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A 3-year field-scale monitoring of foliage-dwelling spiders (Araneae) in transgenic *Bt* maize fields and adjacent field margins

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Abstract

Concerns have been raised that genetically modified Bt maize may harm non-target organisms, and there is a general call and need for a risk assessment of Bt maize. Spiders are important pest predators in agroecosystems and in maize, and can be exposed to the Bttoxin by herbivorous or pollen-collecting prey, by active Bt maize pollen feeding, and by ingesting their pollen-dusted webs. The foliage-dwelling spider fauna of Bt maize fields and adjacent margins was monitored and compared to non-transgenic maize fields. The study took place during the vegetation seasons of 2001–2003 in Bavaria, South Germany. Maize fields and adjacent nettle field margins were colonized by a typical spider assemblage, dominated by space-web spiders (Theridiidae and Linyphiidae). Abundance and species richness of spiders was higher in nettle margins than in maize fields. The proportion of hunting spiders tended to be higher in nettle margins, whereas space-web spiders tended to be more frequent in maize fields. Bt maize showed no consistent effect on individual numbers, species richness and guild structure of spiders in maize fields and adjacent nettle field margin strips. The spider abundance was higher in Bt treatments in 2003, whereas in 2001 and 2002 no significant differences were found. The results provide an important contribution for the implementation of case-specific and general surveillance of transgenic plants to be employed due to the regulations of the European Community.

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1. Introduction

Genetically modified *Bt* maize commercially available in Europe expresses the activated and truncated protein Cry1Ab of the insect pathogen *Bacillus thuringiensis*. Cry1Ab is toxic for Lepidoptera and thus *Bt* maize is described as being protected specifically and effectively against lepidopteran pests such as the European corn borer Ostrinia nubilalis (Hübner) (Lepidoptera, Crambidae) (Gill et al., 1992; Burkness et al., 2001). Due to its specificity, the *Bt* toxin has been considered as relatively safe for non-target organisms (Glare and O'Callaghan, 2000), but some adverse effects on non-targets have been reported (e.g. Hilbeck et al., 1998; Losey et al., 1999; Felke et al., 2002). As the area being cropped with Bt maize is increasing rapidly worldwide (James, 2003), there is a general need and call for an assessment of possible environmental effects on non-target organisms associated with the commercial cultivation of transgenic crops in the field (e.g. European Parliament and Council, 2001; Züghart and Breckling, 2003). Relevant indicator species to be evaluated should be selected based on the exposure of species to the transgenic product, the degree of the adverse effect of the transgenic product, the economic importance of species, the ecological and functional role of the species, and

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on biomass or abundance of species in the field (Jepson et al., 1994; Dutton et al., 2003; Andow and Hilbeck, 2004).

In Europe, spiders are prominent invertebrate predators in agroecosystems showing high population densities and species richness in arable land in general (Samu and Szinetár, 2002), and belong to the most abundant arthropod predators in maize fields in particular (e.g. Katz, 1993; Lang et al., 1999; Albajes et al., 2003). Spiders are a very diverse group with different lifestyles, and feed on a wide variety of prey including most pest species (Marc et al., 1999; Nyffeler, 1999). Hence, they play a vital role in agroecosystems as predators, and so are of economic value due to their pest control function in various crops including maize (e.g. Marc and Canard, 1997; Lang et al., 1999). Further, spiders are among the first predators arriving in newly established crop habitats and thus provide an early season protection against pests (Bishop and Riechert, 1990). Spiders are potentially exposed to the Cry1Ab toxin of Bt maize in various ways: Spiders may actively forage for the maize pollen (Vogelei and Greissl, 1989; Ludy, 2004). Spiders may consume maize pollen indirectly when recycling their pollen-dusted webs (Smith and Mommsen, 1984). Spiders may ingest maize pollen when feeding on prey which has collected or consumed pollen, or is dusted with it (Gregory, 1989). Spiders prey on large quantities of herbivores (Nyffeler, 1999), and herbivores take up Bt toxin when feeding on Bt maize tissue and can pass it on to their predators (Dutton et al., 2002). Not only spiders within the maize field are potentially influenced via these pathways, but also populations occurring in field margins along the maize fields may be affected, in particular by wind drifted pollen, and by herbivorous and pollen collecting prey. Despite their ecological significance and potential exposure to Cry1Ab toxin of Bt maize, studies considering the effect of *Bt* maize on spiders are limited in number and scope (Hassell and Shepard, 2002; Jasinski et al., 2003; Volkmar and Freier, 2003; Candolfi et al., 2004; Meissle and Lang, 2005; Poza et al., 2005).

A number of studies in peer-reviewed journals have evaluated the effect of *Bt* maize on arthropod non-target communities in the field (Orr and Landis, 1997; Pilcher et al., 1997; Wold et al., 2001; Bourguet et al., 2002; Hassell and Shepard, 2002; Musser and Shelton, 2003; Jasinski et al., 2003; Pons and Starý, 2003; Volkmar and Freier, 2003; Candolfi et al., 2004; Lumbierres et al., 2004; Tóth et al., 2004; Meissle and Lang, 2005; Pons et al., 2005; Poza et al., 2005). Three of these included the effect on spiders on species level (Volkmar and Freier, 2003; Candolfi et al., 2004; Meissle and Lang, 2005). Out of these, two studies were conducted in fields of a commercial size (Volkmar and Freier, 2003; Candolfi et al., 2004) and only one study lasted longer than one season (Volkmar and Freier, 2003).

Our study adds to the available work as it provides data on the effect of *Bt* maize over a three year period with an exclusive focus on spider communities both within the crop, as well as in adjacent vegetation.

2. Materials and methods

2.1. Study sites

The study was carried out on three research farms located in Swabia, Frankonia and Upper Bavaria (South Germany) during the seasons 2001-2003. At each experimental site, a pair of Bt maize and conventional maize fields was established each two hectares large the fields being apart between 500 and 1000 m. For the *Bt* maize the event 176 "Navares" was cropped, and for control the nearisogenic variety "Antares" (both from Syngenta). On average, ten maize plants covered one square meter (distance between maize rows was 75 cm, and 15 cm between single maize plants within a row). Herbicides but no insecticides were applied once or twice in May. Field margin strips $(50 \times 7 \text{ m})$ were established on the northern edge of each maize field. Each margin included a plot of stinging nettles (Urtica dioica Linné), which was used for the survey of spiders in field margins. Stinging nettles were chosen, because these plants are abundant in agricultural landscapes and grow often along field margins. The nettle plot measured 10×6 m in 2001, and 18×7 m in both 2002 and 2003. In 2001, 40 nettle shrubs were planted in each plot (about 0.6 shrubs per m²), and in 2002/03 400 shrubs per plot (about 3 shrubs per m²). In 2001 and 2002 nettle shrubs were obtained from local field populations nearby, and were then planted in the concerning plots of each nettle field margin strip. In 2003, nettles were first sown and reared in the glasshouse and then planted in the margin strips. Nettles were regularly supplied with water and fertilized with nitrogen to secure proper growth.

2.2. Sampling dates and sampling methods

Foliage-dwelling spiders were recorded with a suction sampler, which was a modified small vacuum cleaner with a suction hole opening area of 3.0×0.6 cm (Ludy and Lang, 2004). Both maize and nettle plants were sampled from the top to the bottom by holding and moving the suction sampler directly on the plant. In 2001, the mean suction time was 35 s per plant (both maize plant and nettle), and in 2002/03 suction time was increased to 2 min per plant.

In maize fields, 10 maize plants were selected randomly per field and sampling occasion in 2001, and spiders on these plants were collected. This resulted in an overall number of 150 sampled maize plants per *Bt* maize field or conventional maize field, respectively (3 sites × 10 plants per field × 5 sampling dates). The sampled maize plants had at least a distance of 20 m to the field edge. In 2002 and 2003, each maize field was divided into 10 subplots each consisting of 50 maize plants (at least 20 m distance to field edge). On each sampling occasion, one maize plant per subplot was chosen randomly resulting in an overall number of 150 (2002) or 120 (2003) sampled maize plants per *Bt* maize field or conventional field, respectively (3 sites × 10 subplots × 1 maize plant per plot × 4–5 sampling dates). In nettle field margins, 10 nettle shrubs were selected randomly per margin strip and sampling occasion in 2001, and spiders on these plants were sampled. This resulted in an overall number of 150 sampled nettles per margin neighboring *Bt* maize fields or conventional maize fields, respectively (3 sites \times 10 nettles per margin \times 5 sampling dates). In 2002 and 2003, each nettle plot was divided into eight subplots (each 4.5 \times 3.5 m). On each sampling occasion, one nettle shrub per subplot was chosen randomly resulting in an overall number of 120 (2002) or 96 (2003) sampled nettles per margin neighboring *Bt* maize fields or conventional fields, respectively (3 sites \times 8 subplots \times 1 nettle shrub per subplot \times 4–5 sampling dates). For further statistical analysis average values were calculated per maize field and nettle field margin, respectively.

2.3. Identification of spiders

Sampled spiders were fixed in 70% ethanol, brought to the laboratory and identified according to Heimer and Nentwig (1991) and Roberts (1985, 1987, 1995). Species were classified according to Platnick (2005). Juvenile spiders were identified to genus or family level, if possible. Additionally, the recorded spiders were divided in three main guilds (after Nyffeler, 1982): space-web spiders (Dictynidae, Theridiidae, and Linyphiidae), orb-web spiders (Araneidae and Tetragnathidae), and hunting spiders (Lycosidae, Pisauridae, Miturgidae, Corinnidae, Anyphaenidae, Clubionidae, Philodromidae, Thomisidae, and Salticidae).

2.4. Statistical analyses

A repeated measures analysis of variance (ANOVA) was used to analyze the effect of the factor "Bt-status" (i.e. "Bt maize" and "conventional maize") on the dependent variable "number of individuals per plant" (means per sampling date), including the factor "sampling date" (four sampling dates, analysis 1). The first sampling date in 2001 and 2002 was not included in the repeated measures ANOVA, because this analysis required an identical number of sampling dates in all years. For analyzing species richness, an analysis of covariance (ANCOVA) was conducted with the dependent variable "total number of species per plot" (seasonal sums; analysis 2) and the covariate "number of individuals per plot." The covariate "number of individuals per plot" was included in order to correct a potential effect of spider abundance on species richness. A multivariate analysis of variance (MANOVA) was conducted to analyze a possible effect of "Bt-status" on the composition of spider guilds (proportions of three different spider guilds per plot; seasonal sums of guilds; analysis 3). Subsequently, one-way ANOVA was conducted to specify effects on different spider guilds. Additionally, the factors "year" (2001-2003) and "habitat type" ("maize field" and "field margin") were included in all analyses to detect potential interactions with the main factor "Bt-status."

To test the homogeneity of variances, Sen and Puris non-parametric test was conducted. Kolmogorov–Smirnov one-sample test was used for testing the normal distribution of data. Spider abundance and species number of analyses 1 and 2 were $\log x + 1$ transformed and guild proportions of analysis 3 were arcsin-transformed to create normal distribution and/or homogeneity of variance of the data set. Post hoc comparisons were conducted with the least significance differences (LSD) test.

Standardised effect sizes, Cohen's d (Cohen, 1988), were calculated together with the corresponding 95% confidence intervals of d for pair wise comparisons of dependent variables. Effect size d is a dimensionless measurement of the magnitude of an effect recorded and allows the comparison of effects among different results and studies, hence facilitating meta-analysis (Colegrave and Ruxton, 2003; Nakagawa and Foster, 2004). A SPSS script written by Smithson (2001) was used to calculate d and non-central confidence intervals on base of the observed value of a t-statistic of the concerned treatment comparison. The SPSS script can also be downloaded from the internet at http://www.anu.edu.au/ psychology/people/smithson/details/CIstuff/CI.html (October 19, 2005). All other statistical analyses were carried out using STATISTICA for Windows, version 5.0. All average values presented are arithmetic means ± 1 SD and all tests are two-sided.



Fig. 1. Proportion of spider guilds in *Bt* and control maize fields (A) and adjacent nettle field margins (B) of three years (seasonal sums + SD), n = 3 each column.

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3. Results

3.1. The spider community of maize fields and adjacent field margin strips

Overall, 50 foliage-dwelling spider species and 1811 individuals were recorded in three years in both maize fields and nettle field margins (see Appendix A). Generally, spaceweb spiders (Linyphiidae and Theridiidae) dominated the spider community in both habitat types (Fig. 1). The most abundant species in both habitat types were *Theridion impressum* L. Koch (Theridiidae, space-web spiders), *Meioneta rurestris* (C.L. Koch), and *Oedothorax apicatus* (Blackwall) (both Linyphiidae, space-web spiders).

In maize fields, a total of 33 spider species and 868 individuals were found (see Appendix A). The average spider density pooled over three years was 1.05 ± 0.65 spiders per maize plant. *Erigone atra* Blackwall was often recorded in maize fields. In nettle margin strips, 44 species and 943 individuals were found (see Appendix A). The average spider density over three years was 1.39 ± 0.93 spiders per stinging nettle shrub. *Tenuiphantes tenuis* (Blackwall) (Linyphiidae, space-web spiders), *Aculepeira ceropegia* (Walckenear) (Araneidae, orb-web spiders), *Pachygnatha degeeri* Sundevall (Tetragnathidae, orb-web spiders), and *Ebrechtella tricuspidata* (Fabricius) (Thomisidae, hunting spiders) were often recorded in nettle margins.

The spider abundance and the species richness were higher in margins than in maize fields (abundance: Table 1a, factor "habitat type," Fig. 2; species richness: Table 1b, factor "habitat type," Fig. 3). The covariate "number of individuals" had a positive effect on the spider species richness (F = 5.45; dfs = 1,12; P = 0.03).

The relative abundance of the different spider guilds changed from year to year and habitat type tended to affect guild proportions (Table 1c, factors "year" and "habitat type," Fig. 1). In 2001 and 2002, the proportion of space web spiders was lower (ANOVA: factor "year," F=8.89; dfs=2,24; P<0.01; LSD test: P<0.05 each comparison) and the proportion of orb web spiders was higher than in 2003 (ANOVA: factor "year," F=11.98; dfs=2,24; P<0.001;

Table 1

Repeated measures ANOVA on the number of spider individuals (a), ANCOVA on the number of spider species (b), and MANOVA on proportion of guilds (c) for the effect of year, habitat type and *Bt*-status

Source of variation	df	MS	F	Р
(a) Number of spider individuals				
Year	2	1.20	65.42	< 0.01
Habitat type	1	0.10	5.29	0.03
Bt-status	1	0.01	0.29	0.59
Year \times habitat type	2	0.03	1.82	0.18
Year \times <i>Bt</i> -status	2	0.06	3.26	0.05
Habitat type \times <i>Bt</i> -status	1	0.02	1.35	0.26
Year \times habitat type \times <i>Bt</i> -status	2	0.02	0.82	0.45
Error	24	0.02		
Sampling date	3	0.10	10.44	< 0.01
Sampling date × year	6	0.03	3.11	0.01
Sampling date \times habitat type	3	0.01	1.01	0.39
Sampling date \times <i>Bt</i> -status	3	0.00	0.35	0.79
Sampling date \times year \times habitat type	6	0.02	1.79	0.11
Sampling date \times year \times <i>Bt</i> -status	6	0.01	0.63	0.70
Sampling date \times habitat type \times <i>Bt</i> -status	3	0.01	0.77	0.51
Sampling date \times year \times habitat type \times <i>Bt</i> -status	6	0.01	1.10	0.37
Error	72	0.03		
(b) Number of spider species				
Year	2	0.03	4.69	0.02
Habitat type	1	0.16	24.15	< 0.01
Bt-status	1	0.01	1.43	0.24
Year \times habitat type	2	0.02	2.96	0.07
Year \times <i>Bt</i> -status	2	0.01	1.33	0.28
Habitat type \times <i>Bt</i> -status	1	0.00	0.32	0.58
Year \times habitat type \times <i>Bt</i> -status	2	0.01	0.86	0.44
Error	23	0.01		
(c) Proportion of spider guilds	df	Wilk's Λ	$F(\text{Rao's } \mathbf{R})$	Р
Year	6,44	0.39	4.36	< 0.01
Habitat type	3,22	0.71	2.92	0.06
Bt-status	3,22	0.85	1.31	0.29
Year \times habitat type	6,44	0.63	1.91	0.09
Year $\times Bt$ -status	6,44	0.79	0.90	0.50
Habitat type $\times Bt$ -status	3,22	0.80	1.84	0.17
Year \times habitat type \times <i>Bt</i> -status	6,44	0.88	0.49	0.81



Fig. 2. Number of spider individuals per plant recorded in Bt and control maize fields and adjacent nettle field margins of three years (seasonal means + SD), n = 3 each column.



Fig. 3. Number of spider species per plot recorded in Bt and control maize fields and adjacent nettle field margins of three years (seasonal sums + SD), n = 3 each column.

LSD test: P < 0.05 each comparison). The proportion of hunting spiders did not change over the years (ANOVA: factor "year," P > 0.05, Fig. 1). The proportions of space-web spiders tended to be higher in maize fields than in nettle field margins, whereas hunting spiders seemed to be more frequent in nettle margins, and proportions of orb-web spiders appeared to be higher in maize fields in 2003 (Fig. 1).

The numbers of spider individuals changed over the season in both habitat types during all years (Table 1a, factor "sampling date," Fig. 4). In general, spider populations increased by the end of July or the beginning of August, and declined by the end of the season. Spider densities were also different between years, with the lowest densities in 2001 and the highest in 2002 (Table 1a, factor "year," Fig. 2).

3.2. Effect of Bt maize on foliage-dwelling spiders

A total of 24 spider species and 478 individuals were recorded in *Bt* maize fields, and in conventional maize fields 26 species and 390 individuals (see Appendix A). The overall frequency of the different spider guilds were $69 \pm 12\%$

space-web spiders, $23 \pm 8\%$ orb-web spiders, and $8 \pm 8\%$ hunting spiders in *Bt* maize fields. The corresponding values of conventional maize fields were $71 \pm 11\%$ space-web spiders, $23 \pm 8\%$ orb-web spiders, and $6 \pm 4\%$ hunting spiders (Fig. 1).

A total of 36 spider species and 427 individuals were caught in nettle margin strips neighbouring *Bt* maize fields, while in nettle strips neighbouring conventional maize fields 35 spider species and 516 individuals were recorded (see Appendix A). In nettle margins neighbouring *Bt* fields, the overall frequency of the different spider guilds were $62 \pm 14\%$ space-web spiders, $27 \pm 13\%$ orb-web spiders, and $11 \pm 6\%$ hunting spiders. The corresponding values of nettle margins along conventional maize fields were $66 \pm 12\%$ space-web spiders, $17 \pm 12\%$ orb-web spiders, and $17 \pm 12\%$ hunting spiders (Fig. 1). The proportion of guilds never differed between *Bt* maize fields and conventional maize fields or between corresponding neighbouring nettle margin strips, respectively (Table 1c, factor "*Bt*-status," Fig. 1).

There was a trend that the effect of Bt maize on spider densities was different in the successive years (Table 1a, interaction "year × Bt-status"). In 2003, spider numbers were higher in Bt maize fields than in conventional maize fields, whereas this difference was not recorded between Bt nettle margins and non-Bt nettle margins (LSD-test: P < 0.05, Table 2a, Fig. 2). Sampling dates within one year had no significant interaction with the Bt treatment in both habitat types (Table 1a, interaction "sampling date × Bt-status," Fig. 4).

In all years, ANCOVA revealed no effect of Bt-status on species number (Table 1b, factor "Bt-status," Fig. 3). However, in 2003 species number was higher in Bt maize fields as compared to control maize fields (LSD-test: P < 0.01, Table 2b), which was related to the increased abundance of spiders in Bt fields.

Observed standardised effect sizes (Cohen's d) and the corresponding 95% confidence intervals for pair wise comparisons (LSD test) of each dependent variable are shown in Table 2. The majority of the observed differences were rather small indicating that possible effects may be of minor magnitude. Medium to large effect sizes (Cohen's $d \ge 0.50$, sensu Cohen, 1988) were recorded for Bt maize fields for a decrease in abundance in 2001, and in 2003 for an increase of both abundance and species richness. In nettle margins along Bt maize fields, effect sizes were medium to large for a decrease in species richness in 2001, increased proportions of orb-web spiders (2002, 2003) and decreased proportions of hunters (2003). The proportions of space-web spiders showed the strongest effect for decreases in Bt nettle field margins in 2002 and in Bt maize fields in 2003 (Table 2).

4. Discussion

4.1. The spider community of maize fields and adjacent margins

Baseline data about spiders in maize field are very scarce, and refer mainly to activity densities (and not pop-



Fig. 4. Seasonal dynamics of spider individuals in Bt- and control maize fields (A, C, and E) and in adjacent nettle field margins (B, D, and F) of three years (means per sampling date), n = 3 each point. Note different ranges of y-axes. The first sampling dates in 2001 and 2002 were not included in the statistical analysis.

ulation densities) of ground-dwelling species (e.g. Alderweireldt, 1989; Frank and Nentwig, 1995). In all years (2001–2003), the foliage-dwelling spider fauna in maize fields and adjacent nettle field margins strips mainly consisted of space-web spiders (Theridiidae and Linyphiidae). The dominance pattern of spider families and species of this three-year study was quite similar to two one-year studies of maize fields in 2001 (Ludy and Lang, 2004; Meissle and Lang, 2005). This indicates that a typical and relatively steady spider community exists in maize fields in terms of prevailing species and families, which is not prone to high year-to-year changes. This is further corroborated by other studies also reporting the dominance of species of Theridiidae and Linyphiidae in higher strata in maize fields and on field margins (maize fields: Nyffeler and Benz, 1979; Katz, 1993; margin strips: Wyss, 1996; Denys and Tscharntke, 2002). Species number of foliage-dwelling spiders was within the range expected for habitats in arable

land (Luczak, 1979; Barthel, 1997). Generally, more species were found in nettle margins strips, which was mainly due to a higher species number of orb-web spiders and hunting spiders (see also Ludy and Lang, 2004). The dominance of space-web spiders in maize fields and artificial sown nettle field margins could be due to the good aerial dispersal ability (ballooning) of these spiders (e.g. Plagens, 1986). Both habitat types, maize fields and sown nettle margin strips, were habitats created anew each year. So, most spiders had to immigrate into these habitats each season, and spider families such as Linyphiidae and Theridiidae, which frequently disperse by ballooning, have an advantage in the colonization of newly created habitats compared to ground dispersal spiders (Bishop and Riechert, 1990; Frank and Nentwig, 1995). Population densities of foliage-dwelling spiders were fairly higher than detected in some other studies (Nyffeler and Benz, 1979; Barthel, 1997), and peaked in August.

Table 2

Significances (*P*), observed (d obs.) and standardised effect sizes (Cohen's *d*) with the corresponding 95% confidence intervals for Cohen's *d* (95% CI) for pair wise comparisons (LSD test) of the dependent variables "number of spider individuals" (a), "number of spider species" (b) and "proportion of spider guilds" (c) in *Bt* and control plots of different habitat types and during the years 2001-2003

Dependent variable	Habitat type	Р	d obs.	Cohen's d	95% CI
(a) Number of spider individuals					
2001					
Control-Bt	Maize field	0.29	0.21	0.50	-0.40 to 1.34
Control-Bt	Nettle field margin	0.50	0.14	0.30	-0.54 to 1.11
2002					
Control-Bt	Maize field	0.64	-0.07	0.20	-0.62 to 1.00
Control-Bt	Nettle field margin	0.54	0.62	0.27	-0.56 to 1.08
2003					
Control-Bt	Maize field	0.01	-0.79	1.76	0.32 to 3.15
Control- <i>Bt</i>	Nettle field margin	0.59	-0.18	0.24	-0.59 to 1.04
(b) Number of spider species 2001					
Control-Bt	Maize field	0.69	-0.33	0.17	-0.64 to 0.97
Control-Bt	Nettle field margin	0.26	2.00	0.53	-0.37 to 1.38
2002	c				
Control-Bt	Maize field	0.59	1.00	0.23	-0.60 to 1.03
Control-Bt	Nettle field margin	0.92	0.67	0.04	-0.76 to 0.84
2003	-				
Control-Bt	Maize field	< 0.01	-3.00	1.94	0.40 to 3.44
Control-Bt	Nettle field margin	0.15	-2.67	0.71	-0.25 to 1.61
(c) Proportion of spider guilds 2001					
Control- <i>Bt</i> Hunters	Maize field	0.76	-2.39	0.13	-0.68 to 0.93
Control-Bt Orb-web spiders	Maize field	0.98	-0.13	0.07	-0.28 to 0.28
Control-Bt Space-web spiders	Maize field	0.76	2.52	0.13	-0.68 to 0.93
Control-Bt Hunters	Nettle field margin	0.35	7.58	0.43	-0.45 to 1.25
Control-Bt Orb-web spiders	Nettle field margin	0.47	-5.66	0.33	-0.52 to 1.14
Control-Bt Space-web spiders	Nettle field margin	0.74	-1.92	0.14	-0.67 to 0.94
2002	-				
Control-Bt Hunters	Maize field	0.92	0.76	0.04	-0.76 to 0.84
Control-Bt Orb-web spiders	Maize field	0.60	4.31	0.23	-0.60 to 1.03
Control-Bt Space-web spiders	Maize field	0.36	-5.07	0.42	-0.45 to 1.25
Control-Bt Hunters	Nettle field margin	0.98	-0.13	0.01	-0.24 to 0.25
Control-Bt Orb-web spiders	Nettle field margin	0.15	-11.25	0.73	-0.24 to 1.63
Control-Bt Space-web spiders	Nettle field margin	0.08	11.38	0.93	-0.12 to 1.90
2003					
Control-Bt Hunters	Maize field	0.83	-1.72	0.09	-0.72 to 0.89
Control-Bt Orb-web spiders	Maize field	0.58	-4.48	0.24	-0.59 to 1.05
Control-Bt Space-web spiders	Maize field	0.17	6.21	0.67	-0.28 to 1.55
Control-Bt Hunters	Nettle field margin	0.26	9.21	0.53	-0.37 to 1.38
Control-Bt Orb-web spiders	Nettle field margin	0.19	-10.85	0.64	-0.30 to 1.52
Control-Bt Space-web spiders	Nettle field margin	0.74	1.64	0.14	-0.67 to 0.94

4.2. Effect of Bt maize on the spider community

The three-year study showed inconsistent effects of Bt maize on population densities of foliage-dwelling spiders. There was no negative impact detected of Bt corn on spiders in the field, neither on population densities, species numbers or guild proportions. The only significant result was an increase of spider abundance in Bt maize fields in 2003. However, a direct positive effect of the Cry1Ab protein itself on invertebrate predators is unknown, and can probably be ruled out as an explanation. Possibly characteristics of the Bt maize associated with the transformation of the Bt gene may be responsible for effects on non-target organisms. The transformation of maize with new genes can lead to pleiotropic effects, i.e., may

alter the physiological parameters of the transformed plants in addition to the introduced genetic construct (Saxena and Stotzky, 2001). For instance, *Bt* maize is often larger in height and green for longer than the nearisogenic variety, and may also have a differential plant development rate (Hassell and Shepard, 2002; Ma and Subedi, 2005). Also, *Bt* maize plants are not attacked by lepidopteran pests, and therefore *Bt* maize plants stay undamaged. In consequence, *Bt* maize may harbor more non-target herbivores as spider prey later in the season, which may also lead to a higher spider abundance. Possibly, this plays a role under dry climatic conditions such as during the exceptionally hot summer in 2003, where *Bt* maize plants had less dry leaves than conventional maize plants (Lang, unpublished data). Hence, the higher spider numbers in 2003 may have been mediated by plant characteristics rather than by the *Bt* construct itself.

In 2001 spider abundance was decreased in Bt maize (medium effect size, sensu Cohen, 1988), but not significantly (P > 0.05). The lack of a significant result of Bt maize may indicate that there is no effect, or that the effect was masked by interfering factors, or could not be detected due to inadequate methods, design or statistical power (Marvier, 2002). The fact that consumption of Bt maize pollen seems not to harm garden spiders (Araneus diadematus Clerck) supports the no-effect interpretation (Ludy and Lang, 2006). However, it is acknowledged that with a sample size of three fields and margins resulting statistical power of the tests was relatively small in this study. Several other field studies also found no or no consistent effect of Bt maize on invertebrate predators, e.g. on spiders, anthocorid bugs and coccinellid beetles (Orr and Landis, 1997; Pilcher et al., 1997; Wold et al., 2001; Bourguet et al., 2002; Hassell and Shepard, 2002; Musser and Shelton, 2003; Jasinski et al., 2003; Volkmar and Freier, 2003; Meissle and Lang, 2005; Poza et al., 2005). Comparably to our study, high variation of these data sets as well as small effect sizes and/or low replication

may have been responsible for missing an existing direct or indirect effect (cf. Bourguet et al., 2002; Perry et al., 2003; Lang, 2004). Therefore, future field studies should be conducted on longer temporal and larger spatial scales and in higher replication. In addition, laboratory experiments are needed to clarify direct and indirect field effects of the Cry1Ab protein on spiders.

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Appendix A

Summary of spiders captured in Bt maize fields and control maize fields as well as in adjacent nettle margin strips (3 years *4-5 sampling dates *3 locations *10 maize plants or 8-10 stinging nettle shrubs, respectively)

Habitat types	Maize		Field margin	
Bt-status	Bt	Control	Bt	Control
Species				
Araneae				
Unidentified	1	1		1
Theridiidae				
Achaearanea spec.	2	2	3	2
Achaearanea riparia (Blackwall, 1834)			1	1
Enoplognatha spec.	1			
Enoplognatha latimana Hippa and Oksala, 1982			1	1
Episinus spec.		1	1	2
Episinus angulatus (Blackwall, 1836)				1
Neottiura bimaculata (Linneus, 1767)		1		
Robertus neglectus (O. PCambridge, 1871)		1		
Theridion spec.	1		2	4
Theridion impressum L. Koch, 1881	2	4	4	74
Juveniles	216	135	74	110
Linyphiidae				
Araeoncus humilis (Blackwall, 1841)	1			1
Bathyphantes gracilis (Blackwall, 1841)			1	
Diplocephalus cristatus (Blackwall, 1833)		1		
Diplostyla concolor (Wider, 1834)			1	
Eperigone trilobata (Emerton, 1882)			1	1
Erigone atra Blackwall, 1833	15	3	3	3
Erigone dentipalpis (Wider, 1834)	5		3	2
Linyphia triangularis (Clerck, 1757)		1		
Meioneta spec.		2	2	3
Meioneta fuscipalpa (C.L. Koch, 1836)				1
Meioneta rurestris (C.L. Koch, 1836)	4	5	14	7
Microlinyphia spec.		1	7	3
Microlinyphia pusilla (Sundevall, 1830)		1	3	
			(cont	inued on next page

Appendix A (continued)

Habitat types	Maize		Field margin	
<i>Bt</i> -status	Bt	Control	Bt	Control
Neriene spec. Oedothorax apicatus (Blackwall, 1850) Oedothorax fuscus (Blackwall, 1834)	3 15	8 30	4 18 1	3 22
Porrhomma microphthalmum (O.PCambridge, 1871) Porrhomma oblitum (O. PCambridge, 1871) Tenuiphantes tenuis (Blackwall, 1852) Juveniles	3 82	1 1 76	1 7 102	1 4 100
Tetragnathidae Pachygnatha spec. Pachygnatha degeeri Sundevall, 1830 Tetragnatha spec.	2 1 61	2 58	5 8 78	2 13 52
Araneidae Aculepeira ceropegia (Walckenaer, 1802) Araniella spec. Araniella curcubitina (Clerck, 1757) Argiope bruennichi (Scopoli, 1772) Cyclosa conica (Pallas, 1772)	1 8 1	1 5	9 7 2	4 3 1
Cyclosa oculata (Walckenaer, 1802) Larinioides spec. Larinioides cf. cornutus (Clerck, 1757) Nuctenea spec.	63	1 1	3 1	10 1
Singa spec. Juveniles	1 19	26	20	3 27
Lycosidae Pardosa spec. Pardosa agrestris (Westring, 1861)	5	5	8	10 1
Pisauridae Pisaura mirabilis (Clerck, 1757)			1	6
Dictynidae Dictyna spec. Nigma spec.	1			1 1
Miturgidae Cheiracanthium spec.			2	1
Anyphaenidae Anyphaena accentuata (Walckenaer, 1802)				1
Clubionidae Clubiona spec. Clubiona terrestris Westring, 1851	3 1	2	2	1 1
Corinnidae Phrurolithus spec.			1	
Gnaphosidae <i>Micaria</i> spec. Juveniles			2	2 2
Philodromidae <i>Philodromus</i> spec. <i>Tibellus</i> spec. <i>Tibellus oblongus</i> (Walckenaer, 1802)	I	3	3 1	3
Thomisidae <i>Ebrechtella tricuspidata</i> (Fabricius, 1775) <i>Ozyptila</i> spec. <i>Xysticus</i> spec. Juveniles	2 11	1 7 2	8 7 1	4 1 15 1
Salticidae Euophrys spec. Heliophanus spec. Phlegra spec. Juveniles		1	1	1

Appendix A (continued)

Habitat types	Maize	Maize		Field margin	
<i>Bt</i> -status	Bt	Control	Bt	Control	
Individual number	478	390	427	516	
Species number	24	26	36	35	

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