EFFICACY OF CURRENT MAIZE BT TRAITS AGAINST CORN ROOTWORMS IN NORTH DAKOTA

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The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

MASTER OF SCIENCE

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ABSTRACT

Field experiments were conducted to determine the efficacy of current Bt traits in maize (*Zea mays* L.) hybrids and soil-applied insecticide for corn rootworm (*Diabrotica* spp.) management. Experiments were conducted as a randomized complete block design with a split-plot arrangement. The whole plot was no insecticide or soil-applied insecticide. The subplots were different Bt maize hybrids and treatments: non-Bt (untreated control), seed-applied insecticide, Cry3Bb1, Cry34/35Ab1, and Cry3Bb1 + Cry34/35Ab1. Root injury was evaluated using the Iowa State University rating system. Hybrids expressing any Cry protein averaged 0.12 nodes pruned. Hybrids without Cry proteins averaged 0.37 nodes pruned. Seed-applied insecticide reduced root pruning compared to the untreated control. Both had greater root injury than hybrids with Cry proteins. Soil-applied insecticide did not always reduce root pruning. Hybrid yield and test weight were highly variable, depending on the hybrid’s genetics. This research indicates the Bt-traits tested are effective in managing corn rootworm.

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INTRODUCTION

Corn rootworms (CRWs), *Diabrotica* spp., comprise a major insect pest complex of maize (*Zea mays* L.) in the United States. Crop losses and control interventions associated with CRW cost U.S. maize producers $1 billion annually (Metcalf, 1986). For this reason, CRW has been referred to as “the billion dollar pest”. Prior to 2003, CRW was controlled by using foliar and soil-applied insecticides, and crop rotation. In 2003, genes from *Bacillus thuringiensis* (Bt) were transgenically inserted into maize hybrids to induce the production of a crystalline (Cry) protein in the plants, that when ingested, is lethal to CRW (Sanahuja et al., 2011). This technology has helped alleviate yield reduction from root feeding by rootworm larvae. It also has reduced the amount of conventional insecticide applied by producers and the number of pesticide applications that are made (Fernandez-Cornejo et al., 2014). This has resulted in fuel savings, reduced exposure to pesticides, and safer working environments for producers (Marra et al., 2012).

Maize has a large economic impact on the economy of North Dakota and the U.S. The United States Department of Agriculture reports that maize is grown on 32 million hectares in the U.S. (Fernandez-Cornejo et al., 2014). Maize expressing Cry proteins for CRW control were estimated to involve 52% of all hybrids planted in 2012 (Marra et al., 2012). Because of its widespread adoption and use, recent research indicates that western corn rootworm (WCR), *Diabrotica virgifera virgifera* LeConte, has developed resistance to this technology in Iowa, Illinois, Nebraska, and Minnesota (Brooks, 2014). It is not known if resistant populations have developed in North Dakota.

The research reported here was conducted to determine the effectiveness of different Bt traits commonly used in maize hybrids and seed-applied insecticide, combined with soil-applied insecticides, against CRW in North Dakota.

By evaluating the effectiveness of soil insecticide, seed-applied insecticide, and different Bt-trait combinations, best pest management recommendations can be developed to help producers achieve acceptable control. Knowing which combinations of control strategies reduce the producer’s risk to CRW damage will help minimize the risk of yield loss.

OBJECTIVE

Western corn rootworm resistance to the Cry3Bb1 protein has been confirmed in Iowa in 2011 and in Illinois, Minnesota, and Nebraska in 2012 (Gassmann et al., 2011; Brooks, 2014). In 2012, increasing WCR populations were observed in southeastern North Dakota, prompting concern that this may have occurred in North Dakota (Knodel, 2012). The objective of this study was to evaluate the efficacy of maize hybrids with differing Bt traits, including the Cry3Bb1. Hybrids expressing different Bt proteins were combined with an in-furrow soil insecticide treatment to determine if there is a benefit to including insecticides with Bt hybrids in fields with a known history of CRW pressure. Data from these experiments were used to help develop an Integrated Pest Management recommendation that producers can use to manage CRW in maize in North Dakota.

LITERATURE REVIEW

Maize was grown on only 2% of North Dakota's total crop hectares in 1970 (Taylor and Koo, 2013). Today, maize accounts for 10% of the cropland with most of this growth occurring in the last 10 years (NASS, 2014). The economic impact of maize production to the state of North Dakota is approximately $1.42 billion per year (Taylor and Koo, 2013). Maize hectares are continuing to expand into areas that historically have not produced maize. Maize production is expanding west and north in North Dakota, and along with the crop, CRW populations appear to be increasing.

The maize industry in North Dakota has recently expanded to support ethanol and high fructose maize syrup production, in addition to animal feed and export markets. Government programs supporting the production of renewable fuels with mandates such as the 2005 Energy Policy Act have increased the profitability of ethanol (Capehart, 2014). Import fees, duties, and import quotas for sugar have made domestically produced high fructose maize syrup economically attractive. This increased demand has influenced the northern and western expansion of maize hectares. Improved genetics and other production technologies have also greatly increased the yield potential of maize and the number of hybrids available to producers.

Western and northern corn rootworms (NCRs), *Diabrotica barberi* Smith and Lawrence, are the two main CRW species that can be found in North Dakota (Knodel, 2012). Western corn rootworm and NCR are commonly found in the same fields, and both are known to cause root pruning injury to maize plants. Together, they are the most serious insect pests that cause damage to maize fields in the north central corn-producing states and Canada (Levine and Oloumi-Sadeghi, 1991). Producers in all areas use similar strategies to control both species.

Western Corn Rootworm

Western corn rootworm (WCR), *Diabrotica virgifera virgifera* LeConte, is a common insect pest of maize (Sutter, 1999). It is distributed throughout maize growing regions in the U.S. and southeastern Canada. It is not typically found in areas in the southeastern U.S., or west of the Rocky Mountains however, WCR was not considered a major pest until the 1950s, when continuous maize production became a more common practice. At that time, it began expanding its distribution at a rate of 64 to 80 km per year (Sutter, 1999).

Western corn rootworm adults, both male and female, are beetles that are ¾-cm long, yellow in color with three black stripes on their elytra (Sutter, 1999). Males have a more solid marking on their elytra. Western corn rootworms only produce one generation per year and eggs overwinter in the soil. Eggs are oval, creamy white, and measure 0.3- by 0.5-mm in diameter. Eggs are deposited in the top 10 cm of soil, but can be deposited as deep as 30 cm. Females will lay eggs deeper if cracks are present in the soil. Soil cracks form during dry conditions when soil moisture is low. Cold soil temperatures during the winter can reduce egg viability. Temperatures of -7.5 to -10°C for 2 to 3 weeks will generally have a negative impact on the viability of eggs the following spring (Sutter,1999).

Larvae hatch when the soil temperature rises, typically from late-May to early June. The development of WCR is fastest when air temperatures are 21 to 24 °C, and soil temperatures are between 18 and 27°C (Sutter, 1999). Research suggests that 50 percent of the larval hatch will occur after the accumulation of between 684 and 767 degree days, using 11 °C as the base soil temperature (Hodgson and Sission, 2011). Larvae range from 0.3-cm to 1.25-cm in length, depending on instar. They are creamy white with a brown head and brown spot on top of the terminal end of their body. Larvae feed for 30 to 45 days and develop through three instars before pupating, and later emerge as adults. Larval feeding generally takes place until late-July, but it is heavily influenced by seasonal fluctuations in temperature and soil conditions, and can also be influenced by latitude (Tinsley et al., 2012).

Feeding by newly hatched larvae primarily occurs on root hairs and small roots. As larvae mature, they may tunnel into large roots and cause root pruning. Damaged roots are pruned with brown feeding scars on the tips. Pruning is normally the most noticeable and severe on the secondary root system (Sutter, 1999).

After larval root feeding occurs, the larvae pupate for 6 to 13 days, depending on soil temperature. Thereafter, adult beetles emerge between early July and emergence can continue through October. Adults mate and females lay 300 to 400 eggs in the soil from late-summer to fall. During this time, the adults feed on silks, leaves, and pollen of the maize plant (Drees et al., 1999). Pollination can be inhibited if adult feeding injury reduces silk length to less than 1.25 cm protruding from the husk.

Late-maturing maize fields are the most susceptible to damage by adults migrating to feed and lay eggs. These fields are still in the pollination phase and provide fresh silks as a food source for the CRW beetles. Feeding damage on silks potentially inhibits pollination that growing season. Economically significant silk feeding is not generally observed in North Dakota (Knodel, 2012). This makes management very difficult from year to year, as producers may not realize that eggs have been laid in their field. As a result, some producers do not plant maize with Bt traits or use a soil insecticide the following growing season to control a potential CRW infestation.

Western corn rootworm larvae can cause severe damage to maize plants already under stress caused by poor fertility, drought, or soil compaction. The weakened root system often leads to plant lodging. The likelihood of lodging after root pruning occurs is increased with moist soil conditions and strong winds (Drees et al., 1999). Lodging reduces the plant's ability to photosynthesize efficiently, leading to yield losses. Lodged plants also create difficult harvest conditions, resulting in many cobs not being harvested. This contributes to additional yield losses for producers. Tinsley et al. (2012) estimated that for every node of maize roots that is pruned back within 3.8 cm of the stalk or soil line, there is a 15% reduction in yield. Severe plant lodging can contribute additional yield losses of 11 to 34%.

Controlling WCR is difficult because the adult beetles can travel long distance. Depending on the growth stage of the maize, adults may migrate to a different field than the one from which they emerged (Drees et al., 1999). Beetles will travel to a new field in search of a food source or to lay their eggs.

In some areas of the U.S. Corn Belt, producers have seen an increase in the number of WCR eggs deposited in soybean (*Glycine max* L. Merr.) fields (Levine et al., 2002). Western corn rootworm adults emerge in maize fields after larval root feeding and pupation have occurred, and continue to feed on silks and pollen until they need to oviposit. Female WCR then travel to neighboring soybean fields to lay their eggs. This causes major issues for unsuspecting producers the following year, since viable WCR eggs are in the soil where soybeans have been previously planted and will have the correct host crop available for feeding after larval hatch (Levine et al., 2002). It can pose an increased risk to producers where there is a long history of maize-soybean rotations. This is an example of CRW's adapted behavior and its potential to overcome Integrated Pest Management (IPM) strategies that producers commonly use. Integrated pest management is defined as "the continuum of pest management practices that provide for the evaluation, decision making, and managing of insect pests" (Knodel and McMullen, 2012).

Soil-applied insecticides became popular to control CRW in the 1950s (Drees et al., 1999). Most soil insecticide products need a 6 to 10-week residual to be effective against CRW. Larval hatch occurs 4 to 6-weeks after planting, and feeding takes place for another 3 to 4-weeks after hatch. Generally, insecticides can reduce the extent of larval root feeding injury, but adult beetle survival is observed even when insecticides are used (Boetel et al., 2003, Gray et al., 1992). Soil insecticide efficacy can be attributed to insects not coming in contact with the insecticide, exposure to a sub lethal dose of the active ingredient, or insect resistance to the active ingredient.

Insecticides only protect a portion of the root system. Feeding damage can occur beyond the insecticide-treated band, but generally the main root system and brace roots have protection from larval feeding. This can help prevent lodging and major yield losses. Soil-applied insecticides are not used as an adult population management tool, but they can help lessen root damage and yield losses associated with feeding under heavy CRW pressure (Boetel et al., 2003, Gray et al., 1992).

Control of WCR depends heavily on an IPM system. The system should include crop rotation, soil-applied insecticides, and foliar-applied insecticides. Foliar-applied insecticides were used to control adults prior to the introduction of the Bt trait (Drees et al., 1999). These foliar applications would reduce adult populations in the field, resultantly reducing the number of eggs that could be laid in the field. This reduced the number of potential larvae the following year, but did not offer any control of larval root pruning in the current production year. These practices were paired with cultural methods, such as adjusting planting and harvesting dates. Producers also chose hybrids with large root systems and the ability to initiate new root growth after larval feeding (Drees et al., 1999).

Scouting fields is also a critical element of the IPM system. Producers can sample for larvae by digging up maize plants starting at 684 to 767 GDD (based on an 11 °C base soil temperature) and washing the roots in a bucket (Fisher et al., 1990, Hodgson and Sisson, 2011). Generally, larvae will float to the water surface. Sampling plants from different areas of the field can give producers an estimation of the infestation in the field. Rescue applications of foliar-applied insecticides are often ineffective or impractical, but being aware of infestation levels can help determine the need for an effective management plan the following year (Hodgson and Sisson, 2011). All of these practices are still considered effective when paired with the use of Bt traits to create an IPM strategy for effectively managing CRW and delaying resistance to Cry proteins.

Northern Corn Rootworm

The northern corn rootworm (NCR), *Diabrotica barberi* Smith and Lawrence, is native to North America, and can be found from the Rocky Mountains to the east coast of the U.S., south into Tennessee and as far north as Ontario, Canada (Tollefson and Levine, 1999). Northern corn rootworms are frequently found in the same fields as WCR. Control methods for NCR are very similar to WCR, and are based on IPM principles. Generally, NCR completes one generation per year, but some populations have been shown to exhibit extended diapause (French et al., 2012). When extended diapause occurs, eggs pass through two or more winters before hatching (Drees et al., 1999). It was first documented in 1965, and was not a common occurrence until the 1980s, when it became more prevalent in Minnesota, South Dakota, and Iowa. Extended diapause is very variable, and can range from 1 to 4 years. It has also been found to depend on genetics, suggesting that only certain populations are able to undergo extended diapause to overcome annual crop rotation (French et al., 2012). This phenomenon is not responsible for serious widespread economic damage, but causes problems annually in areas inhibited by these populations. Extended diapause has not yet been observed in North Dakota.

Adult NCR beetles lay eggs 20- to 30-cm deep in the soil in late-August to September (Tollefson and Levine, 1999). Eggs begin hatching in late-May to early June. Larvae generally feed on maize roots for three weeks followed by a two week pupation period. This time period is temperature-dependent. Adults will begin to emerge from the soil in late-July or early August.

Northern corn rootworm larvae are white with brown heads and a brown plate on the last abdominal segment. The larvae are approximately 1.6-mm long when they hatch, growing to 16-mm in length at maturity. Adults are approximately 7 mm long and are oval shaped and vary in color from pale to dark green. The beetles do not have any distinct markings on their elytra (Tollefson and Levine, 1999).

Seed Treatments

Commercially applied neonicotinoid seed treatments have replaced older, persistent, contact synthetics and inorganic insecticidal seed treatments (Van Rozen and Ester, 2010). They have become more widely used on maize hybrids since 2004 (Jarvi et al., 2006).

The three main active ingredients of neonicotinoid seed treatments applied on maize seeds are clothianidin, thiamethoxam, and imidacloprid (Van Rozen and Ester, 2010). Neonicotinoids work on the insect’s central nervous system by binding nicotinic acetylcholine receptors, which causes paralysis and death (Jarvi et al., 2006).

Imidacloprid was introduced in 1991. It offered producers photostability and residual activity. It also has high intrinsic insecticidal potency against a broad spectrum of insects, as well as excellent systemic properties, plant compatibility, and low mammalian toxicity (Van Rozen and Ester, 2010). Thiamethoxam was introduced as a seed treatment in 1997 and clothianidin in

2003. These materials are effective as contact and stomach poison insecticides, and are systemically translocated in the plant. Together, they are the most common seed treatments used on maize to protect plants against insects in the U.S. and Europe (Van Rozen and Ester, 2010). Some common insect pests of maize seedlings that are controlled by neonicotinoid seed treatments include: black cutworm (*Agrotis ipsilon* Hufnagel), wireworm (*Melanotus* spp., *Agriotes mancus* Say, and *Limonius dubitans* LeConte), white grub (*Phyllophaga* spp.), seedcorn maggot (*Delia platura* Meigen), and various others.

Increasing the amount of active ingredient of the chemical per kernel can provide some control of CRW larvae in the soil (Jarvi et al., 2006). The use of seed treatments to control CRW can result in erratic root protection (Jarvi et al., 2006). They are viewed as good supplemental products to use in an IPM strategy, but not as the primary control strategy in high-pressure conditions (Gassmann et al., 2011). Neonicotinoid seed treatments are recommended for use when the CRW population is expected to be low, and are considered a root protection method and not a population reduction tool (Van Rozen and Ester, 2010)

Commercially applied seed treatments have some additional advantages. They ensure uniform product coverage on the seed. They also help lower human exposure to insecticides because the product is already on the seed, and there is no need to mix or measure the product. Seed treatments also have low rates of active ingredients compared with soil-applied insecticides, and can be systemic in the plant to increase insect control in the foliage. However, seed treatments do not provide season long protection. They also do not protect plants from all insects (Jarvi et al., 2006).

Bt Trait

The Bt trait is derived from *Bacillus thuringiensis*, a gram-positive, soil-borne bacterium that forms spores (Jurat-Fuentes, 2013). The *B. thuringiensis* produces crystalline proteins, commonly referred to as Cry proteins. Cry proteins have insecticidal properties that are lethal to certain insect orders and species (Sanahuja et al., 2011). Scientists were able to transgenically insert the gene that controls the expression of these proteins into the DNA of plants to enable the control of specific insect pests.

The segment of DNA that is known to produce the protein of interest is inserted into the maize plant's DNA (Witkowski et al., 2013). The primary components of the inserted DNA include a protein gene, a promoter, and a genetic marker. The protein gene contains the Bt genes that express the production of the Cry proteins. The promoter controls the amount of Cry protein that is produced by the plant and where in the plant the protein will be produced. The genetic marker allows scientists to identify if the transformation is successful (Witkowski et al., 2013).

When susceptible insects ingest the plant material from plants expressing the Bt trait, the protein travels down the peritrophic matrix and binds to specific cadherin receptors in the gut of the insect (Sanahuja et al., 2011). The toxin is released when an insect feeds on plant tissue. The toxin accumulates in the gut of the insect after feeding. Accumulation of the toxin within the insect results in osmotic cell shock, as well as pore formation and tumor development. The combination of these effects results in insect death after consumption.

Utilization of the Bt protein for insect control dates back to the early 1900s and was commonly used by producers in granular and sprayable liquid formulations (Sanahuja et al., 2011). These pesticides were safe to mammals and beneficial insects, pest specific, potent to target insects, and biodegradable when compared with traditional insecticides. However, the effectiveness of these pesticides was inconsistent. Applications were only effective if the insect pest ingested plant tissue that had been treated with the protein. In the 1980s, the Bt trait was introduced into tobacco (*Nicotiana tabacum* L.) and tomato (*Solanum lycopersicum* L.). Results were not favorable, prompting a modification to the sequence to be inserted in 1991. Modifications lead to the introduction of the Cry1Ab and Cry1Ac proteins that would later be used as traits in commercial transgenic crops.

Transgenic maize, potato (*Solanum tuberosum* L.), and cotton (*Gossypium hirsutum* L.) with Bt traits were first introduced to the marketplace in 1995 by Monsanto™ (Sanahuja et al., 2011). The first maize hybrids were only resistant to the European corn borer (*Ostrinia nubilalis* L.) (Fernandez-Cornejo et al., 2014). Transgenic potatoes were very effective in controlling the Colorado potato beetle (*Leptinotarsa decemlineata* L.), but were removed from the market in 2002 due to human consumption safety concerns. A second generation of Bt in maize was released in 2002 consisting of a synthetic variant of the Cry3Bb1 gene, targeted at controlling CRW.

In 2003, Monsanto™ released maize hybrids that combined the Cry1Ab1 and Cry3Bb1 traits to control against the European corn borer and CRW (Tabashnik and Gould, 2012). In 2005, a new trait was released for control of CRW. Monsanto released Cry34/35Ab1, followed by mCry3A from Syngenta in 2006, which offered producers additional options for CRW management. The Cry34/35Ab1 toxin was combined with the Cry3Bb1, creating hybrids referred to as “pyramid” trait packages. A trait pyramid is defined as a combination of two toxins that act independently to kill the same pest (Tabashnik and Gould, 2012). Pyramiding the traits is also a strategy for reducing the risk of pests developing resistnace to a single trait (Sanahuja et al., 2011). A fourth CRW Bt trait, eCry3.1Ab, was deregulated by the USDA and registered by the EPA in 2013 for commercial launch by Syngenta in 2014 (Cullen et al., 2013). This Bt trait is currently only available as a pyramid with mCry3A.

Utilization of hybrids with Bt traits for CRW has resulted in major reductions in insecticide use by U.S. producers. Producers in the U.S. used an average of 0.24 kg of insecticide per planted hectare of maize in 1995 (Fernandez-Cornejo et al., 2014). After the introduction of Bt-traited hybrids, the amount decreased to 0.07 kg in 2005 and 0.02 kg in 2010. Use of Bt-traited crops also reduced the number of pesticide applications, resulting in fuel savings for producers (Marra et al., 2012). It has also resulted in a safer working environment for producers, with reduced exposure to soil- and foliar- applied insecticides, while offering convenience and simplicity at planting (Ostlie, 2001). Previously, producers would delay planting dates to avoid the larval hatch, but Bt-traits for CRW allowed them to plant as soon as conditions are optimal for germination and crop growth. This technology has also reduced mortality of beneficial insects from broad-spectrum soil-applied and foliar insecticides (Ostlie, 2001).

Resistance to Bt

Field-evolved resistance is defined as “a genetically-based decrease in susceptibility of a population to a toxin caused by exposure of the population to the toxin in the field” (Tabashnik et al., 2009, 2011). Field-evolved resistance to the Cry3Bb1 protein by WCR populations has been confirmed in Iowa (Gassmann et al., 2011). Resistance to Cry3Bb1 has also been confirmed in Nebraska, Illinois, and Minnesota (Brooks, 2014). Colorado, Kansas, Missouri, New York, South Dakota, North Dakota, and Wisconsin have reported significant damage to fields planted to Bt-traited maize, and analysis is being done to evaluate if Bt resistant CRW populations are present. Cry3Bb1 has been the most dominant Bt trait used to control CRW in the U.S. (Cullen et al., 2013). It was planted on 200,000 ha in 2003 and increased to 12 million ha by 2008. This widespread use has likely contributed to the selection of target insects to develop resistance to this control tool (Cullen et al., 2013).

Gassmann et al. (2011) were able to conclude that WCR survival was three times higher in the problem fields when compared to control fields. This indicated resistance was present to the Cry3Bb1 protein, even though in non-traited maize, WCR survival was much higher than in fields with this Bt trait. When the Cry34/35Ab1 traits were present in the field, there was significantly lower larval survival. They concluded that the longer a field was planted to the Cry3Bb1 trait, the higher the level of WCR survival. They discovered this resistance to the Cry3Bb1 trait was common in some populations and in localized areas in Iowa. They attributed this to the Cry3Bb1 protein acting as a low-dose event, making resistance a more heritable trait in CRWs.

Delaying Resistance

Another major contribution to the evolution of resistance could be producers planting insufficient refuge (Tabashnik and Gould, 2012). A refuge is defined as an area without the Bt traits planted in it. This area is usually within the same field when using Bt-traited hybrids. This refuge can be planted in a block adjacent to or inside the field, or interplanted with the traited hybrid within the same field. The goal of a refuge is to ensure the survival of Bt-susceptible CRW individuals. This would provide resistant insects a susceptible insect to mate with and increase the likelihood of susceptibility persisting in localized populations, thus avoiding resistance development (Tabashnik and Gould, 2012). When planting a single Bt-traited hybrid, producers are required to plant 20% of an untraited refuge on their farm.

Refuge in the bag (RIB) was federally approved and made commercially available as an insect resistance management strategy soon after the introduction of pyramided traits (Cry3Bb1

+ Cry34/35Ab1). Previously, producers had to purchase separate hybrids to be planted in their refuge acres. Now, they can simply purchase a RIB hybrid and have their refuge and Bt-traited hybrids blended to the correct percentage in the same bag. Pyramiding traits lowered the required refuge area from 20% to 5% of non-traited area for some Bt-traited products. Refuge incorporation guarantees that the refuge will be planted in the same field and improves producer compliance with the regulations. The goal of the introduction of RIB was to delay resistance by pyramiding, paired with a built-in refuge requirement that producers cannot avoid (Tabashnik and Gould, 2012).

When producers purchase Bt-traited hybrids, they sign an agreement with the company providing the technology stating that they will comply and plant a refuge to help reduce the risk of resistant population development (Fernandez-Cornejo et al., 2014). The company is required to monitor and enforce grower compliance. However, this requirement is not always followed. Producer failure to comply with refuge requirements was found to be the likely cause of resistance formation in the three best-documented cases of confirmed insect resistance to the Bt trait (Tabashnik and Gould, 2012).

While the new traits and built-in refuge implement a system to delay resistance, recent research suggests refuge areas are not large enough. Tabashnik and Gould (2012) suggest single trait mode of action should be planted with a 50% refuge, and pyramided traits should have a 20% refuge. Modeling of this proposed increase showed that a 50% refuge requirement would double the time for WCR to develop resistance. They cite the primary reason for the proposed increase in the refuge requirement to the fact that Cry3Bb, Cry34/35Ab1, and mCry3A producing maize hybrids do not meet a high-dose strategy standard against WCR.

A high-dose strategy is defined as the use of plants that produce a substantially higher toxin concentration to delay resistance by reducing survival of insects heterozygous for resistance, which are expected to carry most of the resistance alleles when resistant homozygotes are rare (Tabashnik and Gould, 2012). The Environmental Protection Agency (EPA) guidelines for high-dose specificity are that the Bt-traited plant must kill 99.99% of susceptible insects in the field, based on the survival of ≤0.01% on Bt-traited plants compared with non-Bt-traited plants (EPA, 1998). A low-dose strategy plant would have substantially lower toxin concentrations and delay resistance by increasing survival of susceptible homozygotes, maintaining susceptible alleles in the population (Tabashnik and Gould, 2012).

Plants expressing the Cry3Bb1 toxin only had mortality rates of 98.5, 97.5, and 96.2 percent in three different observations. These percentages are not much lower than the high-dose standard, but the failure to kill hybrid progeny indicates an inheritance of the non-recessive resistance. This inheritance, paired with the continued use of single toxins in hybrids, is expected to accelerate the development of Bt-resistant WCR populations.

Maize hybrids producing both Cry3Bb1 and Cry34/35Ab1 proteins do not meet the high-dose standard either. Populations of WCR resistant to Cry3Bb1 are not effectively controlled by pyramids with Cry34/35Ab1 because of the reduced efficacy of Cry3Bb1. Because of the high risk of evolution of resistance, Tabashnik and Gould (2012) proposed increasing the refuge requirements of producers to 50 and 20%, respectively, to help delay resistance. The authors also noted that one of the best approaches producers can use is IPM (Tabashnik and Gould, 2012). The authors discussed how before the introduction of the Bt technology producers were able to control WCR with insecticides and crop rotations. They suggest that producers should increase refuge acres and utilize insecticides, in addition to the Bt technology, to delay the buildup of resistance. This does create a problem, however. The market supply has shifted to offering more hybrids with Bt traits. This means there may not be enough hybrids without Bt traits to meet the increased refuge demand of the growing number of maize hectares. The article did suggest IPM could be implemented in areas with the highest risk of developing resistance, or in those with resistance already occurring (Tabashnik and Gould, 2012).

MATERIALS AND METHODS

This study was conducted at four different locations in North Dakota in 2013: Arthur, Forman, Lidgerwood, and Wyndmere. In 2014, three North Dakota locations were used: Arthur, Page, and Hope. Locations were selected based on previously observed CRW feeding pressure. The past use of Bt traits and crop rotation were also considered when selecting the field locations. All of the locations were planted on fields with a history of continuous maize with the same Bt trait and previous CRW infestations.

Treatments were laid out in a randomized complete block design with a split-plot arrangement, with four replications at each location. The whole plot was insecticide (2 levels), either with insecticide or without soil-applied insecticide in-furrow. The subplots consisted of different maize hybrids and five treatments: non-Bt (untreated control), clothianidin insecticide seed treatment, Cry3Bb1, Cry34/35Ab1, and Cry3Bb1 + Cry34/35Ab1.

The five different hybrids planted in 2013 were: a hybrid expressing no Cry proteins (Pioneer 9675R, DuPont Pioneer, Johnston, IA); a hybrid expressing Cry3Bb1 (Event MON-88Ø17-3, DKC43-27, Monsanto Co., St. Louis, MO); a hybrid expressing Cry34/35Ab1 (Event DAS-59122-7, Pioneer 9675AMRW, DuPont Pioneer); a pyramided hybrid expressing both Cry3Bb1 and Cry34/35Ab1 (Events MON-88Ø17-3 and DAS-59122-7, DKC44-13, Monsanto Co.); and the non-Bt hybrid (Pioneer 9675R, DuPont Pioneer) treated with clothianidin (Poncho 1250®,Bayer CropScience, Durham, NC) at the rate of 1.25 mg a.i. kernel^{-1} (DiFonzo and Cullen, 2013). Hybrids with RIB had all refuge kernels removed prior to planting. They were easily distinguishable, as they were dyed a different color. This was done to ensure that all seeds planted for evaluation contained the Bt trait for CRW and the plots contained no refuge (non-Bt seed). All of the hybrids were glyphosate-resistant.

The five different hybrids planted in 2014 varied from those selected in 2013 because some hybrids were discontinued and no longer available from the seed companies. Some of the 2013 hybrids were discontinued for the 2014 growing season. While the hybrids were different, they included the same Bt traits as those planted in 2013. The hybrids planted in 2014 were: a hybrid expressing no Cry proteins (Pioneer P8640R, DuPont Pioneer, Johnston, IA); a hybrid expressing Cry3Bb1 (Event MON-88017-3, DKC43-48, Monsanto Co., St. Louis, MO); a hybrid expressing Cry34/35Ab1 (Event DAS-59122-7, Pioneer P9526AMX, DuPont Pioneer); a pyramided hybrid expressing both Cry3Bb1 and Cry34/35Ab1 (Events MON-88017-3 and DAS-59122-7, DKC44-13, Monsanto Co.); and the non-Bt hybrid (Pioneer P9526AM, DuPont Pioneer) treated with clothianidin (Poncho 1250® Bayer CropScience, Durham, NC) at the rate of 1.25 mg a.i. kernel^{-1}, (DiFonzo and Cullen, 2013). The same previously mentioned procedure was used to remove the refuge kernels from the RIB hybrids.

The soil applied insecticide used in both years was tefluthrin (Force3G®, Syngenta Crop Protection, Greensboro, NC) placed in-furrow with the seed at planting at a rate of 0.19 kg a.i. ha^{-1}. The plots were planted using a two-row vacuum Monosem NG Plus 4, 5x5-mounted planter (Seed Research Equipment Solutions [SRES], Hutchinson, Kansas).

Herbicides were applied to manage the weed population in all plots during both study years. In 2013, an application of a tank mix consisting of: 0.8 kg a.e. ha^{-1} of glyphosate (Roundup Powermax®, Monsanto Co., St. Louis, MO), tembotrione and isoxadifen safener, (Laudis®, Bayer CropScience, Durham, NC) at 4 g of a.i. ha^{-1} and a non-ionic surfactant (NIS), (ammonium sulfate, corn syrup, alkyl polyglucoside, Class Act NG®, Winfield Solutions, St. Paul, MN) at 2.5% v/v was made with a total water volume of 141 L ha^{-1} on 12 June. In 2014, all locations received one application of glyphosate at 0.8 kg a.e. ha^{-1} and a non-ionic surfactant at 2.5% v/v with a total water volume of 141 L ha^{-1} on 9 June.

Each main plot was a minimum of 38 m long and 7 m wide, depending on location and the amount of land available. Four rows of each hybrid were planted using 76 cm row spacing. Plot lengths were 6 m, with 1.5-m alleys between replicates. Experiments were planted at a plant density of 86,100 plants ha^{-1} with 15.2 cm within-row seed spacing. All plots were fertilized for a yield potential of 10 Mg ha^{-1}. Application rates varied based on soil test results obtained from the individual cooperators at each of the locations. Fertilizer applications were spread as granular urea at the V4 stage in the plots that had not been fertilized completely before planting.

Maize roots were evaluated for rootworm larval feeding injury after the feeding period had ceased. Plants were dug up when the CRW adults began emerging from the soil. This ensured that the highest percentage of feeding damage had already occurred to maize plants, but minimal root regrowth had occurred. Scoring plants before the majority of feeding has occurred can create a false representation of the efficacy of the treatments, but waiting too long after feeding can allow for increased regrowth and root compensation (Gray and Steffey, 2000). This stage normally occurs from late-July to early August, but is variable depending on accumulated degree days. In 2013, root digs were conducted on August 1, and in 2014 digs took place on September 8.

Ten plants were selected from the outside rows of each plot. Five plants were randomly selected, at least 1 m apart, from rows one and four of each plot. Allowing space between plants helps to decrease potential damage from digging into the root mass and maintain the integrity of the roots for accurate scoring. Two people, one on each side of the plant, dug a 15-cm diameter circle around the stalk to a depth of 30 cm and then carefully removed a complete root ball. Before scoring took place, the roots were washed to remove all soil and other debris.

Once roots were cleaned, the complete root ball was evaluated using the Iowa State University 0 to 3 node-injury scale (Oleson et al., 2005). This scale quantifies the amount of cumulative root pruning of the total root mass. An individual root is considered pruned back if it is within 3.8 cm of the stalk or soil lines, depending on which node is being rated (Oleson et al., 2005). The number of pruned roots in each node was counted and divided by the total number of roots in that node to get an accurate root feeding score in each of the treatments. An injury score of zero signified there was no feeding damage to the roots. A score of one indicates one node had been pruned back to within 3.8 cm of the stalk or soil line, depending on its origin. A score of two indicates that two nodes were pruned back and a score of three describes a root that had three nodes pruned back completely. For example, if one node is completely damaged, it would receive a score of one. The second node would then be evaluated and added to the first. If the second node has six of 12 roots pruned (0.5), the cumulative root score (node one score added to node two score) would equal 1.5. This method results in a linear relationship between the numerical value and the amount of root pruning. The economic threshold for insect feeding is a score of 0.25 (Oleson et al., 2005).

The previous scoring scales were qualitative or less linear in nature, while the Iowa State University node injury scale is a more linearly quantitative index. This has been very helpful to evaluate minor root injury, especially in evaluating transgenic maize hybrids, and quantifying differences in feeding among treatments (Oleson et al., 2005).

In the locations where severe root feeding took place, lodging notes were taken before harvest on the different treatments. Plants were considered lodged if they had an incline greater than 45 degrees from the upright position of 90 degrees in relation with the soil line. The number of lodged plants in the inner two rows was divided by the total number of plants in those rows to determine the percentage of lodged plants.

Because lodging creates very difficult harvest conditions, plots with severe lodging were hand-harvested and harvested ears were manually placed in to a combine to obtain the total harvested yield.

Plot lengths and number of harvestable ears in each harvested row of every plot were obtained and recorded before harvest took place. Plots were harvested mechanically with a Wintersteiger Classic™ plot combine (Wintersteiger Ag, Ried, Austria) with a two-row Geringhoff™ corn header (Geringhoff, Minot, ND) when severe lodging was not present. The two center rows were harvested and used for yield, and a subsample was taken to measure the moisture and test weight of the grain for each plot. Yield, percent moisture, and test weight for each plot were recorded using a Harvest Master™ (Juniper Systems, Logan, UT) yield monitor on the combine. Subsample grain was dried to 15.5 g hg^{-1} moisture before measuring test weight to compare treatments. Plots with severe lodging were hand-harvested, and then threshed using the combine, ensuring all recorded data was obtained the same way.

In Forman, in 2013, maize was not harvested, and data were not recorded because of poor stand establishment and development. The Lidgerwood and Wyndmere sites did not have sufficient root pruning to record, but plots were still harvested for yield to determine if there were any significant differences among the hybrids and insecticide treatments.

Root ratings for the 10 plants were averaged and tested for normality. If the data were found to be normal they were analyzed statistically to determine if the treatments were effectively protecting plants from CRW injury and associated yield losses.

Data were subjected to an ANOVA using the general linear models (GLM) procedure in SAS 9.3 (SAS Institute Inc.; Cary, NC, USA). The node injury scale used to evaluate roots is a continuous scale; therefore it can be statistically analyzed using ANOVA. Comparisons were made between hybrids and treatments based on the means for root pruning and yield using *F*-protected least significant differences (LSDs) at a 0.10 level of confidence.

An evaluation on the estimated economic return on investment for hybrids with and without Bt traits was also completed. The cost of hybrids is calculated based on a bag of maize containing 80,000 kernels and a planting population of 86,100 plants ha^{-1} (Table 1). The average cost of a hybrid with no Bt traits for resistance to CRW was $293 per bag. Average hybrid cost with a single Bt trait was $358 per bag. Hybrids expressing pyramid Bt traits cost $369 per bag. The insecticide application was applied to the trial at a rate of 6.25 kg ha^{-1}. The product cost on average $3 kg^{-1} costing the producer around $18 ha^{-1}. These data were not subject to statistical analysis.

Table 1. Estimated cost per hectare of hybrids (80,000 kernel bag) and insecticide (per kilogram) for the 2015 season as of December 1, 2014.

Product	Trait	2015 Price†	Cost ha^{-1}
P8640	None	276	298
P9526AM	None + Poncho 1250	310	335
DKC 43-48	Cry3Bb1	357	386
P9526AMX	Cry34/35Ab1	360	389
DKC 44-13	Cry3Bb1 + Cry34/35Ab1	369	399
Force3G	Tefluthrin	3	18

† Estimated price is suggested retail price for hybrids, and does not include discounts.
(D. Anderson, personal communication, 2014) (J. Ehlers, personal communication, 2014) (B. Frolek, personal communication, 2014)

Yields from the Arthur location in 2013 and 2014 were used to compare yields under heavy and low CRW infestations (Table 1 and 2). Profit was based on a market price of $157/metric ton of maize, and the 2015 prices for maize hybrids. Net return of each hybrid with and without insecticide in-furrow was compared to the hybrid with no Cry proteins and no seed or soil-applied insecticide.

RESULTS AND DISCUSSION

Weather Conditions

The weather data, as recorded by automated weather stations near the various locations, from September 2012 to June 2013, showed the winter air temperatures were slightly colder than average, along with extended low bare soil temperatures (NDAWN, 2014). Precipitation in June 2013 was also above average, which could have increased larval mortality during hatch at some locations (Tables 3 and 4). The fall of 2013 had above average rainfall going into the winter. Research has shown that saturated topsoil can reduce larval establishment on host plant roots, and reduce beetle emergence by 50 percent (Riedell and Sutter, 1995).

Corn rootworm populations were greatly reduced in 2014 across the U.S., as well in North Dakota (Table 2) (Potter and Ostlie, 2014). The weather data from North Dakota supports that the winter of 2013-2014 had colder than normal minimum air temperatures, as well as very cold bare soil temperatures (Table 3 and 4) (NDAWN, 2014). These factors, paired with minimal snow coverage (Figure 1), resulted in suboptimal overwintering conditions for CRW eggs (Potter and Ostlie, 2014). Poor overwintering conditions were followed by above-average rainfall in the spring of 2014 at the time of larval hatch. Saturated topsoil conditions could have attributed to a reduction in larval establishment on the plant roots, and a reduction in the emergence of adult beetles (Riedell and Sutter, 1995).

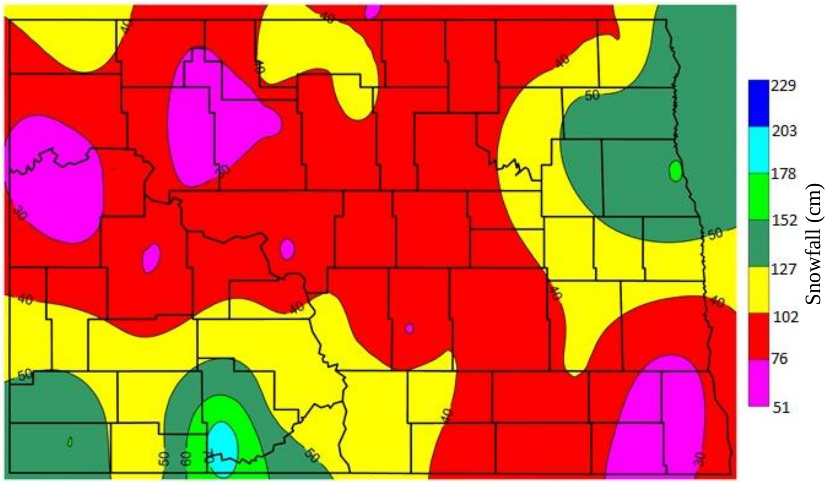

Figure 1. Total snowfall in North Dakota during the winter of 2013-2014 (Data from National Weather Service Cooperative Network; Image from ND State Climate Office).

Northern corn rootworms have been found to have better survival under sub-zero temperatures than WCR (Ellsbury and Lee, 2004). This is consistent with the fact that NCR are indigenous to the tall grass prairies of North America. Northern corn rootworm eggs are able to better resist freezing and chilling injury than WCR eggs. Western corn rootworm eggs experience significant mortality when exposed to temperatures below -10 °C to -13 °C for a time period of 5 weeks or longer (Ellsbury and Lee, 2004). The NCR population was higher than that of the WCR in 2014, supporting that NCR are usually better able to survive cold winter conditions (Table 2) (Potter and Ostlie, 2014). This is likely what impacted the CRW populations that were observed in emergence traps from the experimental plots.

Table 2. Combined total capture of adult CRWs from emergence traps in all treatment plots, Arthur and Wyndmere, ND (2013) and Arthur and Hope, ND (2014).

Year	Northern corn rootworm	Western corn rootworm	Total
2013	1277	1163	2440
2014	554	12	566
Total	1831	1175	3006

(V. Calles Torrez, unpublished data).

There were no significant differences in the amount of root pruning injury in the presence or absence of soil-applied insecticide in 2014.There are multiple factors that could have contributed to the lack of efficacy of the soil-applied insecticide. June rainfall in 2014 was above average (Table 4). Studies have shown that heavy rainfall can reduce the concentration of insecticide at seeding depth by leaching through the soil profile (Levine and Oloumi-Sadeghi, 1991). Degradation of soil-applied insecticide can increase with warm temperatures. June temperatures were above normal (Table 4). Larval emergence in 2014 was observed approximately 45 days after planting, and feeding occurred for approximately another 30 days (Drees et al., 1999). The residual effects of soil-applied insecticides generally only last 30 to 90 days. This would have put stress on the residual effects of the soil insecticide, and may have impacted its effectiveness in preventing root pruning injury.

Table 3. Monthly average temperatures and rainfall recorded by automated weather stations near Prosper and Wyndmere, ND.

Month	Year	Min. air temp.	Normal min. air temp.	Bare† soil temp.	Rainfall	Avg. rainfall
		°C	°C	°C	mm	mm
			Prosper			
Sept.	2012	5.1	7.6	17.0	14.7	65.5
Oct.	2012	0.3	0.8	7.3	44.5	61.7
Nov.	2012	-6.3	-7.2	1.3		23.4
Dec.	2012	-14.7	-15.2	-1.7		15.7
Jan.	2013	-18.6	-18.7	-2.4		15.2
Feb.	2013	-16.4	-15.7	-2.7		14.2
Mar.	2013	-15.3	-7.7	-1.7		29.5
April	2013	-3.9	-0.5	0.8	2.8	36.8
May	2013	7.5	6.3	13.3	105.2	77.5
June	2013	14.0	12.1	20.5	192.5	100.3
			Wyndmere			
Sept.	2012	4.8	8.6	18.3	9.6	62.2
Oct.	2012	0.3	1.7	9.0	46.0	55.6
Nov.	2012	-5.8	-6.2	1.8		22.9
Dec.	2012	-15.2	-14.4	-0.4		16.3
Jan.	2013	-18.0	-17.5	-1.3		16.3
Feb.	2013	-17.0	-14.6	-1.1		16.5
Mar.	2013	-15.0	-7.4	-0.8		30.7
April	2013	-4.7	0.2	0.4	37.3	43.2
May	2013	7.5	7.3	13.3	66.2	74.9
June	2013	13.9	13.0	19.0	149.3	98.6

† Bare soil temperature is the temperature of bare soil with no vegetation or cover as measured at a depth of 10 cm below the soil surface.

Table 4. Monthly average temperatures and rainfall recorded by automated weather stations near Galesburg and Prosper, ND.

Month	Year	Min. air temp.	Normal air min. temp.	Bare† soil temp.	Rainfall	Avg. rainfall
		°C	°C	°C	mm	mm
			Galesburg			
Sept.	2013	9.8	7.1	18.0	83.1	62.2
Oct.	2013	1.5	-0.4	6.9	96.0	46.2
Nov.	2013	-9.6	-8.7	-0.6		17.5
Dec.	2013	-21.2	-17.0	-4.0		14.2
Jan.	2014	-21.7	-20.4	-4.9		10.9
Feb.	2014	-21.3	-17.6	-7.1		18.8
Mar.	2014	-11.5	-9.5	-2.8		19.6
April	2014	-2.2	-1.2	3.3	58.7	23.9
May	2014	6.4	6.0	12.0	34.8	71.4
June	2014	13.0	12.8	19.3	141.5	80.5
			Prosper			
Sept.	2013	10.5	7.6	17.4	92.5	65.5
Oct.	2013	2.0	0.8	7.7	84.3	61.7
Nov.	2013	-8.7	-7.2	0.4		23.4
Dec.	2013	-21.1	-15.2	-1.2		15.7
Jan.	2014	-22.3	-18.7	-1.8		15.2
Feb.	2014	-21.5	-15.7	-2.2		14.2
Mar.	2014	-10.6	-7.7	-1.2		29.5
April	2014	-0.8	-0.5	4.0	79.9	36.8
May	2014	7.2	6.3	13.5	52.1	77.5
June	2014	13.7	12.1	20.5	107.2	100.3

† Bare soil temperature is the temperature of bare soil with no vegetation or cover as measured at a depth of 10 cm below the soil surface.

Root Pruning Injury

Plants from the check plots containing hybrids without Cry proteins and soil- or seed-applied insecticide for root protection were dug to evaluate root injury at the Lidgerwood, Wyndmere, and Arthur sites in 2013, and at the Arthur, Hope, and Page sites in 2014. Insufficient root injury was observed at the following field locations and years, and thus, those sites were not scored: Lidgerwood in 2013, Wyndmere in 2013, and Page in 2014. The Arthur location in 2013 and Arthur and Hope locations in 2014 had sufficient CRW populations present that resulted in sufficient root pruning injury. Hybrid roots were dug, washed, and evaluated to determine the amount of root pruning present.

At the Arthur location in 2013, the hybrid x insecticide interaction for root pruning injury was statistically significant (Table 5). The significant interaction was likely the result of the hybrid with Cry3Bb1 having significantly more root pruning injury when insecticide was present in-furrow, while in the hybrid with Cry34/35Ab1, the opposite effect was observed. The injury score was increased from 0.13 nodes pruned without soil-applied insecticide to 0.5 nodes pruned with soil-applied insecticide. The hybrid with Cry34/35Ab1 had an opposite result, showing a significant decrease in root pruning injury when insecticide was applied in-furrow. The hybrid had 0.45 nodes pruned without insecticide and only 0.15 nodes pruned when insecticide was applied in-furrow. The other hybrids had similar root pruning injury regardless of in-furrow insecticide application.

Table 5. Hybrid x insecticide interaction comparing subplot treatment means and whole plot treatment for root pruning injury, Arthur, ND, 2013.

Hybrid	Node injury scale (0-3)	
	Without insecticide	With insecticide
P9675R (No Cry proteins)	1.07	0.91
P9675 (No Cry proteins + Poncho 1250)	0.75	0.77
DKC43-27 (Cry3Bb1)	0.13	0.50
P9675AMRW(Cry34/35Ab1)	0.45	0.15
DKC44-13 (Cry3Bb1 + Cry34/35Ab1)	0.16	0.02
LSD_1 ($p \leq 0.1$)†	0.28	
LSD_2 ($p \leq 0.1$)‡	0.29	

†LSD_1 was calculated to compare two subplot treatment means for the same whole plot treatment.

‡LSD_2 was calculated to compare two whole plot treatments at the same or different sub plot treatments.

These results support in-furrow insecticide application can have erratic root protection, and should not be used as the primary method of protection against CRW (Head et al, 2014). The results indicate that soil-applied insecticide does not always decrease the amount of root pruning observed. Overall, hybrids with Bt-traits had less root pruning injury than those without Bt-traits in the presence or absence of insecticide in-furrow. These results indicate that all traits were effective in controlling CRW.

Root scores in 2014 reflected the lower CRW populations that were present. In Hope in 2014, there was significantly more root pruning in the hybrid with no Cry protein than in the other hybrids either expressing a Cry protein, or treated with Poncho 1250 (Table 6). There were no significant differences among the treatments expressing Cry3Bb1, Cry34/35Ab1, and Cry3Bb1 + Cry34/35Ab1 and the treatment with no Cry proteins + Poncho 1250. This indicates that under low CRW feeding pressure, seed-applied insecticides can provide some protection against larval feeding.

In Arthur, in 2014, root injury scores were very low. There were no significant differences between the hybrid with no Cry proteins and the hybrid with no Cry proteins + Poncho 1250. Both treatments did have significantly more root pruning than the hybrids expressing some form of Cry protein, but again the amount of pruning was still below the economic threshold associated with yield losses. The hybrids expressing Cry proteins in the form of Cry3Bb1, Cry34/35Ab1, and Cry3Bb1 + Cry34/35Ab1 did not differ significantly from each other, but had significantly less root pruning when compared to the hybrids expressing no Cry proteins or no Cry proteins + Poncho 1250.

The combined analysis across locations and years indicated the hybrid with no Cry proteins had significantly more root pruning than all other treatments, with an average of 0.42 nodes pruned (Table 6). The hybrid with no Cry proteins + Poncho 1250 was also significantly different from all other treatments, and had an average of 0.32 roots pruned. It was significantly lower than the hybrid with no Cry proteins, but was still significantly higher than the hybrids expressing some form of Cry proteins. The hybrid expressing Cry3Bb1 and the hybrid expressing Cry34/35Ab1 were not significantly different from each other, but they did have significantly more root pruning then the hybrid with Cry3Bb1 + Cry34/35Ab1. The hybrid with both traits, Cry3Bb1 + Cry 34/35Ab1, had significantly less root pruning than the other treatments, and had the least amount of root pruning injury overall with an average of 0.06 nodes pruned.

Table 6. Effect of different treatments on the average amount of root pruning injury based on the Iowa State University node injury scale averaged across hybrids Arthur, ND (2013) and Hope and Arthur, ND (2014) and combined locations.

Hybrid	Arthur 2013	Hope 2014	Arthur 2014	Combined
	Node injury scale (0-3)			
P8640 (No Cry proteins)	0.99	0.12	0.16	0.42
P9526AM (No Cry proteins + Poncho 1250)	0.76	0.07	0.14	0.32
DKC43-48 (Cry3Bb1)	0.32	0.06	0.06	0.15
P9526AMX (Cry34/35Ab1)	0.30	0.05	0.05	0.14
DKC44-13 (Cry3Bb1 + Cry34/35Ab1)	0.09	0.07	0.03	0.06
LSD ($p \leq 0.1$)†	0.20	0.03	0.04	0.06
CV %	47.1	72.7	55.7	63.6

†LSD ($p \leq 0.1$) is a Fisher's Protected LSD.

The combined analysis of the locations with root pruning injury resulted in the hybrid x insecticide interaction being statistically significant. When insecticide was not present, the hybrid with Cry3Bb1 + Cry34/35Ab1 had the least root pruning injury with a score of 0.08, but it was not significantly different from the hybrid with Cry3Bb1 or the hybrid with Cry34/35Ab1 (Table 7). The hybrid with Cry34/35Ab1 was not statistically different from the hybrid with no Cry proteins + Poncho 1250, but it did have less root pruning injury with a score of 0.19, compared to 0.31 nodes pruned in the hybrid with no Cry proteins. The hybrid with no Cry proteins + Poncho 1250 was not statistically different from the hybrid with no Cry proteins. This shows that hybrids with Cry proteins had less damage than those hybrids without Cry proteins.

The hybrids with Cry proteins remained below the economic threshold for damage under varying levels of CRW infestations.

In the presence of soil-applied insecticide, the hybrid with Cry3Bb1 + Cry34/35Ab1 was not statistically different from the hybrid with Cry34/35Ab1 or the hybrid with Cry3Bb1. The hybrid with Cry3Bb1 was not statistically different from the hybrid with no Cry proteins + Poncho 1250. The hybrid with no Cry protein + Poncho 1250 was not statistically different from the hybrid with no Cry proteins, but did have less root pruning injury. The hybrid with no Cry proteins and no seed-applied insecticide had the highest amount of root pruning injury with 0.40 nodes pruned.

There were no statistical differences between the any of the hybrids when compared with and without soil-applied insecticide.

These results do not support the application of insecticide in-furrow to reduce the amount of root pruning injury for any of the hybrids tested. Under high populations of corn rootworms, it might be beneficial to apply insecticide in-furrow to help reduce the amount of root pruning injury, but these data are not sufficient to support that conclusion since the reduction of CRW due to insecticide was observed only in one hybrid at one location. Under low populations like those observed in 2014, there was no obvious reduction in root pruning injury when insecticide was applied in-furrow. In the combined analysis, the hybrids with traits had root pruning injury below 0.25, which is the economic threshold for insect feeding. The hybrids with no Cry proteins were above this level, even when insecticide was applied on seed or in-furrow at planting.

The reduction in root pruning injury was not significantly different from the hybrid with no Cry proteins, however. Seed-applied insecticide provided inconsistent root protection for hybrids, and did not perform as well as Bt-traits in reducing root pruning injury.

Head et al. (2014) had similar results. They observed that hybrids with Cry protein had significantly less root pruning injury than hybrids with no Cry proteins. They also observed that Cry3Bb1 + Cry34/35Ab1 had less root pruning injury when compared to hybrids with only Cry3Bb1 or Cry34/35Ab1 alone. They also reported an increase in CRW efficacy when Cry proteins were pyramided when compared to hybrids with single proteins. Overall, however, their study concluded that hybrids containing any Cry protein had better root protection and CRW efficacy than the control hybrid with no Cry proteins (Head et. al., 2014). The authors also reported inconsistencies with soil-applied insecticide. They attributed their results to environmental conditions at planting that may not have activated the insecticide. These results are consistent with the findings of this study.

Obopile et al. (2013) reported that hybrids with insecticide seed treatment had significantly higher root pruning injury then those hybrids with Bt traits. The hybrids with insecticide seed treatment still had significantly less root pruning injury then the untreated hybrids, however (Obopile et al., 2013). The reduction in root pruning injury did not always translate into a yield advantage for the treated hybrids. This supports that Bt traits provide the most root protection, but using a seed treatment can also reduce the amount of root pruning injury when compared to a hybrid with no traits or seed treatment. However, there is not always a yield advantage when there is a reduction in root pruning injury (Obopile et al., 2013).

Table 7. Hybrid x insecticide interaction comparing subplot treatment means for root pruning injury without and with insecticide, Arthur, ND (2013) and the Hope and Arthur, ND (2014) and combined locations.

Hybrid	Node injury scale (0-3)	
	Without	With
No Cry	0.44	0.40
No Cry + Poncho 1250	0.31	0.33
Cry3Bb1	0.09	0.20
Cry34/35Ab1	0.19	0.08
Cry3Bb1 + Cry34/35Ab1	0.08	0.05
LSD_1 ($p \leq 0.1$)†	0.16	
LSD_2 ($p \leq 0.1$)†	0.17	

†LSD_1 was calculated to compare two subplot treatment means for the same whole plot treatment.

‡LSD_2 was calculated to compare two whole plot treatments at the same or different sub plot treatments.

Yield

Having insecticide applied in-furrow did significantly increase the average yield and test weight of the hybrids in Arthur in 2013 (Table 8). This location had high populations of CRW and root pruning that exceeded threshold numbers. Hybrids with insecticide applied in-furrow yielded 1.1 Mg ha^{-1} greater than those same hybrids in the absence of soil-applied insecticide. This may be the result of the high levels of corn rootworm pressure that were observed at this location, and the substantial amount of root pruning injury that was recorded for the hybrids at this location.

Table 8. Effect of insecticide application on yield and test weight of hybrids, Arthur, ND (2013).

Insecticide	Yield	Test weight
	Mg ha^{-1}	kg/hL
Without	7.1	62.7
With	8.2	64.9
LSD ($p \leq 0.1$)†	0.3	1.5

† LSD ($p \leq 0.1$) is a Fisher's Protected LSD.

There were statistical differences between the hybrids at the different locations. This was to be expected because of different genetics and yield potential of the various hybrids. The environments at the various locations the hybrids were placed in may have also favored the yield potential of some hybrids more than others.

In Lidgerwood in 2013, the hybrid x insecticide interaction for yield was statistically significant (Table 9). When insecticide was not applied in-furrow, there were no differences between the different hybrids. When insecticide was present in-furrow, the hybrids with Cry3Bb1 and with Cry3Bb1+ Cry34/35Ab1 were the highest yielding.

Table 9. Hybrid x insecticide interaction comparing subplot and whole plot treatments for yield Lidgerwood, ND (2013).

Hybrid	Without insecticide	With insecticide
	Mg ha^{-1}	
P9675R (No Cry proteins)	7.2	6.0
P9675R (No Cry proteins + Poncho 1250)	7.6	6.6
DKC43-27 (Cry3Bb1)	6.6	8.0
P9675AMRW (Cry34/35Ab1)	7.2	5.4
DKC44-13 (Cry3Bb1 + Cry34/35Ab1)	6.9	7.7
LSD$_1$ ($p \leq 0.1$)†	1.5	
LSD$_2$ ($p \leq 0.1$)‡	1.8	

†LSD$_1$ was calculated to compare two subplot treatment means for the same whole plot treatment.

‡LSD$_2$ was calculated to compare two whole plot treatments at the same or different sub plot treatments.

The only hybrid with a significantly different yield with and without soil-applied insecticide was the hybrid with Cry34/35Ab1 (Table 9). The yield was significantly higher when insecticide was not applied in-furrow. All of the other hybrids were not significantly different with or without insecticide applied in-furrow.

These results could be variable because CRW root pruning was not present at this location. The use of soil-applied insecticide did not show the same benefit for the hybrids with no Cry proteins that it did when high CRW populations were present, like those observed at the Arthur location in 2013.

At Arthur in 2013 the CRW feeding pressure resulted in substantial root pruning. The root feeding may have resulted in the significant differences observed in grain yield among the different hybrids (Table 10). There was no statistical significance in yield between the hybrids expressing Cry3Bb1 only, Cry34/35Ab1 only, and Cry3Bb1 + Cry34/35Ab1. These hybrids yielded significantly higher than the hybrid with no Cry proteins and the hybrid with no Cry proteins + Poncho 1250. There was no significant difference in yield between the hybrid with no Cry proteins and the hybrid with no Cry proteins + Poncho 1250. The hybrid with no Cry proteins or seed-applied insecticide had the lowest yield of 5.7 Mg ha^{-1}.

Under the heavy infestations of CRW at the Arthur location in 2013, yields were decreased with an increase of root pruning injury, both with and without insecticide applied in-furrow (Figure 2). Individual hybrids did not always show a reduction in root pruning injury when insecticide was applied in-furrow. The overall trend from this location, however, is a reduction in yield as the amount of root pruning injury is increased, without or with insecticide applied in-furrow. These data illustrate the potential for yield reduction associated with the severity level of root pruning injury observed in the field.

The regression model for the data in Hope and Arthur in 2014 was not as predictive for the yield loss associated with increased root pruning injury (data not shown). The average root pruning injury score for the hybrids at Arthur was 0.09, and 0.07 at Hope, resulting in injury below the economic threshold.

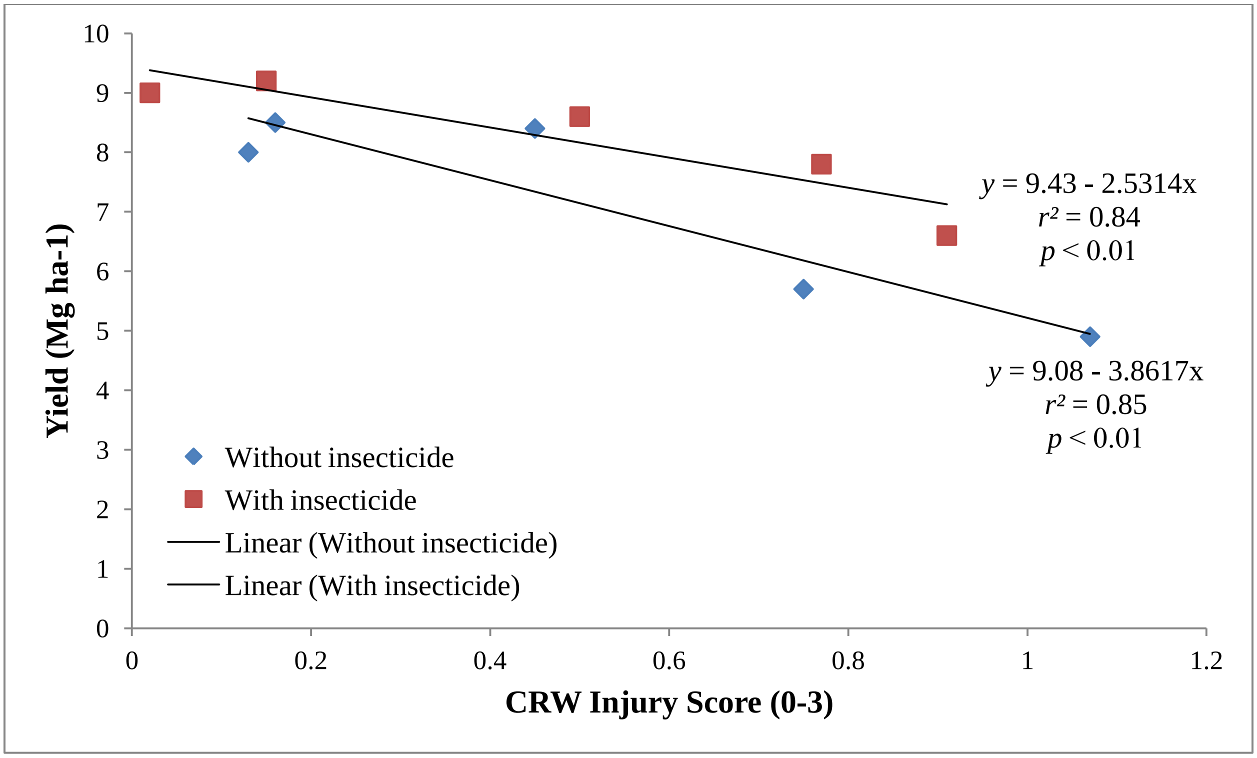

Figure 2. Comparison of CRW injury and yield for hybrids with and without soil insecticide at Arthur, ND 2013 location.

Gray and Steffey (1998) conducted a similar study on hybrids with no Cry proteins and soil-applied insecticide in-furrow. They had similar results when CRW feeding was present. They recorded a significant increase in yield for the insecticide treated plots when compared to the untreated plots. Insecticide also reduced the amount of root pruning injury observed (Gray and Steffey, 1998).

A regression analysis for the impact of CRW feeding injury on yield from the Urbana, Illinois location had similar coefficients of determination as those calculated for the Arthur 2013 location (Gray and Steffey, 1998). This can be attributed to the heavy CRW feeding that resulted in severe root pruning injury. Gray and Steffey (1998) found that about 30 percent of yield loss still could not be attributed to CRW feeding damage. Our study found that approximately 15 percent of yield loss could not be attributed to root pruning injury. This could be caused by advancements in hybrid genetics and environmental conditions that may have increased or reduced stress on the hybrids.

At the Wyndmere location, in the absence of CRW feeding, hybrids did not differ significantly in yield (Table 10).

Table 10. Effect of traits on yield averaged over treatment at Wyndmere, Lidgerwood, and Arthur, ND and combined locations (2013).

Hybrid	Wyndmere	Lidgerwood	Arthur	Combined
		Mg ha^{-1}		
P9675R (No Cry proteins)	9.2	6.6	5.7	7.2
P9675R (No Cry proteins + Poncho 1250)	9.8	7.1	6.7	7.9
DKC43-27 (Cry3Bb1)	9.5	7.3	8.3	8.4
P9675AMRW (Cry34/35Ab1)	9.9	6.3	8.8	8.4
DKC44-13 (Cry3Bb1 + Cry34/35Ab1)	10.2	7.3	8.7	8.6
LSD ($p \leq 0.1$)†	ns	ns	1.2	0.7
CV %	16.0	18.2	18.1	17.7

†LSD ($p \leq 0.1$) is a Fisher's Protected LSD.

Combined yield results for the 2013 growing season resulted in significant differences in yield among the treatments (Table 10). The hybrid with Cry3Bb1 + Cry34/35Ab1 had the highest yield of 8.6 Mg ha^{-1}, but was not statistically different from the hybrid with Cry3Bb1 or the hybrid with Cry34/35Ab1. It was significantly different from both the hybrid with no Cry proteins + Poncho 1250 and the hybrid with no Cry proteins.

Our results show that under heavy CRW infestations, like those observed in 2013, the hybrids with Bt traits for CRW exceeded those without Bt traits in yield, even when seed-applied insecticide was used in Lidgerwood and Arthur only. When CRW populations were below economic threshold or absent, the hybrids with Bt traits had no yield advantage.

Significant differences in yield among the hybrids were detected in Arthur in 2014 (Table 11). The hybrid with no Cry proteins had a yield of 8.6 Mg ha^{-1}, but it did not differ significantly from those with Cry proteins. It was significantly greater than the hybrid with no Cry proteins + Poncho 1250.

In Hope in 2014 significant differences in yield among the hybrids were observed (Table 11). The hybrid with no Cry proteins + Poncho 1250 had a yield of 9.5 Mg ha^{-1}, but it was not significantly different from the hybrid with Cry3Bb1 and the hybrid with Cry34/35Ab1. Those hybrids had significantly higher yields than the hybrid with no Cry proteins and the hybrid with Cry3Bb1 + Cry34/35Ab1. The hybrid with Cry3Bb1 + Cry34/35Ab1 had the lowest yield of 7.7 Mg ha^{-1}.

Table 11. Effects of traits on yield averaged over treatment at Arthur, Hope, and Page, ND and combined locations (2014).

Hybrid	Arthur	Hope	Page	Combined
		Mg ha^{-1}		
P8640 (No Cry proteins)	8.6	8.3	8.5	8.5
P9526AM (No Cry proteins + Poncho 1250)	7.7	9.5	8.5	8.6
DKC43-48 (Cry3Bb1)	8.0	9.4	9.0	8.8
P9526AMX (Cry34/35Ab1)	8.0	9.3	8.8	8.7
DKC44-13 (Cry3Bb1 + Cry34/35Ab1)	8.3	7.7	8.8	8.3
LSD ($p \leq 0.1$)†	0.7	0.6	ns	ns
CV %	10.7	8.5	14.8	11.8

†LSD ($p \leq 0.1$) is a Fisher's Protected LSD.

In Page in 2014 and in the combined analysis across locations, significant differences in yield were not observed (Table 11).

In 2014, the Bt traits did not provide clear yield benefits as observed in the previous year's study when the target insects were above economic threshold levels (Potter and Ostlie, 2014). Obopile et al. (2013) also reported that hybrids with Bt traits or insecticidal seed treatments did not always translate into a yield advantage when root pruning injury levels were low. At high levels of root pruning injury, the Bt hybrids and seed treatments had a significant yield advantage over the untreated hybrids (Obopile et al., 2013).

<u>Test Weight</u>

In 2013, in Wyndmere statistical significance for test weight was observed (Table 12). The hybrid with Cry3Bb1 + Cry34/35Ab1 had a test weight of 63.2 kg/hL and have significantly higher test weight than the hybrid with Cry34/35Ab1 and the hybrid with no Cry proteins + Poncho 1250. In Lidgerwood, in 2013, there were no statistical differences in test weight (Table 12). In Arthur, in 2013, the hybrid with Cry3Bb1 + Cry34/35Ab1 had a significantly higher test weight than the hybrid with Cry3Bb1 and the hybrid with no Cry proteins + Poncho 1250 (Table 12). In 2013, the combined analysis for test weights across locations did not detect significant differences among hybrids (Table 12).

Table 12. Effect of traits on test weight averaged over treatment at Wyndmere, Lidgerwood, and Arthur, ND and combined locations (2013).

Hybrid	Wyndmere	Lidgerwood	Arthur	Combined
		kg/hL		
P9675R (No Cry proteins)	62.1	65.6	63.6	63.8
P9675R (No Cry proteins + Poncho 1250)	61.6	66.8	63.3	63.9
DKC43-27 (Cry3Bb1)	62.3	66.4	63.0	63.9
P9675AMRW (Cry34/35Ab1)	61.8	65.3	64.2	63.8
DKC44-13 (Cry3Bb1 + Cry34/35Ab1)	63.2	66.3	64.8	64.8
LSD ($p \leq 0.1$)†	1.3	ns	1.3	ns
CV %	2.5	3.5	2.3	9.8

†LSD ($p \leq 0.1$) is a Fisher's Protected LSD.

In 2014, in Arthur and Hope statistical differences in test weight between hybrids were not observed (Table 13). In 2014, the statistical analysis for test weights detected significant differences among hybrids (Table 13). The hybrid with no Cry proteins had a test weight of 66.7 kg/hL, and was not statistically different from the hybrid with Cry3Bb1 + Cry34/35Ab1 and the hybrid with Cry3Bb1. The hybrid with Cry34/35Ab1 had the lowest test weight of 63.1 kg/hL. In 2014, the combined analysis across locations of average test weights at the 2014 locations showed significant differences between the hybrids (Table 13). The hybrid with no Cry proteins had a test weight of 63.9 kg/hL. It was significantly greater than the hybrid with no Cry proteins + Poncho 1250 and the hybrid with Cry34/35Ab1.

The results did not show a clear test weight advantage to hybrids with or without Bt traits. The test weights from trials were variable among location and years.

Stress from disease, environment, fertility, and insect damage can cause some reductions in test weight (Rankin, 2009). This occurs because the movement of nutrients is impacted during grain fill or the quality of the kernel is compromised once it is filled. In this experiment, the results do not show that under heavy CRW root pruning test weight values were always decreased when compared to locations with no CRW root pruning.

Table 13. Effects of traits on test weight averaged over treatment at Arthur, Hope, and Page, ND and combined locations (2014).

Hybrid	Arthur	Hope	Page	Combined
		kg/hL		
P8640 (No Cry proteins)	59.9	65.1	66.7	63.9
P9526AM (No Cry proteins + Poncho 1250)	60.5	63.6	64.2	62.8
DKC43-48 (Cry3Bb1)	61.0	64.1	65.9	63.7
P9526AMX (Cry34/35Ab1)	60.0	64.1	63.1	62.4
DKC44-13 (Cry3Bb1 + Cry34/35Ab1)	60.5	63.7	66.1	63.4
LSD ($p \leq 0.1$)†	ns	ns	1.0	0.9
CV %	4.1	2.9	1.9	3.0

†LSD ($p \leq 0.1$) is a Fisher's Protected LSD.

Economics

Under heavy CRW infestations, there was an increased return on investment for hybrids with Bt traits when compared with those without Bt traits (Table 14). There was also an economic advantage when insecticide was applied in-furrow for all of the hybrids. Insecticide applied in-furrow had the greatest return on investment for the hybrids without Bt traits.

Table 14. Estimated return on investment for hybrids with and without Bt traits in the presence and absence of soil insecticide based on hybrid yields, Arthur, ND (2013).

Hybrid	Yield	$157/ metric ton	Control cost ha^{-1}	Net return	Net return vers check
	Without insecticide				
P9697R (No Cry proteins)	4.9	772	298	474	
P9675R (No Cry proteins + Poncho 1250)	5.7	898	335	563	+89
DKC 43-27 (Cry3Bb1)	8.0	1260	386	874	+400
P9675AMRW (Cry34/35Ab1)	8.4	1323	389	934	+460
DKC 44-13 (Cry3Bb1 + Cry34/35Ab1)	8.5	1339	399	940	+466
	With insecticide				
P9697R (No Cry proteins)	6.6	1039	316	723	+249
P9675R (No Cry proteins + Poncho 1250)	7.8	1228	353	875	+401
DKC 43-27 (Cry3Bb1)	8.6	1354	404	950	+476
P9675AMRW (Cry34/35Ab1)	9.2	1449	407	1042	+568
DKC 44-13 (Cry3Bb1 + Cry34/35Ab1)	9.0	1417	417	1000	+526

When CRW infestations were low, the advantage to Bt traits in hybrids was lost (Table 15). Based on the yields from the Arthur 2014 location, there is a net loss to planting hybrids with Bt traits. The advantage to using insecticide in-furrow was much more variable in 2014. Not all of the hybrids had a positive yield response to applying insecticide in-furrow like they had in 2013.

These results support that producers need to be aware of the risk of having high populations of CRW in their fields. Planting hybrids with Bt traits offered a large economic gain when heavy amounts of root pruning took place. When populations were lower and root pruning was minimal, the yield advantage of hybrids with Bt traits was reduced and became an economic loss for producers.

Table 15. Estimated return on investment for hybrids with and without Bt traits in the presence and absence of soil insecticide based on hybrid yields, Arthur, ND (2014).

Hybrid	Yield	$157/ metric ton	Control cost ha^{-1}	Net return	Net return vers check
	Without insecticide				
P8640R (No Cry proteins)	8.8	1386	298	1088	
P9526AM (No Cry proteins + Poncho 1250)	7.4	1165	335	830	-258
DKC 43-48 (Cry3Bb1)	7.3	1150	386	764	-324
P9526AMX (Cry34/35Ab1)	7.9	1244	389	855	-233
DKC 44-13 (Cry3Bb1 + Cry34/35Ab1)	8.3	1307	399	908	-180
	With insecticide				
P8640R (No Cry proteins)	8.3	1307	316	991	-97
P9526AM (No Cry proteins + Poncho 1250)	8.1	1276	353	923	-165
DKC 43-48 (Cry3Bb1)	8.6	1354	404	950	-138
P9526AMX (Cry34/35Ab1)	7.9	1244	407	837	-251
DKC 44-13 (Cry3Bb1 + Cry34/35Ab1)	8.2	1291	417	874	-214

CONCLUSIONS

Corn rootworm pressure varied at the different locations during the two years this study took place. Despite having varying amounts of pressure, we could conclude that all the Bt traits currently available still have efficacy against CRW. We observed less root pruning injury in the hybrids with Bt traits verses the hybrids with no Bt traits and those with no Bt traits treated with insecticide on seed. Soil-applied insecticide did not always reduce the amount of root pruning injury that the hybrids had, even under high levels of CRW pressure. There was also no clear advantage to planting Bt-traited hybrids in addition to applying insecticide in-furrow.

Yield or test weight was not consistently increased with the use of Bt-traited hybrids. When heavy CRW infestation is observed, hybrids with Bt traits had a yield advantage over those hybrids without Bt traits. Results from the Arthur 2013 location support that hybrids with Bt traits will have higher yields than non-Bt hybrids when the target insect is present, and insect populations are above economic threshold levels. When CRW populations were low or nonexistent, the yield advantage was not observed. This is the result of the variability in the individual hybrids yield potential and response to environmental conditions when the target pest is not present.

Efficacy of current Bt traits tested were still effective against CRWs; however, it is important to use this tool with other pest management strategies. Producers should scout fields for CRW and plan accordingly the next production season. Our results show that planting hybrids with Bt traits does not always offer a yield or test weight advantage over hybrids without Bt traits when CRW populations are low or nonexistent. The cost per hectare to plant hybrids with Bt traits is much higher than planting hybrids without Bt traits and using seed or soil-applied insecticide if the protection is not necessary in the field.

Scouting fields, in addition to crop rotation, controlling volunteer corn in fields, and rotating modes of action of Bt traits and insecticide to manage CRW creates a more solid IPM strategy that will help delay CRW resistance to the Bt trait.

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APPENDIX

Table A1. Expected mean squares for the ANOVA for a single location.

SOV	df	Observed	Expected	F-ratio
Rep	3	M_1	$\sigma^2 + h\sigma^2_\gamma + ih\sigma^2_R$	M_1/M_5
Insecticide	1	M_2	$\sigma^2 + h\sigma^2_\gamma + r\sigma^2_{IH} + rh\sigma^2_I$	M_2/M_3
Error (a)	3	M_3	$\sigma^2 + h\sigma^2_\gamma$	
Hybrid	4	M_4	$\sigma^2 + r\sigma^2_{IH} + ri\sigma^2_H$	M_4/M_6
Insecticide X Hybrid	12	M_5	$\sigma^2 + r\sigma^2_{IH}$	M_5/M_6
Residual error	16	M_6	σ^2	
Total	39			

Table A2. Expected mean squares for the ANOVA for combined locations.

SOV	df	Observed	Expected	F-ratio
Location	2	M_1		
Rep(Location)	9	M_2		
Insecticide	1	M_3	$\sigma^2 + h\sigma^2_\gamma + hr\sigma^2_{IL} + hlr\sigma^2_I$	M_3/M_4
Insecticide X Location	2	M_4	$\sigma^2 + h\sigma^2_\gamma + hr\sigma^2_{IL}$	M_4/ M_5
Error (a)	9	M_5	$\sigma^2 + h\sigma^2_\gamma$	M_5/ M_{11}
Hybrid	4	M_6	$\sigma^2 + i\sigma^2_\gamma + ir\sigma^2_{HL}$	M_6/ M_7
Hybrid X Location	8	M_7	$\sigma^2 + i\sigma^2_\gamma$	M_7/ M_{11}
Insecticide X Hybrid	4	M_9	$\sigma^2 + r\sigma^2_{IHL} + lr\sigma^2_{IH}$	M_9/ M_{10}
Insecticide X Hybrid X Location	8	M_{10}	$\sigma^2 + r\sigma^2_{IHL}$	M_{10}/ M_{11}
Residual error	72	M_{11}	σ^2	
Total	119			

Table A3. ANOVA for average yield and test weight, Wyndmere, ND, 2013.

		Yield		Test Weight	
SOV	df	MS	F Value	MS	F Value
Rep	3	4092794.34	1.69	3.92	1.66
A [Insecticide]	1	759270.77	0.42	1.04	0.14
Error a	3	1786684.22	0.74	7.62	3.23
B [Hybrid]	4	1227783.24	0.51	3.32	1.41
A x B	4	3377708.59	1.39	3.02	1.28
Error	24	2427643.58		2.36	
Total	39				

Table A4. ANOVA for average yield and test weight, Lidgerwood, ND, 2013.

		Yield		Test Weight	
SOV	df	MS	F Value	MS	F Value
Rep	3	7483131.88	4.67	10.17	1.93
A [Insecticide]	1	1141753.54	0.30	3.74	1.43
Error a	3	3869496.81	2.42	2.61	0.49
B [Hybrid]	4	1411525.99	0.88	3.34	0.63
A x B	4	3688335.42	2.30	1.81	0.34
Error	23	1601445.25		5.28	
Total	38				

Table A5. ANOVA for average root pruning injury, Hope, ND, 2014.

SOV	df	MS	F Value
Rep	3	0.0019787	1.32
A [Insecticide]	1	0.0000004	0.00
Error a	3	0.00175907	1.18
B [Hybrid]	4	0.00621440	4.16
A x B	4	0.00126778	0.85
Error	24	0.00149419	
Total	39		

Table A6. ANOVA for average root pruning injury, Arthur, ND, 2014.

SOV	df	MS	F Value
Rep	3	0.00435156	1.81
A [Insecticide]	1	0.00069722	0.24
Error a	3	0.00291149	1.21
B [Hybrid]	4	0.02396523	9.95
A x B	4	0.00173285	0.72
Error	24	0.00240784	
Total	39		

Table A7. ANOVA for average yield and test weight, Page, ND, 2014.

		Yield		Test Weight	
SOV	df	MS	F Value	MS	F Value
Rep	3	10439502.20	6.23	11.19	7.51
A [Insecticide]	1	189817.69	0.16	0.16	0.74
Error a	3	1169594.43	0.70	0.21	0.14
B [Hybrid]	4	370154.89	0.22	17.49	11.74
A x B	4	917736.49	0.55	0.37	0.25
Error	24	1674432.80		1.49	
Total	39				

Table A8. ANOVA for average yield, Hope, ND, 2014.

SOV	df	MS	F Value
Rep	3	8050790.82	14.47
A [Insecticide]	1	2920834.99	5.05
Error a	3	578524.15	1.04
B [Hybrid]	4	4760174.16	8.55
A x B	4	542458.66	0.97
Error	23	556545.92	
Total	38		

Table A9. ANOVA for average yield, Arthur, ND, 2014.

SOV	df	MS	F Value
Rep	3	7767690.67	1.03
A [Insecticide]	1	589205.95	0.40
Error a	3	1479408.12	1.96
B [Hybrid]	4	728548.78	0.97
A x B	4	1020607.57	1.35
Error	21	754223.57	
Total	36		

Table A10. ANOVA for average yield, root pruning injury, and test weight, Arthur, ND, 2013.

		Yield		Root Pruning Injury		Test Weight	
SOV	df	MS	F Value	MS	F Value	MS	F Value
Rep	3	1724897.84	0.90	0.05	0.85	5.29	2.39
A [Insecticide]	1	12751863.55	103.60	0.02	0.28	46.87	10.82
Error a	3	123084.30	0.06	0.06	1.06	4.33	1.96
B [Hybrid]	4	14838995.20	7.74	1.09	20.39	4.30	1.95
A x B	4	1147754.08	0.60	0.13	2.50	0.67	0.30
Error	24	1916334.40		0.05		2.21	
Total	39						

Table A11. ANOVA for average test weight, Arthur, ND, 2014.

SOV	df	MS	F Value
Rep	3	0.81	0.13
A [Insecticide]	1	6.01	0.99
Error a	3	6.08	1.00
B [Hybrid]	4	1.69	0.28
A x B	4	6.93	1.14
Error	24	6.09	
Total	39		

Table A12. ANOVA for average test weight, Hope, ND, 2014.

SOV	df	MS	F Value
Rep	3	4.49	1.32
A [Insecticide]	1	0.17	0.13
Error a	3	1.37	0.41
B [Hybrid]	4	2.71	0.80
A x B	4	5.37	1.58
Error	24	3.40	
Total	39		

Table A13. ANOVA for average yield and test weight of combined locations, Arthur, Wyndmere, and Lidgerwood, ND, 2013.

		Yield		Test Weight	
SOV	df	MS	F Value	MS	F Value
Location	2	87982733.7	43.04	54.38	1.41
Rep (Location)	9	5745106.2	2.81	25.82	0.67
Insecticide	1	4955361.0	2.44	66.73	1.66
Insecticide x Location	2	4449983.7	2.18	25.22	0.65
Error (a)	9	2030575.6	0.99	40.32	1.05
Hybrid	4	7628941.5	3.73	13.95	0.36
Hybrid x Location	8	4797444.8	2.35	42.42	1.18
Insecticide x Hybrid	4	2076795.2	1.02	36.33	0.94
Insecticide x Hybrid x Location	8	3697111.3	1.81	42.14	1.09
Residual error	72	2044197.5		38.55	
Total	119				

Table A14. ANOVA for average yield of combined locations, Arthur, Hope, and Page, ND, 2014.

		Yield	
SOV	df	MS	F Value
Location	2	5640934.0	5.57
Rep (Location)	9	6422354.2	6.35
Insecticide	1	568782.2	0.53
Insecticide x Location	2	1502288.3	1.48
Error (a)	9	1075842.2	1.06
Hybrid	4	1053015.4	1.04
Hybrid x Location	8	2384751.4	2.36
Insecticide x Hybrid	4	755442.9	0.75
Insecticide x Hybrid x Location	8	850683.5	0.84
Residual error	68	1012141.7	
Total	115		

Table A15. ANOVA for average test weight of combined locations, Arthur, Hope, and Page, ND, 2014.

		Test Weight	
SOV	df	MS	F Value
Location	2	259.7	70.95
Rep (Location)	9	5.5	1.50
Insecticide	1	2.0	0.77
Insecticide x Location	2	2.2	0.60
Error (a)	9	2.6	0.70
Hybrid	4	9.2	2.51
Hybrid x Location	8	6.4	1.74
Insecticide x Hybrid	4	4.9	1.33
Insecticide x Hybrid x Location	8	3.9	1.07
Residual error	72	3.7	
Total	119		

Table A16. ANOVA for average root pruning injury and test weight of combined locations Arthur, ND, 2013 and Arthur and Hope, ND, 2014.

		Root Pruning Injury		Test Weight	
SOV	df	MS	F Value	MS	F Value
Location	2	2.24	117.24	173.51	44.50
Rep (Location)	9	0.02	0.91	3.53	0.91
Insecticide	1	0.003	0.16	7.71	1.96
Insecticide x Location	2	0.007	0.35	22.67	5.81
Error (a)	9	0.02	1.07	3.93	1.01
Hybrid	4	0.53	27.76	0.90	0.23
Hybrid x Location	8	0.29	15.41	3.89	1.00
Insecticide x Hybrid	4	0.04	2.16	4.46	1.14
Insecticide x Hybrid x Location	8	0.05	2.49	4.26	1.09
Residual error	72	0.02		3.90	
Total	119				

Table A17. ANOVA for average yield of combined locations, Arthur, ND, 2013 and Arthur and Hope, ND, 2014.

		Yield	
SOV	df	MS	F Value
Location	2	13143556.90	11.98
Rep (Location)	9	3517486.11	3.20
Insecticide	1	2198403.81	3.02
Insecticide x Location	2	6924577.63	6.31
Error (a)	9	727005.52	0.66
Hybrid	4	5003960.86	4.56
Hybrid x Location	8	7495050.88	6.83
Insecticide x Hybrid	4	1217905.18	1.11
Insecticide x Hybrid x Location	8	687524.56	0.63
Residual error	68	1097518.8	
Total	115		