DOY when 380 degree days was reached: 2012- DOY 160; 2013- DOY 218; 2014- DOY 202). The combination of a high number of cooling degree-days that extended further into the spring, coupled with a slow increase in soil temperature resulted in high egg mortality which can be viewed in Figs. 2b and 2c.

Total beetle emergence in this study showed a wide range across years. Previous research performed by Bergman and Turpin (1984), indicated a clear reduction in beetle emergence as planting dates extended later into the growing season, presumably resulting from smaller root system at hatch (greater colonization mortality) as well as reduced food availability with a higher proportion of roots with insufficient root diameter for older larvae (increased intra-specific competition for limited food) (Strnad and Bergman 1987). In contrast, beetle emergence in this study did not reveal any consistent reduction with later planting dates across the three years and four locations in this study (Table 3). Weather-induced mortality, variations in cumulative degree-days at the time of planting, and spring population levels of corn rootworms across the locations obscured any pattern.

Beetle emergence data in this study indicated the only modification required was to adjust scouting windows for *Bt*-RW traits. Planting date did not interact with *Bt*-RW traits to further shift adult emergence in the field, which confirmed our null hypothesis. However, a warning is in order; don’t be too quick to shorten scouting windows for later planting dates based on beetle emergence. Later planting dates shift corn phenology; this later silking and pollen-shedding period can be quite attractive to immigrating corn rootworm beetles as silks in earlier-planted surrounding fields dry. Resulting egg densities reflect more than just beetles emerging from the field. Immigrants also bring with them mixed susceptibility / resistance genea to *Bt*-RW traits, which could increase the likelihood of resistance developing to these traits.

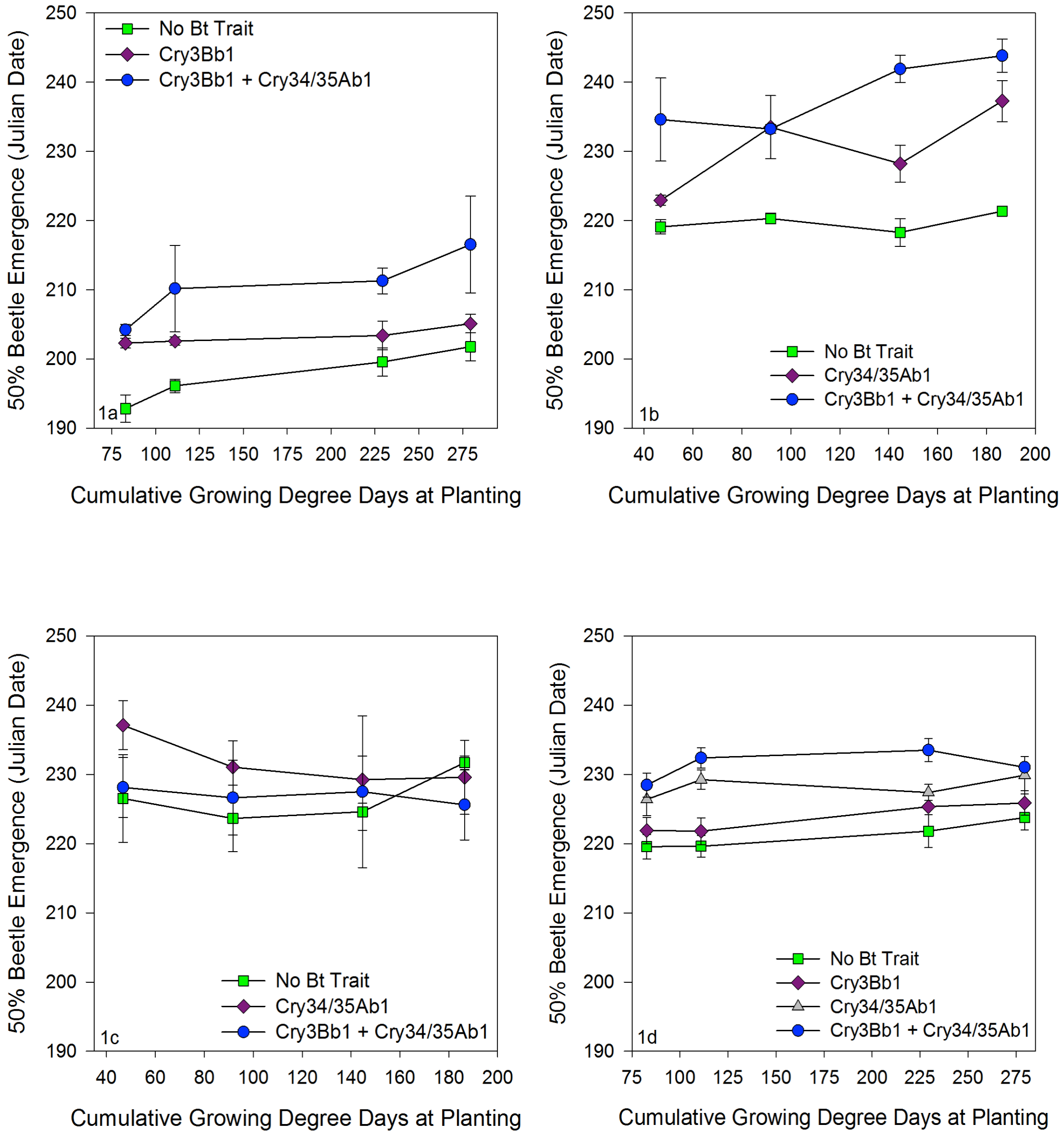
The lack of an emergence delay observed in the VT3 hybrid compared to the non-*Bt*-RW hybrid in 2014 revealed prospective resistance to the Cry3Bb1 trait at this location. Subsequent corn rootworm research in this same field (less than 30 m away) confirmed greater-than-expected root injury occurring on the VT3 hybrid in both 2015 and 2016. The presence of resistance to VT3 in this population would be expected to reduce the emergence delay commonly observed in *Bt*-RW hybrids. This phenomena complicates efforts to define a scouting window for *Bt*-RW hybrids; the scouting window needs to reflect not only the emergence delays induced by the *Bt*-RW trait but also disappearing delays should resistance be present in the field. The combined effects of these factors actually lengthens the prospective scouting window for *Bt*-RW fields.

Table 4. Corn hybrids, relative maturities (in days) and corn rootworm-active *Bt* (*Bt*-RW) traits for each hybrid used in the studies examining planting date x *Bt*-RW hybrid effects on beetle phenology and total emergence.

Year	Hybrid	RM*	*Bt*-RW traits	Trade Name
2012	DKC 44-92	94	None	None
	DKC 46-60	96	Cry3Bb1	VT Triple®
	DKC 46-61	96	Cry3Bb1 +Cry34/35Ab1	Smart Stax®
2013**	DKC 50-67	100	None	None
	2A499	99	Cry34/35Ab1	Herculex® RW
	DKC 49-94	99	Cry3Bb1 +Cry34/35Ab1	Smart Stax®
2014	DKC 43-10	93	None	None
	DKC 43-18	93	Cry3Bb1	VT Triple®
	P9526	95	Cry34/35Ab1	Herculex® RW
	2A399	93	Cry3Bb1 +Cry34/35Ab1	Smart Stax®

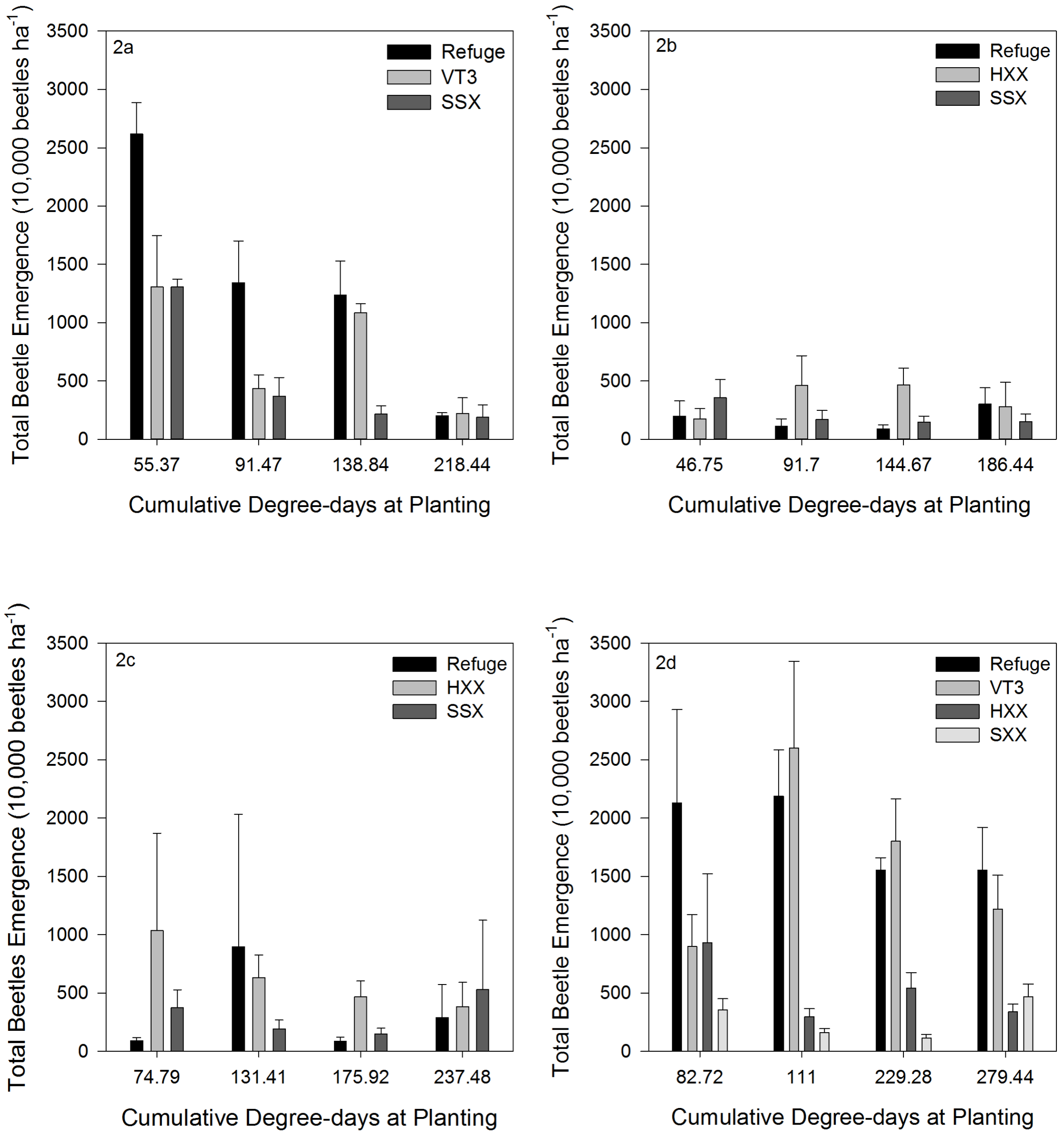
* RM = Relative maturity of the corn hybrid (in days)

** The same hybrids were used at both locations in 2013



Figures 3a-d. Peak beetle emergence (50% emergence) plotted against cumulative growing degree-days at time of planting under different *Bt* protein (Cry3Bb1 [VT3], Cry34/35Ab1 [HXX] and Cry3Bb1 + Cry34/35Ab1 [SSX]) treatments and one non-*Bt* hybrid treatment. Fig. 3a: Planting: F=8.49; df=1, 3; p=0.0618, Hybrid: F=35.06; df=3, 33; p<0.0001. Fig. 3b. Planting: F=0.82; df=1, 3; p=0.4328, Hybrid: F=2.21; df=2, 29;

$p=0.1282$. Fig. 3c. Planting: $F=10.57$; $df=1, 3$; $p=0.0474$, Hybrid: $F=42.28$; $df=2, 34$; $p<0.0001$.Fig. 3d. Planting: $F=2.89$; $df=1, 3$; $p=0.1878$, Hybrid: $F=31.89$; $df=3, 49$; $p<0.0001$. Study performed at the University of Minnesota Research and Outreach Station in Rosemount, MN.



Figures 4a-d. Total beetle emergence (10,000 beetles ha^{-1}) from each hybrid plotted against cumulative growing degree-days at time of planting under different *Bt* protein (Cry3Bb1 [VT3], Cry34/35Ab1 [HXX] and Cry3Bb1 + Cry34/35Ab1[SSX]) treatments and one non-*Bt* (refuge) hybrid treatment: Fig. 4a. Planting: F=17.68; df=1, 3; p=0.0246, Hybrid: F=19.02; df=1, 35; p<0.0001. Fig. 4b. Planting: F=0.08; df=1, 3; p=0.7924, Hybrid: F=2.79; df=2, 36; p=0.0745.Fig. 4c. Planting: F=0.01; df=1, 3; p=0.9106, Hybrid: F=1.01; df=2, 36; p=0.3747. Fig. 4d. Planting: F=0.01; df=1, 3; p=0.9237, Hybrid: F=29.75; df=3, 50; p<0.0001. Study performed at the University of Minnesota Research and Outreach Station in Rosemount, MN.

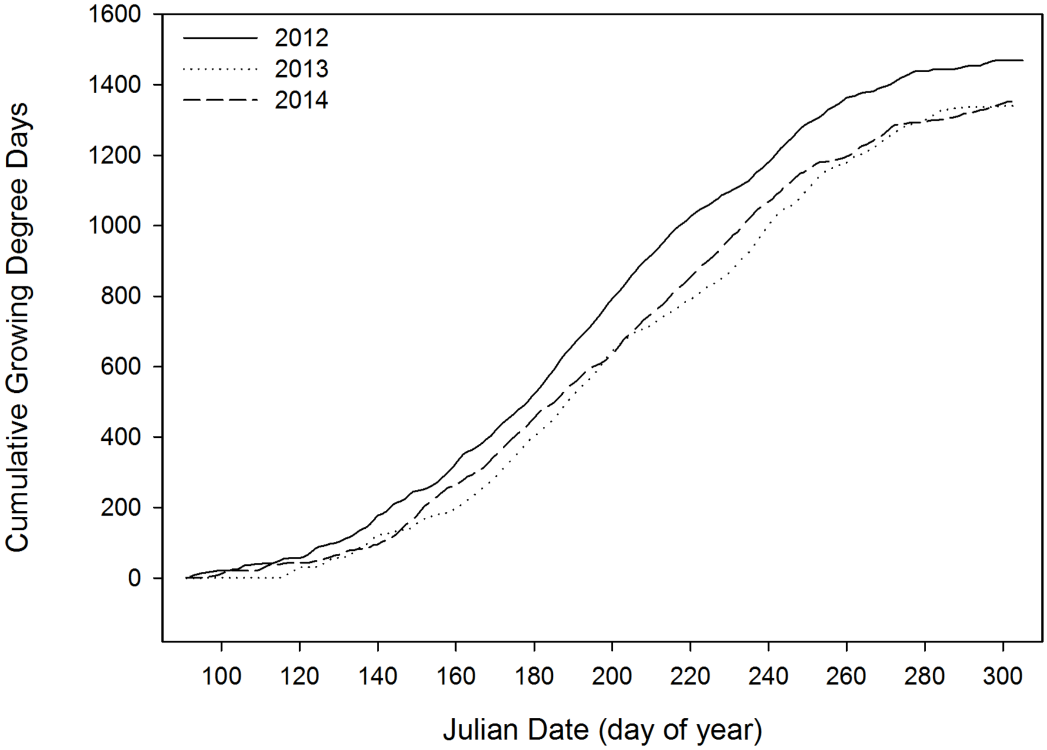


Figure 5. Cumulative growing degree-days from time of planting for each year. Temperature (°C) data is from above ground air temperature at Rosemount, MN.

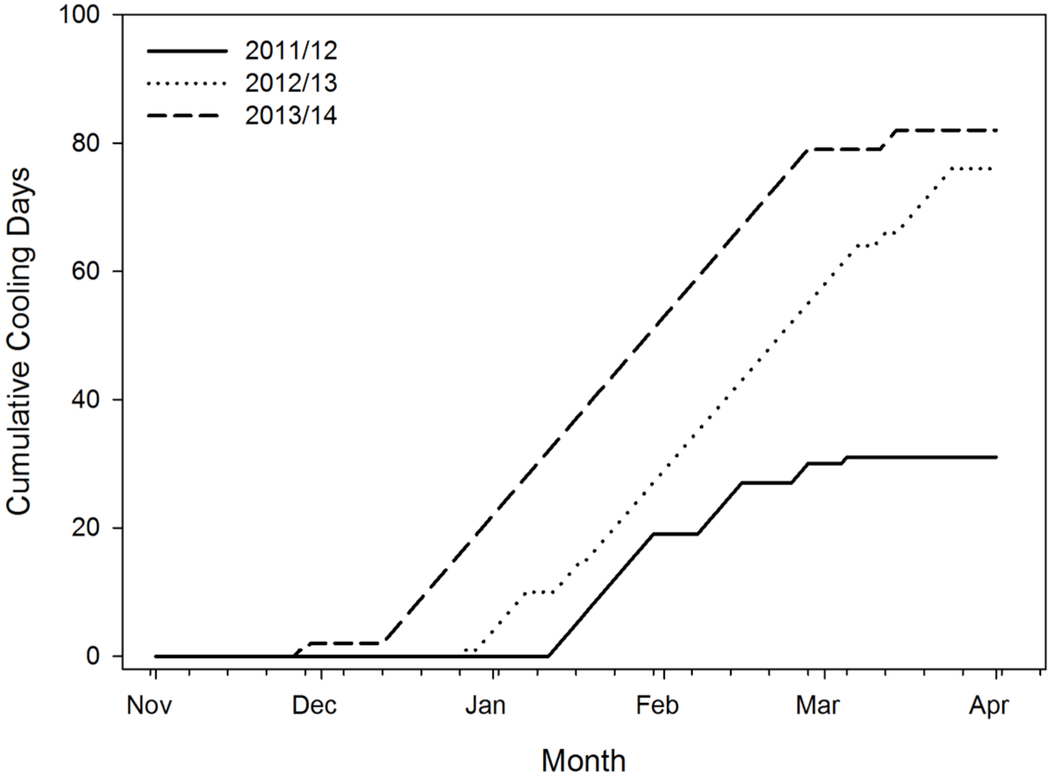


Figure 6. Cumulative cooling degree-days resulting in mortality. Upper limit: -1°C. Temperature data is soil temperature at 5cm below soil surface at Rosemount, MN.

Chapter IV.

Predicting Corn Root Injury Based on Un-Baited Sticky Trap Captures For Corn Rootworm

Introduction

Corn rootworms (*Diabrotica* spp.) have been pests of economic importance on corn (*Zea mays* L.) for over a century in the United States. Damage to the plant occurs in the form of lodging, yield loss, and secondary attack by pathogens as a result of root injury due to larval feeding (Kahler et al. 1985, Spike and Tollefson 1989). For the past fifteen years, growers have been relying primarily upon highly effective, corn-rootworm-active *Bacillus thuringiensis* Berliner (*Bt*) transgenic corn hybrids to control these pests, to the detriment of classic integrated pest management (IPM) practices (Moellenbeck et al. 2001, Crowder et al. 2006). While the re-adoption of IPM is occurring in some locations, growers are using outdated sticky trap scouting thresholds, developed for outdated genetics and outmoded corn production practices from the 1980s, to make management decisions for control of corn rootworms.

Corn rootworm-active *Bt* corn hybrids provided effective control for almost a decade and remain an effective form of control across much of the United States. Beginning in 2009, however, signs of performance problems with *Bt* hybrids appeared in the form of greater-than-expected root injury and lodging. These performance problems suggested resistance to one of the *Bt* traits, expressed in the corn plant, may have formed. Since 2009, performance problems have arisen numerous times and have now been documented for all of the available traits (Cry3Bb1, Cry34/35Ab1, mCry3A, eCry3.1) across a broad geography in the U.S. (Gassmann et al. 2011, Gassman et al. 2016, Ludwick et al. 2017). Due to the high cost of *Bt* hybrid seed, the added difficulties that can occur with chemical corn rootworm control (via the need for additional equipment, storage, and application risks), and the need to slow development of resistance in additional growing areas, the demand for revised sticky trap scouting thresholds has resurfaced (Crowder et al. 2006).

Corn rootworm thresholds based on sticky trap capture of adult western corn rootworm beetles were originally developed in the 1980s, using the Pherocon AM□ un-baited sticky trap (Hein and Tollefson 1985). A threshold of 42 beetles per trap per week determined to accurately predict root injury the following season. While the predictive ability of the Pherocon AM sticky trap was found to accurately predict root injury 73% of the time, only 26% of the variability in their model for resulting root injury was explained with the sticky trap captures (Hein and Tollefson 1985). A little over a decade later, Kuhar and Youngman (1998) evaluated the use of a second sticky trap, the Olson sticky trap, for its ability to predict root injury the following season. A threshold of 20 western corn rootworms per trap per week was determined to result in significant injury the following season (Kuhar and Youngman 1998). These traps were able to explain a large portion of the resulting root injury with 65% of the variability explained with the regression model (Kuhar and Youngman 1998). The Olson sticky trap, however, was never widely adopted as a sticky trap for scouting corn rootworms due to its handling difficulty. While the thresholds developed for both of these sticky traps provided useful scouting tools for decades, neither of the studies addressed populations that were a mixture of northern corn rootworm and western corn rootworm, or primarily northern corn rootworm. In addition, follow up studies were never performed to identify source of variability not explained by the model. Possible sources of variability due to mixed populations containing both species of corn rootworm or edaphic factors that result in mortality of the corn rootworm while in the soil (Turpin et al. 1972).

Corn rootworms spend the majority of their life cycle in the subterranean soil habitat within fields. The mortality to multiple, soil-active life stages from numerous edaphic, anthropological, climactic and biological factors is extremely high. One source of mortality for corn rootworm is winter mortality during the egg stage (Gustin 1983, Godfrey et al. 1995, Ellsbury and Lee 2004). Winter mortality occurs when soil temperatures drop below 1°C for prolonged periods of time. Western corn rootworm and northern corn rootworms suffer from different rates of winter-induced mortality due to differences in egg-depth and cold tolerance (Gustin 1983, 1986, Ellsbury et al. 1998, Ellsbury and Lee 2004). This mortality affects the ability to relate populations in the summer to expected injury to corn after corn the following season.

Additional mortality can occur during the larval stage due to desiccation, drowning, and inability to reach the root system (Spike and Tollefson 1988, Riedell and Sutter 1995, Hibbard et al. 2008). Due to the long duration of time that is spent in the soil, the effect of soil conditions on larvae is important to note. Soil bulk density restricts larval movement to less than 5 cm in highly dense soils, which can inhibit their ability to migrate to the root system (Strnad and Bergman 1987, Gustin and Schumacher 1989). Soil moisture levels (very wet or very dry) have also been found to play a major role in the mortality of the corn rootworm larvae (Brust and House 1990, Macdonald and Ellis 1990). Previous research has found drainage and percent clay in the soil to be correlated with increased corn rootworm-induced root injury (Turpin et al. 1972). Greater injury occurs with a higher percentage of clay in the soil where larval survival is better (Turpin and Peters 1971). This increase in larval survival in these heavy soil environments is most likely due to lower desiccation rates because of the soil's ability to retain moisture (Turpin and Peters 1971). Certain soil textures can retain moisture, inhibit or slow down migration, increase winter mortality for the eggs, or prevent desiccation of corn rootworms.

To date, these numerous sources of mortality have resulted in failed or weak relationships between sticky trap scouting and resulting corn root injury. By accounting for predicted mortality through the use of soil parameters, a stronger relationship can be found between sticky trap scouted beetles and resulting root injury the following year, which will aid in management decisions. This study evaluated peak populations levels obtained via sticky trap scouting, plus additional parameters representative of beetle mortality, as a way to predict root injury the following season. Our hypothesis is that two sticky traps (Multiguard and Olson) serve as better predictors of root injury (due to their high trap capture rates) compared to the traditionally used trap, Pherocon AM, when a parameter that represents mortality was included.

Materials and Methods

Trials were planted at two locations in 2014, four locations in 2015, and one location in 2016 -differing in their climactic and edaphic conditions and species composition: Litchfield, MN; Rosemount, MN (2014 and 2015); Rushford, MN; Lanesboro, MN; and St. Joseph, MN (2015 and 2016). Locations used were scouted the year prior to the trial with four different un-baited yellow sticky traps: Olson™(Olson Products Inc., Medina, OH), Multigard ™ (Scentry, Billings, MT), Pherocon AM No-Bait™ (Trece, Adair, OK), IPM Yellow™(Great Lakes IPM, Vestaburg, MI) (Table 1), in order to gain insight into the insect pressure that might be present during the following growing season.

Sticky traps were placed in three different locations within each of four transects within the field. Transects were approximately 15-20 m apart and locations within transects were approximately 8-10 m apart. Traps were changed weekly over the course of four – five weeks in order to prevent trap saturation (Karr and Tollefson 1987). Trap capture was quantified (for *Diabrotica virgifera virgifera* LeConte and *Diabrotica barberi* Smith and Lawrence), summarized as trap capture per week (in order to account for differences in scouting periods) and pooled across all twelve traps within a site and week. The week with the greatest trap capture was chosen for use in the generalized linear model due to the indication from previous research demonstrating that this week aligns most closely with peak egg laying as well (Foster et al. 1986, Park and Tollefson 2005). Total average trap capture per week was also explored as an explanatory variable.

Trials featured a factorial treatment arrangement containing all available *Bt*-RW traits and their non-*Bt*-RW counterparts: Cry3Bb1, Cry34/35Ab1, Cry3Bb1

+Cry34/35Ab1, mCry3A, mCry3A + Cry34/35Ab1, and mCry3A + eCry3.1, plus a non-*Bt*-RW hybrids. Due to the low number of Sites available for analyses in this study, only data from the non-*Bt* hybrids are presented here. An additional set of treatments in the study existed comprising of the previously listed hybrids with a granular insecticide (Aztec 4.67 G) layered over the top, however this data is not presented in this paper. Each plot contained four rows (at Lanesboro and Rushford locations) or eight rows (at Rosemount, St. Joseph, and Litchfield) (15.2 m length, 76 cm row-spacing) seeded at a rate of 85,000 seeds per ha^{-1}.

Climate data was analyzed for each Site by using data retrieved from NOAA. Cumulative negative degree-days were calculated using air temperatures and were determined by using the sine-wave method in DegDay program (Higley et al. 1986). Precipitation, measured as mm rainfall, was also explored from post planting until the typical time when pupation occurs as a possible source of mortality.

Soil samples were collected from each Site for use in determining soil properties by collecting ten cores from a depth of 20 cm from the study location, mixing the cores and removing a 0.47 L subsample. Soil samples were dried for approximately 72 hours at 30°C and passed through a 2-mm sieve. Soil was then analyzed for particle size determination using the hydrometer method (Miller et al. 1997). Percent organic matter from each Site was analyzed using the LOI method (Day 1965).

Corn roots were dug in mid-August from ea. plot (five roots per plot) in order to determine the amount of injury resulting from corn rootworm larval feeding. Roots were selected at random with the criteria of them being evenly spaced, tiller-free, and at least

0.6 m from the end of the plot to prevent edge affects. Plants were removed by placing a shovel approximately 20 cm away from the base of the plant and digging straight down in order to retain as much of the root system as possible. Roots were then washed using a pressurized water sprayer in order to remove as much of the soil and debris as possible. Evaluations of the root system were performed by quantifying the number of roots pruned to 3.8 cm or less. Total number of roots were converted to the 0-3 Nodal Injury Scale (Oleson et al. 2005). Root injury from each plot was pooled to obtain an average injury rating per plot for use in analyses.

Results were analyzed using mixed effects, generalized linear regression model (GLM) with a binomial family using package lme4 (Bates et al. 2015) in R (R Core Team 2013). When too much residual variance was detected in the model, the response variable of root injury was set up as a series of Bernoulli trials (probability trials with a finite number of samples) for each root evaluated and then re-run with the binomial family. Generalized linear models were chosen in order to set bounds for the response variable which is root injury rated with the 0-3 Nodal Injury Scale. Models were evaluated with Site and replication as random effects and trap capture (natural log of beetles per week) for each trap and percent clay as fixed effects to account for the original design and to provide greater power for determining the model parameters. As a means of comparing generalized linear regression models containing different sticky traps, the Akaike information criterion (AIC) were evaluated to determine model fit. A two-point difference in the AIC value denotes an improvement in the model fit with the lowest value providing the best overall fit.

All sites were evaluated for characteristic responses and biological fit to determine whether they should be omitted from the final data set used in the analysis, due to observed abnormalities during the time of the trials. The Litchfield site had to be removed from the final analysis due to abnormal trap capture (Fig. 8a-d). The Site experienced very high trap capture and low root injury. During the scouting season (2013), it was observed that the phenology of the corn at that location was ahead of the surrounding cornfields. We believe beetles migrated out of our field to others as fresh food became available (Darnell et al. 2000). This movement would reduce oviposition within our Site resulting in a poor relationship between sticky trap capture and subsequent injury. In addition to Litchfield, Rushford data was also removed from the data set used in the final analysis. This location was removed due to atypical synchrony between corn and beetle development corn planting was delayed as a result of wet conditions. The soil temperatures, however, were warm enough to support ongoing corn rootworm development preceding corn planting. The loss of synchrony resulted in a lack of root system development and scare food-source availability for the corn rootworm larvae upon initially reaching the corn root system.

Results

Initial examinations of possible explanatory variables, such as negative cumulative degree days, rainfall, cumulative degree days at time of planting, and cumulative growing degree days between planting and pupation, revealed weak relationships that were difficult to rationalize and explain. It became clear that an underlying explanatory variable was likely the culprit so soil texture was investigated. Soil texture, due to its ability to effect soil moisture infiltration, saturation, water retention, and temperature, directly affects soil-dwelling insects. Percent clay in the soil was revealed to explain a large portion of variability in the model and was, therefore, used in all of the models (Table 5).

Peak sticky trap capture (the week when the greatest number of beetles were captured) serves as the key predictor for root injury (Park and Tollefson 2005). Corn rootworm counts for both species were combined into average weekly counts. Analyses for each trap revealing that Multiguard sticky traps captured the most beetles, followed by Olson sticky traps ($F=31.35$; $df=3, 1392$; $p<0.0001$) (Fig. 7). In addition to evaluating the week with peak beetle capture, the total beetle capture per week over the entire scouting period was evaluated as well. After examining both models, it was determined that using the peak sticky trap capture as the estimate for the beetle population provided the best fit within the model.

Corn root injury ratings, as they relate to beetle capture for each trap revealed a large amount of variability across Sites (Fig. 8a-d). High variability at two locations, due to loss of developmental synchrony between corn and corn rootworms (Rushford), and presumed migration as a response to delayed corn phenology in surrounding fields (Litchfield) are thought to be the reasons behind high trap capture resulting in low root injury the following season (Naranjo 1991) at these sites. Due to the biologically uncharacteristic results from two locations, they were omitted from the final analyses.

Strong relationships between injury and sticky trap capture for corn rootworms were found for all sticky traps when a soil texture parameter, indicative of water infiltration and holding capacity, was included in the model (Table 6, Figs. 9a-d). Percent clay was determined to account for a large portion of the variance present in the model at each Site and was therefore included in the model as a covariate. While all of the models had similar AICs (Table 6), the Olson sticky trap and Multiguard sticky traps reveled the best fit due to their lower AIC values, and Chi square results from model comparisons. Pherocon AM and IPM Yellow sticky traps also demonstrated predictive power with AIC values similar to that of Olson and Multiguard, however, their models did not account for as much of the variability in the data and were slightly weaker in their predictive capabilities (Table 6, Figs. 9a-d). While all traps resulted in models that fit the data well, the Olson, Pherocon AM and IPM Yellow sticky traps predicted a negative relationship with root injury, which is contradictory to previous literature and common sense.

Beetle capture was set at a fixed rate, with percent clay set as a continuous vector in order to determine the response of root injury (Fig. 10a-d). The models for Olson, Pherocon AM, and IPM Yellow sticky traps revealed uncharacteristic predictions of root injury in response to increasing beetle capture. This abnormal response can be viewed in Fig. 10a (for example), where at 20% clay, 50 beetles/week resulted in a predicted nodal injury rating of 1.3, while 200 beetles per week resulted in a predicted nodal injury rating of 0.4. This inversion likely reflects highly variable conditions with a small sample size for determining correct coefficients in the model. The Multiguard sticky trap model predicted an increase with root injury with increasing beetle emergence and percent clay (Fig. 10b).

Previous research by Tinsley et al. (2013), determined a nodal injury rating of 1 (1 node pruned to 3.8 cm or less) resulted in a 15% yield loss. By plugging this injury rating in for Y and solving for a, we were able to determine the threshold for Multiguard, which would warrant management decisions to be made for a Site. With 15% clay at a Site and resulting injury score of 1.0, approximately 213 beetles per week (30 beetles per day) would have been required the previous season in order to result in 15% yield loss. If the location has 20% clay (more typical clay percentage for many corn-growing areas), in order to result in a nodal injury score of 1.0, 113 beetles per week (16 beetles per day) would have been necessary the previous season (Fig. 10b). Both of these thresholds are higher than previously determined for the Pherocon AM and Olson sticky traps, suggesting more beetles are required to result in injury on corn hybrids grown today.

Discussion

The ability to predict root injury the following season based on easily accessible scouting tools and methods are crucial for proper IPM implementation. Multiple studies have previously been performed in attempt to determine the best relationship and predictive ability via a model between sticky traps and root injury (Hein and Tollefson 1985, Kuhar and Youngman 1998). While some studies have succeeded, this tool remained under-utilized due to new technologies and unexplained variability. Previous literature suggested using the scouting week with the highest beetle capture to determine the probable root injury for the following season (Bergman and Turpin 1984). The study performed here confirmed this previous finding with the peak week of beetle capture strongly predicting root injury in the following season.

The strong relationships observed between expected root injury for each of the sticky traps and resulting root injury suggests that multiple traps may serve as reliable tools for scouting a field, once the models are strengthened with additional data. The inclusion of percent clay in the soil demonstrated the need for a parameter, which characterizes the expected mortality due to the subterranean habitat that corn rootworm eggs and larvae experience. The significant relationship between root injury and percent clay is consistent with previous research by Turpin and Peters (1971), where a positive relationship between clay and root injury was demonstrated. More data is necessary to correctly identify the coefficients for beetles per week for the Olson, Pherocon AM and IPM Yellow sticky traps due to the high variability in trap capture at the locations used in this study. Models could be improved with the addition of more locations, which vary in beetle population levels, species composition, and edaphic conditions such as percent clay in the soil.

Previous literature revealed Pherocon AM and Olson sticky traps both had predictive capabilities for root injury. While these traps demonstrated strong relationships with root injury in this study, the models provided inaccurate coefficients for beetle capture due to variability and limited sample size. Multiguard sticky trap had the highest beetle capture as well as the best model for predicting root injury due to beetle captured with sticky traps and percent clay in the soil. The ability of Multiguard to provide realistic root injury and beetle capture estimates with a model fit similar to the Olson sticky trap, for our data, may reflect differences in NCR capture abilities by the Olson sticky trap (Figs. 9a and 9b). Chapter one explored differences in trap capture abilities for both WCR and NCR. While Multiguard and Olson sticky traps provided similar estimates of WCR populations, the Olson sticky trap estimated lower NCR population levels in comparison to the Multiguard sticky trap. Two of the locations (St. Joseph 2015 and 2016) used in the modeling portion of this study contained high ratios of NCRs in comparison to WCRs. This difference in species mix may have resulted in increased variability in the Olson sticky trap predictions.

As expected, differences in model strength were not observed between the IPM Yellow sticky traps and Pherocon AM sticky traps. This similarity may be explained by the use of the same sticky adhesive in both traps. The differences between the peak trap capture (Fig. 7), however, could signal differences in spectral reflectance between the traps, which may result in differences in the sex ratio for the beetles captured with each trap (Fig. 7). Given the difference between the Pherocon AM and IPM Yellow sticky traps for trap capture, a likely explanation is that while the Pherocon AM trap was able to capture a larger number of beetles, the IPM Yellow sticky trap captured more females. Further studies will be needed to explore the differences in sex ratio for these sticky traps.

The resulting thresholds for the Multiguard sticky trap are contradictory to previous reported thresholds for the Pherocon AM and Olson sticky traps. Our results suggest the threshold is not fixed and instead is a sliding scale dependent on the percent clay in the soil. Clay content can have a strong influence on larval survival by decreasing desiccation (Turpin et al. 1972). However, this threshold needs to be validated with additional data. Since the Multiguard sticky trap did not have a developed threshold previously, use of this trap was limited and new data will need to be generated for use in model validations.

Areas of weakness in the model likely result from not accounting for density-dependent mortality, immigration, and emigration during scouting, which were not evaluated in this study. Additionally, a more robust samples size may have revealed additional relevant explanatory variables such as organic matter, rainfall totals, and soil temperatures. Follow-up studies are needed to further evaluate the relationships determined here as well as validate the model for the Multiguard sticky trap. While many of the fields scouted currently will not contain primarily non-*Bt* corn, the model can still accurately predict injury. When injury is predicted and confirmed to be above acceptable limits for *Bt* corn, resistance mitigation can occur through rotation or the choice of different *Bt* traits, seed, or insecticide. The ability to choose a management plan based on reliable predictions of injury can lessen the financial burden that comes with prophylactic trait and insecticide use as well as provide environmental benefits. The benefit of using sticky traps to scout can be demonstrated through reduced time spent in a field and hard evidence that can be shown to the grower when providing recommendations (Losey et al. 2003). With verification of the Multiguard trap model and additional data for strengthening the other traps, a range of sticky traps with established and updated thresholds will be available for use in IPM programs. This will hopefully provide an incentive for increased adoption of sticky trap scouting for use in management decisions.

Table 5. Soil particle size analysis results obtained using the hydrometer method for seven different study locations in Minnesota from 2014-2016. Soil classifications determined using the Web Soil Survey tool.

Site	% Clay	% Silt	% Sand	Soil Texture^{+}	Soil Classification*
Rosemount 2014	25	62.5	12.5	Silt loam	Garvin silty clay loam (60%), Klinger silt loam (40%)
Rosemount 2015	22.5	56.25	21.25	silt loam	Waukegan Silt Loam
Rushford 2015	21.25	58.75	20	silt loam	Tama-Downs complex, driftless
Lanesboro 2015	17.5	45	37.5	loam	Dakota fine sandy loam
Litchfield 2014	20	25	55	sandy clay loam	Kanaranzi loam (70%), Cylinder loam (30%)
St. Joseph 2015	15	50	35	silt loam	Cushing sandy loam
St. Joseph 2016	15	43.75	41.25	loam	Dakota loam

+ Soil texture determination based on soil texture triangle

* Soil classification determined using the Web Soil Survey. NRCS-USDA.

Table 6. Parameters resulting from a generalized linear mixed effects regression analyzed with a binomial family between resulting root injury (non-corn rootworm-active *Bt* corn roots) and beetle sticky trap capture with varying ratios of *Diabrotica virgifera virgifera* (western corn rootworm) and *Diabrotica barberi* (northern corn rootworm) per week and percent clay in the soil. Coefficients for the equation are: a- natural log of beetles per week; b- quadratic term for the natural log of beetles per week; and c- percent clay in the soil. Studies took place at n=5 locations across Minnesota between 2013 and 2016.

Root Injury[+]	Intercept (± SE)	a Beetles/Week (± SE)	b Beetles/Week (± SE)	c Percent Clay (± SE)	AIC
Olson	-15.66** (4.44)	5.05** (2.20)	-0.67* (0.27)	0.30** (0.06)	3098.7
Multiguard	19.13** (6.84)	-11.77** (3.41)	1.37** (0.42)	0.26** (0.04)	3099.9
Pherocon	-31.80** (7.87)	13.04** (3.88)	-1.63** (0.50)	0.28** (0.90)	3100.1
IPM	-29.13** (9.15)	12.22** (4.66)	-1.55** (0.58)	0.24** (0.06)	3101.2

+ Non-*Bt* roots only

* Significant at the P=0.05 level

** Significant at the P=0.01 level

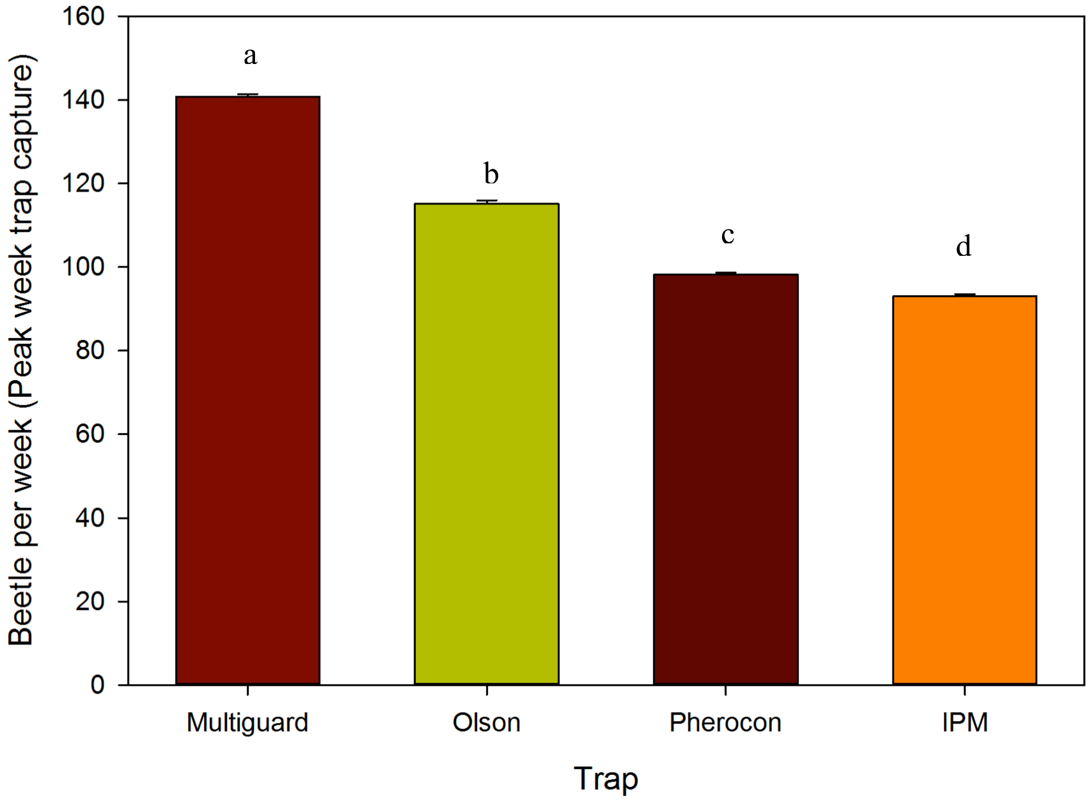
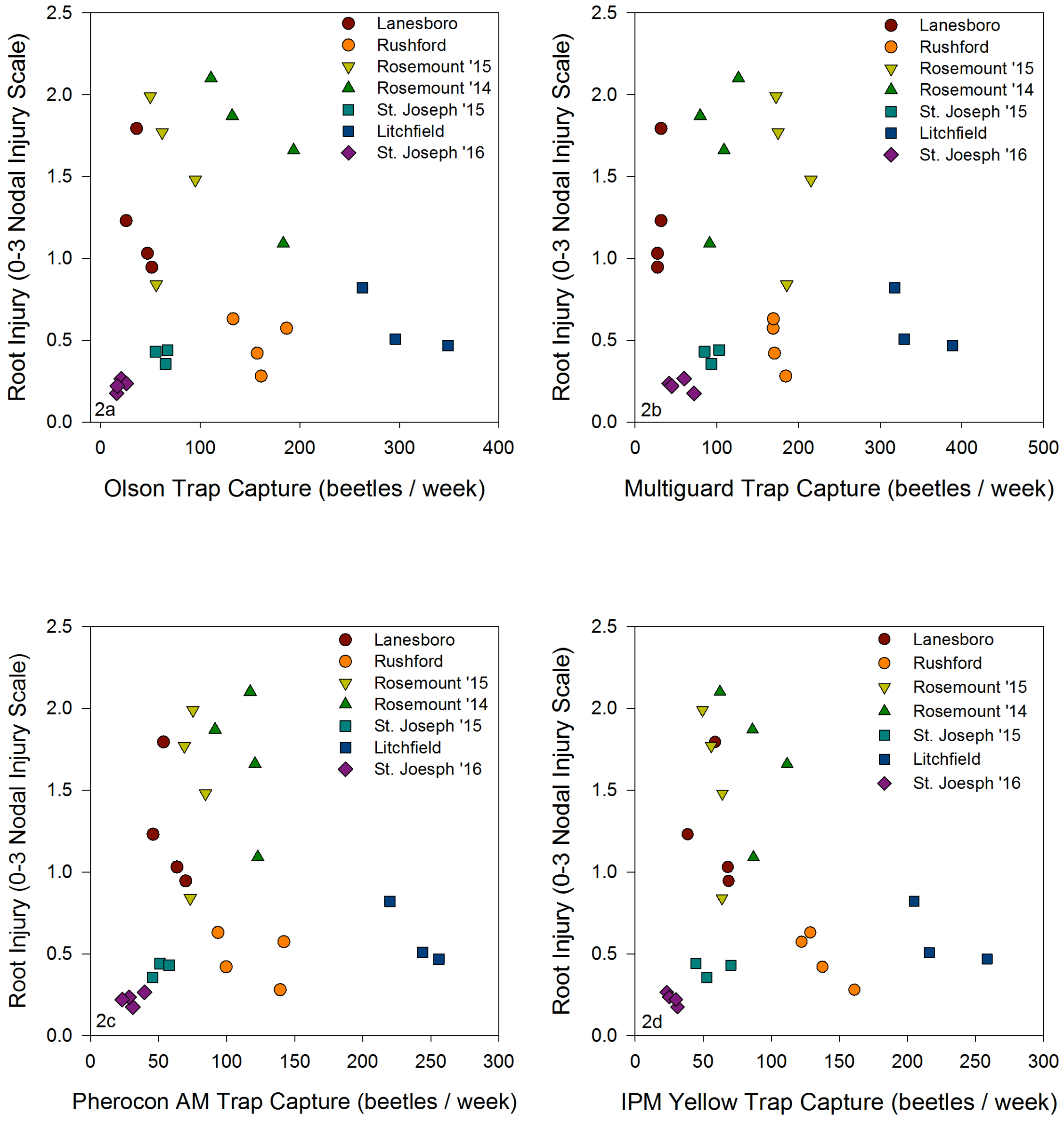
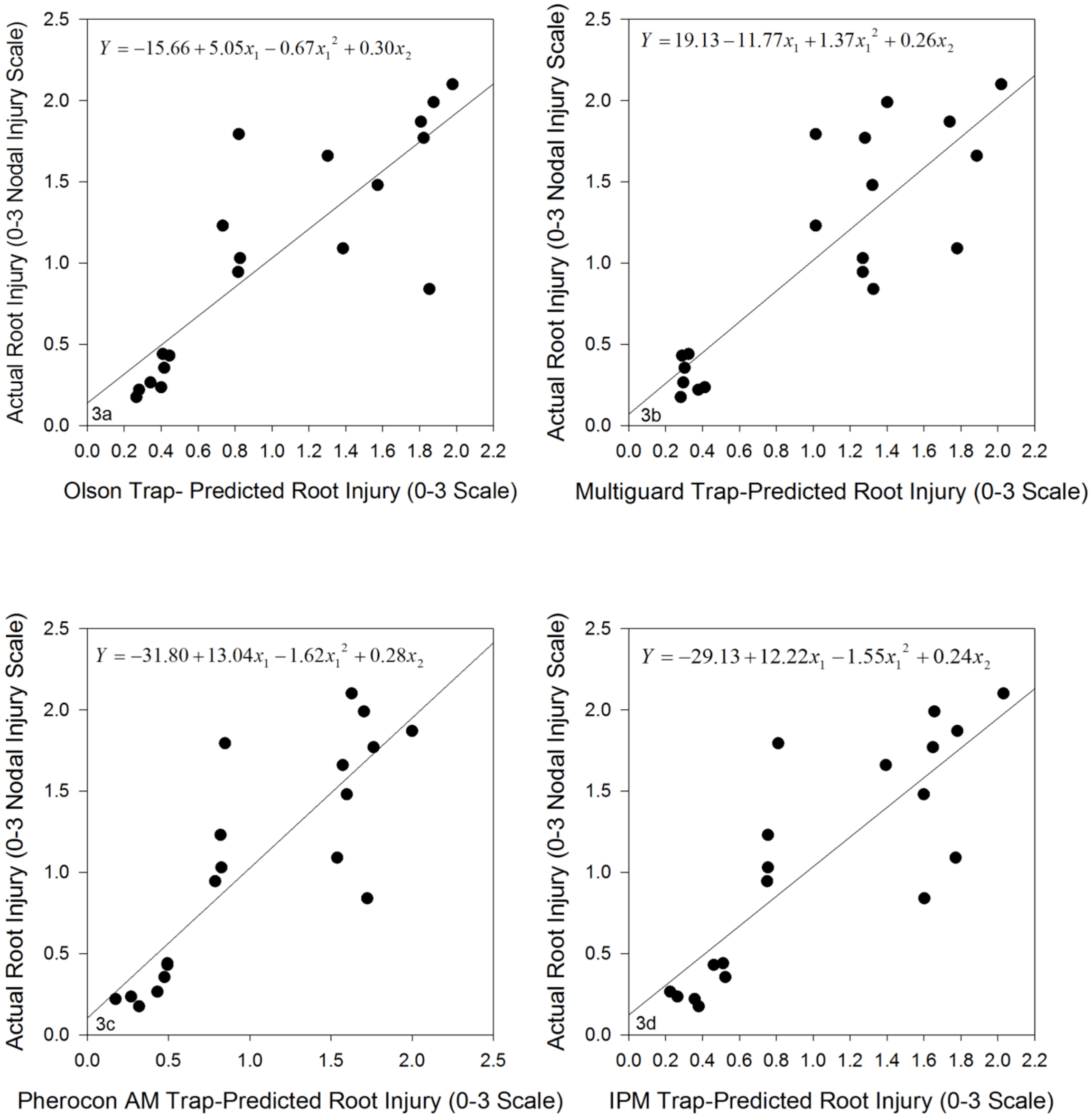


Figure 7. Comparison of beetles per week (peak capture week) for each trap used in the sticky trap – root injury model (F=16.48; df=3, 455; p<0.0001). Four different sticky traps containing a mixture of *Diabrotica virgifera virgifera* (western corn rootworm) and *Diabrotica barberi* (northern corn rootworm): Olson sticky trap, Multiguard sticky trap, Pherocon AM No-bait sticky trap, IPM Yellow sticky trap. Error bars represent standard errors of the means. Letters represent mean separation results. Data obtained from n=7 different locations across Minnesota from 2013-2015.

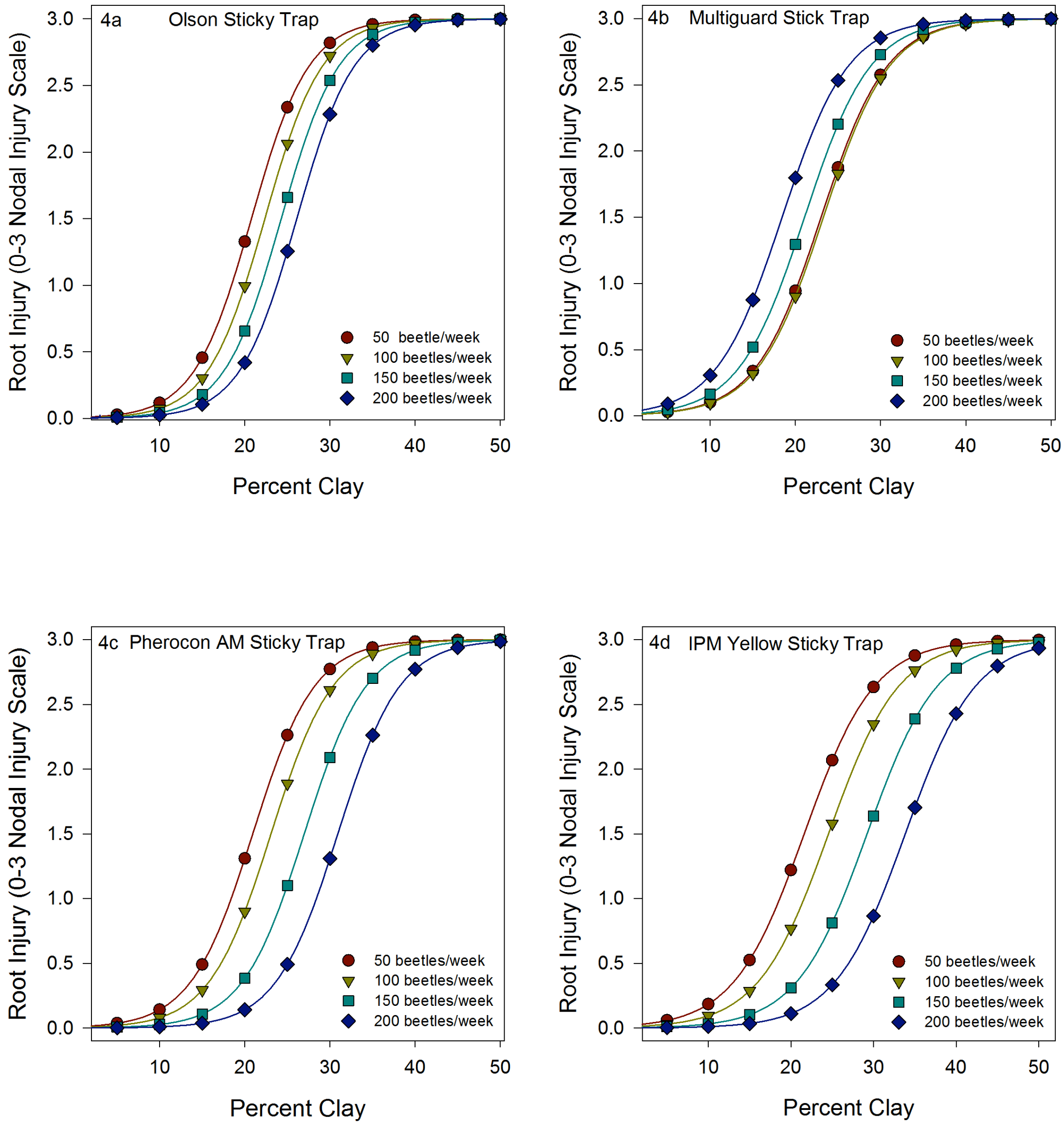


Figures 8 a-d. Root injury as it relates to trap capture (beetles/ week) for all Sites (n=7) and replications (n=4 per Site): 8a. Olson sticky trap, 8b. Multiguard sticky trap, 8c. Pherocon AM No-bait sticky trap, 8d. IPM Yellow sticky trap. Studies took place at seven sites in Minnesota from 2013 to 2016.



Figures 9 a-d. Predicted root injury for all Sites (n=5) and replications (n=4 per Site) for the root injury prediction study with four different sticky traps: 9a. Olson sticky trap, 9b. Multiguard sticky trap, 9c. Pherocon AM No-bait sticky trap, 9d. IPM Yellow sticky trap, where X_1 = the natural log of beetles per week and the quadratic term for beetles per week and X_2= the percent clay in the soil. Equations based studies that took place at n=

five locations in Minnesota from 2013 to 2016. Regression line representing the equations based on sticky trap capture for northern corn rootworm and western corn rootworm trap capture across the entire scouting period.



Figures 10 a-d. Predicted root injury based on percent clay (0-50%) and trap capture for each trap at fixed rates (beetles/ week). 10a. Olson sticky trap (y=-15.66+5.05a-0.67b^2+ 0.30c); 10b. Multiguard sticky trap (y=19.13-11.77a+1.37b^2+0.26c) 10c. Pherocon AM No-bait sticky trap (y=-31.80+13.04a-1.63b^2+0.28c) 10d. IPM Yellow sticky trap (y=-29.13+12.22a-1.55b2+0.24c), where a = the natural log of beetles per week and b = the quadratic term for beetles per week and c= the percent clay in the soil. Equations based studies that took place at five sites in Minnesota from 2013 to 2016.

Bibliography

Abendroth, L. J., R. W. Elmore, M. J. Boyer, and S. K. Marlay. 2011. Corn Growth and Development. Iowa State University Extension, Ames, IA PMR 1009.

Bergman, M. K., and F. T. Turpin. 1984. Impact of corn planting date on the population dynamics of corn rootworms (Coleoptera: Chrysomelidae). Environ. Entomol. 13: 898-901.

Bernklau, E. J., L. B. Bjostad, L. N. Meihls, T. A. Coudron, E. Lim, and B. E. Hibbard. 2009. Localized search cues in corn Roots for western corn rootworm (Coleoptera: Chrysomelidae) larvae. J. Econ. Entomol. 102: 558-562.

Binns, M. R., and J. P. Nyrop. 1992. Sampling insect populations for the purpose of IPM decision making. Annual Review of Entomology 37: 427-453.

Boetel, M. A., and B. W. Fuller. 1997. Seasonal emergence-time effects on adult longevity, fecundity, and egg viability of northern and western corn rootworms (Coleoptera : Chrysomelidae). Environ. Entomol. 26: 1208-1212.

Boetel, M. A., D. D. Walgenbach, G. L. Hein, B. W. Fuller, and M. E. Gray. 1992. Oviposition site selection of the northern corn-rootworm (Coleoptera, Chrysomelidae). J. Econ. Entomol. 85: 246-249.

Branson, T. F. 1982. Olfactory response of larvae of *Diabrotica-virgifera- virgifera* to plant-roots. Entomol Exp Appl 31: 303-307.

Branson, T. F. 1987. The contribution of prehatch and posthatch development to protandry in the Chrysomelid, *Diabrotica-virgifera-virgifera*. Entomol Exp Appl 43: 205-208.

Branson, T. F., and J. L. Krysan. 1981. Feeding and oviposition behavior and life-cycle strategies of Diabrotica - an evolutionary view with implications for pest-management (Coleoptera, Chrysomelidae). Environ. Entomol. 10: 826-831.

Branson, T. F., and G. R. Sutter. 1985. Influence of population-density of immatures on size, longevity, and fecundity of adult *Diabrotica-virgifera-virgifera* (Coleoptera, Chrysomelidae). Environ. Entomol. 14: 687-690.

Brust, G. E., and G. J. House. 1990. Influence of soil texture, soil-moisture, organic cover, and weeds on oviposition preference of southern corn-rootworm (Coleoptera, Chrysomelidae). Environ. Entomol. 19: 966-971.

Capinera, J. L., N. D. Epsky, and D. C. Thompson. 1986. Effects of adult western corn-rootworm (Coleoptera, Chrysomelidae) ear feeding on irrigated field corn in Colorado. J. Econ. Entomol. 79: 1609-1612.

Chaddha, S. 1990. Influence of placement of western corn rootworm eggs on survivorship, root injury and yield. MS thesis, University of Minnesota, Minneapolis, MN.

Chaddha, S., K.R. Ostlie, and J.R. Fisher. 1993. Design for an improved adult emergence trap for corn-rootworm (Coleoptera, Chrysomelidae). J. Kans. Entomol. Soc. 66:338-344.

Chege, P. G., T. L. Clark, and B. E. Hibbard. 2009. Initial larval feeding on an alternate host enhances western corn rootworm (Coleoptera: Chrysomelidae) beetle emergence on Cry3Bb1-expressing maize. Journal of the Kansas Entomological Society 82: 63-75.

Chiang, H. C. 1973. Bionomics of the northern and western corn rootworms. Annual review of entomology 18: 47-72.

Chiang, H. C., L. K. French, and D. E. Rasmussen. 1980. Quantitative relationship between western corn-rootworm population and corn yield. J. Econ. Entomol. 73: 665-666.

Ciampitti, I. A., and T. J. Vyn. 2012. Physiological perspectives of changes over time in maize yield dependency on nitrogen uptake and associated nitrogen efficiencies: A review. Field Crops Research 133: 48-67.

Crowder, D. W., D. W. Onstad, and M. E. Gray. 2006. Planting transgenic insecticidal corn based on economic thresholds: consequences for integrated pest management and insect resistance management. J. Econ. Entomol. 99: 899-907.

Darnell, S. J., L. J. Meinke, and L. J. Young. 2000. Influence of corn phenology on adult western corn rootworm (Coleoptera: Chrysomelidae) distribution. Environ. Entomol. 29: 587-595.

Dunbar, M. W., and A. J. Gassman. 2013. Abundance and distribution of western and northern corn rootworm (*Diabrotica* spp.) and prevalence of rotation resistance in eastern Iowa. J. Econ. Entomol. 106: 168-180.

Elliott, N. C., R. D. Gustin, and S. L. Hanson. 1990. Influence of adult diet on the reproductive biology and survival of the western corn rootworm, *Diabrotica* virgifera-virgifera. Entomol Exp Appl 56: 15-21.

Ellis, R. T., B. A. Stockhoff, L. Stamp, H. E. Schnepf, G. E. Schwab, M. Knuth, J. Russell, G. A. Cardineau, and K. E. Narva. 2002. Novel *Bacillus thuringiensis* binary insecticidal crystal proteins active on western corn rootworm, *Diabrotica virgifera virgifera* LeConte. Applied and Environmental Microbiology 68: 1137-1145.

Ellsbury, M. M., and R. E. Lee. 2004. Supercooling and cold-hardiness in eggs of western and northern corn rootworms. Entomol Exp Appl 111: 159-163.

Ellsbury, M. M., T. E. Schumacher, R. D. Gustin, and W. D. Woodson. 1994. Soil compaction effect on corn rootworm populations in maize artificially infested with eggs of western corn rootworm (Coleoptera, Chrysomelidae). Environ. Entomol. 23: 943-948.

Fisher, J. R. 1985. Comparison of controlled infestations of *Diabrotica virgifera virgifera* and *Diabrotica barberi* (Coleoptera, Chrysomelidae) on corn. J. Econ. Entomol. 78: 1406-1408.

Foster, R. E., W. G. Ruesink, and W. H. Luckmann. 1979. Northern corn rootworm (Coleoptera, Chrysomelidae) egg sampling. J. Econ. Entomol. 72: 659-663.

Foster, R. E., J. J. Tollefson, J. P. Nyrop, and G. L. Hein. 1986. Value of adult corn rootworm (Coleoptera, Chrysomelidae) population estimates in pest management decision making. J. Econ. Entomol. 79: 303-310.

Frank, D. L., R. Kurtz, N. A. Tinsley, A. J. Gassman, L. J. Meinke, D. J. Moellenbeck, M. E. Gray, L. W. Bledsoe, C. Krupke, R. E. Estes, P. Weber, and B. E. Hibbard. 2015a. Effect of seed blends and soil-insecticide on western and northern corn rootworm emergence from mCry3A + eCry3.1Ab *Bt* maize. J. Econ. Entomol. 108: 1260-1270.

French, B. W., and L. Hammack. 2012. Spermatophore size in relation to body size and pairing duration in northern corn rootworm (Coleoptera: Chrysomelidae). Annals of the Entomological Society of America 105: 506-511.

Gassmann, A. J., J. L. Petzold-Maxwell, R. S. Keweshan, and M. W. Dunbar. 2011. Field-evolved resistance to *Bt* maize by western corn rootworm. PloS ONE 6: e22629.

Godfrey, L. D., L. J. Meinke, R. J. Wright, and G. L. Hein. 1995. Environmental and edaphic effects on western corn rootworm (Coleoptera, Chrysomelidae) overwintering egg survival. J. Econ. Entomol. 88: 1445-1454.

Gray, M. E., and J. J. Tollefson. 1988. Influence of tillage systems on egg populations of western and northern corn rootworms (Coleoptera: Chrysomelidae). Journal of the Kansas Entomological Society 61: 186-194.

Gustin, R. D. 1983. *Diabrotica longicornis barberi* (Coleoptera, Chrysomelidae) - cold hardiness of eggs. Environ. Entomol. 12: 633-634.

Gustin, R. D., and T. E. Schumacher. 1989. Relationship of some soil pore parameters to movement of first-instar western corn rootworm (Coleoptera, Chrysomelidae). Environ. Entomol. 18: 343-346.

Hein, G. L., and J. J. Tollefson. 1984. Comparison of adult corn rootworm (Coleoptera, Chrysomelidae) trapping techniques as population estimators. Environ. Entomol. 13: 266-271.

Hein, G. L., and J. J. Tollefson. 1985. Use of the Pherocon AM trap as a scouting tool for predicting damage by corn rootworm (Coleoptera, Chrysomelidae) larvae. J. Econ. Entomol. 78: 200-203.

Hein, G. L., M. K. Bergman, R. G. Bruss, and J. J. Tollefson. 1985. Absolute sampling technique for corn rootworm (Coleoptera, Chrysomelidae) adult emergence that adjusts to fit common row spacing. J. Econ. Entomol. 78: 1503-1506.

Hibbard, B. E., Y. M. Schweikert, M. L. Higdon, and M. R. Ellersieck. 2008. Maize phenology affects establishment, damage, and development of the western corn rootworm (Coleoptera: Chrysomelidae). Environ. Entomol. 37: 1558-1564.

Hibbard, B. E., L. N. Meihls, M. R. Ellersieck, and D. W. Onstad. 2010. Density-dependent and density-independent mortality of the western corn rootworm: impact on dose calculations of rootworm-resistant *Bt* Corn. J. Econ. Entomol. 103: 77-84.

Higley, L. G.; Pedigo, L.P; Ostlie, K.R. **1986.** Degday- A program for calculating degree-days, and assumptions behind the degree-day approach. Environ. Entomol. 15: 999-1016.

Hill, R. E. 1975. Mating, oviposition patterns, fecundity and longevity of western corn rootworm Coleoptera Chrysomelidae. J. Econ. Entomol. 68: 311-315.

Hill, R. E., and Z. B. Mayo. 1980. Distribution and abundance of corn rootworm species (Coleoptera, Chrysomelidae) as influenced by topography and crop rotation in eastern Nebraska. Environ. Entomol. 9: 122-127.

Hitchon, A. J., J. L. Smith, B. W. French, and A. W. Schaafsma. 2015. Impact of the corn proteins Cry34/35Ab1 and Cry3Bb1, alone or pyramided, on western corn rootworm (Coleoptera: Chrysomelidae) beetle emergence in the field. J. Econ. Entomol. 108: 1986-1993.

Kahler, A. L., A. E. Olness, G. R. Sutter, C. D. Dybing, and O. J. Devine. 1985. Root damage by western corn rootworm and nutrient content in maize. Agronomy Journal 77: 769-774.

Kang, J., and C. H. Krupke. 2009. Influence of weight of male and female western corn rootworm (Coleoptera: Chrysomelidae) on mating behaviors. Annals of the Entomological Society of America 102: 326-332.

Karr, L. L., and J. J. Tollefson. 1987. Durability of the Pherocon AM trap for adult wtern and nrthern corn rootworm (Coleoptera, Chrysomelidae) sampling. J. Econ. Entomol. 80: 891-896.

Krysan, J. L. 1978. Diapause, quiescence, and moisture in egg of western corn rootworm, *Diabrotica virgifera*. Journal of Insect Physiology 24: 535-540.

Krysan, J. L. 1982. Diapause in the nearctic species of the virgifera group of Diabrotica - evidence for tropical origin and temperate adaptations. Annals of the Entomological Society of America 75: 136-142.

Krysan, J. L., R. F. Smith, T. F. Branson, and P. L. Guss. 1980. New subspecies of Diabrotica virgifera (Coleoptera, Chrysomelidae) - description, dstribution, and sexual compatibility. Annals of the Entomological Society of America 73: 123-130.

Krysan, J. L., and T. A. Miller (eds.). 1986. Methods for study of pest *Diabrotica*. Springer, New York.

Krysan, J. L., D. E. Foster, T. F. Branson, K. R. Ostlie, and W. S. Cranshaw. 1986. Two years before the hatch: rootworms adapt to crop rotation. Bulletin of Entomological Research 32: 250-253.

Kuhar, T. P., and R. R. Youngman. 1995. Sex ratio and sexual dimorphism in western corn rootworm (Coleoptera: Chrysomelidae) adults on yellow sticky traps in corn. Environ. Entomol. 24: 1408-1413.

Kuhar, T. P., and R. R. Youngman. 1998. Olson yellow sticky trap: decision-making tool for sampling western corn rootworm (Coleoptera : Chrysomelidae) adults in field corn. J. Econ. Entomol. 91: 957-963.

Levine, E., and H. Oloumisadeghi. 1991. Management of diabroticite rootworms in orn. Annual Review of Entomology 36: 229-255.

Levine, E., and H. Oloumisadeghi. 1996. Western corn rootworm (Coleoptera: Chrysomelidae) larval injury to corn grown for seed production following soybeans grown for seed production. J. Econ. Entomol. 89: 1010-1016.

Losey, J. E., L. L. Allee, and V. Zbarsky. 2003. Transect sampling to enhance efficiency of corn rootworm (Coleoptera : Chrysomelidae) monitoring in corn. J J. Econ. Entomol. 96: 1420-1425.

Macdonald, P. J., and C. R. Ellis. 1990. Survival time of unfed, first instar western corn rootworm (Coleoptera, Chrysomelidae) and the effects of soil type, moisture, and compaction on their mobility in soil. Environ. Entomol. 19: 666-671.

Meihls, L. N., M. L. Higdon, M. R. Ellersieck, B. E. Tabashnik, and B. E. Hibbard. 2012. Greenhouse-selected resistance to Cry3Bb1-producing corn in three western corn rootworm populations. PloS ONE 7: e51055.

Meinke, L. J., B. D. Siegfried, R. J. Wright, and L. D. Chandler. 1998. Adult susceptibility of Nebraska western corn rootworm (Coleoptera : Chrysomelidae) populations to selected insecticides. J. Econ. Entomol. 91: 594-600.

Moellenbeck, D. J., M. L. Peters, J. W. Bing, J. R. Rouse, L. S. Higgins, L. Sims, T. Nevshemal, L. Marshall, R. T. Ellis, P. G. Bystrak, B. A. Lang, J. L. Stewart, K. Kouba, V. Sondag, V. Gustafson, K. Nour, D. P. Xu, J. Swenson, J. Zhang, T. Czapla, G. Schwab, S. Jayne, B. A. Stockhoff, K. Narva, H. E. Schnepf, S. J. Stelman, C. Poutre, M. Koziel, and N. Duck. 2001. Insecticidal proteins from *Bacillus thuringiensis* protect corn from corn rootworms. Nature Biotechnology 19: 668-672.

Mohammad, A. J., M. A. Al-Deeb, and G. E. Wilde. 2005. Effect of *Bt* Corn expressing the Cry3Bb1 toxin on western corn rootworm (Coleoptera: Chrysomelidae) biology. J. Kans. Entomol. Soc. 78: 142-152.

Mulock, B. S., C. R. Ellis, and G. H. Whitfield. 1995. Evaluation of an oviposition trap for monitoring egg populations of *Diabrotica spp.* (Coleoptera: Chrysomelidae) in field corn. Can Entomol 127: 839-849.

Murphy, A. F., and C. Krupke. 2011. Mating success and spermatophore composition in western corn rootworm (Coleoptera: Chrysomelidae). Environ. Entomol. 40: 1585-1594.

Musick, G. L., H. C. Chiang, W. H. Luckmann, Z. B. Mayo, and F. T. Turpin. 1980. Impact of planting dates of field corn on beetle emergence and damage by the western and the northern corn rootworms in the Corn Belt. Annals of the Entomological Society of America 73: 207-215.

Nafziger, E. D. 2012. Corn. Illinois Agronomy Handbook.

Naranjo, S. E. 1991. Movement of corn rootworm beetles, *Diabrotica spp*. (Coleoptera, Chrysomelidae), at cornfield boundaries in relation to sex, reproductive status, and crop henology. Environ. Entomol. 20: 230-240.

Obopile, M., R. B. Hammond, and P. R. Thomison. 2012. Interaction among planting dates, transgenic maize, seed treatment, corn rootworm damage and grain yield. Journal of Applied Entomology 137: 45-55.

Oleson, J. D., Y. L. Park, T. M. Nowatzki, and J. J. Tollefson. 2005. Node-injury scale to evaluate root injury by corn rootworms (Coleoptera : Chrysomelidae). J. Econ. Entomol. 98: 1-8.

Ostlie, K. R. 2009. Transgenic hybrid performance probelms occurring in fields in Minnesota following heavy rainfall and cool weather.

Palmer, D. F., M. B. Windels, and H. C. Chiang. 1976. Changes in specific gravity of developing western corn rootworm (Coleoptera: Chrysomelidae) eggs. Environ. Entomol. 5: 621-622.

Park, Y. L., and J. J. Tollefson. 2005. Spatial prediction of corn rootworm (Coleoptera : Chrysomelidae) adult emergence in Iowa cornfields. J. Econ. Entomol. 98: 121-128.

Park, Y. L., and J. J. Tollefson. 2006. Spatial distributions of corn rootworm (Coleoptera : Chrysomelidae) eggs and larvae: implications for sampling. Journal of the Kansas Entomological Society 79: 129-135.

Pierce, C. M. F., and M. E. Gray. 2007. Population dynamics of a western corn rootworm (Coleoptera : Chrysomelidae) variant in east central Illinois commercial maize and soybean fields. J. Econ. Entomol. 100: 1104-1115.

Schaafsma, A. W., G. H. Whitfield, and C. R. Ellis. 1991. A Temperature-dependent model of egg development of the western corn rootworm, *Diabrotica virgifera virgifera* Leconte (Coleoptera: Chrysomelidae). Can Entomol 123: 1183-1197.

Shelton, A. M., S. E. Naranjo, J. Romeis, R. L. Hellmich, J. D. Wolt, B. A. Federici, R. Albajes, F. Bigler, E. P. J. Burgess, G. P. Dively, J. A. Gatehouse, L. A. Malone, R. T. Roush, M. K. Sears, F. Sehnal, N. Ferry, and H. A. Bell. 2009. Appropriate analytical methods are necessary to assess nontarget effects of insecticidal proteins in GM crops through meta-analysis (response to Andow et al. 2009). Environ. Entomol. 38: 1533-1538.

Siegfried, B. D., T. T. Vaughn, and T. Spencer. 2005. Baseline susceptibility of western corn rootworm (Coleoptera : Crysomelidae) to Cry3Bb1 *Bacillus thuringiensis* toxin. J. Econ. Entomol. 98: 1320-1324.

Smith, R. F. 1966. The distribution of diabroticites in western North America. Bulletin of the Entomological Society of America 12: 108-110.

Spencer, J. L., B. E. Hibbard, J. Moeser, and D. W. Onstad. 2009. Behaviour and ecology of the western corn rootworm (*Diabrotica virgifera virgifera* LeConte). Agricultural and Forest Entomology 11: 9-27.

Spike, B. P., and J. J. Tollefson. 1991. Yield response of corn subjected to western corn rootworm (Coleoptera: Chrysomelidae) infestation and lodging. J. Econ. Entomol. 84: 1585-1590.

Steffey, K. L., J. J. Tollefson, and P. N. Hinz. 1982. Sampling plan for population estimation of northern and western corn rootworm adults in Iowa cornfields. Environ. Entomol. 11: 287-291.

Strnad, S. P., and M. K. Bergman. 1987. Distribution and orientation of western corn rootworm (Coleoptera: Chrysomelidae) larvae in corn roots. Environ. Entomol. 16: 1193-1198.

Strnad, S. P., M. K. Bergman, and W. C. Fulton. 1986. First-instar western corn rootworm (Coleoptera: Chrysomelidae) response to carbon dioxide. Environ. Entomol. 15: 839-842.

Tabashnik, B. E., and F. Gould. 2012. Delaying Corn rootworm resistance to *Bt* corn. J. Econ. Entomol. 105: 767-776.

Tinsley, N. A., R. E. Estes, and M. E. Gray. 2012. Validation of a nested error component model to estimate damage caused by corn rootworm larvae. Journal of Applied Entomology 137: 161-169.

Turpin, F. T., and D. C. Peters. 1971. Survival of southern and western corn rootworm larvae in relation to soil texture. J. Econ. Entomol. 64: 1448-1451.

Turpin, F. T., L. C. Dummenil, and D. C. Peters. 1972. Edaphic and agronomic characters that afect potential for rootworm damage to corn in Iowa. J. Econ. Entomol. 65: 1615-1619.

Vaughn, T., T. Cavato, G. Brar, T. Coombe, T. DeGooyer, S. Ford, M. Groth, A. Howe, S. Johnson, K. Kolacz, C. Pilcher, J. Purcell, C. Romano, L. English, and J. Pershing. 2005. A method of controlling corn rootworm feeding using a *Bacillus thuringiensis* protein expressed in transgenic maize. Crop Sci 45: 931-938.

Wangila, D. S. and Meinke, L. J. 2017. Effects of adult emergence timing on susceptibility and fitness of Cry3Bb1-resistant western corn rootworms. J. Appl. Entomol., 141: 372–383. doi:10.1111/jen.12346

Whitworth, R. J., G. E. Wilde, R. A. Shufran, and G. A. Milliken. 2002. Comparison of adult corn rootworm (Coleoptera : Chrysomelidae) sampling methods. J. Econ. Entomol. 95: 96-105.

Woodson, W. D. 1993. Effect of species composition on the survival and development of western and northern corn rootworm (Coleoptera: Chrysomelidae). Journal of the Kansas Entomological Society 66: 377-382.

Woodson, W. D., J. J. Jackson, and M. M. Ellsbury. 1996. Northern corn rootworm (Coleoptera: Chrysomelidae) temperature requirements for egg development. Annals of the Entomological Society of America 89: 898-903.

Wright, R. J., M. E. Scharf, L. J. MEinke, X. G. Zhou, B. D. Siegfried, and L. D. Chandler. 2000. Larval susceptibility of an insecticide-resistant western corn rootworm (Coleoptera : Chrysomelidae) population to soil insecticides: laboratory bioassays, assays of detoxification enzymes, and field performance. J. Econ. Entomol. 93: 7-13.

Appendices

Appendix A. Analysis of variance values for the effects of trap type, week, and site (fixed sources of variation) on western corn rootworm trap capture rates at multiple sites in Minnesota. Results discussed in Chapter II.

Explanatory variables	**df**	**Sum Sq**	**Mean Sq**	**F value**	**P>F**
Site	6	708.4	118.07	959.72	<0.0001
Week (site)	25	67.2	2.69	21.86	<0.0001
Trap Type	3	11.8	3.92	31.86	<0.0001
Transect	3	0.2	0.05	0.44	0.723
Site x Trap Type	18	32.2	1.79	14.55	<0.0001
Site x Week x Trap Type	75	20.1	0.27	2.18	<0.0001
Residuals	340	41.8	0.12		

Appendix B. Analysis of variance values for the effects of trap type, week, and site (fixed sources of variation) on northern corn rootworm trap capture rates at multiple sites in Minnesota. Results discussed in Chapter II.

Explanatory variables	**df**	**Sum Sq**	**Mean Sq**	**F value**	**P>F**
Site	6	293.07	48.85	300.99	<0.0001
Week (Site)	25	71.31	2.85	17.58	<0.0001
Trap Type	3	30.43	10.14	62.5	<0.0001
Transect	3	0.29	0.1	0.6	0.615
Site x Trap Type	18	30.84	1.71	10.56	<0.0001
Site x Week x Trap Type	75	32.25	0.43	2.65	<0.0001
Residuals	340	55.17	0.16		

Appendix C. Analysis of variance values for the effects of the log mean of capture rates, trap type, week, and site (fixed sources of variation) on western corn rootworm trap capture variance at multiple sites in Minnesota. Results discussed in Chapter II.

Explanatory variables	**Df**	**Sum Sq**	**Mean Sq**	**F value**	**P>F**
Log Capture Rate	1	114.47	114.47	1773.58	<0.0001
Site	6	2.05	0.34	5.3	0.0001
Week (Site)	25	3.77	0.15	2.34	0.0021
Trap Type	3	0.43	0.14	2.22	0.0913
Log Capture Rate x Site	6	2.12	0.35	5.48	<0.0001
Residuals	86	5.55	0.06		

Appendix D. Analysis of variance values for the effects of the log mean of capture rates, trap type, week, and site (fixed sources of variation) on northern corn rootworm trap capture variance at multiple sites in Minnesota. Results discussed in Chapter II.

Explanatory variables	Df	Sum Sq	Mean Sq	F value	P>F
Log Capture Rate	1	55.99	55.99	1458.51	<0.0001
Site	6	0.13	0.02	0.56	0.7649
Week (Site)	25	1.74	0.07	1.82	0.0215
Trap Type	3	0.41	0.14	3.52	0.0182
Residuals	92	3.53	0.04		

Appendix E. Analysis of variance values for the effects of planting date and hybrid (fixed sources of variation) on the date when 50 percent of the total corn rootworms emergence. Study performed in 2012. Results discussed in Chapter III.

Explanatory variables	Df	Sum Sq	Mean Sq	F value	P>F
Rep	3	0.003	0.001	2.27	0.1102
Date	3	0.006	0.002	5.64	0.0188
Hybrid	2	0.027	0.01	30.58	<0.0001
Rep x Date	9	0.003	0.0004	0.79	0.6315
Date x Hybrid	6	0.002	0.0004	0.83	0.5598
Residuals	21	0.009	0.0004		

Appendix F. Analysis of variance values for the effects of planting date and hybrid (fixed sources of variation) on the total corn rootworms to emerge per hectare. Study performed in 2012. Results discussed in Chapter III.

Explanatory variables	Df	Sum Sq	Mean Sq	F value	P>F
Rep	3	5.35	1.78	4.41	0.0136
Date	3	20.48	6.83	16.92	<0.0001
Hybrid	2	20.36	10.18	25.22	<0.0001
Rep x Date	9	5.08	0.56	1.4	0.2454
Date x Hybrid	6	7.17	1.2	2.96	0.0272
Residuals	23	9.28	0.4		

Appendix G. Analysis of variance values for the effects of planting date and hybrid (fixed sources of variation) on the date when 50 percent of the total corn rootworms emergence. Study performed in 2013, site 1. Results discussed in Chapter III.

Explanatory variables	**Df**	**Sum Sq**	**Mean Sq**	**F value**	**P>F**
Rep	3	0.01	0.002	3.95	0.0209
Date	3	0.01	0.002	2.73	0.1061
Hybrid	2	0.06	0.03	52.68	<0.0001
Rep x Date	9	0.01	0.001	1.32	0.2793
Date x Hybrid	6	0.01	0.001	1.51	0.2208
Residuals	23	0.01	0.001		

Appendix H. Analysis of variance values for the effects of planting date and hybrid (fixed sources of variation) on the total corn rootworms to emerge. Study performed in 2013, site 1. Results discussed in Chapter III.

Explanatory variables	**Df**	**Sum Sq**	**Mean Sq**	**F value**	**P>F**
Rep	3	1.41	0.47	0.29	0.8292
Date	3	0.14	0.05	0.10	0.9594
Hybrid	2	2.75	1.37	0.86	0.4366
Rep x Date	9	4.20	0.47	0.29	0.9703
Date x Hybrid	6	9.26	1.54	0.96	0.4698
Residuals	24	38.41	1.60		

Appendix I. Analysis of variance values for the effects of planting date and hybrid (fixed sources of variation) on the date when 50 percent of the total corn rootworms emergence. Study performed in 2013, site 2. Results discussed in Chapter III.

Explanatory variables	**Df**	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
Rep	3	0.002	0.001	0.58	0.6381
Date	3	0.002	0.001	3.86	0.0502
Hybrid	2	0.005	0.003	1.89	0.1856
Rep x Date	9	0.015	0.002	1.28	0.3238
Date x Hybrid	6	0.001	0.0002	0.17	0.9823
Residuals	15	0.02	0.001		

Appendix J. Analysis of variance values for the effects of planting date and hybrid (fixed sources of variation) on the total corn rootworms to emerge. Study performed in 2013, site 2. Results discussed in Chapter III.

Explanatory variables	**Df**	**Sum Sq**	**Mean Sq**	**F value**	**P>F**
Rep	3	14.28	4.76	2.51	0.0826
Date	3	0.92	0.31	0.45	0.7263
Hybrid	2	8.43	4.22	2.23	0.1298
Rep x Date	9	6.19	0.69	0.36	0.9417
Date x Hybrid	6	6.45	1.07	0.57	0.7522
Residuals	24	45.47	1.89		

Appendix K. Analysis of variance values for the effects of planting date and hybrid (fixed sources of variation) on the date when 50 percent of the total corn rootworms emergence. Study performed in 2014, site 2. Results discussed in Chapter III.

Explanatory variables	**Df**	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
Rep	3	0.0006	0.0002	0.96	0.4234
Date	3	0.002	0.0007	2.05	0.178
Hybrid	3	0.02	0.006	29.19	<0.0001
Rep x Date	9	0.003	0.0003	1.51	0.1804
Date x Hybrid	9	0.001	0.0001	0.74	0.6707
Residuals	35	0.01	0.0002		

Appendix L. Analysis of variance values for the effects of planting date and hybrid (fixed sources of variation) on the total corn rootworms to emerge. Study performed in 2014, site 2. Results discussed in Chapter III.

Explanatory variables	**Df**	**Sum Sq**	**Mean Sq**	**F value**	**P>F**
Rep	3	0.74	0.25	0.68	0.5718
Date	3	0.13	0.04	0.12	0.9481
Hybrid	3	46.06	15.35	42.38	<0.0001
Rep x Date	9	5.15	0.57	1.58	0.1586
Date x Hybrid	9	10.36	1.15	3.18	0.0063
Residuals	36	13.04	0.36		