

University of Nebraska - Lincoln

DigitalCommons@University of Nebraska - Lincoln

Faculty Publications: Department of
Entomology

Entomology, Department of

2021

Characterizing the Relationship Between Western Corn Rootworm (Coleoptera: Chrysomelidae) Larval Survival on Cry3Bb1-Expressing Corn and Larval Development Metrics

Jordan D. Reinders

University of Nebraska - Lincoln, jreinders2@unl.edu

David S. Wangila

Bayer East Africa Ltd, Nairobi, dwangila2@unl.edu

Emily Robinson

University of Nebraska-Lincoln, emily.robinson@huskers.unl.edu

B. Wade French

USDA-ARS, Brookings, SD

Lance Meinke

University of Nebraska-Lincoln, lmeinke1@unl.edu

Follow this and additional works at: <https://digitalcommons.unl.edu/entomologyfacpub>



Part of the [Entomology Commons](#)

Reinders, Jordan D.; Wangila, David S.; Robinson, Emily; French, B. Wade; and Meinke, Lance, "Characterizing the Relationship Between Western Corn Rootworm (Coleoptera: Chrysomelidae) Larval Survival on Cry3Bb1-Expressing Corn and Larval Development Metrics" (2021). *Faculty Publications: Department of Entomology*. 953.

<https://digitalcommons.unl.edu/entomologyfacpub/953>

This Article is brought to you for free and open access by the Entomology, Department of at DigitalCommons@University of Nebraska - Lincoln. It has been accepted for inclusion in Faculty Publications: Department of Entomology by an authorized administrator of DigitalCommons@University of Nebraska - Lincoln.

Field and Forage Crops

Characterizing the Relationship Between Western Corn Rootworm (Coleoptera: Chrysomelidae) Larval Survival on Cry3Bb1-Expressing Corn and Larval Development Metrics

Jordan D. Reinders,^{1,✉} David S. Wangila,² Emily A. Robinson,^{3,✉} B. Wade French,⁴ and Lance J. Meinke^{1,5,✉}

¹Department of Entomology, University of Nebraska, Lincoln, NE 68583, USA, ²Bayer East Africa Ltd, Crop Science Division, Nairobi 00100, Kenya, ³Department of Statistics, University of Nebraska, Lincoln, NE 68583, USA, ⁴North Central Agricultural Research Laboratory, United States Department of Agriculture–Agricultural Research Service, Brookings, SD 57006, USA, and ⁵Corresponding author, e-mail: lmeinke1@unl.edu

Subject Editor: Aaron Gassmann

Received 8 March 2021; Editorial decision 6 July 2021

Abstract

The western corn rootworm, *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae), is a significant pest of field corn, *Zea mays* L. (Poales: Poaceae), across the United States Corn Belt. Widespread adoption and continuous use of corn hybrids expressing the Cry3Bb1 protein to manage the western corn rootworm has resulted in greater than expected injury to Cry3Bb1-expressing hybrids in multiple areas of Nebraska. Single-plant bioassays were conducted on larval western corn rootworm populations to determine the level of resistance present in various Nebraska counties. The results confirmed a mosaic of susceptibility to Cry3Bb1 across Nebraska. Larval development metrics, including head capsule width and fresh weight, were measured to quantify the relationship between the level of resistance to Cry3Bb1 and larval developmental rate. Regression and correlation analyses indicate a significant positive relationship between Cry3Bb1 corrected survival and both larval development metrics. Results indicate that as the level of resistance to Cry3Bb1 within field populations increases, mean head capsule width and larval fresh weight also increase. This increases our understanding of western corn rootworm population dynamics and age structure variability present in the transgenic landscape that is part of the complex interaction of factors that drives resistance evolution. This collective variability and complexity within the landscape reinforces the importance of making corn rootworm management decisions based on information collected at the local level.

Key words: western corn rootworm, transgenic corn, Cry3Bb1, resistance management, sublethal effect

The western corn rootworm, *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae), is an economically important pest of field corn, *Zea mays* L., in the United States, costing growers in excess of \$2 billion annually in yield losses and control costs (Wechsler and Smith 2018). The modern agronomic practice of planting continuous corn (corn after corn for \geq two consecutive years) in large monocultures creates conditions suitable for rapid western corn rootworm population growth, facilitating annual western corn rootworm management challenges (Meinke et al. 2009). Larval feeding may destroy individual roots or root nodes, which can reduce nutrient uptake,

plant growth, stability, and yield (Riedell and Kim 1990, Kahler et al. 1985, Spike and Tollefson 1991, Godfrey et al. 1993, Hou et al. 1997, Urias-López et al. 2000, Urias-López and Meinke 2001). Yield loss from larval injury can be highly variable because it is caused by the complex interaction of western corn rootworm density, corn hybrid genetics, and environmental conditions (Gray and Steffey 1998, Urias-López and Meinke 2001). However, several meta-analyses of the rootworm injury-yield relationship indicate that grain yield may be reduced by 15–17% for each node of root injury (Dun et al. 2010, Tinsley et al. 2013).

Historically, three main tactics have been used in western corn rootworm management programs to reduce population densities: crop rotation, soil- and foliar-applied insecticides, and transgenic corn. Crop rotation from corn to a non-host crop such as soybean [*Glycine max* (L.) Merr. (Fabales: Fabaceae)], prevents larval development and eliminates the western corn rootworm population present in a field. This management approach is a viable cultural control method due to the strong affinity of western corn rootworm females to oviposit in corn (Branson and Krysan 1981, Spencer et al. 2009). However, in areas of the eastern Corn Belt, a rotation-resistant strain evolved that exhibited less affinity to oviposit in corn and therefore circumvented crop rotation by laying enough eggs outside of corn in non-host crops, causing subsequent injury to first-year corn (Levine et al. 2002, Rondon and Gray 2004). Soil- or foliar-applied insecticides are common management tactics that have been used in continuous corn to manage western corn rootworm larval or adult stages, respectively, since the late 1940s (Muma et al. 1949, Pruess et al. 1974, Souza et al. 2020, Meinke et al. 2021). Multiple applications per growing season and/or continued use of a single mode of action have facilitated resistance evolution to four different classes of insecticides; i.e., cyclodienes, organophosphates, carbamates, and pyrethroids (Ball and Weekman 1962, Meinke et al. 1998, Pereira et al. 2015, Souza et al. 2019, Meinke et al. 2021).

Transgenic corn expressing the insecticidal Cry3Bb1 protein, derived from the soil bacterium *Bacillus thuringiensis* Berliner (Bt) (Bacillales: Bacillaceae), was commercially introduced and marketed as a single-trait product beginning in 2003 for western corn rootworm larval management (USEPA 2003, Vaughn et al. 2005). This technology was widely adopted by growers and continuous cultivation of Cry3Bb1-expressing hybrids has led to documented cases of field-evolved resistance to Cry3Bb1 in Nebraska (Wangila et al. 2015, Reinders et al. 2018) and other areas of the U.S. Corn Belt (Gassmann et al. 2011, Gray and Spencer 2015, Ludwick et al. 2017, Schrader et al. 2017, Calles-Torrez et al. 2019). A standard single-plant larval bioassay (Gassmann et al. 2011, Wangila et al. 2015, Reinders et al. 2018) has been an effective tool to detect resistance within field populations.

Because Cry3Bb1 is not a high dose trait as defined by the U.S. Environmental Protection Agency (i.e., does not produce 25× the concentration of Bt protein necessary to kill 99.99% of susceptible insects; USEPA 1998), some survival to the adult stage occurs (Gassmann et al. 2011, Clark et al. 2012). Sublethal dietary exposure

of western corn rootworms to Cry3Bb1 in the larval stage can reduce subsequent beetle size (Murphy et al. 2011, Keweshan et al. 2015) and commonly contributes to delays in development, leading to later mean adult emergence patterns in susceptible populations (Crowder et al. 2005, Becker 2006, Murphy et al. 2010, Clark et al. 2012, Petzold-Maxwell et al. 2012, Hitchon et al. 2015, Hughson and Spencer 2015, Keweshan et al. 2015). This can lead to an increased likelihood of assortative mating of resistant beetles that emerge from Cry3Bb1 plants, which may accelerate resistance evolution (Murphy et al. 2010, Petzold-Maxwell et al. 2013, Spencer et al. 2013, Andow et al. 2016).

When western corn rootworm populations highly resistant to Cry3Bb1-expressing corn occur, the delayed development often seen in susceptible populations after dietary exposure to Cry3Bb1 disappears. This has been demonstrated with single-plant larval bioassays when resistant populations were reared on Cry3Bb1 versus isoline corn (Wangila and Meinke 2017, Gassmann et al. 2020). This raises the question: Is the sublethal effect of larval exposure to Cry3Bb1 on larval developmental rate positively correlated with the level of resistance present in a population? Oswald et al. (2012) provided some evidence of this in laboratory colonies placed under different levels of selection with Cry3Bb1, but this relationship has not been evaluated with field populations exhibiting a range of western corn rootworm susceptibility levels to this Bt protein. Because a mosaic of western corn rootworm resistance to Cry3 traits occurs in Nebraska (Wangila et al. 2015, Wangila 2016, Reinders et al. 2018) and cross-resistance between Cry3 traits is also variable (Gassmann et al. 2016, Zukoff et al. 2016, Reinders et al. 2018), mean larval development rates among populations may be different. Therefore, to address this, a data set of western corn rootworm field and lab populations was compiled from single-plant larval bioassays that exhibited a range of susceptibility to Cry3Bb1 among populations to evaluate if a direct positive relationship occurs between larval survival on Cry3Bb1-expressing corn and development as characterized by larval head capsule width and larval weight.

Materials and Methods

Insect Populations

A series of adult western corn rootworm field collections was made from 2013 to 2016 for use in this study. Beetles were collected directly from corn plants using aspiration techniques. In total, 54 western corn rootworm populations were collected across 11 different Nebraska

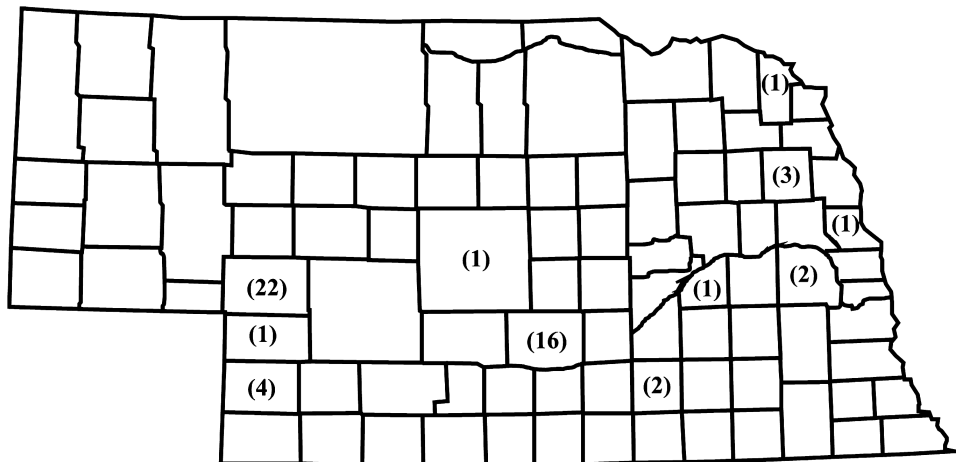


Fig. 1. Nebraska county map showing number of adult western corn rootworm collections per county in parentheses. From left to right, top to bottom: Keith (22), Perkins (1), Chase (4), Custer (1), Buffalo (16), Clay (2), Polk (1), Dixon (1), Cuming (3), Saunders (2), and Washington (1) counties.

counties (Fig. 1). Detailed information on field history, resistance categories, and larval development is presented in Tables 1–3.

In 2013 and 2014, western corn rootworm populations were collected ($n = 10$) from fields exhibiting greater than expected damage as defined by the U.S. Environmental Protection Agency (i.e., node injury scale rating ≥ 1.0 (Oleson et al. 2005) on $\geq 50\%$ of transgenic roots rated (USEPA 2014)) during the collection year or

a previous year (Wangila 2016). These problem fields were characterized by 3–6 consecutive years of Cry3Bb1 protein use. Additional western corn rootworm populations were collected from randomly selected cornfields in three counties with confirmed Cry3Bb1 resistance ($n = 7$). Field histories among these fields were variable and included 3–4 consecutive years of Cry3Bb1 protein use, conventional non-Bt corn cultivation, and first-year corn following

Table 1. Resistance category, corrected survival, and proportion in third instar for field populations collected in 2013 and 2014 from various Nebraska counties used in single-plant larval bioassays

Year collected	County (Nebraska)	Population code ^a	Resistance category	Corrected survival	No. consecutive years Cry3 ^b	No. years continuous corn ^c	3rd Instar ^d Stone 6021RR2	3rd Instar ^d Stone 6021VT3
2013	Keith	PF1	Highly resistant	0.76	5	5	0.35 (0.06)	0.23 (0.06)
	Cuming	PF3	Highly resistant	0.68	4	4	0.23 (0.06)	0.32 (0.11)
	Chase	PF4	Highly resistant	1.01	7	7	0.38 (0.09)	0.11 (0.05)
	Clay	PF5	Susceptible field	0.26	0	5	0.14 (0.06)	0.00 (0.00)*
	Custer	PF6	Moderately resistant	0.58	3	6	0.28 (0.05)	0.32 (0.08)
	Clay	PF8	Moderately resistant	0.54	4	4	0.65 (0.05)	0.50 (0.07)
	Cuming	SF1	Highly resistant	1.23	0	0	0.29 (0.08)	0.38 (0.07)
2014	Washington	FP10	Highly resistant	0.70	5	7+	0.32 (0.07)	0.24 (0.07)
	Keith	SF2	Moderately resistant	0.70	4	7	0.54 (0.07)	0.32 (0.07)
	Perkins	SF3	Moderately resistant	0.39	3	4	0.30 (0.05)	0.40 (0.09)
	Chase	SF5	Highly resistant	0.70	4	4	0.74 (0.05)	0.43 (0.08)*
	Chase	SF6	Susceptible field	0.15	Unknown	Unknown	0.25 (0.06)	0.11 (0.10)
	Keith	SF7	Susceptible field	0.02	0	7	0.44 (0.08)	1.00 (0.00)

^a Complete trait history information per field population codes listed can be found in Wangila (2016), Chapter 4.

^b Number of consecutive years planted with Cry3 hybrids from 2007 to 2013 or 2014.

^c Number of years planted with continuous corn (corn after corn).

^d Mean (\pm SE) proportion of larvae in 3rd instar (i.e., head capsule $\geq 410\mu\text{m}$ according to Hammack et al. 2003) for the non-Bt (Stone 6021RR2) and Cry3Bb1-expressing (Stone 6021VT3) hybrids. Asterisks indicate that exact 95% confidence levels around means do not overlap between hybrids within a population.

Table 2. Resistance category, corrected survival, and proportion in third instar for field populations collected in 2015 from various Nebraska counties used in single-plant larval bioassays

County (Nebraska)	Population code ^a	Resistance category	Corrected survival	No. consecutive years Cry3 ^b	No. years continuous corn ^c	3rd Instar ^d Stone 6021RR2	3rd Instar ^d Stone 6021VT3
Keith	1	Moderately resistant	0.51	0	7	0.97 (0.02)	0.33 (0.08)*
Keith	2	Susceptible Field	0.38	0	7	1.00 (0.00)	0.18 (0.08)*
Keith	3	Susceptible Field	0.17	0	7	1.00 (0.00)	0.25 (0.11)*
Keith	4	Highly Resistant	0.68	2	2	1.00 (0.00)	0.32 (0.08)*
Keith	5	Moderately Resistant	0.67	2	3	1.00 (0.00)	0.72 (0.06)*
Keith	6	Susceptible Field	0.37	6	6	1.00 (0.00)	0.72 (0.09)*
Keith	7	Highly Resistant	0.69	3	4	1.00 (0.00)	0.66 (0.07)*
Keith	8	Highly Resistant	1.01	4	6	0.95 (0.03)	0.68 (0.06)*
Keith	9	Moderately Resistant	0.64	3	3	0.97 (0.02)	0.47 (0.06)*
Buffalo	1	Susceptible Field	0.26	0	6	0.90 (0.04)	0.55 (0.11)*
Buffalo	2	Susceptible Field	0.20	0	2	0.93 (0.03)	0.48 (0.11)*
Buffalo	3	Highly Resistant	0.71	0	4	0.95 (0.03)	0.63 (0.07)*
Buffalo	4	Highly Resistant	0.70	0	1	0.96 (0.02)	0.57 (0.07)*
Buffalo	5	Moderately resistant	0.40	0	3	0.85 (0.05)	0.39 (0.10)*
Buffalo	6	Moderately resistant	0.58	2	4	0.76 (0.06)	0.62 (0.09)
Buffalo	7	Moderately resistant	0.71	Unknown	Unknown	0.78 (0.05)	0.60 (0.07)
Buffalo	8	Susceptible Field	0.35	Unknown	Unknown	0.94 (0.04)	0.25 (0.13)*
Saunders	1	Susceptible Field	0.18	Unknown	7	0.95 (0.02)	0.59 (0.12)*

^a Complete trait history information per field population codes listed can be found in Reinders et al. (2018).

^b Number of consecutive years planted with Cry3 hybrids from 2010 to 2015.

^c Number of years planted with continuous corn (corn after corn).

^d Mean (\pm SE) proportion of larvae in 3rd instar (i.e., head capsule $\geq 410\mu\text{m}$ according to Hammack et al. 2003) for the non-Bt (Stone 6021RR2) and Cry3Bb1-expressing (Stone 6021VT3) hybrids. Asterisks indicate that exact 95% confidence levels around means do not overlap between hybrids within a population.

Table 3. Resistance category, corrected survival, and proportion in third instar for field populations collected in 2016 from various Nebraska counties used in single-plant larval bioassays

County (Nebraska)	Population code ^a	Resistance category	Corrected survival	No. consecutive years Cry3 ^b	No. years continuous corn ^c	3 rd Instar ^d Stone 6021RR2	3 rd Instar ^d Stone 6021VT3
Keith	1	Susceptible field	0.03	0	7	0.68 (0.05)	0.00 (0.00)*
Keith	2	Moderately resistant	0.25	0	7	0.99 (0.01)	0.41 (0.09)*
Keith	10	Moderately resistant	0.27	0	7	0.87 (0.03)	0.70 (0.08)
Keith	11	Susceptible Field	0.20	0	7	1.00 (0.00)	0.71 (0.10)*
Keith	12	Moderately resistant	0.25	4	5	0.86 (0.03)	0.40 (0.09)*
Keith	13	Moderately resistant	0.28	7	7	0.78 (0.04)	0.70 (0.08)
Keith	14	Moderately resistant	0.52	6	7	0.90 (0.03)	0.57 (0.06)*
Keith	15	Moderately resistant	0.69	6	7	0.96 (0.02)	0.55 (0.06)*
Keith	16	Highly Resistant	1.08	6	7	0.98 (0.02)	0.93 (0.03)
Keith	17	Moderately resistant	0.70	6	7	0.90 (0.03)	0.62 (0.06)*
Buffalo	9	Moderately resistant	0.25	1	7	0.79 (0.04)	0.67 (0.09)
Buffalo	10	Susceptible Field	0.22	1	2	0.89 (0.03)	0.52 (0.11)*
Buffalo	11	Moderately resistant	0.25	2	2	0.87 (0.03)	0.48 (0.09)*
Buffalo	12	Moderately resistant	0.39	2	3	0.79 (0.04)	0.52 (0.08)*
Buffalo	13	Moderately resistant	0.48	2	3	0.93 (0.03)	0.45 (0.09)*
Buffalo	14	Moderately resistant	0.35	2	7	0.75 (0.05)	0.28 (0.08)*
Buffalo	15	Highly Resistant	0.76	6	7	0.92 (0.03)	0.57 (0.07)*
Buffalo	16	Moderately resistant	0.44	3	7	0.49 (0.06)	0.36 (0.08)*
Saunders	1	Susceptible Field	0.18	Unknown	7	0.91 (0.03)	0.12 (0.08)*

^a Complete trait history information per field population codes listed can be found in [Reinders et al. \(2018\)](#).

^b Number of consecutive years planted with Cry3 hybrids from 2010 to 2016.

^c Number of years planted with continuous corn (corn after corn).

^d Mean (\pm SE) proportion of larvae in 3rd instar (i.e., head capsule $\geq 410\mu\text{m}$ according to [Hammack et al. 2003](#)) for the non-Bt (Stone 6021RR2) and Cry3Bb1-expressing (Stone 6021VT3) hybrids. Asterisks indicate that exact 95% confidence levels around means do not overlap between hybrids within a population.

soybean. Western corn rootworm populations were collected between 8 August–7 October 2013 and 23 July–3 September 2014.

Additional adult western corn rootworm populations were collected from cornfields in Keith ($n = 19$) and Buffalo ($n = 16$) counties between 6–25 August 2015 and 3 August–2 September 2016 ([Reinders et al. 2018](#)). Many of the fields in Keith County were characterized by continuous cultivation of single-trait or pyramided corn hybrids containing Cry3Bb1 or mCry3A proteins. Fields in Buffalo County were characterized by use of multiple integrated pest management tactics (i.e., use of insecticides, crop rotation, Bt protein rotation) or continuous cultivation of Cry3Bb1- or mCry3A-expressing hybrids. Five fields in Keith County and four fields in Buffalo County had never been planted with hybrids expressing Cry3Bb1 or mCry3A prior to adult collections in 2015 and 2016. A western corn rootworm population was also collected in Saunders County from the Eastern Nebraska Research and Extension Center, Ithaca, Nebraska, during 2015 and 2016. Rootworm-active Bt corn hybrids were periodically planted in small plots at this site, although large areas of continuous corn without rootworm-Bt traits surrounded the site to maintain a refuge and reduce western corn rootworm exposure to Bt proteins.

Four diapausing western corn rootworm colonies maintained at the USDA-ARS North Central Agricultural Research Laboratory in Brookings, South Dakota, were used as susceptible laboratory populations in 2014, 2015, 2016, and 2017 larval bioassays. These populations were collected from cornfields in Butler County, Nebraska (1990), Potter County, South Dakota (1995), Finney County, Kansas (2000), and Centre County, Pennsylvania (2000). Because collections occurred prior to the commercialization of Bt proteins in 2003, these populations remain susceptible to rootworm-active transgenic corn.

Additional information for susceptible laboratory populations can be found in [Table 4](#).

Single-Plant Larval Bioassays

Western corn rootworm adults collected in the field were transported to the laboratory at the University of Nebraska-Lincoln and maintained by population in 28 cm³ plexiglass cages to obtain eggs for use in larval bioassays. Procedures used to maintain adults, collect and process eggs, and temperature regimes to facilitate diapause and post-diapause development are described in [Wangila et al. \(2015\)](#). F₁ neonate progeny from each population were used in single-plant larval bioassays as described by [Gassmann et al. \(2011\)](#) and adapted by [Wangila et al. \(2015\)](#) and [Reinders et al. \(2018\)](#). In total, 54 field-collected populations and 16 susceptible laboratory populations were assayed ($n = 70$).

A transgenic corn hybrid expressing Cry3Bb1 protein (Stone 6021VT3) and its respective non-Bt isolate (Stone 6021RR2) were used in bioassays. Twelve seeds of each corn hybrid ($n = 24$ plants) were planted and grown to the V4-V5 growth stage ([Abendroth et al. 2011](#)) in individual 1L plastic containers. Twelve neonate western corn rootworm larvae were infested onto the roots of each plant and allowed to feed for 17 d in growth chambers maintained at 24°C with a 14:10 (L:D) h photoperiod. After 17 d, plant foliage was cut at the soil line and the root mass/soil was placed into a Berlese funnel for four days to collect larval survivors in jars of 70% ethyl alcohol to determine survival. Proportional survival per plant was calculated by dividing the number of larval survivors by 12 (i.e., number of larvae infested per plant). Mean proportional survival on the non-Bt isolate for this standardized bioassay often ranges from 0.25 to 0.5 (e.g., [Gassmann et al. 2011, 2016](#); [Reinders et al. 2018](#)). Corrected survival for each population was calculated as the complement of corrected mortality using Abbott's correction ([Abbott 1925](#)).

Table 4. Corrected survival on Cry3Bb1 and proportion in third instar for susceptible laboratory populations used in single-plant larval bioassays

County (USA)	Population ^a	Bioassay year	Corrected survival	3 rd Instar ^b Stone 6021RR2	3 rd Instar ^b Stone 6021VT3
Butler, NE	Linwood	2014	0.00	0.47 (0.08)	No survival
Potter, SD	Whitlock	2014	0.05	0.23 (0.05)	0.00 (0.00)*
Finney, KS	Kansas	2014	0.00	0.06 (0.03)	No survival
Centre, PA	Penn I	2014	0.03	0.36 (0.06)	0.00 (0.00)*
Butler, NE	Linwood	2015	0.03	0.67 (0.08)	0.00 (0.00)*
Potter, SD	Whitlock	2015	0.03	0.65 (0.09)	0.00 (0.00)*
Finney, KS	Kansas	2015	0.00	0.69 (0.08)	No survival
Centre, PA	Penn I	2015	0.02	0.40 (0.07)	0.00 (0.00)*
Butler, NE	Linwood	2016	0.06	0.91 (0.03)	1.00 (0.00)*
Potter, SD	Whitlock	2016	0.08	0.86 (0.05)	0.40 (0.22)
Finney, KS	Kansas	2016	0.05	1.00 (0.00)	0.25 (0.22)*
Centre, PA	Penn I	2016	0.01	1.00 (0.00)	0.00 (0.00)*
Butler, NE	Linwood	2017	0.09	0.90 (0.04)	0.20 (0.18)*
Potter, SD	Whitlock	2017	0.02	0.65 (0.05)	0.50 (0.35)
Finney, KS	Kansas	2017	0.00	0.81 (0.05)	No survival
Centre, PA	Penn I	2017	0.02	0.92 (0.03)	0.00 (0.00)*

^a All populations were collected prior to the commercialization of Bt proteins in 2003 and have been continuously reared on non-Bt corn under standard laboratory conditions.

^b Mean (\pm SE) proportion of larvae in 3rd instar (i.e., head capsule \geq 410 μ m according to Hammack et al. 2003) for the non-Bt (Stone 6021RR2) and Cry3Bb1-expressing (Stone 6021VT3) hybrids. Asterisks indicate that exact 95% confidence levels around means do not overlap between hybrids within a population.

Larval development of bioassay survivors was indirectly measured by characterizing head capsule width and fresh weight. Both metrics increase as larvae progress through each instar (Hammack et al. 2003). Individual larval head capsule widths (i.e., distance between eyes on head) were measured for each population on the transgenic and non-transgenic hybrid. Larval instar was determined according to Hammack et al. (2003). Western corn rootworm larvae from each plant were air-dried on a Kimwipe (Kimberly-Clark Worldwide, Inc., Roswell, GA) for three minutes and then weighed using an OHAUS Voyager Pro VP413CN precision balance (OHAUS Corporation, Pine Brook, NJ) to determine the collective fresh weight of larval survivors. Mean larval fresh weight per plant was calculated by dividing the total weight by the number of larval survivors on a given plant.

Data Analysis

SAS 9.4 software (SAS Institute, Cary, NC) was used to analyze all data in this manuscript. Populations exhibiting <20% survival on the isohybrid were excluded from all analyses because survival was deemed too low to make adequate treatment comparisons. Note that all populations were not included in each analysis due to a lack of survival on Cry3Bb1 and/or unavailable data on larval head capsule width or fresh weight. Because the same corn hybrids, rearing and larval bioassay procedures, and susceptible laboratory populations were used each year bioassays were conducted, data were pooled across years to conduct final analyses with larger data sets. Additionally, proportional survival of susceptible laboratory populations on Cry3Bb1 was not significantly different across years, indicating a similar control response regardless of the year in which the bioassay was conducted (Supp. Table S1 [online only]).

Appropriate statistical analyses were conducted based on each research question being addressed. First, all field populations were assigned to Cry3Bb1 resistance categories (susceptible field, moderately resistant, highly resistant) based on bioassay survival relative to the laboratory control (category = susceptible laboratory) populations (Tables 1–4) to determine whether significant differences

in western corn rootworm larval development occurred among categories. Then, the effect of resistance category on western corn rootworm larval development metrics (i.e., head capsule width and fresh weight) was analyzed. Next, corrected survival for each population by hybrid combination was calculated. Regression and correlation analyses were then used to define the relationship between corrected larval survival and population mean head capsule width as well as corrected larval survival and population mean fresh weight. Finally, the proportion of larvae in third instar for each population by hybrid combination was compared. Details of each analysis approach are presented below.

Resistance Category Assignment

A two-step approach was taken to place field populations into Cry3Bb1 resistance categories based upon proportional survival data from single-plant larval bioassays. The first step was to determine which populations fit within the susceptible field category. Then, the remaining populations were assigned to either the moderately resistant or highly resistant categories. For each step, a generalized linear mixed model (implemented using PROC GLIMMIX) following a binomial distribution with a logit link function (Stroup 2015, Stroup et al. 2018) was used to analyze bioassay survival data within each bioassay year. Generalized χ^2/df values and conditional residual plots were used to evaluate model fit.

Differences in mean survival among populations on the Cry3Bb1-expressing corn hybrid were compared to determine which populations met the criteria for placement in the susceptible field category. Population was included in the model as a fixed factor with plant observation nested within population included as a random statement to control for an overdispersion of variance (Stroup et al. 2018). Pairwise comparisons of Cry3Bb1 survival between populations were conducted using Tukey's HSD test to control for type I error rates. Field populations were placed in the susceptible field category if mean Cry3Bb1 survival was not significantly different than mean Cry3Bb1 survival of all susceptible laboratory populations.

Proportional survival between the isoline and Cry3Bb1-expressing hybrids was compared to assign the remaining field populations to the moderately resistant or highly resistant categories. The generalized linear mixed model included population, corn hybrid, and the population by corn hybrid interaction as fixed factors and plant observation nested within the population by corn hybrid interaction was included as a random statement to control for an overdispersion of variance (Stroup et al. 2018). Simple effect comparisons of proportional survival on isoline versus Cry3Bb1 within individual populations were evaluated. Based on these comparisons, field populations not previously assigned to the susceptible field category were placed in the moderately resistant (i.e., significant difference in mean larval survival between isoline and Cry3Bb1, $P < 0.05$) or highly resistant (i.e., no significant difference in mean larval survival between isoline and Cry3Bb1, $P > 0.05$) category.

Resistance Category and Larval Development Metrics

After assigning populations to resistance categories, head capsule width was averaged for survivors on each bioassay plant. Mean larval fresh weight was calculated for each individual plant and considered as an experimental unit. A linear mixed model (implemented using PROC GLIMMIX) with a 4×2 factorial treatment design (4 resistance categories: susceptible field, moderately resistant, highly resistant, susceptible laboratory; 2 corn hybrids: isoline, Cry3Bb1) and split plot experimental design (whole plot factor: resistance category assigned to populations; split plot factor: corn hybrid assigned to individual plants) was used to analyze the effect of resistance category and hybrid on each larval development metric. Separate analyses were conducted for mean head capsule width and mean fresh weight. Type I error rates were controlled using Satterthwaite degrees of freedom adjustment and Tukey's HSD test (Stroup et al. 2018). Conditional residuals were used to evaluate model fit.

Cry3Bb1 Corrected Survival

Because the proportional survival response of susceptible laboratory populations on Cry3Bb1 was similar across bioassay years (Supp. Table S1 [online only]), proportional survival data was pooled across years and a generalized linear mixed model (implemented using PROC GLIMMIX) following a binomial distribution (Stroup 2015, Stroup et al. 2018) was used to evaluate proportional survival on the isoline and Cry3Bb1-expressing hybrids for each population. Population, corn hybrid, and the population by corn hybrid interaction were included in the model as fixed factors and plant observation nested within the population by corn hybrid interaction was included as a random statement to control for an overdispersion of variance (Stroup et al. 2018). The results from the population by hybrid LSMEANS were used to calculate corrected survival (Tables 1–4) based on the following equation:

$$\text{Corrected survival} = 1 - \frac{(\text{survival on isoline} - \text{survival on Bt})}{(\text{survival on isoline})}$$

Corrected Survival and Larval Development Metrics

A linear regression model (implemented using PROC GLIMMIX) was used to determine the intercept and slope of the relationship between corrected larval survival and mean development metric of larval survivors on Cry3Bb1 for each population with the following model:

$$\text{Population Mean Larval Development Metric} = \beta_0 + \beta_1 \cdot \text{Corrected Survival} + e$$

where β_0 is the intercept β_1 is the slope associated with the corrected survival of western corn rootworm larvae on Cry3Bb1 corn, and errors (denoted e) are assumed to be independent and normally distributed with a variance of σ^2 . Pearson's correlation coefficient was used to measure the strength of association between corrected larval survival and mean larval development metric using PROC CORR. The same analysis technique was used to analyze both mean head capsule width and mean fresh weight.

Proportion Third Instar

A binomial distribution was used to obtain an estimated value and asymptotic standard error for the proportion of larvae in third instar (according to Hammack et al. 2003) for each population and hybrid using PROC FREQ in SAS 9.4 (Tables 1–4). Clopper–Pearson was used to calculate an exact 95% confidence interval for each population and hybrid combination to account for extreme proportions (Bilder and Loughin 2014). A correlation analysis as previously described was also conducted to estimate the strength of association between corrected survival and mean proportion third instar of survivors on Cry3Bb1 in bioassays.

Results

Resistance Category and Larval Development Metrics

The interaction between corn hybrid and Cry3Bb1 resistance category significantly affected mean western corn rootworm larval head capsule width ($F = 9.54$; $df = 3, 1280$; $P < 0.0001$) (Fig. 2; Table 5). When reared on isoline corn, mean head capsule width was not significantly different among resistance categories ($F = 0.87$, $df = 3, 71.95$, $P = 0.4602$). Within categories, mean larval head capsule width was always significantly greater when reared on isoline than Cry3Bb1 (Fig. 2). Significant differences in mean head capsule width among resistance categories (e.g., susceptible field, moderately resistant, highly resistant, susceptible laboratory) when exposed to Cry3Bb1 ($F = 3.83$, $df = 3, 101.5$, $P = 0.0121$) contributed to the significant interaction. Larvae in susceptible laboratory populations exhibited a significantly smaller mean head capsule width than the resistant field populations when reared on Cry3Bb1 (Fig. 2).

The interaction between corn hybrid and Cry3Bb1 resistance category also significantly affected mean western corn rootworm larval fresh weight ($F = 3.92$; $df = 3, 898.8$; $P = 0.0085$) (Fig. 3; Table 5). Within individual categories, mean fresh weight on the isoline hybrid was significantly greater than mean fresh weight on Cry3Bb1 (Fig. 3). Significant differences in mean fresh weight among resistance categories on the Cry3Bb1 ($F = 8.17$, $df = 3, 90.59$, $P < 0.0001$) hybrid contributed to the significant interaction. Within the Cry3Bb1 hybrid, mean fresh weight of susceptible laboratory populations was significantly lower than means of the highly and moderately resistant categories, but not significantly different than the susceptible field population mean (Fig. 3).

Corrected Survival and Larval Development Metrics

The correlation analyses revealed a significant positive relationship between corrected survival on Cry3Bb1 with mean head capsule width ($r = 0.451$, $P = 0.0003$, $n = 61$; Fig. 4) and mean fresh weight ($r = 0.690$, $P < 0.0001$, $n = 50$; Fig. 5), respectively, of larval survivors. Results of the linear regression analyses present the clear positive numerical change in mean head capsule width and larval fresh weight, respectively, with increases in corrected larval survival

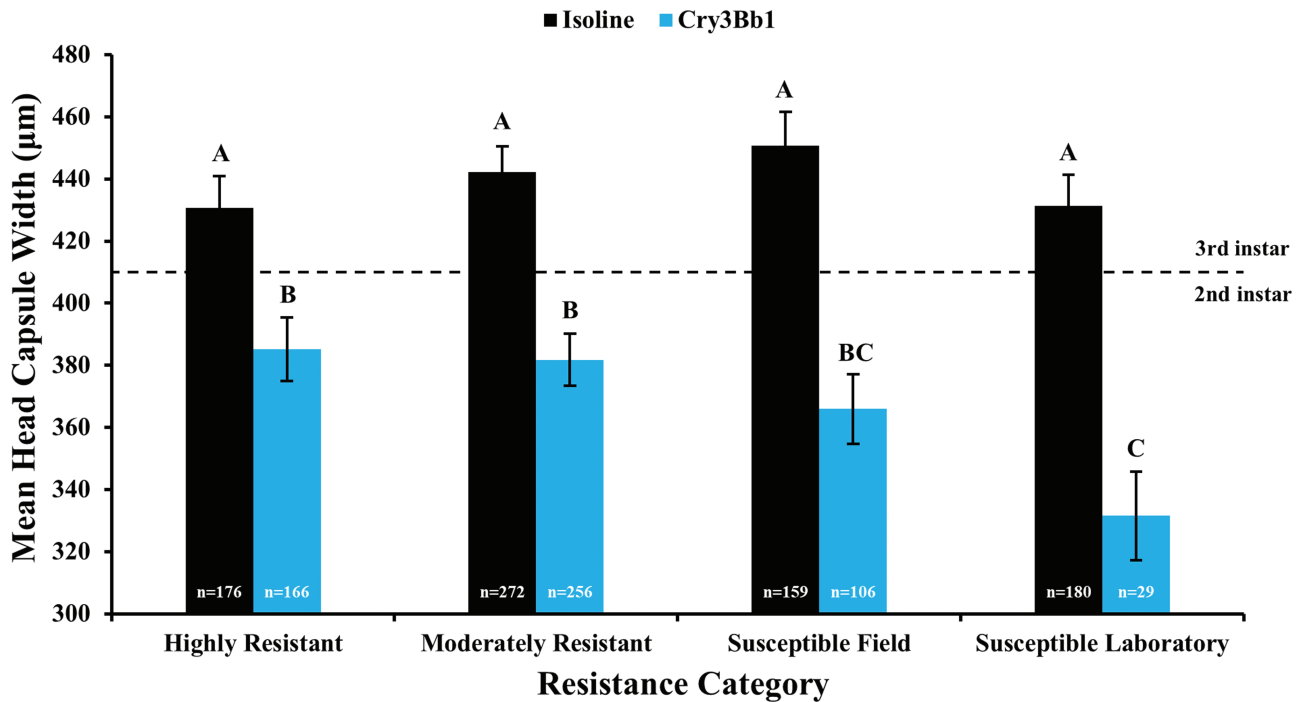


Fig. 2. Mean (±SE) western corn rootworm larval head capsule width (µm) within different resistance categories on isoline and Cry3Bb1-expressing hybrids. Means with the same letter were not significantly different by Tukey's HSD ($P > 0.05$); (n) represents the number of plants per resistance category and hybrid. The black dashed line indicates the separation between 2nd and 3rd instar larvae according to Hammack et al. (2003).

Table 5. F-test statistics, degrees of freedom, and P-values for model effects (hybrid, resistance category) and the interaction of hybrid and resistance category on head capsule width or fresh weight

Developmental Factor	Model Effect		
	Hybrid	Resistance category	Hybrid × resistance category
Head Capsule Width	352.48	1.88	9.54
	[1, 286]	[3, 68.83]	[3, 1280]
	<0.0001	0.1413	<0.0001
Fresh Weight	904.7	45.84	3.92
	[1, 184.79]	[7.06, 0.0005]	[3, 898.8]
	<0.0001	0.0005	0.0085

Significant effects at $\alpha=0.05$. Satterthwaite adjustment of degrees of freedom used to control for Type I error rates.

(Figs. 4 and 5; Table 6). A significant positive correlation was also obtained between corrected survival and mean proportion third instar ($r = 0.390, P = 0.0019, n = 61$).

Discussion

The results of this study strongly support the working hypothesis that a direct positive relationship exists between the level of Cry3Bb1 resistance present in western corn rootworm populations and the rate of larval development. This was clearly demonstrated by the strength of association between corrected larval survival and both mean larval head capsule width or fresh weight, which were used to characterize developmental time in this study (Figs. 4 and 5).

In Cry3Bb1 bioassays, mean larval head capsule width in all categories did not exceed the third instar threshold (Fig. 2). However, the composite means presented for moderately and highly resistant categories included populations that exhibited high proportions of third instar on both Cry3Bb1 and isoline hybrids as

well as populations with much lower survival on Cry3Bb1 than the isoline (Tables 1–3). All categories had some larvae that developed to third instar during Cry3Bb1 bioassays (Tables 1–3), but the significantly smaller mean head capsule width recorded in the susceptible laboratory category compared to the moderately and highly resistant categories, plus the positive correlation between corrected survival and proportion third instar, strongly indicate that the resistant categories had a higher frequency of larvae in an advanced stage of development.

The significantly larger mean weight of larvae in the highly resistant category compared to the susceptible field and susceptible laboratory categories complements the mean head capsule width and proportion third instar results (Figs. 2 and 3). This is consistent with the discrete head capsule width ranges that characterize western corn rootworm instars which get larger and heavier with each molt (Hammack et al. 2003). The mean weight of larvae from the highly resistant category during Cry3Bb1 bioassays was not significantly different than 3 of 4 non-Bt isoline treatments, similar to results

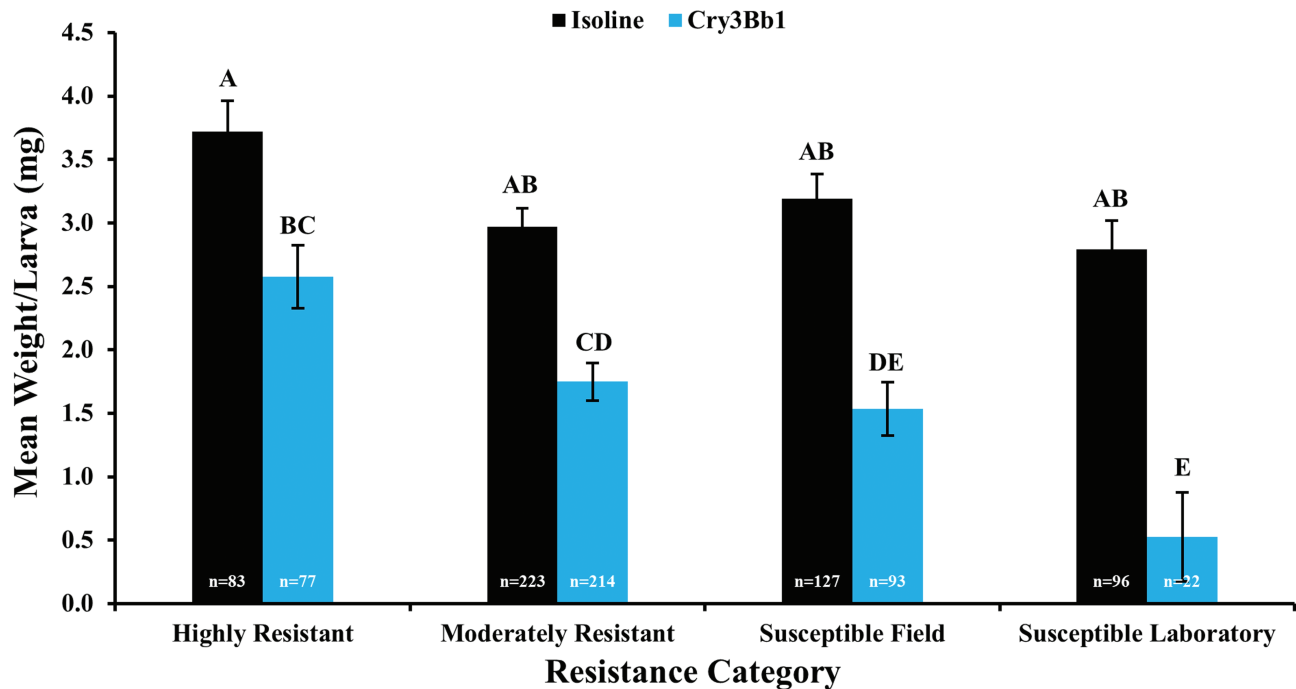


Fig. 3. Mean (\pm SE) western corn rootworm larval fresh weight (mg) within different resistance categories on isoline and Cry3Bb1-expressing hybrids. Means with the same letter were not significantly different by Tukey's HSD ($P > 0.05$); (n) represents the number of plants per resistance category and hybrid.

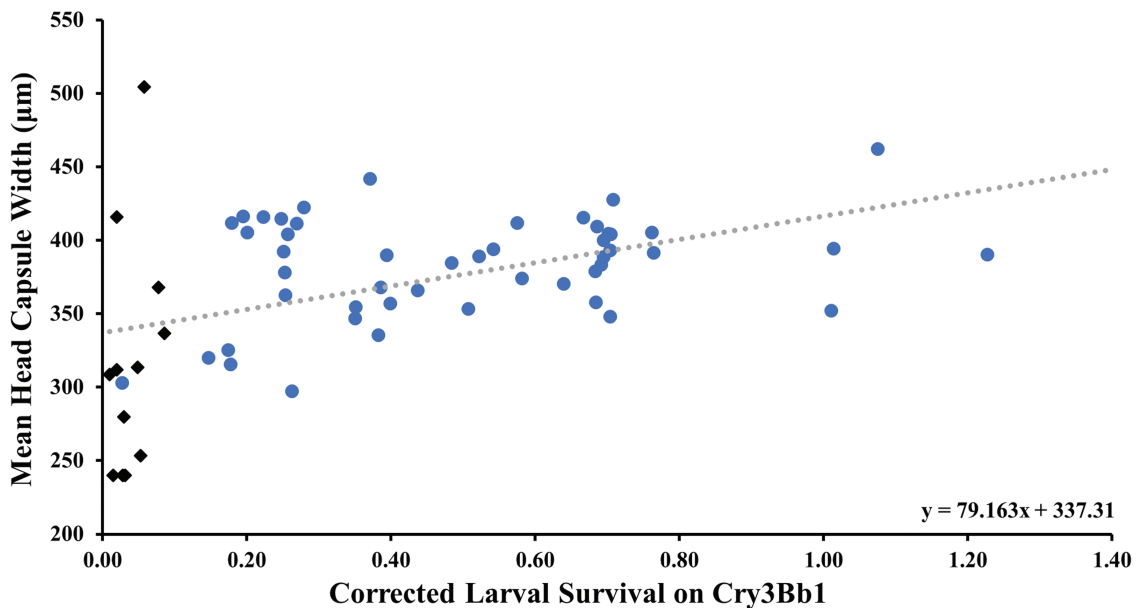


Fig. 4. Relationship between corrected survival of western corn rootworm larvae on Cry3Bb1 and the average head capsule width of larval survivors. Each dot represents an individual population. Diamonds represent susceptible laboratory populations. Circles indicate field-collected populations. The gray dashed line represents the estimated regression equation.

reported by Wangila and Meinke (2017) in populations exhibiting complete resistance.

Western corn rootworm populations within the susceptible field category appeared to be in early stages of selection for resistance to Cry3Bb1 as mean development metrics were not significantly different from the susceptible laboratory or moderately resistant categories (Figs. 2 and 3). The susceptible laboratory category metrics were typical of susceptible populations exhibiting extended development time after sublethal dietary exposure to Cry3Bb1 while metrics of populations in the moderately resistant category clearly

indicated faster development than the susceptible laboratory category. Relatively high background Cry3Bb1 resistance allele frequencies in western corn rootworm field populations are common in the landscape (Onstad and Meinke 2010, Andow et al. 2016), promoting rapid selection for western corn rootworm resistance to Cry3Bb1 under laboratory (Meihls et al. 2008, 2012; Oswald et al. 2012) and field conditions (Gassmann et al. 2011, 2014; Wangila et al. 2015). In general, the use of non-rootworm Bt hybrids or use of Cry3 hybrids for 0–2 consecutive years was often associated with lower corrected survival on Cry3Bb1 while fields with moderately or

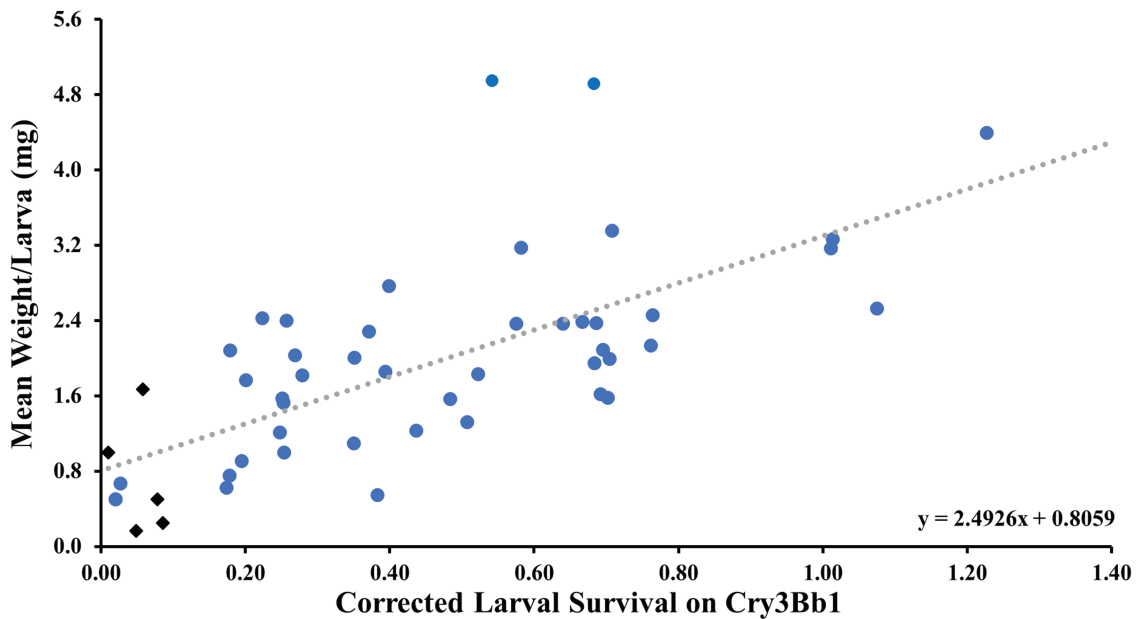


Fig. 5. Relationship between corrected survival of western corn rootworm larvae on Cry3Bb1 and the average fresh weight of larval survivors. Each dot represents an individual population. Diamonds represent susceptible laboratory populations. Circles indicate field-collected populations. The gray dashed line represents the estimated regression equation.

Table 6. Results of linear regression analyses between corrected survival and larval head capsule width or fresh weight

Analysis	<i>n</i>	β_0 (\pm SE)	β_1 (\pm SE)	σ^2
Cry3Bb1 head capsule width	61	337.31 (10.38)	79.16 (20.40)	2311.97
Cry3Bb1 fresh weight	50	0.8059 (0.20)	2.4926 (0.38)	0.6348

highly resistant populations were often characterized by continuous cultivation of Cry3-expressing hybrids for ≥ 3 yr (Tables 1–3).

However, there were exceptions to this general trend (especially apparent at some sites collected in 2015, Table 2). The data set presented in this paper is very diverse and includes year, region, and landscape effects potentially acting on populations which may contribute to the variability observed. For example, Cuming SF1 and Buffalo 4 (Tables 1 and 2) were collected from first-year and second-year cornfields, respectively, after crop rotation was imposed as a Cry3Bb1 resistance mitigation tactic. Both were surrounded by continuous corn planted with Cry3Bb1-expressing hybrids for many years. Bioassays revealed a high level of Cry3Bb1 resistance in each field, which suggests they were reinfested by Cry3Bb1-resistant adults from neighboring fields (Wangila 2016, Reinders et al. 2018). A reverse scenario was observed in Keith 6 and Keith 12 (Tables 2 and 3). These fields had been planted to Cry3Bb1-expressing hybrids for six and four consecutive years, respectively, but corrected survival was much lower than expected (Tables 2 and 3). Both fields were near clusters of continuous cornfields with no Cry3Bb1 history, which suggests that gene flow among fields may have diluted the selection pressure in Keith 6 and Keith 12 (Reinders et al. 2018). The extended time many fields remain in continuous corn in this production system (Tables 1–3) also facilitates gene flow, which may have contributed to the variability in local susceptibility to Cry3Bb1 observed. These findings complement previous research that documented localized selection pressure is a key driver of susceptibility to Cry3Bb1 in Nebraska western corn rootworm populations (Reinders et al. 2018).

The differences in western corn rootworm development associated with various Cry3 susceptibility levels contributes to variability in western corn rootworm population dynamics and age structure across the landscape. This variability and the relative densities present can potentially affect mating patterns. Results from this study suggest that as the frequency of resistant individuals increases in a population and the negative effects of dietary exposure to Cry3Bb1 decrease, developmental rates and the time of adult emergence from Bt or refuge plants will be more synchronous which could lead to more random mating between resistant and susceptible individuals. The potential impact of this more synchronous adult emergence pattern on the rate of resistance evolution would be somewhat dependent on the density of susceptible individuals present, the inheritance of resistance within a population, and the fitness of resistant individuals. Research conducted to date has shown that fitness costs of Cry3Bb1 resistance are often minimal and inheritance of resistance to Cry3Bb1 is variable across populations but is often non-recessive (Gassmann 2021). These two factors have contributed to the rapid evolution of western corn rootworm resistance to the less than high dose Cry3Bb1 protein that has been deployed in the field using the high dose refuge strategy (Andow et al. 2016, Gassmann 2016, Gassmann 2021). The reduction in developmental fitness cost with increasing frequency of Cry3Bb1-resistant individuals identified in this study provides another example indicating fitness costs are minimal in Cry3Bb1-resistant populations. As a population shifts more toward a highly resistant state, synchronous emergence within a population may not be that beneficial to resistance management because the frequency of susceptible individuals will be greatly

reduced and the proportion of R × R matings will likely increase. This will contribute to the refuge becoming functionally ineffective.

Aerial application of foliar insecticides to reduce adult western corn rootworm densities is commonly used in Nebraska and the western Corn Belt in continuous corn as a complementary tactic in the transgenic era (Souza et al. 2019, Meinke et al. 2021). Variability in Cry3 susceptibility and associated population age structure among western corn rootworm populations makes timing of foliar insecticide applications more difficult. If the goal is to reduce adult density and oviposition to help mitigate potential resistance when greater than expected injury occurs in a Bt cornfield, or as part of a holistic rootworm management strategy, insecticide applications need to target as much of the female adult population as possible to be effective. The extended adult emergence and oviposition periods associated with the Bt and non-Bt refuge system observed in Cry3Bb1-susceptible or moderately resistant populations makes this challenging (Meinke et al. 2021). Monitoring adult population dynamics in individual fields is recommended to determine the need for intervention and to appropriately time insecticide applications (Meinke 2014).

Overall, this study provides empirical evidence from field populations that the rate of larval development is positively associated with western corn rootworm corrected survival on Cry3Bb1 corn. Results confirm the hypothesis of Wangila and Meinke (2017) that developmental delays associated with sublethal exposure to Cry3Bb1 diminish as the level of resistance increases within a population. Clarifying this relationship of susceptibility and larval development increases our understanding of western corn rootworm population dynamics and age structure variability present in the transgenic landscape that is part of the complex interaction of factors that drives resistance evolution. This collective variability and complexity within the landscape reinforces the importance of making corn rootworm management decisions based on information collected at the local level.

Supplementary Data

Supplementary data are available at *Journal of Economic Entomology* online.

Acknowledgments

We thank James Brown for his aid in collecting western corn rootworm field populations and Emily Reinders for her help in conducting bioassays and laboratory rearing. We also thank numerous summer interns for aid in collecting western corn rootworm populations, measuring larval head capsule widths, and weighing larvae. Thank you to Nebraska corn growers for allowing us to collect western corn rootworm populations from their fields and Nebraska crop consultants for providing field history data. Seed was provided by Monsanto Company. We thank Chad Nielson for rearing the control western corn rootworm colonies at the USDA-ARS NCARL. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture. USDA is an equal opportunity employer. This research was partially supported by The Nebraska Agricultural Experiment Station with funding from the Hatch Act (Accession Number 1007272) through the USDA National Institute of Food and Agriculture and

The USDA-NIFA Biotechnology Risk Assessment Competitive Grant Program Grant 2012-33522-20010.

References Cited

- Abbott, W. S. 1925. A method of computing the effectiveness of an insecticide. *J. Econ. Entomol.* 18: 265–267.
- Abendroth, L. J., R. W. Elmore, M. J. Boyer, and S. K. Marlay. 2011. Corn growth and development. Iowa State University Extension and Outreach, Ames, IA.
- Andow, D. A., S. G. Pueppke, A. W. Schaafsma, A. J. Gassmann, T. W. Sappington, L. J. Meinke, P. D. 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.
- Ball, H. J., and G. T. Weekman. 1962. Insecticide resistance in the adult western corn rootworm in Nebraska. *J. Econ. Entomol.* 55: 439–441.
- Becker, S. C. 2006. Stage-specific development and mortality of western and northern corn rootworm reared on transgenic event MON863 and on a non-transgenic isolate field corn hybrid. M.S. Thesis, University of Nebraska, Lincoln.
- Bilder, C. R., and T. M. Loughin. 2014. Clopper-Pearson confidence interval, pp. 14–16. *In* Analysis of categorical data with R, 1st ed. CRC Press, Boca Raton, FL.
- Branson, T. F., and J. L. Krysan. 1981. Feeding and oviposition behavior and life cycle strategies of *Diabrotica*: an evolutionary view with implications for pest management. *Environ. Entomol.* 10: 826–831.
- Calles-Torrez, V., J. J. Knodel, M. A. Boetel, B. W. French, B. W. Fuller, and J. K. Ransom. 2019. 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. *J. Econ. Entomol.* 112: 1875–1886.
- 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.
- Gassmann, A. J. 2016. Resistance to Bt maize by western corn rootworm: insights from the laboratory and the field. *Curr. Opin. Insect Sci.* 15: 111–115.
- Gassmann, A. J. 2021. Resistance to Bt maize by western corn rootworm: effects of pest biology, the pest-crop interaction and the agricultural landscape on resistance. *Insects* 12: 136. doi: [10.3390/insects12020136](https://doi.org/10.3390/insects12020136)
- 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, 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. U. S. A.* 111: 5141–5146.
- Gassmann, A. J., R. B. Shrestha, S. R. 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.
- Gassmann, A. J., R. B. Shrestha, A. L. Kropf, C. R. St Clair, and B. D. Brenizer. 2020. Field-evolved resistance by western corn rootworm to Cry34/35Ab1 and other *Bacillus thuringiensis* traits in transgenic maize. *Pest Manag. Sci.* 76: 268–276.

- Godfrey, L. D., L. J. Meinke, and R. J. Wright. 1993. Vegetative and reproductive biomass accumulation in field corn: response to root injury by western corn rootworm (Coleoptera: Chrysomelidae). *J. Econ. Entomol.* 86: 1557–1573.
- Gray, M. E., and J. L. Spencer. 2015. Western corn rootworm: *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae) resistance to Bt maize and crop rotation: management challenges and opportunities. *Bull. Royal Entomol. Society; Antenna: ECE Special Edition* 39: 100–101.
- Gray, M. E., and K. L. Steffey. 1998. Corn rootworm (Coleoptera: Chrysomelidae) larval injury and root compensation of 12 maize hybrids: an assessment of the economic injury index. *J. Econ. Entomol.* 91: 723–740.
- Hammack, L., M. M. Ellsbury, R. L. Roehrdanz, and J. L. Pikul, Jr. 2003. Larval sampling and instar determination in field populations of northern and western corn rootworm (Coleoptera: Chrysomelidae). *J. Econ. Entomol.* 96: 1153–1159.
- 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.
- 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.
- Hughson, S. A., and J. L. Spencer. 2015. Emergence and abundance of western corn rootworm (Coleoptera: Chrysomelidae) in Bt cornfields with structured and seed blend refuges. *J. Econ. Entomol.* 108: 114–125.
- 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.
- 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.
- 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.
- 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.
- 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. U. S. A.* 105: 19177–19182.
- Meihls, L. N., M. L. Higdon, M. R. Ellersieck, B. E. Tabashnik, and B. E. Hibbard. 2012. Greenhouse-selected resistance to Cry3Bb1-producing corn in three western corn rootworm populations. *PLoS One.* 7: e51055.
- Meinke, L. J. 2014. Corn rootworm management in the transgenic era: adult corn rootworm suppression. (<http://plantmanagementnetwork.org/edcenter/seminars/corn/AdultRootworm/presentation.html>) (accessed 25 January 2021).
- Meinke, L. J., B. D. Siegfried, R. J. Wright, and L. D. Chandler. 1998. Adult susceptibility of Nebraska western corn rootworm (Coleoptera: Chrysomelidae) populations to selected insecticides. *J. Econ. Entomol.* 91: 594–600.
- Meinke, L. J., T. W. Sappington, D. W. Onstad, T. Guillemaud, N. J. Miller, J. Komáromi, N. Levay, L. Furlan, J. Kiss, and F. Toth. 2009. Western corn rootworm (*Diabrotica virgifera virgifera* LeConte) population dynamics. *Agr. Forest Entomol.* 11: 29–46.
- Meinke, L. J., D. Souza, and B. D. Siegfried. 2021. The use of insecticides to manage the western corn rootworm, *Diabrotica virgifera virgifera*, LeConte: history, field-evolved resistance, and associated mechanisms. *Insects* 12: 112. doi: 10.3390/insects12020112
- Muma, M. H., R. E. Hill, and E. Hixson. 1949. Soil treatments for corn rootworm control. *J. Econ. Entomol.* 42: 822–824.
- 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.
- 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.
- 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.
- Onstad, D. W., and L. J. Meinke. 2010. Modeling evolution of *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae) to transgenic corn with two insecticidal traits. *J. Econ. Entomol.* 103: 849–860.
- Oswald, K. J., B. W. French, C. Nielson, and M. Bagley. 2012. Assessment of fitness costs in Cry3Bb1-resistant and susceptible western corn rootworm (Coleoptera: Chrysomelidae) laboratory colonies. *J. Appl. Entomol.* 136: 730–740.
- Pereira, A. E., H. Wang, S. N. Zukoff, L. J. Meinke, B. W. French, and B. D. Siegfried. 2015. Evidence of field-evolved resistance to bifenthrin in western corn rootworm (*Diabrotica virgifera virgifera* LeConte) populations in Western Nebraska and Kansas. *PLoS One.* 10: e0142299.
- Petzold-Maxwell, J. L., X. Cibils-Stewart, B. W. French, and A. J. Gassmann. 2012. Adaptation by western corn rootworm (Coleoptera: Chrysomelidae) to Bt maize: inheritance, fitness costs, and feeding preference. *J. Econ. Entomol.* 105: 1407–1418.
- 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.
- Pruess, K. P., J. F. Witkowski, and E. S. Raun. 1974. Population suppression of western corn rootworm by adult control with ULV malathion. *J. Econ. Entomol.* 67: 651–655.
- Reinders, J. D., B. D. Hitt, W. W. Stroup, B. W. French, and L. J. Meinke. 2018. Spatial variation in western corn rootworm (Coleoptera: Chrysomelidae) susceptibility to Cry3 toxins in Nebraska. *Plos One.* 13: e0208266.
- 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.
- Rondon, S. L., and M. E. Gray. 2004. Ovarian development and ovipositional preference of western corn rootworm (Coleoptera: Chrysomelidae) variant in east central Illinois. *J. Econ. Entomol.* 97: 390–396.
- SAS Institute. 2013. SAS/STAT 2013 user's guide. SAS Institute, Inc., 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.
- Souza, D., B. C. Vieira, B. K. Fritz, W. C. Hoffmann, J. A. Peterson, G. R. Kruger, and L. J. Meinke. 2019. Western corn rootworm pyrethroid resistance confirmed by aerial application simulations of commercial insecticides. *Sci. Rep.* 9: 6713.
- Souza, D., J. A. Peterson, R. J. Wright, and L. J. Meinke. 2020. Field efficacy of soil insecticides on pyrethroid-resistant western corn rootworms (*Diabrotica virgifera virgifera* LeConte). *Pest Manag. Sci.* 76: 827–833.
- Spencer, J. L., B. E. Hibbard, J. Moeser, and D. W. Onstad. 2009. Behavior and ecology of the western corn rootworm (*Diabrotica virgifera virgifera* LeConte). *Agr. Forest Entomol.* 11: 9–27.
- Spencer, J., D. Onstad, C. Krupke, S. Hughson, Z. Pan, B. Stanley, and L. Flexner. 2013. Isolated females and limited males: evolution of insect resistance in structured landscapes. *Entomol. Exp. Appl.* 146: 38–49.
- Spike, B. P., and J. J. Tollefson. 1991. Response of western corn rootworm-infested corn to nitrogen fertilization and plant density. *Crop Sci.* 31: 776–785.
- Stroup, W. W. 2015. Rethinking the analysis of non-normal data in plant and soil science. *Agron. J.* 107: 811–827.
- Stroup, W. W., G. A. Milliken, E. A. Claassen, and R. D. Wolfinger. 2018. SAS for mixed models: introduction and basic applications. SAS Institute, Cary, NC.

- 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.
- Urías-López, M. A., and L. J. Meinke. 2001. Influence of western corn rootworm (Coleoptera: Chrysomelidae) larval injury on yield of different types of maize. *J. Econ. Entomol.* 94: 106–111.
- 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.
- (USEPA) US Environmental Protection Agency. 1998. Final report of the FIFRA Scientific Advisory Panel Subpanel on *Bacillus thuringiensis* (Bt) plant-pesticides and resistance management (<https://archive.epa.gov/scipoly/sap/meetings/web/pdf/finalfeb.pdf>) (accessed 25 January 2021).
- (USEPA) US Environmental Protection Agency. 2003. Pesticide product label, corn event MON863: corn rootworm-protected corn (https://www3.epa.gov/pesticides/chem_search/ppls/000524-00528-20030224.pdf) (accessed 25 January 2021).
- (USEPA) US Environmental Protection Agency. 2014. SAP minutes no. 2014-01. 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). (<https://www.epa.gov/sites/production/files/2015-06/documents/120413minutes.pdf>) (accessed 25 January 2021).
- Vaughn, T., T. Cavato, G. Brar, T. Coombe, T. DeGooyer, S. Ford, M. Growth, A. Howe, S. Johnson, K. Kolacz, *et al.* 2005. A method of controlling corn rootworm feeding using a *Bacillus thuringiensis* protein expressed in transgenic maize. *Crop Sci.* 45: 931–938.
- Wangila, D. S. 2016. Resistance management of western corn rootworm, *Diabrotica virgifera virgifera* LeConte, to corn traits in Nebraska. PhD Dissertation, University of Nebraska, Lincoln.
- 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.
- Wangila, D. S., A. J. Gassmann, J. L. Petzold-Maxwell, B. W. French, and L. J. Meinke. 2015. Susceptibility of Nebraska western corn rootworm (Coleoptera: Chrysomelidae) populations to Bt corn events. *J. Econ. Entomol.* 108: 742–751.
- Wechsler, S., and D. Smith. 2018. Has resistance taken root in U.S. corn fields? Demand for insect control. *Amer. J. Agr. Econ.* 100: 1136–1150.
- Zukoff, S. N., K. R. Ostlie, B. Potter, L. N. Meihls, A. L. Zukoff, L. French, M. R. Ellersieck, B. Wade 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.