

2-2014

Susceptibility and Aversion of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) to Cry1F Bt Maize and Considerations for Insect Resistance Management

Rachel Renee Binning
DuPont Pioneer, rachel.binning@pioneer.com

Joel R. Coats
Iowa State University, jcoats@iastate.edu

Xiaoxiao Kong
DuPont Pioneer

Richard L. Hellmich
Iowa State University, richard.hellmich@ars.usda.gov

Follow this and additional works at: http://lib.dr.iastate.edu/ent_pubs

 Part of the [Agricultural Science Commons](#), and the [Entomology Commons](#)

The complete bibliographic information for this item can be found at http://lib.dr.iastate.edu/ent_pubs/142. For information on how to cite this item, please visit <http://lib.dr.iastate.edu/howtocite.html>.

This Article is brought to you for free and open access by the Entomology at Iowa State University Digital Repository. It has been accepted for inclusion in Entomology Publications by an authorized administrator of Iowa State University Digital Repository. For more information, please contact digirep@iastate.edu.

Susceptibility and Aversion of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) to Cry1F Bt Maize and Considerations for Insect Resistance Management

Abstract

Bacillus thuringiensis (Bt) maize was developed primarily for North American pests such as European corn borer (*Ostrinia nubilalis* (Hübner)). However, most Bt maize products are also cultivated outside of North America, where the primary pests may be different and may have lower susceptibility to Bt toxins. Fall armyworm (*Spodoptera frugiperda* JE Smith) is an important pest and primary target of Bt maize in Central and South America. *S. frugiperda* susceptibility to Cry1F (expressed in event TC1507) is an example of a pest-by-toxin interaction that does not meet the high-dose definition. In this study, the behavioral and toxic response of *S. frugiperda* to Cry1F maize was investigated by measuring the percentage of time naïve third instars spent feeding during a 3-min exposure. *S. frugiperda* also were exposed as third instars to Cry1F maize for 14 d to measure weight gain and survival. *S. frugiperda* demonstrated an initial, postingestive aversive response to Cry1F maize, and few larvae survived the 14 d exposure. The role of susceptibility and avoidance are discussed in the context of global IRM refuge strategy development for Bt products.

Keywords

event TC1507, larval movement, transgenic corn, antixenosis

Disciplines

Agricultural Science | Entomology

Comments

This article is from *Journal of Economic Entomology*; 107 (2014); 368-374; doi: [10.1603/EC13352](https://doi.org/10.1603/EC13352)

Rights

Works produced by employees of the U.S. Government as part of their official duties are not copyrighted within the U.S. The content of this document is not copyrighted.

Susceptibility and Aversion of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) to Cry1F Bt Maize and Considerations for Insect Resistance Management

Author(s): Rachel R. Binning , Joel Coats , Xiaoxiao Kong , and Richard L. Hellmich

Source: Journal of Economic Entomology, 107(1):368-374. 2014.

Published By: Entomological Society of America

URL: <http://www.bioone.org/doi/full/10.1603/EC13352>

BioOne (www.bioone.org) is a nonprofit, online aggregation of core research in the biological, ecological, and environmental sciences. BioOne provides a sustainable online platform for over 170 journals and books published by nonprofit societies, associations, museums, institutions, and presses.

Your use of this PDF, the BioOne Web site, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at www.bioone.org/page/terms_of_use.

Usage of BioOne content is strictly limited to personal, educational, and non-commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

Susceptibility and Aversion of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) to Cry1F Bt Maize and Considerations for Insect Resistance Management

RACHEL R. BINNING,^{1,2} JOEL COATS,³ XIAOXIAO KONG,⁴ AND RICHARD L. HELLMICH⁵

J. Econ. Entomol. 107(1): 368–374 (2014); DOI: <http://dx.doi.org/10.1603/EC13352>

ABSTRACT *Bacillus thuringiensis* (Bt) maize was developed primarily for North American pests such as European corn borer (*Ostrinia nubilalis* (Hübner)). However, most Bt maize products are also cultivated outside of North America, where the primary pests may be different and may have lower susceptibility to Bt toxins. Fall armyworm (*Spodoptera frugiperda* JE Smith) is an important pest and primary target of Bt maize in Central and South America. *S. frugiperda* susceptibility to Cry1F (expressed in event TC1507) is an example of a pest-by-toxin interaction that does not meet the high-dose definition. In this study, the behavioral and toxic response of *S. frugiperda* to Cry1F maize was investigated by measuring the percentage of time naïve third instars spent feeding during a 3-min exposure. *S. frugiperda* also were exposed as third instars to Cry1F maize for 14 d to measure weight gain and survival. *S. frugiperda* demonstrated an initial, postingestive aversive response to Cry1F maize, and few larvae survived the 14 d exposure. The role of susceptibility and avoidance are discussed in the context of global IRM refuge strategy development for Bt products.

KEY WORDS event TC1507, larval movement, transgenic corn, antixenosis

Bt maize has been grown commercially in the United States since the introduction of lepidopteran-resistant maize in 1996. Since then, many Bt maize products have been developed that confer protection against lepidopteran and coleopteran maize pests in North America. The U.S. Environmental Protection Agency (EPA) identified the preservation of Bt efficacy as “in the public good” (US EPA 1996, 1998a). At the request of EPA, a Scientific Advisory Panel (SAP) considered the topic of insect resistance management (IRM) and refuge strategy as a means to extend the durability of Bt transgenic crops. At the time, all maize events on the market were highly toxic to the primary lepidopteran maize pests of North America, namely, European corn borer (*Ostrinia nubilalis* (Hübner)) and Southwestern corn borer (*Diatraea grandiosella* Dyar). The SAP defined high dose as “25 times the toxin concentration needed to kill a susceptible larva” and further indicated that this dose should kill 95% of heterozygous larvae with one resistance allele (US EPA 1998b). A structured refuge of non-Bt maize was recommended based on the assumption that if the Bt maize product is high dose, resistance will be func-

tionally recessive and rare. The SAP clearly identified size, configuration, and placement of the refuge relative to the Bt field as critical components of an IRM plan. The SAP’s recommendations were specific to a high-dose product; however, high-dose refuge strategy has been broadly applied to Bt transgenic crops in instances where the product may not be high dose against the primary pest(s).

Bt maize is currently developed primarily for North American pests such as *O. nubilalis* and *D. grandiosella*. However, many Bt maize products are cultivated both in and outside of North America, where the primary pests may be different and may have lower susceptibility to the toxins. As Bt maize products are commercialized in new geographies, it is important that IRM plans for those geographies consider relevant pest by toxin interactions, instead of assuming the high-dose refuge strategy applies to all pests in all geographies. Before implementing an IRM plan that includes size and placement of refuge, it is useful to understand the biology and susceptibility of the primary pest(s) for each geography. Recent developments of field resistance to Bt maize by African maize stalk borer (*Busseola fusca* Fuller) in South Africa (Cry1Ab) and fall armyworm (*Spodoptera frugiperda* (JE Smith)) in Puerto Rico (Cry1F) highlight the need to characterize the pest-by-toxin interaction (van Rensburg 2007, Matten et al. 2008). There are many important factors to consider when developing an IRM plan for a new geography, including the pest complex, cultivation and cultural practices, and crop

¹ DuPont Pioneer, 7250 NW 62nd Ave., PO Box 552, Johnston, IA 50131.

² Corresponding author, e-mail: rachel.binning@pioneer.com.

³ Department of Entomology, Iowa State University, 116 Insectary, Ames, IA 50011-3140.

⁴ DuPont Pioneer, 2450 SE Oak Tree Ct., Ankeny, IA 50021.

⁵ USDA-ARS, Iowa State University, 110 Genetics Laboratory, Ames, IA 50011-3222.

biology (MacIntosh 2009). When considering only the pest-related factors of IRM, understanding the biology and susceptibility of the primary pest(s) for each geography and how a pest might develop resistance to the toxin will help to develop an IRM plan with the appropriate size and placement of refuge.

Although a Bt maize product may not meet the definition of a high dose, Bt maize may still be efficacious (i.e., protect yield). This could be a result of a lower, yet effective, level of toxicity or a behavioral response that causes the insect to reject the Bt maize as a food source. Insect rejection of a toxic compound is not rare (Zhang et al. 2004, Men et al. 2005, Li et al. 2006). However, rejection sometimes occurs in the absence of toxicity (Gould et al. 1991, Gore et al. 2005, Prasifka et al. 2007).

The initial rejection of a food source may be the beginning of a process that ends in acceptance of that food source, that is, loss of aversion (Glendinning and Gonzalez 1995, Glendinning and Slansky 1995). Loss of aversion may be the result of desensitization to the mechanism that causes the aversive response (e.g., taste-mediated) (Glendinning et al. 2001), increased (or induced) detoxification of the aversive compound (Glendinning and Slansky 1995, Snyder and Glendinning 1996), or a combination of both desensitization and detoxification (Szentesi and Bernays 1984, Glendinning and Gonzalez 1995).

S. frugiperda susceptibility to Cry1F (event TC1507) maize is a good example of a pest-by-toxin interaction that does not meet the high dose definition (Storer et al. 2012). *S. frugiperda* is an important maize pest and a primary pest of Bt maize in Central and South America. Even though *S. frugiperda* cannot overwinter in the North American Corn Belt, this species migrates every year and can cause significant damage to unprotected maize in the United States. Aversion to Cry1F by a maize pest such as *S. frugiperda*, and the ability of that pest to overcome aversion, may have implications for placement and size of a Bt maize refuge.

Accordingly, a series of laboratory studies were conducted to evaluate the behavioral response of *S. frugiperda* to the Cry1F protein as expressed in event TC1507 maize. Two separate experiments, of short and long durations, were designed to examine: 1) if *S. frugiperda* exhibit an initial aversive response to Cry1F maize and 2) if *S. frugiperda* can overcome aversion and develop on Cry1F maize.

Materials and Methods

For both experiments, eggs from a susceptible laboratory population of *S. frugiperda* were obtained from a commercial source (Benzon Research Inc., Carlisle, PA). The colony was initiated from field collected populations in the United States before the introduction of Bt maize. Storer et al. (2010) report the LC_{50} (concentration required to kill 50% of the insects) and GIC_{50} (concentration required to cause 50% growth inhibition) for the *S. frugiperda* Benzon colony as 428 and 19.7 ng Cry1F/cm² diet, respec-

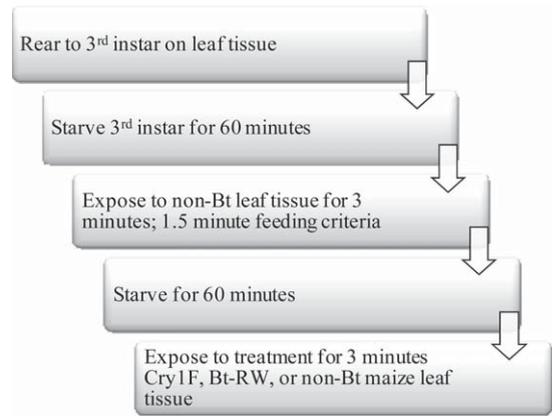


Fig. 1. Sequence of events for the short duration study.

tively. Larvae were individually maintained on non-Bt maize leaf material until they reached the third instar.

Three maize types were used for each experiment and all were Pioneer brand hybrids. The hybrids included maize that contained Bt event 1507 (Cry1F maize), maize that contained Bt event 59122 (Bt maize targeting larval rootworms [Bt-RW maize]), and maize that was a near-isoline non-Bt hybrid that did not express any insecticidal proteins (non-Bt maize). All maize tissues used for these studies were obtained by removing fully formed individual leaves from plants (approximately growth stages V6–V10) grown in pots in a walk-in environmental growth chamber maintained using standardized parameters for maize production (a photoperiod of 17:7 [L:D] h, $24 \pm 3^\circ\text{C}$). Leaves were rinsed with tap water to remove surface debris and stored in resealable plastic bags in the refrigerator ($\approx 4^\circ\text{C}$) or on wet ice until use, and not longer than 48 h. Insects were exposed to plant tissue instead of artificial diet to maximize the field-relevance of the experiment and to reduce confounding effects that nutrition or water content might have on behavior (Glendinning and Slansky 1994).

Short Duration Study. To identify how *S. frugiperda* detects Bt (preingestively or postingestively), Glendinning and Slansky (1994) used a 3-min exposure assay. The short duration study described in this paper is modeled after their methods. The sequence of events for the short duration study is outlined in Fig. 1.

The short duration study is divided into two phases, namely, screening and testing. For the screening phase, *S. frugiperda* that were within the first 24 h of the third stadium were individually removed from the rearing material, placed in an empty petri dish (100 by 25 mm, NUNC #4031), and deprived of food for ≈ 60 min. After starvation, a piece of non-Bt leaf material (≈ 3 cm²) was placed within 1 cm of the larva's head. Data collection began when the larva started feeding. Time spent feeding was recorded using the event tracking portion of a video tracking software program (EthoVision XT, Noldus Information Technology, The Netherlands) using keystrokes to indicate when

the larva stopped and started feeding. Observation continued for 3 min, after which the larva was allowed to continue feeding for an additional 7 min to allow for a full feeding bout and avoid any potential for extreme hunger that might affect test results. To ensure that only larvae that readily fed on maize were used in the next phase, larvae that had not fed for at least 90 of the 180 s observation period were discarded (Glendinning and Slansky 1994). Approximately 15% of larvae tested during the screening phase did not meet the criteria and were discarded.

For the testing phase, the larva was food-deprived for a second time in an empty petri dish for 60 min. Then, a piece of leaf material from one of the three treatments (non-Bt, Cry1F, or Bt-RW) was placed within 2 cm of the larva's head. Data collection began when the larva started feeding, and time spent feeding was recorded for 3 min. Twenty larvae per treatment were tested. Finally, each larva was placed in an individual well of a six-well bioassay tray (BD Falcon #353046, BD-Falcon Biosciences, Lexington, TN) and provided with non-Bt leaf material. Larvae were checked for mortality after 72 h.

Validity of this test system was determined by comparing the amount of time spent feeding on non-Bt leaf tissue in the screening stage to the amount of time spent feeding on non-Bt leaf tissue in the testing phase. If the time spent feeding in the testing phase is shorter than that in the screening phase, it would indicate that 60 min of starvation is not long enough to account for the normal gap between *S. frugiperda* feeding bouts on maize tissue.

If rejection of Cry1F maize is because of (at least in part) a deterrent, there will be an immediate significant decrease in time spent feeding compared with non-Bt maize. Glendinning and Slansky (1994) observed *S. frugiperda* decreased time spent feeding within the first 15–30 s of exposure to the deterrent compounds linamarin, a cyanogenic glycoside, and caffeine. Even if deterrence is not observed, there may still be rejection related to a postingestive effect. Rejection because of a postingestive effect of Bt would likely take longer than 60 s, especially if it is because of toxicity of Bt. The Bt protein must be ingested, must pass through the foregut into the midgut, must bind to receptors, must insert into the membrane, and finally must form pores that lead to gut lysis and septicemia (Whalon and Wingerd 2003). Any delayed response (>60 s), similar to that observed by Glendinning and Slansky (1994) to nicotine hydrogen tartrate, will indicate that a reduction in feeding is because of a postingestive effect. If there is no rejection of Cry1F maize leaf disks, the larvae should feed for the same amount of time as larvae on the non-Bt leaf disk.

Long Duration Study. The long duration study was designed to investigate the ability of *S. frugiperda* to overcome aversion to Bt maize by monitoring daily growth and survival. Third instars were chosen because they are generally less susceptible to Bt and will be more likely to survive the toxin long enough to show a loss of aversion. The sequence of events for the long duration study is outlined in Fig. 2.

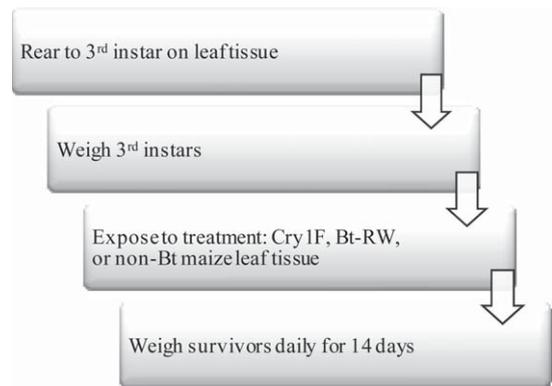


Fig. 2. Sequence of events for the long duration study.

During the first 24 h of the third stadium, each larva was individually removed from the rearing material, placed in a well of a six-well bioassay tray, and deprived of food for 60 min. Next, each larva was individually weighed to the nearest 0.1 mg, returned to the bioassay tray, and provided with leaf cuttings of non-Bt, Cry1F, or Bt-RW maize.

This experiment employed a randomized complete block design containing 16 replications per treatment, and two larvae per replication. Each donor plant provided leaf tissue for one replication per treatment. Mortality and weight of survivors were recorded daily. The experiment ended on Day 14, where Day 1 was the day of infestation. A switch from rejection to acceptance was indicated by survival and weight gain.

Data Analysis. For the short duration study, statistical analyses were conducted using SAS software, Version 9.3 (SAS Institute Inc. 2011) to compare the cumulative feeding time of *S. frugiperda* on the three treatments. SAS PROC MIXED was used to fit the analysis of variance model. A two-tailed *t*-test was conducted at 15-s intervals, where a significant difference was identified if the *P* value (of the *t*-test) for difference between treatments was <0.01, because of multiple pair-wise comparisons.

For the long duration study, the total weight gain of *S. frugiperda* fed each of the three treatments was compared. A heterogeneous variance model was used to compare treatment effects. SAS PROC MIXED was used to fit the model. A two-tailed *t*-test was conducted and a significant difference was identified if the *P* value (of the *t*-test) for difference between treatments was <0.05.

Results

Short Duration Study. Average time spent feeding on non-Bt maize in the screening phase was not different from time spent feeding on non-Bt maize in the testing phase ($t = 1.52$; $df = 73$; $P = 0.13$). This validates 60 min as an adequate gap between feeding bouts for *S. frugiperda* on maize leaf tissue.

In the testing phase, mean time spent feeding on Bt-RW maize was not significantly different from

Table 1. Mean time *S. frugiperda* third instars spent feeding during a 3-min exposure to maize leaf tissue

Treatment	n	LS-mean time feeding (sec) (95% CI) ^a
Cry1F	20	133 (117-149)a
Bt-RW	20	165 (149-181)b
Non-Bt	20	173 (157-189)b

^a Treatments with different letters were statistically different ($P < 0.05$).

non-Bt maize ($t = -0.70$; $df = 57$; $P = 0.48$; Table 1). Third-instar *S. frugiperda* spent significantly less time feeding on Cry1F maize compared with either non-Bt ($t = -3.51$; $df = 57$; $P = 0.001$) or Bt-RW ($t = -2.80$; $df = 57$; $P = 0.01$) maize. Although this indicates that *S. frugiperda* reject Cry1F maize, examination of the cumulative feeding was needed to evaluate whether this rejection was preingestive or postingestive. Figure 3 compares the cumulative time spent feeding on all three treatments. A significant difference between Cry1F and non-Bt maize first occurs at 105 s ($t = -2.61$; $df = 57$; $P = 0.01$), indicating that *S. frugiperda* aversion to Cry1F is likely postingestive. No mortality was observed in any treatment 72 h after the short duration exposure study.

Long Duration Study. Mortality was high in the Cry1F treatment, with only 11% (two larvae) surviving after 14 d. However, these survivors did gain weight (Table 2). Average total weight gain of survivors on Cry1F maize was significantly less than on either Bt-RW ($t = -6.54$; $df = 54$; $P < 0.0001$) or non-Bt maize ($t = -5.49$; $df = 54$; $P < 0.0001$). The insects that were exposed Cry1F maize but died before the end of the assay lived an average of 4.3 d, with a median of 3.5 d, and lost an average total weight of 2.2 mg before death.

Frequency distributions of daily weight gain show that 56 and 63% of the weight gain for insects fed non-Bt and Bt-RW maize, respectively, was ≥ 31 mg per day (Fig. 4). Conversely, 62% of the daily weight gain for insects fed Cry1F leaf tissue were ≤ 0 mg for the entire cohort tested (including survivors and those that died during the experiment). Some (38%) larvae

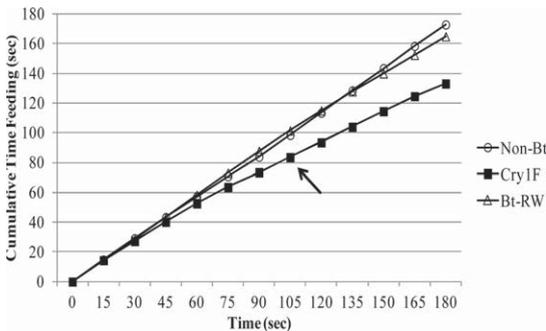


Fig. 3. Cumulative time spent feeding by third instar *S. frugiperda* on Cry1F, Bt-RW, and non-Bt maize leaf tissue. The earliest significant difference between Cry1F and non-Bt is indicated by an arrow ($P = 0.01$).

Table 2. Mean weight gain of surviving *S. frugiperda* larvae after 14-d exposure to maize leaf tissue

Treatment	n ^a	LS-mean weight gain (mg) (95% CI) ^b
Cry1F	2, 0, 16 ^c	127 (73-181)a
Bt-RW	0, 20, 0	317 (297-338)b
Non-Bt	0, 19, 1	311 (271-350)b

If pupation occurred before the end of the assay, final larval wt before the prepupal stage was used to calculate means. Cry1F, event 1507 maize; Bt-RW, event 59122 maize; non-Bt, near-isoline non-Bt maize.

^a n: larvae, pupae, dead.

^b Treatments with different letters were statistically different ($P < 0.05$).

^c Two individuals were missing from the Cry1F treatment at the end of the assay.

that were fed Cry1F maize did gain weight on one or more days; the daily weight gain for these larvae was typically low (between 1 and 10 mg). The Cry1F treatment also can be separated into insects that survived exposure to Cry1F (Fig. 5) and insects that did not survive exposure to Cry1F (Fig. 5). Insects that survived exposure to Cry1F maize averaged a daily weight gain of 9.8 mg, and 77% of daily weight gains were >0 mg (Fig. 5a). Of the daily weight gains for those that did not survive Cry1F exposure, 80% were ≤ 0 mg (Fig. 5b).

When daily weight gain was averaged by treatment, insects exposed to non-Bt and Bt-RW maize showed similar trends in average daily weight gain (Fig. 6). All insects in these two treatments had pupated by Day 8 and there was a distinct weight loss across Days 6 and

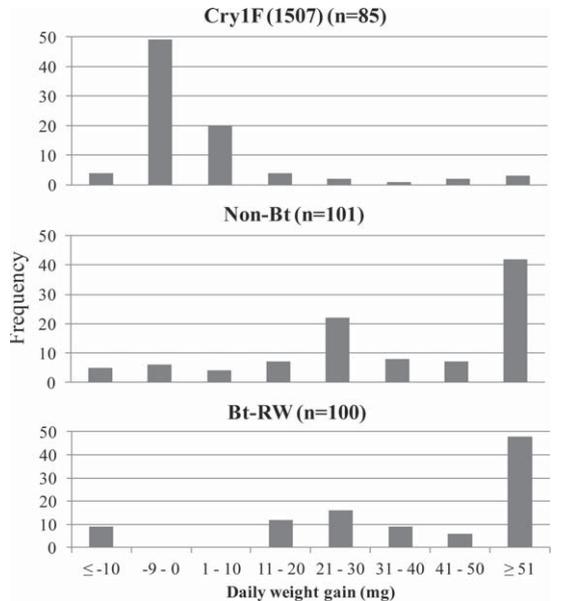


Fig. 4. Frequency of weight gain values (mg) for each of three treatments (Cry1F, non-Bt, and Bt-RW). All observed weights from all insects were included. Insects that did not survive for the entire 14 d of exposure were weighed every day until death. Insects were not weighed after death. N is the total number of days that weight gain was measured across all insects.

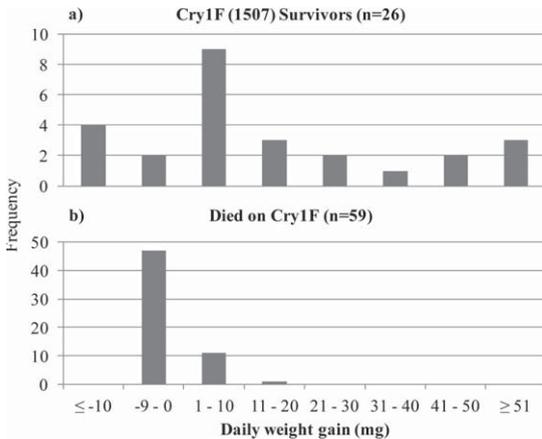


Fig. 5. Frequency of weight gain values (mg) for the insects that survived (a) and did not survive (b) exposure to Cry1F leaf material. Insects that did not survive for the entire 14 d of exposure were weighed every day until death. Insects were not weighed after death. *N* is the total number of days that weight gain was measured across all insects.

7, suggesting that the larvae stopped eating in preparation for pupation. Average weight gain for larvae that survived Cry1F exposure was generally positive but relatively flat over time, although there was a distinct loss of weight on Days 13 and 14, which appeared to mirror the prepupation weight loss for non-Bt and Bt-RW treatments on Days 6 and 7. Average weight gain of individuals that eventually died after exposure to Cry1F was minimal, ranging from -1.6 to 10.5 mg, with 30% of the changes positive and 70% negative (Fig. 6).

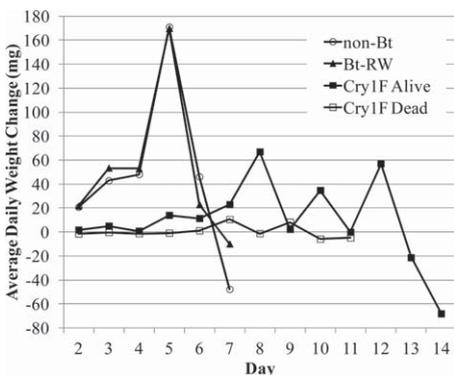


Fig. 6. Average daily weight gain of *S. frugiperda* when exposed to Bt-RW, non-Bt, and Cry1F maize. For Cry1F Alive, only weight gain of those insects that survived exposure to Cry1F for the entire length of the assay were included in the calculation. Cry1F Dead represents insects that did not survive for the duration of the experiment. Sample size varies from $n = 16$ to $n = 1$ across days for the Cry1F Dead line, and all insects were dead after day 11. $N = 2$ for all points on the Cry1F Alive line. Day 1 was the first day larvae were weighed, therefore there is no weight change to report for that day.

Discussion

The treatments of non-Bt and Bt-RW maize did not differ from each other in any analysis. Neither treatment caused an aversive response or significant mortality. This is expected because the proteins expressed in Bt-RW (59122) maize are generally acknowledged to have no toxic effect against Lepidoptera.

Mallet and Porter (1992) identify larval movement from Bt plants to non-Bt plants as a primary reason to avoid blending refuge in the field. This is based on the survival advantage blended refuge would confer to heterozygous resistant insects when movement occurs from a Bt plant to a non-Bt plant. However, if there is no selection for resistance (i.e., no mortality) before Bt to non-Bt larval movement, and no survival disadvantage (i.e., fitness effects) after movement, then there is no heterozygote advantage. The short duration study indicates that the initial response of third-instar *S. frugiperda* to Cry1F maize is aversion (Table 1). The analysis shows that it takes 105 s for the response to be significant, suggesting that it is postingestive (Fig. 3). Although the larvae are consuming Cry1F leaf tissue, the observation that all 20 insects in the short duration assay survived exposure to Cry1F indicates that the larvae are not consuming a toxic dose before rejection occurs. This is not the first study to conclude high survival after tasting exposure to Bt maize. Binning et al. (2010) described essentially 100% survival of western corn rootworm (*Diabrotica virgifera virgifera* LeConte) when exposed to Bt-RW maize for 17 d and then moved to non-Bt maize. These data were later used by Pan et al. (2011) to inform the predispersal tasting survival parameter in a simulation model to compare the durability of block and blended refuge for *D. v. virgifera*. Data from the study reported here could be used in a similar fashion. However, it is difficult to infer whether the larvae would abandon the maize plant after the initial aversive response demonstrated in the short duration study. Larval movement and survival studies with whole plants could help address this question of host plant abandonment.

The alternative to host abandonment after initial rejection is that larvae remain on the Bt plant until they either 1) starve, 2) consume enough plant material to cause mortality, or 3) overcome both aversion and the toxic effect of Cry1F. The high mortality and median time to death after exposure of 3.5 d in the long duration experiment indicate that most *S. frugiperda* either starve or succumb to Cry1F toxicity (Table 2); however, the two responses cannot be separated with these data. The insects that survive Cry1F maize are significantly smaller, and therefore, less fit than those fed non-Bt or Bt-RW maize. The high mortality and median time to death after exposure of 3.5 d in the long duration experiment indicate that most *S. frugiperda* either starve or succumb to Cry1F toxicity (Table 2); however, the two responses cannot be separated with these data. The insects that survive Cry1F maize are significantly smaller, and therefore, less fit than those fed non-Bt or Bt-RW maize. Several possibilities could explain the survival of a few *S. frugiperda* on Cry1F maize, including detoxification or a heterogeneous genetic response. However, the simplest explanation is that these insects were less susceptible because of

natural variation in the population. This, combined with reduced feeding due to the aversive response, could account for survival plus reduced growth and development in this no-choice assay.

Larval movement is only one component of the insect-plant interaction that impacts the durability of blended refuge for Bt maize. Number and fitness of susceptible insects produced from refuge plants, adult mating, dispersal, and oviposition are some of the additional parameters that may be considered before broad adoption of blended refuge strategy for Bt maize. Conclusions about larval movement after the initial aversive response cannot easily be drawn from the studies reported in this article. Larvae may immediately abandon the host or move to a different part of the plant and continue to sample until it overcomes rejection or dies from toxicity. Additional studies are needed to investigate if host abandonment occurs, and if additional sampling after the initial tasting leads to selection for resistance. However, if the initial aversion does equate to host abandonment, then blended refuge could be a viable refuge deployment option for *S. frugiperda*. This could be critical information for countries outside of North America, where planting refuge may not be a regulatory requirement and *S. frugiperda* is a primary pest with continuous generations. This study is one piece of evidence that can inform the development of an effective IRM strategy for *S. frugiperda* outside North America to reduce selection pressure and extend the life of Bt traits such as Cry1F.

Acknowledgments

We thank Aaron Gassmann, Erin Hodgson, Jeff Wolt, Lindsey Flexner, Clint Pilcher, and two anonymous reviewers for improvements to this manuscript resulting from their effort. We also thank Kris Sturtz, Rachel Woods, Katie Helbing, and Ashley Young for their assistance implementing the experiments, and DuPont Pioneer for providing maize seed, laboratory space, and supplies. Mention of a proprietary product does not constitute an endorsement or a recommendation by Iowa State University or USDA for its use.

References Cited

- Binning, R. R., S. A. Lefko, A. Y. Millsap, S. D. Thompson, and T. M. Nowatzki. 2010. Estimating western corn rootworm (Coleoptera: Chrysomelidae) larval susceptibility to event DAS-59122-7 maize. *J. Appl. Entomol.* 134: 551-561.
- Glendinning, J. I., and F. Slansky. 1994. Interactions of allelochemicals with dietary constituents: effects on deterrence. *Physiol. Entomol.* 19: 173-186.
- Glendinning, J. I., and N. A. Gonzalez. 1995. Gustatory habituation to deterrent allelochemicals in a herbivore: concentration and compound specificity. *Anim. Behav.* 50: 915-927.
- Glendinning, J. I., and F. Slansky. 1995. Consumption of a toxic food by caterpillars increases with dietary exposure: support for a role of induced detoxification enzymes. *J. Comp. Physiol. A* 176: 337-445.
- Glendinning, J. I., H. Brown, M. Capoor, A. Davis, A. Gbedemah, and E. Long. 2001. A peripheral mechanism for behavioral adaptation to specific "bitter" taste stimuli in an insect. *J. Neurosci.* 21: 3688-3696.
- Gore, J., J. J. Adameczyk, and C. A. Blanco. 2005. Selective feeding of tobacco budworm and bollworm (Lepidoptera: Noctuidae) on meridic diet with different concentrations of *Bacillus thuringiensis* proteins. *J. Econ. Entomol.* 98: 88-94.
- Gould, F., A. Anderson, D. Landis, and H. Van Mellaert. 1991. Feeding behavior and growth of *Heliothis virescens* larvae on diets containing *Bacillus thuringiensis* formulations or endotoxins. *Entomol. Exp. Appl.* 58: 199-210.
- Li, Y. X., S. M. Greenberg, and T. X. Liu. 2006. Effects of Bt cotton expressing Cry1Ac and Cry2Ab and non-Bt cotton on behavior, survival and development of *Trichoplusia ni* (Lepidoptera: Noctuidae). *Crop Prot.* 25: 940-948.
- MacIntosh, S. C. 2009. Managing the risk of insect resistance to transgenic insect control traits: practical approaches in local environments. *Pest Manage. Sci.* 66: 100-106.
- Mallet, J., and P. Porter. 1992. Preventing insect adaptation to insect-resistant crops: are seed mixtures or refugia the best strategy? *Proc. R. Soc. B* 250: 165-169.
- Matten, S. R., G. P. Head, and H. D. Quemada. 2008. How governmental regulation can help or hinder the integration of Bt crops within IPM programs, pp. 27-39. In J. Romeis, A. M. Shelton, and G. G. Kennedy (eds.), *Integration of Insect-Resistant Genetically Modified Crops Within IPM Programs*. Springer, Amsterdam, The Netherlands.
- Men, X., F. Ge, E. N. Yardim, and M. N. Parajulee. 2005. Behavioral response of *Helicoverpa armigera* (Lepidoptera: Noctuidae) to cotton with and without expression of the Cry1Ac d-endotoxin protein of *Bacillus thuringiensis* Berliner. *J. Insect Behav.* 18: 33-50.
- Pan, Z., D. W. Onstad, T. M. Nowatzki, B. H. Stanley, L. J. Meinke, and J. L. Flexner. 2011. Western corn rootworm (Coleoptera: Chrysomelidae) dispersal and adaptation to single-toxin transgenic corn deployed with block or blended refuge. *Environ. Entomol.* 40: 964-978.
- Prasifka, P. L., R. L. Hellmich, J. R. Prasifka, and L. C. Lewis. 2007. Effects of Cry1Ab-expressing corn anthers on the movement of monarch butterfly larvae. *Environ. Entomol.* 36: 228-233.
- SAS Institute. 2011. SAS/STAT 9.3 user's guide. SAS Institute, Cary, NC.
- Snyder, M. J., and J. I. Glendinning. 1996. Causal connection between detoxification enzyme activity and consumption of a toxic plant compound. *J. Comp. Physiol. A* 179: 255-261.
- Storer, N. P., J. M. Babcock, M. Schlenz, T. Meade, G. D. Thompson, J. W. Bing, and R. M. Huckaba. 2010. Discovery and characterization of field resistance to Bt maize: *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in Puerto Rico. *J. Econ. Entomol.* 103: 1031-1038.
- Storer, N. P., M. E. Kubiszak, J. Ed King, G. D. Thompson, and A. C. Santos. 2012. Status of resistance to Bt maize in *Spodoptera frugiperda*: lessons from Puerto Rico. *J. Invertebr. Pathol.* 110: 294-300.
- Szentesi, A., and E. A. Bernays. 1984. A study of behavioural habituation to a feeding deterrent in nymphs of *Schistocerca gregaria*. *Physiol. Entomol.* 9: 329-340.
- (US EPA) U. S. Environmental Protection Agency. 1996. Pesticide Program Dialogue Committee Meeting Summary. (<http://www.epa.gov/pesticides/ppdc/july96.htm>).
- (US EPA) U. S. Environmental Protection Agency. 1998a. The Environmental Protection Agency's white paper on Bt plant-pesticide resistance management. (<http://www.epa.gov/EPA-PEST/1998/January/Day-14/paper.pdf>).

- (US EPA) U. S. Environmental Protection Agency. 1998b. Scientific advisory panel: subpanel on *Bacillus thuringiensis* (Bt) plant-pesticides and resistance management, February 9–10, 1998. Docket No. OPPTS-00231. (<http://www.epa.gov/scipoly/sap/meetings/1998/february/finalfeb.pdf>).
- van Rensburg, J.B.J. 2007. First report of field resistance by the stem borer, *Busseola fusca* (Fuller) to *Bt*-transgenic maize. S. Afr. J. Plant Soil. 24: 147–151.
- Whalon, M. E., and B. A. Wingerd. 2003. Bt: mode of action and use. Arch. Insect Biochem. Physiol. 54: 200–211.
- Zhang, J. H., C. Z. Wang, J. D. Qin, and S. D. Guo. 2004. Feeding behavior of *Helicoverpa armigera* larvae on insect-resistant transgenic cotton and non-transgenic cotton. J. Appl. Entomol. 128: 218–225.

Received 8 August 2013; accepted 12 December 2013.
