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Three-Year Field Monitoring of Cry1F, Event DAS-Ø15Ø7–1, Maize Hybrids for Nontarget Arthropod Effects

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ABSTRACT Field studies were conducted over a 3-yr period to investigate the potential effects of cultivating transgenic maize hybrids containing a Cry1F insect-resistant protein on nontarget arthropod abundance. The narrow spectrum of activity of Cry1F against a subset of lepidopteran pest species would not suggest broad-spectrum effects on nontarget arthropods. However, because of the insecticidal nature of *Bt* proteins, an alternate hypothesis is that some nontargets may be affected by exposure to the protein. To examine this hypothesis at the field level, monitoring for nontarget organism abundance was initiated at four locations across the U.S. Corn Belt from 2004 through 2006. At each location, paired fields (≈0.8 ha each) of commercial Cry1F maize hybrids and isogenic nontransgenic control hybrids were planted. Sampling methods used to monitor nontarget organisms included visual surveillance, sticky cards, pitfall traps, and litterbags. Data were analyzed using multivariate analyses to look for a general community level response to the treatments. Analysis of variance was conducted on individual taxa to detect differences distinct from the primary community response. Community level analyses of the nontarget arthropod abundance showed no significant impact on community abundance when comparing *Bt* with non-*Bt* maize fields. Analyses of the individual taxa also showed no significant differences in abundance between *Bt* and non-*Bt* fields. Results of these studies confirm earlier laboratory testing and support the hypothesis that Cry1F maize does not produce adverse effects on nontarget arthropods occurring in maize fields.

KEY WORDS Cry1F, *Bt* corn, nontarget arthropods, field monitoring

Maize hybrids containing event DAS-Ø15Ø7–1 expressing the *cry1F* gene for control of lepidopteran maize pests were approved for commercial sale in the United States in 2001 under the trade name Herculex I. Event DAS-Ø15Ø7–1 maize was developed through the insertion of a synthetic truncated *cry*1F gene from *Bacillus thuringiensis* (*Bt*) variety *aizawai*. Accumulation of Cry1F protein in maize provides effective plant protection against the larval stage of many lepidopteran maize pests including European corn borer, *Ostrinia nubilalis* (Hübner); southwestern corn borer, *Diatraea grandiosella* Dyar; fall armyworm, *Spodoptera frugiperda* (J. E. Smith); western bean cutworm, *Striacosta albicosta* (Smith); black cutworm, *Agrotis ipsilon* (Hufnagel); and corn earworm *Helicoverpa zea* (Boddie). Event DAS-Ø15Ø7–1 maize also contains the selectable marker phosphinothricin acetyltransferase (*pat*), which confers tolerance to glufosinate-ammonium (Liberty Herbicide; Bayer CropScience, Research Triangle Park, NC). The PAT protein is not a known toxin and/or pathogen of plant or animal species (U. S. EPA 2005). Although some field studies suggest shifts in arthropod abundance based on changes in weed management and tillage (Thorbek and Bilde 2004, Brooks et al. 2005), this study involved standard weed management practices in all treatments and did not involve the application of glufosinate-ammonium herbicides.

Bt Cry proteins have been shown to have a narrow range of insecticidal activity within a few insect orders (Metz 2003). Since the introduction of *Bt* crops for commercial use in the United States in the 1990s, a large body of studies designed to evaluate their effect on nontarget organisms has been generated. O'Callaghan et al. (2005) reviewed the effects of *Bt* plants (primarily maize and cotton) on aerial (pollinators and natural enemies) and soil-borne nontarget biota. They did not detect significant adverse effects in plant feeding and beneficial nontarget insects or in soil-dwelling organisms such as earthworms, collembolans, and other soil microflora. Romeis et al. (2006) summarized >50 field studies conducted to examine the impact of *Bt* plant incorporated proteins on the natural enemies of crop pests. These studies covered a variety of genetically modified crops including maize, cotton, potato, tobacco, and eggplant. Nearly one half of these studies were conducted on maize expressing the Cry1Ab protein, the first *Bt* protein to

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be commercialized in maize and which currently constitutes the majority of *Bt* maize acreage in the United States. These studies ranged in size, duration, and sampling methodology and mainly focused on comparing the abundance of beneficial arthropods found in *Bt* and non-*Bt* plots (Daley and Buntin 2005, Dively 2005, Pilcher et al. 2005, Lopez et al. 2005). Overall, these studies indicated no major effects against natural enemies in *Bt* fields compared with non-*Bt* fields, with the occasional exception of taxa that were dependent on *Bt*-susceptible pests as hosts. These data support the conclusions of lower tier (laboratory) safety tests for *Bt* maize, which generally have concluded that few negative effects on nontarget organisms are expected because of the narrow spectrum of insecticidal activity of *Bt* proteins. More recently, Marvier et al. (2007) conducted a meta-analysis of the effects of *Bt* cotton and maize on nontarget invertebrates using data from 42 field experiments. Their analyses suggest that nontarget invertebrates are generally more abundant in *Bt* cotton and *Bt* maize fields compared with nontransgenic fields treated with conventional insecticides. Some differences in abundance were detected for certain taxa when *Bt* fields were compared with non-*Bt* fields without insecticides, although these differences were event specific and may have been caused (either directly or indirectly) by control of the target pests that serve as prey for the affected beneficial species.

To date, no field scale evaluations of nontarget arthropods in Cry1F maize have been published in the literature. As with other lepidopteran-active *Bt* proteins, the Cry1F protein seems to have a narrow range of insecticidal activity (U.S. EPA 2001). However, because of the insecticidal nature of *Bt* proteins, an alternate hypothesis is that some nontargets may be affected by exposure to the protein. To evaluate this hypothesis, field trials were conducted from 2004 to 2006 to compare nontarget arthropod abundance in fields containing commercial Cry1F maize hybrids with abundance in paired non-*Bt* maize fields.

Materials and Methods

Nontarget arthropod abundance was monitored at four locations across the U.S. Corn Belt with field sites in Nebraska, Iowa, Indiana, and Wisconsin. Table 1 lists field locations, field characteristics, maize hybrids, and planting dates for each study. At each test location, a pair of Cry1F *Bt* maize and isogenic (maize line of similar genetic background but lacking the DAS-Ø15Ø7–1 event) control maize fields were planted in plots of ≈0.8 ha using cultivation practices typical of each area. Each field was bordered with non-*Bt* hybrid maize. No conventional insecticides were applied to the fields during the growing season. Test plots in this study were not sprayed with glufosinate-ammonium herbicides, and weed control practices were uniformly applied across test and control plots.

Data collection within the fields was conducted from randomly selected points assigned in the "sampling area" (area excluding the outermost ≈30.5 m) of

Table 1. Field and crop characterization summary for 2004–2006 nontarget arthropod field monitoring

Field location	Year	Field characterization						Crop		
		Soil						Maize hybrids		
		Type	Series	Percent organic	Previous crop	Seeding rate[a]	Irrigated	*Bt*	non-*Bt*	Planting date
York, NE	2004	Silt loam	Filmore	2.3	Soybean	79,040	Yes	Pioneer 33B55	Pioneer 33B50	21 Apr. 2004
	2005	Silt loam	Filmore	2.0	Soybean	69,160	Yes	Pioneer 33B55	Pioneer 33B50	17 May 2005
	2006	Silt loam	Filmore	Not available	Soybean	86,450	Yes	Pioneer 33B55	Pioneer 33B50	15 Apr. 2006
Scott County, IA	2004	Silty clay loam	Tama	3–4	Soybean	74,100	No	Pioneer 33B55	Pioneer 33B50	14 May 2004
	2005	Silty clay loam	Tama	3–4	Soybean	74,100	No	Pioneer 33B55	Pioneer 33B50	4 May 2005
	2006	Silty clay loam	Tama	3–4	Soybean	71,630	No	Pioneer 33B55	Pioneer 33B50	5 May 2006
Fowler, IN	2004	Clay loam	Selma	3.2	Winter wheat	68,419	No	Mycogen 2G768	Mycogen 2784	24 May 2004
Frankfort, IN	2005	Silty clay loam	Brookston	6.0	Soybean	79,040	No	Mycogen 2G768	Mycogen 2784	17 May 2005
	2006	Silty clay loam	Brookston	4.3	Soybean	74,100	No	Mycogen 2G768	Mycogen 2784	24 May 2006
Arkansaw, WI	2004	Sandy loam	Burkhardt	2.3	Corn	81,510	Yes	Mycogen 2E390	Mycogen 2395	7 May 2004
	2005	Sandy loam	Burkhardt	2.6	Vegetables	74,100	Yes	Mycogen 2E214	Mycogen 2E212	25 May 2005
	2006	Sandy loam	Burkhardt	2.0	Potato	83,980	Yes	Mycogen 2E214	Mycogen 2E212	11 May 2006

[a] Seeds per hectare.

Table 2. Monitored taxa summary, 2004–2006

Taxa	Life stage(s)	Functional group	Sampling method
Ladybird beetles (Coleoptera: Coccinellidae)	Eggs, larvae, pupae, adults	Predators	Visual observation
Lacewings (Neuroptera: Chrysopidae)			
Parasitic wasps (Hymenoptera: Braconidae and Ichneumonidae)	Adults	Parasitoids	Sticky cards
Rove beetles (Coleoptera: Staphylinidae)		Predators	
Long-legged flies (Diptera: Dolichipodidae)			
Insidious flower bugs (Hemiptera: Anthocoridae)	Nymphs and adults		
Phytophagus thrips (Thysanoptera: Thripidae)		Herbivores	
Leafhoppers (Hemiptera: Cicadellidae)			
Aphids (Homoptera: Aphididae)			
Ground beetles (Coleoptera: Carabidae)	Larvae and adults	Predators and herbivores	Pitfall and litterbag
Rove beetles (Coleoptera: Staphylinidae)		Predators	
Globular springtails (Collembola: Sminthuridae)	Immatures and adults	Detritivores	
Elongate springtails (Collembola: Entomobryidae, Isotomidae, Hypogasturidae)			
Oribatid mites (Acari: Oribatei)			
Spiders (Araneae)		Predators	
Centipedes/Millipedes (Diplopoda/Chilopoda)		Predators and detritivores	

each field. Arthropod abundance was monitored using multiple sampling techniques: visual observations to monitor taxa occurring on maize plants, sticky cards to monitor aerial taxa, pitfall traps to monitor surface and ground dwelling taxa, and litterbags to measure taxa colonizing ground litter. Table 2 lists the taxa monitored during the study by sampling method, as well as the functional groups they represent. Sampling occurred at three time points during the growing season for visual observations, sticky cards, and pitfall traps: late vegetative stage (V-stage), immediately after anthesis (pollen shed, R1), and postanthesis (R3) (Ritchie et al. 1993). Litterbags were set in the field during the late vegetative growth stage, and subsets were collected after anthesis and again near crop maturity (R5).

Visual Observations. Visual counts of ladybird beetle and lacewing abundance were taken from 10 observation points within each plot at each sampling time. At each sampling point, 10 adjacent plants were selected, and visual counts were made of all life stages of ladybird beetles and lacewings present on the plants.

Sticky Cards. At each sampling time, 10 sticky cards were set in the sampling area of each plot. Cards were 7.6 by 12.7-cm unbaited yellow sticky cards attached to a stake embedded in the soil between rows. Cards were placed at canopy level in vegetative stage maize and at ear height on subsequent sampling dates. Cards were removed 24 h after placement in 2004 and 72 h after placement in 2005 and 2006. Cards were placed in labeled clear plastic bags for storage until identification of trapped arthropods.

Pitfall Traps and Litterbags. Ten pitfall traps per plot were set at each sampling date. Pitfall traps consisted of plastic cups (ranging from 300 to 473 ml) buried upright to the rim containing a small amount of ethylene glycol. Pitfall traps were set for 24 h, and the contents were collected for arthropod identification. Two sets of 10 litterbags were set in each plot at the late vegetative growth stage. Litterbags consisted of plastic 0.9- to 1.4-kg mesh onion bags (General Bag, Cleveland, OH) with a mesh width (unstretched) of ≈1 cm. Litterbags were filled half full with mulched sterile wheat straw. Litterbags were placed between rows on the soil surface and held in place with a stake or wire flag. At each sampling date, 10 litterbags were removed from each plot (one per sample point) and placed in resealable plastic storage bags for transportation to the extraction units. The contents of each litterbag were placed in a Burlese-Tullgren funnel for extraction of arthropods inhabiting the litter.

Climate data (average temperatures and rainfall) were collected for each field location from the nearest available weather station. Thirty-year averages (1971–2000) were taken from National Oceanic and Atmospheric Administration weather stations located closest to the test sites.

Data Analysis. Software programs used for statistical analyses were SAS V. 9.1 (SAS Institute 2002–2003) and CANOCO V. 4.5 (Microcomputer Power, Ithaca, NY).

The PROC MIXED (SAS) procedure was used to test for treatment and interaction effects on individual taxa. Treatment effects were analyzed by averaging data from all years because sample timing was synchronized by crop phenology. Treatments and sampling times were modeled as fixed factors, whereas location was treated as a random effect. A mixed model with repeated measures and the most appropriate covariance structure was used to account for correlations among the observations. Before the analysis, the data were transformed by using the common logarithm of the counts plus 1, and residual plots and Shapiro-Wilks' W test were performed to examine for data normality. Only individual or groups of taxa with sufficient data that satisfied the assumptions of analysis of variance (ANOVA) were analyzed.

Statistical power to detect a 50% impact on population abundance was calculated for the key taxa for each sampling method. The power was computed for detecting a 50% change in the control mean for the difference of means using the estimated variance of a difference of means from the mixed model analysis.

To determine the 50% change, the least squares mean of the control treatment was back transformed to counts. The counts were divided by two (50% change), and this count change was transformed back to log(count + 1) to determine the change in transformed means that correspond to 50% change in the counts. The power was computed by solving for the *t*-value for a type II error rate from the following sample size equation (Meinert 1986), where the type I error rate or α was set to 0.05:

$$n = \frac{2\sigma^2}{\Delta^2}[t_{\frac{\alpha}{2},df} + t_{\beta,df}]^2$$

where Δ^2 is the change (in this case 50% of control mean), σ^2 is the estimate of the variance, α is the type I error rate, and β is the type II error rate. This equation can be solved for $t_{\beta,df}$ as

$$t_{\beta,df} = \frac{n\Delta^2}{2\sigma^2} - t_{\frac{\alpha}{2},df}$$

where $\frac{2\sigma^2}{n}$ represents the SE of the difference of two means. Let $s.e.diff = \sqrt{\frac{2\hat{\sigma}^2}{n}}$ denote the estimate of the standard of the difference of the control mean minus the comparison mean. Then $t_{\beta,df}$ can be expressed as

$$t_{\beta,df} = \frac{\Delta^2}{s.e.diff} - t_{\frac{\alpha}{2},df}$$

where df corresponds to the degrees of freedom used to estimate the standard error of the difference.

Multivariate analysis was used to look for a general community response to the treatments; this level of analysis was implemented to detect trends that occur in the community and to also identify the key taxa influencing those trends. Every taxon for which data were collected for a particular sampling method was included in the community level analysis. The method of principal response curves (Van den Brink and ter Braak 1999) was used to investigate and describe treatment effects at the community level. The advantage of using a multivariate method is that it summarizes all information on the investigated populations simultaneously, and in doing so, it evaluates the effects of a test substance at the community level (Van den Brink and ter Braak 1999). Principal response curves provide an intuitive graphical summarization of the community abundance relative to a specific control. For each year and trap type, the following model was used:

$$log(y_{djtk} + 1) = r_{jk} + g_{tk} + b_k c_{dt} + \varepsilon_{djtk},$$

for treatment d ($d = 1,2$), replication (location) j ($j = 1, \ldots, 4$), growth stage t ($t = 1, \ldots, Ng$), and taxa k ($k = 1, \ldots, Ns$) and for Ng equal to the number of growth stages and Ns equal to the number of taxa present. In the above equation, y_{djtk} represents the average abundance per trap of the sampling sites in each plot, and it is the log (of these average counts plus 1) that is modeled. In this model example, treatment 2 is taken to be the control treatment and $c_{2t} = 0$. The terms r_{jk}, g_{tk}, b_k, and c_{dt} are estimated model parameters, and ε_{djtk} represents a random error term.

The above equation consists of a set of taxa weights (b_k) and a set of canonical coefficients (c_{dt}), which together define the estimated abundance of taxa (k) in treatment (d) at growth stage (t), expressed as a difference with the control abundance in treatment (d) at growth stage (t). The value $\exp(b_k \times c_{dt})$ gives the estimated relative abundance of the treatment group (d) to the control group for a particular taxa (k) and stage (t). For the analysis of data, the canonical coefficients correspond to the first principal response curve (PRC) for each treatment and are plotted (*y1*-axis) against time (*x*-axis), whereas the taxa weights are presented on the *y2*-axis. When the canonical coefficient (*y*-axis) is greater than zero and a taxon has a positive weight (or when both the canonical coefficient and the taxon weight are negative), the taxon abundance is expected to be greater in the treatment group than in the untreated control. Van den Brink and ter Braak (1999) reported the taxa weights only when they are above +0.5 or less than −0.5, because taxa having weights with an absolute value <0.5 are unlikely to show a meaningful response that is similar to the overall community response captured in the first principal response curve. All taxa weights are reported in these analyses. Analyses were conducted across all sampling dates in each year using a statistical significance level of $P = 0.05$.

Estimates of taxon weights and canonical coefficients were obtained using CANOCO V. 4.5. In addition, permutation tests (499 permutations) for significance of the first canonical axis were performed in CANOCO using data from all of the sampling dates.

Results

Climatic Conditions Summary. A summary of average high and low temperatures and rainfall during the 2004–2006 growing seasons for each location is presented in Table 3. The average temperatures for each growing season from April to October were within 2°C of the 30-yr average for all locations. Rainfall averages were within 20% of the 30-yr averages for each location with the following exceptions: York, NE, in 2004, which received 68% of the 30-yr average rainfall during April–October; Scott County, IA, in 2005, which received 41% of the April–October 30-yr average rainfall; and the 2006 Wisconsin location, which received 78% of the April–October 30-yr average rainfall.

Community Level Analyses. The method of PRCs (Van den Brink and ter Braak 1999) was used to study and describe treatment effects at the community level. Taxa counts were summarized and analyzed across visual observations and sticky and pitfall traps; the litterbag analysis was conducted separately because of the asynchrony of sample collection compared with other trap types and the large taxa counts observed by that sampling method. Figure 1 gives the canonical coefficients and taxa weights for the first PRCs for the

Table 3. Monthly climate summary of field locations monitored from 2004 to 2006

Site	Month	2004 weather data				2005 weather data				2006 weather data				Historical weather data[a]			
		Temperature (°C)			Precipitation (mm)	Temperature (°C)			Precipitation (mm)	Temperature (°C)			Precipitation (mm)	Temperature (°C)			Precipitation (mm)
		Max	Min	Avg		Max	Min	Avg		Max	Min	Avg		Max	Min	Avg	
Nebraska[b]	April	20	5	12	22.9	19	6	12	147.3	21	6	13	96.5	19	4	12	68.6
	May	25	11	18	127.0	24	11	18	233.7	26	12	19	43.2	24	11	17	114.3
	June	27	15	21	55.9	30	18	24	73.7	31	17	24	45.7	30	16	23	94.0
	July	29	17	23	71.1	32	19	26	88.9	33	19	26	33.0	32	19	26	88.9
	Aug.	28	15	22	17.8	30	17	24	55.9	30	18	24	182.9	31	17	24	83.8
	Sept.	29	14	21	73.7	29	14	21	20.3	24	10	17	91.4	27	12	19	68.6
	Oct.	19	6	13	17.8	20	6	13	61.0	17	4	10	25.4	21	6	13	50.8
Iowa[c]	April	18	4	11	40.6	19	6	12	25.4	20	6	13	134.6	17	4	10	96.5
	May	23	11	17	235.0	22	8	15	50.8	22	11	16	61.0	23	10	17	109.2
	June	26	15	20	81.3	30	17	24	30.5	27	14	21	66.0	28	15	22	116.8
	July	27	16	21	104.1	30	17	23	40.6	31	19	24	101.6	30	18	24	101.6
	Aug.	24	13	18	99.1	29	16	22	66.0	27	17	22	144.8	29	17	23	111.8
	Sept.	26	11	18	20.3	27	13	21	43.2	22	10	16	22.9	25	12	18	81.3
	Oct.	17	6	11	96.5	18	6	12	25.4	15	3	9	61.0	18	5	12	71.1
Indiana[d]	April	18	4	11	30.5	27	-1	13	101.1	19	6	13	101.6	16	4	10	91.4
	May	24	12	18	106.7	31	-1	15	60.7	21	10	16	180.3	22	10	16	104.1
	June	26	11	19	33.0	33	11	22	89.2	26	15	21	91.4	27	15	21	114.3
	July	27	14	21	61.0	33	12	23	167.6	29	19	24	154.9	29	17	23	104.1
	Aug.	25	12	19	213.4	32	12	22	79.2	28	18	23	121.9	28	16	22	101.6
	Sept.	27	11	19	17.8	32	4	18	114.3	23	12	17	68.6	24	12	18	76.2
	Oct.	18	5	12	71.1	30	-2	14	26.9	17	4	11	94.0	18	6	12	73.7
Wisconsin[e]	April	16	1	8	38.1	17	3	10	50.8	18	4	11	50.8	14	1	7	73.7
	May	19	7	13	223.5	18	7	13	73.7	22	9	16	68.6	21	8	14	94.0
	June	24	12	18	129.5	28	16	22	99.1	28	13	21	45.7	26	13	19	109.2
	July	27	14	21	99.1	29	16	22	76.2	32	18	25	45.7	28	16	22	99.1
	Aug.	24	11	17	61.0	27	14	21	71.1	27	16	22	160.0	27	14	21	119.4
	Sept.	25	12	18	86.4	24	11	18	188.0	21	8	14	76.2	21	9	15	94.0
	Oct.	15	4	10	106.7	17	4	11	12.7	13	1	7	55.9	14	3	8	55.9

[a] Historic weather data (1971–2000) taken from National Oceanic and Atmospheric Administration weather stations located closest to the test sites (source: http://cdo.ncdc.noaa.gov/cgi-bin/climatenormals/climatenormals.pl?directive=prod_select2&prodtype=CLIM81&submum=): Seward, NE (York, NE); Quad Cities, IL (Scott County, IA); Frankfort, IN; and Eau Claire, WI (Arkansaw, WI).

[b] York, NE, weather data taken from the Pioneer Hi-Bred Research Center weather station, York, NE.

[c] Scott County weather data taken from the NOAA weather station, Davenport, IA.

[d] 2004 weather data taken from the Fowler IN, weather station, 2005 from Tipton, IN, weather station, and 2006 data from Frankfort, IN, weather station.

[e] 2004–2005 weather data collected from the Alma, WI, weather station with the exception of April, Sept. and Oct. 2004 and April 2005 weather data from Eau Claire NWS Station (included for completeness). 2006 weather data collected from the Eau Claire NWS Station.

visual, sticky, and pitfall trap analysis. *P* values for a test of significance of the first canonical axis are included by year, and taxa weights were calculated based on a meta-analysis of all 3 yr of data. No statistically significant differences were detected between the Cry1F maize hybrids and the non-*Bt* controls in any year of the study. The first canonical axis accounted for 62.3% of the total variation of the species–environment relationship in 2004, 47.0% in 2005, and 71.7% in 2006. Taxon weights for 16 of the monitored taxa fell between −0.5 and 0.5, indicating no change or an unrelated response pattern than depicted by the PRC.

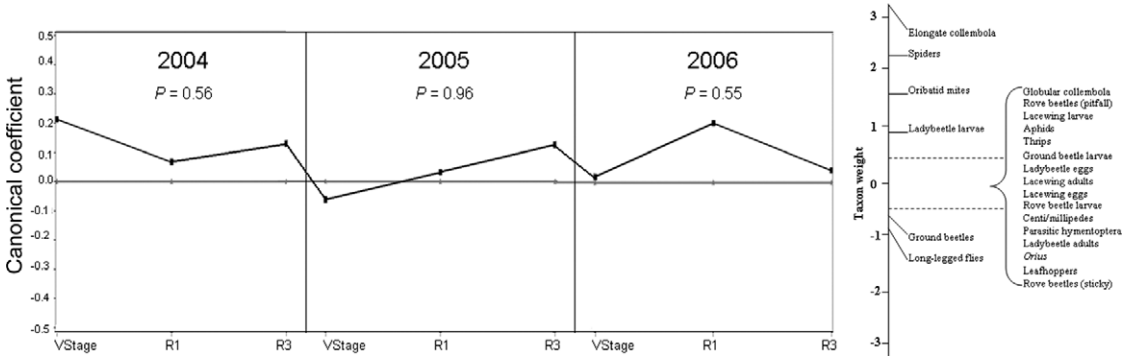

Fig. 1. PRCs and taxon weights of invertebrate communities exposed to Cry1F maize compared with isogenic non-*Bt* control maize as measured by visual observation, sticky cards, and pitfall traps. Responses of taxa with positive weights and positive canonical coefficients (*y*-axis) showed greater abundance than the control; taxa with negative weights and positive canonical coefficients were less abundant. Organisms with taxon weights between −0.5 and 0.5 generally either show no response or a response that is unrelated to the PRC pattern.

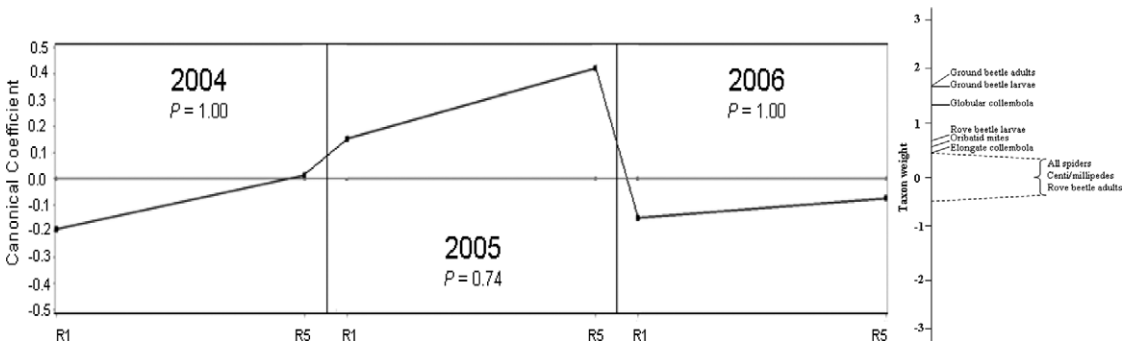

Fig. 2. PRCs and taxon weights of invertebrate communities exposed to Cry1F maize compared with isogenic non-*Bt* control maize as measured by litterbags. Responses of taxa with positive weights and positive canonical coefficients (*y*-axis) showed greater abundance than the control; taxa with negative weights and positive canonical coefficients were less abundant. Organisms with taxon weights between −0.5 and 0.5 generally either show no response or a response that is unrelated to the PRC pattern.

The PRC graph and corresponding taxon weights indicate a general increase in abundance relative to the non-*Bt* control in collembola, spiders, oribatid mites, and ladybird beetle larvae. A slight decrease in carabid adults and long-legged flies is suggested by the PRC; however, the canonical coefficients and taxa weights are both small, indicating neither were primary drivers of the community response.

Figure 2 gives the canonical coefficients and taxa weights for the first PRCs for the litterbag analysis. *P* values for a test of significance of the first canonical axis are included by year, and taxa weights were calculated based on a meta-analysis of all 3 yr of data. No statistically significant differences were detected between the Cry1F maize hybrids and the non-*Bt* controls in any year of the study. The first canonical axis accounted for 61.9% of the total variation of the species–environment relationship in 2004, 88.3% in 2005, and 83.2% in 2006. Species weights for three of the monitored taxa fell between −0.5 and 0.5, indicating no change or an unrelated response pattern than depicted by the PRC. The PRC graph and corresponding table of species weights show that the largest drivers of the PRC were ground beetles in 2005, which gen-

Table 4. Statistical summary of individual taxon abundance data 2004 (Nebraska, Iowa, Indiana, and Wisconsin) as monitored by visual surveillance, sticky cards, and pitfall traps

Functional group	Taxon	Mean counts per plot						*P* values			Power analyses	
		Non-*Bt*	*Bt*	Diff	SE diff	Lower CI	Upper CI	Treatment	Stage	Treatment × stage	Delta	Power
Predators/ detritivores	Centipedes and millipedes	0.06	0.00	−0.06	0.06	−0.24	0.13	0.39	0.40	0.40	0.03	0.04
Detritivores	Elongated collembola	16.73	42.94	0.91	0.52	−0.74	2.55	0.18	0.48	0.79	2.24	0.83
Detritivores	Globular collembola	2.96	3.71	0.17	0.64	−1.87	2.21	0.81	0.31	0.75	0.91	0.09
Detritivores	Oribatid mites	4.79	5.96	0.18	0.43	−1.18	1.55	0.70	0.02	0.39	1.22	0.38
Herbivores	Corn leaf aphid	1.50	1.46	−0.02	0.29	−0.94	0.90	0.95	0.04	1.00	0.56	0.15
Herbivores	Leafhoppers	17.20	15.43	−0.10	0.24	−0.87	0.67	0.70	0.01	0.74	2.26	1.00
Herbivores	Thrips	10.32	11.39	0.09	0.27	−0.78	0.96	0.76	0.00	0.78	1.82	0.98
Parasitoids	Parasitic hymenoptera	6.31	6.96	0.09	0.29	−0.83	1.00	0.79	0.00	0.72	1.42	0.91
Predators	All spiders	20.09	41.30	0.70	0.43	−0.66	2.05	0.20	0.57	0.44	2.40	0.95
Predators	Ground beetle adults	13.15	10.48	−0.21	0.29	−1.12	0.71	0.52	0.19	0.66	2.02	0.98
Predators	Ground beetle larvae	0.16	0.37	0.17	0.21	−0.49	0.83	0.48	0.39	0.26	0.08	0.03
Predators	Lacewing adults	0.10	0.26	0.14	0.15	−0.33	0.61	0.41	0.38	0.95	0.05	0.03
Predators	Lacewing eggs	3.07	2.72	−0.09	0.17	−0.62	0.44	0.62	0.80	0.47	0.93	0.95
Predators	Lacewing larvae	0.06	0.12	0.06	0.10	−0.25	0.37	0.60	0.38	0.71	0.03	0.03
Predators	Ladybird beelte adults	4.23	4.51	0.05	0.34	−1.02	1.12	0.89	0.17	0.46	1.14	0.57
Predators	Ladybird beetle egg clutches	0.26	0.41	0.12	0.23	−0.60	0.83	0.64	0.38	0.38	0.12	0.04
Predators	Ladybird beetle eggs	0.57	1.75	0.56	0.58	−1.29	2.41	0.40	0.55	0.57	0.25	0.04
Predators	Ladybird beetle larvae	11.47	12.51	0.08	0.23	−0.66	0.82	0.75	0.00	0.64	1.91	0.99
Predators	Insidious flower bug (*Orius*)	3.77	2.81	−0.22	0.34	−1.30	0.85	0.55	0.00	0.77	1.06	0.48
Predators	Rove beetle adult (pitfall)	3.54	3.40	−0.03	0.45	−1.45	1.39	0.95	0.15	0.50	1.02	0.22
Predators	Rove beetle larvae (pitfall)	0.65	0.51	−0.09	0.18	−0.67	0.50	0.67	0.31	0.21	0.28	0.10
Predators	Rove beetles adults (sticky)	0.53	0.30	−0.16	0.18	−0.73	0.41	0.44	0.80	0.54	0.23	0.08

Lower and upper confidence intervals (CIs) are 95% confidence intervals about the difference of the mean counts for *Bt* minus the mean counts for non-*Bt;* intervals that contain zero indicate there are no significant differences between the non-*Bt* and *Bt* mean counts. For the power analyses, delta corresponds to the 50% change in the control mean and is one half of the mean counts for the non-*Bt* group transformed to log(50% counts + 1). Power describes the probability of detecting a 50% change (or larger) when in fact it occurs.

Table 5. Statistical summary of individual taxon abundance data 2005 (Nebraska, Iowa, Indiana, and Wisconsin) as monitored by visual surveillance, sticky cards, and pitfall traps

Functional group	Taxon	Mean counts per plot						*P* values			Power analyses	
		Non-*Bt*	*Bt*	Diff	SE diff	Lower CI	Upper CI	Treatment	Stage	Treatment × stage	Delta	Power
Predators/ detritivores	Centipedes and millipedes	0.00	0.51	0.41	0.20	−0.23	1.06	0.14	0.68	0.68	0.00	0.03
Detritivores	Elongated collembola	21.72	23.85	0.09	0.43	−1.27	1.45	0.85	0.58	0.69	2.47	0.96
Detritivores	Globular collembola	4.55	7.04	0.37	0.45	−1.06	1.80	0.47	0.67	0.59	1.19	0.31
Detritivores	Oribatid mites	4.05	3.44	−0.13	0.27	−1.00	0.74	0.67	0.37	0.76	1.11	0.77
Herbivores	Corn leaf aphid	0.91	0.57	−0.20	0.20	−0.84	0.45	0.40	0.02	0.17	0.37	0.14
Herbivores	Leafhoppers	28.87	25.80	−0.11	0.27	−0.98	0.76	0.72	0.46	0.57	2.74	1.00
Herbivores	Thrips	75.31	61.64	−0.20	0.22	−0.90	0.51	0.44	0.04	0.96	3.65	1.00
Parasitoids	Parasitic hymenoptera	17.22	15.93	−0.07	0.34	−1.15	1.00	0.84	0.26	1.00	2.26	0.98
Predators	All spiders	4.60	5.53	0.15	0.32	−0.87	1.18	0.67	0.44	0.67	1.19	0.68
Predators	Ground beetle adults	3.48	5.59	0.39	0.30	−0.56	1.34	0.29	0.01	0.70	1.01	0.57
Predators	Ground beetle larvae	0.55	0.89	0.20	0.29	−0.71	1.11	0.54	0.38	0.50	0.24	0.05
Predators	Lacewing adults	0.06	0.00	−0.06	0.06	−0.24	0.13	0.39	0.40	0.40	0.03	0.04
Predators	Lacewing eggs	4.41	4.44	0.00	0.22	−0.70	0.71	0.98	0.12	0.65	1.16	0.93
Predators	Lacewing larvae	0.06	0.00	−0.06	0.06	−0.24	0.13	0.39	0.40	0.40	0.03	0.04
Predators	Ladybird beelte adults	1.17	1.18	0.00	0.29	−0.93	0.94	0.99	0.85	0.66	0.46	0.10
Predators	Ladybird beetle egg clutches	0.19	0.35	0.13	0.18	−0.43	0.68	0.53	0.18	0.51	0.09	0.04
Predators	Ladybird beetle eggs	0.73	0.99	0.14	0.49	−1.41	1.69	0.79	0.10	0.74	0.31	0.04
Predators	Ladybird beetle larvae	0.89	1.57	0.31	0.40	−0.98	1.60	0.50	0.03	0.79	0.37	0.05
Predators	Insidious flower bug (*Orius*)	9.31	8.92	−0.04	0.25	−0.82	0.74	0.88	0.19	0.50	1.73	0.98
Predators	Rove beetle adult (pitfall)	1.48	0.84	−0.30	0.36	−1.45	0.85	0.47	0.88	0.56	0.55	0.10
Predators	Rove beetle larvae (pitfall)	0.00	0.17	0.15	0.07	−0.06	0.37	0.11	0.04	0.04	0.00	0.03
Predators	Rove beetles adults (sticky)	0.26	0.63	0.26	0.18	−0.31	0.83	0.24	0.70	0.52	0.12	0.04

Lower and upper confidence intervals (CIs) are 95% confidence intervals about the difference of the mean counts for *Bt* minus the mean counts for non-*Bt;* intervals that contain zero indicate there are no significant differences between the non-*Bt* and *Bt* mean counts. For the power analyses, delta corresponds to the 50% change in the control mean and is one half of the mean counts for the non-*Bt* group transformed to log(50% counts + 1). Power describes the probability of detecting a 50% change (or larger) when in fact it occurs.

erally had higher abundance in 2005 in the Cry1F maize treatment than the untreated control.

Individual Taxa Analyses: By-year Analyses. Summaries of the statistical analyses for each taxon monitored by visual observations, sticky cards, and pitfall traps (by year) are provided in Tables 4–6. There were no statistically significant treatment main effects detected for any of the monitored taxa between the Cry1F maize hybrids and the isogenic controls in the single year analyses. Investigation of the treatment by stage interaction by individual years showed no statistically significant effects in any taxon with the exception of rove beetle larvae (pitfall) in 2005 ($P = 0.04$), where abundance was greater in the Cry1F maize hybrids. Average counts for rove beetles in 2005 were very low (mean count of less than one organism per plot) in both treatments, and this result does not seem to be biologically significant. Significant sampling stage effects were noted in some of the monitored taxa when studied by year, particularly thrips, which had a significant sampling stage main effect in all 3 yr of study. Examination of the data showed that approximately two to three times more thrips were caught on sticky traps at the vegetative stage sampling period than were caught at the R1 and R3 stage.

Summaries of the statistical analyses for each taxon monitored by litterbags (by year) are provided in Tables 7–9. There were no statistically significant treatment main effects or treatment by stage interactions detected for any of the monitored taxa between the Cry1F maize hybrids and the isogenic controls in the single year analyses. Significant sampling stage effects were noted for several of the monitored taxa, reflecting the increase in colonization of the litterbags that were left in the field until crop maturity.

Meta-analysis. A summary of the meta-analysis of visual observations, sticky card, and pitfall trap abundance data across all 3 yr of study is provided in Table 10. There were no statistically significant treatment main effects detected for any of the monitored taxa between the Cry1F maize hybrids and the isogenic controls in the meta-analysis. There were no statistically significant treatment interactions (treatment × stage, treatment × year, or treatment × stage × year) detected between the Cry1F maize hybrids and the isogenic controls. Statistically significant sampling stage effects were detected in the herbivorous taxa, the parasitoid taxon, and five of the monitored predators. Investigation of the predator data showed that ladybird beetle larvae, ladybird beetle adults, and *Orius* counts were very low at the first (v-stage) sampling and increased substantially through the R1 and R3 sampling dates. Conversely, ground beetle adult and spider numbers were higher in the early sampling stage (v-stage) and lower at the last sampling stage (R3). This may relate biologically to the abundance trends of the herbivorous taxa monitored in this study. Ground and plant-dwelling predators like ground beetles and spiders were in greater numbers during the sampling stage where thrips and leafhopper abundance was highest (v-stage, with diminishing abundance through the R1 and R3 sam-

Table 6. Statistical summary of individual taxon abundance data 2006 (Nebraska, Iowa, Indiana, and Wisconsin) as monitored by visual surveillance, sticky cards, and pitfall traps

Functional group	Taxon	Mean counts per plot						*P* values			Power analyses	
		Non-*Bt*	*Bt*	Diff	SE diff	Lower CI	Upper CI	Treatment	Stage	Treatment × stage	Delta	Power
Predators/ detritivores	Centipedes and millipedes	0.38	0.35	−0.02	0.22	−0.73	0.68	0.92	0.50	0.59	0.17	0.05
Detritivores	Elongated collembola	14.42	18.25	0.22	0.23	−0.52	0.96	0.41	0.28	0.67	2.11	1.00
Detritivores	Globular collembola	3.59	3.54	−0.01	0.21	−0.69	0.67	0.97	0.02	0.78	1.03	0.90
Detritivores	Oribatid mites	3.22	4.90	0.33	0.30	−0.62	1.29	0.35	0.84	0.74	0.96	0.50
Herbivores	Corn leaf aphid	0.93	1.52	0.26	0.32	−0.77	1.30	0.47	0.94	0.99	0.38	0.07
Herbivores	Leafhoppers	12.09	12.63	0.04	0.22	−0.67	0.75	0.87	0.03	0.98	1.95	0.99
Herbivores	Thrips	19.62	19.40	−0.01	0.21	−0.68	0.66	0.96	0.03	0.94	2.38	1.00
Parasitoids	Parasitic hymenoptera	5.99	4.53	−0.23	0.34	−1.33	0.86	0.54	0.16	0.63	1.39	0.77
Predators	All spiders	9.13	8.02	−0.12	0.31	−1.12	0.88	0.73	0.00	0.55	1.72	0.95
Predators	Ground beetle adults	10.61	12.31	0.14	0.41	−1.16	1.43	0.76	0.16	0.84	1.84	0.86
Predators	Ground beetle larvae	0.16	0.12	−0.03	0.13	−0.44	0.38	0.81	0.36	0.93	0.08	0.04
Predators	Lacewing adults	0.46	0.35	−0.08	0.20	−0.71	0.55	0.71	0.24	0.60	0.21	0.06
Predators	Lacewing eggs	7.28	11.59	0.42	0.43	−0.96	1.80	0.40	0.13	0.60	1.53	0.63
Predators	Lacewing larvae	0.23	0.86	0.41	0.31	−0.58	1.41	0.28	0.45	0.99	0.11	0.03
Predators	Ladybird beetle adults	2.14	2.32	0.05	0.32	−0.98	1.09	0.88	0.08	0.71	0.73	0.21
Predators	Ladybird beetle egg clutches	0.62	0.77	0.09	0.30	−0.86	1.05	0.78	0.51	0.95	0.27	0.05
Predators	Ladybird beetle eggs	1.98	2.03	0.02	0.58	−1.83	1.86	0.98	0.60	0.79	0.69	0.07
Predators	Ladybird beetle larvae	1.41	2.35	0.33	0.55	−1.42	2.08	0.59	0.10	0.72	0.53	0.06
Predators	Insidious flower bug (*Orius*)	3.92	3.73	−0.04	0.28	−0.92	0.84	0.90	0.11	0.95	1.09	0.75
Predators	Rove beetle adult (pitfall)	0.94	1.56	0.28	0.34	−0.81	1.36	0.47	0.08	0.63	0.38	0.07
Predators	Rove beetle larvae (pitfall)	0.33	0.43	0.07	0.19	−0.54	0.68	0.75	0.13	0.88	0.15	0.05
Predators	Rove beetles adults (sticky)	0.79	0.20	−0.40	0.26	−1.22	0.42	0.22	0.42	0.74	0.33	0.08

Lower and upper confidence intervals (CIs) are 95% confidence intervals about the difference of the mean counts for *Bt* minus the mean counts for non-*Bt;* intervals that contain zero indicate there are no significant differences between the non-*Bt* and *Bt* mean counts. For the power analyses, delta corresponds to the 50% change in the control mean and is one half of the mean counts for the non-*Bt* group transformed to log(50% counts + 1). Power describes the probability of detecting a 50% change (or larger) when in fact it occurs.

pling stages). Likewise, aphid predators such as ladybird beetles and *Orius* increased in density over sampling stages as did aphid abundance. Some of these predators are also facultative pollen feeders, which also may explain their increased numbers during the R1 and R3 sampling stages. Ladybird beetle larvae also showed a significant year effect and sampling stage × year interaction, driven by high larval abundance in the R1 and R3 sampling stages in 2004. Ladybird beetle larvae counts were consistently lower in 2005 and 2006.

A summary of the meta-analysis of litterbag abundance data across all 3 yr of study is provided in Table 11. There were no statistically significant treatment main effects detected for any of the monitored taxa between the Cry1F maize hybrids and

Table 7. Statistical summary of individual taxon abundance data 2004 (Nebraska, Iowa, Indiana, and Wisconsin) as monitored by litterbags

Functional group	Taxon	Mean counts per plot						*P* values			Power analyses	
		Non-*Bt*	*Bt*	Diff	SE diff	Lower CI	Upper CI	Treatment	Stage	Treatment × stage	Delta	Power
Predators/ detritivores	Centipedes and millipedes	1.68	1.36	−0.13	0.26	−0.96	0.70	0.66	0.01	0.92	0.61	0.23
Detritivores	Elongated collembola	559.90	616.89	0.10	0.57	−1.72	1.91	0.88	0.17	0.62	5.64	1.00
Detritivores	Globular collembola	12.15	12.14	0.00	0.35	−1.13	1.13	1.00	0.04	0.57	1.96	0.95
Detritivores	Oribatid mites	120.92	139.04	0.14	0.37	−1.03	1.30	0.73	0.02	0.80	4.12	1.00
Predators	Wolf spiders	5.09	6.90	0.26	0.19	−0.35	0.87	0.27	0.60	0.69	1.27	0.98
Predators	Other spiders	3.20	3.84	0.14	0.26	−0.68	0.96	0.62	0.03	0.92	0.96	0.68
Predators	All spiders	24.57	37.19	0.40	0.28	−0.49	1.29	0.25	0.02	0.71	2.59	1.00
Predators	Rove beetle adults	8.33	9.42	0.11	0.40	−1.17	1.40	0.80	0.98	0.73	1.64	0.78
Predators	Rove beetle larvae	6.43	3.89	−0.42	0.42	−1.76	0.93	0.40	0.17	0.45	1.44	0.58
Predators and herbivores	Ground beetle adults	12.29	12.07	−0.02	0.35	−1.15	1.11	0.97	0.48	0.76	1.97	0.95
Predators and herbivores	Ground beetle larvae	15.58	15.74	0.01	0.96	−3.06	3.08	0.99	0.66	0.99	2.17	0.21

Lower and upper confidence intervals (CIs) are 95% confidence intervals about the difference of the mean counts for *Bt* minus the mean counts for non-*Bt;* intervals that contain zero indicate there are no significant differences between the non-*Bt* and *Bt* mean counts. For the power analyses, delta corresponds to the 50% change in the control mean and is one half of the mean counts for the non-*Bt* group transformed to log(50% counts + 1). Power describes the probability of detecting a 50% change (or larger) when in fact it occurs.

Table 8. Statistical summary of individual taxon abundance data 2005 (Nebraska, Iowa, Indiana, and Wisconsin) as monitored by litterbags

Functional group	Taxon	Mean counts per plot						*P* values			Power analyses	
		Non-*Bt*	*Bt*	Diff	SE diff	Lower CI	Upper CI	Treatment	Stage	Treatment × stage	Delta	Power
Predators/ detritivores	Centipedes and millipedes	6.48	8.54	0.24	0.49	−1.30	1.79	0.65	0.21	0.29	1.44	0.42
Detritivores	Elongated collembola	647.35	773.56	0.18	0.44	−1.21	1.56	0.71	0.00	0.81	5.78	1.00
Detritivores	Globular collembola	12.16	25.43	0.70	0.73	−1.62	3.02	0.41	0.02	0.92	1.96	0.33
Detritivores	Oribatid mites	117.34	136.13	0.15	0.53	−1.53	1.82	0.80	0.03	0.58	4.09	0.99
Predators	Wolf spiders	2.74	3.03	0.08	0.29	−0.85	1.00	0.81	0.52	0.39	0.86	0.42
Predators	Other spiders	34.83	40.80	0.15	0.24	−0.59	0.90	0.56	0.00	0.91	2.91	1.00
Predators	All spiders	39.45	45.81	0.15	0.27	−0.72	1.01	0.63	0.01	0.86	3.03	1.00
Predators	Rove beetle adults	18.16	18.49	0.02	0.46	−1.46	1.49	0.97	0.01	0.93	2.31	0.91
Predators	Rove beetle larvae	4.05	7.32	0.50	0.61	−1.45	2.45	0.48	0.46	0.44	1.11	0.13
Predators and herbivores	Ground beetle adults	13.86	30.16	0.74	0.58	−1.11	2.59	0.29	0.00	0.57	2.07	0.64
Predators and herbivores	Ground beetle larvae	21.04	35.79	0.51	0.70	−1.73	2.75	0.52	0.76	0.62	2.44	0.60

Lower and upper confidence intervals (CIs) are 95% confidence intervals about the difference of the mean counts for *Bt* minus the mean counts for non-*Bt;* intervals that contain zero indicate there are no significant differences between the non-*Bt* and *Bt* mean counts. For the power analyses, delta corresponds to the 50% change in the control mean and is one half of the mean counts for the non-*Bt* group transformed to log(50% counts + 1). Power describes the probability of detecting a 50% change (or larger) when in fact it occurs.

the isogenic controls in the meta-analysis. There were no statistically significant treatment interactions (treatment × stage, treatment × year, or treatment × stage × year) detected between the Cry1F maize hybrids and the isogenic controls. Significant sampling stage effects were noted for many of the taxa, driven primarily by the increase in litterbag colonization over time.

Discussion

Experimental Design. The experimental design of this study evaluated paired fields at four distinct geographies over 3 yr of study. A perceived weakness is the lack of replication at each location. However, it was considered more important to sample in larger fields to eliminate potential effects of neighboring plots that may occur in smaller research plot settings. This design has been used successfully in other field monitoring studies (Perry et al. 2003, Torres and Ruberson 2005), although the scale of treatments (e.g., independent fields) is debatable and likely influenced by geography and the biology of the taxa of interest (Duffield and Aebischer 1994, Kennedy et al. 2001, Perry et al. 2003). Treating location as a random effect assumes the locations

Table 9. Statistical summary of individual taxon abundance data 2006 (Nebraska, Iowa, Indiana, and Wisconsin) as monitored by litterbags

Functional group	Taxon	Mean counts per plot						*P* values			Power analyses	
		Non-*Bt*	*Bt*	Diff	SE diff	Lower CI	Upper CI	Treatment	Stage	Treatment × stage	Delta	Power
Predators/ detritivores	Centipedes and millipedes	4.26	4.21	−0.01	0.31	−1.00	0.98	0.98	0.00	0.43	1.14	0.67
Detritivores	Elongated collembola	157.95	144.38	−0.09	0.18	−0.68	0.50	0.66	0.10	0.80	4.38	1.00
Detritivores	Globular collembola	4.93	6.11	0.18	0.22	−0.51	0.88	0.47	0.05	0.77	1.24	0.96
Detritivores	Oribatid mites	60.33	47.03	−0.24	0.37	−1.43	0.94	0.56	0.32	0.75	3.44	1.00
Predators	Wolf spiders	4.77	6.44	0.25	0.32	−0.75	1.26	0.48	0.95	0.96	1.22	0.73
Predators	Other spiders	101.57	77.73	−0.26	0.26	−1.39	0.86	0.42	0.04	0.73	3.95	1.00
Predators	All spiders	38.11	36.93	−0.03	0.25	−0.81	0.75	0.91	0.01	0.47	3.00	1.00
Predators	Rove beetle adults	13.99	12.34	−0.12	0.17	−0.66	0.43	0.55	0.07	0.96	2.08	1.00
Predators	Rove beetle larvae	4.73	5.59	0.14	0.55	−1.60	1.88	0.82	0.20	0.88	1.21	0.20
Predators and herbivores	Ground beetle adults	5.28	6.08	0.12	0.37	−1.06	1.30	0.77	0.02	0.75	1.29	0.61
Predators and herbivores	Ground beetle larvae	11.10	10.78	−0.03	0.43	−1.39	1.34	0.96	0.02	0.85	1.88	0.84

Lower and upper confidence intervals (CIs) are 95% confidence intervals about the difference of the mean counts for *Bt* minus the mean counts for non-*Bt;* intervals that contain zero indicate there are no significant differences between the non-*Bt* and *Bt* mean counts. For the power analyses, delta corresponds to the 50% change in the control mean and is one half of the mean counts for the non-*Bt* group transformed to log(50% counts + 1). Power describes the probability of detecting a 50% change (or larger) when in fact it occurs.

Table 10. Statistical summary of individual taxon abundance data 2004–2006 (Nebraska, Iowa, Indiana, and Wisconsin) as monitored by visual surveillance, sticky cards, and pitfall traps

Functional group	Taxon	Mean counts per plot						*P* values							Power analyses	
		Non-*Bt*	*Bt*	Diff	SE Diff	Lower CI	Upper CI	Treatment	Stage	Treatment × stage	Year	Treatment × year	Stage × year	Treatment × stage × year	Delta	Power
Predators/ detritivores	Centipedes and millipedes	0.13	0.27	0.11	0.10	−0.11	0.33	0.29	0.33	0.90	0.14	0.16	0.90	0.90	0.07	0.07
Detritivores	Elongated collembola	17.22	26.35	0.41	0.24	−0.13	0.94	0.12	0.23	0.96	0.91	0.36	0.84	0.96	2.26	1.00
Detritivores	Globular collembola	3.65	4.56	0.18	0.27	−0.44	0.80	0.53	0.22	0.62	0.88	0.86	0.43	0.62	1.04	0.92
Detritivores	Oribatid mites	3.92	4.60	0.13	0.18	−0.27	0.53	0.48	0.45	0.82	0.90	0.59	0.10	0.82	1.08	1.00
Herbivores	Corn leaf aphid	1.10	1.13	0.02	0.16	−0.34	0.38	0.92	0.01	0.73	0.82	0.52	0.39	0.73	0.44	0.68
Herbivores	Leafhoppers	18.23	17.17	−0.06	0.14	−0.38	0.27	0.70	0.02	0.62	0.80	0.89	0.01	0.62	2.31	1.00
Herbivores	Thrips	25.12	24.11	−0.04	0.14	−0.35	0.27	0.78	0.00	0.88	0.24	0.69	0.14	0.88	2.61	1.00
Parasitoids	Parasitic hymenoptera	8.76	8.07	−0.07	0.19	−0.50	0.35	0.70	0.00	0.62	0.64	0.79	0.02	0.62	1.68	1.00
Predators	All spiders	9.73	12.70	0.24	0.20	−0.22	0.71	0.26	0.00	0.80	0.11	0.28	0.26	0.80	1.77	1.00
Predators	Ground beetle adults	8.17	9.19	0.10	0.20	−0.35	0.56	0.61	0.00	0.90	0.24	0.51	0.10	0.90	1.63	1.00
Predators	Ground beetle larvae	0.24	0.39	0.11	0.14	−0.20	0.42	0.44	0.24	0.29	0.47	0.75	0.82	0.29	0.11	0.09
Predators	Lacewing adults	0.19	0.19	0.00	0.08	−0.19	0.19	1.00	0.13	0.81	0.16	0.53	0.40	0.81	0.09	0.14
Predators	Lacewing eggs	4.67	5.34	0.11	0.17	−0.28	0.50	0.53	0.14	0.95	0.69	0.47	0.08	0.95	1.20	1.00
Predators	Lacewing larvae	0.11	0.28	0.14	0.11	−0.11	0.39	0.24	0.36	0.86	0.12	0.25	0.54	0.86	0.06	0.06
Predators	Ladybird beetle adults	2.29	2.41	0.04	0.18	−0.38	0.45	0.85	0.02	0.78	0.15	0.99	0.62	0.78	0.76	0.95
Predators	Ladybird beetle egg clutches	0.34	0.50	0.11	0.14	−0.21	0.43	0.45	0.06	0.68	0.25	0.99	1.00	0.68	0.16	0.14
Predators	Ladybird beetle eggs	1.01	1.55	0.24	0.32	−0.48	0.96	0.47	0.15	0.45	0.61	0.77	0.56	0.45	0.41	0.18
Predators	Ladybird beetle larvae	2.84	3.88	0.24	0.23	−0.29	0.77	0.33	0.00	0.85	0.00	0.89	0.00	0.85	0.88	0.92
Predators	Insidious flower bug (*Orius*)	5.23	4.64	−0.10	0.14	−0.42	0.22	0.49	0.00	0.93	0.71	0.83	0.34	0.93	1.29	1.00
Predators	Rove beetle adult (pitfall)	1.76	1.72	−0.02	0.23	−0.53	0.50	0.94	0.08	0.96	0.17	0.61	0.34	0.96	0.63	0.69
Predators	Rove beetle larvae (pitfall)	0.30	0.36	0.05	0.10	−0.18	0.27	0.65	0.46	0.42	0.56	0.62	0.07	0.42	0.14	0.21
Predators	Rove beetles adults (sticky)	0.51	0.37	−0.10	0.12	−0.36	0.16	0.41	0.54	0.86	0.98	0.12	0.71	0.86	0.23	0.38

Lower and upper confidence intervals (CIs) are 95% confidence intervals about the difference of the mean counts for *Bt* minus the mean counts for non-*Bt;* intervals that contain zero indicate there are no significant differences between the non-*Bt* and *Bt* mean counts. For the power analyses, delta corresponds to the 50% change in the control mean and is one half of the mean counts for the non-*Bt* group transformed to log(50% counts + 1). Power describes the probability of detecting a 50% change (or larger) when in fact it occurs.

Table 11. Statistical summary of individual taxon abundance data 2004–2006 (Nebraska, Iowa, Indiana, and Wisconsin) as monitored by litterbags

Functional group	Taxon	Mean counts per plot						*P* values							Power analyses	
		Non-*Bt*	*Bt*	Diff	SE diff	Lower CI	Upper CI	Treatment	Stage	Treatment × stage	Year	Treatment × year	Stage × year	Treatment × stage × year	Delta	Power
Predators/detritivores	Centipedes and millipedes	3.72	3.89	0.04	0.20	−0.43	0.50	0.86	0.00	0.55	0.35	0.76	0.53	0.55	1.05	0.99
Detritivores	Elongated collembola	385.65	410.30	0.06	0.25	−0.50	0.62	0.81	0.00	0.75	0.62	0.90	0.03	0.75	5.27	1.00
Detritivores	Globular collembola	9.08	12.51	0.29	0.28	−0.34	0.93	0.32	0.00	0.93	0.85	0.59	0.03	0.93	1.71	1.00
Detritivores	Oribatid mites	95.00	96.34	0.01	0.25	−0.55	0.58	0.96	0.00	0.50	0.70	0.77	0.22	0.50	3.88	1.00
Predators	Wolf spiders	4.08	5.19	0.20	0.16	−0.16	0.55	0.24	0.50	0.66	0.88	0.86	0.90	0.66	1.11	1.00
Predators	Other spiders	23.89	24.15	0.01	0.15	−0.33	0.35	0.95	0.00	0.83	0.07	0.49	0.27	0.83	2.56	1.00
Predators	All spiders	33.33	39.77	0.17	0.15	−0.17	0.52	0.29	0.00	0.78	0.96	0.54	0.66	0.78	2.87	1.00
Predators	Rove beetle adults	12.89	12.94	0.00	0.19	−0.43	0.44	0.98	0.01	0.85	0.61	0.89	0.05	0.85	2.01	1.00
Predators	Rove beetle larvae	4.99	5.45	0.07	0.30	−0.61	0.76	0.81	0.43	0.45	1.00	0.49	0.19	0.45	1.25	0.95
Predators and herbivores	Ground beetle adults	9.75	13.24	0.28	0.24	−0.27	0.83	0.28	0.00	0.82	0.61	0.43	0.06	0.82	1.77	1.00
Predators and herbivores	Ground beetle larvae	15.41	18.36	0.17	0.42	−0.79	1.12	0.71	0.13	0.84	0.73	0.85	0.53	0.84	2.16	0.99

Lower and upper confidence intervals (CIs) are 95% confidence intervals about the difference of the mean counts for *Bt* minus the mean counts for non-*Bt*; intervals that contain zero indicate there are no significant differences between the non-*Bt* and *Bt* mean counts. For the power analyses, delta corresponds to the 50% change in the control mean and is one half of the mean counts for the non-*Bt* group transformed to log(50% counts + 1). Power describes the probability of detecting a 50% change (or larger) when in fact it occurs.

sampled are representative of U.S. Corn Belt maize growing environments.

The statistical power to detect a 50% change in taxa abundance is included in the "by year" and "across years" summary tables (Tables 4–11). When considered over sampling stages and all years of the study, analyses of taxa with high abundance such as elongate collembola, mites, leafhoppers, thrips, parasitic hymentoptera, spiders, ground beetles, lacewing eggs, and *Orius* had very little probability of a type II error. Other taxa such as globular collembola, ladybird beetle adults, and larvae also had high power values, indicating little chance of erroneously failing to reject the null hypothesis. As might be expected, those taxa with the lowest abundance (centipedes/millipedes, ground beetle larvae, lacewing larvae, ladybird beetle eggs, and rove beetles) had the lowest statistical power in the study. However, the 95% confidence intervals reported in the tables are for the differences of the control and *Bt* means on the transformed scale, all of which contain zero, indicating there are no significant differences between the control and *Bt* mean counts.

Taxa Grouping and Abundance. Because laboratory safety testing does not suggest Cry1F protein has broad spectrum insecticidal activity (U.S. EPA 2001), these field monitoring studies were designed to conduct general surveillance of nontarget arthropod abundance. The selected taxa and subsequent groupings were based on their abundance in corn (Bitzer et al. 2005, Dively 2005, Prasifka et al. 2005, Rose and Dively 2007) and their functional roles to serve as indicators of treatment effects. Although the taxa groupings in this study likely would not detect impacts on individual species, monitoring the abundance of functional groups or family-level monitoring can be an effective means of assessing impacts of insecticidal compounds (Bitzer et al. 2005; Dively 2005, Rose and Dively 2007).

The results of this study are consistent with other field studies evaluating the nontarget effects of Cry1 proteins in maize. Field studies such as Dively (2005) and Daley and Buntin (2005) evaluated maize containing Cry1Ab in field plots using similar nontarget collection techniques (e.g., plant evaluations, sticky traps, pitfall traps) and found few differences between *Bt* and non-*Bt* plots. The differences that were noted were attributed to indirect causes such as control of target pests that serve as hosts or prey. This corroborates the conclusions of Pilcher et al. (2005), where no significant differences in generalist predators were noted between *Bt* and non-*Bt* plots; however, a reduction in *Macrocentrus cingulum*, a specialist parasitoid of European corn borer, was noted in *Bt* plots. Meta analyses conducted on *Bt* maize containing Cry1 proteins such as those reported in Marvier et al. (2007) and Wolfenbarger et al. (2008) also support the conclusion that Cry1 activity is limited to selected lepidopteran insects, and impacts to nontarget organisms are largely attributable to reductions in host/prey.

In summary, the multiple test locations used in this study are representative of the major maize cultiva-

tion regions of the United States. These studies were conducted in commercial scale agricultural environments over 3 yr using commercially available Cry1F maize hybrids. Nontarget arthropod abundance showed similar dynamics between maize fields containing Cry1F maize and non-*Bt* isogenic maize hybrids. No differences were detected at either the community level or by individual taxa. The probability of detecting at least a 50% impact in abundance was high for most monitored taxa, especially when considered over all 3 yr of monitoring. Results of these field studies support the hypothesis that maize hybrids containing Cry1F protein are not expected to negatively impact nontarget arthropods. These field studies also confirm the expectation from laboratory experiments that no significant undesirable effects on nontarget arthropods are expected from the cultivation of event DAS-Ø15Ø7–1 Cry1F maize hybrids.

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