GEOGRAPHIC DISTRIBUTION AND EFFECTS OF TRANSGENIC CORN HYBRIDS AND CHEMICAL INSECTICIDES ON NORTHERN AND WESTERN CORN ROOTWORMS (COLEOPTERA: CHRYSOMELIDAE) IN NORTH DAKOTA

A Dissertation
Submitted to the Graduate Faculty
of the
North Dakota State University
of Agriculture and Applied Science

By

Veronica Calles Torrez

In Partial Fulfillment of the Requirements
for the Degree of
DOCTOR OF PHILOSOPHY

Major Department:
Entomology

April 2018

Fargo, North Dakota

North Dakota State University
Graduate School

Title

GEOGRAPHIC DISTRIBUTION AND EFFECTS OF TRANSGENIC CORN HYBRIDS AND CHEMICAL INSECTICIDES ON NORTHERN AND WESTERN CORN ROOTWORMS (COLEOPTERA: CHRYSOMELIDAE) IN NORTH DAKOTA

By

Veronica Calles Torrez

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

DOCTOR OF PHILOSOPHY

SUPERVISORY COMMITTEE:

Dr. Janet J. Knodel
Co-Chair

Dr. Mark A. Boetel
Co-Chair

Dr. B. Wade French

Dr. Billy W. Fuller

Dr. Joel K. Ransom

Approved:

April 13, 2018
Date

Frank Casey
Department Chair

ABSTRACT

Northern (*Diabrotica barberi* Smith & Lawrence) and western (*D. virgifera virgifera* LeConte) corn rootworms are major corn (*Zea mays* L.) pests in North America. Integrated pest management (IPM) strategies used for corn rootworm control include crop rotation, chemical insecticides, and corn hybrids expressing *Bacillus thuringiensis* (Bt) toxins. Field and laboratory experiments were conducted between July 2013 and October 2017 to address the following objectives: 1) determine the geographic distribution and densities of *D. barberi* and *D. v. virgifera* in North Dakota (ND) and compare the effectiveness of unbaited green Scentry^{TM} Multigard and yellow Pherocon® AM/NB sticky traps for monitoring both species; 2) access the impacts of corn hybrids expressing the Cry3Bb1, Cry34/35Ab1, and Cry3Bb1 + Cry34/35Ab1 proteins, tefluthrin soil insecticide, and clothianidin insecticidal seed treatment on beetle emergence, larval feeding injury, and corn grain yield; and 3) evaluate if either species has developed resistance to these Bt-proteins. Both species were commonly distributed in east-central and southeastern ND, and capture rates ranged from 0 to >10 beetles per trap per week. Green Scentry^{TM} sticky traps captured greater mean numbers of *D. barberi* beetles in 68% of fields containing this species, while the yellow Pherocon® AM/NB trap captured higher mean numbers of *D. v. virgifera* in 57% of fields containing this species, but their relative trap efficiency was inconclusive. Overall beetle emergence was significantly lower in Bt-protected than in non-Bt corn. Bt hybrids produced higher grain yield and sustained consistently lower levels of larval root-feeding injury than non-Bt in fields with higher rootworm densities. Tefluthrin and clothianidin did not significantly impact beetle emergence or larval root injury. Results also documented the first confirmed field-evolved incomplete resistance in *D. barberi* populations to Cry3Bb1 and Cry34/35Ab1 proteins. The Ransom population of *D. v. virgifera* was confirmed as resistant to the Cry3Bb1 toxin. These findings indicate reduced efficacy of corn rootworm control using tefluthrin, clothianidin, and Bt corn. Continuously scouting for rootworms using sticky traps or other methods prior to making rootworm management decisions, and the use of an IPM approach to their control is needed to mitigate the development of Bt-resistance rootworms in ND.

ACKNOWLEDGEMENTS

My most sincere appreciation is extended to Dr. Janet J. Knodel, my advisor, for being my mentor, for giving me the opportunity to be her student, for trusting in my abilities, for encouraging me when I felt discouraged, for watching over me, for teaching me techniques to develop skills in science, and for all her guidance in this step of my professional career. I would also like to acknowledge Dr. Mark A. Boetel, my co-advisor, for helping and teaching me tools and strategies for being a better writer. In addition, I thank him for his dedication and patience for editing, commenting, and providing suggestions for overall improvement of this dissertation. Likewise, I would like to acknowledge Dr. B. Wade French, Dr. Billy W. Fuller, and Dr. Joel K. Ransom for being my committee members and for all their contributions to the dissertation.

Special thanks are also due to Dr. B. Wade French and Chad Nielson from the U.S. Department of Agriculture, Agriculture Research Service, North Central Agricultural Research Laboratory (Brookings, SD) for training me on how to handle rootworms and for providing this project with Bt-susceptible corn rootworm eggs. I also thank DuPont Pioneer and Monsanto Companies for providing corn seed used in this research. I immensely appreciate everyone (names are provided in the acknowledgments section of chapters two, three, and four) who helped me with field, greenhouse, or laboratory work.

I also thank all my friends, families (from Bolivia and U.S.), and my husband, Alexander H. Knudson, for sharing all types of memories with me, but especially for their support when the sun was not out (during the difficult times). Love you all!!

My grateful appreciation is also expressed to the faculty of the Department of Entomology for sharing their knowledge inside and outside of the classroom. I thank the Graduate Center for Writers for providing one-on-one consultation in order to improve my writing skills during my tenure at NDSU. I also thank NDSU for supporting graduate students by means of providing scholarships, workshops, and trainings, and the NDSU Agricultural Experiment Station Research Greenhouse Complex for letting us conduct research in their facilities.

This project was mainly funded by the North Dakota Corn Utilization Council, and also partially funded by the National Institute of Food and Agriculture, U.S. Department of Agriculture, Hatch project accession no. 0223202.

DEDICATION

This dissertation is dedicated to my family who lives in Bolivia. My father Francisco Calles de Torrez and my late mother Exalta Torrez de Calles, and to my siblings: Tomas, Arminda, Wilfredo, Jeivi, and Zulma. It is also dedicated to all my nephews and nieces in hope that each one of them can have an opportunity to go to college and pursue a degree.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I. LITERATURE REVIEW

Many North American corn growers annually face major production problems associated with corn rootworms (Coleoptera: Chrysomelidae). Corn, *Zea mays* L. (Poales: Poaceae), is an annual grass that originated in Mexico in North America, and may have been domesticated more than 5,000 years ago (Mangelsdorf et al. 1964, Piperno and Flannery 2001). Corn is the most widely grown crop, and probably one of the three most important cereal crops in the world (Mejía 2003, Awika 2011). The United States produces more than one-third of all the corn grown in the world, which is the largest production and exportation of any country [National Corn Growers Association (NCGA) 2017, United States Department of Agriculture-Foreign Agricultural Service (USDA-FAS) 2018]. Corn is used in human consumption, forage and silage for livestock feed, and ethanol fuel production. In North Dakota, the average annual corn cultivation has been about 12.9 million ha from 2013 to 2016, and was valued at greater than US$1.5 billion in 2016 (NCGA 2017). The increased market value of corn in some years, as well as the marketplace demands for corn as a feedstock and biofuel source, are partially responsible for increased production in the state. As a result, many producers have shifted from corn-soybean rotations to continuous corn production, which can promote damaging infestations of northern corn rootworm (*Diabrotica barberi* Smith & Lawrence) and western corn rootworm (*D. virgifera virgifera* LeConte). The estimated cost due to crop losses and control practices (i.e., insecticide) for corn rootworms was estimated to be more than US$1 billion annually in the United States (Metcalf 1986).

Diabrotica barberi* and *D. v. virgifera

Distribution

The greatest diversity of *Diabrotica* Chevrolat species occurs in tropical areas, and the greatest proportion of *Diabrotica* pest species occur north of Mexico (Krysan 1986). In the United States, the following *Diabrotica* species occur: *D. balteata* LeConte, *D. barberi*, *D. cristata* Harris, *D. lemniscata* LeConte, *D. longicornis* (Say), *D. tibialis* Jacoby, *D. undecimpunctata howardi* Barber, *D. u. tenella* LeConte, *D. u. undecimpunctata* Mannerheim, *D. v. virgifera*, and *D. virgifera zeae* Krysan & Smith (Riley et al. 2003). Northern and western corn rootworms are the most serious insect pests of corn in the north central region of the United States and Canada (Levine and Oloumi-Sadeghi 1991). The northern corn rootworm is restricted to the Nearctic region, including the United States and Canada (Krysan 1986), and occurs primarily in the north central region of the U.S. (Chiang 1973). Whereas, *D. v. virgifera* occurs from Panama in Central America to the southern rim of Canadian provinces in North America, (Krysan 1986). The highest densities of *D. v. virgifera* occur mostly in Iowa, Illinois, Indiana, Missouri, and Ohio (Edwards and Kiss 2012). In 1992, *D. v. virgifera* was first detected in a corn field near the Belgrade Airport in Europe (Baca 1994). Since then, infestations of *D. v. virgifera* population have been reported as increasing in countries of Europe (Gray et al. 2009, Rak Cizej and Škerbot 2015).

Identification

Female and male *D. barberi* beetles are tan to lime green in color, with a body length of 4.8 to 5.6 mm and width 2.0 to 2.5 mm (Krysan and Smith 1987, Derunkov et al. 2015). The teneral specimen is usually pale green or light brown in color, and eventually turns green with age. Female and male *D. v. virgifera* beetles are yellow to green and black; their body lengths vary from 4.4 to 6.6 mm in males and from 4.2 to 6.8 mm in females. Male *D. v. virgifera* wings are usually heavily black with yellow pigmentation at the apex and outer margin. In contrast, most *D. v. virgifera* female wings have three distinctly black stripes on the dorsal view (Krysan and Smith 1987, Kuhar and Youngman 1995). Females of both species have larger abdomens than males. The apex of the abdomen is pointed in females versus rounded with an extra sclerite at the posterior tip of the abdomen in males. The second segment of the antenna in females is longer than the third segment, but these segments are the same length in males (Krysan 1986). Also, males have a hairless planar ovoid patch in the ventral surfaces of the first tarsomere (i.e., basitarsus) of the prothoracic and mesothoracic legs (Hammack and French 2007). Corn rootworm eggs are oval and white to cream in color, and about 0.3 by 0.5 mm long. The larva, with a brown head capsule and brown anal plate, is slender and white- to cream-colored. The full-grown third-instar larva can measure 13 mm in length. Pupae are translucent white to cream in color, and somewhat resemble adults.

Biology and Life Cycle

The biology and life cycles of *D. barberi* and *D. v. virgifera* are very similar. Both species have one generation per year. Female beetles deposit their eggs within the top 15.24 cm of the soil profile in corn fields from mid-summer until fall (Ball 1957, Patel and Apple 1967). The optimal temperature for oviposition is 15.6-18.3°C during evenings (Ball 1957, Chiang 1973). Both pests overwinter in the egg stage in an obligate diapause. A period of cold temperature is required to induce and break egg, and larvae subsequently hatch from the eggs during the following spring. Egg hatch occurs from late-May to early June in the Midwest corn producing area; however, timing varies according to latitude and soil temperatures. Larvae go through three instar stages prior to pupation. Pupation occurs in the soil in earthen cells (Steffey et al. 1999), and beetle emergence from the puparium typically begins in early to mid-July, but this also varies according to environmental (Levine and Oloumi-Sadeghi 1991, Levine et al. 2002).

Feeding Behavior and Corn Damage

Corn rootworm feeding behavior varies with larval instars. First-instar larvae mainly feed on seminal roots (i.e., initial roots that emerge from seeds) or at root tips where cell formation occurs, thus causing root pruning. Second instars feed on developing root tissue and lateral roots, as well as tunneling into the cortex of the roots. Third instars continue feeding on roots, and often burrow into the cortical parenchyma cells, making a channel inside the roots. Larval tunneling scars the root cortex (Apple and Patel 1963, Chiang 1973, Riedell and Kim 1990, Steffey et al. 1999, Clark et al. 2006). Clark et al. (2006) observed severe feeding injury on roots by day 12 after larval feeding began. A key above-ground corn rootworm symptom of larval feeding injury includes plant lodging, or ‘goosenecking’, which results from a loss of major nodal roots and brace roots (Steffey et al. 1999, Levine et al. 2002, Obopile et al. 2013). Since the root system can be severely injured by larval feeding activity, plants can also display drought-like symptoms due to a reduction in capacity to assimilate water and nutrients (Kahler et al. 1985, Riedell 1990, Levine et al. 2002, Clark et al. 2006). Therefore, corn rootworm larval root-feeding injury can cause substantial yield losses (Branson et al. 1980, Spike and Tollefson 1991).

Corn rootworm beetles feed on leaves, pollen, silks, and immature kernels of the corn plant, although *D. barberi* beetles can be found feeding on the pollen of several plant species (Ludwig and Hill 1975). As a consequence of silk-feeding injury, corn rootworm beetles can also reduce corn pollination, which can contribute to yield loss.

Management Strategies and Corn Rootworm Adaptation

Crop rotation, foliar insecticides, and soil insecticides have been used effectively to manage *D. barberi* and *D. v. virgifera* for over multiple decades. From 1910 through the mid-1990s, a corn-soybean or non-host crop rotation was an effective strategy to control *D. v. virgifera* (Levine et al. 2002). However, in the late 1990s, *D. v. virgifera* exhibited a behavioral shift in that females laid eggs in soybean fields instead of corn in the eastern Corn Belt of the Midwestern United States (Levine and Oloumi-Sadeghi 1991, Spencer et al. 1998, Levine et al. 2002, Pierce 2003). Furthermore, *D. barberi* has adapted to rotation through prolonged egg diapause, in which eggs remain in diapause and hatch after two or more winters (Chiang 1965, Levine et al. 1992, Geisert and Meinke 2013, French et al. 2014). As such, corn growers that used a corn-soybean-corn rotation could find crop damage when corn was planted in the third year.

In addition to *D. barberi* or *D. v. virgifera* developing behavioral and physiological adaptations to crop rotation, these pests have also developed resistance to several insecticides (e.g., methyl parathion, dimethoate, and carbaryl) during the past several decades (Meinke et al. 1998, Scharf et al. 1999, Wright et al. 2000, Levine et al. 2002). However, researchers also have developed new strategies to control these pests, such as corn hybrids expressing *Bacillus thuringiensis* Berliner (Bt) (Bacillales: Bacillaceae) insecticidal Cry proteins. Bt is a soil bacterium that occurs naturally on dead or decaying soil organic matter (Roh et al. 2007). It was first described by Berliner (1911); however, the first isolation of a Bt strain was from a diseased silkworm larva in 1901 by Ishiwata Shigetane (Roh et al. 2007). Currently, Bt has widespread use as a biopesticide in agriculture [National Academies of Sciences (NAS) 2016]. The Bt var. *tenebrionis* produces Cry proteins that are effective for killing some beetle pest species. Effective for the 2003 growing season, Monsanto Company received federal registration for grower use of the first corn rootworm-resistant corn trait that expressed the Cry3Bb1 Bt toxin. This toxin is considered “not high-dose”, as it only provides moderate toxicity against corn rootworms [U.S. Environmental Protection Agency (USEPA) 2003].

In recent years, producers have increased continuous corn production (i.e., consecutive annual planting of corn in the same fields), which has led to increased problems with corn rootworms. To combat this situation, producers were quick to adopt the use of Bt corn hybrids for *D. barberi* and *D. v. virgifera* control. Corn rootworm-protected Bt corn hybrids are genetically modified to express single or multiple Cry proteins that function as toxins to protect plants from these pests. Hybrids with corn rootworm-active Cry proteins are marketed under several trade names, including AcreMax, Agrisure, Herculex, Intrasect, YieldGard, and SmartStax Refuge Advanced (DiFonzo et al. 2018), and they are commonly used by North Dakota producers. The Bt corn hybrid expressing the Cry3Bb1 toxin has been widely used for well over a decade in many corn-growing areas throughout the United States. Planting Bt corn hybrids for rootworm control also requires a refuge (i.e., non-Bt corn) planting in a structured block or strip within or near the Bt corn field or a seed blend, in which refuge seeds are blended with the Bt corn seeds in the bag. Use of this “refuge in the bag” approach does not require planting a separate structured refuge for appropriately labeled and packaged seed (DiFonzo et al. 2018).

In addition to these pests having adapted to cultural and chemical control tools, they also have recently become resistant to Bt corn management strategies, resulting in several cases of poor control. In 2009, just six years after Bt corn became commercially available to growers for corn rootworm management, the first cases of Cry3Bb1-resistant *D. v. virgifera* field populations were detected in Iowa (Gassmann et al. 2011). The initial detections in Iowa involved *D. v. virgifera* resistance to Cry3Bb1-expressing corn; however, the phenomenon expanded to include cross-resistance in this species to Bt corn expressing the mCry3A protein, which was observed in 2011 (Gassmann et al. 2014). Western corn rootworm resistance to these or other Bt Cry toxins is now also confirmed or suspected within other U.S. states (Gassmann et al. 2012, 2016, Gassmann 2012, Gray 2012, Porter et al. 2012, Wangila et al. 2015, Zukoff et al. 2016, Schrader et al. 2017, Ludwick et al. 2017).

Corn Rootworm Fitness

It is conceivable that insecticidal proteins expressed by different Bt corn hybrids could induce fitness cost effects on *Diabrotica* species. Head capsule width has been used to partially characterize the fitness of corn rootworm larvae (Branson and Sutter 1985, Ellsbury et al. 2005), because head capsule size remains stable during the insect's development (Li et al. 2009). Branson and Sutter (1985) and Branson et al. (1988) determined that laboratory-reared and field-collected *D. v. virgifera* beetles have a mean head capsule width of 1.2 mm. However, head capsule width in *D. v. virgifera* varies and is affected by several factors, such as diet (Ellsbury et al. 2005), environmental conditions (Woodson and Jackson 1996), population density (Branson and Sutter 1985), host plant species (Clark and Hibbard 2004, Gacii Chege et al. 2005). Additionally, host plant infection with a plant-pathogenic fungus can also impact fitness. For example, head capsule width of *D. v. virgifera* larvae was shown to be reduced by feeding on corn infected with *Fusarium verticillioides* Sheldon (Kurtz et al. 2010). Smaller head capsule width was also reported in male and female *D. v. virgifera* emerging from corn hybrids expressing the Cry3Bb1 toxin when compared to individuals that emerged from non-Bt refuge corn plots (Murphy et al. 2011). Use of transgenic corn technology has benefited corn producers by increasing corn yields (Haegele and Below 2013), as well as reducing insecticide and herbicide costs and pesticide loads on the environment (NAS 2016); however, the safety of Bt corn for the environment and non-target organisms is largely unknown (Makoni and Mohamed-Katerere 2013). A better understanding of all implications of this exiting and economically beneficial technology will be essential to maintaining and sustaining its use for the future.

References Cited

Apple, J. W., and K. K. Patel. 1963. Sequence of attack by northern corn rootworms on the crown roots of corn. Proc. N. Cent. Br. Entomol. Soc. Am. 18: 80–81.

Awika, J. M. 2011. Major cereal grains production and use around the world, pp. 1-13. *In*: J. M. Awika, V. Piironen, and S. Bean (eds), Advances in cereal science: implications to food processing and health promotion. Am. Chem. Soc. Washington, DC.

Baca, F. 1994. New member of the harmful entomofauna of Yugoslavia *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae). Zast. Bilja 45: 125–31.

Ball, H. J. 1957. On the biology and egg-laying habits of the western corn rootworm. J. Econ. Entomol. 50: 126–128.

Berliner, E. 1911. Über de schlaffsucht der mehlmottenraupe. Zeitschrift f*ü*r das Gesamstadt 252: 3160–3162.

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.

Branson, T. F., G. R. Sutter, and J. R. Fisher. 1980. Plant response to stress induced by artificial infestations of western corn rootworm. Environ. Entomol. 9: 253–257.

Branson, T. F., J. J. Jackson, and G. R. Sutter.1988. Improved method for rearing *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae). J. Econ. Entomol. 81: 410–414.

Chiang, H. C. 1965. Survival of northern corn rootworm eggs through one and two winters. J. Econ. Entomol. 58: 470–472.

Chiang, H. C. 1973. Bionomics of the northern and western corn rootworms. Annu. Rev. Entomol. 18: 47–72.

Clark, P. L., T. T. Vaughn, L. J. Meinke, and J. Molina-Ochoa, and J. E. Foster. 2006. *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae) larval feeding behavior on transgenic maize (MON 863) and its isoline. J. Econ. Entomol. 99: 722–727.

Clark, T. L., and B. E. Hibbard. 2004. Comparison of nonmaize hosts to support western corn rootworm (Coleoptera: Chrysomelidae) larval biology. Environ. Entomol. 33: 681–689.

Derunkov, A., A. Konstantinov, and A. Tishechkin. 2015. *Diabrotica* ID: identification of *Diabrotica* species (Coleoptera: Chrysomelidae) from North and Central America; *D. barberi* Smith & Lawrence. (http://idtools.org/id/beetles/diabrotica/factsheet.php?name=6740) (accessed 18 February 2018).

DiFonzo, C., P. Porter, and K. Tilmon. 2018. The handy Bt trait table for U.S. corn production (updated January 2018). Michigan State University, East Lansing, Mich. Field Crops Entomol. (//www.texasinsects.org/bt-corn-trait-table.html) (accessed 20 Februry 2018).

Edwards C. R., and J. Kiss. 2012. *Diabrotica virgifera virgifera* LeConte in North America 2012. (https://extension.entm.purdue.edu/wcr/images/jpg/2012/NORTHAMERICA2012.jpg) (accessed 15 February 2018).

Ellsbury, M. M., K. R. Banken, S. A. Clay, and F. Forcella. 2005. Interactions among western corn rootworm (Coleoptera: Chrysomelidae), yellow foxtail, and corn. Environ. Entomol. 34: 627–634.

French, B. W., B. S. Coates, and T. W. Sappington. 2014. Inheritance of an extended diapause trait in the northern corn rootworm, *Diabrotica barberi* (Coleoptera: Chrysomelidae). J. Appl. Entomol. 138: 213–221.

Gacii Chege, P., T. L. Clark, and B. E. Hibbard. 2005. Alternate host phenology affects survivorship, growth, and development of western corn rootworm (Coleoptera: Chrysomelidae) larvae. Environ. Entomol. 34: 1441–1447.

Gassmann, A. J. 2012. Field-evolved resistance to Bt maize by western corn rootworm: predictions from the laboratory and effects in the field. J. Invertebr. Pathol. 110: 287–293.

Gassmann, A. J., J. L. Petzold-Maxwell, E. H. Clifton, M. W. Dunbar, A. M. Hoffmann, D. A. Ingber, and R. S. Keweshan. 2014. Field-evolved resistance by western corn rootworm to multiple *Bacillus thuringiensis* toxins in transgenic maize. Proc. Natl. Acad. Sci. 111: 5141–5146.

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.

Gassmann, A. J., J. L. Petzold-Maxwell, R. S. Keweshan, and M. W. Dunbar. 2012. Western corn rootworm and Bt maize: challenges of pest resistance in the field. GM Crops and Food 3: 235–244.

Gassmann, A. J., R. B. Shrestha, S. R. K. Jakka, M. W. Dunbar, E. H. Clifton, A. R. Paolino, D. A. Ingber, B. W. French, K. E. Masloski, J. W. Dounda, et al. 2016. Evidence of resistance to Cry34/35Ab1 corn by western corn rootworm (Coleoptera: Chrysomelidae):

root injury in the field and larval survival in plant-based bioassays. J. Econ. Entomol. 109: 1872–1880.

Geisert, R. W., and L. J. Meinke. 2013. Frequency and distribution of extended diapause in Nebraska populations of *Diabrotica barberi* (Coleoptera: Chrysomelidae). J. Econ. Entomol. 106: 1619–1627.

Gray, M. E. 2012. Continuing evolution confirmed of field resistance to Cry3Bb1 in some Illinois fields by western corn rootworm. The Bulletin: pest management and crop development information for Illinois. Univ. Ill. Ext., Urbana, IL.

Gray, M. E., T. W. Sappington, N. J. Miller, J. Moeser, and M. O. Bohn. 2009. Adaptation and invasiveness of western corn rootworm: intensifying research on a worsening pest. Annu. Rev. Entomol. 54: 303–21.

Haegele, J. W., and F. E. Below. 2013. Transgenic corn rootworm protection increases grain yield and nitrogen use of maize. Crop Sci. 53: 585–594.

Hammack, L., and B. W. French. 2007. Sexual bimorphism of basitarsi in pest species of *Diabrotica* and *Cerotoma* (Coleoptera: Chrysomelidae). Ann. Entomol. Soc. Am. 100: 59–63.

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. Agron. J. 77: 769–774.

Krysan, J. L. 1986. Introduction: biology, distribution, and identification of pest *Diabrotica*, pp. 1-23. *In*: J. L. Krysan and T. A. Miller (eds.), Methods for the study of pest *Diabrotica*. Springer-Verlag, NY.

Krysan, J. L., and R. F. Smith. 1987. Systematics of the *virgifera* species group of *Diabrotica* (Coleoptera: Chrysomelidae: Galerucinae). Entomography 5: 375–484.

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.

Kurtz, B., P. Karlovsky, and S. Vidal. 2010. Interaction between western corn rootworm (Coleoptera: Chrysomelidae) larvae and root-infecting *Fusarium verticillioides*. Environ. Entomol. 39: 1532–1538.

Levine, E., and H. Oloumi-Sadegh. 1991. Management of diabroticite rootworms in corn. Annu. Rev. Entomol. 36: 229–255.

Levine, E., H. Oloumi-Sadeghi, and J. R. Fisher. 1992. Discovery of multiyear diapause in Illinois and South Dakota northern corn rootworm (Coleoptera: Chrysomelidae) eggs and incidence of the prolonged diapause trait in Illinois. J. Econ. Entomol. 85: 262–267.

Levine, E., J. L. Spencer, S. A. Isard, D. W. Onstad, and M. E. Gray. 2002. Adaptation of the western corn rootworm to crop rotation: evolution of a new strain in response to a management practice. Am. Entomol. 48: 94–107.

Li, H., S. Toepfer, and U. Kuhlmann. 2009. Relationship between phenotypic traits and selected fitness components of *Diabrotica virgifera virgifera*. Entomol. Exp. Appl. 131: 254–263.

Ludwick, D. C., L. N. Meihls, K. R. Ostlie, B. D. Potter, L. French, and B. E. Hibbard. 2017. Minnesota field population of western corn rootworm (Coleoptera: Chrysomelidae) shows incomplete resistance to Cry34Ab1/Cry35Ab1 and Cry3Bb1. J. Appl. Entomol. 141: 28–40.

Ludwig, K. A., and R. E. Hill. 1975. Comparison of gut contents of adult western and northern corn rootworms in northeast Nebraska. Environ. Entomol. 4: 435–438.

Makoni, N., and J. Mohamed-Katerere. 2013. Genetically modified crops. Africa Environ. Outlook 2: 300–330.

Mangelsdorf, P. C., R. S. MacNeish, and W. C. Galinat. 1964. Domestication of Corn. Science 143: 538–545.

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

Mejía, D. 2003. Maize: post-harvest operation. Food and Agriculture Organization of the United Nations. (http://www.fao.org/in-action/inpho/crop-compendium/cereals-grains/en/) (accessed 23 February 2018).

Metcalf, R. L. 1986. Foreword, pp. vii–xi. *In* J. L. Krysan and T. A. Miller (eds.), Methods for the study of pest *Diabrotica.* Springer, New York.

Murphy, A. F., N. J. Seiter, and C. H. Krupke. 2011. The impact of Bt maize as a natal host on adult head capsule width in field populations of western corn rootworm. Entomol. Exp. Appl. 139: 8–16.

(NAS) National Academies of Sciences. 2016. Genetically engineered crops: experiences and prospects. The National Academies Press, Washington, DC.

(NCGA) National Corn Growers Association. 2017. World of corn. (http://www.worldofcorn.com/#/) (accessed 21 February 2018).

Obopile, M., R. B. Hammond, and P. R. Thomison. 2013. Interaction among planting dates, transgenic maize, seed treatment, corn rootworm damage and grain yield. J. Appl. Entomol. 137: 45–55.

Patel, K. K., and J. W. Apple. 1967. Ecological studies on the eggs of northern corn rootworm. J. Econ. Entomol. 60: 496–500.

Pierce, C. F. 2003. Case study of a variant of western corn rootworm, *Diabrotica virgifera virgifera* LeConte, in east-central Illinois. Ph.D. dissertation, University of Illinois, Urbana-Champaign.

Piperno, D. R., and K.V. Flannery. 2001. The earliest archaeological maize (*Zea mays* L.) from highland Mexico: new accelerator mass spectrometry dates and their implications. Proc. Natl. Acad. Sci. 98: 2101–2103.

Porter, P., E. Cullen, T. Sappington, A. Schaafsma, S. Pueppke, D. Andow, J. Bradshaw, L. Buschman, Y. Cardoza, C. DiFonzo, et al. 2012. Comment submitted by Patrick Porter, North Central Coordinating Committee NCCC46 (22 members). 5 March 2012. (http://www.regulations.gov/#!documentDetail; ID: EPA-HQ-OPP-2011-0922-0013) (accessed 19 February 2018).

Rak Cizej, M., and I. Škerbot. 2015. Monitoring of western corn rootworm (*Diabrotica virgifera virgifera* LeConte) on the area of the Celje region from 2004 to 2014. Novi izzivi v agronomiji 252–259.

Riedell, W. E., and A. Y. Kim. 1990. Anatomical characterization of western corn rootworm damage in adventitious roots of maize. J. Iowa Acad. Sci. 97: 15–17.

Riley, E. G., S. M. Clark, and T. N. Seeno. 2003. Catalog of the leaf beetles of America north of Mexico (Coleoptera: Megalopodidae, Orsodacnidae and Chrysomelidae, excluding Bruchinae). Coleopterists Society, Special Publication no. 1, pp. 78-79. Sacramento, CA.

Roh, J. Y., J. Y. Choi, M. S. Li, B. R. Jin, and Y. H. Je. 2007. *Bacillus thuringiensis* as a specific, safe, and effective tool for insect pest control. J. Microbiol. Biotechnol. 17: 547–59.

Scharf, M. E., L. J. Meinke, B. D. Siegfried, R. J. Wright, and L. D. Chandler. 1999. Carbaryl susceptibility, diagnostic concentration determination and synergism for U.S. populations of western corn rootworm. J. Econ. Entomol. 92: 33–39.

Schrader, P. M., R. E. Estes, N. A. Tinsley, A. J. Gassmann, and M. E. Gray. 2017. Evaluation of adult emergence and larval root injury for Cry3Bb1-resistant populations of the western corn rootworm. J. Appl. Entomol. 141: 41–52.

Spencer, J. L., S. A. Isard, and E. Levine. 1998. Western corn rootworms on the move: monitoring beetles in corn and soybeans, pp. 10–23. *In* 1998 Proc. Illinois Agric. Pestic. Conf., Coop. Ext. Serv., University of Illinois, Urbana-Champaign.

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: 1585–1591.

Steffey, K. L., M. E. Rice, J. All, D. A. Andow, M. E. Gray, and J. W. Van Duyn. 1999. Handbook of corn insects: pest information. Entomological Society of America, Lanham, MD.

(USEPA) U.S. Environmental Protection Agency. 2003. *Bacillus thuringiensis* Cry3Bb1 protein and the genetic material necessary for its production (vector ZMIR13L) in event MON863 corn (006484) fact sheet. (https://search.epa.gov/epasearch/epasearch?querytext=Bacillus+thuringiensis+Cry3Bb1+protein+and+the+genetic+material+necessary+for+its+production+%28vector+zmir13L%29+in+event+MON863+corn+%28006484%29+fact+sheet&areaname=&areacontacts=&areasearchurl=&typeofsearch=epa&result_template=2col.ftl) (accessed 24 February 2018).

(USDA–FAS) U. S. Department of Agriculture–Foreign Agricultural Service. 2018. Coarse grains: world markets and trade. (https://www.fas.usda.gov/data/grain-world-markets-and-trade) (accessed 23 February 2018).

Wangila, D. S., A. J. Gassmann, J. L. Petzold-Maxwell, B. W. French, and L. J. Meinke. 2015. Susceptibility of Nebraska western worn rootworm (Coleoptera: Chrysomelidae) populations to Bt corn events. J. Econ. Entomol. 108: 742–751.

Woodson, W. D., and J. J. Jackson. 1996. Developmental rate as a function of temperature in northern corn rootworm (Coleoptera: Chrysomelidae). Ann. Entomol. Soc. Am. 89: 226–230.

Wright, R. J., M. E. Scharf, L. J. Meinke, X. 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.

Zukoff, S. N., K. R. Ostlie, B. Potter, L. N. Meihls, A. L. Zukoff, L. French, M. R. Ellersieck, B. W. French, and B. E. Hibbard. 2016. Multiple assays indicate varying levels of cross resistance in Cry3Bb1-selected field populations of the western corn rootworm to mCry3A, eCry3.1Ab, and Cry34/35Ab1. J. Econ. Entomol. 109: 1387–1398.

CHAPTER II. CORN ROOTWORMS (COLEOPTERA: CHRYSOMELIDAE) IN NORTH DAKOTA: A STATE-WIDE TRAP SURVEY AND A COMPARISON OF TWO STICKY TRAPS FOR MONITORING BEETLE ACTIVITY

Abstract

Northern, *Diabrotica barberi* Smith & Lawrence, and western, *D. virgifera virgifera* LeConte, corn rootworms are major economic pests of corn, *Zea mays* L., in the U.S. This research was conducted to determine the geographic distribution, abundance, and species composition of *Diabrotica* species in North Dakota (ND), and to compare effectiveness of green Scentry^{TM} Multigard and yellow Pherocon® AM/NB sticky traps for monitoring these pests. During 2013 to 2015, 51 North Dakota corn fields were monitored for corn rootworm beetle activity between July and October by using the two sticky trap models. The overall species ratio of *Diabrotica* spp. captured was 61% *D. barberi* and 39% *D. v. virgifera*. Both species were most commonly distributed in southeastern ND. Corn fields with densities of >10 beetles per trap per week were only found in the southeastern portion of the state. Low densities (i.e., <0.1 beetles per trap per week) of *D. barberi* were present in areas further north, but no *D. v. virgifera* were captured in those fields. Most corn fields were below the economic threshold for trapping. Although comparisons of trap efficiency were inconclusive, green Scentry^{TM} Multigard traps captured numerically more *D. barberi* than yellow Pherocon® AM/NB traps in 68% of surveyed fields, while the Pherocon AM/NB traps captured numerically more *D. v. virgifera* beetles in 57% of the locations. Our findings indicate that corn rootworm populations are not at economically significant infestation levels throughout all corn-producing areas of ND. Therefore, regular scouting, mainly in southeastern and east-central counties of the state, will be necessary to assist producers with decisions for corn rootworm IPM.

Key Words: *Diabrotica barberi*, *Diabrotica virgifera virgifera*, sticky traps, adults, densities, *Zea mays*

Introduction

Northern (*Diabrotica barberi* Smith & Lawrence) and western corn rootworms (*D. virgifera virgifera* LeConte) (Coleoptera: Chrysomelidae) are major economic pests of corn (*Zea mays* L.) in the United States (U.S.). Infestations of *D. v. virgifera* have also been reported as increasing and spreading quickly throughout several European countries since its detection in 1992 (Baca 1994, Gray et al. 2009, Rak Cizej and Škerbot 2015). Increased infestation levels of *D. barberi* and associated larval root-feeding injury have been observed in North Dakota (ND) in recent years (Knodel 2012, Calles-Torrez et al. 2018).

The northern corn rootworm is restricted and adapted to the Nearctic Region (Krysan 1986). In the U.S., the distribution of this species ranges from eastern Colorado to northern Alabama, Georgia, and South Carolina, and extends northward into the provinces of Canadian New Brunswick, Ontario, and Quebec (Krysan and Smith 1987). Latitude distribution of *D. v. virgifera* extends from Panama in Central America to Canada in North America (Krysan 1986). Within the U.S., *D. v. virgifera* occurs from Arizona, Utah, and Idaho towards the eastern coast, including northern Alabama, Georgia, and South Carolina (Krysan and Smith 1987, Krysan 1986, Edwards and Kiss 2012).

The larval stages of both *D. barberi* and *D. v. virgifera* cause the most significant damage to corn by feeding on roots, which often includes tunneling into roots (Apple and Patel 1963, Chiang 1973, Riedell and Kim 1990, Steffey et al. 1999). Larval feeding injury to roots can interfere with corn plant water assimilation, nutrient uptake, and photosynthesis rates (Kahler et al. 1985, Riedell 1990, Hou et al. 1997, Godfrey et al. 1993, Urías-López et al. 2000, Levine et al. 2002). This injury is also negatively correlated with yield (Hills and Peters 1971), and can also adversely affect corn grain nutrient content (Kahler et al. 1985). In addition, extensive root injury can cause plant lodging, or also referred to as ‘goosenecking’ (Steffey et al. 1999, Obopile et al. 2013), which can complicate harvest operations and thus further reduce yield (Spike and Tollefson 1991). Adults feed on vegetative (i.e., leaves) and reproductive (i.e., silks, pollen, and immature kernels) parts of the plant (Ludwig and Hill 1975). Feeding injury to reproductive tissues can reduce kernel size and number of kernels per ear, thus contributing to yield reduction, and reduced seed quality (Culy et al. 1992). The economic yield losses and control costs associated with corn rootworm damage in the United States has been estimated at over US$1 billion annually (Metcalf 1986).

Several strategies are used by corn producers to manage corn rootworms in the U.S. Historically, these strategies have largely included soil-applied and foliar insecticides and rotation of corn with nonhost crops. The innovative technology of corn hybrids expressing *Bacillus thuringiensis* Berliner (Bt) insecticidal proteins has been widely adopted to manage these pests since 2003 (U.S. Department of Agriculture–Economic Research Service [USDA–ERS] 2016). However, some corn rootworm populations have developed resistance to crop rotation (Chiang 1965, Levine et al. 1992, French et al. 2014) and chemical insecticides in several states (Meinke et al. 1998, Wright et al. 2000). Field-evolved resistance of *D. v. virgifera* to Cry3Bb1-expressing Bt corn was first reported by Gassmann et al. (2011) in Iowa populations, and the occurrence of this phenomenon has subsequently expanded from Cry3Bb1 to other Bt proteins and in other states (primary in the north central U.S.) (Gassmann et al. 2012, 2014, Gray 2012, Wangila et al. 2015, Zukoff et al. 2016).

Scouting for corn rootworm adults to accurately identify the insect species present and determine the population density in fields is essential for implementation of the most appropriate integrated pest management (IPM) strategy to protect the crop from economic loss. Whole-plant beetle counts and sticky traps are two methods that have frequently been used to estimate corn rootworm beetle infestation levels, and economic thresholds (ET) have been developed for both techniques to anticipate larval damage potential in the following year (Godfrey and Turpin 1983, Hein and Tollefson 1985). Before Bt corn hybrids were commercially available, Hein and Tollefson (1985) estimated an ET of more than six beetles (i.e., regardless of *Diabrotica* species) per trap per day using unbaited Pherocon® AM traps. They also suggested sampling of beetles during the last three weeks of August in Iowa (Hein and Tollefson 1985). Dunbar and Gassmann (2013) calculated an ET of two beetles for either *D. barberi* or *D. v. virgifera* beetles (individually or in combination) per sticky trap per day during the week of peak abundance within a field. This ET was calculated based on efficacies of Bt corn hybrids and soil insecticides in first-year corn and the use of unbaited Pherocon AM yellow sticky traps (Dunbar and Gassmann 2013).

The geographic distribution, species composition, and abundance of corn rootworm populations in ND corn fields are currently unknown. Therefore, we carried out this research to assess the following: 1) the geographic distribution, abundance, and species composition of *D. barberi* or *D. v. virgifera* beetles in ND; and 2) the comparative effectiveness of yellow Pherocon® AM/NB and green Scentry^{TM} Multigard sticky traps for both sexes of these two corn rootworm species.

Materials and Methods

Corn rootworm beetle surveys were conducted between early July and October of 2013 through 2015 in 51 fields representing 20 ND counties. Totals of 29, 3, and 19 corn fields were surveyed in 2013, 2014, and 2015, respectively (Table 2.1). Grower contact information was obtained with the assistance of several local agronomists and North Dakota State University county extension agents. Latitude and longitude were recorded for each corn field.

Table 2.1. Monitoring sites, sampling periods, and combined total numbers of *D. barberi* and *D. v. virgifera* beetles captured per field by using unbaited yellow Pherocon® AM/NB and green Scentry^{TM} Multigard sticky traps in North Dakota, 2013 – 2015

Year	County	Latitude N	Longitude W	Field site ID	Trapping period	Total beetles captured[a] *D. barberi*	Total beetles captured[a] *D. v. virgifera*
2013							
	Barnes	47.152620	-97.705980	B1	7 Aug-28 Aug	4	9
	Barnes	46.963380	-97.722200	B2	7 Aug-28 Aug	160	71
	Barnes	47.031900	-98.011600	B3	13 Aug-10 Sept	3	3
	Benson	48.029950	-99.197690	BE1	19 Aug-1 Oct	0	0
	Cass	47.167995	-97.360519	C1[b]	1 Aug-17 Oct	1608	1940
	Cass	47.151980	-97.451220	C2[et]	7 Aug-28 Aug	1203	198
	Cass	46.920300	-97.598070	C3	7 Aug-28 Aug	5	8
	Cavalier	48.696590	-98.274870	CA1	9 Aug-10 Sept	0	0
	Dunn	47.039250	-102.34288	D1	8 Aug-10 Sept	94	14
	Foster	47.522700	-99.147600	F1	8 Aug-9 Sept	1	0
	Grand Forks	47.700967	-97.066589	G1	13 Aug-20 Sept	1	0
	Griggs	47.471300	-98.063570	GR1	14 Aug-11 Sept	6	0
	LaMoure	46.310700	-98.234117	L1	8 Aug-6 Sept	127	22
	McLean	47.508200	-101.04280	M1	18 Aug-12 Sept	0	0
	Mercer	47.493600	-101.57520	ME1	13 Aug-10 Sept	0	0
	Pembina	48.847620	-97.819620	P1	9 Aug-6 Sept	2	0
	Renville	48.777910	-101.62967	R1	13 Aug-3 Sept	0	0
	Richland	46.442130	-97.141370	RI1	30 July-18 Oct	238	1198
	Richland	46.209620	-97.239780	RI2	8 Aug-27 Aug	26	13
	Richland	46.239330	-97.087080	RI3	8 Aug-27 Aug	67	10
	Richland	46.282350	-97.073150	RI4	8 Aug-27 Aug	7	10
	Richland	46.252432	-97.010466	RI5	8 Aug-27 Aug	12	2
	Richland	45.992738	-97.099240	RI6	1 Aug-29 Aug	24	1
	Richland	46.080720	-96.845850	RI7	8 Aug-29 Aug	4	0
	Richland	46.036420	-96.783720	RI8	8 Aug-29 Aug	22	5
	Sargent	46.094989	-97.638562	S1	1 Aug-29 Aug	2	0
	Steele	47.268710	-97.677000	ST1	7 Aug-28 Aug	0	2
	Traill	47.396250	-97.113348	T1	7 Aug-28 Aug	6	15
	Ward	48.178880	-101.28868	W1	13 Aug-4 Sept	0	0
2014							
	Cass	47.164847	-97.367889	C4	6 Aug-8 Oct	129	42
	Cass	47.166271	-97.499015	C5	6 Aug-8 Oct	69	1
	Steele	47.397457	-97.704470	ST2	6 Aug-1 Oct	383	0

Table 2.1. Monitoring sites, sampling periods, and combined total numbers of *D. barberi* and *D. v. virgifera* beetles captured per field by using unbaited yellow Pherocon® AM/NB and green Scentry^{TM} Multigard sticky traps in North Dakota, 2013 – 2015 (continued)

Year	County	Latitude N	Longitude W	Field site ID	Sampling period	Total beetles captured[a]	
						D. barberi	*D. v. virgifera*
2015							
	Barnes	47.066720	-98.193730	B4	28 July-27 Aug	0	0
	Barnes	46.956630	-97.954200	B5	28 July-27 Aug	1	0
	Cass	47.167995	-97.360519	C6	30 July-1 Oct	186	1
	Cass	46.977630	-96.950880	C7	27 July-24 Aug	0	0
	Cass	46.763880	-97.200130	C8	29 July-24 Aug	6	0
	Cass	47.137459	-97.611517	C9	6 Aug.-1 Oct	493	0
	Cass	47.137476	-97.611086	C10	30 July-17 Sept	547	4
	Dickey	46.022100	-98.318480	DI1	28 July-28 Aug	0	0
	Grand Forks	47.759880	-97.559370	G2	28 July-3 Sept	0	0
	Grand Forks	47.802570	-97.120860	G3	28 July-3 Sept	1	0
	Griggs	47.428040	-98.131200	GR2	28 July-27 Aug	0	0
	LaMoure	46.394620	-98.662070	L2	28 July-24 Aug	5	0
	Ransom	46.572610	-97.813280	RA1	29 July-24 Aug	12	0
	Richland	46.245760	-97.134960	RI9	28 July-28 Aug	15	0
	Sargent	46.650170	-97.674930	S2	28 July-28 Aug	3	0
	Sargent	46.107250	-97.301250	S3	28 July-28 Aug	131	0
	Steele	47.312580	-97.731990	ST3	28 July-3 Sept	1	0
	Stutsman	47.240710	-98.551910	SU1	28 July-27 Aug	0	0
	Traill	47.403340	-97.134550	T2	28 July-3 Sept	3	0

[a] Cumulative total of beetles captured on yellow Pherocon® AM/NB and green Scentry^{TM} Multigard sticky traps for each species.

[b] C1 and C6 are the same corn fields surveyed in 2013 and 2015.

[et] Fields that exceeded the economic threshold (i.e., six beetles per trap per day, calculated as *D. barberi* plus *D. v. virgifera*) as developed by Hein and Tollefson (1985).

In each individual field, two linear transects of unbaited sticky traps were arranged parallel to each other, and between 40 and 100 m apart (Hein and Tollefson 1985, Dunbar et al. 2013). Orange field flags were used to mark the location of each transect. Five yellow Pherocon® AM/NB (Trécé Incorporated, Adair, OK) sticky traps were deployed in one transect, and five green Scentry^{TM} Multigard (Scentry Biologicals Inc., Billings, MT) traps were deployed in the other transect. Sticky traps were separated by 20 m (Hein and Tollefson 1985). The first of each trap type within a transect was placed within the field headland (i.e., field margin with rows running perpendicular to reminder of the field rows). Each trap was attached to the stalk of a corn plant at ear height (Hein and Tollefson 1984). Traps were mostly collected and replaced at weekly intervals and transported into the laboratory where they were stored in a conventional freezer at 0 ± 2°C pending processing. Captured beetles were identified according to sex and species using the key developed by Krysan (1986) and a 10x magnification microscope (10 x/23, Nikon SMZ-2T, Nikon USA, Melville, NY).

ArcMap 10.3.1 (2015) (ESRI, Redland, CA) software was used with field coordinates and the trapping data to generate distribution, density, and peak abundance maps of both *D. barberi* and *D. v. virgifera* throughout the corn-producing counties of ND. To generate density maps, combined beetle counts of both species for both trap models were summed for each corn field across the survey period and divided by the number of traps (i.e., 10), producing an average number of beetles per trap. The average number of beetles per trap was subsequently divided by the number of sampling days to calculate the average number of beetles per trap per day. Due low beetle collection rates in some fields during the surveys, maps were generated using numbers of beetles per trap per week. Peak abundance maps for both species were generated using the week with the highest number of captured beetles for each field. The resulting maps display the average capture rate for each species on a beetles-per-trap-per-day basis. Symbols and colors on maps are used to represent distribution and relative population density data. Beetle capture rates of the two sticky trap models were compared for each species by using a two-sample *t*-test (Student 1908) at an alpha level of 0.05 (SAS Institute 2013). Due to the variability in beetle capture rates and the number of trapping days among fields, individual analyses were conducted for each corn field. If the null hypothesis of homogeneity between two variances was not rejected at our *a priori* 95% confidence level, then a *t*-test with equal variance was used. However, if the two error variances were not equal, a *t'* test with unequal variance was used.

Seasonal beetle emergence patterns by species and trap model were created using the mean ± SEM of beetles captured per trap type for each collection date in the following site years: C1 and RI1 in 2013; C4, C5, and ST2 in 2014; and C6 and C9 in 2015. In these particular fields, traps were deployed before the onset of beetle emergence (first collection times displayed as zero) and maintained until emergence ceased. Sticky trap models were compared within collection date by species using a two-sample *t*-test or *t'* test (i.e., depending on resulting of variance comparison as previously described) at an alpha level of 0.05 in SAS (SAS Institute 2013). In addition, the sex ratios in these seven corn fields were compared between the two trap types for both species using a Chi-square 2 X 2 contingency table (Zar 1984) at an alpha level of 0.05 using SAS. Sex ratio analyses were conducted within each field by using total captured beetles within trap type across the entire collection period.

Results

Totals of 5,607 *D. barberi* and 3,569 *D. v. virgifera* beetles were captured throughout this three-year study. Capture rates among the 51 surveyed corn fields ranged from 0 to 1,608 *D. barberi* and 0 to 1,940 *D. v. virgifera* beetles per field during the survey period (Table 2.1). Corn rootworm beetles were detected in 76% (i.e., 39) of the surveyed fields. No beetles of either species were captured in 24% (i.e., 12) of the surveyed corn fields. The absence of these species was most commonly observed in the northern part of the state (Fig. 2.1). No *D. barberi* were found in 25% (i.e., 13 corn fields) of all surveyed fields, and no *D. v. virgifera* were captured in 59% (i.e., 30 fields) of the fields (Fig. 2.1 and Table 2.1).

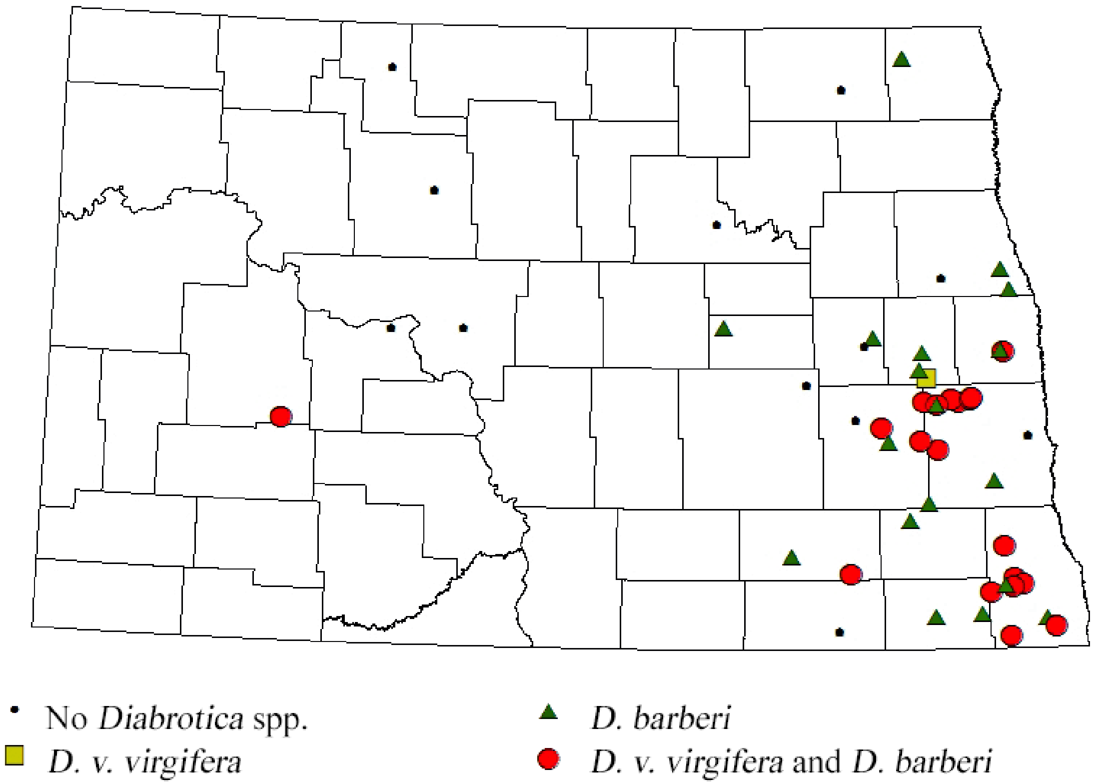


Fig. 2.1. Distribution and species composition of *D. barberi* and *D. v. virgifera* beetles captured on yellow Pherocon® AM/NB and green Scentry^{TM} Multigard sticky traps in North Dakota from 2013 – 2015.

The distribution map (Fig. 2.1) shows that 51% of the 39 corn fields where rootworm beetles were detected contained both species. Those fields were mostly distributed in southeastern and east-central parts of the state. Of the 39 corn fields in which rootworm beetles were detected, 46% contained only *D. barberi*. Those fields were found primarily in southeastern counties, but detections of *D. barberi* also extended northward within the state. Only 3% of the 39 corn fields in which rootworms beetles were found contained solely *D. v. virgifera*.

Densities of >10 beetles per trap per week were captured in only 5% (i.e., C1 and C2) of the 38 corn fields where *D. barberi* beetles were detected (Fig. 2.2). Capture rates of 5.1 to 10.0 and 2.1 to 5.0 *D. barberi* beetles per trap per week were observed in 8% and 16% of those fields, respectively; whereas, low capture rates of 0.1 to 2.0 and <0.1 *D. barberi* beetles per trap per week were obtained in 45% and 26% of surveyed fields, respectively. Respective densities of >10.0, 5.1-10.0, 2.1-5.0, 0.1-2.0, and <1.0 beetles per trap per week were captured in 10%, 5%, 5%, 48%, and 33% of the 21 fields that contained *D. v. virgifera* (Fig. 2.3). The fields in which captures of >10 *D. v. virgifera* beetles per trap per week occurred in fields C1 and RI1. An average of seven beetles per trap per day were captured in field C2 in 2013 (Table 2.1). That was the only field in the 3-yr study in which a capture rate exceeded the ET of six beetles per trap per day as established by Hein and Tollefson (1985).

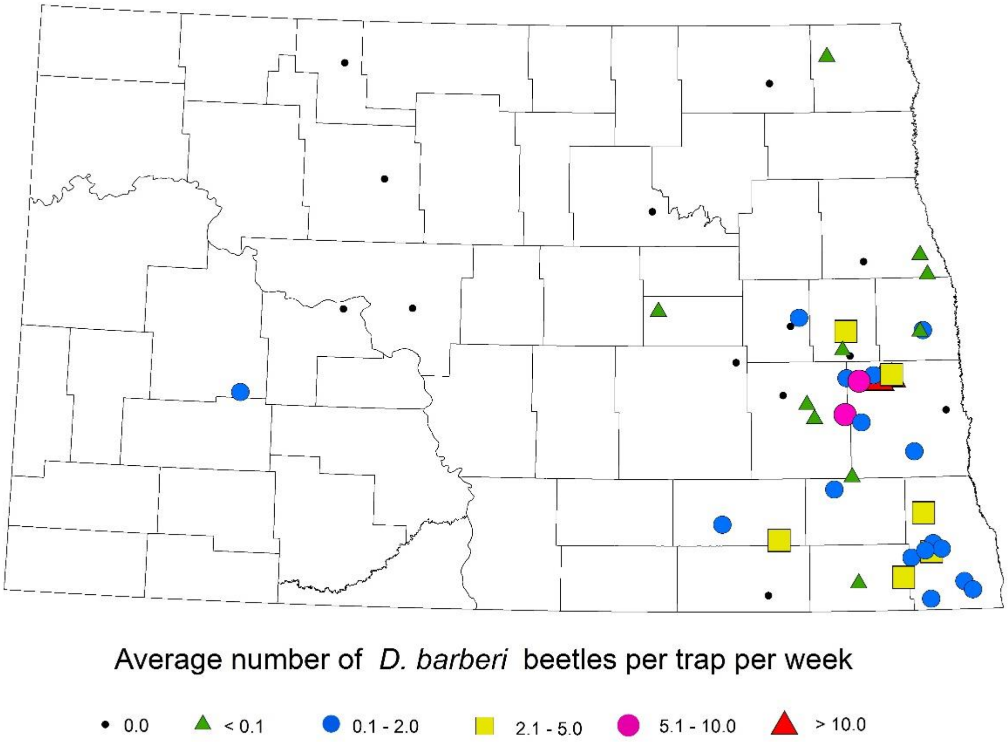


Fig. 2.2. Average densities of *D. barberi* beetles captured per week on yellow Pherocon® AM/NB and green Scentry^{TM} Multigard sticky traps in North Dakota from 2013 – 2015.

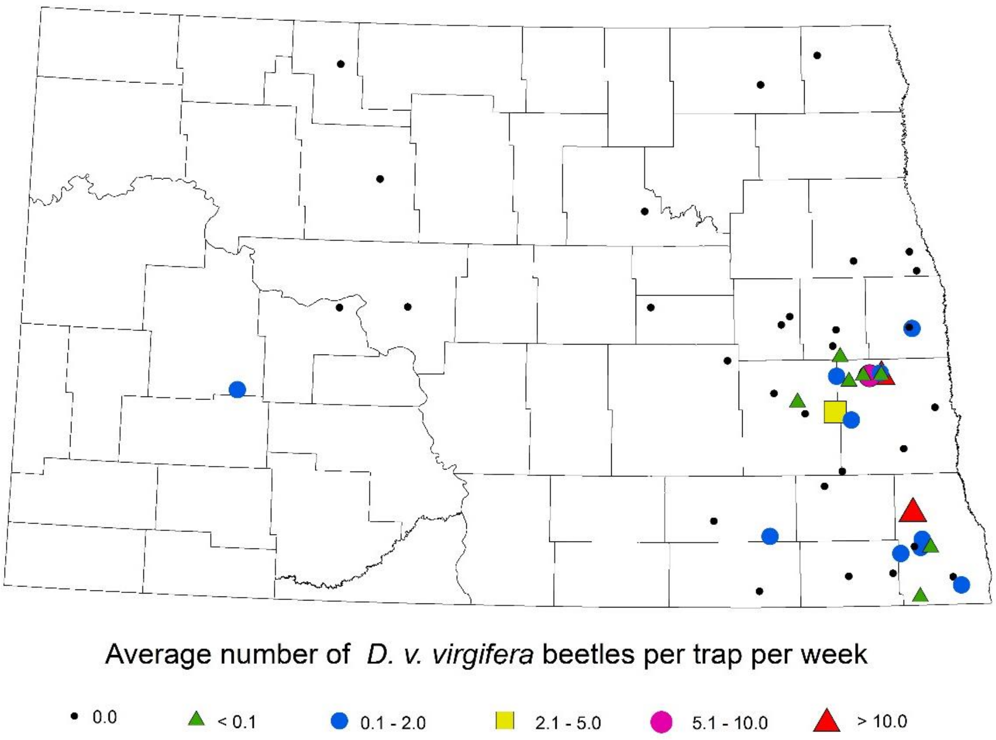


Fig. 2.3. Average density of *D. v. virgifera* beetles captured per week on yellow Pherocon® AM/NB and green Scentry^{TM} Multigard sticky traps in North Dakota from 2013 – 2015.

Capture rates of *D. barberi* during peak abundance week (Fig. 2.4 and Table 2.2) exceeded two beetles per trap per day in 8% of the 38 corn fields where *D. barberi* were present. Additionally, densities of 1.1-1.5 beetles per trap per day were observed in another 8% of those fields. Densities of 0.51-1.0 and <0.5 *D. barberi* were captured in 16% and 68% of the *D. barberi*-inhabited corn fields, respectively. Average capture rate ranges of >2.1, 1.1-1.5, 0.51-1.0, and <0.5 *D. v. virgifera* beetles per trap per day during the peak abundance week were observed in 10%, 5%, 5%, and 81% of the 21 corn fields containing *D. v. virgifera*, respectively (Fig. 2.5 and Table 2.2). Capture rates during peak abundance periods either reached or surpassed the economic threshold in 10% (fields B2, C1, C2, and RI1 in 2013) and 3% (field C10 in 2015) of the 39 corn fields where beetles of either species were detected; whereas all remaining corn fields were below the ET (Table 2.2).

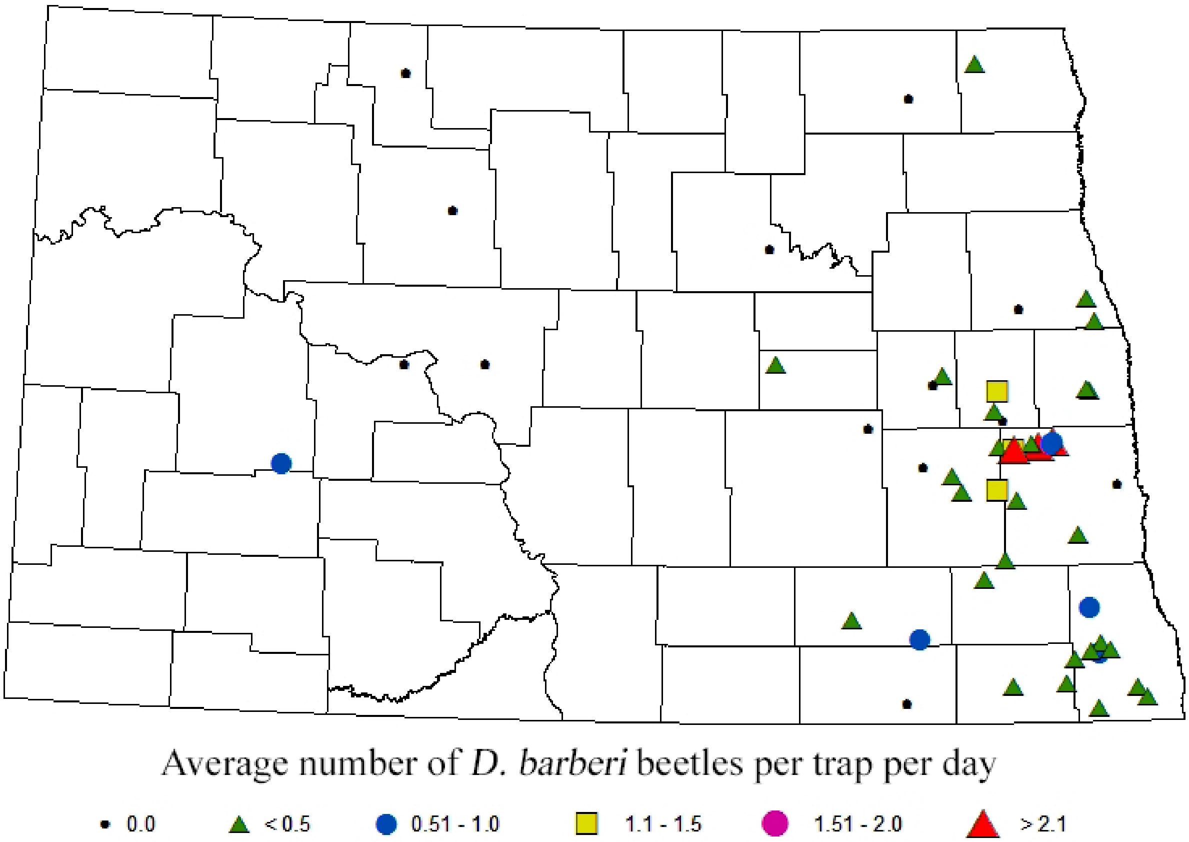


Fig. 2.4. Density of *D. barberi* beetles captured on yellow Pherocon® AM/NB and green Scentry^{TM} Multigard sticky traps during peak abundance week in North Dakota from 2013 – 2015.

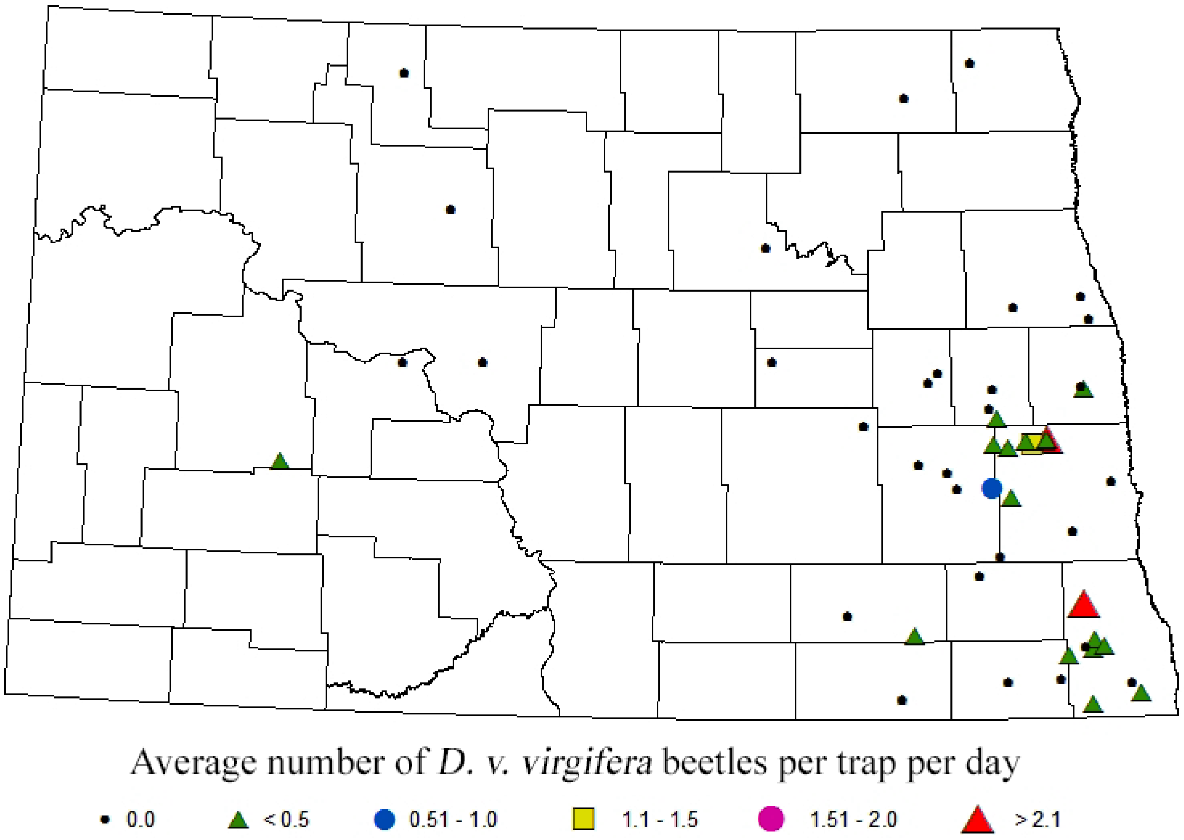


Fig. 2.5. Density of *D. v. virgifera* beetles captured on yellow Pherocon® AM/NB and green Scentry^{TM} Multigard sticky traps during peak abundance week in North Dakota from 2013 – 2015.

Table 2.2. Combined capture rates of *D. barberi* and *D. v. virgifera* beetles captured on yellow Pherocon® AM/NB and green Scentry™ Multigard sticky traps during the week of peak abundance in North Dakota, 2013 – 2015

Year	Field site ID	Peak abundance per trap per day	
		D. barberi	*D. v. virgifera*
2013			
	B1	0.03	0.07
	B2[et]	1.18	0.55
	B3	0.03	0.03
	C1[a,et]	6.23	3.98
	C2[et]	8.88	1.43
	C3	0.04	0.07
	D1	0.73	0.20
	F1	0.01	0.00
	G1	0.01	0.00
	GR1	0.06	0.00
	L1	0.86	0.07
	P1	0.03	0.00
	RI1[et]	0.61	2.96
	RI2	0.31	0.19
	RI3	0.63	0.11
	RI4	0.10	0.16
	RI5	0.22	0.04
	RI6	0.34	0.01
	RI7	0.04	0.00
	RI8	0.24	0.06
	S1	0.03	0.00
	ST1	0.00	0.03
	T1	0.03	0.10
2014			
	C4	0.66	0.16
	C5	0.49	0.01
	ST2	1.33	0.00
2015			
	B5	0.01	0.00
	C6	0.77	0.01
	C8	0.07	0.00
	C9	1.46	0.00
	C10[et]	3.26	0.06
	G3	0.01	0.00
	L2	0.07	0.00
	RA1	0.13	0.00
	RI9	0.03	0.00
	S2	0.04	0.00
	S3	0.43	0.00
	ST3	0.00[b]	0.00
	T2	0.02	0.00

[et] Peak abundance of *D. barberi* and *D. v. virgifera* was at or above the economic threshold of two beetles per trap per day (*D. barberi* and *D. v. virgifera* combined) based defined by Dunbar and Gassmann (2013).

[a] Sites C1 and C6 were the same corn field, but surveyed in 2013 and 2015.

[b] The capture rate at field site ST3 was 0.0048 *D. barberi* beetles per trap per day.

Statistically significant differences in *D. barberi* captures were detected between yellow and green sticky traps for capturing *D. barberi* beetles in fields C2, D1, RI3, C4, ST2, C8, and RA1 (Table 2.3). Green Scentry^{TM} Multigard sticky traps captured significantly greater numbers of *D. barberi* beetles than yellow Pherocon® AM/NB sticky traps in fields R13, C4, ST2, C8, and RA1, although the yellow traps captured significantly more *D. barberi* than green traps in fields C2 and D1. The green Multigard traps also captured numerically more *D. barberi* than yellow traps in 21 corn fields; however, no significant differences between the two trap models were detected in those fields.

Significant differences were also detected between the two trap models in relation to captures of *D. v. virgifera* beetles in fields C2, RI1, RI3, and RI4 (Table 2.4). Mean numbers of *D. v. virgifera* captured on green Multigard traps were significantly greater than those captured on Pherocon AM/NB traps in fields C2, RI3, and RI4. The only instance of yellow sticky traps capturing significantly more *D. v. virgifera* beetles than green traps occurred in field RI1; however, yellow sticky traps captured numerically more *D. v. virgifera* than green traps in 11 fields.

Table 2.3. Mean (± SEM) captures of *D. barberi* beetles on yellow Pherocon® AM/NB and green Scentry^{TM} Multigard sticky traps by field site in North Dakota, 2013 – 2015

Year	Field site ID	*D. barberi* beetles per trap (mean ± SEM)[a]		df	*t*	*P*
		Green sticky trap	Yellow sticky trap			
2013						
	B1	0.07 ± 0.07	0.20 ± 0.11	28	1.06[t]	0.299
	B2	5.93 ± 1.05	4.73 ± 1.49	28	0.66[t]	0.515
	B3	0.00 ± 0.00	0.17 ± 0.09	17	1.84	0.083
	C1[b]	13.35 ± 1.84	15.89 ± 2.23	108	0.88[t]	0.381
	C2	25.67 ± 2.45	54.53 ± 10.73*	15	2.62	0.019
	C3	0.13 ± 0.09	0.20 ± 0.11	28	0.48[t]	0.638
	D1	0.75 ± 0.23	4.78 ± 1.10*	20	3.05	0.007
	F1	0.05 ± 0.05	0.00 ± 0.00	19	1.00	0.330
	G1	0.00 ± 0.00	0.07 ± 0.07	14	1.00	0.334
	GR1	0.05 ± 0.05	0.25 ± 0.12	25	1.51	0.145
	L1	2.80 ± 0.81	3.55 ± 0.72	38	0.69[t]	0.493
	P1	0.10 ± 0.10	0.00 ± 0.00	19	1.00	0.330
	RI1	2.25 ± 0.35	2.07 ± 0.37	108	0.35[t]	0.724
	RI2	1.53 ± 0.68	0.20 ± 0.14	15	1.91	0.075
	RI3	3.80 ± 0.92	0.67 ± 0.29*	17	3.26	0.005
	RI4	0.47 ± 0.24	0.00 ± 0.00	14	1.97	0.068
	RI5	0.90 ± 0.38	0.30 ± 0.21	18	1.38[t]	0.184
	RI6	1.00 ± 0.56	0.20 ± 0.13	21	1.39	0.178
	RI7	0.27 ± 0.21	0.00 ± 0.00	14	1.29	0.217
	RI8	1.33 ± 0.60	0.13 ± 0.09	15	1.98	0.067
	S1	0.10 ± 0.07	0.00 ± 0.00	19	1.45	0.163
	T1	0.20 ± 0.11	0.20 ± 0.14	28	0.00[t]	1.000
2014						
	C4	2.09 ± 0.52	0.78 ± 0.23*	61	2.31	0.024
	C5	1.09 ± 0.28	0.44 ± 0.19	78	1.91	0.059
	ST2	7.40 ± 1.37	2.18 ± 0.37*	45	3.69	0.001
2015						
	B5	0.07 ± 0.07	0.00 ± 0.00	14	1.00	0.334
	C6	1.98 ± 0.46	2.16 ± 0.45	88	0.27[t]	0.787
	C8	0.30 ± 0.13	0.00 ± 0.00*	19	2.35	0.030
	C9	6.35 ± 0.85	5.98 ± 0.80	78	0.32[t]	0.749
	C10	7.46 ± 1.25	8.17 ± 1.07	68	0.34[t]	0.736
	G3	0.07 ± 0.07	0.00 ± 0.00	14	1.00	0.334
	L2	0.20 ± 0.12	0.05 ± 0.05	26	1.18	0.249
	RA1	0.60 ± 0.26	0.00 ± 0.00*	19	2.35	0.030
	RI9	0.60 ± 0.35	0.15 ± 0.11	23	1.22	0.234
	S2	0.10 ± 0.10	0.05 ± 0.05	28	0.45	0.658
	S3	4.05 ± 0.73	2.50 ± 0.52	38	1.72[t]	0.093
	ST3	0.07 ± 0.07	0.00 ± 0.00	14	1.00	0.334
	T2	0.15 ± 0.11	0.00 ± 0.00	19	1.37	0.186

[a] Asterisks indicate significant differences between sticky trap models according to a two-sample *t*-test ($P < 0.05$).

[t] A *t*-test with an equal variance was used when the two error variances were homogenous; for all remaining corn field analyses, a *t’* test with unequal variance was used due to lack of homogeneity between the error variances.

[b] C1 and C6 are the same corn fields surveyed in 2013 and 2015.

Table 2.4. Mean (± SEM) captures of *D. v. virgifera* beetles on yellow Pherocon® AM/NB and green Scentry^{TM} Multigard sticky traps by field site in North Dakota, 2013 – 2015

Year	Field site ID	*D. v. virgifera* per trap (mean ± SEM)[a]		df	*t*	*P*
		Green sticky trap	Yellow sticky trap			
2013						
	B1	0.27 ± 0.18	0.33 ± 0.13	28	0.30[ł]	0.7653
	B2	3.00 ± 0.72	1.73 ± 0.65	28	1.30[ł]	0.2037
	B3	0.00 ± 0.00	0.17 ± 0.09	17	1.84	0.0827
	C1[b]	16.89 ± 2.09	18.38 ± 1.97	108	0.52[ł]	0.6055
	C2	8.53 ± 1.10	4.67 ± 1.35*	28	2.20[ł]	0.0352
	C3	0.20 ± 0.11	0.33 ± 0.19	28	0.62[ł]	0.5407
	D1	0.20 ± 0.12	0.53 ± 0.43	21	0.73	0.4706
	L1	0.45 ± 0.57	0.65 ± 0.22	38	0.59[ł]	0.5579
	RI1	6.95 ± 1.01	14.84 ± 2.25*	75	3.20	0.0020
	RI2	0.87 ± 0.50	0.00 ± 0.00	14	1.75	0.1027
	RI3	0.67 ± 0.25	0.00 ± 0.00*	14	2.65	0.0192
	RI4	0.67 ± 0.29	0.00 ± 0.00*	14	2.32	0.0359
	RI5	0.20 ± 0.13	0.00 ± 0.00	9	1.50	0.1679
	RI6	0.00 ± 0.00	0.05 ± 0.05	19	1.00	0.3299
	RI8	0.00 ± 0.00	0.33 ± 0.27	14	1.23	0.2377
	ST1	0.00 ± 0.00	0.13 ± 0.13	14	1.00	0.3343
	T1	0.53 ± 0.27	0.47 ± 0.32	28	0.16[ł]	0.8757
2014						
	C4	0.62 ± 0.17	0.31 ± 0.09	69	1.61	0.1126
	C5	0.00 ± 0.00	0.02 ± 0.02	44	1.00	0.3228
2015						
	C6	0.00 ± 0.00	0.02 ± 0.02	44	1.00	0.3228
	C10	0.00 ± 0.00	0.11 ± 0.11	34	1.00	0.3244

[a] Asterisks indicate significant differences between sticky trap models according to a two-sample *t*-test ($P < 0.05$).

[ł] A *t*-test with an equal variance was used when the two error variances were homogenous; for all remaining corn field analyses, a *t'* test with unequal variance was used due to lack of homogeneity between the error variances.

[b] C1 and C6 are the same corn fields surveyed in 2013 and 2015.

In relation to seasonal emergence, significant differences between yellow Pherocon® AM/NB and green Scentry^{TM} Multigard sticky traps were occasionally observed in fields C1, RI1, C4, and C6 (Table 2.5 and Fig. 2.6). In 2013, at field site C1, green sticky traps captured significantly greater numbers of *D. barberi* beetles than yellow traps on 15 August, but significantly lower numbers of this species on 22 and 28 August (corn growth stage was around

R2 stage) (Table 2.5 and Fig. 2.6A). In the same field and year, green sticky traps captured significantly greater numbers of *D. v. virgifera* beetles than yellow sticky traps on 15 August, whereas yellow traps captured more *D. v. virgifera* than the green traps on 1 October (corn growth stage was around R5 stage). At field RI1 in 2013, yellow traps captured significantly more *D. barberi* beetles than green sticky traps on 18 September (Table 2.5 and Fig. 2.6B). In the same field and year, green sticky traps captured significantly greater numbers of *D. v. virgifera* beetles than the yellow traps on 27 August; however, from 18 September to the end of the sampling season that year, when corn fields were in R4, R5, and R6 stages of development, yellow sticky traps captured significantly more *D. v. virgifera* beetles than green traps. In 2014, green sticky traps captured significantly greater numbers of *D. barberi* than yellow sticky traps on 24 September and 8 October in field C4 (Table 2.5 and Fig. 2.6C). In 2015, yellow sticky traps captured significantly greater numbers of *D. barberi* beetles than the green sticky traps on 24 September in field C6 (Table 2.5 and Fig. 2.6F). There were no significant differences in captures of *D. barberi* among the trap models in fields C5 and ST2 in 2014 and field C9 in 2015 (Figs. 2.6D, 2.6E, and 2.6G), irrespective of sampling date. Captures of *D. v. virgifera* beetles ranged from very low to zero in field C4 in 2014 (Fig. 2.6C), C5 in 2014 (Fig. 2.6D), ST2 in 2014 (Fig. 2.6E), C6 in 2015 (Fig. 2.6F), and C9 in 2015 (Fig. 2.6G).

Table 2.5. Significance testing for comparisons of yellow Pherocon® AM/NB and green Scentry$^{\text{TM}}$ Multigard sticky traps on selected collection dates and by species (trapping data presented in Figs. 6A, B, C, F)

Field site ID	Sampling date	*D. barberi* beetles			*D. v. virgifera* beetles		
		df	*t*	*P*	df	*t*	*P*
C1[a] (2013)							
	15 Aug	4	2.68	0.0492*[b]	4	3.11	0.0292*
	22 Aug	8	2.73[t]	0.0257*	8	1.92[t]	0.0913
	28 Aug	8	2.38[t]	0.0443*	8	0.99[t]	0.3504
	1 Oct	8	0.52[t]	0.6170	8	3.08[t]	0.0152*
RI1 (2013)							
	27 Aug	4	2.56	0.0566	5	3.04	0.0307*
	18 Sept	8	3.73[t]	0.0058*	8	2.53[t]	0.0351*
	25 Sept	8	1.70[t]	0.1271	4	3.82	0.0174*
	1 Oct	8	0.80[t]	0.4458	8	3.16[t]	0.0133*
	9 Oct	5	2.20	0.0850	8	5.85[t]	0.0004*
	18 Oct	8	1.39[t]	0.2029	8	3.36[t]	0.0010*
C4 (2014)							
	24 Sept	8	2.47[t]	0.0390*	8	1.77[t]	0.1151
	8 Oct	4	3.16	0.0341*	N/A[c]	N/A	N/A
C6 (2015)							
	24 Sept	8	2.91[t]	0.0195*	N/A	N/A	N/A

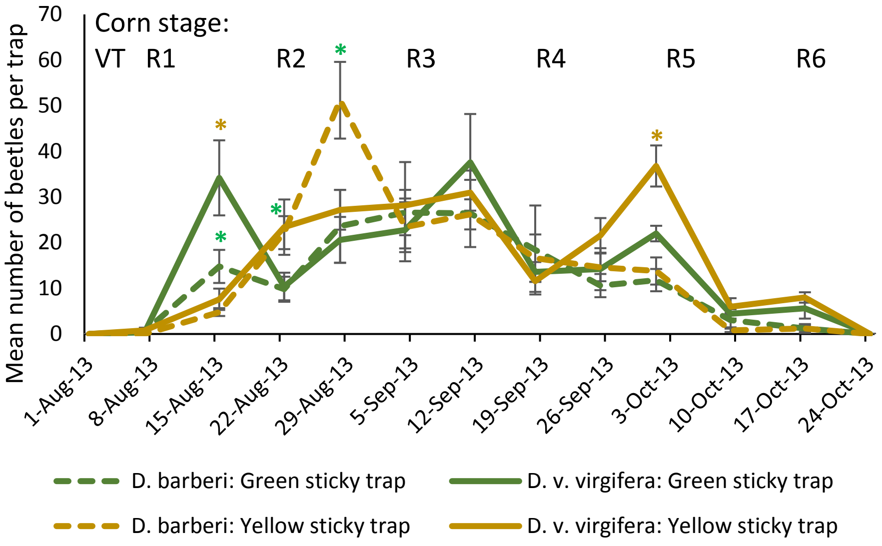
[a] C1 and C6 are the same corn fields surveyed in 2013 and 2015.

[b] Asterisks indicate significant differences between sticky trap models according to a two-sample *t*-test ($P < 0.05$).

[t] A *t*-test with an equal variance was used when the two error variances were homogenous; for all remaining corn field analyses, a *t'* test with unequal variance was used due to lack of homogeneity between the error variances.

[c] No *D. v. virgifera* beetles were captured.

A. Field C1 (2013)



B. Field RI1 (2013)

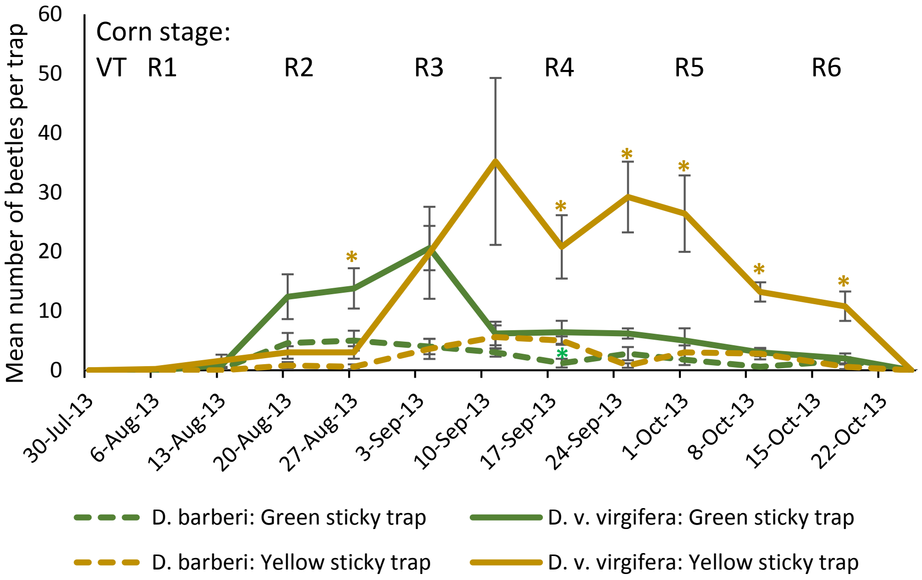
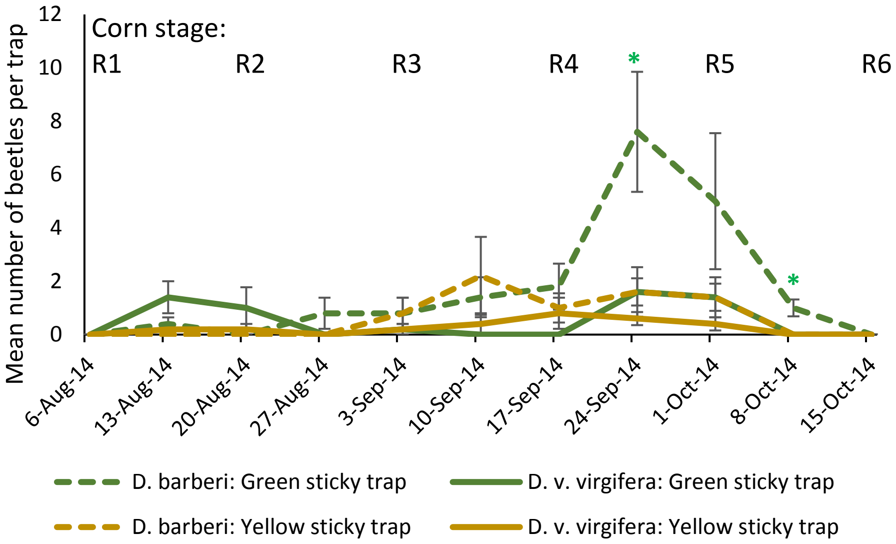


Fig. 2.6. (A-G) Seasonal captures of *D. barberi* and *D. v. virgifera* on yellow Pherocon® AM/NB and green Scentry^{TM} Multigard sticky traps (mean number of beetles per trap per sampling date) at seven corn field sites in North Dakota. Green lines indicate green sticky traps; yellow lines indicate yellow sticky traps. Green asterisks (*) indicate significant differences between green and yellow sticky traps for *D. barberi* (denoted by the broken lines) for each sampling date. Yellow asterisks (*) indicate significant differences between green and yellow sticky traps for *D. v. virgifera* (denoted by solid lines) for each sampling date.

C. Field C4 (2014)



D. Field C5 (2014)

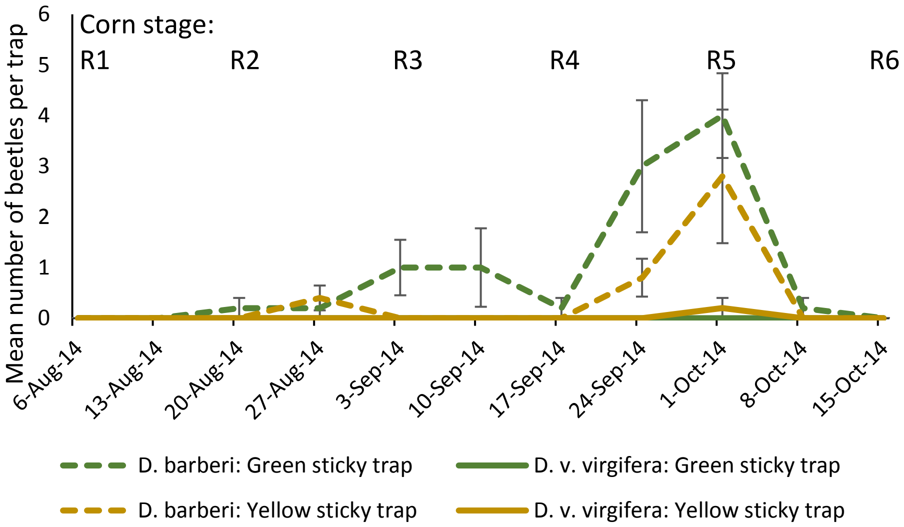
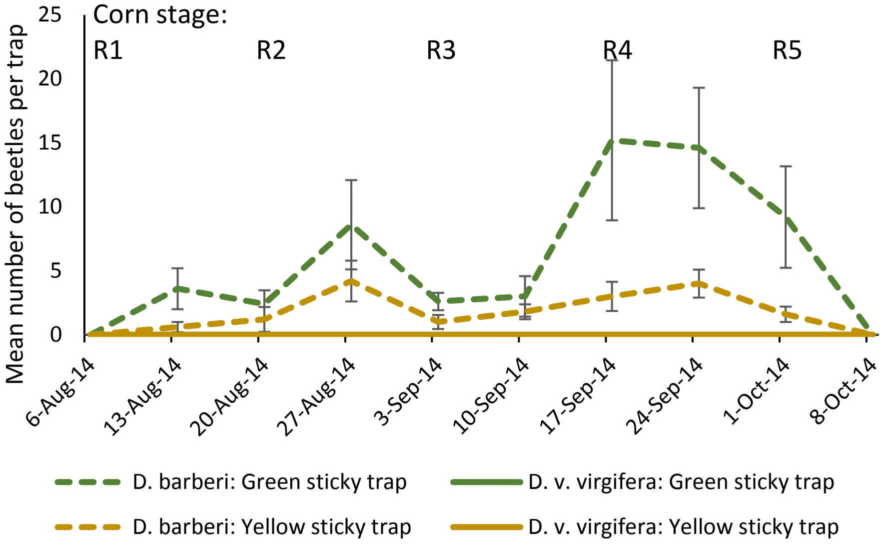


Fig. 2.6. (A-G) Seasonal captures of *D. barberi* and *D. v. virgifera* on yellow Pherocon® AM/NB and green Scentry^{TM} Multigard sticky traps (mean number of beetles per trap per sampling date) at seven corn field sites in North Dakota (continued). Green lines indicate green sticky traps; yellow lines indicate yellow sticky traps. Green asterisks (*) indicate significant differences between green and yellow sticky traps for *D. barberi* (denoted by the broken lines) for each sampling date. Yellow asterisks (*) indicate significant differences between green and yellow sticky traps for *D. v. virgifera* (denoted by solid lines) for each sampling date.

E. Field ST2 (2014)



F. Field C6 (2015)

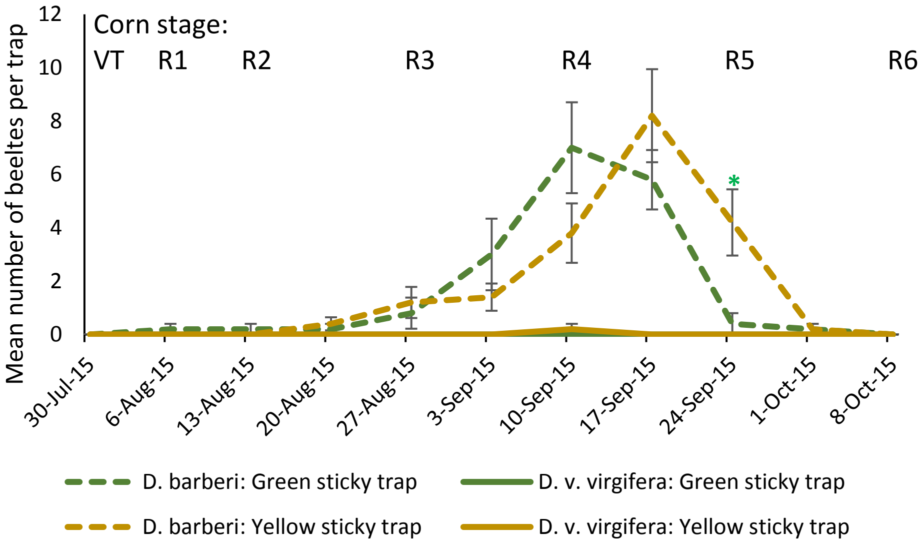


Fig. 2.6. (A-G) Seasonal captures of *D. barberi* and *D. v. virgifera* on yellow Pherocon® AM/NB and green Scentry^{TM} Multigard sticky traps (mean number of beetles per trap per sampling date) at seven corn field sites in North Dakota (continued). Green lines indicate green sticky traps; yellow lines indicate yellow sticky traps. Green asterisks (*) indicate significant differences between green and yellow sticky traps for *D. barberi* (denoted by the broken lines) for each sampling date. Yellow asterisks (*) indicate significant differences between green and yellow sticky traps for *D. v. virgifera* (denoted by solid lines) for each sampling date.

G. Field C9 (2015)

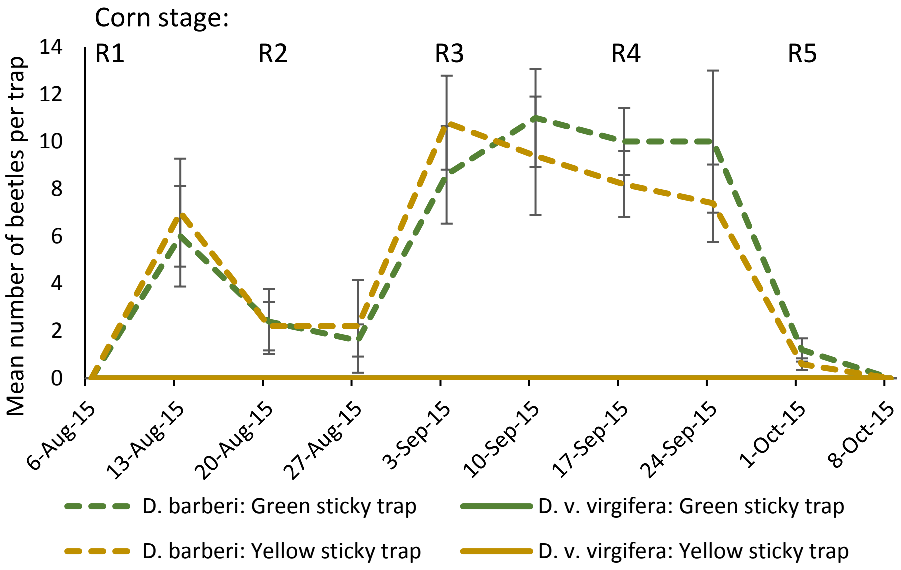


Fig. 2.6. (A-G) Seasonal captures of *D. barberi* and *D. v. virgifera* on yellow Pherocon® AM/NB and green Scentry^{TM} Multigard sticky traps (mean number of beetles per trap per sampling date) at seven corn field sites in North Dakota (continued). Green lines indicate green sticky traps; yellow lines indicate yellow sticky traps. Green asterisks (*) indicate significant differences between green and yellow sticky traps for *D. barberi* (denoted by the broken lines) for each sampling date. Yellow asterisks (*) indicate significant differences between green and yellow sticky traps for *D. v. virgifera* (denoted by solid lines) for each sampling date.

The only instances of significant differences regarding sex ratios between green and yellow sticky traps were observed at field ST2 for *D. barberi* and in C4 for *D. v. virgifera* (both in 2014) (Table 2.6). In both cases, green traps captured a significantly lower proportion of females to males than the yellow sticky traps.

Table 2.6. Sex ratios of *D. barberi* and *D. v. virgifera* captured on yellow Pherocon® AM/NB and green Scentry$^{\text{TM}}$ Multigard sticky traps in North Dakota, 2013 – 2015

Species	Year	Field site ID	Beetle sex ratios (male: female)[a] green Scentry trap	Pherocon trap	χ^2	*P*
D. barberi						
	2013					
		C1[b]	451: 283	570: 304	2.4505	0.1175
		RI1	88: 36	83: 31	0.0993	0.7526
	2014					
		C4	69: 25	21: 14	2.1725	0.1405
		C5	33: 16	12: 8	0.338	0.5610
		ST2	267: 29	65: 22*	13.9768	0.0002
	2015					
		C6	72: 17	73: 24	0.8595	0.3539
		C9	208: 46	195: 44	0.0074	0.9314
D. v. virgifera						
	2013					
		C1	661: 268	698: 313	1.0286	0.3105
		RI1	275: 107	587: 229	0.0004	0.9847
	2014					
		C4	23: 5	7: 7*	4.7250	0.0297

One degree of freedom for each comparison.

[a] An asterisk indicates significant differences between the two trap colors according to a Chi-square 2 X 2 contingency table test ($P < 0.05$).

[b] C1 and C6 are the same corn fields surveyed in 2013 and 2015.

Discussion

Both *D. barberi* and *D. v. virgifera* were most common in the southeastern part of North Dakota where the majority of corn production occurs in the state (USDA–NASS 2016); however *D. barberi* was commonly found farther northward in the state. In 24% of surveyed fields, corn rootworm beetles were not detected. However, at least one beetle of either species was captured in the remaining 76% of the fields in our study. Of the 39 corn fields in which beetles were detected, 51% contained both *Diabrotica* species. Overall, 61% of captured beetles were *D. barberi* and 39% were *D. v. virgifera*. Ellsbury et al. (1998) reported greater proportions of *D. barberi* (i.e., 74% and 86% in 1995 and 1996, respectively) using emergence cages near

Brookings, SD. In contrast, Ordosch et al. (2016) captured significantly greater numbers of *D. v. virgifera* than *D. barberi* beetles using sticky traps near Chester and Milbank, SD in 2013 and 2014. Those authors suggested that *D. barberi* was probably at higher densities prior to the widespread adoption of Bt corn hybrid in South Dakota.

As indicated in our distribution and density maps, numbers of both *D. barberi* and *D. v. virgifera* in North Dakota differed greatly among field sites and years. Previous authors have suggested that other factors such as soil type and other edaphic factors, climate conditions, and cultural practices can negatively influence corn rootworm larval survival and, as such, can influence abundance and distribution of both *D. barberi* and *D. v. virgifera* (Johnson and Turpin 1985, Fisher and Bergman 1986, Riedell and Sutter 1995, Ellsbury et al. 1996, 1997, 1998, Beckler et al. 2004, Ma et al. 2009). Distribution and abundance of *D. barberi* can also be affected by structural characteristics of the landscape (French et al. 2004). Those authors observed greater abundances of *D. barberi* at elevations ranging between 500 and 509 m. Previous research has also demonstrated that *D. barberi* is more prevalent in corn fields managed by using crop rotation practices, but *D. v. virgifera* is most commonly found in continuous corn production systems (Hill and Mayo 1980, Ellsbury et al. 1998). Chiang (1973) and Krysan (1986) documented the range of *D. barberi*, and suggested that it is adapted to the northern ecoclimate of the U.S. This species was collected in central North Dakota near Bismarck at least 60 years before the first recorded observation of *D. v. virgifera* in the state (Krysan and Smith 1987). That species is more prevalent and has a greater economic impact than *D. barberi* in Midwestern states of the U.S. (e.g., Iowa, Illinois, Indiana, etc.) (Edwards and Kiss 2012). Our findings support those of previous authors in that *D. barberi* is more prevalent in North Dakota and other northern areas of the U.S. Whether the higher incidence of this species in North Dakota corn fields was related to farmers actively practicing crop rotation cannot be assessed from the results of this study, because corn field history was difficult to obtain from land owners. However, we have been observing that crop rotation is probably the most commonly used cultural control practice being used to manage corn rootworms, which is likely a response to recent warnings about insecticide resistance.

In our study, only one corn field (C2) exceeded the economic threshold of six beetles per trap per day, while all remaining corn fields were below the ET. Also, peak abundances of both *Diabrotica* species in the majority of corn fields in our study were well below the ET, and only a few fields had beetle densities which reached or exceeded the ET during peak seasonal abundance. Similar findings were reported by Dunbar and Gassmann (2013) for eastern Iowa, and those authors suggested that scouting is an effective tool for use in a corn rootworm IPM program.

In previous research, unbaited Pherocon AM traps and cylindrical sticky traps were found to be the best corn rootworm scouting methods (Hein and Tollefson 1984); however, green sticky traps were not evaluated in that study. Those authors found that unbaited Pherocon AM traps and whole-plant counts served as good predictors for larval damage in the following year (Hein and Tollefson 1985). In our study, green Scentry^{TM} Multigard sticky traps captured either numerically or significantly greater numbers of *D. barberi* beetles than yellow Pherocon® AM/NB traps in 68% of the 38 corn fields where *D. barberi* was present. However, the Pherocon® traps captured numerically or significantly more *D. v. virgifera* beetles than the green traps in 57% of the 21 corn fields inhabited by this species. Gray et al. (2009) observed that *D. v. virgifera* beetles are attracted to the yellow color of unbaited Pherocon® AM traps. Agee et al. (1983) found that both species are sensitive to colors within the green-yellow, and to a lesser extent ultraviolet regions of the electromagnetic spectrum. In our experiment, captures of both species varied among trap models and by sampling date. As such, drawing any definitive conclusions about relative efficiency of the two trap models we tested from these variable results is difficult, especially given the relatively low numbers of beetles captured in many of the corn fields we surveyed.

Kuhar and Youngman (1995) reported a significantly female-biased sex ratio of *D. v. virgifera* captured when using an aspirator; however, they reported no significant differences in sex ratios (i.e., females to males) between two yellow sticky trap models (i.e., the Olson trap and Modified Pherocon® AM sticky traps). Hesler and Sutter (1993) observed that male and female *D. v. virgifera* capture rates changed with both trap color and attractant type, but neither appeared to affect captures of *D. barberi*. Hein and Tollefson (1984) reported that baited Pherocon® 1C traps captured a significantly greater proportion of females than both Pherocon® sticky traps without apple maggot bait and cylindrical sticky traps. In our study, the only cases of significant sex ratio biases in relation to trap color were that yellow sticky traps captured greater proportions of males to females for *D. barberi* at the field ST2 and for *D. v. virgifera* at the C4 field. These findings of infrequent sex ratio biases are also likely the result of our using unbaited traps for this research.

Scouting for corn rootworm beetles by using yellow or green sticky traps in North Dakota corn fields should be helpful for making decisions about managing these pests. If a corn field is known to be mostly infested with *D. barberi*, green sticky traps could be more effective for monitoring this species. Considering our findings of mostly low to moderate corn rootworm infestations for most corn-producing areas within North Dakota, scouting for these pests will be a helpful tool in the decision-making process regarding corn rootworm management for many producers in the state.

Acknowledgments

The authors gratefully thank the local agronomists, seed company representatives, and NDSU County Extension Agents for their assistance with contacting local corn growers to identify research sites and for help with trapping efforts. We also thank P. B. Beauzay, B. G. Berghuis, E. Miller, T. Rock, T. Senger, and J. Uriell for their assistance with field and laboratory work. Finally, we acknowledge J. Jyoti for his very helpful contributions to the data analyses. This research was partially funded by the North Dakota Corn Utilization Council, and it was also supported by the National Institute of Food and Agriculture, U.S. Department of Agriculture, Hatch project accession no. 0223202.

References Cited

Agee, H. R., V. M. Kirk, and J. C. Davis. 1983. Comparative spectral sensitivity and some observations on vision related behavior of northern and western com rootworm adults *(Diabrotica:* Coleoptera). J. Ga. Entomol. Soc. 18: 240–245.

Apple, J. W., and K. K. Patel. 1963. Sequence of attack by northern corn rootworms on the crown roots of corn. Proc. N. Cent. Br. Entomol. Soc. Am. 18: 80–81.

Baca, F. 1994. New member of the harmful entomofauna of Yugoslavia *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae). Zast. Bilja 45: 125–31.

Beckler, A. A., B. W. French, and L. D. Chandler. 2004. Characterization of western corn rootworm (Coleoptera: Chrysomelidae) population dynamics in relation to landscape attributes. Agric. For. Entomol. 6: 129–139.

Calles-Torrez, V., J. J. Knodel, M. A. Boetel, C. D. Doetkott, K. K. Podliska, J. K. Ransom, P. Beauzay, B. W. French, and B. W. Fuller. 2018. Transgenic Bt corn, soil insecticide, and insecticidal seed treatment effects on corn rootworm (Coleoptera: Chrysomelidae) beetle emergence, larval feeding injury, and corn yield in North Dakota. J. Econ. Entomol. 111: 348–360.

Chiang, H. C. 1965. Survival of northern corn rootworm eggs through one and two winters. J. Econ. Entomol. 58: 470–472.

Chiang, H. C. 1973. Bionomics of the northern and western corn rootworms. Annu. Rev. Entomol. 18: 47–72.

Culy, M. D., C. R. Edwards, and J. R. Cornelius. 1992. Effect of silk feeding by western corn rootworm (Coleoptera: Chrysomelidae) on yield and quality of inbred corn in seed corn production fields. J. Econ. Entomol. 85: 2440–2446.

Dunbar, M. W., and A. J. Gassmann. 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.

Edwards C. R., and J. Kiss. 2012. *Diabrotica virgifera virgifera* LeConte in North America 2012. Available at https://extension.entm.purdue.edu/wcr/images/jpg/2012/NORTHAMERICA2012.jpg (accessed 20 March 2017).

Ellsbury, M. M., W. D. Woodson, S. A. Clay, and C. G. Carlson. 1996. Spatial characterization of corn rootworm populations in continuous and rotated corn, pp. 487–494. *In* P. C. Robert, R. H. Rust, and W. E. Larson (eds.), Proc., 3rd Internat. Conf. Prec. Agric., 23-26 June 1996. Minneapolis, MN.

Ellsbury, M. M., W. D. Woodson, L. D. Chandler, S. A. Clay, D. E. Clay, D. Malo, T. Schumacher, J. Schumacher, and C. G. Carlson. 1997. Spatial characterization of adult emergence patterns and oviposition for corn rootworm populations in continuous and rotated corn, pp. 227–234. *In* J. V. Stafford (eds.), Proc, 1st Internat. Conf. Prec. Agric., 7-10 September 1997. Warwick University Conference Centre, Coventry, UK.

Ellsbury, M. M., W. D. Woodson, S. A. Clay, D. Malo, J. Schumacher, D. E. Clay, and C. G. Carlson. 1998. Geostatistical characterization of the spatial distribution of adult corn rootworm (Coleoptera: Chrysomelidae) emergence. Environ. Entomol. 27: 910–917.

(ESRI) Environment Systems Research Institute. 2015. ArcMap release 10.3.1. ESRI, Redlands, CA.

Fisher, J. R, and M. K. Bergman. 1986. Field sampling of larvae and pupae, pp. 101–121. *In*: J. L. Krysan, and T. A. Miller (eds.), Methods for the study of pest *Diabrotica*. Springer-Verlag, New York.

French, B. W., A. A. Beckler, and L. D. Chandler. 2004. Landscape features and spatial distribution of adult northern corn rootworm (Coleoptera: Chrysomelidae) in the South Dakota areawide management site. J. Econ. Entomol. 97: 1943–1957.

French, B. W., B. S. Coates, and T. W. Sappington. 2014. Inheritance of an extended diapause trait in the northern corn rootworm, *Diabrotica barberi* (Coleoptera: Chrysomelidae). J. Appl. Entomol. 138: 213–221.

Gassmann, A. J., J. L. Petzold-Maxwell, E. H. Clifton, M. W. Dunbar, A. M. Hoffmann, D. A. Ingber, and R. S. Keweshan. 2014. Field-evolved resistance by western corn rootworm to multiple *Bacillus thuringiensis* toxins in transgenic maize. Proc. Natl. Acad. Sci. 111: 5141–5146.

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.

Gassmann, A. J., J. L. Petzold-Maxwell, R. S. Keweshan, and M. W. Dunbar. 2012. Western corn rootworm and Bt maize: challenges of pest resistance in the field. GM Crops and Food 3: 235–244.

Godfrey, L. D., L. J. Meinke, and R. J. Wright. 1993. Affects of larval injury by western corn rootworm (Coleoptera: Chrysomelidae) on gas exchange parameters of field corn. J. Econ. Entomol. 86: 1546–1556.

Godfrey, L. D., and F. T. Turpin. 1983. Comparison of western corn rootworm (Coleoptera: Chrysomelidae) adult populations and economic thresholds for first-year and continuous corn fields. J. Econ. Entomol. 76: 1028–1032.

Gray, M. E. 2012. Continuing evolution confirmed of field resistance to Cry3Bb1 in some Illinois fields by western corn rootworm. The Bulletin: pest management and crop development information for Illinois. 20 (12) University of Illinois Extension, Urbana. (http://bulletin.ipm.illinois.edu/article.php?id=1704) (accessed 5 March 2016).

Gray, M. E., T. W. Sappington, N. J. Miller, J. Moeser, and M. O. Bohn. 2009. Adaptation and invasiveness of western corn rootworm: intensifying research on a worsening pest. Annu. Rev. Entomol. 54:303–21.

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.

Hesler, L. S., and G. R. Sutter. 1993. Effect of trap color, volatile attractants, and type of toxic bait dispenser on captures of adult corn rootworm beetles (Coleoptera: Chrysomelidae). Environ. Entomol. 22: 743–750.

Hill, R. E., and Z B Mayo. 1980. Distribution and abundance of corn rootworm species as influenced by topography and crop rotation in eastern Nebraska. Environ. Enlomol. 9: 122–127.

Hills, T. M., and D. C. Peters, 1971. A method of evaluating postplanting insecticide treatments for control of western corn rootworm larvae. J. Econ. Entomol. 64: 764–765

Hou, X., L. J. Meinke, and T. J. Arkebauer. 1997. Soil moisture and larval western corn rootworm injury: influence on gas exchange parameters in corn. Agron. J. 89: 709–717.

Johnson, T. B., and F. T. Turpin. 1985. Northern and western corn rootworm (Coleoptera: Chrysomelidae) oviposition in corn as influenced by foxtail populations and tillage systems. J. Econ. Entomol. 78: 57–60.

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. Agron. J. 77: 769–774.

Knodel, J. J. 2012. Western corn rootworm in corn in North Dakota. Corn Talk. Oct-Nov.2012. ND Corn Growers Association, pp. 12–13. Fargo, ND.

Krysan, J. L. 1986. Introduction: biology, distribution, and identification of pest *Diabrotica*, pp. 1–23. *In*: J. L. Krysan, and T. A. Miller (eds.), Methods for the study of pest *Diabrotica*. Springer-Verlag, New York.

Krysan, J. L., and R. F. Smith. 1987. Systematics of the *virgifera* species group of *Diabrotica* (Coleoptera: Chrysomelidae: Galerucinae). *Entomography* 5: 375–484.

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.

Levine, E., and H. Oloumi-Sadeghi. 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.

Levine, E., H. Oloumi-Sadeghi, and J. R. Fisher. 1992. Discovery of multiyear diapause in Illinois and South Dakota northern corn rootworm (Coleoptera: Chrysomelidae) eggs and incidence of the prolonged diapause trait in Illinois. J. Econ. Entomol. 85: 262–267.

Levine, E., J. L. Spencer, S. A. Isard, D. W. Onstad, and M. E. Gray. 2002. Adaptation of the western corn rootworm to crop rotation: evolution of a new strain in response to a management practice. Am. Entomol. 48: 94–107.

Ludwig, K. A., and R. E. Hill. 1975. Comparison of gut contents of adult western and northern corn rootworms in northeast Nebraska. Environ. Entomol. 4: 435–438.

Ma, B. L., F. Meloche, and L. Wei. 2009. Agronomic assessment of Bt trait and seed or soil-applied insecticides on the control of corn rootworm and yield. Field Crops Res. 111: 189–196.

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

Metcalf, R. L. 1986. Foreword, pp. vii–xi. *In* J. L. Krysan, and T. A. Miller (eds.), Methods for the study of pest *Diabrotica.* Springer, New York.

Obopile, M., R. B. Hammond, and P. R. Thomison. 2013. Interaction among planting dates, transgenic maize, seed treatment, corn rootworm damage and grain yield. J. Appl. Entomol. 137: 45–55.

Ordosch, D., R. Narem, and A. Szczepaniec. 2016. Effectiveness of Bt maize against corn rootworm (Coleoptera: Chrysomelidae) and species composition in South Dakota ten years following the introduction of transgenic maize. J. Agric. Urban Entomol. 32: 59–70.

SAS Institute. 2013. SAS Version 9.4. SAS Institute, Cary, NC.

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: 1585–1591.

Steffey, K. L., M. E. Rice, J. All, D. A. Andow, M. E. Gray, and J. W. Van Duyn. 1999. Handbook of corn insects: pest information. Entomological Society of America, Lanham, MD.

Student. 1908. The probable error of a mean. Biometrika 6: 1–25.

Rak Cizej, M., and I. Škerbot. 2015. Monitoring of western corn rootworm (*Diabrotica virgifera virgifera* LeConte) on the area of the Celje region from 2004 to 2014. Novi izzivi v agronomiji, pp. 252–259.

Riedell, W. E. 1990. Rootworm and mechanical damage effects on root morphology and water relations in maize. Crop Sci. 30: 628–631.

Riedell, W. E., and A. Y. Kim. 1990. Anatomical characterization of western corn rootworm damage in adventitious roots of maize. J. Iowa Acad. Sci. 97: 15–17.

Riedell, W. E., and G. R. Sutter. 1995. Soil moisture and survival of western corn rootworm larvae in field plots. J. Kans. Entomol. Soc. 68: 80–84.

Urías-López, M. A., L. J. Meinke, L. G. Higley, and F. J. Haile. 2000. Influence of western corn rootworm (Coleoptera: Chrysomelidae) larval injury on photosynthetic rate and vegetative growth of different types of maize. Environ. Entomol. 29: 861–867.

(USDA–ERS) U.S. Department of Agriculture–Economic Research Service. 2016. Adoption of genetically engineered crops in the U.S.: recent trends in GE adoption. (https://www.ers.usda.gov/data-products/adoption-of-genetically-engineered-crops-in-the-us/recent-trends-in-ge-adoption/) (accessed 18 April 2017).

(USDA–NASS) U.S. Department of Agriculture–National Agricultural Statistics Service. 2015. North Dakota Agricultural Statistics 2015. Ag. Statistics No. 84. ND.

Wangila, D. S., A. J. Gassmann, J. L. Petzold-Maxwell, B. W. French, and L. J. Meinke. 2015. Susceptibility of Nebraska western worn rootworm (Coleoptera: Chrysomelidae) populations to Bt corn events. J. Econ. Entomol. 108: 742–751.

Wright, R. J., M. E. Scharf, L. J. Meinke, X. 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.

Zukoff, S. N., K. R. Ostlie, B. Potter, L. N. Meihls, A. L. Zukoff, L. French, M. R. Ellersieck, B. W. French, and B. E. Hibbard. 2016. Multiple assays indicate varying levels of cross resistance in Cry3Bb1-selected field populations of the western corn rootworm to mCry3A, eCry3.1Ab, and Cry34/35Ab1. J. Econ. Entomol. 109: 1387–1398.

Zar, J. H. 1984. Biostatistical analysis, 2nd ed. Prentice-Hall, Englewood Cliffs, NJ.

CHAPTER III. TRANSGENIC Bt CORN, SOIL INSECTICIDE, AND INSECTICIDAL SEED TREATMENT EFFECTS ON CORN ROOTWORM (COLEOPTERA: CHRYSOMELIDAE) BEETLE EMERGENCE, LARVAL FEEDING INJURY, AND CORN YIELD IN NORTH DAKOTA[1]

Abstract

Northern, *Diabrotica barberi* Smith & Lawrence, and western, *D. virgifera virgifera* LeConte, corn rootworms are economic pests of corn, *Zea mays* L., in North America. We measured the impacts of corn hybrids incorporated with Cry3Bb1, Cry34/35Ab1, and pyramided (Cry3Bb1 + Cry34/35Ab1) *Bacillus thuringiensis* Berliner (Bt) proteins, tefluthrin soil insecticide, and clothianidin insecticidal seed treatment on beetle emergence, larval feeding injury, and corn yield at five locations from 2013 to 2015 in eastern North Dakota. In most cases, emergence was significantly lower in Bt-protected corn than in non-Bt corn hybrids. Exceptions included Wyndmere, ND (2013), where *D. barberi* emergence from Cry34/35Ab1 plots was not different from that in the non-Bt hybrid, and Arthur, ND (2013), where *D. v. virgifera* emergence from Cry3Bb1 plots did not differ from that in the non-Bt hybrid. Bt hybrids generally produced increased grain yield compared to non-Bt corn where rootworm densities were high, and larval root-feeding injury was consistently lower in Bt-protected plots than in non-Bt corn. The lowest overall feeding injury and emergence levels occurred in plots planted with the Cry3Bb1 + Cry34/35Ab1 hybrid. Time to 50% cumulative emergence of both species was 5 to 7 d later in Bt-protected than in non-Bt hybrids. Tefluthrin and clothianidin were mostly inconsequential in

---

[1] The material in this chapter was co-authored by Calles-Torrez, V., J. J. Knodel, M. A. Boetel, C. D. Doetkott, K. K. Podliska, J. K. Ransom, P. Beauzay, B. W. French, and B. W. Fuller; and was published in the Journal of Economic Entomology 111(1): 348–360, 2018. V. Calles-Torrez had primary responsibility for collecting samples in the field and for processing samples in the laboratory. V. Calles-Torrez was the primary developer of the conclusions that are advanced here. Co-authors served as proofreader and checked all content of this experiment conducted by V. Calles-Torrez.

relation to beetle emergence and larval root injury. Our findings could suggest that some North Dakota populations could be in early stages of increased tolerance to some Bt toxins; however, Bt corn hybrids currently provide effective protection against rootworm injury in the state.

Key Words: *Diabrotica barberi, Diabrotica virgifera virgifera,* Bt corn, Cry protein, soil insecticide, emergence, root injury, yield, resistance

Introduction

United States producers contributed over one-third of the world’s corn production in 2015 (U.S. Department of Agriculture–Foreign Agriculture Service [USDA–FAS] 2016). According to the National Corn Growers Association (NCGA), the U.S. corn crop was valued at greater than US$48 billion in 2015 (NCGA 2016). In North Dakota, corn plantings exceeded one million ha, and the crop was valued at more than US$1 billion that year. Sixty-three percent of the corn production in North Dakota occurs in southeastern and east central areas of the state (U.S. Department of Agriculture–National Agricultural Statistics Service [USDA–NASS] 2016).

Northern, *Diabrotica barberi* Smith & Lawrence, and western corn rootworms, *D. virgifera virgifera* LeConte, (Coleoptera: Chrysomelidae) are major economic pests of corn, *Zea mays* L., in North America. In recent years, increasing corn rootworm infestation levels and associated larval root-feeding injury have been observed in North Dakota corn production fields (Knodel 2012), thus increasing its profile in the state as a serious economic corn pest. Corn rootworm larvae injure corn plants by feeding on seminal, lateral, and nodal roots. Severe injury that results in destruction of nodal roots can cause plant lodging or ‘goosenecking’ symptomology and complicate harvest operations (Chiang 1973, Steffey et al. 1999, Levine et al. 2002), and can also reduce yield (Spike and Tollefson 1991). Plants that sustain extensive feeding injury to the root system also can display drought-like symptoms due to a diminished capacity of roots to assimilate water and nutrients (Kahler et al. 1985, Riedell 1990, Levine and Oloumi-Sadeghi 1991, Levine et al. 2002). Root-feeding injury by corn rootworm larvae also can negatively impact the nutrient content in grain and yield (Kahler et al. 1985). Corn rootworm beetles feed on corn leaves, silks, pollen, and developing kernels (Ludwig and Hill 1975). Beetle feeding injury on silks can reduce pollination, and both silk and kernel feeding injury can contribute to yield loss. Larval and beetle feeding sites can also provide an entry point for fungal infections (Palmer and Kommedahl 1969). Corn rootworms have been estimated to cost U.S. producers over US$1 billion annually in damage and control costs (Metcalf 1986, Mitchell 2011).

Corn hybrids expressing *Bacillus thuringiensis* Berliner (Bt) insecticidal proteins have been widely used by U.S. corn producers to manage corn rootworms since 2003. Thus far, the rootworm-active Bt toxins incorporated into corn are expressed at “less than high-dose” concentrations (U.S. Environmental Protection Agency [USEPA 2002], Siegfried et al. 2005, Storer et al. 2006, Hibbard et al. 2011, Gassmann 2012), which is believed to increase the risk of resistance development in target pests (Gould 1998, Tabashnik et al. 2013, Andow et al. 2016). The first field-evolved resistance in *D. v. virgifera* to Bt insect-protected corn involved Iowa populations of 2009 and hybrids expressing the Cry3Bb1 protein (Gassmann et al. 2011). Subsequently, Gassmann et al. (2014) reported on cross-resistance between Cry3Bb1- and mCry3A-expressing corn in Iowa populations of *D. v. virgifera*. Complete or incomplete cross-resistance has also been documented between Cry3Bb1 and both mCry3A and eCry3.1Ab proteins in Minnesota and Iowa populations of *D. v. virgifera* (Jakka et al. 2016, Zukoff et al. 2016). Since the initial detections in 2009, the distribution of *D. v. virgifera* field populations exhibiting resistance to Bt proteins has expanded considerably within in the north central U.S., principally in fields with a history of continuous Bt corn plantings (Gassmann et al. 2012, 2014, Gray 2012, Wangila et al. 2015, Zukoff et al. 2016). Oyediran et al. (2016) conducted diet bioassays determined that *D. barberi* from a laboratory colony remained susceptible to mCry3A and eCry3.1Ab proteins. As such, field resistance to Bt corn has not yet been reported in *D. barberi* populations. The impacts of corn rootworm-active Bt proteins on North Dakota populations of *D. barberi* and *D. v. virgifera* have not been previously investigated.

We conducted field studies between 2013 and 2015 in eastern North Dakota to compare corn rootworm beetle emergence rates, larval root-feeding injury levels, and resulting grain yields in corn hybrids expressing single or pyramided Bt proteins with that in a non-Bt corn hybrid. The potential impacts of including a soil insecticide for additive root protection and corn rootworm population management were also assessed to optimize rootworm management recommendations for North Dakota producers. Our specific research objectives included the following: 1) assess the impacts of Bt corn hybrids expressing Cry3Bb1, Cry34/35Ab1, and pyramided Cry3Bb1 + Cry34/35Ab1 proteins on emergence of *D. barberi* and *D. v. virgifera* beetles in North Dakota; 2) determine if the deployment of Bt corn hybrids, with and without tefluthrin insecticide, provide effective protection from root feeding injury by corn rootworm larvae; 3) evaluate the impacts of clothianidin (i.e., Poncho 1250) insecticidal seed treatment on root protection and corn rootworm beetle emergence; and 4) estimate the impacts of these management tools on corn grain yield and emergence timing for both species in North Dakota.

Materials and Methods

Field Sites

Research was conducted at five locations in southeastern and east central North Dakota from 2013 to 2015: 1) Arthur (Cass Co.; 47.167995 N, -97.360519 W) in 2013 and 2014 (i.e., same field both years); 2) Wyndmere (Richland Co.; 46.442222 N, -97.141389 W) in 2013; 3) Hope (Steele Co.; 47.400833 N, -97.7025 W) in 2014; 4) Page (Cass Co.; 47.166389 N, -97.498889 W) in 2014; and 5) Page (Cass Co.; 47.1375 N, -97.611111 W) in 2015. All field sites had been in continuous corn production for at least three years before our research, and all were reported by the respective growers as experiencing economic loss resulting from corn rootworm feeding injury. The Arthur site was planted with Cry3Bb1-expressing Bt corn hybrids for three consecutive years immediately prior to our study. Additional detailed field history information was not available from corn growers for the other study sites.

Corn Hybrids and Insecticides

Five corn hybrids were tested in this study. Three of the hybrids expressed the following Cry toxin proteins: 1) toxin protein Cry3Bb1 expressed in the event MON88017 (planted Dekalb DKC43-27 VT3 in 2013 and DKC43-48 in 2014 and 2015 [Monsanto Company, St. Louis, MO]); 2) toxin protein Cry34/35Ab1 expressed in the event DAS 59122-7 (planted Pioneer P9675AMRW with seed treatment coating Poncho 1250 in 2013 and P9526AMX without seed treatment in 2014 and 2015 [DuPont Pioneer, Johnston, IA]); and 3) pyramided toxin proteins Cry3Bb1 + Cry34/35Ab1 expressed in the event MON89034 x TC1507 x MON88017 x DAS 59122-7 (planted Dekalb DKC44-13 all three years [Monsanto Company]). The other two hybrids were non-Bt corn hybrids that were both agronomically similar (i.e., of common parental lineage and physiological maturity class) to the Bt hybrids. Pioneer P9675R seed (DuPont Pioneer), with and without Poncho 1250 insecticidal seed treatment coating was used as a non-Bt hybrid in 2013, whereas Pioneer P9526AM with Poncho 1250 and Pioneer P8640AM without an insecticidal seed treatment were used as non-Bt hybrids in 2014 and 2015). Although hybrids planted in 2013 varied from the other Bt hybrids in the following two years due to seed unavailability, the same active Bt toxins were present in the hybrids used each year.

Seeds of one of the non-Bt hybrids planted in all years and Pioneer P9675AMRW planted only in 2013, were coated with seed treatment Poncho 1250 (PONCHO/VOTiVO [clothianidin, 1.25 mg active ingredient [a.i] per kernel; Bayer CropScience, Research Triangle Park, NC]), a rate at which clothianidin is considered to provide some protection against rootworms. Due to difficulties obtaining untreated seed from seed vendors, other seed hybrids were coated with Cruiser 250 (thiamethoxam, 0.25 mg a.i. per kernel; Syngenta Crop Protection, Greensboro, NC) or Acceleron (clothianidin, 0.5 mg a.i. per kernel; Monsanto Company) seed treatments; these rates are insufficient for corn rootworm control and are, thus, not labeled as such (Knodel et al. 2016). All hybrids were Roundup Ready® (Monsanto Company), a genetic modification that confers resistance to plant injury that would otherwise result from direct application of glyphosate herbicide (National Academies of Sciences [NAS] 2016).

Experimental Design and Plot Management

Experimental plots were arranged in a randomized complete block design with a split plot arrangement (Steel and Torrie 1980) and four replications at each site. The main factors tested were corn hybrids and soil insecticide. Corn hybrids were assigned to 20 whole plots, and soil insecticide was assigned to 40 subplots. Twenty of the 40 subplots were treated at planting with soil insecticides and 20 subplots remained untreated. Whole-plot dimensions were 6 m long by 6 m wide. Subplots were half of the width of whole plots, and each contained four corn rows of an individual hybrid that were spaced 0.8 m apart.

Corn hybrids were planted on 24 May at Wyndmere and Arthur in 2013; on 22 May at Hope, on 23 May at Page, and on 27 May at Arthur in 2014; and on May 22 at Page 2015. Corn was planted at 85,251 seeds per hectare with 15.2 cm interplant spacing. All plots were fertilized with 46-0-0 (i.e., 46% urea nitrogen, 0% phosphate, and 0% potash, respectively) granular urea when plants were at the V4 stage of physiological development (Ritchie et al. 1986) for a yield potential of 11,000 kg/ha. The fertilizer rate varied by location based on results of cooperator soil testing at each site. One subplot of each whole plot was treated with tefluthrin (Force® 3G [3% a.i. granular]; Syngenta Crop Protection, Greensboro, NC) at planting time at 0.47 g (product)/m by using in-furrow placement, and delivery rate was controlled by using Gandy Junior (Gandy Co., Owatonna, MN) insecticide metering devices. Glyphosate-based postemergence herbicide applications were made in mid-June of each year to control weeds in plot alleys at each field site plots. Within-plot weed management was achieved by hoeing and hand-weeding.

During mid-July of each year, leaf tissue from two to four randomly selected plants per subplot was tested in the field by using toxin-specific QuickStick™, EnviroLogix™, (EnviroLogic, Portland, ME) test kits to confirm the expression of Bt endotoxins. All Bt plants tested positive for presence of the respectively intended Cry protein; similarly, non-Bt plants were tested with QuickStick™ combo kits designed for pyramided Bt hybrids, and all displayed a negative reaction.

Emergence Cages and Beetle Collection

One black soil emergence cage (i.e., 96 x 26 mesh, 0.6 x 0.6 x 0.6 m) (MegaView Science Co., Ltd. Taichung, Taiwan) was placed per subplot at the center of the second row in early July (plants were in V6 to V7 stage). A total of 40 cages were used per field site. Each cage was positioned to cover four plants that were cut off at between 30 and 45 cm above the soil surface immediately before cage placement as described by Frank et al. (2015). Each cage body had four loops (i.e., one at each corner) for connecting the tent pegs, and flaps around its base that were covered with soil to keep emerged beetles inside of the cage. One side of each cage had a large dome-shaped zipper for access to the interior. Two vials were connected to each other at the cage top; the upper vial had a hole that joined with the wrapped mesh of the trap, allowing entrance of beetles into the vial after being trapped inside of the emergence cage.

Beetles were collected from traps once per week by using a black handheld D-cell insect aspirator (Fulton MX-pp1/U, Gempler's, Janesville, WI). The aspirator was comprised of a handle (200 mm length x 49 mm diameter), suction tube (153 mm length and 9 mm internal diameter), and collector container (45 mm length and 25 mm internal diameter). Collector containers containing beetles were returned to the laboratory and stored in a conventional freezer pending processing. Beetles were identified to species using an identification key (Krysan 1986) and microscope at 10x magnification (10 x/23, Nikon SMZ-2T). Beetles were also identified by sex (Krysan 1986) for the Arthur 2013, Wyndmere 2013, and Page 2014 and 2015 field sites, but not for Arthur and Hope 2014 because those beetles were maintained in the laboratory for another experiment. Voucher specimens of both species were deposited in the North Dakota Insect Museum at North Dakota State University.

Root Injury Ratings

During late August and early September (plants were in R3 or R4 stages), roots of 10 randomly selected plants were excavated from each subplot for Arthur 2013 and 2014 and Page 2015; root injury was not evaluated at the other sites due to low beetle populations. Distance between sampled plants within each plot row was at least 1 m. Excavation consisted of a distance of approximately 20 cm outward from the stalk in all directions and a soil depth of 30 cm. Subsequently, roots were washed, and rated for rootworm feeding injury in accordance with the node-injury scale of Oleson et al. (2005).

Corn Grain Yield

Corn was harvested from the two center rows of each subplot by using a plot combine on 22 October at Wyndmere and Arthur in 2013, on 24 October at Arthur, Page, and Hope in 2014, and on 22 October at Page in 2015. If corn lodging was evident in plots, then corn ears were hand-harvested and placed into the combine for threshing. Grain mass was converted to metric tons per hectare and adjusted to 15.5% moisture content.

Timing of Emergence Probability

The appropriate Julian day was assigned to the timing of each beetle collection date from each field site and year. Beetles from the same Julian date for each hybrid and soil insecticide were summed across six sites (Wyndmere 2013, Arthur 2013 and 2014, Hope 2014, and Page 2014 and 2015) for *D. barberi* and five sites (Wyndmere 2013, Arthur 2013 and 2014, Hope 2014, and Page 2015) for *D. v. virgifera*.

Data Analysis

Numbers of emerged beetles for each species were counted per subplot for each field site. All beetle count data were square-root (x + 0.5) transformed prior to conducting analyses of variance to meet the assumption of normality and equal variances. All data were analyzed using the General Linear Models (GLM) procedure for a randomized complete block design with a split plot arrangement ($P \leq 0.05$). Hybrid and soil insecticide were considered as fixed effects, and replications were considered as random effects for beetle emergence, root injury, and corn yield analysis. Statistical analysis was conducted by field site because the assumption of homogeneity at a 95% confidence level was violated using either Bartlett's Chi-Square Test (Bartlett 1937) or Levene's Test (Levene 1960). As such, a combined analysis could not be performed on the data. Larval root-feeding injury and corn grain yield data were analyzed per field site using the GLM procedure at an alpha level of 0.05). Root injury rating data from Arthur 2014 and Page 2015 were transformed using log 10 to meet the assumption of normality (Shapiro and Wilk 1965) and constant variance (Levene 1960) before analysis. Root injury data from Arthur 2013 and corn yield data from all sites were not transformed because data met the required normality and variance assumptions. The LS-Means / Lines procedure (SAS Institute 2013) was used to compare treatment means for beetle emergence, root injury rating, and corn yield at an alpha level of 0.05.

Analysis of variance ($P < 0.05$) was performed across four field sites (i.e., Wyndmere 2013, Arthur 2013, and Page 2014 and 2015) for emergence of *D. barberi* by sex and by plots with and without tefluthrin. Tukey's Honestly Significant Difference (HSD) test (Tukey 1953) was used for multiple comparison procedures at an alpha level of 0.05. Similar analysis was performed for *D. v. virgifera*, but only on data from the Arthur 2013 site because beetle emergence at the other study sites was either low, nonexistent, or the beetles could not be handled for sexing due to needs for another experiment. All means and standard errors presented in tables are based on untransformed data. The GLM procedure in SAS (SAS Institute 2013) was used to analyze that data.

Kaplan-Meier survival curves with 95% Hall-Wellner confidence interval bands (Kaplan and Meier 1958, Hall and Wellner 1980) were used to construct emergence probability curves plotted against Julian date using the LIFETEST procedure in SAS (SAS Institute 2013). Emergence probability curves were compared pairwise among hybrids and between insecticides using the Log-Rank test. Tukey-Kramer adjusted P-values were used to control type I error across multiple pairwise comparisons between hybrids at alpha = 0.05. Numbers of male and female *D. barberi* collected from six site years and *D. v. virgifera* collected from five site years were used for the analyses.

Results

Beetle Emergence

Dates and duration of *D. barberi* and *D. v. virgifera* beetle emergence varied by site and year (Table 3.1). The earliest emergence of *D. barberi* beetles occurred during the seven-day period ending on 13 July 2013 at Wyndmere, and the latest onset began during 13 August collection period at Page in 2014. The last week of *D. barberi* emergence ranged from 2 October 2013 at Wyndmere to 18 October 2013 at Arthur. A total of 4,164 *D. barberi* beetles were trapped across six site years, with the highest populations observed at Arthur in 2013 and Page in 2015. For *D. v. virgifera*, the first week of beetle emergence was recorded on the 20 July collection at Wyndmere in 2013, and the latest emergence was observed on the 9 October collection date at Arthur in 2013. A total of 2,263 *D. v. virgifera* beetles were captured across five site years during the study; however, the majority (i.e., 97%) of populations were observed at the Arthur site in 2013. Emergence of *D. v. virgifera* from other site years was low to undetectable. Duration of the emergence period ranged from 65 to 103 days for *D. barberi* and 12 to 86 days for *D. v. virgifera*.

Table 3.1. Emergence period onset, duration, and total number of *D. barberi* and *D. v. virgifera* beetles emerged from all treatments at field sites in southeastern and east central of North Dakota, 2013–2015

Species	Site	Emergence dates		Emergence duration (days)	Total emerged beetles
		First[a]	Last[b]		
D. barberi					
	Arthur 2013	15-Jul	18-Oct	103	1833
	Wyndmere 2013	13-Jul	2-Oct	88	70
	Arthur 2014	4-Aug	7-Oct	71	373
	Page 2014	13-Aug	10-Oct	65	180
	Hope 2014	4-Aug	7-Oct	71	378
	Page 2015	30-Jul	7-Oct	76	1330
D. v. virgifera					
	Arthur 2013	22-Jul	9-Oct	86	2198
	Wyndmere 2013	20-Jul	2-Oct	81	16
	Arthur 2014	23-Aug	7-Oct	51	41
	Page 2014	N/A[c]	N/A	0	0
	Hope 2014	17-Aug	23-Aug	12	6
	Page 2015	30-Jul	20-Aug	28	2

[a] Corn was in vegetative growth stages.

[b] Most corn was in R5 (dent) or R6 (maturity) stages of physiological development (Ritchie et al. 1986).

[c] No *D. v. virgifera* beetles were captured at the Page, ND location in 2014.

No significant interactions between hybrid and soil insecticide were observed ($P > 0.05$) for *D. barberi* or *D. v. virgifera* emergence for any study site or year. Significant differences were detected for hybrid effect for *D. barberi* at all sites: Arthur 2013 ($F = 22.20$; df = 4, 12; $P < 0.0001$), Wyndmere 2013 ($F = 3.26$; df = 4, 12; $P = 0.0500$), Arthur 2014 ($F = 7.79$; df = 4, 12; $P = 0.0025$), Page 2014 ($F = 23.05$; df = 4, 12; $P < 0.0001$), Hope 2014 ($F = 8.11$; df = 4, 12; $P = 0.0021$), and Page 2015 ($F = 147.46$; df = 4, 12; $P < 0.0001$). Emergence of *D. barberi* beetles (Table 3.2) from Bt hybrid treatments was significantly lower than that in non-Bt hybrids, regardless of whether non-Bt seeds were coated with Poncho 1250, except for Cry34/35Ab1 at Wyndmere 2013. Significantly fewer *D. barberi* adults emerged from the pyramided (Cry3Bb1 + Cry34/35Ab1) hybrid than from all other treatments at Arthur in both 2013 and 2014, and numerically less beetles emerged from the pyramided hybrid than all other treatments at Wyndmere in 2013. The only instance of a significant difference in *D. barberi* emergence between the single-toxin Bt hybrids was at Page in 2015, where emergence of *D. barberi* from Cry3Bb1 was lower than that from plots planted with the Cry34/35Ab1 hybrid. Tefluthrin did not have a significant impact on *D. barberi* emergence rate at any study location in either year of the experiment.

Table 3.2. Effect of Bt corn hybrid, soil insecticide, and insecticidal treatment on *D. barberi* beetle emergence (mean ± SEM) per cage in southeastern and east central North Dakota, 2013–2015

Hybrid	Beetles emerged (mean ± SEM)					
	Arthur 2013[a]	Wyndmere 2013	Arthur 2014	Page 2014	Hope 2014	Page 2015
Non-Bt	84.00 ± 10.28a	3.63 ± 1.53a	21.13 ± 6.12a	8.63 ± 2.40a	24.00 ± 9.65a	81.25 ± 7.34a
Non-Bt + Poncho 1250	69.00 ± 8.33a	3.00 ± 1.07a	12.00 ± 2.22b	12.00 ± 3.11a	19.75 ± 4.45a	76.75 ± 10.28a
Cry3Bb1	32.88 ± 3.15b	0.25 ± 0.25b	6.75 ± 1.13c	1.38 ± 0.98b	0.25 ± 0.25b	1.25 ± 0.49c
Cry34/35Ab1[b]	28.88 ± 3.76b	1.75 ± 1.21ab	5.25 ± 2.00c	0.13 ± 0.13b	2.75 ± 1.22b	5.25 ± 1.13b
Cry3Bb1 + Cry34/35Ab1	14.38 ± 4.11c	0.13 ± 0.13b	1.50 ± 0.46d	0.38 ± 0.26b	0.50 ± 0.27b	1.75 ± 0.59c
Insecticide						
Without tefluthrin	48.05 ± 7.12a	2.25 ± 0.65a	9.75 ± 2.10a	5.55 ± 1.88a	11.95 ± 4.69a	36.90 ± 10.54a
With tefluthrin	43.60 ± 7.28a	1.25 ± 0.71a	8.90 ± 2.73a	3.45 ± 1.11a	6.95 ± 2.30a	29.60 ± 7.66a

Means sharing a letter within site and within main effect indicate no significant differences. ANOVA was performed at $P \leq 0.05$ using **sqrt** (x + 0.5) transformed data, and LS-Means / Lines was used for multiple comparisons at an alpha level of 0.05. Untransformed means are shown.

[a] The same field site was used at Arthur in 2013 and 2014.

[b] Cry34/35Ab1 coated with Poncho 1250 seed treatment in 2013.

Emergence of *D. v. virgifera* beetles was significantly impacted by hybrid at the Arthur site in 2013 (F = 23.22; df = 4, 12; $P < 0.0001$), but not at Wyndmere in 2013 or Arthur in 2014 (Table 3.3). Significantly fewer *D. v. virgifera* adults emerged from Cry34/35Ab1 and the pyramided hybrid than from plots planted with Cry3Bb1 seed and both non-Bt hybrids (i.e., with and without Poncho 1250) at Arthur in 2013. Although not statistically significant, emergence of *D. v. virgifera* beetles from the pyramided Cry3Bb1 + Cry34/35Ab1 hybrid was 43.5% lower than that from the single Cry34/35Ab1-expressing hybrid at Arthur in 2013. As observed with *D. barberi*, tefluthrin soil insecticide did not significantly impact the numbers of *D. v. virgifera* beetles that emerged from corn in our study, regardless of hybrid treatment or site. Beetle emergence rates at Wyndmere in 2013 and Arthur in 2014 were extremely low when compared to those at Arthur in 2013, and no statistical differences were detectable among hybrid or soil insecticide treatments. Emergence of *D. v. virgifera* ranged from very low to undetected across treatments at Hope in 2014 and Page in both 2014 and 2015. Therefore, statistical analyses were not performed on those data.

Table 3.3. Effect of Bt corn hybrid, soil insecticide, and insecticidal treatment on *D. v. virgifera* beetle emergence (mean ± SEM) per cage at Arthur and Wyndmere, North Dakota in 2013 and 2014

Hybrid	Beetles emerged (mean ± SEM)		
	Arthur 2013[a]	Wyndmere 2013	Arthur 2014
Non-Bt	80.75 ± 5.24a	0.50 ± 0.27a	2.00 ± 0.76a
Non-Bt + Poncho 1250	63.25 ± 13.56a	0.88 ± 0.52a	1.13 ± 0.85a
Cry3Bb1	106.50 ± 23.67a	0.25 ± 0.25a	1.75 ± 0.37a
Cry34/35Ab1[b]	15.50 ± 2.73b	0.38 ± 0.26a	0.13 ± 0.13a
Cry3Bb1 + Cry34/35Ab1	8.75 ± 2.46b	0.00 ± 0.00a	0.13 ± 0.13a
Insecticide			
Without tefluthrin	65.10 ± 13.43a	0.25 ± 0.14a	0.75 ± 0.29a
With tefluthrin	44.80 ± 8.58a	0.55 ± 0.23a	1.30 ± 0.44a

Means sharing a letter within site and within main effect indicate no significant differences. ANOVA was performed at $P < 0.05$ using **sqrt** (x + 0.5) transformed data, and LS-Means / Lines was used for multiple comparisons at an alpha level of 0.05, untransformed means are shown.

[a] The same field site was used at Arthur in 2013 and 2014.

[b] Cry34/35Ab1 coated with Poncho 1250 seed treatment in 2013.

Female and Male Beetle Emergence

Hybrid had a significant impact on the number of male *D. barberi* that emerged from plots planted without soil insecticide ($F = 8.05$; df = 4, 72; $P < 0.0001$) and those treated at planting with tefluthrin ($F = 7.55$; df = 4, 72; $P < 0.0001$) (Table 3.4). Similarly, hybrid also significantly impacted the numbers of emerged *D. barberi* females from plots established without ($F = 8.91$; df = 4, 72; $P < 0.0001$) and with a planting-time application of tefluthrin ($F = 6.61$; df = 4, 72; $P = 0.0001$). In the absence of planted tefluthrin soil insecticide applications, mean numbers of female and male *D. barberi* beetles that emerged from non-Bt hybrid plots were significantly greater than those from Cry3Bb1, Cry34/35Ab1, and the pyramided Cry3Bb1 + Cry34/35Ab1 plots. There were no significant differences between Bt hybrids in female or male *D. barberi* emergence, irrespective of whether tefluthrin insecticide was applied.

In plots treated at planting with tefluthrin, the number of *D. barberi* males that emerged from Cry34/35Ab1 were not significantly different than the non-Bt hybrid with Poncho 1250.

However, Cry3Bb1 and the pyramided hybrid produced significantly lower numbers of *D. barberi* males than both non-Bt hybrid plots. Female *D. barberi* emergence from plots established without a soil-applied insecticide was significantly lower in all Bt hybrid treatments when compared to emergence from non-Bt corn, irrespective of whether Poncho 1250 seed treatment was included. In plots that received the planting-time application of tefluthrin, female *D. barberi* emergence was significantly lower in all Bt hybrid treatments than in the non-Bt hybrid without Poncho 1250. However, emergence of *D. barberi* females from the non-Bt hybrid that included Poncho 1250 did not differ significantly from that in the Cry34/35Ab1 or Cry3Bb1 treatments when tefluthrin was applied.

Table 3.4. Mean (± SEM) number of emerged male and female *D. barberi* beetles per cage from Bt corn hybrids planted with and without tefluthrin soil insecticide in southeastern and east central North Dakota, 2013–2015

Hybrid	Without tefluthrin (mean ± SEM)		With tefluthrin (mean ± SEM)	
	Male	Female	Male	Female
Non-Bt	22.25 ± 5.52a	23.69 ± 6.03a	22.75 ± 5.88a	20.06 ± 4.81a
Non-Bt + Poncho 1250	23.81 ± 5.51a	23.56 ± 5.41a	17.25 ± 4.71ab	15.75 ± 3.60ab
Cry3Bb1	4.25 ± 1.84b	4.13 ± 1.55b	3.44 ± 1.64c	6.06 ± 2.74bc
Cry34/35Ab1	5.19 ± 1.97b	3.88 ± 1.19b	4.56 ± 1.96bc	4.38 ± 1.82bc
Cry3Bb1 + Cry34/35Ab1	2.25 ± 1.39b	2.94 ± 1.32b	1.50 ± 0.68c	1.63 ± 0.68c

Means sharing a letter within a column indicate no significant differences. ANOVA was performed at $P < 0.05$ using **sqrt** (x + 0.5) transformed data, and Tukey's Honestly Significant Difference (HSD) test was used for multiple comparison procedures at an alpha level of 0.05. Untransformed means are shown.

Hybrid had a significant impact on emergence of *D. v. virgifera* males from plots without an at-plant insecticide ($F = 10.65$; df = 4, 12; $P = 0.0006$) and those treated at planting with tefluthrin ($F = 4.16$; df = 4, 12; $P = 0.0242$) (Table 3.5). Female *D. v. virgifera* emergence was also significantly impacted by hybrid in plots without a planting-time insecticide application ($F = 19.41$; df = 4, 12; $P < 0.0001$) in plots that received tefluthrin at planting ($F = 4.61$; df = 4, 12; $P = 0.0175$). In the absence of tefluthrin, significantly fewer *D. v. virgifera* males and females emerged from Cry34/35Ab1 and Cry3Bb1 + Cry34/35Ab1 plots when compared to the numbers that emerged from Cry3Bb1 plots and those planted with non-Bt seed that was not treated with Poncho 1250. In plots treated with tefluthrin, significantly fewer *D. v. virgifera* males and females emerged from the pyramided Cry3Bb1 + Cry34/35Ab1 hybrid when compared to the non-Bt hybrid without Poncho 1250. In tefluthrin-treated plots, there were no significant differences between any of the Bt-expressing hybrids in relation to either male or female *D. v. virgifera* emergence.

Table 3.5. Mean (± SEM) number of male and female emerged *D. v. virgifera* beetles per cage from Bt corn hybrids planted with and without tefluthrin soil insecticide, Arthur, North Dakota, 2013

Hybrid	Without tefluthrin (mean ± SEM)		With tefluthrin (mean ± SEM)	
	Male	Female	Male	Female
Non-Bt	18.75 ± 2.17a	68.00 ± 1.87a	16.75 ± 2.75a	58.00 ± 9.57a
Non-Bt + Poncho 1250	12.25 ± 3.92ab	53.00 ± 14.46a	14.75 ± 6.85ab	46.50 ± 16.54ab
Cry3Bb1	33.50 ± 6.95a	114.75 ± 26.13a	13.25 ± 3.58ab	51.50 ± 17.04ab
Cry34/35Ab1	3.50 ± 1.50b	12.25 ± 2.78b	4.25 ± 1.89ab	11.00 ± 3.08ab
Cry3Bb1 + Cry34/35Ab1	3.50 ± 1.55b	6.00 ± 2.68b	1.25 ± 0.75b	6.75 ± 2.46b

Means sharing a letter within a column indicate no significant differences. ANOVA was performed at $P < 0.05$ using **sqrt** (x + 0.5) transformed data, and Tukey’s Honestly Significant Difference (HSD) test was used for multiple comparison procedures at an alpha level of 0.05. Untransformed means are shown.

Root Injury Ratings

There was no significant interaction between hybrid and soil insecticide for root injury ratings at any location or year. The hybrid effect for root injury was significant at Arthur in 2013 ($F = 54.38$; df = 4, 12; $P < 0.0001$) and 2014 ($F = 14.11$; df = 4, 12; $P = 0.0002$), and at Page in 2015 ($F = 30.92$; df = 4, 12; $P = 0.0001$) (Table 3.6). Root injury ratings at all sites were moderate to low, and ranged from 0.09 to 0.99 at Arthur in 2013, 0.03 to 0.16 at Arthur in 2014, and 0.01 to 0.35 at Page in 2015. Rootworm feeding injury ratings did not differ significantly among the three Bt corn hybrids at Arthur in 2013 or Page in 2015. However, the pyramided (i.e., Cry3Bb1 + Cry34/35Ab1) hybrid had significantly lower levels of root injury than both of the single-toxin Bt hybrids at Arthur in 2014, and numerically lower root injury ratings at Arthur in 2013 and at Page in 2015. At Arthur in both years, and at Page in 2015, the two non-Bt hybrids had significantly greater levels of rootworm feeding injury than all Bt hybrids, regardless of whether Poncho 1250 seed treatment was included. Roots in tefluthrin-treated plots at Page in 2015 had significantly (F = 13.57; df = 1, 15; P = 0.0022) lower rootworm feeding injury than those that did not receive a planting-time application of the insecticide; however, that was the only site or year in which a significant impact of tefluthrin was detected in relation to larval feeding injury.

Table 3.6. Mean (± SEM) root injury rating in Bt corn hybrids caused by corn rootworm larvae at Arthur and Page, North Dakota, 2013–2015

Hybrid	Root injury rating [a] (mean ± SEM)		
	Arthur 2013[b]	Arthur 2014	Page 2015[c]
Non-Bt	0.99 ± 0.13a	0.16 ± 0.03a	0.35 ± 0.08a
Non-Bt + Poncho 1250	0.76 ± 0.06a	0.14 ± 0.02a	0.27 ± 0.11a
Cry3Bb1	0.32 ± 0.10b	0.06 ± 0.01b	0.02 ± 0.00b
Cry34/35Ab1[d]	0.30 ± 0.07b	0.05 ± 0.01b	0.02 ± 0.00b
Cry3Bb1 + Cry34/35Ab1	0.09 ± 0.05b	0.03 ± 0.01c	0.01 ± 0.00b
Insecticide			
Without tefluthrin	0.51 ± 0.10a	0.09 ± 0.02a	0.19 ± 0.06a
With tefluthrin	0.47 ± 0.08a	0.09 ± 0.02a	0.07 ± 0.03b

Means sharing a letter within column and main effect indicate no significant differences. ANOVA was performed at $P < 0.05$ using **log 10** transformed data for Arthur 2014 and Page 2015 sites, and LS-Means / Lines was used for multiple comparisons at an alpha level of 0.05. Untransformed means are shown.

[a] Roots were rated in accordance to the node-injury scale of Oleson et al. (2005).

[b] The same field site was used at Arthur in 2013 and 2014.

[c] Field site infested only with *D. barberi*.

[d] Cry34/35Ab1 coated with Poncho 1250 seed treatment planted in 2013.

Corn Grain Yield

Interactions between hybrid and insecticide for corn grain yield were not significant for any study site or year. The hybrid effect was significant at Arthur in 2013 (*F* = 6.52; 4, 12; *P* = 0.0050), Hope in 2014 (*F* = 5.07; 4, 12; *P* = 0.0126), and Page in 2015 (*F* = 6.07; 4, 12; *P* = 0.0066). As shown in Table 3.7, the three Bt hybrids produced significantly greater grain yields than the non-Bt hybrids at Arthur in 2013 and Page in 2015. However, at Hope in 2014, yield produced by the non-Bt hybrid that included Poncho 1250 seed treatment was not significantly different from that produced by the Cry3Bb1 and Cry34/35Ab1 hybrids. Interestingly, plots planted with the non-Bt hybrid that included Poncho 1250 yielded significantly more grain than the pyramided Cry3Bb1 + Cry34/35Ab1 hybrid at Hope in 2014; however, that was the only such occurrence among all six study locations. There was no significant hybrid effect on grain yield at Wyndmere in 2013, Arthur in 2014, or Page in 2014. Additionally, there were no significant differences in grain yield between the two non-Bt hybrid treatments (i.e., with and without Poncho 1250) at Arthur in 2013, Hope in 2014, or Page in 2015. The impact of tefluthrin on grain yield was significant at Arthur in 2013 (*F* = 10.05; 1, 15; *P* = 0.0063), Arthur in 2014 (*F* = 5.62; 1, 15; *P* = 0. 0047), and Hope in 2014 (*F* = 5.62; 1, 15; *P* = 0.0316). Planting-time applications of tefluthrin insecticide significantly increased grain yield production at Arthur in both 2013 and 2014.

Table 3.7. Mean (± SEM) grain yield from Bt corn hybrids planted with and without tefluthrin soil insecticide or Poncho 1250 insecticidal seed treatment for corn rootworm control in southeastern and east central North Dakota, 2013–2015

Hybrid	Grain yield in metric tons per hectare (mean ± SEM)					
	Arthur 2013[a]	Wyndmere 2013	Arthur 2014	Page 2014	Hope 2014	Page 2015[b]
Non-Bt	5.88 ± 0.38b	10.75 ± 0.41a	8.76 ± 0.25a	8.67 ± 0.45a	8.50 ± 0.35bc	10.45 ± 0.38c
Non-Bt + Poncho 1250	6.91 ± 0.78b	11.35 ± 0.81a	7.31 ± 0.47a	8.74 ± 0.80a	9.12 ± 0.67ab	10.71 ± 0.39c
Cry3Bb1	8.52 ± 0.52a	10.08 ± 0.26a	8.16 ± 0.40a	9.20 ± 0.47a	9.67 ± 0.46a	11.71 ± 0.23b
Cry34/35Ab1[c]	9.02 ± 0.36a	10.11 ± 0.65a	8.03 ± 0.41a	9.05 ± 0.34a	9.49 ± 0.51ab	11.54 ± 0.34b
Cry3Bb1 + Cry34/35Ab1	8.95 ± 0.37a	11.53 ± 0.71a	8.47 ± 0.23a	9.00 ± 0.55a	7.89 ± 0.38c	12.65 ± 0.34a
Insecticide						
Without tefluthrin	7.28 ± 0.46b	11.00 ± 0.37a	7.78 ± 0.22b	9.00 ± 0.34a	9.31 ± 0.26a	11.19 ± 0.26a
With tefluthrin	8.43 ± 0.33a	10.53 ± 0.40a	8.51 ± 0.25a	8.86 ± 0.32a	8.55 ± 0.37b	11.62 ± 0.28a

Means followed by same letters within site and within main effect indicate no significant differences. ANOVA was performed at $P < 0.05$, and LS-Means / Lines was used for multiple comparisons at an alpha level of 0.05.

[a] The same field site was used at Arthur in 2013 and 2014.

[b] Field site infested only with *D. barberi*.

[c] Cry34/35Ab1 coated with Poncho 1250 seed treatment planted in 2013.

Timing of Emergence Probability

Emergence trapping resulted in the captures of 4,164 *D. barberi* and 2,263 *D. v. virgifera* beetles for calculation of emergence timing probabilities. Probability curves for emergence timing were significantly (χ^2: 276.59; df = 4; $P < 0.0001$) different among hybrid treatments for *D. barberi*. Significant differences were observed in most pairwise comparisons between hybrid treatments for timing of emergence, except for pairwise comparisons between the two non-Bt (i.e., with and without Poncho 1250) hybrids, and between Cry3Bb1 and Cry34/35Ab1. Emergence of *D. barberi* beetles occurred earlier from non-Bt hybrids than from Bt hybrids (Fig. 3.1A-F). The probable date for 50% emergence of *D. barberi* beetles in non-Bt hybrids occurred on Julian day 234 (i.e., August 22); whereas, it occurred in Bt hybrids on Julian day 240 (i.e., 28 August) (Fig. 3.1A-F and Table 3.8).

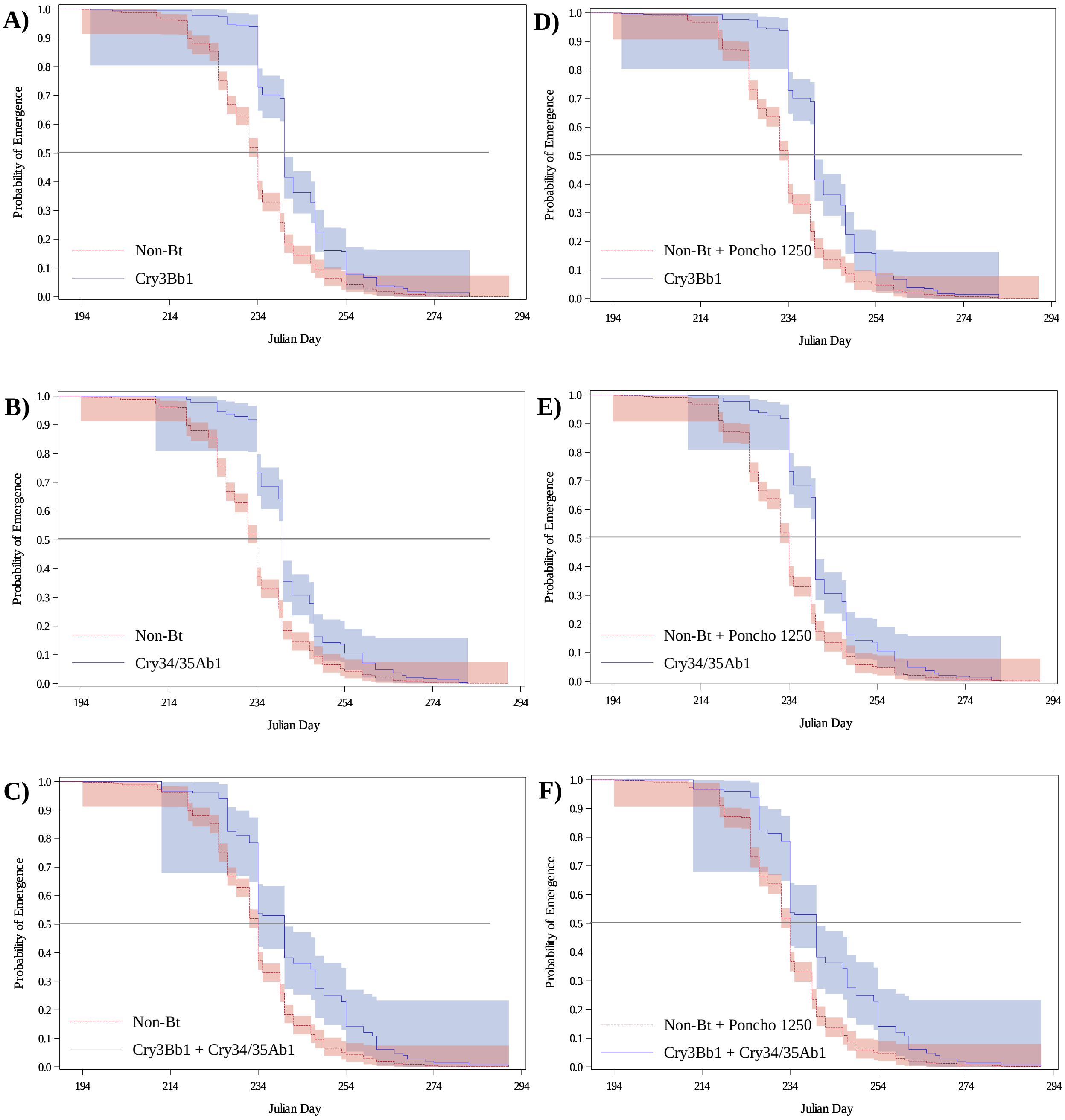


Fig. 3.1. (A-F) Probability of *D. barberi* beetle emergence across six site years in North Dakota. Emergence time at 50% was on Julian Day 234 for Non-Bt and Non-Bt + Poncho 1250 hybrids and on Julian Day 240 for Cry3Bb1, Cry34/35Ab1, and Cry3Bb1 + Cry34/35Ab1 Bt hybrids. Non-Bt and Bt corn hybrids are indicated by red and blue colors, respectively. A Kaplan-Meier estimator with a 95% Hall-Wellner confidence interval bands was used to construct emergence probability curves plotted against Julian days. Horizontal line indicates 50% emergence.

Table 3.8. Julian and Calendar dates for three cumulative percentages of probable *D. barberi* and *D. v. virgifera* emergence timing from Bt corn hybrids in North Dakota

Species	Hybrid	Date of emergence					
		25%		50%		75%	
		Julian day	Calendar day	Julian day	Calendar day	Julian day	Calendar day
D. barberi							
	Non-Bt	227	15-Aug	234	22-Aug	240	28-Aug
	Non-Bt + Poncho 1250	225	13-Aug	234	22-Aug	239	27-Aug
	Cry3Bb1	234	22-Aug	240	28-Aug	247	4-Sep
	Cry34/35Ab1	234	22-Aug	240	28-Aug	247	4-Sep
	Cry3Bb1 + Cry34/35Ab1	234	22-Aug	240	28-Aug	249	6-Sep
D. v. virgifera							
	Non-Bt	234	22-Aug	240	28-Aug	247	4-Sep
	Non-Bt + Poncho 1250	240	28-Aug	242	30-Aug	254	11-Sep
	Cry3Bb1	242	30-Aug	247	4-Sep	254	11-Sep
	Cry34/35Ab1	240	28-Aug	247	4-Sep	254	11-Sep
	Cry3Bb1 + Cry34/35Ab1	240	28-Aug	247	4-Sep	254	11-Sep

The emergence timing probability pattern for *D. v. virgifera* was also significantly (χ^2: 150.84; df = 4; $P < 0.0001$) impacted by the treatments. Most pairwise comparisons generated significant differences between hybrid treatments for *D. v. virgifera* emergence, except for the comparison between Cry34/35Ab1 and the Cry3Bb1 + Cry34/35Ab1 Bt pyramid. Emergence of *D. v. virgifera* beetles from non-Bt hybrids occurred earlier than from Bt hybrids (Fig. 3.2A-F). The timing of 50% *D. v. virgifera* emergence from non-Bt hybrids took place between Julian day 240 and 242 (i.e., 28 to 30 August); in Bt hybrids, it occurred on Julian day 247 (i.e., 4 September) (Fig. 3.2A-F and Table 3.8). Tefluthrin did not have a significant influence on emergence timing of either species.

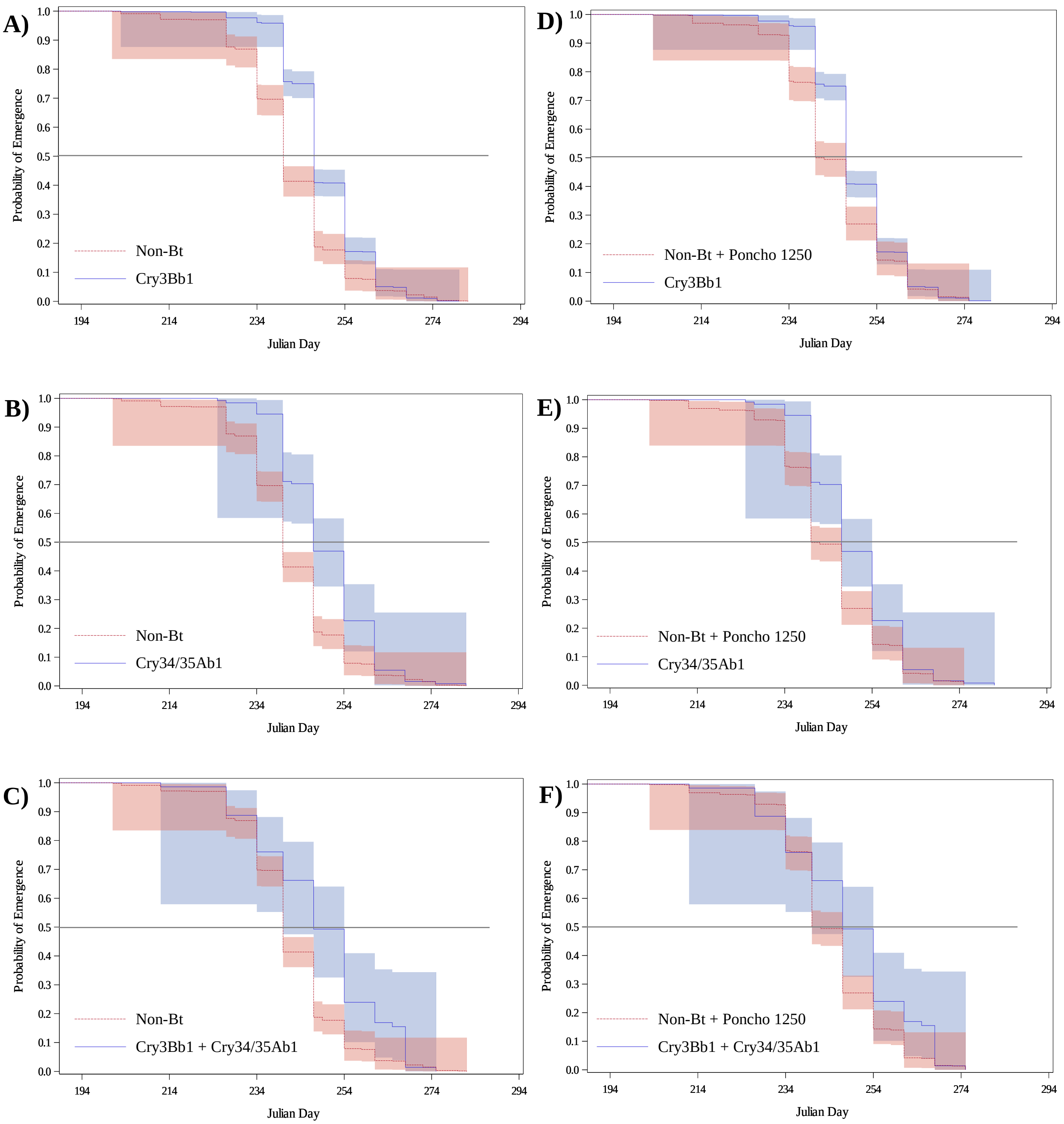


Fig. 3.2. (A-F) Probability of *D. v. virgifera* beetle emergence across five site years in North Dakota. Emergence time at 50% was on Julian Day 240 and 242 for Non-Bt and Non-Bt + Poncho 1250 hybrids, respectively, and on Julian Day 247 for Cry3Bb1, Cry34/35Ab1, and Cry3Bb1 + Cry34/35Ab1 Bt hybrids. Non-Bt and Bt corn hybrids are indicated by red and blue colors, respectively. A Kaplan-Meier estimator with a 95% Hall-Wellner confidence interval bands was used to construct emergence probability curves plotted against Julian days. Horizontal line indicates 50% emergence.

Discussion

The three Bt corn hybrids tested in this experiment generally provided adequate protection from corn rootworm feeding injury when compared to the injury that occurred in non-Bt hybrids. Significantly greater numbers of *D. barberi* emerged from the Cry34/35Ab1 hybrid than from the Cry3Bb1 + Cry34/35Ab1 pyramid at three of six study sites. Similarly, *D. barberi* emergence from Cry3Bb1 hybrid plots was significantly greater than that from pyramided hybrid plots at two sites. The mean number of *D. v. virgifera* beetles that emerged from Cry3Bb1 corn plots was also significantly greater than that for the pyramid, and it was numerically greater than the number that emerged from non-Bt corn at Arthur in 2013, thus suggesting that this population of *D. v. virgifera* could potentially be developing increased tolerance to Cry3Bb1. This particular field had a three-year history of continuous deployment of Cry3Bb1-expressing hybrids for corn rootworm management immediately before this study. Keweshan et al. (2015) observed that emergence of *D. v. virgifera* and *D. barberi* beetles from corn hybrids expressing Cry3Bb1 and pyramided (i.e., Cry3Bb1 + Cry34/35Ab1) hybrids was significantly lower than that from non-Bt corn hybrids, which supports our results for *D. barberi*. Conversely, Ordosch et al. (2016) found no difference in emergence of *D. v. virgifera* beetles between non-Bt and Cry3Bb1 corn hybrids in eastern South Dakota, which suggested the possibility of some level of tolerance or resistance to Cry3Bb1 in that population. Gassmann et al. (2011) provided the first documented case of field-evolved resistance in a *D. v. virgifera* population to Cry3Bb1 in continuous Bt corn plantings in Iowa. Since then, Cry3Bb1-resistant populations of *D. v. virgifera* have been reported in Illinois, Minnesota, Nebraska and South Dakota (Porter et al. 2012, Wangila et al. 2015, Zukoff et al. 2016, Ludwick et al. 2017, Schrader et al. 2017). Moreover, *D. v. virgifera* populations have shown a remarkable propensity to quickly develop resistance to Cry3Bb1, Cry34/35Ab1, mCry3A, and eCry3.1Ab after only a few successive generations of exposure in laboratory selection studies (Lefko et al. 2008, Meihls et al. 2008, 2011, Frank et al. 2013). Therefore, it is reasonable to expect that tolerance to Cry3Bb1 could be increasing in some eastern North Dakota corn rootworm populations. Factors contributing to Bt resistance development include the following: rootworm-active Bt corn hybrids are typically considered low-dose-expressing products (USEPA 2002, Siegfried et al. 2005, Storer et al. 2006, Meihls et al. 2008, Hibbard et al. 2011, Gassmann 2012); continuous corn plantings (i.e., lack of crop rotation); and repeated use of Bt corn hybrids employing the same mode of action. As such, adherence to integrated pest management principles will be essential to extending the utility of Bt toxins to control corn rootworms, as well as insect pests targeted with this technology in other cropping systems. Tabashnik and Gould (2012) proposed that increasing the proportion of refuge corn to Bt corn acreage could help delay the development of resistance in *D. v. virgifera* populations to rootworm-active Bt corn, and recommended the practice for both single-toxin and pyramided corn. Additionally, using predictive modeling, Tabashnik et al. (2004) suggested that the deployment of Bt crop plants expressing high-dose toxins to control lepidopteran insect pests would provide greater efficacy of Bt toxins against cotton pests.

Gassmann et al. (2016) detected incomplete (i.e., early stage) resistance to Cry34/35Ab1 in Iowa populations of *D. v. virgifera*, and Ludwick et al. (2017) reported on incomplete resistance to Cry34/35Ab1 in a Minnesota population of *D. v. virgifera*. Additionally, Zukoff et al. (2016) reported on incomplete resistance to Cry34/35Ab1 in Cry3Bb1-resistant populations of *D. v. virgifera* from Minnesota. In our study, *D. barberi* emergence from Cry34/35Ab1plots was significantly lower than that from non-Bt hybrids at all sites, except Wyndmere in 2013. Although there were no significant differences among treatments at Wyndmere, the

Cry34/35Ab1 plots produced about 50% fewer *D. barberi* beetles than the non-Bt hybrid without Poncho 1250 seed treatment. These findings suggest that hybrids expressing the Cry34/35Ab1 protein were effective managing *D. barberi* populations in the eastern North Dakota sites chosen for our research. The pyramided (i.e., Cry3Bb1 + Cry34/35Ab1) corn hybrid used in our testing was the most effective Bt-expressing treatment overall for reducing emergence of both *D. barberi* and *D. v. virgifera* beetles. Those findings were consistent with the results of previous researchers on pyramided Bt corn for rootworm control (Head et al. 2013, Keweshan et al. 2015).

Root injury ratings for non-Bt hybrids (i.e., with and without Poncho 1250) were significantly higher in comparison to those for all Bt hybrids at all three sites where larval feeding pressure was sufficient for statistical analyses (i.e., Arthur in 2013, Arthur in 2014, and Page in 2015). Also, grain yields of all Bt-corn hybrids were significantly greater than those of non-Bt hybrids without Poncho 1250 at the sites that had the highest, albeit moderate, rootworm populations (i.e., 44.9 to 53% increases at Arthur in 2013 and 10.4 to 21.1% increases at Page in 2015). The highest root injury ratings in this study were recorded for the non-Bt hybrid at Arthur in 2013. Those roots averaged nearly 1.0 on the node-injury scale of Oleson et al. (2005), which equates to a complete node of roots pruned. Conversely, root injury ratings in the Bt hybrids averaged one-third or less of one node pruned, which is below a level that would cause economic damage according to the economic injury level matrix developed by Oleson et al. (2005). Root injury ratings of 1.0 in corn hybrids expressing a single Bt protein and 0.5 in pyramided hybrids are used as respective thresholds for regulatory triggers for follow-up investigations on cases of unexpected damage (USEPA 2014). Tinsley et al. (2013) and Dun et al. (2010) found that an average of one node of roots pruned by corn rootworm can result in 15% to 17.9% corn yield loss. In our study, the Cry3Bb1 corn hybrid had significantly lower rootworm feeding injury and numerically greater yield than the non-Bt corn hybrids at sites where the hybrid effect was significant. Petzold-Maxwell et al. (2013) also reported that yield produced by Cry3Bb1 corn was significantly greater than a non-Bt hybrid. Ma et al. (2009) concluded that Cry3Bb1 controlled *Diabrotica* spp. larvae and protected yield under severe infestations. No yield differences were observed among hybrids in our study at Wyndmere in 2013, Arthur in 2014, or Page in 2014. This was probably mainly due to the low rootworm infestations that occurred in these site years. However, it supports the conclusions of previous researchers that yield tends to be more adversely impacted by corn rootworm larval feeding injury under high-stress environments than in those characterized as low- or moderate-stress environments (Oleson et al. 2005, Tinsley et al. 2015). Spike and Tollefson (1991) also concluded that corn rootworm infestations did not consistently reduce grain yield. Obopile et al. (2013) found that rootworm impacts on grain yield can vary significantly with planting date, Bt hybrid, and insecticidal seed treatment, and also can be variable among years and locations. In our study, Bt corn hybrids reduced rootworm feeding injury at all sites, and produced an average of 8% more grain yield than the two non-Bt corn hybrids.

In previous research conducted in Iowa, Nebraska, and Illinois, Petzold-Maxwell et al. (2013) found that both Poncho 1250 and Aztec® soil insecticide (tebupirimphos + cyfluthrin, AMVAC Chemical Corporation, Newport Beach, CA) resulted in reduced rootworm larval feeding injury in non-Bt corn; however, neither of the insecticides affected non-Bt corn grain yield. Those authors also reported that, although both insecticides delayed emergence of *D. barberi* and *D. v. virgifera* beetles, reductions in survival were only observed with *D. v. virgifera* in Aztec-treated plots. Our findings indicated that corn rootworm larval feeding injury was not reduced by Poncho 1250 when included with non-Bt hybrids at sites where a significant hybrid effect was detected. The addition of Poncho 1250 to non-Bt seed had no significant effect on numbers of emerged *D. barberi* and *D. v. virgifera* beetles, except at Arthur in 2014, where *D. barberi* emergence from untreated non-Bt corn was about 76% greater than that from non-Bt corn treated with Poncho 1250. Despite our findings, those of Petzold-Maxwell et al. (2013) suggest that insecticidal seed treatments such as Poncho 1250 could augment root protection to some degree when higher corn rootworm infestations occur. The results of van Rozen and Ester (2010) could suggest that the slight differences between impacts of Poncho 1250 on rootworm survival in our study and that of Petzold-Maxwell et al. (2013) could have been influenced by soil characteristics, as well as soil temperature and/or moisture.

Tefluthrin soil insecticide applications had no significant impact on emergence of *D. barberi* or *D. v. virgifera* beetles at any of our eastern North Dakota study sites. These results are similar to those of Boetel et al. (2003) who observed equivalent numbers of emerged beetles between tefluthrin-treated and untreated control plots of conventional (i.e., non-Bt) corn in a mixed population of *D. barberi* and *D. v. virgifera* in South Dakota. Similarly, Gray et al. (1992) observed greater numbers of *D. v. virgifera* beetles emerging from soil insecticide- (i.e., carbofuran, chlorpyrifos, and terbufos) treated plots than from untreated non-Bt corn in certain years. Gray et al. (1992) and Boetel et al. (2003) both concluded that the use of soil insecticides can provide significant levels of root protection, but the practice does not always result in management of corn rootworm populations. Petzold-Maxwell et al. (2013) and Frank et al. (2015) evaluated the impacts of tefluthrin and Aztec 2.1G soil insecticides on corn rootworm survival, and both found no significant impact from either insecticide on *D. barberi* emergence. Petzold-Maxwell et al. (2013) further concluded that planting-time applications of Aztec did not impact rootworm feeding injury levels or grain yields in Cry3Bb1-protected corn. We only observed significant differences in corn rootworm feeding injury ratings between tefluthrin and non-tefluthrin plots at the Page site in 2015. The feeding injury at Page was likely imposed by *D. barberi*, because no *D. v. virgifera* beetles were collected at that location in 2015.

Grain yield was significantly increased by using tefluthrin soil insecticide at Arthur in 2013 and 2014, as well as Hope in 2014. The Arthur 2013 site had the highest corn rootworm populations of any site year in our study. There was no significant impact of tefluthrin on grain yield at Wyndmere in 2013 or Page in either 2014 or 2015, all of which had low to moderate corn rootworm infestation levels. Tinsley et al. (2015) indicated that the addition of tefluthrin to Bt corn hybrid seed blends did not consistently provide a yield benefit under moderate to heavy corn rootworm larval pressure. Johnson et al. (2017) found that planting-time applications of tefluthrin with Cry34Ab1/Cry35Ab1, both alone and pyramided with Cry3Bb1, resulted in improved root protection from corn rootworm feeding injury when high larval root feeding pressure was present. In contrast, those authors saw no significant root protection or yield benefit under moderate larval feeding pressure. Our varied findings regarding the effects of tefluthrin applications on root injury and yield, as well as those reported by Tinsley et al. (2015) and Johnson et al. (2017), could have been impacted by multiple environmental factors. This suggestion is supported by the findings of Levine and Oloumi-Sadeghi (1991) and van Rozen and Ester (2010) who concluded that insecticide efficacy, in relation to corn rootworm management, can be significantly affected by both biotic and abiotic factors, such as soil pH and soil moisture.

Our combined analysis of data from four site years in eastern North Dakota showed that, in the absence of an at-plant soil insecticide, significantly fewer male and female *D. barberi* emerged from Cry3Bb1, Cry34/35Ab1, and pyramided hybrid plots than from non-Bt plots. This was also mostly the case for *D. v. virgifera*; however, planting the Cry3Bb1 hybrid did not result in significant reductions in emergence of either sex of this species when deployed without a soil insecticide at Arthur in 2013. Our findings on *D. v. virgifera* are in slight contrast to those of Hitchon et al. (2015) who observed significant reductions in emergence of both female and male *D. v. virgifera* from Cry3Bb1 and pyramided Cry3Bb1 + Cry34/35Ab1 plots than from non-Bt hybrid plots in Ontario, Canada.

In tefluthrin-treated plots, there were no significant differences in emergence of either male or female *D. v. virgifera* beetles among the three Bt hybrids we tested; however, emergence rates from both Cry3Bb1 and Cry34/35Ab1 were not different from the untreated non-Bt hybrid. Boetel et al. (2003) observed significant (i.e., 10-day) delays in emergence onset and 37% reductions in total emergence of female *D. barberi* from non-Bt hybrid plots treated at planting with tefluthrin when compared to that from untreated non-Bt corn. Similarly, Frank et al. (2015) observed reductions in *D. barberi* (sexes combined) and both male and female *D. v. virgifera* emergence by applying tefluthrin at planting with 100% pyramided (i.e., mCry3A + eCry3.1Ab) corn, a 95:5% (i.e., pyramid:isoline) seed blend, and a near-isoline hybrid when compared to the same seed types without tefluthrin. Frank et al. (2015) also observed delayed emergence of *D. v. virgifera* by using tefluthrin in the above-mentioned pyramid, pyramid/isoline blend, and isoline corn treatments. Our findings regarding the impacts of tefluthrin on corn rootworm emergence suggest that inclusion of a soil insecticide with Bt corn for rootworm control could potentially mask otherwise detectable cases of what would be classified as unexpected damage.

Onset and duration of *D. barberi* and *D. v. virgifera* beetle emergence were not consistent among site years in this study. General patterns observed were that *D. barberi* emerged between mid-July and mid-October, and *D. v. virgifera* emergence took place from late July to the first week of October. However, previous research indicates that corn rootworm beetle emergence can vary among years and locations. Murphy et al. (2010) reported similar results to ours for *D. v. virgifera* in Indiana, as emergence occurred from 25 June to 30 August in 2007 and from 10 July to 2 October in 2008. Beetle emergence has also been shown to be impacted by planting date (Bergman and Turpin 1984), while flight activity in the field was observed by Naranjo and Sawyer (1988) and Elliott et al. (1991) as being closely synchronized with timing of corn flowering. As such, both emergence and activity of corn rootworm adults can vary considerably due to differing environmental conditions. Regardless of this variability, the corn rootworm beetle emergence patterns observed on North Dakota populations in this study provide valuable information to assist crop consultants and corn producers in determining when to scout fields for beetles.

Emergence timing of both *Diabrotica* species in this study differed significantly between non-Bt and Bt hybrids. The timing of 50% emergence probability for beetles of both species was delayed by about one week (i.e., 5 to 7 days) in Bt hybrids when compared to non-Bt hybrids (Table 3.8). Timing of 25% and 75% beetle emergence were also delayed by Bt hybrids in *D. barberi*. These findings support the those of previous researchers who observed delayed emergence in *Diabrotica* populations that were susceptible to rootworm-specific Cry proteins (Crowder et al. 2005, Becker 2006, Murphy et al. 2010, Clark et al. 2012, Oswald et al. 2012, Petzold–Maxwell et al. 2013, Hitchon et al. 2015, Keweshan et al. 2015). The results of Wangila and Meinke (2017) further indicated that delays in larval development and resulting emergence delays can occur in Cry3Bb1-susceptible populations of *D. v. virgifera*, or those in early stages of evolving resistance to Cry3Bb1, and that the delays can become shorter as a population completes resistance development. Previous authors have also concluded that corn rootworm beetle emergence delays can facilitate assortative matings between Bt-selected individuals, which can accelerate the evolution of resistance to Bt endotoxins (Liu et al. 1999, Murphy et al. 2010, Spencer et al. 2012, Petzold-Maxwell et al. 2013, Andow et al. 2016).

The results of our study provide a better understanding regarding the status of *D. barberi* and *D. v. virgifera* ecology in eastern North Dakota, and the relative impacts of Bt hybrids expressing Cry3Bb1, Cry34/35Ab1, and pyramided combinations of these two endotoxins for controlling them in this corn production area. Although we cannot confirm incomplete resistance in the populations tested, our findings suggest at least the possibility that some could be in early stages of decreased sensitivity to these toxins. Sustaining the effectiveness of Bt corn technology to mitigate damage caused by corn rootworms is a worthy pursuit for corn production in areas affected by these pests. As such, the implementation of an IPM approach for corn rootworm management that employs alternative pest management tools (e.g., crop rotation) can assist with this effort. Further research involving field collections and laboratory bioassays will be necessary to quantify the relative susceptibility of these eastern North Dakota corn rootworm populations to Bt endotoxins and determine if increased tolerance to these materials is occurring in this corn production area.

Acknowledgments

We would like to acknowledge the late J. J. Hammond, as well as J. L. Jyoti and E. Brockmann, for their contributions to data analyses. We thank DuPont Pioneer and Monsanto Company for providing the corn seed used for this research. We also appreciate B. Berghuis, D.

Carey, E. Miller, J. Rikhus, T. Rock, A. Schroeder, and J. Uriell for their assistance with field and laboratory work. Appreciation is also extended to two anonymous reviewers whose efforts contributed to significant improvements on an earlier manuscript of this paper. Major funding support was provided by the North Dakota Corn Utilization Council. This work was also partially supported by the National Institute of Food and Agriculture, U.S. Department of Agriculture, Hatch project accession no. 0223202.

References Cited

Andow, D. A., S. G. Pueppke, A. W. Schaafsma, A. J. Gassmann, T. W. Sappington, L. J. Meinke, P. Mitchell, T. M. Hurley, R. L. Hellmich, and R. P. Porter. 2016. Early detection and mitigation of resistance to Bt maize by western corn rootworm (Coleoptera: Chrysomelidae). J. Econ. Entomol. 109: 1–12.

Bartlett, M. S. 1937. Properties of sufficiency and statistical tests. Proceedings of the Royal Society of London, Ser. A, 160: 268–282.

Becker, S. C. 2006. Stage-specific development and mortality of western and northern corn rootworm reared on transgenic event MON863 and a non-transgenic isoline field corn hybrid. M. Sc. Thesis. University of Nebraska, Lincoln.

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.

Boetel, M. A., B. W. Fuller, and P. D. Evenson. 2003. Emergence of adult northern and western corn rootworms (Coleoptera: Chrysomelidae) following reduced soil insecticide applications. J. Econ. Entomol. 96: 714–729.

Chiang, H. C. 1973. Bionomics of the northern and western corn rootworms. Annu. Rev. Entomol. 18: 47–72.

Clark, T. L., D. L. Frank, B. W. French, L. J. Meinke, D. Moellenbeck, T. T. Vaughn, and B. E. Hibbard. 2012. Mortality impact of MON863 transgenic maize roots on western corn rootworm larvae in the field. J. Appl. Entomol. 136: 721–729.

Crowder, D. W., D. W. Onstad, M. E. Gray, C. M. F. Pierce, A. G. Hager, S. T. Ratcliffe, and K. L. Steffey. 2005. Analysis of the dynamics of adaptation to transgenic corn and crop rotation by western corn rootworm (Coleoptera: Chrysomelidae) using a daily time-step model. J. Econ. Entomol. 98: 534–551.

Dun, Z., P. D. Mitchell, and M. Agosti. 2010. Estimating *Diabrotica virgifera virgifera* damage functions with field trial data: applying an unbalanced nested error component model. J. Appl. Entomol. 134: 409–419.

Elliott, N. C., J. J. Jackson, G. R. Sutter, and D. A. Beck. 1991. A descriptive study of the population dynamics of adult *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae) in artificially infested corn fields. Great Lakes Entomol. 24: 159–167.

Frank, D. L., A. Zukoff, J. Barry, M. L. Higdon, and B. E. Hibbard. 2013. Development of resistance to eCry3.1Ab-expressing transgenic maize in a laboratory-selected population of western corn rootworm (Coleoptera: Chrysomeldae). J. Econ. Entomol. 106: 2506–2513.

Frank, D. L., R. Kurtz, N. A. Tinsley, A. J. Gassmann, L. J. Meinke, D. Moellenbeck, M. E. Gray, L. W. Bledsoe, C. H. Krupke, R. E. Estes, P. Weber, and B. E. Hibbard. 2015. 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.

Gassmann, A. J. 2012. Field-evolved resistance to Bt maize by western corn rootworm: predictions from the laboratory and effects in the field. J. Invertebr. Pathol. 110: 287–293.

Gassmann, A. J., J. L. Petzold-Maxwell, E. H. Clifton, M. W. Dunbar, A. M. Hoffmann, D. A. Ingber, and R. S. Keweshan. 2014. Field-evolved resistance by western corn rootworm to multiple *Bacillus thuringiensis* toxins in transgenic maize. Proc. Natl. Acad. Sci. 111: 5141–5146.

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.

Gassmann, A. J., J. L. Petzold-Maxwell, R. S. Keweshan, and M. W. Dunbar. 2012. Western corn rootworm and Bt maize: challenges of pest resistance in the field. GM Crops and Food Food Chain 3: 235–244.

Gassmann, A. J., R. B. Shrestha, S. R. K. Jakka, M. W. Dunbar, E. H. Clifton, A. R. Paolino, D. A. Ingber, B. W. French, K. E. Masloski, J. W. Dounda, and C. R. St. Clair. 2016. Evidence of resistance to Cry34/35Ab1 corn by western corn rootworm (Coleoptera: Chrysomelidae): root injury in the field and larval survival in plant-based bioassays. J. Econ. Entomol. 109: 1872–1880.

Gould, F. 1998. Sustainability of transgenic insecticidal cultivars: integrating pest genetics and ecology. Annu. Rev. Entomol. 43: 701–726.

Gray, M. E. 2012. Continuing evolution confirmed of field resistance to Cry3Bb1 in some Illinois fields by western corn rootworm. The Bulletin: pest management and crop development information for Illinois. 20 (12) University of Illinois Extension, Urbana. (http://bulletin.ipm.illinois.edu/article.php?id=1704) (accessed 5 March 2016).

Gray, M. E., A. S. Felsot, K. L. Steffey, and E. Levine. 1992. Planting time application of soil insecticides and western corn rootworm (Coleoptera: Chrysomelidae) emergence: implications for long-term management programs. J. Econ. Entomol. 85: 544–553.

Hall, W. J., and J. A. Wellner. 1980. Confidence bands for a survival curve from censored data. *Biometrika* 67: 133–143.

Head, G., M. Carroll, T. Clark, T. Galvan, R. M. Huckaba, P. Price, L. Samuel, and N. P. Storer. 2013. Efficacy of SmartStax® insect-protected corn hybrids against corn rootworm: the value of pyramiding the Cry3Bb1 and Cry34/35Ab1 proteins. Crop Protection 57: 38–47.

Hibbard, B. E., D. L. Frank, R. Kurtz., E. Boudreau, M. R. Ellersieck, and J. F. Odhiambo. 2011. Mortality impact of Bt transgenic maize roots expressing eCry3.1Ab, mCry3A, and eCry3.1Ab plus mCry3A on western corn rootworm larvae in the field. J. Econ. Entomol. 104: 1584–1591.

Hitchon, A. J., J. L. Smith, B. W. French, and A. W. Schaafsma. 2015. Impact of the Bt 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.

Jakka, S. R., R. B. Shrestha, and A. J. Gassmann. 2016. Broad-spectrum resistance to *Bacillus thuringiensis* toxins by western corn rootworm (*Diabrotica virgifera virgifera*). Scientific Reports 6: 27860. DOI: 10.1038/srep27860.

Johnson, K. D., L. A. Campbell, M. D. Lepping, and D. M. Rule. 2017. Field trial performance of Herculex XTRA (Cry34Ab1/Cry35Ab1) and SmartStax (Cry34Ab1/Cry35Ab1 + Cry3Bb1) hybrids and soil insecticides against western and northern corn rootworms (Coleoptera: Chrysomelidae). J. Econ. Entomol. 110: 1062–1069.

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. Agron. J. 77: 769–774.

Kaplan, E. L., and P. Meier. 1958. Nonparametric Estimation from Incomplete Observations. J. Amer. Stat. Assoc. 53: 457–481.

Keweshan, R. S., G. P. Head, and A. J. Gassmann. 2015. Effects of pyramided Bt corn and blended refuges on western corn rootworm and northern corn rootworm (Coleoptera: Chrysomelidae). J. Econ. Entomol. 108: 720–729.

Knodel, J. J. 2012. Western corn rootworm in corn in North Dakota. Corn Talk. Oct-Nov.2012. ND Corn Growers Association, pp. 12–13. Fargo, ND.

Knodel, J. J., P. Beauzay, and M. Boetel. 2016. 2017 North Dakota Field Crop Insect Management Guide. Bull. E–1143 (revised), Dec. 2016. North Dakota State Univ. Ext. Serv., Fargo.

Krysan, J. L. 1986. Introduction: biology, distribution, and identification of pest *Diabrotica*, pp. 1–23. *In*: J. L. Krysan and T. A. Miller (eds.), Methods for the study of pest *Diabrotica*. Springer-Verlag, New York, N.Y.

Lefko, S. A., T. M. Nowatzki, S. D. Thompson, R. R. Binning, M. A. Pascual, M. L. Peters, E. J. Simbro, and B. H. Stanley. 2008. Characterizing laboratory colonies of western corn rootworm (Coleoptera: Chrysomelidae) selected for survival on maize containing event DAS-59122-7. J. Appl. Entomol. 132: 189–204.

Levene, H. 1960. Robust tests for equality of variances pp. 278–292. *In*: I. Olkin, S. G. Ghurye, W. Hoeffding, W. G. Madow, and H. B. Mann (eds.), Contributions to probability and statistics: essays in honor of Harold Hotelling. Stanford Univ. Press, Stanford, CA.

Levine, E., and H. Oloumi-Sadegh. 1991. Management of diabroticite rootworms in corn. Annu. Rev. Entomol. 36: 229–255.

Levine, E., J. L. Spencer, S. A. Isard, D. W. Onstad, and M. E. Gray. 2002. Adaptation of the western corn rootworm to crop rotation: evolution of a new strain in response to a management practice. Am. Entomol. 48: 94–107.

Liu, Y. B., B. E. Tabashnik, T. J. Dennehy, A. L. Patin, and A. C. Bartlett. 1999. Development time and resistance to Bt crops. Nature 400: 519.

Ludwig, K. A., and R. E. Hill. 1975. Comparison of gut contents of adult western and northern corn rootworms in northeast Nebraska. Environ. Entomol. 4: 435–438.

Ludwick, D. C., L. N. Meihls, K. R. Ostlie, B. D. Potter, L. French, and B. E. Hibbard. 2017. Minnesota field population of western corn rootworm (Coleoptera: Chrysomelidae) shows incomplete resistance to Cry34Ab1/Cry35Ab1 and Cry3Bb1. J. Appl. Entomol. 141: 28–40.

Ma, B. L., F. Meloche, and L. Wei. 2009. Agronomic assessment of Bt trait and seed or soil-applied insecticides on the control of corn rootworm and yield. Field Crops Res. 111: 189–196.

Meihls, L. N., M. L. Higdon, B. D. Siegfried, N. J. Miller, T. W. Sappington, M. R. Ellersieck, T. A. Spencer, and B. E. Hibbard. 2008. Increased survival of western corn rootworm on transgenic corn within three generations of on-plant greenhouse selection. Proc. Natl. Acad. Sci. USA. 105: 19177–19182.

Meihls L. N., M. L. Higdon, M. R. Ellersieck, and B. E. Hibbard. 2011. Selection for resistance to mCry3A-Expressing transgenic corn in western corn rootworm. J. Econ. Entomol. 104: 1045–1054.

Metcalf, R. L. 1986. Foreword, pp. vii–xi. *In* J. L. Krysan and T. A. Miller (eds.), Methods for the study of pest *Diabrotica.* Springer, New York.

Mitchell, P. 2011. Costs and benefits of controlling pest *Diabrotica* in maize in the United States Corn Belt. 24th IWGO Conference. Freiburg, Germany.

Murphy, A. F., M. D. Ginzel, and C. H. Krupke. 2010. Evaluating western corn rootworm (Coleoptera: Chrysomelidae) emergence and root damage in a seed mix refuge. J. Econ. Entomol. 103: 147–157.

(NAS) National Academies of Sciences. 2016. Genetically engineered crops: experiences and prospects. The National Academies Press, Washington, DC.

(NCGA) National Corn Growers Association. 2016. World of corn: U.S. Corn Crop Value. (http://www.worldofcorn.com/#/) (accessed 7 March 2016).

Naranjo, S. E., and A. J. Sawyer. 1988. Impact of host plant phenology on the population dynamics and oviposition of northern com rootworms, *Diabrotica barberi* (Coleoptera: Chrysomelidae) in field com. Environ. Entomol. 17: 508–52l.

Obopile, M., R. B. Hammond, and P. R. Thomison. 2013. Interaction among planting dates, transgenic maize, seed treatment, corn rootworm damage and grain yield. J. Appl. Entomol. 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.

Ordosch, D., R. Narem, and A. Szczepaniec. 2016. Effectiveness of Bt maize against corn rootworm (Coleoptera: Chrysomelidae) and species composition in South Dakota ten years following the introduction of transgenic maize. J. Agric. Urban Entomol. 32: 59–70.

Oswald, K. J., B. W. French, C. Nielson, and M. Bagley M. 2012. Assessment of fitness costs in Cry3Bb1-resistant and susceptible western corn rootworm (Coleoptera: Chrysomelidae) laboratory colonies. J. Appl. Entomol. 136: 730–740.

Oyediran, I. O., P. Matthews, N. Palekar, W. French, J. Conville, and T. Burd. 2016. Susceptibility of northern corn rootworm *Diabrotica barberi* (Coleoptera: Chrysomelidae) to mCry3A and eCry3.1Ab *Bacillus thuringiensis* proteins. Insect Sci. 23: 913–917.

Palmer, L. T., and T. Kommedahl. 1969. Root-infecting *Fusarium* species in relation to rootworm infestations in corn. Phytopathology 59: 1613–1617.

Petzold-Maxwell, J. L., L. J. Meinke, M. E. Gray, R. E. Estes, and A. J. Gassmann. 2013. Effect of Bt maize and soil insecticides on yield, injury, and rootworm survival: implications for resistance management. J. Econ. Entomol. 106: 1941–1951.

Porter, P., E. Cullen, T. Sappington, A. Schaafsma, S. Pueppke, D. Andow, J. Bradshaw, L. Buschman, Y. Cardoza, C. DiFonzo, et al. 2012. Comment submitted by Patrick Porter, North Central Coordinating Committee NCCC46. (http://www.regulations.gov/#!documentDetail; D_EPA-HQ-OPP-2011-0922-0013). 5 March 2012.

Riedell, W. E. 1990. Rootworm and mechanical damage effects on root morphology and water relations in maize. Crop Sci. 30: 628–631.

Ritchie, S. W., J. J. Hanway, and G. O. Benson. 1986. How a corn plant develops. Iowa State University of Science and Technology Coop. Ext. Serv. Special Rept. 48, vol. 48. Iowa State University, Ames.

SAS Institute. 2013. SAS Version 9.4. SAS Institute, Cary, NC.

Schrader, P. M., R. E. Estes, N. A. Tinsley, A. J. Gassmann, and M. E. Gray. 2017. Evaluation of adult emergence and larval root injury for Cry3Bb1-resistant populations of the western corn rootworm. J. Appl. Entomol. 141: 41–52.

Shapiro, S. S., and M. B. Wilk. 1965. An analysis of variance test for normality (complete samples). Biometrika 52: 591-611.

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

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: 1585–1591.

Steel, R. G. D., and J. H. Torrie. 1980. Principles and procedures of statistics: a biometrical approach, McGraw-Hill, New York, N.Y.

Steffey, K. L., M. E. Rice, J. All, D. A. Andow, M. E. Gray, and J. W. Van Duyn. 1999. Handbook of corn insects: pest information. Entomological Society of America, Lanham, MD.

Storer, N. P., J. M. Babcock, and J. M. Edwards. 2006. Field measures of western corn rootworm (Coleoptera: Chrysomelidae) mortality caused by Cry34/35Ab1 proteins expressed in maize event 59122 and implications for trait durability. J. Econ. Entomol. 99: 1381–1387.

Tabashnik, B. E., T. Brévault, and Y. Carrière. 2013. Insect resistance to Bt crops: lessons from the first billion acres. Nat. Biotech. 31: 510–521.

Tabashnik, B. E., and Croft, B. A. 1982. Managing pesticide resistance in crop-arthropod complexes: interactions between biological and operational factors. Environ. Entomol. 11: 1137–1144.

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

Tabashnik, B. E., F. Gould, and Y. Carrière. 2004. Delaying evolution of insect resistance to transgenic crops by decreasing dominance and heritability. J. Evol. Biol. 17: 904–912.

Tinsley, N. A., R. E. Estes, and M. E. Gray. 2013. Validation of a nested error component model to estimate damage caused by corn rootworm larvae. J. Appl. Entomol. 137: 161–169.

Tukey, J. W. 1953. The problem of multiple comparisons. Department of Statistics, Princeton Univ., NJ.

Tinsley, N. A., R. E. Estes, P. M. Schrader, and M. E. Gray. 2015. Evaluating multiple approaches for managing western corn rootworm larvae with seed blends. J. Appl. Entomol. 139: 76–86.

(USDA–FAS) U.S. Department of Agriculture–Foreign Agriculture Service. 2016. Grain: world markets and trade. (http://www.fas.usda.gov/) (accessed 2 March 2016).

(USDA–NASS) U.S. Department of Agriculture–National Agricultural Statistics Service. 2016. North Dakota Agricultural Statistics 2016. Ag. Statistics No. 85. ND.

(USEPA) U.S. Environmental Protection Agency. 2002. A set of scientific issues being considered by the Environmental Protection Agency regarding: corn rootworm plant-incorporated protectant non-target insect and insect resistance management issues. FIFRA Scientific Advisory Panel Meeting Minutes No. 2002-05A.

(USEPA) U.S. Environmental Protection Agency. 2014. A set of scientific issues being considered by the Environmental Protection Agency regarding scientific uncertainties associated with corn rootworm resistance monitoring for Bt corn plant incorporated protectants (PIPs). FIFRA Scientific Advisory Panel Meeting Minutes No. 2014-01. (https://www.regulations.gov/document?D=EPA-HQ-OPP-2013-0490-0044) (accessed 15 June 2017).

van Rozen, K., and A. Ester. 2010. Chemical control of *Diabrotica virgifera virgifera* LeConte. J. Appl. Entomol. 134: 376–384.

Wangila, D. S., A. J. Gassmann, J. L. Petzold-Maxwell, B. W. French, and L. J. Meinke. 2015. Susceptibility of Nebraska western worn rootworm (Coleoptera: Chrysomelidae) populations to Bt corn events. J. Econ. Entomol. 108: 742–751.

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

Zukoff, S. N., K. R. Ostlie, B. Potter, L. N. Meihls, A. L. Zukoff, L. French, M. R. Ellersieck, B. W. French, and B. E. Hibbard. 2016. Multiple assays indicate varying levels of cross resistance in Cry3Bb1-selected field populations of the western corn rootworm to mCry3A, eCry3.1Ab, and Cry34/35Ab1. J. Econ. Entomol. 109: 1387–1398.

CHAPTER IV. FIELD-EVOLVED RESISTANCE OF NORTHERN AND WESTERN CORN ROOTWORM (COLEOPTERA: CHRYSOMELIDAE) POPULATIONS TO CORN HYBRIDS EXPRESSING SINGLE AND PYRAMIDED Cry3Bb1 AND Cry34/35Ab1 Bt PROTEINS IN NORTH DAKOTA

Abstract

Northern, *Diabrotica barberi* Smith & Lawrence, and western, *D. virgifera virgifera* LeConte, corn rootworms (Coleoptera: Chrysomelidae) are major economic pests of corn, *Zea mays* L., in North America. Corn hybrids expressing *Bacillus thuringiensis* Berliner (Bt) toxins are commonly used by growers to manage these pests. Many cases of field evolved-resistance to insecticidal proteins expressed by Bt corn hybrids have been documented, but only for populations of *D. v. virgifera*. In 2016, corn rootworm beetles were collected from five North Dakota corn fields and reared in a growth chamber. In 2017, larvae reared from field-collected populations were subjected to single-plant bioassays to screen North Dakota populations of *D. barberi* and *D. v. virgifera* for potential resistance to three Bt toxins (Cry3Bb1, Cry34/35Ab1, and pyramided Cry3Bb1 + Cry34/35Ab1) and to explore the potential existence of cross-resistance between these toxins in *D. barberi* populations. Our results demonstrated the first documented cases of field-evolved incomplete resistance in *D. barberi* (Arthur population) to Cry3Bb1 and Cry34/35Ab1 [Arthur, Page (problem documented area), Ransom, and Sargent populations]. Complete resistance to Cry3Bb1 was observed in the Ransom population of *D. v. virgifera*. Increased larval survival on the pyramided Cry3Bb1 + Cry34/35Ab1 hybrid was observed in both species. Cross-resistance between Cry3Bb1 and Cry34/35Ab1 was not evident; however, a positive correlation was found between Cry3Bb1 and the pyramided toxins. These findings indicate that field-evolved resistance to Bt toxins is occurring in North Dakota populations of *D. barberi*, and strongly underscore the importance of grower adoption of and adherence to Bt resistance management strategies.

Key Words: *Diabrotica barberi, Diabrotica virgifera virgifera,* Cry protein, resistance, maize

Introduction

In North America, northern, *Diabrotica barberi* Smith & Lawrence, and western, *D. virgifera virgifera* LeConte, corn rootworms (Coleoptera: Chrysomelidae) can cause major economic damage to corn, *Zea mays* L., (Poales: Poaceae). The economic losses due to corn rootworm feeding injury and management costs have been estimated at over US$1 billion per year in the U.S. (Metcalf 1986). Corn rootworm larvae injure plants by feeding on the root system (Apple and Patel 1963, Chiang 1973, Riedell and Kim 1990, Steffey et al. 1999), while adults feed on leaves, silks, pollen, and immature kernels (Ludwig and Hill 1975). Extensive larval feeding injury to roots can lead to corn plant lodging and can reduce yield (Branson et al. 1980, Spike and Tollefson 1991). For example, an average of 15% to 18% yield loss can occur for each node of roots pruned (Dun et al. 2010, Tinsley et al. 2013). Beetle feeding injury to silks can reduce kernel numbers and grain quality (Culy et al. 1992).

For several decades, growers have used various pest management strategies, including crop rotation and insecticides, and more recently, corn hybrids expressing *Bacillus thuringiensis* Berliner (Bt) (Bacillales: Bacillaceae) insecticidal Cry proteins, to control corn rootworms. However, both *D. barberi* and *D. v. virgifera* have shown a propensity to adapt to some of these strategies. *Diabrotica barberi* has adapted to crop rotation through prolonged egg diapause, in which eggs remain in diapause and hatch in the spring after two or more winters (Chiang 1965, Levine et al. 1992, Geisert and Meinke 2013, French et al. 2014). Another adaptation occurs in some *D. v. virgifera* populations, where variant females in corn-soybean rotations leave corn and lay eggs in soybean, with the resulting hatch occurring in the succeeding corn crop (Levine and Oloumi-Sadeghi 1991, Spencer et al. 1998, Levine et al. 2002, Pierce 2003, Gray et al. 2009). Some *D. v. virgifera* populations have also developed resistance to many chemical insecticides (e.g., methyl parathion, dimethoate, and carbaryl) used for corn rootworm management (Meinke et al. 1998, Miota et al. 1998, Scharf et al. 1999, Wright et al. 2000, Zhu et al. 2001).

The commercialization of Bt corn hybrids expressing insecticidal Cry proteins for rootworm control in the U.S. market was met by rapid grower adoption and wide-speed reliance on this technology to reduce or prevent corn rootworm damage. However, the extensive adoption of this technology did not provide a complete corn rootworm management solution. In 2009, just six years after the first corn hybrid expressing the Cry3Bb1 toxin received federal registration [U.S. Environmental Protection Agency (USEPA) 2003], the first cases of field-evolved resistance to Cry3Bb1 corn were reported in *D. v. virgifera* populations in Iowa (Gassmann et al. 2011). Gassmann et al. (2014) also found that field-evolved resistance of *D. v. virgifera* extended to cross-resistance between Cry3Bb1- and mCry3A-expressing corn hybrids. Recently, in Iowa and Minnesota, complete or incomplete cross-resistance of *D. v. virgifera* between Cry3Bb1 and both mCry3A and eCry3.1Ab proteins has also been reported (Jakka et al. 2016, Zukoff et al. 2016). Incomplete field-evolved resistance to Cry34/35Ab1 was also documented for *D. v. virgifera* populations (Gassmann et al. 2016, Ludwick et al. 2017) as well as in Cry3Bb1-resistant populations of *D. v. virgifera* in Minnesota (Zukoff et al. 2016). Several single-plant bioassay studies have since demonstrated considerable expansion of *D. v. virgifera* field-evolved resistance to different Cry proteins within the north central U.S. (Iowa, Illinois, Minnesota, Nebraska, and South Dakota) for populations collected from continuous Bt corn fields

(Gassmann et al. 2011, 2012, 2014, Gassmann 2012, Gray 2012, Porter et al. 2012, Wangila et al. 2015, Zukoff et al. 2016, Schrader et al. 2017, Ludwick et al. 2017).

The potential of *D. v. virgifera* to develop resistance to Cry3Bb1, mCry3A, Cry34/35Ab1, and eCry3.1Ab proteins has also been demonstrated in laboratory-selected colonies after only three generations (Lefko et al. 2008, Meihls et al. 2008, 2011, Oswald et al. 2011, Frank et al. 2013). Oyediran et al. (2016) found that *D. barberi* from a laboratory colony remained susceptible by exposing the neonates to mCry3A and eCry3.1Ab endotoxins incorporated into an artificial diet. Tabashnik et al. (1998) and Heckel et al. (2007) explained that, in the most common type of resistance to Bt, the genetic-basis of Cry proteins resistance is carried by recessive genes and reduced Cry protein binding to midgut receptors. The concentrations of rootworm-active Cry proteins in all current registered Bt corn hybrids are at ‘less than high-dose’ concentrations (USEPA 2002, Siegfried et al. 2005, Storer et al. 2006, Meihls et al. 2008, Hibbard et al. 2011, Gassmann 2012), which can raise the potential for rapid resistance evolution to Bt crops in target pests if these crops are not used appropriately (Gould 1998, Tabashnik et al. 2013, Andow et al. 2016). Moreover, continuous corn plantings, repeated use of Bt corn hybrids with the same mode of action, and misuse or exclusion of refuge corn can hasten the evolution of resistance.

Corn production in North Dakota was valued at more than US$1.5 billion in 2016 [U.S. Department of Agriculture–National Agricultural Statistics Service (USDA–NASS) 2017]. However, increased infestation levels of *D. barberi* and associated larval root-feeding injury have recently been observed (Knodel 2012, Calles-Torrez et al. 2018). Although, field-evolved resistance to Bt corn expressing Cry proteins by *D. v. virgifera* populations has been demonstrated in some U.S. states, resistance to Bt corn by *D. barberi* populations has not yet been documented. Additional, field-evolved resistance to Bt corn has not yet been confirmed in North Dakota populations of *D. v. virgifera*. Therefore, this research was carried out to determine if North Dakota populations of *D. barberi* and *D. v. virgifera* have developed resistance to Cry3Bb1, Cry34/35Ab1, and pyramided (i.e., Cry3Bb1 + Cry34/35Ab1) proteins expressed in Bt corn hybrids. Another objective was to assess the potential of *D. barberi* cross-resistance between Cry3Bb1 and Cry34/35Ab1 proteins, and to determine if there was an association between survivals of larvae exposed to Cry3Bb1 or Cry34/35Ab1 proteins and that of larvae surviving exposure to the Cry3Bb1 + Cry34/35Ab1 pyramid. The data generated by this research will be beneficial to corn producers, integrated pest management (IPM) scouts, crop consultants, seed companies, and the scientific community, as it provides important information regarding the use of Bt corn for corn rootworm management.

Materials and Methods

Beetle Source Populations and Production of Larvae

Beetles of *D. barberi* were randomly collected between 25 July and 20 September in 2016 from five corn fields in southeastern and east central North Dakota. Locations chosen for collections included the following: Arthur in Cass County, Page in Cass County [i.e., two corn fields at this location: one field with a history of economic losses resulting from corn rootworm larval feeding injury (collected beetles are referred to as a problem population), and the other field without a history of economic losses (referred to as the control population)], Sargent in Sargent County, and Ransom in Ransom County. The Arthur field had also been reported as incurring economic losses due to corn rootworm larval feeding injury and continuous Bt corn planting previous to 2013. Beetles collected from this field also were referred to as a problem population. In Table 4.1, the following parameters are summarized: field history of sites from

2013 through 2016, beetle collecting period, total numbers of *D. barberi* beetles collected, and approximate numbers of eggs obtained per population. In addition, 304 *D. v. virgifera* beetles were collected from the Ransom field, and 51,050 eggs were obtained from those beetles.

Table 4.1. Collection period, numbers of beetles collected, approximate numbers of eggs collected from source field populations of *Diabrotica barberi* beetles in 2016, and cropping history of corn fields

Site	Beetle collecting period	Numbers of beetles collected	Numbers of eggs obtained	Field history			
				2013	2014	2015	2016
Arthur (Problem population)[a]	25 July -19 Sept.	9,909	53,270	Non-Bt corn	Soybean	VT Triple PRO RIB[b]	VT Triple PRO RIB
Page (Problem population)[c]	5 Aug. - 19 Sept.	5,473	13,000	N/A[d]	N/A	Smartstax RIB[e]	Smartstax RIB
Sargent population	25 Aug. - 20 Sept.	5,311	12,020	N/A	N/A	Soybean	VT Double Pro[f]
Ransom population	25 Aug. - 20 Sept.	6,479	12,020	N/A	N/A	N/A	Corn[g]
Page (Control population)[h]	5 Aug. - 15 Sept.	3,200	20,500	N/A	N/A	N/A	VT Double Pro

[a] This field had a 3-yr history of continuous deployment of Cry3Bb1 toxin-expressing corn hybrid plantings for corn rootworm control immediately previous to 2013. From 2013-2014, trials on corn hybrids expressing Cry3Bb1 and Cry34/35Ab1 proteins were conducted within the field.

[b] Corn hybrid expressing Cry3Bb1 protein for corn rootworm control.

[c] This field had a history of unexpected corn rootworm damage previous to 2013. In 2015, Smartstax RIB corn was planted in half of the field, and wheat was grown in the other half.

[d] Cropping history not available from participating grower.

[e] Corn hybrid expressing Cry3Bb1 and Cry34/35Ab1 proteins for corn rootworm control.

[f] Corn hybrid does not express rootworm-active Cry proteins.

[g] Corn hybrid planted was not available from the grower.

[h] This field did not have a history of previous corn rootworm damage.

Beetles were captured using a handheld black insect aspirator (Watkins and Doncaster, Herefordshire, UK) and to avoid overcrowding and mechanical injury to the insects, no more than 60 beetles were held in the aspirator at the same time. Containers (i.e., of the aspirator) enclosing the beetles were placed in a cooler (Igloo Island Breeze 48-Quart); however, before placing beetles in the cooler, six ice packs (17.9 x 4.3 x 17.2 cm) (Rubbermaid Blue Ice Weekender, Gott Corporation, Windfield, Kansas, USA) were positioned at the bottom of the cooler. The ice packs were covered with a cloth towel to prevent direct contact between the ice packs and the beetle containers. The cooler containing collected beetles was transported to the North Dakota State University (NDSU) Agricultural Experiment Station Research Greenhouse Complex (Fargo, ND) where beetles were placed into separate insect cages (BugDorm-4454F, MegaView Science Co., Ltd. Taichung, Taiwan) by collection site and held in a growth room (wide range room, Biocold Environmental Chamber, Ellisville, MO) at 25°C, 50% humidity, and 14:10 (L:D) h photoperiod. Each insect cage held between approximately 800 and 1,500 *D. barberi* beetles (i.e., mixture of females and males). Female and male *D. v. virgifera* beetles collected from the Ransom site were held in a separate cage.

With some adjustments, the laboratory procedures of personnel at the U.S. Department of Agriculture – Agricultural Research Service – North Central Agricultural Research Laboratory (USDA-ARS-NCARL) in Brookings, SD was used to rear the beetles in cages and to obtain eggs (B. W. French and C. N. Nielson, personal communication). Caged beetles were supplied with an artificial corn diet and with two to four ice cubes of water agar solid per cage was at least every other day. The artificial diet was provided by personnel from the USDA-ARS-NCARL in Brookings, SD. The agar was prepared in ice cube trays using their protocol (B. W. French and C. N. Nielson, personal communication). One or two plastic petri dishes (Kord–Valmark

Labware Products, BioPlast Manufacturing, L.L.C., Bristol, PA) containing fine sieved moist soil were provided per cage as oviposition substrate. Petri dishes were covered with lids for maintaining moisture of the oviposition substrate. Each lid had five circular holes (i.e., each hole was 1.8 cm in diameter) to allow beetles to enter to lay eggs and then exit. Oviposition dishes were then covered with fluted roof-like structures (10 cm by 12 cm) made from layers of aluminum foil. Oviposition dishes were replaced at least twice per week, depending on the number of beetles per cage. Upon removal from a cage, each petri dish containing eggs was sprinkled with tap water, powdered with fine sieved soil, covered with a petri dish lid (i.e., without holes), labeled, and held for two weeks. Petri dishes inside and outside of the cages were regularly sprinkled with water on an as-needed basis. Cages were cleaned every four days.

After maintaining the petri dishes outside of the cages for two weeks, eggs were separated from soil and counted using the protocol of B. W. French and C. N. Nielson (personal communication). Counted eggs were then placed in new petri dishes containing fine sieved soil. Afterward, eggs were powdered with the same soil, sealed with parafilm (Laboratory Film, Neenah, WI), labeled, and stored in partially open 4.4 L plastic containers that were then placed in a growth chamber (Conviron PGW40 Controlled Environments Ltd., Winnipeg, Manitoba, Canada) at 8°C and 24 h darkness pending use for May 2017. Susceptible *D. barberi* and *D. v. virgifera* eggs used for this study were obtained from laboratory colonies maintained by the USDA-ARS-NCARL. These eggs served as control populations in this experiment because they are susceptible rootworm-active to Cry protein toxins. These colonies were established by collecting beetles from fields near Brookings, SD before rootworm-active Bt corn hybrids were commercially available. As such they have never been exposed to these novel insecticidal proteins. Eggs were shipped on 23 May 2017 in accordance with a United States Department of

Agriculture - Animal and Plant Health Inspection Service (USDA-APHIS) permit (i.e., permit number P526P-15-02757).

Bioassays

Corn hybrids expressing the following Cry proteins were tested in our experiment: 1) Cry3Bb1; 2) Cry34/35Ab1; and 3) pyramided Cry3Bb1 + Cry34/35Ab1. In addition, two non-Bt corn hybrids were used as control treatments: 1) a non-Bt corn hybrid agronomically similar to the Cry3Bb1 and pyramided Cry3Bb1 + Cry34/35Ab1 corn hybrids; and 2) a non-Bt corn hybrid agronomically similar to the Cry34/35Ab corn hybrid. The Cry34/35Ab1 seeds and those of the non-Bt hybrid that was agronomically similar to Cry34/35Ab were coated with Cruiser 250 seed treatment (thiamethoxam, 0.25 mg a.i. per kernel; Syngenta Crop Protection, Greensboro, NC). Although this rate is not labeled for corn rootworm control (Knodel et al. 2016), the seed treatment insecticide was removed following the procedure of Gassmann et al. (2011).

Seed planting to establish plants for bioassays was initiated in early May 2017. Plants for bioassays were grown as described by Gassmann et al. (2011) with minor adjustments made to carry out our experiment. Corn plants were grown on 80 cm high benches from early May through September in 2017 in the greenhouse at 25°C, 60% humidity, and 16:8 (L:D) h photoperiod; these greenhouses, controlled by Argus Control Systems Ltd. (Surrey, British Columbia, Canada), are equipped with 600W high-pressure sodium lamps (P.L. Light Systems, Inc., Beamsville, Ontario, Canada). In the greenhouse, containers with seeds (for each bioassay) were arranged in a randomized complete block design with 12 replications (Steel and Torrie, 1980), and a total of 60 containers per bioassay. Two bioassays (runs) were completed for each *D. barberi* and *D. v. virgifera* population, with bioassays being conducted separately by species. Each individual seed was planted in a 0.95-L clear plastic container (height: 14.5 cm and diameter: 11.5 cm) (HomeFresh RD 32 oz Placon Corporation, Madison, WI). All containers used in the bioassays were absent of drainage holes. Each container was filled to three-fourths capacity with the following potting soil mixture: 50% Metro Mix 902 Professional growing mix (Sun Gro Horticulture Distribution Inc. F1971, Agawam, MA) and 50% Pro-Mix® LP15 Flexible purpose growing medium (Premier Tech Horticulture, Quakertown, PA). Plants were fertilized with 100 mL of a prepared solution of 4 mg of Jack's Professional LX 15-5-15 4Ca 2Mg (J.R. Peters, Inc., Allentown, PA) per mL. These fertilizer applications were first made at 10 days after planting, and were repeated every seven days during plant development. Each container also received 100 mL of distilled water on an as-needed basis.

Two to three days before plants were infested with neonate larvae, seven plants per corn hybrid (i.e., treatment) within each bioassay were randomly selected and tested to ensure the expression of their respective Cry protein by using toxin-specific QuickStick EnviroLogix (EnviroLogic, Portland, ME) test kits. All tested plants confirmed a positive reaction for the presence of their corresponding Cry protein. QuickStick combo kits (EnviroLogic), designed for pyramided Bt hybrids, were used for testing the non-Bt corn plants, and all tested plants displayed negative Cry protein reactions.

After two to three weeks of corn plant growth (V3 stage), eggs were incubated in a growth chamber set at 25°C and 65% humidity (Gassmann et al. 2011), and 24 h darkness. The first hatched egg was observed on incubation day 11 or 12 for *D. barberi* and on day 14 or 15 for *D. v. virgifera*. After a sufficient number of eggs had hatched, the neonate larvae were collected for infesting corn plants grown in the greenhouse. Prior to infestations, when corn was in V5 stage of physiological development (Ritchie et al. 1986), leaves were held above the stalk and trimmed to achieve a plant height of approximately 65 cm from the base of the stalk. The two lower leaves also were trimmed to 15 cm in length. The each root system of each plant was then infested with 12 neonate larvae using a camel hair brush (round No. 2, Linzer Products Corp., Wyandanch, NY). Infested plants were transferred to a growth chamber (Conviron GR64, µmoles/m²/s light intensity, Controlled Environments Ltd., Winnipeg, Manitoba, Canada) set at 25°C and 14:10 (L:D) h photoperiod, and held for 12 days. Plants were watered with 100 mL of distilled water as needed, and no fertilizer was applied after infestations.

Twelve days after infestations, the stalk of each plant was excised from the collar, and the cups were sprinkled with 25 mL of distilled water. All soil containing the root and larvae was then transferred into a separate Berlese funnel apparatus for each cup. All Berlese funnels were maintained at 23 ± 0.03°C for four days to extract larvae. Several pre-constructed Berlese funnels were available at NDSU, and more were assembled by using 8.5 L galvanized steel tractor fuel funnels (Behrens, LLC Winona, MN). The heat source used to drive larvae out of soil and through each funnel consisted of a 26.7 cm brooder house clamp light equipped with a porcelain socket (Bayco Products Inc., Wylie, TX) and a 15W soft-white light bulb (GE Lighting, Cleveland, OH, USA). A larval collection basin, comprised of a 0.95 L plastic jar, was positioned immediately below each funnel to recover surviving larvae as they exited the root/soil mass. A 12 cm diameter wire mesh (0.6 x 0.6 cm mesh) circle was positioned inside of each funnel to prevent soil particles falling into the collection basin. Each collection basin was filled with a 200-mL solution of soapy water at a concentration of 10 mL Palmolive dish detergent (Colgate-Palmolive Co., New York, NY) per five liters of water. All basins were wrapped with aluminum foil to exclude any light during day-time hours. Surviving larvae were counted and recorded independently for each sample after being maintained in the Berlese systems for four days.

Data Analysis

The proportion of surviving larvae (i.e., the number of recovered larvae divided by the initial number of larvae at infestation time) was calculated before data analysis. The initial analysis indicated that the bioassay run effect in the model was not significantly different ($P > 0.05$). Accordingly, run was removed from the model. Proportional larval survival data for both species were subjected to a two-way mixed-model analysis of variance (ANOVA) ($P < 0.05$) by using the MIXED procedure in SAS (SAS Institute 2013). Larval survival from assays on data of Cry3Bb1, pyramided Cry3Bb1 + Cry34/35Ab1, and their agronomically similar non-Bt hybrid were analyzed separately from the survival data for larvae exposed to Cry34/35Ab1 and its agronomically similar non-Bt hybrid. Fixed effects in the ANOVA table were comprised of corn hybrid, corn rootworm (*D. barberi* or *D. v. virgifera*) population site, and their interactions. Replication was considered as a random factor in the experiment. Pairwise comparisons were made by using the PDIFF option in PROC MIXED, and alpha values were adjusted for multiple comparisons using a Bonferroni correction in SAS (SAS Institute 2013). Before conducting the analysis, all data arcsine square-root were transformed to improve normality of the residuals and homogeneity of variances within hybrids. All means and standard errors presented in the results herein are from untransformed data.

Corrected proportional larval survival was calculated within population (i.e., site) for each Bt-corn hybrid. The Abbott (1925) corrected proportional mortality formula was applied to each replicate of our data as follows: [(% corrected mortality = % larval survival on non-Bt corn – % larval survival on Bt corn) ÷ % larval survival on non-Bt corn]. Result was applied as a complementary of the corrected survival (e.g., 1 – % corrected mortality based on Abbott’s correction). The non-Bt hybrid that was agronomically similar to Cry3Bb1 and pyramided

Cry3Bb1 + Cry34/35Ab1 was used for comparison with both Bt hybrids. The corrected proportional larval survival of *D. barberi* was subjected to a one-way mixed-model ANOVA ($P < 0.05$) by using PROC MIXED in SAS (SAS Institute 2013). The LSMEANS procedure and its PDIFF option, and alpha values were adjusted based on a Bonferroni correction and were used for mean comparisons of corrected larval survival among populations. A Student's *t*-test with equal variances at an alpha level of 0.05 was used (Student 1908) to compare the corrected proportional larval survival between the Ransom and laboratory control populations of *D. v. virgifera* for each Bt corn hybrid by using SAS software (SAS Institute 2013).

Pearson's correlation coefficients (r) were calculated to identify potential cross-resistance as evidence by between the mean corrected larval survival on Cry3Bb1 and Cry34/35Ab1 for *D. barberi*, and to measure the intensity of the association between corrected larval survival on Cry3Bb1 and pyramided Cry3Bb1 + Cry34/35Ab1 and between Cry34/35Ab1 and pyramided Cry3Bb1 + Cry34/35Ab1 among all *D. barberi* populations. Pearson's correlation coefficient, which tests the null hypothesis of ρ (Rho) = 0, was calculated using the CORR procedure in SAS (SAS Institute 2013).

Results

Proportional Larval Survival

A significant ($F = 10.55$; df =10, 403; $P < 0.0001$) hybrid x population interaction was detected for *D. barberi* reared on Cry3Bb1, pyramided Cry3Bb1 + Cry34/35Ab1, and their non-Bt corn hybrid counterparts. Larval survival was similar on the non-Bt corn hybrid among all six populations. Larval survival was significantly lower on Cry3Bb1 and pyramided Cry3Bb1 + Cry34/35Ab1 treatments when compared to the non-Bt corn for all *D. barberi* populations (Fig. 4.1A). Survival of the Arthur problem population was significantly greater than in all other populations, when assayed on either Cry3Bb1 or pyramided Cry3Bb1 + Cry34/35Ab1 corn. Whereas, larval survival of the Arthur problem population on pyramided Cry3Bb1 + Cry34/35Ab1 was not significantly different from larval survival of the Sargent population on Cry3Bb1. Survival of *D. barberi* larvae from the Arthur problem population was significantly lower on pyramided Cry3Bb1 + Cry34/35Ab1 corn hybrid than on the Cry3Bb1 corn hybrid. Larval survival was not significantly different among the laboratory control, Page (problem and control populations), Sargent, and Ransom populations when assayed on Cry3Bb1 and pyramided Cry3Bb1 + Cry34/35Ab1 corn hybrids.

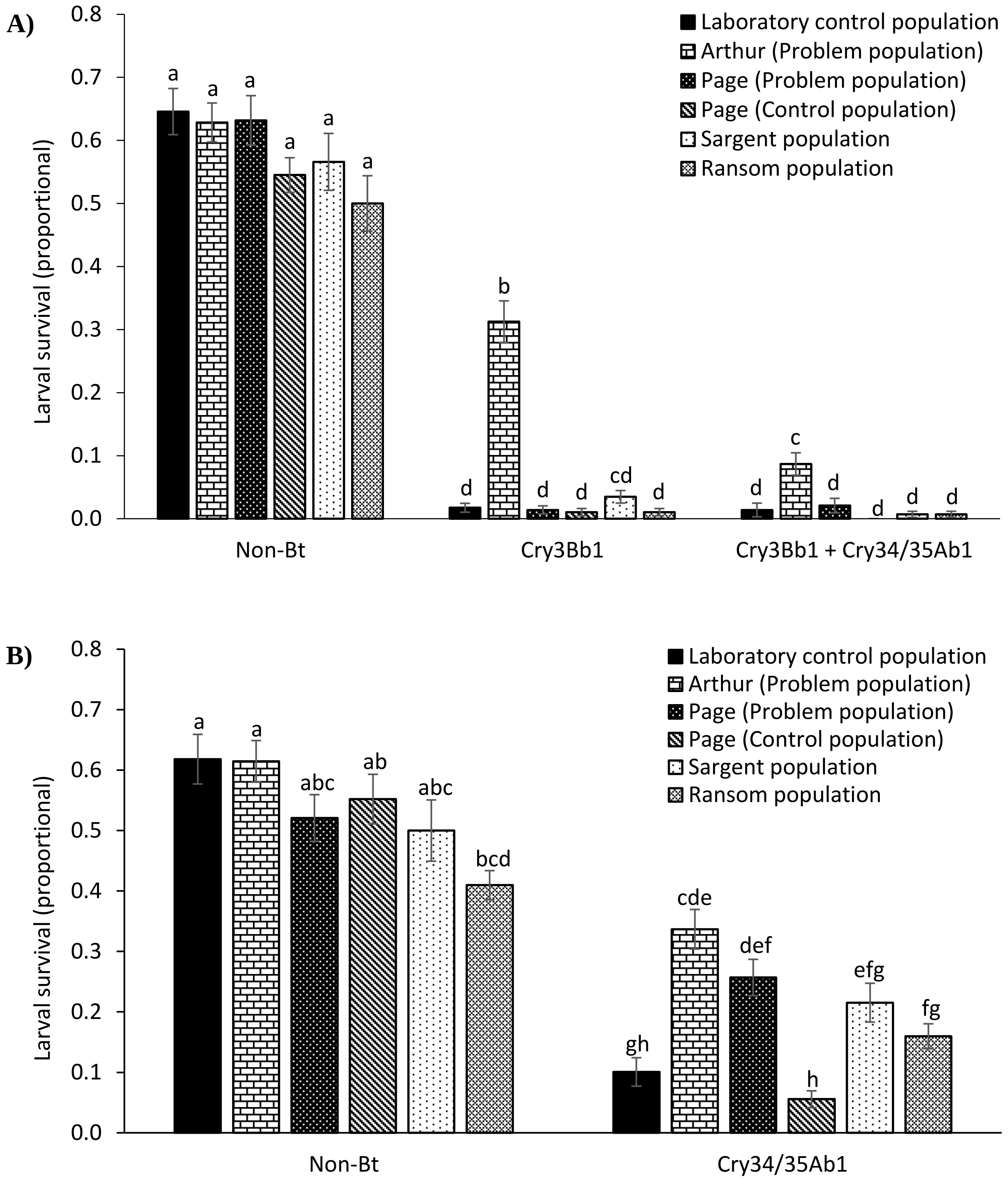


Fig. 4.1. (A-B) Mean (± SEM) proportional larval survival of southeastern and east central North Dakota *D. barberi* populations on **(A)** Cry3Bb1 and Cry3Bb1 + Cry34/35Ab1 hybrids and agronomically similar non-Bt corn, and **(B)** Cry34/35Ab1 and its similar non-Bt hybrid in 2017. Bars sharing a letter are not significantly different based on a two-way mixed-model ANOVA ($P < 0.05$) and LSMEANS (with the PDIFF option). Alpha values were adjusted by using a Bonferroni correction.

The hybrid by population interaction significantly ($F = 9.19$; df = 5, 265; $P < 0.0001$) impacted mean larval survival of *D. barberi* reared on Cry34/35Ab1 and its corresponding non-Bt corn hybrid. Mean survival in the non-Bt corn hybrid was not significantly different among most *D. barberi* populations, with the exception of Ransom population (Fig. 4.1B). Also, as indicated in Figure 4.1B, survival of the Ransom population in non-Bt was significantly different from just two (i.e., the Arthur problem population and the laboratory population) of the five other populations. Mean survival of larvae from the Arthur and Page problem populations assayed on Cry34/35Ab1 and Ransom larvae reared on non-Bt did not differ significantly; however, survival of these populations (Arthur and Page problem populations) was significantly different from the laboratory and Page control populations reared on the Cry34/35Ab1 hybrid. Survival of the Sargent population on Cry34/35Ab1 was not significantly different from that of Arthur (problem population), Page (problem population), Ransom, and laboratory populations, but numerically higher than the laboratory control and Ransom populations. The lowest mean *D. barberi* larval survival on Cry34/35Ab1 occurred in the Page (control population), laboratory control, and Ransom populations.

The interaction between corn hybrid and population was significantly different ($F = 77.32$; df = 2, 127; $P < 0.0001$) for *D. v. virgifera* populations reared on Cry3Bb1, the Cry3Bb1 + Cry34/35Ab1 pyramid, and their non-Bt corn counterpart hybrids. The hybrid x population interaction was also significant ($F = 5.15$; df = 1, 81; $P = 0.0259$) for larvae reared on Cry34/35Ab1 and its similar non-Bt corn. Mean survival of *D. v. virgifera* on Cry3Bb1 and pyramided Cry3Bb1 + Cry34/35Ab1 corn was significantly greater in the Ransom population than in the laboratory control population (Fig. 4.2A). In contrast, survival on Cry34/35Ab1 was not significantly different between these two populations (Fig. 4.2B). Interestingly, mean proportional larval survival of the Ransom population on Cry3Bb1 was not significantly different than on the non-Bt corn hybrid (Fig. 4.2A). Mean survival of the two *D. v. virgifera* populations (Ransom and laboratory control populations) was not significantly different when compared to the corresponding non-Bt corn hybrid (Fig. 4.2A-B).

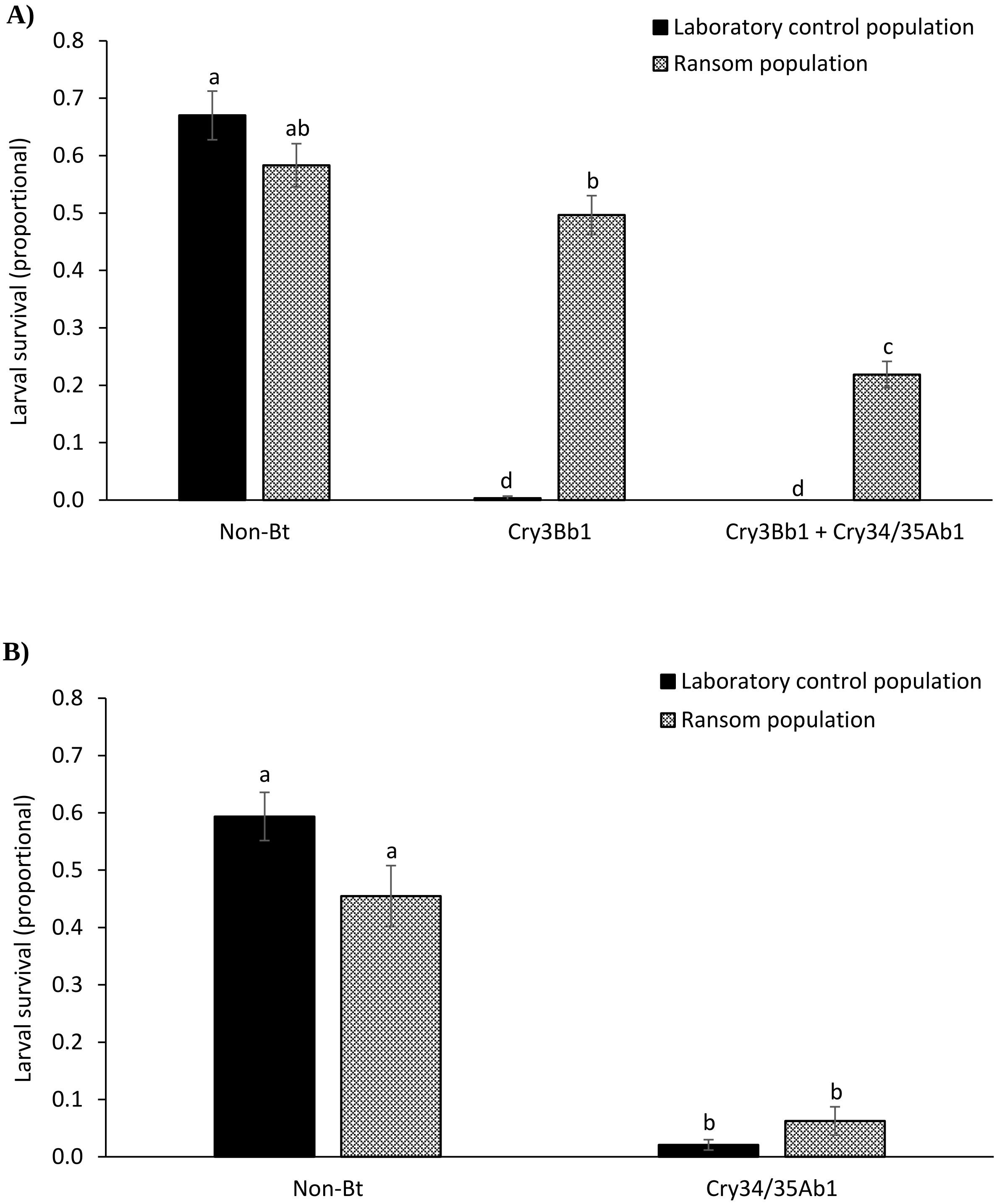


Fig. 4.2. (A-B) Mean (± SEM) proportional larval survival of Ransom, North Dakota, and laboratory control (Brookings, SD) *D. v. virgifera* populations on **(A)** Cry3Bb1 and Cry3Bb1 + Cry34/35Ab1 hybrids and agronomically similar non-Bt corn, and **(B)** Cry34/35Ab1 and its similar non-Bt hybrid in 2017. Bars sharing a letter are not significantly different based on a two-way mixed-model ANOVA (*P* < 0.05) and LSMEANS (with the PDIFF option). Alpha values were adjusted by using a Bonferroni correction.

Corrected Proportional Larval Survival

Mean corrected larval survival was significantly different among *D. barberi* populations assayed on Cry3Bb1 ($F = 40.63$; df = 5, 127; $P < 0.0001$), Cry34/35Ab1 ($F = 11.58$; df = 5, 127; $P < 0.0001$), and pyramided Cry3Bb1 + Cry34/35Ab1 ($F = 8.05$; df = 5, 127, $P < 0.0001$) corn hybrids. As shown in Table 4.2, corrected proportional larval survival of the Arthur (problem population) on Cry3Bb1 was significantly greater than the other five *D. barberi* populations on corn roots expressing this toxin. While proportional corrected survivals of these five populations were very low (i.e., ranging from 0.02 to 0.09), proportional corrected survival was 0.51 for the Arthur problem population. Larval mean corrected survivals on Cry34/35Ab1 from Arthur (problem population), Page (problem population), Sargent, and Ransom populations (ranging from 0.41 to 0.54) were significantly greater than in the laboratory and Page control populations (range: 0.13 to 0.18). Corrected *D. barberi* larval survival on pyramided Cry3Bb1 + Cry34/35Ab1 corn was significantly lower in the Page (problem and control), Sargent, Ransom, and laboratory populations compared to Arthur (problem population). Means ranged from 0.00 to 0.04 lower than that of the Arthur problem population, which averaged 0.13 corrected larval survival.

Table 4.2. Mean (± SEM) proportional corrected larval survival for different North Dakota populations of *D. barberi* on corn hybrids expressing Cry3Bb1, Cry34/35Ab1, and pyramided Cry3Bb1 + Cry34/35Ab1 in single-plant bioassays conducted in 2017

	Proportional corrected larval survival (mean ± SEM)		
Population	Cry3Bb1	Cry34/35Ab1	Cry3Bb1 + Cry34/35Ab1
Arthur (Problem population)	0.51 ± 0.05a	0.54 ± 0.04a	0.13 ± 0.03a
Page (Problem population)	0.02 ± 0.01b	0.51 ± 0.05a	0.04 ± 0.02b
Sargent	0.09 ± 0.04b	0.45 + 0.06a	0.01 ± 0.01b
Ransom	0.03 ± 0.02b	0.41 ± 0.05a	0.02 ± 0.02b
Laboratory control	0.03 ± 0.02b	0.18 ± 0.05b	0.02 ± 0.02b
Page (Control population)	0.03 ± 0.01b	0.13 ± 0.05b	0.00 ± 0.00b

Means sharing a letter within a Bt corn hybrid are not significantly different based on a one-way mixed-model ANOVA ($P < 0.05$) and LSMEANS (with PDIFF option). Alpha values were adjusted by using a Bonferroni correction test.

Corrected larval survival of the *D. v. virgifera* laboratory control was significantly lower than that of the Ransom population of *D. v. virgifera* when assayed on Cry3Bb1 ($t = 14.83$; df = 46; $P < 0.0001$) and on the Cry3Bb1 + Cry34/35Ab1 ($t = 5.35$; df = 46; $P < 0.0001$) pyramid (Table 4.3). Whereas, corrected survival on the Cry34/35Ab1 corn was not significantly ($t = 0.77$; df = 46; $P = 0.4446$) different between these two populations (i.e., laboratory control and the Ransom populations).

Table 4.3. Mean (± SEM) proportional corrected larval survival between a Ransom, North Dakota field population, and a susceptible laboratory control population (Brookings, SD) of *D. v. virgifera* on corn hybrids expressing single and pyramided Bt proteins in 2017

	Corrected larval survival (mean ± SEM)				
Corn hybrid	Ransom	Laboratory control	df	*t*	*P*
Cry3Bb1	0.86 ± 0.04	0.05 ± 0.04*	46	14.83	<0.0001
Cry34/35Ab1	0.13 ± 0.05	0.08 ± 0.04	46	0.77	0.4446
Cry3Bb1 + Cry34/35Ab1	0.41 ± 0.05	0.04 ± 0.04*	46	5.35	<0.0001

Asterisks indicate significant differences between the Ransom and control populations according to a *t*-test with equal variances ($P < 0.05$).

Correlations in D. barberi Larval Survival Between Single- and Pyramided-Toxin Corn

There was no statistically significant correlation in corrected larval survival of *D. barberi* between Cry3Bb1 and Cry34/35Ab1 corn hybrids ($r = 0.50928$; df = 4; $P = 0.3021$) (Fig. 4.3A). Similarly, larval survival rates on Cry34/35Ab1 and pyramided Cry3Bb1 + Cry34/35Ab1 corn were not significantly ($r = 0.62101$; df = 4; $P = 0.1882$) correlated (Fig. 4.3C). However, a positive correlation ($r = 0.95479$; df = 4; $P = 0.0030$) was observed in *D. barberi* corrected larval survival between Cry3Bb1 and pyramided Cry3Bb1 + Cry34/35Ab1 hybrids (Fig. 4.3B).

A)

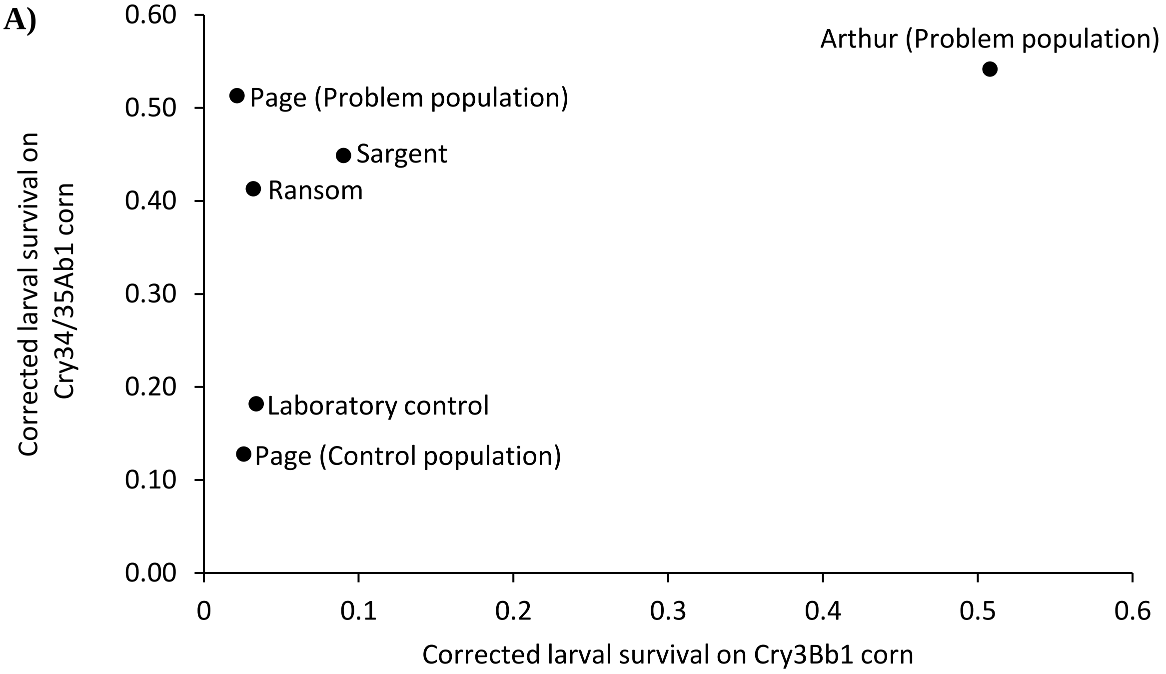


Fig. 4.3. (A-C) Correlation for mean corrected *D. barberi* larval survival between (**A**) Cry3Bb1 and Cry34/35Ab1, **(B)** Cry3Bb1 and pyramided Cry3Bb1 + Cry34/35Ab1, and **(C)** Cry34/35Ab1 and pyramided Cry3Bb1 + Cry34/35Ab1 corn hybrids among all populations. No significant correlations were detected between corrected larval survival between Cry3Bb1 and Cry34/35Ab1 ($r = 0.50928$; df = 4; $P = 0.3021$) (Fig. 3A) or between Cry34/35Ab1 and Cry3Bb1 + Cry34/35Ab1 ($r = 0.62101$; df = 4; $P = 0.1882$) (Fig. 3C). A significant positive correlation was observed for corrected larval survival between Cry3Bb1 and the Cry3Bb1 + Cry34/35Ab1 pyramid ($r = 0.95479$; df = 4; $P = 0.0030$) (Fig. 3B).

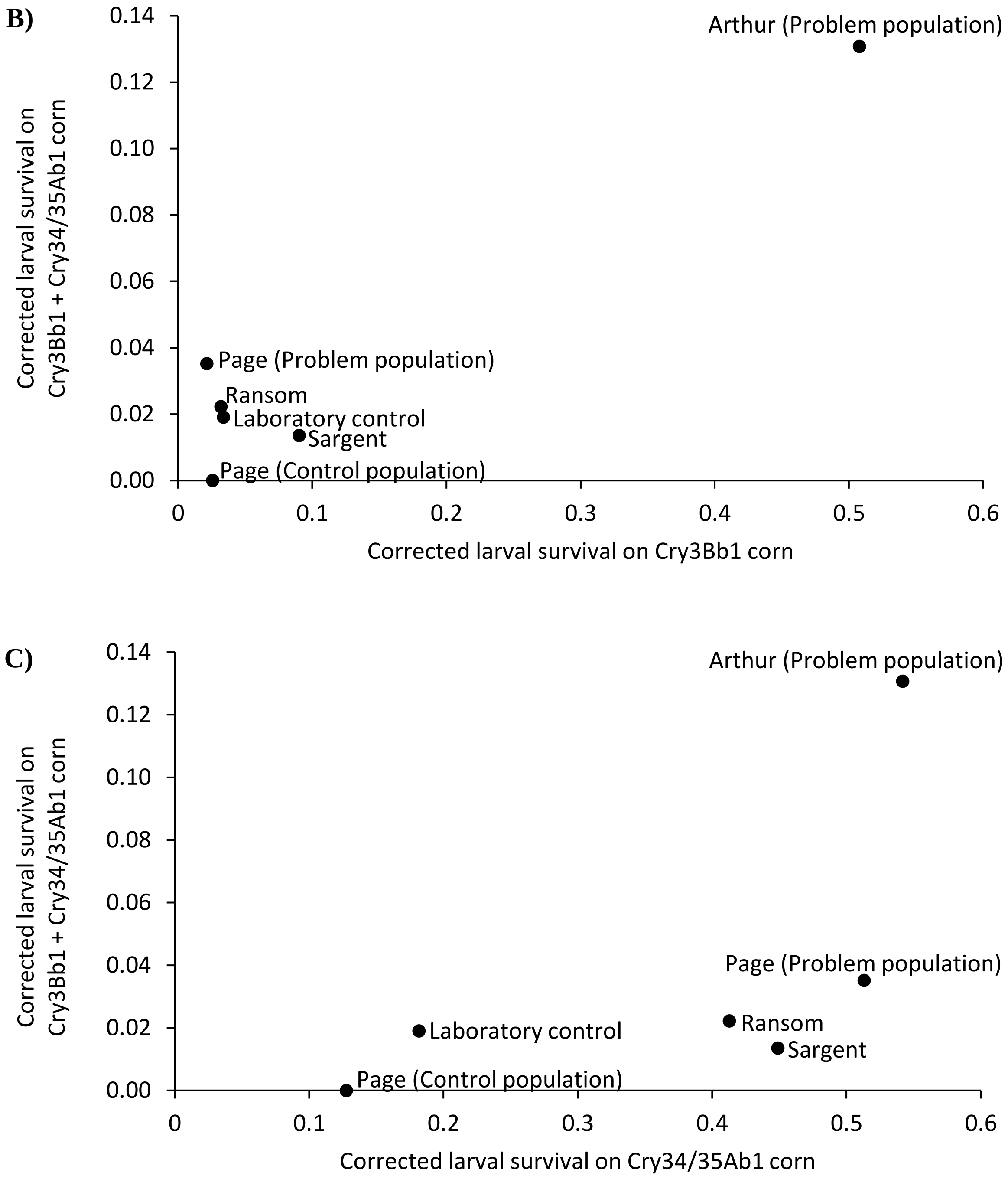


Fig. 4.3. (A-C) Correlation for mean corrected *D. barberi* larval survival between (**A**) Cry3Bb1 and Cry34/35Ab1, **(B)** Cry3Bb1 and pyramided Cry3Bb1 + Cry34/35Ab1, and **(C)** Cry34/35Ab1 and pyramided Cry3Bb1 + Cry34/35Ab1 corn hybrids among all populations (continued). No significant correlations were detected between corrected larval survival between Cry3Bb1 and Cry34/35Ab1 (r = 0.50928; df = 4; P = 0.3021) (Fig. 3A) or between Cry34/35Ab1 and Cry3Bb1 + Cry34/35Ab1 (r = 0.62101; df = 4; P = 0.1882) (Fig. 3C). A significant positive correlation was observed for corrected larval survival between Cry3Bb1 and the Cry3Bb1 + Cry34/35Ab1 pyramid (r = 0.95479; df = 4; P = 0.0030) (Fig. 3B).

Discussion

This series of single-plant bioassays demonstrated varying levels of field-evolved resistance and increased larval survival to corn rootworm-active Cry proteins in certain *D. barberi* populations in eastern North Dakota. Field-evolved resistance and increased larval survival were also evident in the Ransom population of *D. v. virgifera*. However, some populations of *D. barberi* remained susceptible to the Cry proteins tested in this experiment. This is the first report of field-evolved incomplete resistance in *D. barberi* to Cry3Bb1 and Cry34/35Ab1 toxins, increased levels of larval survival on pyramided Cry3Bb1 + Cry34/35Ab1 Bt toxins in both *D. barberi* and *D. v. virgifera*, and complete resistance to Cry3Bb1 in *D. v. virgifera* in North Dakota.

Mean proportional and corrected larval survival of the Arthur problem population of *D. barberi* when assayed on Cry3Bb1 corn were significantly greater than that of laboratory control population. In addition, survival rates of the Page (problem and control populations), Sargent, and Ransom populations were also significantly lower than that of the Arthur *D. barberi* population, thus indicating that the Arthur population is resistant to the Cry3Bb1 toxin. In a closely related species (i.e., *D. v. virgifera*), Gassmann et al. (2011) also reported resistance to Cry3Bb1 because proportional larval survival and corrected survival were significantly greater in problem than in control fields. In spite of this, those authors concluded that this significance was likely compromised because of incomplete field-evolved resistance to Cry3Bb1 by *D. v. virgifera* among tested populations. Gassmann et al. (2011) founded this conclusion (incomplete field-evolved resistance to Cry3Bb1 by *D. v. virgifera*) on the fact that larval survival was significantly lower on Cry3Bb1 corn than on non-Bt corn. In a similar case, Gassmann et al. (2016) reported incomplete resistance of *D. v. virgifera* to the Cry34/35Ab1 toxin in Iowa. Our study also showed that field-evolved resistance by *D. barberi* to Cry3Bb1 was incomplete because proportional larval survival of the Arthur problem population was 50.3% lower on Cry3Bb1 corn when compared to the non-Bt hybrid. As Gassmann et al. (2011) discussed in their original study, an occurrence of incomplete resistance might involve a mixture of both resistant and susceptible individuals within the same problem field. Thus, given our findings, larval survival of *D. barberi* in Cry3Bb1 corn fields could be on the rise in some areas of North Dakota.

We also observed, incomplete resistance to Cry34/35Ab1 in the Arthur and Page problem populations of *D. barberi*. Proportional and corrected larval survival rates were significantly greater in those two populations than in the laboratory and Page control populations; however, larval survival of both problem populations when exposed to Cry34/35Ab1 were significantly lower than that of non-Bt corn. Our study also confirmed incomplete resistance to Cry34/35Ab1 in the Sargent and Ransom populations of *D. barberi*. Although, mean proportional survival rates of these two populations on Cry34/35Ab1 were numerically greater than that of the laboratory control, but the differences were not statistically significant. In contrast, corrected larval survival of the Sargent and Ransom populations on Cry34/35Ab1 was 150% and 127.8% significantly greater, respectively, than that of the laboratory control population, and significantly greater than the Page control population. Additionally, proportional survival of the Sargent and Ransom populations was significantly greater than survival in the Page control field. The corrected mortality described by Abbott (1925) takes into account test organisms that die from natural causes, in addition to the treatment applied. Therefore, our findings that the proportional larval survival of the Sargent and Ransom populations did not differ significantly from the laboratory control population could have resulted from some larvae of these two populations dying due to other, unknown causes. Gassmann et al. (2011) observed 11% corrected survival in a susceptible *D. v. virgifera* population from problem fields reared on Cry34/35Ab1. In our study, the mean corrected survival of *D. barberi* larvae was much higher, ranging from 41% to 45% for the Sargent and Ransom populations, respectively. Therefore, we suggest that *D. barberi* larval survival rates from Cry34/35Ab1-expressing corn could also be increasing in some North Dakota populations.

On pyramided Cry3Bb1 + Cry34/35Ab1 in our study, the proportional (8.7%) and corrected (13%) larval survival rates of the Arthur problem population of *D. barberi* were significantly greater than in the other tested populations. However, the proportional survival from the pyramid was 86.2% lower than the survival from non-Bt corn. Incomplete resistance by *D. barberi* to pyramided Cry3Bb1 + Cry34/35Ab1 cannot be confirmed from our results, because the proportional and corrected larval survival rates (i.e., 8.7% to 13.0%) were low. In similar research, Wangila et al. (2015) concluded that *D. v. virgifera* was not resistant to Cry34/35Ab1 because the corrected survival (i.e., from 14% to 27%) from all problem populations was low, although the mean corrected survival was significantly greater in the problem population than in the control population and was not significantly higher as compared to the laboratory control population. However, we cannot overlook the possibility of increasing larval survival by some *D. barberi* populations in pyramided Cry3Bb1 + Cry34/35Ab1 corn fields in eastern North Dakota.

Cross-resistance between Cry3Bb1 and Cry34/35Ab1 toxins could not be confirmed in the *D. barberi* populations tested in our study; however, a significant positive correlation was observed between Cry3Bb1 and the Cry3Bb1 + Cry34/35Ab1 Bt pyramid. In contrast, a positive correlation was absent between Cry34/35Ab1 and the pyramided Cry3Bb1 + Cry34/35Ab1 Bt toxins. Roush (1998) suggested that in the presence of cross-resistance, pyramided hybrids will likely provide similar protection to that of a single-toxin hybrid, because individuals resistant to one Bt toxin are killed by the second toxin. Tabashnik (1994) and Tabashnik et al. (2009) stated that populations develop cross-resistance to Bt toxins when decreased susceptibility to one toxin leads to decreased susceptibility to another toxin. The positive correlation in larval survival of *D. barberi* between Cry3Bb1 and pyramided Cry3Bb1 + Cry34/35Ab1 Bt proteins in our study could be a natural progression phase following the increased tolerance by populations to Cry3Bb1, Cry34/35Ab1, and Cry3Bb1 + Cry34/35Ab1 toxins. Several other studies on *D. v. virgifera*, a closely related species, cross-resistance was not observed between Cry3Bb1 and Cry34/35Ab1 (Gassmann et al. 2011, 2012, 2014, Wangila et al. 2015, Schrader et al. 2017) or mCry3A and Cry34/35Ab1 (Gassmann et al. 2014, Wangila et al. 2015). In contrast, cross-resistance was found between Cry3Bb1 and mCry3A (Gassmann et al. 2014, Wangila et al. 2015, Zukoff et al. 2016) and between Cry3Bb1 and eCry3.1Ab (Zukoff et al. 2016). Schrader et al. (2017) suggested that, with the absence of cross-resistance between Cry34/35Ab1 and Cry3Bb1, Cry34/35Ab1 will continue to provide effective control against *D. v. virgifera* larvae in fields with a history of Cry3Bb1 resistance or suspected resistance. The hypothesis that Cry34/35Ab1 will continue to be effective against *D. v. virgifera* arises based on the fact that Cry34Ab1/Cry35Ab1, which belongs to the binary-like Cry protein family, has a distinct mode of action from that of the Cry3 proteins (i.e., Cry3Bb, mCry3A, and eCry3.1Ab; belong to the three-domain Cry family) (Ellis et al. 2002, Bravo et al. 2012, Pardo-López et al. 2013, Wei et al. 2017). Although cross-resistance between Cry3Bb1 and mCry3A could develop, cross-resistance between Cry3Bb1 and Cry34/35Ab1 is not likely, because the Cry34Ab1/Cry35Ab1 and Cry3Aa proteins do not share a common binding site on the *D. v. virgifera* midgut brush border membrane vesicles (Li et al. 2013). As such, Li et al. (2013) suggested that combining the Cry34Ab1/Cry35Ab1 with Cry3Aa insecticidal proteins would be appropriate as a pyramid for insect resistance management in relation to *D. v. virgifera* control. Contrary to previous studies, Gassmann et al. (2016) suggested that *D. v. virgifera* populations which have developed resistance to Cry3Bb1 or mCry3A may be at higher risk of developing resistance to Cry34/35Ab1 pyramiding with the aforementioned Bt toxins. Our findings on *D. barberi* support the suggestion by Gassmann et al. (2016) because some *D. barberi* populations in our study had increased survival on the Cry3Bb1 + Cry34/35Ab1 pyramided corn hybrid. Therefore, delaying the development of resistance in *D. barberi* to the Cry3Bb1 + Cry34/35Ab1 pyramid could be limited, and there could also be a greater risk of these populations becoming resistant to Cry34/35Ab1, especially in fields where *D. barberi* has developed resistance to Bt corn hybrids expressing the Cry3Bb1 and Cry34/35Ab1 toxins.

Diabrotica barberi populations in the Page (problem population), Sargent, and Ransom populations of our experiment remained susceptible to the Cry3Bb1 and pyramided Cry3Bb1 + Cry34/35Ab1 corn hybrids. Interestingly, the Arthur field (i.e., where the *D. barberi* population has developed incomplete resistance) was located 19 km and 17 km from the Page control and problem populations, respectively, within Cass County, North Dakota. The Sargent and Ransom populations were not located in Cass County. The distance between the Page control and Page problem population fields was 9 km; this indicates that the incidence of Bt resistance in *D. barberi* may be localized and, as a result, susceptible and resistant populations can co-occur within the same county. Previous studies also have found that Cry3Bb1-resistant and susceptible *D. v. virgifera* populations can co-occur within the same county (Gassmann et al. 2011, Wangila et al. 2015). For example, research conducted by Wangila et al. (2015) included a Cry3Bb1 problem field and a control field that were located <8 km apart from each other within the same Nebraska county. Those authors suggested that *D. v. virgifera* field-evolved resistance in fields with continuous Cry3Bb1 plantings is driven by localized selection.

In our study, complete resistance to Cry3Bb1 by *D. v. virgifera* was observed in the Ransom population. Mean proportional survival of that species on Cry3Bb1 and non-Bt corn hybrids did not differ for that population. Moreover, proportional and corrected rates of larval survival on Cry3Bb1 corn were 99.3% and 94.2% lower, respectively, in the laboratory control population than in the Ransom population. Many other authors have reported field-evolved resistance in *D. v. virgifera* to Cry3Bb1, and most of those cases involved fields with a history of continuous Bt corn plantings (Gassmann et al. 2011, 2012, 2014, Gassmann 2012, Gray 2012, Porter et al. 2012, Wangila et al. 2015, Zukoff et al. 2016, Ludwick et al. 2017, Schrader et al. 2017). Shrestha et al. (2016) also observed greater emergence of *D. v. virgifera* beetles from Cry3Bb1- and mCry3A-expresing hybrids in problem fields than in control fields. Thus, finding complete resistance to Cry3Bb1 in *D. v. virgifera* in North Dakota corn fields is not surprising, although the field history of the Ransom site is unknown. Resistance to Cry3Bb1 may have been present in some North Dakota populations of *D. v. virgifera* even earlier, because a previous study also demonstrated complete field-evolved resistance in the Arthur *D. v. virgifera* population in 2015 (Calles-Torrez et al., unpublished data). In addition, Calles-Torrez et al. (2018) reported a significantly greater emergence of *D. v. virgifera* beetles from Cry3Bb1 plots than from non-Bt plots in Arthur, ND in 2013; those authors indicated that the Arthur population of *D. v. virgifera* may be less susceptible to the Cry3Bb1 toxin. That Arthur field had a history of 3 yr of continuous Cry3Bb1 corn plantings previous to 2013, which was followed by non-Bt corn in 2013, soybean in 2014, and then two additional years of successive Cry3Bb1 corn use in 2015 and 2016. However, the risk of major economic loss due to this phenomenon in eastern North Dakota in the near future is uncertain because Calles-Torrez et al. (2018) reported markedly decreased densities of *D. v. virgifera* beetles in all surveyed fields beginning in 2014. The decreases were attributed to the exceptionally cold winter of 2013/2014 that increased overwintering mortality of eggs. As such, pest management efforts to delay resistance in this area of the U.S. Corn Belt region should focus on *D. barberi* more than *D. v. virgifera*, since it is the primary corn rootworm species now found in North Dakota.

Proportional and corrected larval survival of the Ransom population of *D. v. virgifera* in the pyramided Cry3Bb1 + Cry34/35Ab1 corn hybrid were significantly greater than those of the laboratory population. Although, a significantly greater proportional survival of the Ransom population was observed in the pyramided Cry3Bb1 + Cry34/35Ab1 hybrid, the survival rate on the pyramid was 62.5% lower than that from the non-Bt corn hybrid. These results suggest that *D. v. virgifera* larval survival is increasing on pyramided Cry3Bb1 + Cry34/35Ab1 corn, even though previous research found that pyramided Cry3Bb1 + Cry34/35Ab1 corn was the most effective for reducing beetle emergence of both *D. barberi* and *D. v. virgifera* (Head et al. 2014, Keweshan et al. 2015, Calles-Torrez et al. 2018).

Gassmann et al. (2016) found incomplete resistance in *D. v. virgifera* to Cry34/35Ab1 in fields with severe injury (>2 nodes injured) and to Cry34/35Ab1 pyramided with either Cry3Bb1 or mCry3A (>1 nodes injured). Increased tolerance to Cry34/35Ab1 in Cry3Bb1-resistant *D. v. virgifera* populations also noted by Zukoff et al. (2016). Conversely, in this study, neither complete nor incomplete field-evolved resistance has been confirmed in the Ransom *D. v. virgifera* population to Cry34/35Ab1. The mean proportional and corrected larval survival rates on this toxin were equivalent to or lower than 13%. Therefore, the Ransom *D. v. virgifera* population appears to remain susceptible to the Cry34/35Ab1 toxin in eastern North Dakota.

In populations for which incomplete resistance of *D. barberi* to Cry3Bb1 and Cry34/35Ab1 and increased survival on the pyramided Cry3Bb1 + Cry34/35Ab1 by both *D. barberi* and *D. v. virgifera* were observed, it is likely that these populations will eventually develop complete resistance to these toxins in North Dakota. Similarly, considering the recent research conducted in Iowa and Minnesota (Gassmann et al. 2011, Ludwick et al. 2017), complete resistance of *D. v. virgifera* to Cry34/35Ab1 is also a strong possibility for populations located in those states. Incomplete resistance to Cry3Bb1 was initially observed in Iowa populations of *D. v. virgifera* in 2009. However, in 2010 and 2011, those populations showed complete resistance, which demonstrated that the levels of *D. v. virgifera* resistance to that toxin had increased with time (Gassmann et al. 2011, 2012, 2014). Furthermore, Gassmann et al. (2014) mentioned the condition of that Bt-resistance in *D. v. virgifera* populations persists, if not increases, over time. This strongly suggests that the use of Bt corn technology for corn rootworm management should be integrated with control tactics to slow the speed of resistance development in corn-producing states affected by these pests.

Gassmann et al. (2011) observed a significant positive correlation between larval survival rates and history of Cry3Bb1 Bt corn cultivation, where survival in Cry3Bb1 increased because of re-planting of this hybrid in the same field. Although field history details from growers were not available for some fields and control populations for each location were unavailable for our research, the results offer valuable information, especially in consideration of the overall challenges working with *D. barberi*. In addition, considering the high adoption rate (i.e., 95% of planted acres) of biotechnology for corn production in North Dakota (USDA–NASS 2017), we underscore the importance for corn producers, pest management advisors, and seed companies to take action to delay the development of corn rootworm resistance to Bt toxins in North Dakota.

In addition to continuous Bt corn plantings several other factors, including Bt toxin concentrations, refuge percentage, misuse of refuge, and repeated use of the same Bt modes of action, have been suggested as contributing to the development of field-evolved resistance to this technology by *D. v. virgifera* populations. We believe some of those same factors are also driving the development of *D. barberi* resistance to Bt corn in North Dakota. Current Bt corn hybrids do not meet the criterion of 'high-dose' for rootworm management (USEPA 2002, Siegfried et al. 2005, Storer et al. 2006, Meihls et al. 2008, Hibbard et al. 2011, Gassmann 2012). Tabashnik et al. (2004) indicated that Bt crop plants expressing high-dose concentrations of Bt toxin should be most effective in delaying pest resistance for a longer period than those expressing lower toxin concentrations. This is because high-dose concentrations are more likely to kill all or nearly all heterozygous progeny (Tabashnik et al. 2004). The addition of refuge (non-Bt corn) plantings will further slow resistance development (Gould 1998, Meihls et al. 2008). In contrast, low Bt toxin concentrations allow more heterozygous progeny to survive (Tabashnik et al. 2004).

In addition to the direct benefits of root protection and yield increases, using Bt technology for corn rootworm management is also beneficial to U.S. corn producers because it also offers other benefits, such as reduced reliance on synthetic insecticides, lower labor costs, promotion of soil health (by allowing for reduced-tillage farming), and promotion of natural enemies [Cattaneo et al. 2006, Hunt et al. 2007, Buntin 2008, Haegele and Below. 2013, Vincelli 2016, National Academies of Sciences (NAS) 2016]. Many researchers have suggested several strategies to delay field-evolved resistance to Bt crops. For example, Tabashnik and Gould

(2012) suggested that increasing the proportion of refuge corn in Bt corn fields could aid in delaying the evolution of *D. v. virgifera* resistance to Bt toxins. However, Deitloff et al. (2016) recently indicated that *D. v. virgifera* resistance to Bt corn is not always delayed by non-Bt corn refuges. Also, pyramiding (combining two or more insecticidal toxins targeting the same insect pest) of Bt crops (Li et al. 2013), refuge abundance (Carrière et al. 2010, Tabashnik et al. 2013), and refuge with pyramided strategies (Deitloff et al. 2016) should delay evolution of pest resistance to Bt crops for more generations. As suggested by other researchers (Tabashnik and Gould 2012, Gassmann et al. 2011, 2016, Andow et al. 2016, Deitloff et al. 2016), we also propose that delaying Bt corn hybrids within an IPM framework that also includes other management tactics such as crop rotation, soil insecticide use with non-Bt corn hybrids, and planting corn treated with insecticidal seed treatments, to delay or prevent the development of field-evolved resistance by corn rootworm populations in North Dakota. New, alternative insect resistance management strategies will be crucial to maintaining the efficacy and durability of the currently used Bt corn hybrids and for the coming novel insecticidal protein events. Therefore, proper use and stewardship of biotechnology must be striven to sustain the effective use of this approach for corn rootworm pest management (Shrestha et al. 2016). Additionally, further research will be needed to fully understand and characterize the expansion of *D. barberi* and *D. v. virgifera* resistance to Bt corn technology in North Dakota.

Acknowledgments

We gratefully acknowledge the guidance of Curt D. Doetkott, Consulting Statistician, and Jawahar L. Jyoti, Postdoctoral Research Fellow, in our data analyses. We also appreciate DuPont Pioneer and Monsanto Company for providing seed used in this experiment. We thank P. Beauzay, D. Carey, A. Knudson, E. Miller, J. Rikhus, A. Schroeder, and T. Senger for assistance with field, greenhouse, and laboratory aspects of this research. Gratitude is also extended to the corn producers who allowed us to conduct this research on their land, and to J. Knott of Arthur Companies Inc., for helping identify field sites. Our appreciation is also extended to several other local agronomists who helped locate fields with corn rootworm infestations. We also express sincere appreciation to the anonymous reviewers for their time and effort to improve the quality of this paper. Major funding support was provided by the North Dakota Corn Utilization Council. This work was also partially supported by the U.S. Department of Agriculture, National Institute of Food and Agriculture, Hatch project accession no. 0223202.

References Cited

Abbott, W. S. 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18: 265–267.

Andow, D. A., S. G. Pueppke, A. W. Schaafsma, A. J. Gassmann, T. W. Sappington, L. J. Meinke, P. Mitchell, T. M. Hurley, R. L. Hellmich, and R. P. Porter. 2016. Early detection and mitigation of resistance to Bt maize by western corn rootworm (Coleoptera: Chrysomelidae). J. Econ. Entomol. 109: 1–12.

Apple, J. W., and K. K. Patel. 1963. Sequence of attack by northern corn rootworms on the crown roots of corn. Proc. N. Cent. Br. Entomol. Soc. Am. 18: 80–81.

Branson, T. F., G. R. Sutter, and J. R. Fisher. 1980. Plant response to stress induced by artificial infestations of western corn rootworm. Environ. Entomol. 9: 253–257.

Bravo, A., I. Gómez, H. Porta, B. I. García-Gómez, C. Rodriguez-Almazan, L. Pardo, and M. Soberón. 2012. Evolution of *Bacillus thuringiensis* Cry toxins insecticidal activity. Microb. Biotechnol. 6: 17–26.

Buntin, G. D. 2008. Corn expressing Cry1Ab or Cry1F endotoxin for fall armyworm and corn earworm (Lepidoptera: Noctuidae) management in field corn for grain production. Fl. Entomol. 91: 523–530.

Calles-Torrez, V., J. J. Knodel, M. A. Boetel, C. D. Doetkott, K. K. Podliska, J. K. Ransom, P. Beauzay, B. W. French, and B. W. Fuller. 2018. Transgenic Bt corn, soil insecticide, and insecticidal seed treatment effects on corn rootworm (Coleoptera: Chrysomelidae) beetle emergence, larval feeding injury, and corn yield in North Dakota. J. Econ. Entomol. 111: 348–360.

Carrière, Y, D. W. Crowder, and B. E. Tabashnik. 2010. Evolutionary ecology of insect adaptation to Bt crops. Evol. Appl. 3: 561–573.

Cattaneo, M. G., C. Yafuso, C. Schmidt, C. -Y. Huang, M. Rahman, C. Olson, C. Ellers-Kirk, B. J. Orr, S. E. Marsh, L. Antilla, et al. 2006. Farm-scale evaluation of the impacts of transgenic cotton on biodiversity, pesticide use, and yield. Proc. Natl. Acad. Sci. 103: 7571–7576.

Chiang, H. C. 1965. Survival of northern corn rootworm eggs through one and two winters. J. Econ. Entomol. 58: 470–472.

Chiang, H. C. 1973. Bionomics of the northern and western corn rootworms. Annu. Rev. Entomol. 18: 47–72.

Culy, M. D., C. R. Edwards, and J. R. Cornelius. 1992. Effect of silk feeding by western corn rootworm (Coleoptera: Chrysomelidae) on yield and quality of inbred corn in seed corn production fields. J. Econ. Entomol. 85: 2440–2446.

Deitloff, J., M. W. Dunbar, D. A. Ingber, B. E. Hibbard, and A. J. Gassmann. 2016. Effects of refuges on the evolution of resistance to transgenic corn by the western corn rootworm, *Diabrotica virgifera virgifera* LeConte. Pest Manag. Sci. 72: 190–198.

Dun, Z., P. D. Mitchell, and M. Agosti. 2010. Estimating *Diabrotica virgifera virgifera* damage functions with field trial data: applying an unbalanced nested error component model. J. Appl. Entomol. 134: 409–419.

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. App. Environm. Microbiol. 68: 1137–1145.

Frank, D. L., A. Zukoff, J. Barry, M. L. Higdon, and B. E. Hibbard. 2013. Development of resistance to eCry3.1Ab-expressing transgenic maize in a laboratory-selected population of western corn rootworm (Coleoptera: Chrysomelidae). J. Econ. Entomol. 106: 2506–2513.

French, B. W., B. S. Coates, and T. W. Sappington. 2014. Inheritance of an extended diapause trait in the northern corn rootworm, *Diabrotica barberi* (Coleoptera: Chrysomelidae). J. Appl. Entomol. 138: 213–221.

Gassmann, A. J. 2012. Field-evolved resistance to Bt maize by western corn rootworm: predictions from the laboratory and effects in the field. J. Invertebr. Pathol. 110: 287–293.

Gassmann, A. J., J. L. Petzold-Maxwell, E. H. Clifton, M. W. Dunbar, A. M. Hoffmann, D. A. Ingber, and R. S. Keweshan. 2014. Field-evolved resistance by western corn rootworm to multiple *Bacillus thuringiensis* toxins in transgenic maize. Proc. Natl. Acad. Sci. 111: 5141–5146.

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.

Gassmann, A. J., J. L. Petzold-Maxwell, R. S. Keweshan, and M. W. Dunbar. 2012. Western corn rootworm and Bt maize: challenges of pest resistance in the field. GM Crops and Food 3: 235–244.

Gassmann, A. J., R. B. Shrestha, S. R. K. Jakka, M. W. Dunbar, E. H. Clifton, A. R. Paolino, D. A. Ingber, B. W. French, K. E. Masloski, J. W. Dounda, and C. R. St. Clair. 2016. Evidence of resistance to Cry34/35Ab1 corn by western corn rootworm (Coleoptera: Chrysomelidae): root injury in the field and larval survival in plant-based bioassays. J. Econ. Entomol. 109: 1872–1880.

Geisert, R. W., and L. J. Meinke. 2013. Frequency and distribution of extended diapause in Nebraska populations of *Diabrotica barberi* (Coleoptera: Chrysomelidae). J. Econ. Entomol. 106: 1619–1627.

Gould, F. 1998. Sustainability of transgenic insecticidal cultivars: integrating pest genetics and ecology. Annu. Rev. Entomol. 43: 701–726.

Gray, M. E. 2012. Continuing evolution confirmed of field resistance to Cry3Bb1 in some Illinois fields by western corn rootworm. The Bulletin: pest management and crop development information for Illinois. 20 (12) University of Illinois Extension, Urbana. (http://bulletin.ipm.illinois.edu/article.php?id=1704) (accessed 23 December 2017).

Gray, M. E., T. W. Sappington, N. J. Miller, J. Moeser, and M. O. Bohn. 2009. Adaptation and invasiveness of western corn rootworm: intensifying research on a worsening pest. Annu. Rev. Entomol. 54: 303–21.

Haegele, J. W., and F. E. Below. 2013. Transgenic corn rootworm protection increases grain yield and nitrogen use of maize. Crop Sci. 53: 585–594.

Head, G., M. Carroll, T. Clark, T. Galvan, R. M. Huckaba, P. Price, L. Samuel, and N. P. Storer. 2014. Efficacy of SmartStax® insect-protected corn hybrids against corn rootworm: the value of pyramiding the Cry3Bb1 and Cry34/35Ab1 proteins. Crop Protection 57: 38–47.

Heckel, D. G., L. J. Gahan, S. W. Baxter, J. -Z. Zhao, A. M. Shelton, F. Gould, and B. E. Tabashnik. 2007. The diversity of Bt resistance genes in species of Lepidoptera. J. Invertebr. Pathol. 95: 192–197.

Hibbard, B. E., D. L. Frank, R. Kurtz., E. Boudreau, M. R. Ellersieck, and J. F. Odhiambo. 2011. Mortality impact of Bt transgenic maize roots expressing eCry3.1Ab, mCry3A, and eCry3.1Ab plus mCry3A on western corn rootworm larvae in the field. J. Econ. Entomol. 104: 1584–1591.

Hunt, T. E., L. L. Buschman, and P. E. Sloderbeck. 2007. Insecticide use in Bt and non-Bt field corn in the western corn belt: as reported by crop consultants in a mail survey. Am. Entomol. 53: 86–93.

Jakka, S. R., R. B. Shrestha, and A. J. Gassmann. 2016. Broad-spectrum resistance to *Bacillus thuringiensis* toxins by western corn rootworm (*Diabrotica virgifera virgifera*). Scientific Reports 6: 27860. DOI: 10.1038/srep27860.

Keweshan, R. S., G. P. Head, and A. J. Gassmann. 2015. Effects of pyramided Bt corn and blended refuges on western corn rootworm and northern corn rootworm (Coleoptera: Chrysomelidae). J. Econ. Entomol. 108: 720–729.

Knodel, J. J. 2012. Western corn rootworm in corn in North Dakota. Corn Talk. Oct-Nov. 2012. ND Corn Growers Association, pp. 12–13. Fargo, ND.

Knodel, J. J., P. Beauzay, and M. Boetel. 2016. 2017 North Dakota Field Crop Insect Management Guide. Bull. E–1143 (revised), Dec. 2016. North Dakota State Univ. Ext. Serv., Fargo.

Lefko, S. A., T. M. Nowatzki, S. D. Thompson, R. R. Binning, M. A. Pascual, M. L. Peters, E. J. Simbro, and B. H. Stanley. 2008. Characterizing laboratory colonies of western corn rootworm (Coleoptera: Chrysomelidae) selected for survival on maize containing event DAS-59122-7. J. Appl. Entomol. 132: 189–204.

Levine, E., and H. Oloumi-Sadegh. 1991. Management of diabroticite rootworms in corn. Annu. Rev. Entomol. 36: 229–255.

Levine, E., H. Oloumi-Sadeghi, and J. R. Fisher. 1992. Discovery of multiyear diapause in Illinois and South Dakota northern corn rootworm (Coleoptera: Chrysomelidae) eggs and incidence of the prolonged diapause trait in Illinois. J. Econ. Entomol. 85: 262–267.

Levine, E., J. L. Spencer, S. A. Isard, D. W. Onstad, and M. E. Gray. 2002. Adaptation of the western corn rootworm to crop rotation: evolution of a new strain in response to a management practice. Am. Entomol. 48: 94–107.

Li, H., M. Olson, G. Lin, T. Hey, S. Y. Tan, and K. E. Narva. 2013. *Bacillus thuringiensis* Cry34Ab1/Cry35Ab1 interactions with western corn rootworm midgut membrane binding sites. PLoS ONE 8: e53079.

Ludwick, D. C., L. N. Meihls, K. R. Ostlie, B. D. Potter, L. French, and B. E. Hibbard. 2017. Minnesota field population of western corn rootworm (Coleoptera: Chrysomelidae) shows incomplete resistance to Cry34Ab1/Cry35Ab1 and Cry3Bb1. J. Appl. Entomol. 141: 28–40.

Ludwig, K. A., and R. E. Hill. 1975. Comparison of gut contents of adult western and northern corn rootworms in northeast Nebraska. Environ. Entomol. 4: 435–438.

Meihls, L. N., M. L. Higdon, B. D. Siegfried, N. J. Miller, T. W. Sappington, M. R. Ellersieck, T. A. Spencer, and B. E. Hibbard. 2008. Increased survival of western corn rootworm on transgenic corn within three generations of on-plant greenhouse selection. Proc. Natl. Acad. Sci. 105: 19177–19182.

Meihls L. N., M. L. Higdon, M. R. Ellersieck, and B. E. Hibbard. 2011. Selection for resistance to mCry3A-expressing transgenic corn in western corn rootworm. J. Econ. Entomol. 104: 1045–1054.

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

Metcalf, R. L. 1986. Foreword, pp. vii–xi. *In* J. L. Krysan and T. A. Miller (eds.), Methods for the study of pest *Diabrotica.* Springer, New York.

Miota, F., M. E. Scharf, M. Ono, P. Marçon, L. J. Meinke, R. J. Wright, L. D. Chandler, and B. D. Siegfried. 1998. Mechanisms of methyl and ethyl parathion resistance in the western corn rootworm. Pestic. Biochem. Physiol. 61: 39–52.

(NAS) National Academies of Sciences. 2016. Genetically engineered crops: experiences and prospects. The National Academies Press, Washington, DC.

Oswald, K. J., B. W. French, C. Nielson, and M. Bagley. 2011. Selection for Cry3Bb1 resistance in a genetically diverse population of nondiapausing western corn rootworm (Coleoptera: Chrysomelidae). J. Econ. Entomol. 104: 1038–1044.

Oyediran, I. O., P. Matthews, N. Palekar, W. French, J. Conville, and T. Burd. 2016. Susceptibility of northern corn rootworm *Diabrotica barberi* (Coleoptera: Chrysomelidae) to mCry3A and eCry3.1Ab *Bacillus thuringiensis* proteins. Insect Sci. 23: 913–917.

Pardo-López, L., M. Soberón, and A. Bravo. 2013. *Bacillus thuringiensis* insecticidal three-domain Cry toxins: mode of action, insect resistance and consequences for crop protection. FEMS Microbiol. Rev. 37: 3–22.

Pierce, C. F. 2003. Case study of a variant of western corn rootworm, *Diabrotica virgifera virgifera* LeConte, in east-central Illinois. Ph.D. dissertation, University of Illinois, Urbana-Champaign.

Porter, P., E. Cullen, T. Sappington, A. Schaafsma, S. Pueppke, D. Andow, J. Bradshaw, L. Buschman, Y. Cardoza, C. DiFonzo, et al. 2012. Comment submitted by Patrick Porter, North Central Coordinating Committee NCCC46 (22 members). 5 March 2012. (http://www.regulations.gov/#!documentDetail; ID: EPA-HQ-OPP-2011-0922-0013) (accessed 28 December 2017).

Riedell, W. E., and A. Y. Kim. 1990. Anatomical characterization of western corn rootworm damage in adventitious roots of maize. J. Iowa Acad. Sci. 97: 15–17.

Ritchie, S. W., J. J. Hanway, and G. O. Benson. 1986. How a corn plant develops. Iowa State University of Science and Technology Coop. Ext. Serv. Special Rept. 48, vol. 48. Iowa State University, Ames.

Roush, R. T. 1998. Two-toxin strategies for management of insecticidal transgenic crops: can pyramiding succeed where pesticide mixtures have not? Phil. Trans. R. Soc. Lond. B. Biol. Sci. 353: 1777–1786.

SAS Institute. 2013. SAS Version 9.4. SAS Institute, Cary, NC.

Scharf, M. E., L. J. Meinke, B. D. Siegfried, R. J. Wright, and L. D. Chandler. 1999. Carbaryl susceptibility, diagnostic concentration determination and synergism for U.S. populations of western corn rootworm. J. Econ. Entomol. 92: 33–39.

Schrader, P. M., R. E. Estes, N. A. Tinsley, A. J. Gassmann, and M. E. Gray. 2017. Evaluation of adult emergence and larval root injury for Cry3Bb1-resistant populations of the western corn rootworm. J. Appl. Entomol. 141: 41–52.

Shrestha, R. B., S. R. K. Jakka, B. W. French, and A. J. Gassmann. 2016. Field-based assessment of resistance to Bt corn by western corn rootworm (Coleoptera: Chrysomelidae). J. Econ. Entomol. 109: 1399–1409.

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

Spencer, J. L., S. A. Isard, and E. Levine. 1998. Western corn rootworms on the move: monitoring beetles in corn and soybeans, pp. 10–23. *In* 1998 Proc. Illinois Agric. Pestic. Conf., Coop. Ext. Serv., University of Illinois, Urbana-Champaign.

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: 1585–1591.

Steffey, K. L., M. E. Rice, J. All, D. A. Andow, M. E. Gray, and J. W. Van Duyn. 1999. Handbook of corn insects: pest information. Entomological Society of America, Lanham, MD.

Steel, R. G. D., and J. H. Torrie. 1980. Principles and procedures of statistics: a biometrical approach. McGraw-Hill, New York, NY.

Storer, N. P., J. M. Babcock, and J. M. Edwards. 2006. Field measures of western corn rootworm (Coleoptera: Chrysomelidae) mortality caused by Cry34/35Ab1 proteins expressed in maize event 59122 and implications for trait durability. J. Econ. Entomol. 99: 1381–1387.

Student. 1908. The probable error of a mean. Biometrika 6: 1–25.

Tabashnik, B. E. 1994. Evolution of resistance to *Bacillus thuringiensis*. Annu. Rev. Entomol. 39: 47–79.

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

Tabashnik, B. E., F. Gould, and Y. Carrière. 2004. Delaying evolution of insect resistance to transgenic crops by decreasing dominance and heritability. J. Evol. Biol. 17: 904–912.

Tabashnik, B. E., J. B. J. Van Rensburg, and Y. Carrière. 2009. Field-evolved insect resistance to *Bt* crops: definition, theory, and data. J. Econ. Entomol. 102: 2011–2025.

Tabashnik, B. E., T. Brévault, and Y. Carrière. 2013. Insect resistance to Bt crops: lessons from the first billion acres. Nat. Biotech. 31: 510–521.

Tabashnik, B. E., Y. -B Liu, T. Malvar, D. G. Heckel, L. Masson, and J. Ferré. 1998. Insect resistance to *Bacillus thuringiensis*: uniform or diverse? Phil. Trans. R. Soc. Lond. B. 353: 1751–1756.

Tinsley, N. A., R. E. Estes, and M. E. Gray. 2013. Validation of a nested error component model to estimate damage caused by corn rootworm larvae. J. Appl. Entomol. 137: 161–169.

(USDA–NASS) U.S. Department of Agriculture–National Agricultural Statistics Service. 2017. North Dakota Agricultural Statistics 2017. Ag. Statistics No. 86. ND.

(USEPA) U.S. Environmental Protection Agency. 2002. A set of scientific issues being considered by the Environmental Protection Agency regarding: corn rootworm plant-incorporated protectant non-target insect and insect resistance management issues. FIFRA Scientific Advisory Panel Meeting Minutes No. 2002-05A.

(USEPA) U.S. Environmental Protection Agency. 2003. *Bacillus thuringiensis* Cry3Bb1 protein and the genetic material necessary for its production (vector ZMIR13L) in event MON863 corn (006484) fact sheet. (https://search.epa.gov/epasearch/epasearch?querytext=Bacillus+thuringiensis+Cry3Bb1+protein+and+the+genetic+material+necessary+for+its+production+%28vector+zmir13L%29+in+event+MON863+corn+%28006484%29+fact+sheet&areaname=&areacontacts=&areasearchurl=&typeofsearch=epa&result_template=2col.ftl) (accessed 26 December 2017).

(USEPA) U.S. Environmental Protection Agency. 2014. A set of scientific issues being considered by the Environmental Protection Agency regarding scientific uncertainties associated with corn rootworm resistance monitoring for Bt corn plant incorporated protectants (PIPs). FIFRA Scientific Advisory Panel Meeting Minutes No. 2014-01. (https://www.regulations.gov/document?D=EPA-HQ-OPP-2013-0490-0044) (accessed 27 December 2017).

Vincelli, P. 2016. Genetically engineered crops: emerging opportunities. PPA-47. Univ. of Kentucky Coop. Ext. Serv., Lexington.

Wangila, D. S., A. J. Gassmann, J. L. Petzold-Maxwell, B. W. French, and L. J. Meinke. 2015. Susceptibility of Nebraska western worn rootworm (Coleoptera: Chrysomelidae) populations to Bt corn events. J. Econ. Entomol. 108: 742–751.

Wei, J. -Z., J. O'Rear, U. Schellenberger, B. A. Rosen, Y. -J. Park, M. J. McDonald, G. Zhu, W. Xie, A. Kassa, L. Procyk, et al. 2017. A selective insecticidal protein from

Pseudomonas mosselii for corn rootworm control. Plant Biotechnol. J. DOI: 10.1111/pbi.12806.

Wright, R. J., M. E. Scharf, L. J. Meinke, X. 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.

Zhao, J. -Z., M. A. Oneal, N. M. Richtman, S. D. Thompson, M.C. Cowart, M. E. Nelson, Z. Pan, A. P. Alves, and T. Yamamoto. 2016. mCry3A-selected western corn rootworm (Coleoptera: Chrysomelidae) colony exhibits high resistance and has reduced binding of mCry3A to midgut tissue. J. Econ. Entomol. 109: 1369–1377.

Zhu, K. Y., G. E. Wilde, R. A. Higgins, P. E. Sloderbeck, L. L. Buschman, R. A. Shufran, R. J. Whitworth, S. R. Starkey, and F. He. 2001. Evidence of evolving carbaryl resistance in western corn rootworm (Coleoptera: Chrysomelidae) in areawide-managed corn fields in North Central Kansas. J. Econ. Entomol. 94: 929–934.

Zukoff, S. N., K. R. Ostlie, B. Potter, L. N. Meihls, A. L. Zukoff, L. French, M. R. Ellersieck, B. W. French, and B. E. Hibbard. 2016. Multiple assays indicate varying levels of cross resistance in Cry3Bb1-selected field populations of the western corn rootworm to mCry3A, eCry3.1Ab, and Cry34/35Ab1. J. Econ. Entomol. 109: 1387–1398.

CHAPTER V. CONCLUSION

Overall, this research found that *D. barberi* or *D. v. virgifera* densities varied greatly among corn fields and years in North Dakota. *Diabrotica barberi* and *D. v. virgifera* species were distributed mostly in the southeast and in the east central parts of North Dakota, where the majority of the corn is grown; however, *D. barberi* also extended northward within the state. Beetle emergence was observed generally between mid-July and mid-October for *D. barberi* and from late July to the first week of October for *D. v. virgifera*.

Fifty-one corn fields were surveyed for corn rootworm beetle activity in the state during 2013-2015 using unbaited green Scentry^{TM} Multigard and yellow Pherocon® AM/NB sticky traps, a proportion of 61% *D. barberi* beetles and 39% *D. v. virgifera* beetles were captured. In addition, 76% of corn fields surveyed had either or both *Diabrotica* species, and both insect pests were absent in 24% of the corn fields. Density of beetles varied from 0 to >10 beetles per trap per week, and the majority of the corn fields were below economic thresholds.

Green Scentry^{TM} Multigard traps captured numerically greater numbers of *D. barberi* beetles in 68% of the corn fields containing this species compared to yellow Pherocon® AM/NB traps. In contrast, the yellow Pherocon® AM/NB traps captured numerically greater numbers of *D. v. virgifera* beetles in 57% of the corn fields containing this species than the Green Scentry^{TM} Multigard traps. However, a definitive conclusion on which sticky traps were most efficient was inconclusive because the low densities of beetles captured in most corn fields, which could confound results.

A total of 4,164 *D. barberi* and 2,263 *D. v. virgifera* beetles, across six and five site years, respectively, was captured using emergence cages deployed on Bt and non-Bt corn plots during 2013-2015. Overall, the Bt corn hybrids (Cry3Bb1, Cry34/35Ab1, and the pyramided

Cry3Bb1 + Cry34/35Ab1) provided protection against corn rootworms feeding compared to the non-Bt corn hybrids. *Diabrotica barberi* emergence from Cry34/35Ab1 plots was significantly lower than that from non-Bt hybrids at all sites, except at Wyndmere in 2013 where beetle emergence from Cry34/35Ab1 plots was about 50% fewer than the non-Bt hybrid without Poncho 1250 seed treatment. This suggests that corn hybrid-incorporated with Cry34/35Ab1 protein was effective for managing *D. barberi* populations at our chosen sites. Furthermore, the mean number of *D. v. virgifera* beetles that emerged from Cry3Bb1 corn plots was not significantly different from the non-Bt corn hybrids, but significantly greater than other two Bt corn hybrids in Arthur in 2013, indicating that this *D. v. virgifera* population could potentially be developing increased tolerance to Cry3Bb1. Overall, the pyramided Cry3Bb1 + Cry34/35Ab1 corn hybrid was the most effective Bt toxin for reducing emergence of both *D. barberi* and *D. v. virgifera* beetles in North Dakota.

Root injury ratings at all sites were low to moderate. In all Bt corn hybrids, the root injury ratings were significantly lower in comparison to the non-Bt hybrids at all site years. In contrast, the grain yield of all Bt-corn hybrids were significantly greater than the non-Bt hybrids without Poncho 1250 at the sites that had the highest, albeit moderate, rootworm populations (e.g., at Arthur in 2013 and Page in 2015). Thus, reduction of rootworm feeding injury and increase grain yield by Bt corn hybrids can be expected in some situations.

The addition of Poncho 1250 to non-Bt seed had no significant effect on larval feeding injury or on numbers of emerged beetles of both species, except at Arthur in 2014 for *D. barberi*. Also, tefluthrin soil insecticide applications had no significant impact on emergence of both corn rootworm beetles at any site or on feeding injury at Arthur in 2013 and 2014. On the contrary, the impact of tefluthrin on root feeding could have been significantly affected by both biotic and abiotic factors. On the other hand, grain yield was significantly increased by using tefluthrin soil insecticide at Arthur in 2013 and 2014, and Hope in 2014.

The timing of 50% emergence probability for beetles of both species was delayed by about one week (i.e., 5 to 7 days) in Bt hybrids when compared to non-Bt hybrids. Thus, the preferable mating between beetles emerging from Bt corn and non-Bt corn fields can be negatively impacted by the difference timing of emergence, which can speed the evolution of resistance of both species in North Dakota.

This disquisition also evaluated whether corn rootworm developed resistance in North Dakota. Corn rootworm populations were collected in 2016, and corn rootworm bioassays were run in 2017 to detect resistance. Results demonstrated incomplete field-evolved resistance to Cry3Bb1 by the Arthur *D. barberi* population (problem population). Also, incomplete resistance by *D. barberi* to Cry34/35Ab1 was detected for the Arthur (problem population), Page (problem population), Sargent, and Ransom populations. A significant positive correlation was observed between Cry3Bb1 and pyramided Cry3Bb1 + Cry34/35Ab1 Bt proteins, which may be from the increased tolerance by *D. barberi* populations to the three Bt corn hybrids. For *D. v. virgifera*, complete resistance to Cry3Bb1 was found in the Ransom population. It also was found that *D. barberi* and *D. v. virgifera* larval survival increased in the pyramided Cry3Bb1 + Cry34/35Ab1 corn hybrid.

Overall, larval survival of *D. barberi* and *D. v. virgifera* in Cry3Bb1 or Cry34/35Ab1 corn fields may be on the rise in some areas of North Dakota, although some populations still remain susceptible to Bt corn hybrids. Furthermore, in populations for which incomplete resistance by *D. barberi* to corn expressing a single Bt protein and increased larval survival of corn rootworms on the pyramided Cry3Bb1 + Cry34/35Ab1 were detected, it is likely that these populations may eventually show complete resistance to these Bt corn hybrids in corn fields of North Dakota if prevention strategies are not implemented by producers.

Monitoring corn rootworms in corn fields is crucial to help make proper IPM decisions. Alternative pest management strategies (e.g., crop rotation, chemical insecticides, and insecticidal seed treatments) may reduce or delay *D. barberi* and *D. v. virgifera* field-evolved resistance to Bt corn hybrids and help maintain effective Bt corn technologies. In addition, further research is needed to fully understand the relationship between corn rootworms and Bt corn hybrids, chemical insecticides, or other management strategies, such as crop rotation. Additional work should focus on understanding the ecology of corn rootworm resistance to Bt corn, and the increased risk for the spread of this resistance across North Dakota corn production.