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Comparing methods to evaluate the effects of *Bt* maize and insecticide on spider assemblages

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Abstract

In a field study, potential adverse effects of *Bt* maize on plant-dwelling spiders (Araneae) were assessed in 2001 in Bavaria, South Germany. Spider assemblages were recorded in *Bt* maize fields and conventional maize fields, with and without (pyrethroid) insecticide application. In addition, the efficacy of several sampling techniques to collect plant-dwelling spiders was tested (beating sheets, suction sampling, plant removal, stem eclectors). A total of 29 species and 14 families were identified. Juvenile spiders of the families Theridiidae, Linyphiidae, Tetragnathidae and Araneidae dominated the catch. The sampling methods differed in their capture efficiency with regard to abundance, family composition, species richness and power to detect effects. Suction samplers performed best, and are recommended for monitoring plant-dwelling spiders in maize. *Bt* maize had no substantial effects on species richness and abundance of spiders, whereas insecticide application reduced spider densities.

Keywords: Araneae; *Bacillus thuringiensis*; General surveillance; Non-target arthropods; Pyrethroid; Risk assessment; Sampling techniques; Transgenic crop

1. Introduction

The European corn borer *Ostrinia nubilalis* Hübner (Lepidoptera) is a major pest of maize worldwide (Krattinger, 1997). Chemical insecticides, such as pyrethroids, are widely used to control this and other pests in conventional agriculture. In organic farming, formulations containing Cry1A(b) protein of *Bacillus thuringiensis* (*Bt*) ssp. *kurstaki* de Barjac & Lemille, toxic to lepidopteran larvae, have been applied successfully for several decades (Glare and O'Callaghan, 2000). As a new means of crop protection, genetically modified (GM) maize expressing the Cry1A(b) toxin (Koziel et al., 1993) has been made available commercially in several countries (James, 2002). However, many countries (e.g., the EU member states) are still in the

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assessment and decision process whether or not *Bt* maize and other GM crops should be cropped on a large scale.

Plant protection methods usually not only reduce the target pest, but influence the community of non-target organisms in a direct or indirect way. Chemical broad-spectrum insecticides have severe effects on many groups of non-target arthropods, including spiders (e.g., Krause et al., 1993; Reed et al., 2001; Candolfi et al., 2004; Duan et al., 2004). On the other hand, *Bt* sprays are widely considered as safe for spiders and other beneficial insects (e.g., Glare and O'Callaghan, 2000; Reed et al., 2001; Duan et al., 2004). However, sprayed insecticides remain on the plant surface and have a temporarily limited range of action, as they are sensitive to UV radiation and washed off by rainfall. In contrast, *Bt* maize expresses the toxin over the whole growing season in all green tissues and pollen. A truncated, partly activated form of the toxin is produced, shortcutting the activation process necessary for toxicity of Cry proteins in *Bt* formulations (Koziel et al., 1993). Furthermore, the expressed Cry1A(b) protein might be altered by the complex of plant enzymes. Thus, *Bt* sprays cannot be compared directly to *Bt* crops and a wider range of effects is potentially possible.

Generalist predators and especially spiders belong to the most abundant invertebrate predators in agroecosystems and play an important role in biological pest control in many crops including maize (e.g., Lang et al., 1999; Marc et al., 1999; Symondson et al., 2002; Lang, 2003). Thus, any negative effect on spider populations has potential consequences for biological control. Spiders are likely to be exposed to Cry1A(b) toxin in *Bt* maize fields over the whole season as they are known to prey on herbivores (Nyffeler, 1999; Kiss et al., 2003), and herbivores feeding on *Bt* maize tissue are likely to ingest and pass on the toxin to predators (e.g., Dutton et al., 2002). Furthermore, spiders are likely to be exposed especially during anthesis in *Bt* maize cultivars expressing high toxin concentrations in pollen such as event 176 (Fearing et al., 1997; Lang et al., 2004). Spiders may forage for pollen actively (Vogelei and Greissl, 1989; Ludy, 2004), ingest pollen when recycling their webs (Smith and Mommsen, 1984), or when their prey has collected or consumed pollen or is dusted with it (Gregory, 1989).

In ecological studies and concurrent risk evaluations of *Bt* maize concerning spiders, there is a lack of data with regard to three points: (1) baseline data of spider assemblages in European maize fields (Nyffeler and Sunderland, 2003), (2) effects of *Bt* plants on spider assemblages in relation to common plant protection practice and (3) knowledge of adequate sampling methods for plant-dwelling spiders in maize. All three aspects are fundamental for a risk assessment of and monitoring scheme for present and future cultivars of transgenic maize in Europe. In tiered risk assessment procedures suggested by Poppy and Sutherland (2004) and Dutton et al. (2003), laboratory and semi-field studies are performed to assess potential hazard and exposure for certain indicator species. Additionally, field experiments are important to detect consequences of new GM crops in the environment (Poppy and Sutherland, 2004; Andow and Hilbeck, 2004). Comparing side effects of a new transgenic cultivar to common agricultural practice should be the basis for a decision whether it is an improvement or not (Hails, 2000).

After a GM crop has passed the pre-market risk assessment, regulators usually demand general surveillance over several years accompanying commercial production to ensure long-term environmental safety and sustainability (e.g., EU Directive 2001/18/EC, European Parliament and Council, 2001). Suitable and adequate sampling methods are a requirement for a quantitative and standardized survey of non-target species compositions. Four common methods for sampling plant-dwelling spiders in the field were therefore compared, i.e., stem eclector, beating sheet, suction sampling and plant removal.

The present study aimed to assess and compare the potential effect of *Bt* maize on plant-dwelling spider assemblages during one season in *Bt* maize to those in untreated conventional maize and to pyrethroid treated plots and addressed the following questions: How is the plant-dwelling spider community of maize fields composed? Does the community of plant-dwelling spiders differ between insecticide treated and untreated plots? Does the community of plant-dwelling spiders differ between *Bt* maize fields and fields of a control line? Is the effect of *Bt* maize different from the effect of insecticide treatment? Which is the most efficient method for monitoring potential effects on plant-dwelling spiders?

2. Material and methods

The study was carried out between 1 July and 25 September 2001 in maize fields of four state research farms in Bavaria, South Germany. The farms were situated between 366 and 550 m a.s.l., the mean temperature at 2 m above ground during the experimental period was between 15.9 and 16.4 °C and the average rainfall ranged from 224 to 316 mm. The fields were managed, fertilized, herbicide treated and sown according to standard practice (sowing end of April/beginning of May, herbicide treatment approximately 3 weeks later, harvest in October).

2.1. Treatments

On each farm, a maize field was divided into four 30 m × 50 m plots. Four treatments were applied to the plots in factorial combinations, i.e., *Bt* maize (transgenic variety Navares, event 176) with or without insecticide, and conventional maize (variety Antares, near-isoline to Navares) with or without insecticide. Each field was surrounded by at least 7 m wide strips of other maize cultivars. Insecticide treated plots were sprayed once with the pyrethroid Baythroid® with 750 ml active ingredient (Cyfluthrin) per ha between 6 July and 12 July. The density of maize plants was ca. 10 plants/m^2.

2.2. Sampling

Each sampling method was applied to 10 maize plants on each sampling occasion and plot, and the catch from these plants was pooled separately for each method, plot and sampling date. Each plant was sampled only once, except for stem eclectors, which remained on the same plants.

2.2.1. Stem eclector

Transparent plastic funnels (8 cm diameter) were clipped to maize stems in a way to embrace the stalk completely and attached with adhesive tape. Transparent 100 ml polyethylene flasks fitting into the funnel pipes were filled with 5% acetic acid and attached to the funnels to form a stem trap based on the principle of a pitfall trap (Barber, 1931). The flasks were emptied every fortnight and the catch was transferred into 70% ethanol. The trapping fluid was renewed on each occasion.

2.2.2. Plant removal

Individual maize plants were bent and placed into a large plastic bag. After the stem was cut just above the ground, the bag was closed tightly. The collected plants were stored at 5 °C in the laboratory until examination a few days later. Plastic bags were opened over a white tray filled with water. After shaking out the bag, the plant was cut into pieces. Pieces and water were carefully searched for spiders, which were transferred into ethanol.

2.2.3. Beating sheet

Single plants were beaten for 2.0 ± 0.4 min (mean ± S.D., $n = 29$) with a stick. Spiders dropping from the plant were caught on a round cotton sheet (surface area: 0.44 m^2) and transferred into ethanol.

2.2.4. Suction sampling

A small hand-held vacuum sampler was used (modified 3.6 V rechargeable hoover) to vacuum single maize plants thoroughly for 2.3 ± 0.3 min ($n = 29$), starting with the bottom leaves, and then the inflorescence, upper leaves and the rest of the plant. The suction device was emptied onto a cotton sheet and captured spiders were transferred into ethanol.

The maize fields were sampled on six occasions during the season, i.e., on 4–17 July, 18–30 July, 1–10 August, 14–23 August, 28 August–7 September and 11–25 September 2001. The first sampling was done before insecticide application and maize anthesis, just as the plants were in the BBCH-growth stage 5 (‘‘tassel emergence’’; Meier, 1997). There was no stem eclector catch for the first sampling date, because the traps were installed only then. The second sampling was conducted after insecticide application and during anthesis. The last sampling occurred shortly before harvest (growth stage 8 or 9, ‘‘ripening’’ or ‘‘senescence’’). Sampling took place at least 10 m from plot edges on plants selected at random, while assuring that the different sampling methods were interspersed over the plot, thus spreading possible spatial environmental heterogeneity evenly among methods.

2.3. Data analyses

Spiders were identified to species or to the highest possible taxonomic category according to Heimer and

Nentwig (1991), Roberts (1993, 1995), Nentwig et al. (2002) and Platnick (2002). In analysing species and genus richness, the highest possible number of species and genera was considered. For instance, if only the genus of a collected spider was known, it was counted as a further species in case no other species of the concerned genus was recorded.

For comparing the efficacy of the methods, the different sites served as replicates ($n = 4$). For each site, the spider catches were pooled over all sampling occasions and treatments for each sampling method. Numbers and proportions of species, genera and families were evaluated by a one-way ANOVA followed by Tukey's HSD multiple comparison post hoc test.

A repeated measurement ANOVA (RM ANOVA) was used to test for differences in the main factors *Bt* status (yes/no) and insecticide application (yes/no) and for any interactions between factors and sampling date. In the RM ANOVA, the factor site could be not considered due to a lack of degrees of freedom. In order to account for site effects and to test single date $\times$ treatment interactions, a univariate ANOVA was applied to abundance data for each sampling date and method separately, including site as a third, random factor.

The number of species, genera and families collected with each method was analyzed for the first sampling occasion and for pooled data from the second to the last sampling by univariate ANOVA (fixed factors insecticide and *Bt*, random factor site).

If necessary, data were log 10 or $\log 10(x + 1)$ transformed prior to analyses to normalise distributions of standardized residuals and to ensure the homogeneity of variances. Proportions were ln(arcsine($x + 0.1$)) transformed. All analyses were performed using Statistica (Version 6.1, Statsoft Inc., Tulsa, USA, 2003). Average values presented are arithmetic means $\pm$ 1S.E. of those means, and tests are given with two-tailed probabilities.

The sample size necessary to detect the impact of *Bt* maize on spider abundance was calculated for the two most efficient sampling methods (GPower 2.0; Faul and Erdfelder, 1992) based on hypothetical effects ranging from 10 to 90% difference between *Bt* and untreated control fields. It was assumed that data were normally distributed, sample size equal for both types of fields, and a *t*-test for two independent samples was applied on differences between two treatments, the test being two-sided with a significance level $\alpha = 0.05$, and the power 20, 40, 60 or 80% (i.e., the probability of rejecting the null hypothesis at a given α). The basis of the sample size calculation for each method was the standard deviation (S.D.) of the mean spider abundance of the four untreated conventional maize plots (maize variety "Antares", without insecticides) with spider abundances of sampling occasions pooled for each site.

3. Results

A total of 29 species were identified in 32 genera and 14 families (Table 1). In terms of ecological guilds (Uetz et al., 1999), most spiders were web builders (95.5%). Space web builders (Theridiidae, Dictynidae) dominated (52.9%), followed by orb weavers (Araneidae and Tetragnathidae, 25.5%) and tangle weavers (Linyphiidae, 17.1%). Theridiidae (52.8% of all individuals) dominated the catches, followed by Tetragnathidae (18.6%), Linyphiidae (17.1%) and Araneidae (6.8%). The proportion of adult spiders was 240 out of a total of 2357 individuals (10.2%).

3.1. Comparison of sampling methods

The four methods differed significantly in collection efficiency (one-way ANOVA, d.f. = 3, $F = 43.507$, sum of squares (SS) = 1.925, $p < 0.001$, Fig. 1). Stem eclectors and plant removal recovered less spiders than beating sheet and suction sampling (Tukey's HSD, $p < 0.01$). Fewer spiders were recorded in the stem traps than with plant removal ($p = 0.051$), beating sheets collecting nearly as many spiders as suction sampling. Fewer species and genera were recorded by stem eclectors than by suction sampling (Tukey's HSD, $p < 0.05$), all other differences among methods being not significant.

Different proportions of Araneidae, Linyphiidae, Theridiidae and others were caught by the four methods (one-way ANOVA, d.f. = 3, $p < 0.01$, Fig. 2; Araneidae: $F = 11.326$, SS = 2.784; Linyphiidae: $F = 24.727$, SS = 5.032; Theridiidae: $F = 40.794$, SS = 15.690; others: $F = 23.871$, SS = 14.965).

Table 1
Spiders recorded in Bavarian maize fields in 2001 (4 sites × 4 plots × 10 plants × 5–6 samplings)

Family	Species	Number of individuals recorded with			
		Stem eclector	Beeting sheet	Suction sampling	Plant removal
Anyphaenidae	*Anyphaena accentuata* (Walckenaer)	1	1	1	
Clubionidae	*Clubiona lutescens* Westring		1		
	Gen. sp.		1		7
Lycosidae	*Pardosa* sp.	1			
	Gen. sp.	2			
Gnaphosidae	Gen. sp.				1
Salticidae	*Evarcha arcuata* (Clerck)			1	
	Synageles venator (Lucas)	1			
Philodromidae	*Philodromus cespitum* (Walckenaer)			1	
Thomisidae	Gen. sp.	1	6	16	10
Pisauridae	*Pisaura mirabilis* (Clerck)		1	2	1
Linyphiidae	*Araeoncus humilis* (Blackwall)	3	1	3	1
	Bathyphantes gracilis (Blackwall)	1	1		
	Erigone atra Blackwall	5	7	11	8
	Erigone dentipalpis (Wider)		5	3	4
	Floronia sp.		1		
	Meioneta rurestris (C.L. Koch)	1	1	8	2
	Microlinyphia pusilla (Sundevall)		1	2	1
	Microlinyphia sp.			2	
	Oedothorax apicatus (Blackwall)	22	6	34	19
	Porrhomma microphthalmum (O.P.-Cambridge)	7	2	1	
	Porrhomma oblitum (O.P.-Cambridge)			1	
	Porrhomma sp.	2			
	Tenuiphantes mengei (Kulczynski)		1		
	Tenuiphantes tenuis (Blackwall)			6	
	Tenuiphantes sp.	1	3	6	2
	Gen. sp.	20	65	102	31
Araneidae	*Aculepeira ceropegia* (Walckenaer)	1	5	11	5
	Araniella sp.		5	5	7
	Cyclosa conica (Pallas)		5	3	1
	Cyclosa oculata (Walckenaer)				1
	Larinioides sp.				1
	Mangora acalypha (Walckenaer)		1		
	Gen. sp.	3	49	43	15
Tetragnathidae	*Metellina segmentata* (Clerck)			1	
	Metellina sp.		1	1	
	Pachygnatha degeeri Sundevall	5	4	3	6
	Pachygnatha sp.		1	1	3
	Tetragnatha sp.		5		
	cf. *Tetragnatha* sp.	25	177	164	42
Uloboridae	*Hyptiotes paradoxus* (C.L. Koch)				1
Theridiidae	*Achaearanea riparia* (Blackwall)			1	
	Achaearanea sp.		1	4	2
	Enoplognatha latimana Hippa & Oksala		1		1
	Neottiura bimaculata (Linnaeus)		1	2	
	Paidiscura pallens (Blackwall)		1		

Table 1 (*Continued*)

Family	Species	Number of individuals recorded with			
		Stem eclector	Beeting sheet	Suction sampling	Plant removal
	Theridion impressum L. Koch	1	10	16	7
	Gen. sp.	7	492	621	76
Dictynidae	*Dictyna* sp.				2
	Nigma sp.			1	1
Fam.	Gen. sp.	16	4	8	21

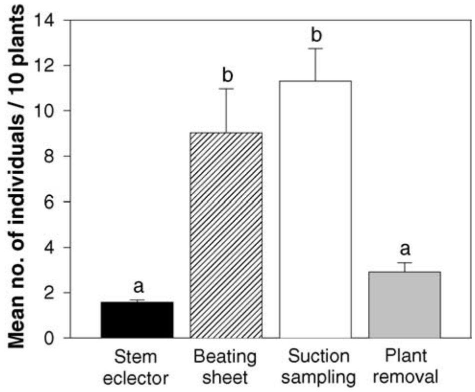

Fig. 1. Average number of spiders per 10 maize plants (mean + S.E.) using different sampling methods (seasonal means, $n = 4$ sites). Different letters denote significant differences ($p < 0.05$, Tukey's HSD test).

Theridiidae were best recorded by beating sheets and suction sampling, whereas stem eclectors recorded relatively more Linyphiidae than all other methods, but less Theridiidae.

3.2. Effects of insecticide application and Bt maize

Fig. 3 shows the seasonal abundance of spiders in the different treatments for each of the sampling methods. Effects of insecticide application were revealed by suction sampling and plant removal (RM ANOVA, d.f. = 1, $p < 0.05$, Fig. 3(B and C); suction sampling: SS = 0.633, $F = 9.990$; plant removal: SS = 0.524, $F = 11.663$). A trend for insecticide effect was observed with beating sheets ($p = 0.071$), but not for stem eclectors ($p > 0.05$). No sampling method detected any *Bt* effect (RM ANOVA, $p > 0.05$). The abundance of spiders increased during the season, as documented by beating sheet, suction sampling and plant removal method (RM ANOVA, d.f. = 5, $p < 0.001$, Fig. 3; beating sheet: SS = 9.872, $F = 38.380$; suction sampling: SS = 9.850, $F = 46.973$; plant removal: SS = 3.671, F = 12.387). A significant date × insecticide interaction was found with suction sampling (RM

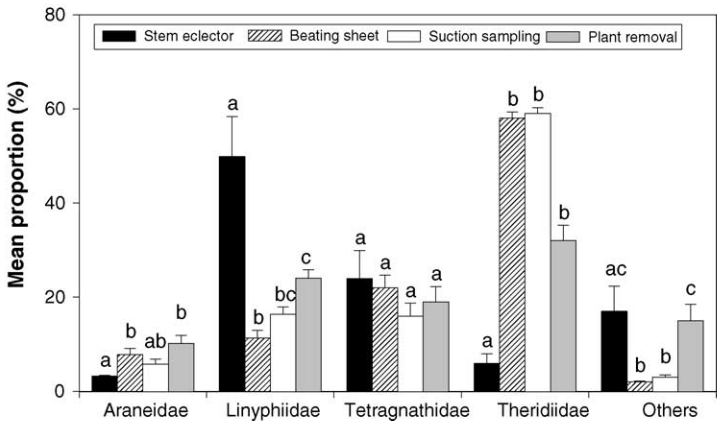

Fig. 2. Family composition (means + S.E.) of plant-dwelling spiders caught in maize with different sampling methods (seasonal means, $n = 4$ sites). Different letters denote significant differences for each spider family ($p < 0.05$, Tukey's HSD test).

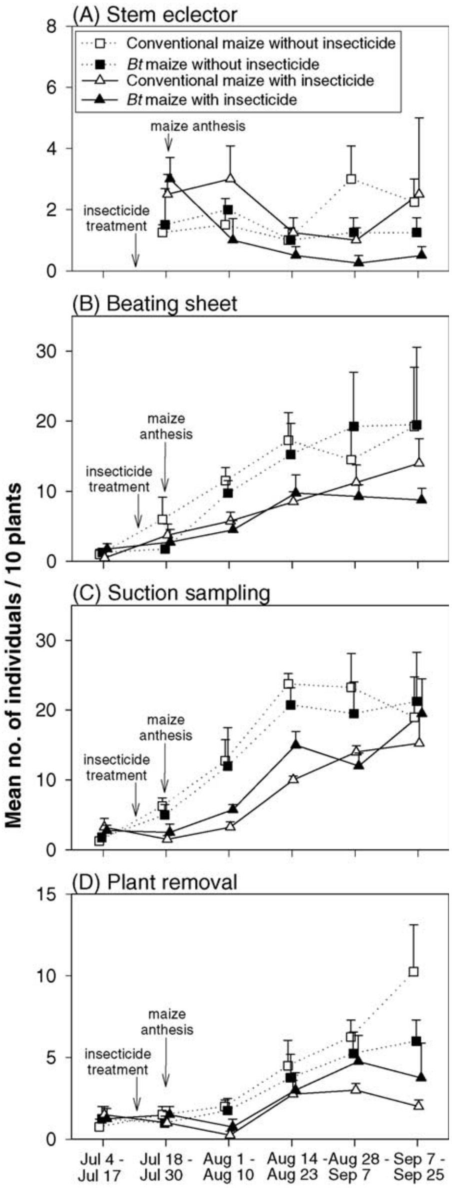

Fig. 3. Seasonal changes (mean catches per 10 plants + S.E., $n = 4$ sites) of abundance of plant-dwelling spiders recorded in maize by different sampling techniques.

ANOVA, d.f. = 5, SS = 0.878, $F = 4.186$, $p < 0.05$), but no date × *Bt* interaction in any method.

As repeated measurement ANOVA revealed date × treatment interactions, each sampling date was tested separately (Table 2). Spider abundance between plots in any method was similar at the first sampling before insecticide application or maize anthesis. Reduced spider numbers in insecticide treated plots were recorded on third and fourth sampling by beating sheets, on second to fifth sampling by suction sampling, and on sixth sampling by plant removal. No *Bt* effect was ever detected. A significant factor site at the last suction sample as well as several site × treatment interactions indicated that magnitude and direction of effects on spider abundance differed between the four sites.

The number of species, genera and families did neither significantly differ before treatment for the factors insecticide and *Bt* when the methods beating sheet, suction sampling and plant removal were pooled nor differ between treatments over the season, when samples 2–6 and all four methods were pooled (univariate ANOVA, $p > 0.05$, Table 3).

3.3. Sample size calculation

Suction sampling recorded 86.25 ± 22.90 spiders over the whole season, and beating sheets 69.50 ± 32.59 (mean of 4 sites ± 1S.D.). Fig. 4 shows the number of fields that should be sampled to detect a certain effect of *Bt* maize on spider abundance with 20–80% power for suction sampling and beating sheets, suction sampling needing lower sample sizes than beating sheets to detect a given effect.

4. Discussion

The spider families recorded in the studied fields generally dominate agroecosystems throughout Europe (Luczak, 1979; Barthel, 1997; Nyffeler and Sunderland, 2003). Spider assemblages consisted mainly of juveniles and small web building spiders, which is in accordance with other studies (Nyffeler et al., 1994; Marc et al., 1999; Ludy and Lang, 2004). Altogether 38 species were distinguished (29 identified to species level). *Erigone atra*, *Oedothorax apicatus*, *Theridion impressum* and Tetragnatha spp. were dominant, as in most European crop fields (Luczak, 1979; Platen, 1996; Nyffeler and Sunderland, 2003; Ludy and Lang, 2004). Nyffeler and Benz (1979) reported a low spider density in European maize fields. However, our findings were similar to the densities found by Pilcher et al. (1997) in the USA, and by Ludy and Lang (2004) in southern Germany.

Table 2
Effects of insecticide application and *Bt* maize on abundance of spiders for each sampling occasion and method (sum of squares, *F*-value and significance level at $p = 0.05$); see footnotes for significant interactions and effects of the factor site

	Stem eclector[a]			Beating sheet			Suction sampling[b]			Plant removal		
	SS	*F*	*p*	SS	*F*	*p*	SS	*F*	*p*	SS	*F*	*p*
Sample 1: July 4–17												
Insecticide				0.002	0.114	n.s.	0.143	8.601	0.061	0.038	0.936	n.s.
Bt				0.119	3.100	n.s.	0.007	0.044	n.s.	0.000	0.006	n.s.
Sample 2: July 18–30												
Insecticide	0.239	6.834	n.s.	0.006	0.130	n.s.	0.512	53.981	0.005	0.002	0.236	n.s.
Bt	<0.001	0.001	n.s.	0.180	4.870	n.s.	0.000	0.000	n.s.	0.002	0.236	n.s.
Sample 3: August 1–10												
Insecticide	<0.001	0.037	n.s.	0.365	62.001	0.004	0.478	24.026	0.016	0.326	6.333	n.s.
Bt	0.032	1.274	n.s.	0.036	0.638	n.s.	0.014	0.513	n.s.	0.001	0.025	n.s.
Sample 4: August 14–23												
Insecticide	0.006	0.272	n.s.	0.191	22.298	0.018	0.238	40.829	0.008	0.043	3.003	n.s.
Bt	0.027	0.638	n.s.	0.002	0.244	n.s.	0.009	1.093	n.s.	0.001	0.035	n.s.
Sample 5: August 28–September 7												
Insecticide	0.239	3.485	n.s.	0.110	3.373	n.s.	0.136	10.289	0.049	0.140	8.340	n.s.
Bt	0.160	2.705	n.s.	0.001	0.091	n.s.	0.021	3.888	n.s.	0.001	0.012	n.s.
Sample 6: September 7–25												
Insecticide	0.143	1.195	n.s.	0.061	0.617	n.s.	0.004	0.313	n.s.	0.596	12.501	0.038
Bt	0.074	2.923	n.s.	0.059	3.722	n.s.	0.023	3.759	n.s.	0.003	0.105	n.s.

[a] Sample 2: *Bt* × site—$p = 0.012$ (SS = 0.326, $F = 25.829$); insecticide × site—$p = 0.058$.
[b] Sample 1: *Bt* × site—$p = 0.028$ (SS = 0.465, $F = 14.386$); sample 4: *Bt* × site—$p = 0.013$ (SS = 0.024, $F = 24.055$); insecticide × site—$p = 0.020$ (SS = 0.018, $F = 17.875$), insecticide × *Bt*—$p = 0.001$ (SS = 0.046, $F = 141.722$); sample 6: site—$p = 0.023$ (SS = 0.857, $F = 20.373$).

Jepson et al. (1994) have proposed to consider *O. apicatus*, *E. atra* and *Tenuiphantes tenuis* amongst other arthropod species for risk assessment studies of *Bt* crops, but emphasized that their list focused on mainly soil-associated species. *Theridion impressum* and *Tetragnatha* spp. are herewith suggested to be included in such studies as representatives of typical plant-dwelling spiders.

Stem eclectors recorded less numbers and species than all methods. Catches were dominated by small Linyphiidae, whereas Theridiidae and Araneidae were underrepresented compared to the other methods. Trapping efficacy could be improved with a higher number of traps, shorter sampling interval and covers to protect against rain and dropping dead spiders, however, at higher cost and time investment.

Plant removal was expensive, time consuming and ineffective for small spiders. Losses in the field (escaping spiders), during transport and storage (crushed individuals), together with overseen spiders in the sorting procedure were responsible for the low efficiency.

Beating sheets recorded a high number of individuals and species. Time effort and costs were acceptable. Beating sheets are an effective method to

Table 3
Spider species richness recorded in the different treatments on second to sixth sampling (all methods pooled, $n = 4$ sites, mean ± S.E.)

Treatment	Species	Genera	Families
Conventional maize without insecticide	11.00 ± 0.58	11.00 ± 0.58	5.75 ± 0.48
Bt maize without insecticide	12.25 ± 2.29	12.00 ± 2.08	7.00 ± 0.71
Conventional maize with insecticide	11.50 ± 1.55	11.25 ± 1.49	6.50 ± 0.50
Bt maize with insecticide	13.25 ± 1.49	13.00 ± 1.29	5.75 ± 0.25

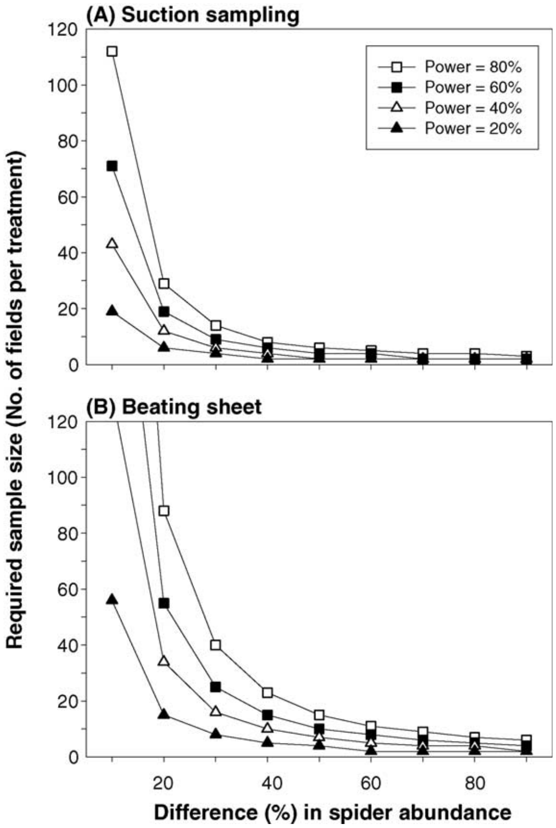

Fig. 4. Sample size for monitoring the effect of *Bt* maize on spider abundance with suction sampling (A) and beating sheet (B) to detect a difference of 10–90% in spider abundance between *Bt* and conventional fields (with differing statistical power of 20–80%). Field numbers apply to *Bt* fields and to non-*Bt* fields (calculations based on the mean number (±1S.D.) of spiders recorded in four plots of conventional maize without insecticide, samplings being pooled for site, two-tailed two-sample *t*-test for independent data, $p < 0.05$, and sample size equally distributed between samples).

collect plant-dwelling spiders, although estimates of absolute spider abundance per plant are biased to some extents and sampling was restricted to dry weather.

Suction sampling recorded the highest number of species and individuals. Small rechargeable hoovers are relatively cheap and light, barely damage the animals collected and are known to adequately collect plant-dwelling spiders (Henschel, 1995; Ludy and Lang, 2004). The larger, fuel-driven D-Vac (Dietrick et al., 1959) samples the vegetation less precisely and there is an edge effect caused by strong lateral airflow (Sunderland et al., 1995; Bell and Wheater, 2001). Our suction device was best to estimate abundance per plant, because plants could be sampled individually and the proportion of escaped or crushed spiders appeared to be relatively low. Also, spider numbers obtained with suction samplers were more even among single collecting events, and the concomitant lower variance of the data would require a smaller sample size than the other tested sampling methods. This sampling, however, depends on dry weather and is limited in time by the battery capacity.

On average, 23–63% less spiders were recorded in pyrethroid treated compared to untreated plots over the whole season. The insecticide effect was significantly recorded by suction sampling and plant removal, and indicated by beating sheets. Suction sampling recorded fewer spiders from just after the insecticide treatment in mid-July up to September, beating sheets only in two consecutive samplings and plant removals only for the last sampling. Stem eclectors were unable to detect insecticide effects.

No significant effect of *Bt* maize on spider abundance could be detected. However, with a sample size of four fields per treatment, suction sampling would only be able to reveal an effect on spider abundance of 70% (with 80% probability), and beating sheets not even a 90% effect. On the other hand, a difference of 50% between control and treatment could be detected with 60% probability by suction sampling and with 20% by beating sheets. Increasing the sample size to 14 or 40 fields per treatment for suction sampling and beating sheet, respectively, would be necessary to detect a 30% difference between *Bt* and control fields with 80% probability. If higher replication is not feasible, *Bt* crops should be compared with common plant protection practice. In the present study, even a relatively small sample size detected an insecticide effect, indicating that any effect of *Bt* maize on spider abundance had to be much lower than that of pyrethroid insecticide. Similarly, Dively and Rose (2002), Volkmar and Freier (2003) and Candolfi et al. (2004) reported no effect of *Bt* maize on spiders, whereas the application of pyrethroid insecticide reduced them significantly. Also, *Bt* potato and *Bt* cotton had no negative effect on spiders, whereas broad-spectrum insecticides including pyrethroids reduced spider populations considerably (e.g., Fitt et al., 1994; Reed et al., 2001; Duan et al., 2004; Men et al., 2004). Pilcher et al. (1997) and Jasinski et al.

(2003) recorded equal numbers of plant-dwelling arachnids in *Bt* and non-*Bt* maize. However, none of these studies exceeded a study period longer than two years and may possibly have missed long-term effects. Moreover, it has to be kept in mind that pyrethroid insecticides are not the only alternative to *Bt* maize for protection against the European corn borer. Alternative strategies with less side effects on non-targets would include *Bt* sprays, mechanical soil treatment after harvest, release of parasitoids or promotion of natural enemies, or even do nothing if corn borer densities are below the economic injury threshold (Hurle et al., 1996).

Concepts for case-specific monitoring and general surveillance will be required in Europe for *Bt* crops to be approved for commercial production (EU directive 2001/18/EC, European Parliament and Council, 2001). Possible long-term effects on non-target populations, which are likely to be missed in 1–2 year field experiments of pre-market studies, can only be discovered using appropriate methods.

The following conclusions can be drawn:

(1) The plant-dwelling spider community recorded in South German maize fields mainly consisted of agrobiont species, frequently found in different crops across Europe.
(2) A single application of a pyrethroid insecticide against the European corn borer in spring had a significant negative impact on the spider community that lasted over the whole growing season.
(3) *Bt* maize did not differ from conventional maize in terms of spider abundance.
(4) *Bt* maize had less effect on spider abundance than the application of a pyrethroid insecticide.
(5) Suction sampling is recommended for the surveillance of plant-dwelling spiders in transgenic maize.

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References

Andow, D.A., Hilbeck, A., 2004. Science-based risk assessment for non-target effects of transgenic crops. BioScience 54, 637–649.

Barber, H., 1931. Traps for cave inhabiting insects. J. Elisha Mitchell Sci. Soc. 46, 259–266.

Barthel, J., 1997. Einfluss von Nutzungsmuster und Habitatkonfiguration auf die Spinnenfauna der Krautschicht (Araneae) in einer süddeutschen Agrarlandschaft. Agrarökologie 25. Verlag Agrarökologie, Bern Hannover.

Bell, J.R., Wheater, C.P., 2001. Analysis of the most popular techniques for sampling spiders in large-scale ecological experiments in grasslands. Newsl. Br. Arachnol. Soc. 91, 10–12.

Candolfi, M.P., Brown, K., Grimm, C., Reber, B., Schmidli, H., 2004. A faunistic approach to assess potential side effects of genetically modified Bt-corn on non-target arthropods under field conditions. Biocontrol Sci. Technol. 14, 129–170.

Dietrick, E.J., Schlinger, E.I., van den Bosch, R., 1959. A new method for sampling arthropods using a suction collecting machine and a modified Berlese funnel separator. J. Econ. Entomol. 52, 1085–1091.

Dively, G.P., Rose, R., 2002. Effects of Bt transgenic and conventional insecticide control on the non-target natural enemy community in sweet corn. In: Proceedings of the First International Symposium on Biological Control of Arthropods, Honolulu, Hawaii, USA, USDA Forest Service FHTET, pp. 265–274.

Duan, J.J., Head, G., Jensen, A., Reed, G., 2004. Effects of transgenic *Bacillus thuringiensis* potato and conventional insecticides for Colorado potato beetle (Coleoptera: Chrysomelidae) management on the abundance of ground-dwelling arthropods in Oregon potato ecosystems. Environ. Entomol. 33, 275–281.

Dutton, A., Klein, H., Romeis, J., Bigler, F., 2002. Uptake of Bt-toxin by herbivores feeding on transgenic maize and consequences for the predator *Chrysoperla carnea*. Ecol. Entomol. 27, 441–447.

Dutton, A., Romeis, J., Bigler, F., 2003. Assessing the risk of insect resistant transgenic plants on entomophagous arthropods: Bt-maize expressing Cry1Ab as a case study. Biocontrol 48, 611–636.

European Parliament and Council, 2001. Directive 2001/18/EC of the European Parliament and Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EC—Commission Declaration. Official Journal L106, pp. 1–39.

Faul, F., Erdfelder, E., 1992. Gpower: A Priori, Post Hoc and Compromise Power Analysis for MS-DOS [Computer Program]. Bonn University, Department of Psychology, Germany.

Fearing, P.L., Brown, D., Vlachos, D., Meghji, M., Privalle, L., 1997. Quantitative analysis of CryIA(b) expression in Bt maize plants, tissue, and silage and stability of expression over successive generations. Mol. Breeding 3, 169–176.

Fitt, G.P., Mares, C.L., Llewellyn, D.J., 1994. Field-evaluation and potential ecological impact of transgenic cottons (*Gossypium hirsutum*) in Australia. Biocontrol Sci. Technol. 4, 535–548.

Glare, T.R., O'Callaghan, M., 2000. *Bacillus thuringiensis*: Biology, Ecology and Safety. Wiley and Sons, Chichester.

Gregory, B.M., 1989. Field observations of *Gasteracantha cancriformis* (Araneae Araneidae) in a Florida mangrove stand. J. Arachnol. 17, 119–120.

Hails, R.S., 2000. Genetically modified plants—the debate continues. Trends Ecol. Evol. 15, 14–18.

Heimer, S., Nentwig, W., 1991. Spinnen Mitteleuropas. Verlag Paul Parey, Berlin, Hamburg.

Henschel, J.R., 1995. Ein handliches Vakuumsammelgerät für die Erfassung von Spinnen und Insekten. Arachnol. Mitt. 9, 67–70.

Hurle, K., Lechner, M., König, K., 1996. Mais—Unkräuter, Schädlinge, Krankheiten. Verlag Th. Mann, Gelsenkirchen.

James, C., 2002. Preview: Global status of Commercialized Transgenic Crops: 2002. ISAAA Briefs No. 27. ISAAA, Ithaca, NY.

Jasinski, J.R., Eisley, J.B., Young, C.E., Kovach, J., Wilson, H., 2003. Select non-target arthropod abundance in transgenic and non-transgenic field crops in Ohio. Environ. Entomol. 32, 407–413.

Jepson, P.C., Croft, B.A., Pratt, G.E., 1994. Test systems to determine the ecological risks posed by toxin release from *Bacillus thuringiensis* genes in crop plants. Mol. Ecol. 3, 81–89.

Kiss, J., Szentkirályi, F., Tóth, F., Szénási, Á., Kádár, F., Árpás, K., Szekeres, D., Edwards, C.R., 2003. *Bt*-corn: impact on non-targets and adjusting to local IPM systems. In: Proceedings of the International OECD Workshop held in Grossrussbach, Austria, September 27–28, 2002, pp. 157–172.

Koziel, M.G., Beland, G.L., Bowman, C., Carozzi, N.B., Crenshaw, R., Crossland, L., Dawson, J., Desai, N., Hill, M., Kadwell, S., Launis, K., Lewis, K., Maddox, D., McPherson, K., Meghji, M.R., Merlin, E., Rhodes, R., Warren, G.W., Wright, M., Evola, S.V., 1993. Field performance of Elite transgenic maize plants expressing an insecticidal protein derived from *Bacillus thuringiensis*. Bio-Technol. 11, 194–200.

Krattinger, A.F., 1997. Insect Resistance in Crops: A Case Study of *Bacillus thuringiensis* (Bt) and its Transfer to Developing Countries. ISAAA Briefs No. 2. ISAAA, Ithaca, NY.

Krause, U., Pfaff, K., Dinter A., Poehling, H.-M., 1993. Nebenwirkungen von Insektiziden, vor allem Pyrethroiden, auf epigäische Spinnen bei der Bekämpfung von Getreideblattläusen. Agrarökologie 9, Verlag Paul Haupt, Bern, Stuttgart, Wien.

Lang, A., 2003. Intraguild interference and biocontrol effects of generalist predators in a winter wheat field. Oecologia 134, 144–153.

Lang, A., Filser, J., Henschel, J.R., 1999. Predation by ground beetles and wolf spiders on herbivorous insects in a maize crop. Agric. Ecosyst. Environ. 72, 189–199.

Lang, A., Ludy, C., Vojtech, E., 2004. Dispersion and distribution of Bt maize pollen in field margins. J. Plant Dis. Prot. 111, 417–428.

Luczak, J., 1979. Spiders in Agrocoenoses. Pol. Ecol. Stud. 5, 151–200.

Ludy, C., 2004. Eat spiders their vegetables? Intentional pollen feeding in the garden spider *Araneus diadematus*. Newl. Br. Arachnol. Soc. 101, 4–5.

Ludy, C., Lang, A., 2004. How to catch foliage-dwelling spiders (Araneae) in maize fields and their margins: a comparison of two sampling methods. J. Appl. Entomol. 128, 501–509.

Marc, P., Canard, A., Ysnel, F., 1999. Spiders (Araneae) useful for pest limitation and bioindication. Agric. Ecosyst. Environ. 74, 229–273.

Meier, U., 1997. Growth Stages of Mono- and Dicotyledonous Plants. Federal Biological Research Centre for Agriculture and Forestry. BBCH-Monograph. Blackwell Wissenschaftsverlag, Berlin, Wien.

Men, X.Y., Ge, F., Edwards, C.A., Yardim, E.N., 2004. Influence of pesticide applications on pest and predatory arthropods associated with transgenic Bt cotton and non-transgenic cotton plants. Phytoparasitica 32, 246–254.

Nentwig, W., Hänggi, A., Kropf, C., Blick, T., 2002. Spinnen Mitteleuropas/Central European Spiders. An Internet Identification Key. Version 21-2-02, http://www.araneae.unibe.ch

Nyffeler, M., 1999. Prey selection of spiders in the field. J. Arachnol. 27, 317–324.

Nyffeler, M., Benz, G., 1979. Zur ökologischen Bedeutung der Spinnen der Vegetationsschicht von Getreide-und Rapsfeldern bei Zürich (Schweiz). Z. Angew. Entomol. 87, 348–376.

Nyffeler, M., Sterling, W.L., Dean, D.A., 1994. Insectivorous activities of spiders in United States field crops. J. Appl. Entomol. 118, 113–128.

Nyffeler, M., Sunderland, K.D., 2003. Composition, abundance and pest control potential of spider communities in agroecosystems: a comparison of European and US studies. Agric. Ecosyst. Environ. 95, 579–612.

Pilcher, C.D., Obrycki, J.J., Rice, M.E., Lewis, L.C., 1997. Preimaginal development, survival, and field abundance of insect predators on transgenic *Bacillus thuringiensis* corn. Environ. Entomol. 26, 446–454.

Platen, R., 1996. Spinnengemeinschaften mitteleuropäischer Kulturbiotope. Arachnol. Mitt. 12, 1–45.

Platnick, N.I., 2002. The World Spider Catalog, Version 2.5. American Museum of Natural History. http://research.amnh.org/entomology/spiders/catalog81-87/index.html; Version January 2002.

Poppy, G.M., Sutherland, J.P., 2004. Can biological control benefit from genetically-modified crops? Tritrophic interactions on insect-resistant transgenic plants. Physiol. Entomol. 29, 257–268.

Reed, G.L., Jensen, A.S., Riebe, J., Head, G., Duan, J.J., 2001. Transgenic Bt potato and conventional insecticides for colorado potato beetle management: comparative efficacy and non-target impacts. Entomol. Exp. Appl. 100, 89–100.

Roberts, M.J., 1993. The Spiders of Great Britain and Ireland, Part 1: Text and Part 2: Colour Plates. Harley Books, Colchester, UK.

Roberts, M.J., 1995. Spiders of Britain and Northern Europe, Collins Field Guide. HarperCollins Publishers, London.

Smith, R.B., Mommsen, T.P., 1984. Pollen feeding in an orb-weaving spider. Science 226, 1330–1332.

Sunderland, K.D., De Snoo, G.R., Dinter, A., Hance, T., Helenius, J., Jepson, P.C., Kromp, B., Lys, J.A., Samu, F., Sotherton, N.W., Toft, S., Ulber, B., 1995. Density estimation for invertebrate predators in agroecosystems. Acta Jutl. 70, 133–162.

Symondson, W.O.C., Sunderland, K.D., Greenstone, M.H., 2002. Can generalist predators be effective biocontrol agents? Ann. Rev. Entomol. 47, 561–594.

Uetz, G.W., Halaj, J., Cady, A.B., 1999. Fuild structure of spiders in major crops. J. Arachnol. 3, 101–111.

Vogelei, A., Greissl, R., 1989. Survival strategies of the crab spider *Thomisus onustus* Walckenaer 1806 (Chelicerata, Arachnida Thomisidae). Oecologia 80, 513–515.

Volkmar, C., Freier, B., 2003. Spinnenzönosen in Bt-Mais und nicht gentechnisch veränderten Maisfeldern. Z. PflKrankh. Pflschutz 110, 572–582.