2015

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Daniel L. Frank
USDA-ARS

Ryan Kurtz
Syngenta Biotechnology, Inc., Cotton Incorporated

N A. Tinsley
University of Illinois, tinsley@illinois.edu

Aaron J. Gassmann
Iowa State University

Lance J. Meinke
University of Nebraska-Lincoln, lmeinke1@unl.edu

See next page for additional authors

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Frank, Daniel L.; Kurtz, Ryan; Tinsley, N A.; Gassmann, Aaron J.; Meinke, Lance J.; Moellenbeck, Daniel; Gray, Michael E.; Bledsoe, Larry W.; Krupke, Christian H.; Estes, Ronald E.; Weber, Patrick; and Hibbard, Bruce E., "Effect of Seed Blends and Soil-Insecticide on Western and Northern Corn Rootworm Emergence from mCry3A1+ eCry3.1Ab Bt Maize" (2015). Faculty Publications: Department of Entomology. 382. https://digitalcommons.unl.edu/entomologyfacpub/382

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Authors
Daniel L. Frank, Ryan Kurtz, N A. Tinsley, Aaron J. Gassmann, Lance J. Meinke, Daniel Moellenbeck, Michael E. Gray, Larry W. Bledsoe, Christian H. Krupke, Ronald E. Estes, Patrick Weber, and Bruce E. Hibbard
INSECTICIDE RESISTANCE AND RESISTANCE MANAGEMENT

Effect of Seed Blends and Soil-Insecticide on Western and Northern Corn Rootworm Emergence from mCry3A + eCry3.1Ab Bt Maize

DANIEL L. FRANK,1,2 RYAN KURTZ,3,4 NICHOLAS A. TINSLEY,5 AARON J. GASSMANN,6 LANCE J. MEINKE,7 DANIEL MOELLENBECK,8 MICHAEL E. GRAY,5 LARRY W. BLEDSOE,9 CHRISTIAN H. KRUPKE,9 RONALD E. ESTES,5 PATRICK WEBER,6 AND BRUCE E. HIBBARD1,10

J. Econ. Entomol. 108(3): 1260–1270 (2015); DOI: 10.1093/jee/tov081

ABSTRACT Seed blends containing various ratios of transgenic Bt maize (Zea mays L.) expressing the mCry3A + eCry3.1Ab proteins and non-Bt maize (near-isoline maize) were deployed alone and in combination with a soil-applied pyrethroid insecticide (Force CS) to evaluate the emergence of the western corn rootworm, Diabrotica virgifera virgifera LeConte, in a total of nine field environments across the Midwestern United States in 2010 and 2011. Northern corn rootworm, Diabrotica barberi Smith & Lawrence emergence was also evaluated in four of these environments. Both western and northern corn rootworm beetle emergence from all Bt treatments was significantly reduced when compared with beetle emergence from near-isoline treatments. Averaged across all environments, western corn rootworm emergence from the 95:5, 90:10, and 80:20 seed blend ratios of mCry3A + eCry3.1Ab: near-isoline were 2.6-, 4.2-, and 6.7-fold greater than that from the 100:0 ratio treatment. Northern corn rootworm emergence from the same seed blend treatments resulted in 2.8-, 3.2-, and 4.2-fold more beetles than from the 100:0 treatment. The addition of Force CS (tefluthrin) significantly reduced western corn rootworm beetle emergence for each of the three treatments to which it was applied. Force CS also significantly delayed the number of days to 50% beetle emergence in western corn rootworms. The time to 50% beetle emergence in the 100% mCry3A + eCry3.1Ab treatment with Force CS was delayed 13.7 d when compared with western corn rootworm beetle emergence on near-isoline corn. These data are discussed in terms of rootworm resistance management.

KEY WORDS Diabrotica virgifera virgifera, Diabrotica barberi, refuge-in-a-bag, MIR604, 5307, insect resistance management, seed mix refuge
et al. 2005; Walters et al. 2008, 2010). In the marketplace, there are three competing Bt maize proteins (Cry3Bb1, Cry3A/35Ab1, and mCry3A) targeting corn rootworms that were originally registered for commercial sale as single events. In 2013, an additional Bt protein, eCry3.1Ab (event 5307), was registered, but only as a pyramid with mCry3A (MIR604) under the trade name Agrisure Duracade. Hibbard et al. (2011) showed that eCry3.1Ab controlled ~99.79% of western corn rootworm larvae when averaged across five Missouri field environments, a greater efficacy than has been reported for the other rootworm-active proteins (Storer et al. 2006, Hibbard et al. 2010a, Clark et al. 2012), but still not quite achieving a “high dose” (a dose that kills 99.99% of the susceptible insects in the field) as defined by the environment protection agency (EPA, Scientific Advisory Panel 1998) for single trait events.

Given the history of adaptation by the western corn rootworm and northern corn rootworm to various management tactics such as crop rotation (Krysan et al. 1956, Levine et al. 2002, Gray et al. 2009) and some conventional insecticides (Ball and Weeckman 1962, Hamilton 1965, Meinke et al. 1998), the mandate of the United States EPA that all registered Bt crops have an insect resistance management (IRM) plan was likely warranted at that time. Under the implemented strategy, a refuge of susceptible plants is maintained near the Bt crop. It is expected that susceptible insects emerging from the refuge will mate with any resistant individuals emerging from the Bt crop to produce heterozygous susceptible offspring and thus delay the evolution of pest resistance. The first IRM plans for rootworm-active Bt maize required at least a 20% block or strips (structured refuges) of non-Bt maize planted within or adjacent to the 80% of the field planted to Bt maize (Tabashnik and Gould 2012). Seed companies have now begun registering Bt products as seed blends where specific percentages of Bt and non-Bt seed are preblended in the bag sold to growers. This ensures that corn rootworm larvae would not move between plots, a 3.05 m buffer containing no vegetation was maintained between each plot within rows and between rows of plots (at the Missouri location buffer rows between plot rows were planted to maize). Maize was hand or machine planted at various locations.

### Insecticide Application

Insecticide treatments consisted of the pyrethroid soil insecticide Force CS (Tefluthrin, Syngenta Crop Protection, Greensboro, NC) applied at the time of planting (Table 1). Equipment varied between locations, but the recommended rate of 11.8 ml of liquid Force CS was applied per 304.8 m (0.46 fluid ounces per 1,000 row ft).

### Materials and Methods

#### Study Sites and Planting

Field studies were conducted in five environments in 2010 and 2011 across the Midwestern United States (Table 1). Seed blends consisted of a ratio of percentage (%) mCry3A plus eCry3.1Ab: % near-isoline. Treatments included: (1) 80:20 seed blend, (2) 90:10 seed blend, (3) 95:5 seed blend, (4) 95:5 seed blend with insecticide treatment, (5) 100:0 seed blend, (6) 100:0 seed blend with insecticide treatment, (7) eCry3.1Ab, (8) mCry3A, (9) near-isoline (hereafter isoline), and (10) isoline with insecticide treatment. Cultural practices (tillage, fertilization, herbicide application, etc.) were typical of that recommended for agricultural procedures for each area; however, soil insecticides were only applied as part of a specific treatment. Each field site consisted of 10 treatments replicated three times (four times in Champaign Co., IL in 2010) in a randomized complete block design.

Each plot consisted of four 3.05 m long rows (3.05 m long by 3.05 m wide) planted with 20 seeds per row (80 seeds per plot) with a 76.2 cm row spacing. To ensure that corn rootworm larvae would not move between plots, a 3.05 m buffer containing no vegetation was maintained between each plot within rows and between rows of plots (at the Missouri location buffer rows between plot rows were planted to maize).

#### Table 1. Important dates associated with the 2010 and 2011 field study

| Environment          | Planting | Tent set-up | First beetle collected |
|----------------------|----------|-------------|------------------------|
| 2010                 |          |             |                        |
| Tippecanoe Co, IN    | April 30 | June 18     | June 25                |
| Champaign Co., IL    | May 28   | July 1      | July 5                 |
| Boone Co., MO        | April 21 | June 29     | July 6                 |
| Story Co., IA        | May 5    | June 29     | July 3                 |
| Kossuth Co., IA      | May 18   | July 16     | July 23                |
| 2011                 |          |             |                        |
| Tippecanoe Co, IN    | May 13   | June 24     | June 27                |
| Champaign Co., IL    | May 12   | June 24     | July 5                 |
| Boone Co., MO        | May 4    | June 29     | July 11                |
| Story Co., IA        | May 11   | June 30     | July 6                 |
| Saunders Co., NE     | May 17   | June 30     | July 6                 |
Insects and Infestation. With the exception of the Missouri field sites, a trap crop of late planted maize (with pumpkins at some sites) was sown in each site in the years prior to the study to ensure a natural infestation of corn rootworm larvae the following year. In Missouri, a nonhost crop consisting of soybean, Glycine max (L.), was planted during both years prior to the study. Because central Missouri does not have rotation-resistant strains of corn rootworm that oviposit outside of maize in soybeans, all plots were artificially infested with western corn rootworm eggs obtained from the main diapausin colony of the USDA-ARS laboratory in Brookings, SD. For each year of the study, plots were infested at a rate of 1,000 eggs per 30.5 cm of row when maize had reached the V2-V3 stage (Ritchie et al. 2008). The total number of eggs needed for each plot was suspended into 400 ml of 0.15% agar solution. Trenches (5 cm deep) were dug on each side of each row using hoes, and 50 ml of egg solution was distributed evenly along the length of each side of each row. Percentage egg hatch was monitored in the laboratory from a subsample of eggs and averaged 83.8% in 2010 and 75.7% in 2011. Total viable eggs for each respective four row plot in the Missouri sites were ~30,840 for 2010 and ~30,280 for 2011.

Placement of Screen Tents and Beetle Collection. Emerging corn rootworm beetles from treatment plots were contained using screen tents (~3.4 by 4.0 m) that were placed over each individual plot. The bottom edges of each tent were buried 8–12 cm below the soil surface to prevent beetle escape and to securely anchor the tents against strong winds. Shortly after the first beetle emergence date (Table 1), all maize plants in tents at Missouri locations were stripped of leaves except for four central plants. The intact plants were left in tents to feed emerging corn rootworm beetles between collection dates and to concentrate beetles for easier collection. At other locations plants were cut just above the growing point of the plant. This height ranged from as short as 30 cm at the Illinois location to ~60 cm at the Nebraska location, where any new ear formation was also removed. Beetles were collected 2–3 times weekly from each tent using either mouth or battery operated aspirators (Catalog #s 1135A and 2820B, respectively, BioQuip, Rancho Dominguez, CA). Upon collection, beetles from isoline treatments compared with transgenic treatments (Fig. 1A and B). Significantly fewer western corn rootworm beetles emerged from the 1000:0 treatment (100% mCry3A + eCry3.1Ab) coupled with Force CS than from all other treatments (Fig. 1A and B). Overall, there was a slight, nonsignificant female bias in the number of beetles recovered from transgenic treatments (Fig. 1B). Gender and the interaction of gender and treatment were significant in the overall analysis (Table 2). The few significant differences between the number of female and male beetles that emerged from specific treatments were from the Bt plus insecticide treatments and the eCry3.1Ab treatment (Fig. 1B). The weight of western corn rootworm beetles that emerged from tents varied significantly among environment, maize treatment, gender, and all possible two-factor interactions (Tables 2 and 3). The three-factor interaction of environment × treatment × gender was marginally significant at \( P = 0.0507 \) (Table 2). When averaged across years, environments, and genders, significantly more western corn rootworm beetles emerged from isoline maize than from all other treatments (Fig. 1A and B). Significantly fewer western corn rootworm beetles emerged from the 1000:0 treatment (100% mCry3A + eCry3.1Ab) coupled with Force CS than from all other treatments (Fig. 1A and B). Overall, mean weights were greater for those beetles recovered from isoline treatments compared with transgenic treatments (Fig. 2A), and females generally weighed all possible effects of the main plot and subplot. Replication within environment was the denominator of \( F \) for the main plot effects, replication within environment and treatment was the denominator of \( F \) for the subplot effects, and the residual mean square was the denominator of \( F \) for the sub-subplot effects. Least squares means (LSMEANS) of fixed effects were calculated separately for each environment and comparisons were performed using the t-test output of the SAS model. The western and northern corn rootworm data were analyzed separately. Results from all tests were considered statistically different at \( P < 0.05 \). Beetle emergence data were analyzed by plotting observed cumulative probabilities versus Julian dates. PROC PROBIT of the SAS statistical package (SAS Institute 2008) was used to model the occurrence of 50% beetle emergence and the 95% confidence intervals (CIs) among maize treatments.

Results
The number of western corn rootworm beetles emerging from tents varied significantly across environment, maize treatment, gender, and all possible two-factor interactions (Tables 2 and 3). The three-factor interaction of environment × treatment × gender was marginally significant at \( P = 0.0507 \) (Table 2). When averaged across years, environments, and genders, significantly more western corn rootworm beetles emerged from isoline maize than from all other treatments (Fig. 1A and B). Significantly fewer western corn rootworm beetles emerged from the 1000:0 treatment (100% mCry3A + eCry3.1Ab) coupled with Force CS than from all other treatments (Fig. 1A and B). Overall, there was a slight, nonsignificant female bias in the number of beetles recovered from transgenic treatments (Fig. 1B). Gender and the interaction of gender and treatment were significant in the overall analysis (Table 2). The few significant differences between the number of female and male beetles that emerged from specific treatments were from the Bt plus insecticide treatments and the eCry3.1Ab treatment (Fig. 1B). The weight of western corn rootworm beetles that emerged from tents varied significantly among environment, maize treatment, gender, and all possible interactions except maize treatment × gender (Table 2). Overall, mean weights were greater for those beetles recovered from isoline treatments compared with transgenic treatments (Fig. 2A), and females generally weighed...
more than males (Fig. 2B), though an estimate of LSMEANS was not possible because of the large number of missing values (beetles were not always recovered, especially from transgenic treatments).

When averaged across years, environments, and gender, there was nearly a 14-d delay in time to 50% emergence from the 100:0 treatment coupled with Force CS compared with isoline maize as calculated by PROC PROBIT (Table 4). CIs (95%) overlapped for just three combinations: the 95:5 seed blend and 90:10 seed blend; 100:0 seed blend and eCry3.1Ab; and 100:0 seed blend and the 95:5 seed blend coupled with Force CS. The 50% emergence date was significantly different for all other treatments in the combined analysis.

The number of northern corn rootworm beetles emerging from tetrads varied significantly among environment, maize treatment, environment × maize treatment, and environment × gender (Tables 5 and 6). When averaged across years, environments, and gender, significantly more northern corn rootworm beetles

| Treatment | 2010 Female no. | Male no. | Total no. | 2011 Female no. | Male no. | Total no. |
|-----------|----------------|----------|-----------|----------------|----------|-----------|
| Tippecanoe Co., IN | 95:5 | 4.0 | 6 | 6 | 6 | 6 |
| 95:5 + Force | 3.7 | 1.2 | 0.7 | 5.0 | 1.7 | 0.7 |
| 100:0 | 4.7 | 1.7 | 0.0 | 5.7 | 1.7 | 0.0 |
| 1000 + Force | 2.0 | 1.5 | 0.0 | 2.0 | 1.5 | 0.0 |
| eCry3.1Ab | 7.0 | 2.5 | 4.7 | 9.0 | 2.5 | 4.7 |
| ncCry3A | 69.3 | 23.5 | 4.0 | 7.0 | 2.5 | 4.7 |
| Isoline + Force | 37.7 | 7.4 | 264 | 74.9 | 614 | 129.5 |
| 129.0 | 23.2 | 88.3 | 11.3 | 217.3 | 30.7 | 15.7 |
| 95:5 | 80:20 | 100:0 | 0.7 | 14.3 | 1.9 | 0.7 |
| 95:5 | 14.3 | 1.9 | 0.7 |
| 95:5 | 3.7 | 1.2 | 0.7 |
| 100:0 | 4.7 | 1.7 | 0.0 |
| 1000 + Force | 2.0 | 1.5 | 0.0 |
| eCry3.1Ab | 7.0 | 2.5 | 4.7 |
| ncCry3A | 69.3 | 23.5 | 4.0 |
| Isoline + Force | 37.7 | 7.4 | 264 |
| 129.0 | 23.2 | 88.3 |
| 95:5 | 80:20 | 100:0 | 0.7 |
| 95:5 | 14.3 | 1.9 | 0.7 |
| 95:5 | 3.7 | 1.2 | 0.7 |
| 100:0 | 4.7 | 1.7 | 0.0 |
| 1000 + Force | 2.0 | 1.5 | 0.0 |
| eCry3.1Ab | 7.0 | 2.5 | 4.7 |
| ncCry3A | 69.3 | 23.5 | 4.0 |
| Isoline + Force | 37.7 | 7.4 | 264 |
| 129.0 | 23.2 | 88.3 |
| 95:5 | 80:20 | 100:0 | 0.7 |
| 95:5 | 14.3 | 1.9 | 0.7 |
| 95:5 | 3.7 | 1.2 | 0.7 |
| 100:0 | 4.7 | 1.7 | 0.0 |
| 1000 + Force | 2.0 | 1.5 | 0.0 |
| eCry3.1Ab | 7.0 | 2.5 | 4.7 |
| ncCry3A | 69.3 | 23.5 | 4.0 |
| Isoline + Force | 37.7 | 7.4 | 264 |
| 129.0 | 23.2 | 88.3 |

Different lowercase letters indicate significant differences (P < 0.05) between treatments within a column and location.
emerged from isoline treatments compared with transgenic treatments (Fig. 3). In addition, significantly fewer northern corn rootworm beetles emerged from the 100:0 treatment and 95:5 seed blend coupled with Force CS than from all other maize treatments except for the 100:0 treatment coupled with Force CS and eCry3.1Ab treatments (Fig. 3). The weight of northern corn rootworm beetles that emerged from tents varied significantly among environment and gender (Table 5). Treatment was marginally significant at $P = 0.0553$ (Table 5). Female beetles weighed significantly more than male beetles overall but this difference was not significant within the 100:0 treatment, 100:0 treatment coupled with Force CS, 90:10 seed blend, mCry3A, and isoline treatments (Fig. 4).

Discussion

Relatively few studies involving comparisons of beetle emergence from seed blend refuges to block refuges have been published in the refereed literature. Petzold-Maxwell et al. (2013b) compared blended refuges of near-isoline and Cry34/35Ab1 to pure stands of non-Bt and Cry34/35Ab1. In their study, rootworm survival in the blended refuge treatments was not significantly greater than survival in the pure stand of Bt maize. Head et al. (2014a) conducted a series of laboratory and field experiments comparing mixtures of non-Bt seed and seed containing Cry3Bb1 + Cry34/35Ab1. Again, there was no significant difference in western corn rootworm beetle emergence between the blended refuge and the pure Bt stand. Both studies also evaluated insecticidal seed treatments, but in general, seed treatments did not significantly impact beetle emergence. Both Cry34/35Ab1 and Cry3Bb1 + Cry34/35Ab1 significantly delayed 50% beetle emergence when compared with the pure non-Bt refuge (Petzold-Maxwell et al. 2013b, Head et al. 2014a). In this study, when averaged across gender and all environments, significantly more beetles of both western and northern
corn rootworm emerged from the 5% seed blend refuges when compared with the pure pyramid treatment (Figs. 1A and 4). For western corn rootworm, an average of 14.1 beetles per plot emerged from 100% eCry3.1Ab + mCry3A. Significantly more western corn rootworm beetles emerged from the 5% seed blend when male and female data were combined (Fig. 1A).

Table 4. Mean Julian date for 50% emergence of WCR beetle emergence when averaged across 2 yr, 5 environments, and 28 total replications

| Treatment    | 50% emergence | 95% confidence interval |
|--------------|---------------|-------------------------|
| Isoline      | 196.23 h      | 196.196–196.26          |
| Isoline + Force | 197.00 g    | 196.938–197.056         |
| 80:20        | 201.64 f      | 201.531–201.737         |
| mCry3A       | 202.88 e      | 202.795–202.964         |
| 95:5         | 203.39 d      | 203.213–203.577         |
| 90:10        | 203.58 d      | 203.425–203.736         |
| 95:5 + Force | 205.67 c      | 205.317–205.030         |
| 100:0        | 205.94 b      | 205.659–206.213         |
| eCry3.1Ab    | 206.29 b      | 206.051–206.528         |
| 100:0 + Force| 209.99 a      | 209.401–210.369         |

Different lowercase letters indicate significant differences (P < 0.05).

Mean (± SE) weight of western corn rootworm beetles recovered from maize treatments (A) data averaged across years, environments, and gender (B) data averaged across years and environment.

Table 5. ANOVA table for factors impacting the number and dry weight of northern corn rootworm (NCR) beetles recovered from tents during the study

| Effect               | NCR no. | NCR wt. |
|----------------------|---------|---------|
| df                   | F       | P       |
| df                   | F       | P       |
| Environment (Env)    | 3, 8    | 16.93   | 0.0008  |
| Treatment (Trt)      | 9, 72   | 14.46   | <0.0001 |
| Env × Trt            | 9, 63   | 1.99    | 0.0553  |
| Gender               | 1, 80   | 0.69    | 0.4080  |
| Gender               | 1, 59   | 41.63   | <0.0001 |
| Environment × gender | 3, 80   | 7.62    | 0.0002  |
| Trt × gender         | 9, 59   | 0.59    | 0.6220  |
| Env × Trt × gender   | 27, 80  | 0.87    | 0.6516  |

(Figs. 1A and 4). For western corn rootworm, an average of 14.1 beetles per plot emerged from 100% eCry3.1Ab + mCry3A. Significantly more western corn rootworm beetles emerged from the 5% seed blend when male and female data were combined (Fig. 1A).
The 5% blended refuge produced 2.6-fold more western corn rootworm beetles than the pure pyramid, while the 10–20% seed blends had 4.2- and 6.7-fold more beetles emerged, respectively (Fig. 1A). To put this in perspective, an average of 832.2 western corn rootworm beetles per tent were produced from 100% near isolate (Fig. 1A). A pure stand of near-isoline refuge corn that was 5% of this area would produce 5% of 832.2 or 41.6 beetles, but these beetles would emerge a considerable distance away from any beetles produced from a pure stand of Bt if block refuges were utilized. In this study, an average of 37.3 western corn rootworm beetles per tent were produced from the 95:5 seed blend (Fig. 1A). This is nearly 90% of the beetles from a similar number of near-isolate plants in a pure stand, but emerging in the same vicinity as any beetles from Bt. Although only 2.6-fold more beetles emerged from 5% near-isolate seed blend than from the 100% eCry3.1Ab + nCry3A, unlike the 5% seed blend for SmartStax (Head et al. 2014a) and Cry34/35Ab alone (Petzold-Maxwell et al. 2013b), this number was significantly more than the number of beetles from the pure pyramid (Fig. 1A). However, some of these actually emerged from the 95% Bt rather than...
the 5% refuge, so the number that likely emerged from just isoline plants might be closer to 24 [37.3 from the 5% seed blend—number expected from Bt plants (0.95 \times 14.1)].

The high dose refuge strategy for IRM in single trait events involves planting Bt crops that produce a high concentration of toxin (a minimum of 99.99% mortality in the field) to ensure that individuals that are heterozygous for resistance do not survive on the Bt crop, thus making resistance functionally recessive (EPA, Scientific Advisory Panel 1998). None of the previously registered individual rootworm-active Bt products express a concentration of Cry proteins considered high-dose (Storer et al. 2006, Hibbard et al. 2010a, Clark et al. 2012, Head et al. 2014b), effectively making the strategy for single trait rootworm products simply a refuge strategy. According to Roush (1998) “much can be gained from pyramiding if the mortality of susceptible insects [to individual components] is consistently >95%.” Previously, Hibbard et al. (2011) showed that eCry3.1Ab and mCry3A caused a reduction in western corn rootworm emergence of 99.79% and 97.83% when compared with emergence from isoline maize in the specific western corn rootworm populations and the environments evaluated, suggesting that pyramiding would be useful. Efficacy of rootworm-active Bt maize can vary with environment and insect population (Storer et al. 2006; Hibbard et al. 2010a, 2011; Clark et al. 2012). In this study, the 100:0 treatment (100% eCry3.1Ab + mCry3A) caused a greater reduction in beetle emergence than each protein used separately (98.3% compared with 97.4% for eCry3.1Ab and 81.9% for mCry3A), but none of the treatments caused as much of a reduction in beetle emergence as from similar treatments in Hibbard et al. (2011). Because egg densities in most locations were unknown in this study, it is possible that density-dependent mortality reduced emergence on isoline corn in some locations resulting in an underestimate of mortality due to the Bt toxins (Hibbard et al. 2010b). It is also possible that the strain used by Hibbard et al. (2010b) was more susceptible than most of the field strains evaluated here. Pyramiding mCry3A and eCry3.1Ab will be effective in delaying resistance as long as cross resistance is not present (Roush 1998).

Petzold-Maxwell et al. (2013a) and Tinsley et al. (2015) concluded that combining insecticide with Bt maize did not increase yield or reduce root injury to Bt maize. Furthermore, Petzold-Maxwell et al. (2013a) concluded that delays in emergence from Bt maize combined with insecticides could promote assortative mating among Bt-selected individuals, which may hasten resistance evolution. We did not collect yield or plant injury information, but in our study, insecticides significantly increased delays in emergence compared with Bt maize alone. The greatest delay in time to 50% emergence was from the 100:0 treatment coupled with Force CS (13.8 d relative to 50% emergence from near-isoline). The delayed emergence of western corn rootworm beetles from Bt fields relative to refuge fields is a commonly observed phenomenon. Hibbard et al. (2011) showed that there was an 8.0 d delay in time to 50% emergence for western corn rootworm beetles from eCry3.1Ab compared with isoline maize, but for eCry3.1Ab + mCry3A the delay was 4.6 d. Although this study similarly showed a greater delay in the time to 50% emergence for eCry3.1Ab (10.1 d) compared with the 100:0 treatment (9.7 d), delays in beetle emergence were greater than those reported previously by Hibbard et al. (2011). The addition of Force CS exacerbated this delay and would make the risk of assortative mating greater if used on pure stand pyramidal maize.
and to a lesser extent, for seed blends as well. The addition of insecticides also increased western corn rootworm mortality compared with Bt maize alone. The 95:5 seed blend and 100:0 treatment coupled with Force CS caused a reduction in western corn rootworm beetle emergence of 98.6–99.3%, respectively, versus 95.5–98.3%, respectively, for these same treatments without insecticide. When averaged across the locations of the present study, the addition of Force CS significantly affected western corn rootworm beetle emergence of 98.6–99.3%, respectively, versus 95.5–98.3%, respectively, for seed blends as well. The 95:5 seed blend and 100:0 treatment coupled with Force CS caused a reduction in western corn rootworm mortality compared with Bt maize alone. The 95:5 seed blend and 100:0 treatment coupled with Force CS caused a reduction in western corn rootworm mortality compared with Bt maize alone.

Acknowledgments

We wish to thank a large number of workers at each location that assisted in the labor-intensive tasks of setting up plots, digging screen tents into the ground, and collecting, counting and sexing beetles. This study was funded, in part by Syngenta and in part by Biotechnology Risk Assessment Grant Program competitive grant 2009-33120-20256 from the USDA National Institute of Food and Agriculture. Finally, we wish to thank two anonymous reviewers whose suggestions improved the writing quality and overall manuscript. This article reports the results of research only. 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) or any of the universities represented in this publication. USDA is an equal opportunity provider and employer.

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*Received 3 November 2014; accepted 13 March 2015.*