Interactions of Alternate Hosts, Postemergence Grass Control, and Rootworm-Resistant Transgenic Corn on Western Corn Rootworm (Coleoptera: Chrysomelidae) Damage and Adult Emergence

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ABSTRACT Field studies were conducted in 2003 and 2004 to determine the effects of grassy weeds, postemergence grass control, transgenic rootworm-resistant corn, *Zea mays* L., expressing the Cry3Bb1 endotoxin and glyphosate herbicide tolerance (Bt corn), and the interactions of these factors on western corn rootworm, *Diabrotica virgifera virgifera* LeConte, damage and adult emergence. Three insect management tactics (Bt corn, its nontransgenic isoline, and isoline plus tefluthrin) were evaluated with two weed species (giant foxtail, *Setaria faberi* Herrm, and large crabgrass, *Digitaria sanquinalis* L. Scop), and four weed management regimes (weed free, no weed management, early [V3–4] weed management and late [V5–6] weed management) in a factorial arrangement of a randomized split split-plot design. In each case, the isoline was also tolerant to glyphosate. Root damage was significantly affected by insect management tactics in both years, but weed species and weed management did not significantly affect damage to Bt corn in either year. Adult emergence was significantly affected by insect management tactics in both years and by weed species in 2003, but weed management and the interaction of all three factors was not significant in either year. The sex ratio of female beetles produced on Bt corn without weeds was generally greater than when weeds were present and this difference was significant for several treatments each year. Average dry weight of male and female beetles emerging from Bt corn was greater than the weights of beetles emerging from isoline or isoline plus tefluthrin in 2003, but there was no difference for females in 2004 and males weighed significantly less than other treatments in 2004. The implications of these results in insect resistance management are discussed.

KEY WORDS glyphosate, herbicide, grassy weeds, Cry3Bb1, *Diabrotica virgifera virgifera*

The western corn rootworm, *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae), is one of the major pests of corn, *Zea mays* L., in the Corn Belt (north central United States), costing growers ≈$1 billion annually in terms of control costs and yield reduction (Metcalf 1986). Effective control tactics for this insect and the closely related northern corn rootworm, *Diabrotica barberi* Smith & Lawrence, are limited in certain regions because of insecticide resistance (Meinke et al. 1998), resistance to crop rotation (Levine et al. 2002), and extended diapause (Krysan et al. 1986). Transgenic corn hybrids expressing insecticidal proteins derived from *Bacillus thuringiensis* (Bt) Berliner is a newer tactic for corn rootworm control. Several insecticidal proteins from different Bt strains have been reported to be toxic to rootworm larvae (Baum et al. 2004, and references therein). The first rootworm-resistant Bt corn commercially available in the United States, YieldGard Rootworm (Monsanto Company, St. Louis, MO), expresses the Cry3Bb1 protein and is active against neonate western and northern rootworm larvae (Vaughn et al. 2005).

Rootworm-resistant Bt corn is available for sale as a "stack," with tolerance to broad-spectrum postemergent herbicides that contain the active ingredient glyphosate. The addition of herbicide tolerance traits creates weed management flexibility that, in some instances, may allow for germination and growth of grassy weeds in cornfields planted with Bt corn during the time of western corn rootworm egg hatch. This is important because western corn rootworm larvae can develop to at least the second instar on 57 of 60 grass species evaluated (Clark and Hibbard 2004, Oyediran et al. 2004, Wilson and Hibbard 2004), and Cry3Bb1 corn is less efficacious against larvae that have developed beyond the first instar (EPA SAP 2002). When grassy weeds were present in greenhouse trials, sig-

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nificantly more western corn rootworm adults emerged from Cry3Bb1 corn than from Cry3Bb1 corn without weeds or the weeds alone (Oyediran et al. 2005). When this information is coupled with the findings of Hibbard et al. (2003, 2004) on postestablishment larval movement, then one could conclude that initial establishment of western corn rootworm larvae on grassy weeds followed by management of weeds could have the potential to impact the remaining Bt corn in the field. Second or third instars that initially develop on grassy weeds could be forced onto Bt corn upon management of the weedy hosts with herbicide application, creating a risk for enhanced damage and yield loss. This is also important because movement of larvae from susceptible to transgenic plants or vice versa could adversely affect resistance management (Mallet and Porter 1992). The Environmental Protection Agency (EPA) requires an insect resistance management plan for all Bt crops before registration. Understanding the role of alternate hosts in interactions of the western corn rootworm and Bt corn is an important component of optimizing such a plan. The objective of this study was to examine the impact of selected grassy weed species and the timing of their management on western corn rootworm root damage and beetle emergence on Bt corn containing the Cry3Bb1 endotoxin in a central Missouri field environment.

Materials and Methods

Experimental Site and Design. The study was conducted in 2003 and 2004 at the University of Missouri, Bradford Research and Extension Center, located 9.6 km east of Columbia, MO. The soil type at this location is a Mexico silt loam made up of 12.5% sand, 65% silt, and 22.5% clay as determined by the University of Missouri Soil Testing Facility, Columbia, MO. The 2003 and 2004 sites had been planted to soybean, *Glycine max* L., in 2002 and 2003, respectively, so it was assumed that there were no feral western corn rootworms.

In 2003, the experimental design was a randomized complete block split-split plot in space in which the treatments were arranged as a three × three × three factorial plus one control (insect management tactics × weed species × weed management plus season-long weed-free) with five replications as outlined in Steele et al. (1997). The main plot was rootworm management tactic (Bt corn, tefluthrin, or none), the subplot was weed species (giant foxtail, large crabgrass, or both), and sub-subplot was timing of weed management (V4, V6, and no weed management) (Ritchie et al. 1992). A season-long weed-free treatment was included as a control. In 2004, the experimental design was a randomized complete block split-split plot in space in which the treatments were arranged as a three × two × four (insect management tactics × weed species × weed management) factorial with five replications as outlined in Steele et al. (1997). The main plot was the same as in 2003, the subplot was weed species (giant foxtail, *Setaria faberi* Herrm, and large crabgrass, *Digitaria sanquinalis* L. Scop), and the sub-subplot was timing of weed management (V3, V5, no management, and season-long weed-free).

Corn was planted 17 April 2003 and 5 May 2004 using a 2-row planter (John Deere MaxEmerge, Deere and Company, Moline IL) at a rate of 59,300 plants per ha. Each plot consisted of four 4.6-m rows with 76-cm row spacing (a beetle emergence row, a root damage row, and border rows on each side of these rows). The Bt corn hybrid expressing the Cry3Bb1 protein was CS304YGRW/RR and its near-isoline was CS304/RR (Monsanto Company). Both hybrids were tolerant to glyphosate. One week after plant emergence, gene checks were conducted on five randomly selected Bt corn and isoline plants from each replication of the experimental plots by using kits provided by Monsanto Company to assay for expressing Cry3Bb1 protein (available from EnviroLogix, Portland, ME).

Rootworm Infestation. During both years, each row was infested with diapausing western corn rootworm eggs suspended in 0.15% agar solution at a rate of 2,400 eggs per m by using an egg infesting tractor described by Moellenbeck et al. (1994) modified after Sutter and Branson (1980). In 2003, eggs were obtained from the USDA–ARS, Northern Grains Insects Laboratory (NGIRL), Brookings, SD, and infested immediately after planting corn on 17 April. In 2004, eggs were again infested just after planting on 5 May, but were a 1:1 mixture from NGIRL and wild-type eggs from French Agricultural Research, Inc., Lamberton, MN. In previous studies, no significant difference was found between a wild-type egg source and the Brookings colony (Hibbard et al. 1999).

Weed Infestation. In 2003, plots assigned for weed infestation were sown with giant foxtail, *Setaria faberi* Herrm; large crabgrass, *Digitaria sanquinalis* L. Scop; or a 1:1 mixture of each by using hand-held broadcast spreaders (model 126, Spyker Spreaders, LLC. Urbana, IN) on 17 April at a rate of 15 kg seed per ha immediately after planting and rootworm egg infestation. In 2004, the field was tilled, and giant foxtail or large crabgrass (the weed mixture was eliminated) was sown on 14 April, 3 wk before corn planting and rootworm infestation.

Pesticide Application. In 2003, plots to be kept weed free were sprayed with glyphosate (Roundup Ultramax, Monsanto Company) in a 2% solution (product, vol:vol) as needed using a knapsack sprayer. In 2004, the weed-free plots also received a preemergence application of atrazine/metolachlor (Bicep II Magnum 5.5 F, Syngenta Corporation, Greensboro, NC) at the rate of 3.2 kg (AI)/ha. The weed-free plots also were sprayed with glyphosate as needed in 2004. The early and late sprays were on 10 and 23 June 2003 and 18 and 28 May 2004, respectively. In 2003, sprays were at V4 and V6 and in 2004 V3 and V5 (Ritchie et al. 1992)

The soil insecticide tefluthrin (Force 3G, Syngenta Corporation) was applied at planting in a T-band over each row designated to receive tefluthrin at the linear rate of 0.014 g (AI)/m.

Root Damage Evaluations. To evaluate the impact of weediness and subsequent weed management on the efficacy of Bt corn, roots were assessed for injury using a linear 0–3 root damage scale (Oleson et al. 2005). When ≈80% of the larvae in the plots had reached pupation, roots were dug, washed to remove soil, and rated on 8 July 2003 and 28 June 2004. The number of growing degree-days (DD) (Wilde 1971) after infestation in the soil was 619 and 617 for 2003 and 2004, respectively. Each year, five roots per replicate were rated for rootworm damage.

Beetle Sampling. Two adult emergence cages were each centered over a severed corn plant and spanned from mid-row to mid-row (Hein and Tollefson 1985). Cages (78 × 36 cm) were modified after Chaddha et al. (1993). Timing of adult emergence from artificial infestations can be estimated fairly accurately with degree-day models (Wilde 1971, Levine et al. 1992). Under our conditions, the average number of degree-days to first beetle emergence is ≈700. Emergence cages were put out at ≈650 DD so that first emergence was not missed and beetles had the maximum time to develop on living plants. In 2003, sampling of beetles started on 7 July, the first beetles were caught on 9 July, and the last beetles were caught on 27 August. In 2004, sampling began on 3 July, first beetles were caught on 7 July, and the last beetle was caught on 2 August. Each year traps were checked for at least 2 wk after the final adult was caught. During the peak emergence periods, traps were checked three times per week and twice per week during times when few adults were caught. All the beetles collected were placed in individually labeled scintillation vials containing 95% ethanol and brought to laboratory for processing. First, beetles collected from each plot were counted and identified to sex. Second, pronotum width of five males and five females randomly selected from all of those emerging from a particular plot was measured using an ocular micrometer (10×/21, Wild Co., Heerbrugg, Switzerland) mounted on a microscope (MsZ, Wild Co.). Upon completion of the pronotum measurements, all beetles were pooled by sex and placed in a desiccating oven (Thelco model 16, GCA/Precision Scientific Co., Chicago, IL) at 80°C for 48 h. The dried beetles were then placed on an analytical scale (SR-182A, A&D Company Limited, Tokyo, Japan), and total dry weight was recorded for each group of beetles. The average dry weight for beetles from a given plot and sex was then calculated.

Statistical Analysis. All data were analyzed as a randomized complete block split-split-plot design using the PROC MIXED procedure of the statistical package SAS (SAS Institute 1990). The model contained the main plot of insect management tactics, the subplots of weed species, the sub-subplot of weed management practices and the interaction of these factors. Beyond the standard analysis of variance (ANOVA), we preplanned a comparison of grass species and weed management within each of the three insect control categories and a comparison of insect control categories within grass species and weed management (within columns and rows of the tables). These were done with an least significant difference (LSD) for a split-split-plot design as described by Steele et al. (1997).

Although untransformed data are shown in the tables, to meet the assumptions of the analysis, all data, except percentage data were transformed by square root (x + 0.5) before analysis. The data were transformed because the residuals of the untransformed data were distributed in a Poisson and not in a normal distribution manner. The square root (x + 0.5) transformation was chosen, because it is the most effective if values are small (Snedecor and Cochran 1989). Percentage data were transformed with arcsine $\sqrt{(x)}$ (Snedecor and Cochran 1989).

Results

All the Bt corn plants that were randomly selected tested positive in the gene check test, and all the isoline plants checked tested negative.

Root Damage. *2003 Field Study.* Root damage ratings were significantly affected by insect management tactics (F = 10.99; df = 2, 8; P = 0.0051) and the interaction of insect management and weed management (F = 2.84; df = 6, 72; P = 0.0153). However, weed species (F = 0.98; df = 2, 36; P = 0.3850), weed management (F = 1.27; df = 3, 36; P = 0.2988), and the interactions among all three factors (F = 1.63; df = 8, 72; P = 0.1324) did not significantly affect root damage. Generally, root damage readings were highest in the isoline treatments and lowest in Bt corn (Table 1). Seven of the nine combinations of weed species and weed management within the isoline plots had significantly less damage than the isoline weed free plots (Table 1). Weed species and weed management did not significantly affect plant damage within the Bt corn or isoline plus tefluthrin treatments.

2004 Field Study. Insect management tactics significantly affected root damage (F = 110.67; df = 2, 8; P < 0.0001). However, weed species (F = 0.26; df = 1, 28; P = 0.6155), weed management (F = 1.93; df = 3, 28; P = 0.1472), and the interaction of all three factors (F = 0.73; df = 6, 56; P = 0.6277) did not significantly affect root damage. Root damage was highest in all the isoline treatments and lowest in all the Bt corn treatments (Table 2). Contrary to 2003 data, isoline plus weed treatments had more damage, on average, than the isoline weed-free plots, and this difference was significant in two of the three giant foxtail isoline treatments (Table 2). On average, slightly more damage was done to Bt corn plants when combined with grassy weeds, but this difference was not significant.

Beetle Emergence. *2003 Field Study.* The total number of beetles that emerged was significantly affected by insect management tactics (F = 67.55; df = 2, 8; P < 0.0001), weed species (F = 4.33; df = 2, 36; P = 0.0206), and the interaction of these two factors (F = 3.91; df = 4, 72; P = 0.0063). However weed management (F = 0.63; df = 3, 36; P = 0.6028) and the interactions among all three factors (F = 0.49; df = 8, 72; P = 0.8589) did not significantly affect emergence of beetles. When looking at the main effect of insect control, significantly more beetles emerged from isoline corn than

Table 1. Mean ± SE root damage ratings for 2003

Weed species	Weed management time	Insect management		
		Bt corn	Isoline	Isoline + tefluthrin
Giant foxtail	V4	0.02 ± 0.01bA	0.63 ± 0.27aAB	0.21 ± 0.10bA
	V6	0.01 ± 0.00bA	0.53 ± 0.27aBC	0.29 ± 0.13abA
	No weeding	0.01 ± 0.00bA	0.39 ± 0.13aBC	0.27 ± 0.13abA
Large crabgrass	V4	0.07 ± 0.05bA	0.68 ± 0.29aAB	0.21 ± 0.15bA
	V6	0.07 ± 0.04aA	0.29 ± 0.14aC	0.11 ± 0.05aA
	No weeding	0.02 ± 0.00bA	0.52 ± 0.18aBC	0.29 ± 0.18aA
Foxtail + crabgrass	V4	0.02 ± 0.01bA	0.30 ± 0.08aC	0.20 ± 0.09abA
	V6	0.02 ± 0.00aA	0.31 ± 0.08aC	0.30 ± 0.13aA
	No weeding	0.02 ± 0.01bA	0.56 ± 0.27aBC	0.07 ± 0.05bA
Weed-free control		0.06 ± 0.05bA	0.84 ± 0.31aA	0.10 ± 0.04bA
Insect management main effect		0.03b	0.50a	0.21b

Significance differences ($P = 0.05$) between weed species and management combinations within a column are indicated by different uppercase letters. Significant differences between insect management types within a row are indicted by different lowercase letters. Although untransformed data are shown, data were analyzed as sqrt (x + 0.5).

isoline corn plus tefluthrin, and significantly more beetles emerged from isoline plus tefluthrin corn than Bt corn (Table 3). Within the Bt corn treatment combinations, eight of nine treatments with weeds produced more beetles than Bt corn kept weed free, but this difference was not statistically significant (Table 3). The first beetle that emerged from the Bt corn treatment kept weed free was 18 d later than the first beetle from isoline treatment kept weed free. The first beetle that emerged from a Bt corn plus weed treatment was only 2 d later than isoline. Within the isoline treatments, there was a trend toward reduced adult emergence when weed treatments were present compared with the weed-free plot, although in most comparisons, this was not significant (Table 3). Within the isoline plus tefluthrin treatments, there were no significant differences between weedy and weed-free plots.

2004 Field Study. The total number of adults that emerged was significantly affected by insect management tactics ($F = 114.17$; df = 2, 8; $P < 0.0001$). However, weed species ($F = 0.01$; df = 1, 28; $P = 0.9338$), weed management ($F = 2.19$; df = 3, 28; $P = 0.1120$), and the interactions among these three factors ($F = 0.61$; df = 6, 56; $P = 0.7190$) did not significantly affect the total number of beetles emerged. When weed species and weed management data were combined into a main effect for insect management tactic, significantly more beetles emerged from isoline treatments than isoline plus tefluthrin or Bt corn treatments (Table 4). Weed species and weed management did not significantly affect adult emergence from within the Bt corn, isoline, or isoline plus tefluthrin treatments (Table 4). Adult emergence also was not as spread out in 2004 and beetles did not emerge earlier from the Bt corn treatments plus weeds than the Bt corn treatments kept weed free in 2004.

Sex Ratio. *2003 Field Study.* Percentage of female emergence was significantly affected by insect management tactics ($F = 32.58$; df = 2, 8; $P < 0.0001$) and the interaction of weed species and weed management ($F = 2.72$; df = 4, 36; $P = 0.0445$). However, weeds species ($F = 0.11$; df = 2, 36; $P = 0.8998$), weed management ($F = 1.43$ df = 3, 36; $P = 0.2499$), and the interactions among these three factors ($F = 1.12$; df = 8, 69; $P = 0.3602$) did not significantly impact percentage of female emergence. The degrees of freedom are slightly different than other analyses because when no adults emerged from a particular plot, the percentage of females from the plot was treated as missing data. Percentage of females was highest in the Bt corn treatments, significantly so when averaged

Table 2. Mean ± SE root damage ratings for 2004

Weed species	Weed management time	Insect management		
		Bt corn	Isoline	Isoline + tefluthrin
Giant foxtail	V3	0.10 ± 0.05cA	0.72 ± 0.07aABC	0.38 ± 0.10bAB
	V5	0.17 ± 0.04cA	0.65 ± 0.10aBC	0.42 ± 0.11bAB
	No weeding	0.13 ± 0.04bA	0.87 ± 0.11aA	0.30 ± 0.05bAB
	Weed free	0.06 ± 0.03cA	0.53 ± 0.06aBC	0.23 ± 0.06bB
Large crabgrass	V3	0.06 ± 0.06bA	0.51 ± 0.08aC	0.45 ± 0.04aA
	V5	0.11 ± 0.05cA	0.64 ± 0.08aBC	0.34 ± 0.04bAB
	No weeding	0.13 ± 0.04bA	0.73 ± 0.03aAB	0.27 ± 0.04bAB
	Weed free	0.06 ± 0.03bA	0.59 ± 0.07aBC	0.42 ± 0.01bA
Insect management main effect		0.10c	0.65a	0.35b

Significance differences ($P = 0.05$) between weed species and management combinations within a column are indicated by different uppercase letters. Significant differences between insect management types within a row are indicted by different lowercase letters. Although untransformed data are shown, data were analyzed as sqrt (x + 0.5).

Table 3. Mean ± SE number of western corn rootworm adults that emerged in 2003

Weed species	Weed management time	Insect management		
		Bt corn	Isoline	Isoline + tefluthrin
Giant foxtail	V4	5.2 ± 1.6cA	58.4 ± 10.4aA	20.6 ± 4.3bA
	V6	7.0 ± 1.6bA	37.6 ± 4.4aAB	17.2 ± 1.5aA
	No weeding	4.2 ± 1.0bA	51.4 ± 16.9aAB	25.8 ± 7.2aA
Large crabgrass	V4	4.2 ± 1.2bA	24.8 ± 4.8aBC	25.8 ± 6.8aA
	V6	6.8 ± 3.4bA	24.0 ± 4.2aBC	36.0 ± 12.8aA
	No weeding	7.4 ± 1.8bA	26.6 ± 4.7aBC	23.4 ± 5.9aA
Foxtail + crabgrass	V4	3.6 ± 1.5bA	32.8 ± 16.6aBC	22.4 ± 3.4aA
	V6	3.8 ± 1.5bA	32.6 ± 12.9aBC	23.8 ± 4.5aA
	No weeding	2.2 ± 0.4bA	19.0 ± 5.5aC	16.2 ± 3.7aA
Weed-free control		2.4 ± 1.0cA	50.0 ± 11.1aAB	18.0 ± 3.6bA
Insect management main effect		4.6c	35.7a	22.9b

Significance differences ($P = 0.05$) between weed species and management combinations within a column are indicated by different uppercase letters. Significant differences between insect management types within a row are indicted by different lowercase letters. Although untransformed data are shown, data were analyzed as sqrt (x + 0.5).

across weed species and weed management for the insect management main effect (Table 5). Within the Bt corn plots, five of the nine combinations of weed species and weed management had significantly lower percentage of females than the Bt corn weed-free plots, and all were nominally lower than the weed-free plots (Table 5). No significant difference was found within isoline or within isoline plus tefluthrin plots.

2004 Field Study. The percentage of females that emerged was not significantly affected by insect management tactics ($F = 4.40$; df = 2, 8; $P = 0.0514$), weed species ($F = 3.83$; df = 1, 28; $P = 0.0605$), weed management ($F = 0.33$; df = 3, 28; $P = 0.8023$), or the interaction of all three factors ($F = 0.77$; df = 6, 48; $P = 0.7709$). All treatments were female biased in 2004, including isoline and isoline plus tefluthrin treatments, but when preplanned, unprotected comparisons were made, the percentage of female emergence was significantly greater for the Bt plots than either the isoline or isoline plus tefluthrin plots in the main effect of insect management (Table 6).

Average Dry Weight of Adults. *2003 Field Study.* The average dry weights of the males were not significantly affected by insect management tactics ($F = 2.21$; df = 2, 8; $P = 0.1723$), weed species ($F = 0.27$; df = 2, 35; $P = 0.7667$), or weed management ($F = 0.67$; df = 3, 35; $P = 0.5732$). However, the interaction of weed species and weed management ($F = 3.60$; df = 4, 35; $P = 0.0146$) and the interaction among all three factors ($F = 2.88$; df = 8, 44; $P = 0.0113$) did significantly affect average dry weight of male beetles. No consistent trends were found within Bt corn, isoline, or isoline plus tefluthrin treatments for males or females. The average dry weights for females varied significantly among insect management tactics ($F = 6.14$; df = 2, 8; $P = 0.0242$) and the interaction of all three factors ($F = 3.25$; df = 8, 62; $P = 0.0038$). However, weed species ($F = 1.36$; df = 2, 36; $P = 0.2690$) and weed management ($F = 0.47$; df = 3, 36; $P = 0.7048$) did not significantly affect average dry weights of female beetles. For both males and females, the dry weight of those recovered from Bt corn was significantly greater than the dry weight of those recovered from isoline or isoline plus tefluthrin when data from all weed species and weed management were combined into a main effect (Table 7). Over all treatment combinations, the females with the second greatest weight were recovered from the weed-free Bt corn plots.

2004 Field Study. The average dry weight of male beetles was significantly affected by insect management tactics ($F = 6.72$; df = 2, 8; $P = 0.0194$) and weed management ($F = 4.51$; df = 3, 28; $P = 0.0105$). How-

Table 4. Mean ± SE number of western corn rootworm adults that emerged in 2004

Weed species	Weed management time	Insect management		
		Bt corn	Isoline	Isoline + tefluthrin
Giant foxtail	V3	2.8 ± 1.9bA	31.2 ± 3.4aA	15.8 ± 2.3bA
	V5	6.6 ± 2.2bA	33.6 ± 10.6aA	26.2 ± 7.2aA
	No weeding	7.8 ± 3.0bA	40.0 ± 11.8aA	33.2 ± 9.9aA
	Weed free	2.2 ± 1.3cA	36.2 ± 8.2aA	20.6 ± 7.0bA
Large crabgrass	V3	1.6 ± 1.2bA	30.8 ± 5.2aA	22.2 ± 3.7aA
	V5	3.8 ± 1.7bA	34.2 ± 3.9aA	19.4 ± 9.2bA
	No weeding	4.8 ± 2.8bA	37.8 ± 4.9aA	34.8 ± 6.4aA
	Weed free	3.6 ± 1.1bA	33.0 ± 5.8aA	29.6 ± 6.1aA
Insect management main effect		4.15c	34.60a	25.22b

Significance differences ($P = 0.05$) between weed species and management combinations within a column are indicated by different uppercase letters. Significant differences between insect management types within a row are indicted by different lowercase letters. Although untransformed data are shown, data were analyzed as sqrt (x + 0.5).

Table 5. Mean ± SE percentage of females emerged in 2003

Weed species	Weed management time	Insect management		
		Bt corn	Isoline	Isoline + tefluthrin
Giant foxtail	V4	55.0 ± 16.6aC	49.2 ± 2.9aA	55.7 ± 4.6aA
	V6	82.4 ± 8.0aAB	52.1 ± 3.8bA	55.0 ± 5.6bA
	No weeding	86.7 ± 6.2aAB	49.9 ± 6.9bA	59.4 ± 3.7bA
Large crabgrass	V4	90.3 ± 6.1aA	57.3 ± 5.3bA	65.6 ± 10.7bA
	V6	63.4 ± 13.6aBC	41.4 ± 6.8aA	53.2 ± 7.1aA
	No weeding	76.9 ± 3.6aBC	55.5 ± 3.4aA	56.2 ± 2.8aA
Foxtail + crabgrass	V4	87.6 ± 8.1aAB	59.2 ± 5.8bA	58.8 ± 7.0bA
	V6	70.8 ± 16.1aBC	47.4 ± 8.2bA	52.2 ± 2.9abA
	No weeding	63.3 ± 18.6aBC	60.5 ± 3.1aA	55.0 ± 6.1aA
Weed-free control		91.7 ± 8.3aA	44.7 ± 2.2bA	61.2 ± 2.5bA
Insect management main effect		72.2a	52.5b	56.8b

Significance differences ($P = 0.05$) between weed species and management combinations within a column are indicated by different uppercase letters. Significant differences between insect management types within a row are indicted by different lowercase letters. Although untransformed data are shown, the analysis was performed on arcsine ($\sqrt{x}$).

ever, male dry weight was not significantly affected by weed species ($F = 0.14$; df = 1, 28; $P = 0.7109$) nor the interaction of all three factors ($F = 0.30$; df = 4, 32; $P = 0.8745$). Note that when no adults emerged from a particular treatment combination, the data point was treated as a missing value. The reduced degrees of freedom are due to the lack of adults from all replications within two treatment combinations and specific replications of other treatment combinations. The average dry weight of female beetles was not significantly affected by insect management tactics ($F = 0.26$; df = 2, 8; $P = 0.7759$), weed species ($F = 0.00$; df = 1, 28; $P = 0.9795$), weed management ($F = 1.18$; df = 3, 28; $P = 0.3343$), or the interactions among these three factors ($F = 0.96$; df = 6, 46; $P = 0.4597$). Male beetles had a significantly lower average dry weight when recovered from Bt corn than when recovered from isoline or isoline plus tefluthrin when the effects of weed species and weed management were combined into the main effect of insect control (Table 8). The average dry weight of males and females recovered from weed free Bt corn was not significantly different than the average weight of any grassy weed treatment.

Pronotum Width of Adults. *2003 Field Study.* Pronotum widths of adults were not significantly affected by insect management tactics for males ($F = 0.55$; df = 2, 8; $P = 0.5972$) or females ($F = 3.16$; df = 2, 8; $P = 0.0976$). Weed species ($F = 0.02$; df = 2, 35; $P = 0.9504$), weed management ($F = 0.91$; df = 3, 35; $P = 0.4480$), and the interactions among these three factors ($F = 0.93$; df = 8, 47; $P = 0.5035$) did not significantly affect pronotum widths of males or females ($F = 1.60$; df = 2, 35; $P = 0.2155$; $F = 1.73$; df = 3, 36; $P = 0.1781$; and $F = 0.65$; df = 8, 63; $P = 0.7295$) for weed species, weed management, and the interactions of these factors, respectively. There was no significant difference between the pronotum widths of male or female beetles between any treatments (data not shown).

2004 Field Study. The pronotum widths of male beetles were significantly affected by insect management tactics ($F = 5.18$; df = 2, 8; $P = 0.0361$). However, nothing else was significant for males, including weed species ($F = 0.04$; df = 1, 8; $P = 0.8481$), weed management ($F = 1.19$; df = 3, 28; $P = 0.3313$), and the interaction of all three factors ($F = 0.73$; df = 4, 32; $P = 0.5786$). None of the factors significantly affected pronotum width for females, including insect management ($F = 1.65$; df = 2, 8; $P = 0.2512$), weed species ($F = 0.93$; df = 1, 28; $P = 0.3436$), weed management ($F = 0.50$; df = 3, 28; $P = 0.6835$), and the interactions

Table 6. Mean ± SE percentage of females emerged in 2004

Weed species	Weed management time	Insect management		
		Bt corn	Isoline	Isoline + tefluthrin
Giant foxtail	V3	88.89 ± 7.86aAB	70.41 ± 3.98bA	81.53 ± 9.23abA
	V5	87.64 ± 6.34aAB	76.34 ± 4.18aA	75.80 ± 6.02aAB
	No weeding	74.91 ± 7.03aB	71.58 ± 6.48aA	79.56 ± 6.73aAB
	Weed free	95.83 ± 4.17aA	73.92 ± 5.31bA	88.21 ± 3.50abA
Large crabgrass	V3	75.00 ± 25.0aAB	75.71 ± 3.33aA	64.26 ± 3.60aB
	V5	75.83 ± 15.8aAB	74.16 ± 5.60aA	73.26 ± 9.87aAB
	No weeding	80.83 ± 9.82aAB	78.01 ± 4.66aA	70.32 ± 5.08aAB
	Weed free	83.78 ± 9.24aAB	70.62 ± 2.91abA	61.64 ± 6.89bB
Insect management main effect		82.64a	74.84b	73.32b

Significance differences ($P = 0.05$) between weed species and management combinations within a column are indicated by different uppercase letters. Significant differences between insect management types within a row are indicted by different lowercase letters. Although untransformed data are shown, the analysis was performed on arcsine ($\sqrt{x}$).

Table 7. Mean ± SE average dry weights (milligrams) of males and females in 2003

Weed species	Weed management time	Insect management					
		Bt corn, males	Isoline, males	Iso/tefluthrin, males	Bt corn, females	Isoline, females	Iso/tefluthrin, females
Giant foxtail	V4	1.76 ± 0.33aBC	1.59 ± 0.09aA	1.60 ± 0.29aA	2.14 ± 0.31aCE	1.93 ± 0.11aA	1.70 ± 0.24aA
	V6	1.36 ± 0.43aC	1.61 ± 0.16aA	1.80 ± 0.25aA	2.42 ± 0.14aBD	2.31 ± 0.36aA	2.03 ± 0.28aA
	No weeding	3.10 ± 1.53aAB	1.72 ± 0.17bA	1.62 ± 0.13bA	2.07 ± 0.39aDE	2.10 ± 0.19aA	1.87 ± 0.08aA
Large crabgrass	V4	2.89 ± 0.25aAB	1.72 ± 0.14bA	2.04 ± 0.05bA	2.76 ± 0.41aAD	2.32 ± 0.19aA	2.06 ± 0.19aA
	V6	1.56 ± 0.33aBC	1.93 ± 0.49aA	1.33 ± 0.14aA	1.54 ± 0.19aE	2.24 ± 0.35aA	2.16 ± 0.36aA
	No weeding	1.43 ± 0.54aC	1.45 ± 0.05aA	1.42 ± 0.14aA	3.09 ± 0.44aAC	1.71 ± 0.14bA	2.23 ± 0.24abA
Foxtail + crabgrass	V4	2.07 ± 0.71aAC	2.11 ± 0.41aA	1.47 ± 0.29aA	3.44 ± 0.35aA	1.90 ± 0.30bA	2.28 ± 0.20bA
	V6	3.16 ± 0.33aA	1.56 ± 0.20bA	1.52 ± 0.23bA	2.44 ± 0.33aAD	2.11 ± 0.35aA	2.12 ± 0.13aA
	No weeding	1.44 ± 0.00aBC	1.50 ± 0.17aA	1.60 ± 0.17aA	1.62 ± 0.43bE	2.41 ± 0.23aA	2.39 ± 0.30abA
Weed-free control		2.50 ± 0.78aAC	1.53 ± 0.08aA	1.87 ± 0.07aA	3.34 ± 0.46aAB	1.96 ± 0.16bA	2.00 ± 0.22bA
Insect management main effect		2.12a	1.67b	1.61b	2.70a	2.09b	2.08b

Significance differences ($P = 0.05$) between weed species and management combinations within a column are indicated by different uppercase letters. Significant differences between insect management types within a row are indicted by different lowercase letters. Although untransformed data are shown, data were analyzed as sqrt (x + 0.5).

among these three factors ($F = 0.43$; df = 6, 46; $P = 0.8543$).

Discussion

Insect management tactics significantly affected plant damage, adult emergence numbers, adult sex ratios, and average dry weight of males and females (Tables 1–8). Fewer adults emerged from Bt corn treatments than from isoline or isoline plus tefluthrin treatments, and the plants that they came from were significantly less damaged. The effects of weeds and weed management depended upon the insect management regime and the environment. In general, weeds and weed management did not significantly affect adult emergence or damage within the isoline plus tefluthrin treatment; they tended to slightly increase the number of beetles emerging within Bt corn treatments; and they had variable effects within the isoline treatments between the 2 yr. The presence of weeds did not significantly affect plant damage or adult emergence numbers either year within Bt corn treatments. However, sex ratio of emerging adults was significantly affected for some treatments.

In a series of greenhouse studies similar to the current field study, Oyediran et al. (2005) documented that significantly more beetles were recovered from Bt corn plus weeds than Bt corn alone or weeds alone. The weeds in that study were sprayed with glyphosate 5 d after adding neonate larvae and seemed dead 4–5 d later. Beetle emergence was delayed fewer days behind the isoline treatment in the Bt corn plus weeds treatment than the Bt corn alone or weeds alone treatments (Oyediran et al. 2005). Oyediran et al. (2005) also recorded fecundity, and more eggs were laid by females reared on Bt corn plus weeds than by females reared on Bt corn alone, and in one of three trials, percentage of hatch was also greater from females reared on Bt corn plus weeds than Bt corn alone. Grassy weeds effected the ability of western corn rootworm larvae to survive Bt corn in the greenhouse. There was no significant difference between the number of beetles emerging from isoline corn, isoline plus weeds, and Bt corn plus weeds in the greenhouse, a result that differs greatly from the current field study.

In the current study, adult emergence was not significantly higher in Bt corn plots with grassy weeds than in Bt corn plots without weeds in either year

Table 8. Mean ± SE average dry weights (milligrams) of males and females in 2004

Weed species	Weed management time	Insect management					
		Bt corn, males	Isoline, males	Iso/tefluthrin, males	Bt corn, females	Isoline, females	Iso/tefluthrin, females
Giant foxtail	V3	1.88 ± 0.71aA	1.47 ± 0.16aA	1.63 ± 0.14aAB	1.87 ± 0.61aA	1.48 ± 0.03aA	1.73 ± 0.14aA
	V5	1.43 ± 0.28aAB	1.38 ± 0.21aA	1.49 ± 0.10aAB	1.83 ± 0.29aA	1.89 ± 0.31aA	2.36 ± 0.33aA
	No weeding	0.89 ± 0.09bB	1.43 ± 0.13aA	1.23 ± 0.15abB	1.79 ± 0.29aA	1.74 ± 0.15aA	1.76 ± 0.26aA
	Weed free	n/a	1.63 ± 0.15aA	1.66 ± 0.04aAB	1.70 ± 0.11aA	1.64 ± 0.12aA	1.88 ± 0.26aA
Large crabgrass	V3	n/a	1.49 ± 0.16aA	1.89 ± 0.14aA	1.39 ± 1.05aA	1.70 ± 0.20aA	1.88 ± 0.19aA
	V5	1.02 ± 0.00bB	1.42 ± 0.13abA	1.83 ± 0.27aA	2.22 ± 0.46aA	1.86 ± 0.19aA	1.65 ± 0.23aA
	No weeding	0.68 ± 0.37bB	1.33 ± 0.06aA	1.39 ± 0.19aAB	1.71 ± 0.55aA	2.01 ± 0.26aA	1.92 ± 0.20aA
	Weed free	1.12 ± 0.14bB	1.70 ± 0.18aA	1.86 ± 0.32aA	1.69 ± 0.26aA	1.88 ± 0.20aA	2.00 ± 0.26aA
Insect management main effect		1.15b	1.48a	1.61a	1.80a	1.77a	1.89a

Significance differences ($P = 0.05$) between weed species and management combinations within a column are indicated by different uppercase letters. Significant differences between insect management types within a row are indicted by different lowercase letters. Although untransformed data are shown, data were analyzed as sqrt (x + 0.5).

(Tables 3 and 4). Plant damage was not evaluated by Oyediran et al. (2005). In the current study, there was no statistically significant difference in damage to Bt corn when kept weed free or combined with grassy weeds in either year. However, in 2004, there was a trend toward increased damage in several of the weed treatments (Table 2). Why the difference between greenhouse and field results? The reason is not clear, but in the greenhouse, the grassy weeds were larger at the time of infestation with neonate larvae than the field plots averaged at the time of egg hatch. In 2003, weeds were especially small at the time of egg hatch. In 2004, great care was taken to establish the weeds earlier relative to the corn. The field was tilled and planted to weeds in mid-April, 3 wk before planting corn, and no tillage on the day that corn was planted. We wanted to create the worst possible scenario, but still there was no significant difference in damage to or adult emergence numbers from Bt corn.

Although the actual number of beetles did not differ among treatments within Bt corn, the percentage of females from the Bt corn weed-free plots was significantly greater than Bt plots with different weed combinations for five of nine treatments in 2003 (Table 5). In 2004, within the giant foxtail subplots of Bt corn treatments, the percentage of females emerged was 95.83 ± 4.17, which was nominally more female biased than any other treatment combination in 2004. Adult emergence from Cry3Bb1 Bt corn has typically been female biased (EPA SAP 2002). That Bt corn with weeds was sometimes less female biased than Bt corn without weeds would seem to indicate that males production was enhanced by the presence of alternate hosts in Bt corn.

Foxtail had previously been shown to have an effect on adult emergence in the field. Johnson et al. (1984) showed that an unspecified density of foxtail species reduced adult emergence in all 3 yr of the study. Ellsbury et al. (2005) found that rootworm adults emerged later when foxtail was present and had smaller head capsule widths than did individuals from areas without foxtail. In the current study, similar results were obtained from treatments that included crabgrass in 2003 (Table 3), but in 2004, no significant difference was found between the weed treatments within isoline corn for adult emergence (Table 4). One of the weed treatments in 2004 had significantly more damage to isoline corn than isoline corn kept weed free (Table 2). This result contradicts the data from 2003 (Table 1) and data from Johnson et al. (1984) and Ellsbury et al. (2005).

Results of previous studies (Clark and Hibbard 2004, Oyediran et al. 2004, Wilson and Hibbard 2004, Chege et al. 2005) have shown that most grass species can support the growth of western corn rootworm larvae. The partial development of corn rootworm larvae on grassy weeds did significantly affect adult emergence from Bt corn in the greenhouse (Oyediran et al. 2005), but in the current study, adult emergence was not significantly effected in either of the 2 yr of the study. Damage to Bt corn also was not significantly affected either year of the current study. However, only one weed density and one corn rootworm egg density was evaluated in the current study. Given the greenhouse data, it is possible that under some field conditions, such as different combinations of weed and rootworm egg densities, the presence of grassy weeds could have a significant effect on damage to Bt corn and adult emergence from Bt corn. In the current field study, grassy weeds played only a minor role. Whether partial development on an alternate host or non-Bt corn plant is positive or negative in terms of long-term resistance management is impossible to determine without evaluation of the progeny of individuals surviving partially on Bt and comparing their performance on Bt corn to the performance of those never exposed to Bt and to those reared on Bt corn.

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