

Effects of Cultivation of Genetically Modified *Bt* Maize on Epigeic Arthropods (Araneae; Carabidae)

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ABSTRACT A field study was conducted in Germany to determine the possible effects of transgenic maize cultivation on nontarget epigeic predator organisms. During the growing period of 2001–2003, the activity abundances of spiders and carabid beetles were recorded and compared in three treatments: (1) *Bt*-maize (Mon 810) expressing the Cry1Ab protein from *Bacillus thuringiensis* (Berliner), (2) an isogenic variety, and (3) the isogenic variety treated with insecticide. All three treatments were replicated in eight plots. The results were evaluated using three different methods. The activity abundances of single species were statistically analyzed by confidence interval methods. In addition, the phenological behaviors of the spider and carabid beetle species were determined, and multivariate statistical evaluation of the community by principal component analysis was conducted. Significantly different activity abundances in *Bt* plots compared with isogenic control plots were observed both for spiders and carabid beetles during 2001. However, in 2002 and 2003, no changes in community structure were detectable in any of the treatments. The change in the first year may have been caused by the influence of a massive cornborer infestation and accompanying large changes in microclimatic factors.

KEY WORDS *Bt* maize, carabid beetles, spiders, epigeic predators, nontarget organisms

The European cornborer (*Ostrinia nubilalis* Hübner), a maize pest, is vastly spread over many parts of Europe, including Germany (Schmitz et al. 2002, Gathmann and Rothmeier 2005). One strategy to protect plants against the cornborer is to cultivate genetically modified (GM) maize expressing a toxic protein (Cry1Ab) derived from *Bacillus thuringiensis* Berliner (*Bt*). Indeed, the cultivation of GM maize has increased from 1996 to 2005 and is now grown on 17.8 million ha worldwide (James 2005). However, concerns remain that the *Bt* protein may harm nontarget organisms (Krebs et al. 1999, Dale et al. 2002, Mullin et al. 2005, O'Callaghan et al. 2005, Harwood et al. 2006).

Epigeic predators, such as spiders and carabid beetles, are the most abundant invertebrates in European agroecosystems and are essential to the control of pest organisms (Eckschmitt et al. 1997, Holland 2002, Brooks et al. 2003, Meissle and Lang 2005). Thus, detrimental effects on this functional group can increase the risk of secondary pest problems (Andow and Zwahlen 2006). These beneficial species are potentially exposed to Cry1Ab because they take up various herbivorous prey and are closely associated with the soil system (Eckschmitt et al. 1997). Cry1Ab can be released through root exudates into the rhizosphere of the soil (Saxena et al. 1999, 2002) and can be

detected in bulk soil, the rhizosphere, and plant residues over several months (Baumgarte and Tebbe 2005). Moreover, it is protected against microbiological degradation by binding to clay minerals and humic acids but remains biologically active in the bound form (Stotzky 2000).

GM plants may have direct or indirect negative effects on predators if *Bt* protein-containing plant tissues or pollen are incorporated in prey organisms (O'Callaghan et al. 2005). Some carabid species (e.g., *Pseudophonus rufipes* De Geer, *Pterostichus melanarius* Illiger) feed directly on maize plants and litter (Thiele 1977) and do not avoid GM maize when conventional maize is available (Meissle et al. 2005). Zwahlen and Andow (2005) showed that some carabid beetle species take up the Cry1Ab protein in the field. Negative effects on some species that were fed susceptible prey have been observed (Meissle et al. 2005). Harwood et al. (2005) found high levels of Cry 1Ab endotoxin in spiders and coleopteran predators. In addition to the possible accumulation of Cry1Ab in soil and its transmission to epigeic predators through the food web (Harwood et al. 2005, Obrist et al. 2006), spiders may take up the protein when recycling their webs during the anthesis of maize (Meissle and Lang 2005, Ludy and Lang 2006). Moreover, systemic modification of the transgenic plant itself, e.g., changes in the content of lignin or in its components, can give rise to additional negative effects (Saxena and Stotzky 2001, Meissle and Lang 2005).

Several field trial studies have examined the impact of *Bt* maize cultivation on nontarget carabids and

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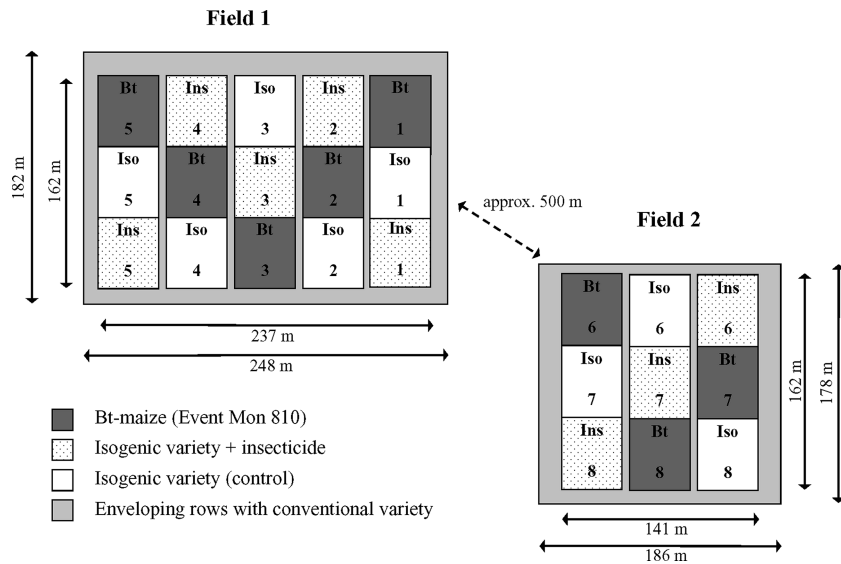


Fig. 1. Plot design of the field trial during 2001–2003. The two fields together contained 24 plots, 8 for each treatment: (1) *Bt*-maize, (2) isogenic variety + insecticide (control), and (3) isogenic variety (control). The total size was 7.8 ha (field 1, 4.5 ha; field 2, 3.3 ha); the two fields were separated by a distance of ≈ 500 m. This arrangement was maintained throughout the study period.

spiders (Lozzia 1999, Hassel and Shepard 2002, Brooks et al. 2003, Hawes et al. 2003, Kiss et al. 2003 Volkmar and Freier 2003 French et al. 2004, Candolfi et al. 2004, Meissle and Lang 2005, Daly and Buntin 2005, de la Poza et al. 2005Bhatti et al. 2005, Harwood et al. 2006, Ludy and Lang 2006, Ludy and Lang 2006). Although in most cases no adverse effects were reported, many questions remain concerning the transmission of *Bt* protein through the food chain and its medium- and long-term effects on the communities of those organisms (Harwood et al. 2005, O'Callaghan et al. 2005). Thus, effective prospective and retrospective methods are needed to assess the risks of transgenic crops to biological diversity (Andow and Zwahlen 2006) both before and after commercialization.

The study described here was based on a field design that met the requirements for statistical evaluation, i.e., randomization and sample size large enough for claiming no meaningful change, at least for selected taxa. In this field trial, the activity abundances of spiders and carabid beetles on *Bt* maize (Novelis, Mon810) plots and insecticide-treated or untreated isogenic control (Nobilis) plots were compared. Samples of these epigeic predators were obtained from the three different maize varieties (eight replicates each) during a period of 3 yr, a time frame sufficient to detect medium-term effects. Because significant alterations for a few species have been reported in some field trial studies (Brooks et al. 2003, Candolfi et al. 2004), the data were analyzed by several different evaluation methods with respect to consistency and power. Tiered ecological risk assessment is a common analytical method used in ecotoxicology studies (HARAP 1999), and it also has been discussed as a feasible stepwise approach to reveal the potential risk of GM

organisms (Romeis et al. 2006, Andow and Zwahlen 2006). The analytical methods used in this study can be implemented both for higher-tier testing on a field scale to prospectively assess the potential risks of GM crops and for retrospectively evaluating their potential long-term effects.

Materials and Methods

Site Properties and Study Design. This 3-yr study was conducted in an area near Bonn, Germany, from 2001 to 2003. The size of the study site, which consisted of two fields, totaled 7.8 ha (field 1: 4.5 ha, field 2: 3.3 ha). The soil type of field 1 was stagnic luvisol (FAO nomenclature). It was a slightly clayey silt soil with balanced air and water condition and a pH between 7.0 and 7.3. The soil type of field 2 was gleyic cambisol. It was a clayey, stony-gritty, silt soil with a tendency to contain standing water. The pH of the soil in the two fields was between 7.3 and 7.4. Both soil types originated from loess. The physical site properties of the fields were comparable.

Three different maize varieties were cultivated on the two fields: (1) *Bt* maize NOVELIS (Event Mon 810): a genetically modified variety that expresses Cry 1Ab, modified from *Bacillus thuringiensis* subsp. kurstaki HD-1 strain (Monsanto Company 2002); (2) the isogenic variety NOBILIS (control): an unchanged variety with properties genetically similar to those of NOVELIS not treated with an insecticide; and (3) NOBILIS treated with an insecticide (control) according to common agricultural practice.

The 24 experimental plots consisted of eight replicates for each of the three varieties (Fig. 1). Each plot was 56 by 46 m and contained 72 rows of maize plants

Table 1. Field and study conditions for 2001–2003

Year	Sowing	Application of herbicide	Application of insecticide	Exposure time of pitfall traps	Harvest
2001	20 May	29 May	13 July	12 June to 25 Sept.	10 Oct.
2002	17 May	10 June	20 July	20 June to 01 Oct.	29 Oct.
2003	28 Apr	28 May	11 July	17 June to 16 Sept.	25 Sept.

with 75 cm between rows and 15 cm between plants. The experimental plots were enclosed by rows of a conventional maize variety (ROMARIO). The arrangement of the test varieties was maintained throughout the study. All varieties were treated with a mixture of the herbicides Callisto (0.9l per ha, active agent: mesotrione) and Gardobuc (0.9 liters per ha, active agent: terbuthylazine and bromoxynil as octanoate/heptanoate). The insecticide Baytroid 50 (750 ml/ha, active agent: cyfluthrin; Bayer CropScience, Germany) was used for the insecticide-treated plots. The time of application was adapted to the phenology of the cornborer (*Ostrinia nubilalis*; Table 1). The seeds of all three maize varieties were treated uniformly with Maxim (Syngenta, Switzerland). The whole plant, except for grain remaining on the field, was harvested. In contrast to usual practice, the fields were not deeply lowed but instead were cultivated only superficially with a grubber to enhance the potential accumulation of *Bt* protein in the upper soil layer.

Sampling of Arthropods (Spiders; Carabid Beetles). The activity abundances of carabid beetles and spiders were sampled with pitfall traps. The pitfall traps consisted of plastic funnels (Ø, 9.5 cm; height, 20 cm) with a trap-glass inserted at the bottom. The trap-glasses were fitted in the funnels and sealed with rubber sleeves. The traps were filled with ≈100 ml of a 70% ethylene-glycol/water solution to immediately kill and preserve the arthropods. Detergent was added to reduce the surface tension of the solution, thereby forcing the arthropods to immediately sink to the bottom of the trap. Each trap was covered with a transparent plastic roof to protect against precipitation. In 2001 and 2002, two pitfall traps per plot (total 48 traps) were installed, whereas in 2003, one pitfall trap per plot (total 24 traps) was used. The traps were placed at stage 1 of leaf development, according to the BBCH code (Lancashire et al. 1991), in rows 18 and 54 at a distance of at least 13.5 m from the edge of the plots. The minimum distance between each trap was >27 m because of staggered arrangement. This arrangement was used to avoid problems with later applications of pesticides and to ensure that the captures of each trap were independent of each other. Capture was completed just before harvesting (Table 1). The traps were emptied once a week.

Pitfall trapping is a standard method (Holland 2002) to catch epigeic predators and also has been used successfully in other comparative studies (Lozzia 1999, Brooks et al. 2003, Hawes et al. 2003, Volkmar and Freier 2003, French et al. 2004, Candolfi et al. 2004, Prasifka et al. 2005, Bhatti et al. 2005, de la Poza et al. 2005, Daly and Buntin 2005, Harwood et al. 2006). In

this study, pitfall traps were chosen because they are able to capture a large number of individuals independent of the time of day (Trautner 1993). The number of trapped individuals depends on their behavior and on other parameters, including species size and the cursorial activities of the arthropods. Pitfall trapping does not provide estimates of real density. Measurement of activity abundance has many advantages and disadvantages, as discussed extensively in the literature (Stammer 1948, 1949, Greenslade 1964, Holopainen 1992, Mühlenberg 1993, Spence and Niemelä 1994, Luff 2002). Because behavior as well as size and cursorial activity can be assumed to be similar for a given species at different sites, the method is useful to compare the relative occurrence of that species at different sites (Roß-Nickoll 2000). Furthermore, because active predators have a better chance to encounter prey than sedentary predators, the activity abundances may be more important than real density in matters of biological control of pests (Lanski 1982, Luff 1990). However, a decrease in abundance of prey organisms as a result of pest management can be followed by increased activity of predators to compensate for the lower prey density (Baars 1979, de Ruiter et al. 1989). However, short-term, sublethal, or indirect effects of the *Bt* protein on activity may be difficult to distinguish from direct effects on mortality (Holland et al. 2002). Moreover, possible effects could be masked by cursorial activity of the larger beetles and the movement of mobile spider species. Lennartz (2003) described this influence of species abundance viz. species with high abundance have greater influence on pitfall trap catches of the surrounding area than those with lower abundance. All these considerations indicate the need for cautious interpretation of relative abundances.

Carabid beetles were examined with reference to Freude et al. (1976) and Borcherding et al. (1994). The nomenclature followed the "Index of German beetles" (Köhler and Klausnitzer 1998). Spiders were identified with reference to Roberts (1985a, b, 1987, 1993, 1995) and Heimer and Nentwig 1991. The nomenclature followed that of Platen et al. (1995).

All individuals were identified to the species level when possible. Adult spiders and larger carabid beetles were counted sex specifically.

Data Analysis and Statistics. A standardized evaluation method to analyze the data independently for each year was developed by one of the authors (L.A.H.). The data were statistically evaluated at the species level using the activity abundances of the different species (endpoint: sum of a year). All of the adult spider species and the larger carabid beetles also

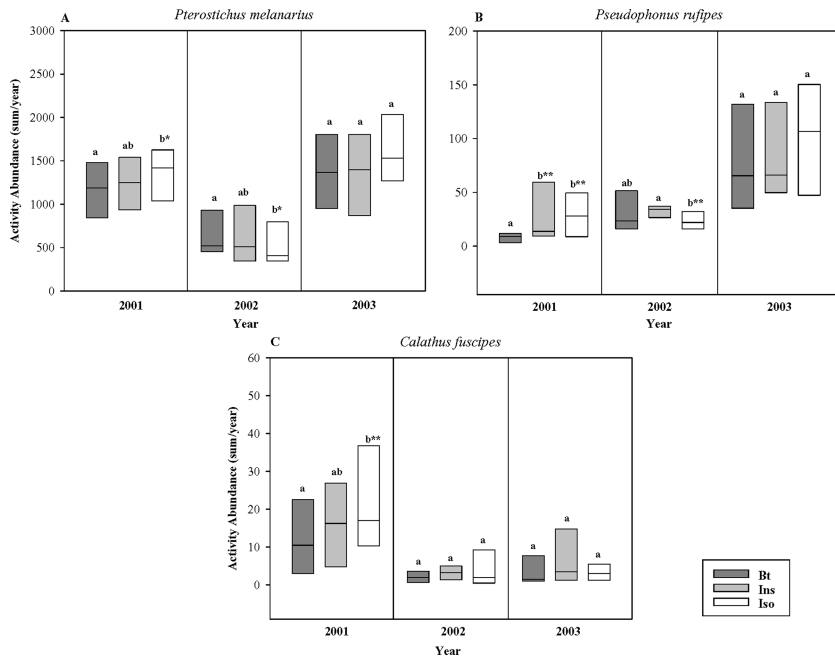


Fig. 2. Variance within the dataset ($n = 8$) of the Carabid species (A) *P. melanarius*, (B) *P. rufipes*, and (C) *C. fuscipes*. The activity abundances (sum per year) for 2001, 2002, and 2003 are shown. In a boxplot, the median value (horizontal line) is between the 25th and 75th percentiles (box). Different letters above the columns indicate significant differences between respective treatments: (1) *Bt*-maize, (2) isogenic variety + insecticide (control), and (3) isogenic variety (control). **Significant difference according to the 95% CI approach; *significant difference according to the 90% CI approach.

were tested sex specifically. From a risk-assessment perspective, proof-of-safety requires a finding of no meaningful change for the selected nontarget species in *Bt* maize compared with the near-isogenic variety (Hauschke and Hothorn 1998). The commonly used nonsignificant P value, i.e., $P > 0.05$, as a criterion for no meaningful change is not appropriate because “absence of evidence is not evidence of absence” (Altman and Bland 1995). Equivalence can be claimed when two-sided $(1-2\alpha)$ confidence limits are within an equivalence region (lower, upper). However, because there were no taxon-specific predefined equivalence regions, both two-sided $(1-\alpha)$ confidence limits (95% intervals) and two-sided $(1-2\alpha)$ confidence limits (90% intervals) were calculated instead. These can be viewed as common 90% and 95% CIs, because in both approaches, the point estimator for the ratio is the same. A lower 95% limit >1 implies a significant increase, and an upper 95% limit <1 a significant decrease.

Therefore, confidence intervals for the ratios of the pairwise comparisons INS (isogenic variety plus insecticide) versus *Bt*, ISO (isogenic variety) versus *Bt*, and ISO versus INS were estimated for 2001, 2002, and 2003. Because many taxa were considered simultaneously, it was easier to interpret the percentage increase or decrease in abundance as the species-specific absolute difference in the number of individuals. Because the assumption of a normal distribution was problematic in analyzing these skewed abundance data (boxplots in Fig. 2), nonparametric confidence

intervals for the ratios were used. Ties in the data were resolved using an exact test method (Hothorn and Oberdoerfer 2006). The computations were done by functions that we wrote using statistical software (R Development Core Team 2005).

Community Structure Analysis. In addition to the above-described analyses, the community structures of the three varieties were studied by principal component analysis (PCA). The precondition for this multivariate statistical method was that the highest “gradient length” within the dataset was <3 , i.e., the gradient was short and assumed to be linear. The gradient was determined by applying a detrended correspondence analysis (DCA). PCA was used to compare the relationship between the different pitfall traps (sites) based on the particular species composition of each. The eigenvalues showed the percentage of variance within a dataset represented by the first two axes (ter Braak and Smlauer 2002). For this evaluation method, only the data from those species that showed significant differences by the 90% CI method ($P < 0.10$) were used. Because of nonconservative adjustment of the statistical tests, even small differences served as input factors.

Boxplot Diagrams. For those species that showed significant differences with respect to maize variety, the activity abundance was represented in boxplot diagrams, which provided information about variability and possible outliers of the dataset. In a boxplot, the median value (horizontal line) is between the 25th and 75th percentiles (box). In this study, input was the

Table 2. Total no. individuals and species of spiders and carabid beetles captured with pitfall traps during 2001–2003

Species	Counts			Carabid beetles				
	2001	2002	2003	Species		Counts		
						2001	2002	2003
<i>Abax parallelepipedus</i> Piller a. Mittepacher	0	9	12	<i>Agelena labyrinthica</i> Clerck	Agelenidae	1	0	0
<i>Acupalpus meridianus</i> L.	0	4	4	<i>Agyneta decora</i> O. P.-Cambridge	Linyphiidae	1	0	0
<i>Agonum marginatum</i> L.	0	0	1	<i>Araeoncus humilis</i> Blackwall	Linyphiidae	4	0	0
<i>Agonum muelleri</i> Herbst	4	12	1	<i>Bathypantes gracilis</i> Blackwall	Linyphiidae	373	235	33
<i>Amara aenea</i> De Geer	0	19	9	<i>Bathypantes parvulus</i> Westring	Linyphiidae	0	1	0
<i>Amara aulica</i> Panzer	1	0	0	<i>Centromerus sylvaticus</i> Blackwall	Linyphiidae	0	1	0
<i>Amara bifrons</i> Gyllenhal	6	1	0	<i>Cicurina cicur</i> F.	Dictynidae	2	10	2
<i>Amara familiaris</i> Duftschmid	1	1	0	<i>Clubiona terrestris</i> Westring	Clubionidae	1	0	0
<i>Amara ovata</i> F.	4	0	0	<i>Coelotes terrestris</i> Wider	Amaurobiidae	2	8	0
<i>Amara plebeja</i> Gyllenhal	1	0	0	<i>Collinsia inerrans</i> O. P.-Cambridge	Linyphiidae	128	71	14
<i>Amara similata</i> Gyllenhal	6	0	2	<i>Diplocephalus latifrons</i> O. P.-Cambridge	Linyphiidae	0	1	0
<i>Anchomenus dorsalis</i>	413	31	22	<i>Diplostyla concolor</i> Wider	Linyphiidae	43	44	4
Pontoppidan								
<i>Anisodactylus binotatus</i> F.	0	23	0	<i>Erigone atra</i> Blackwall	Linyphiidae	907	137	96
<i>Badister bullatus</i> Schrank	0	2	0	<i>Erigone dentipalpis</i> Wider	Linyphiidae	138	20	2
<i>Bembidion lampros</i> Herbst	142	575	146	<i>Erigoneella hiemalis</i> Blackwall	Linyphiidae	0	0	1
<i>Bembidion lunulatum</i> Duftschmid	1	0	1	<i>Gnaphosidae juvenile</i>	Gnaphosidae	4	0	2
<i>Bembidion obtusum</i> Audinet-Serville	272	445	211	<i>Hahnia nava</i> Blackwall	Hahniidae	0	1	0
<i>Bembidion properans</i> Stephens	1	1	0	<i>Histopona torpida</i> C. L. Koch	Agelenidae	1	1	0
<i>Bembidion quadrimaculatum</i> L.	522	2,415	499	<i>juvenile</i>		0	15	0
<i>Bembidion tetracolum</i> Say	121	420	80	<i>Lepthyphantes ericaeus</i> Blackwall	Linyphiidae	0	2	0
<i>Brachinus crepitans</i> L.	2	0	0	<i>Lepthyphantes pallidus</i> O. P.-Cambridge	Linyphiidae	2	1	0
				<i>Lepthyphantes tenuis</i> Blackwall	Linyphiidae	896	827	176
<i>Calathus ambiguus</i> Paykull	86	10	0	<i>Linyphiidae juvenile</i>	Linyphiidae	861	421	108
<i>Calathus fuscipes</i> Goeze	938	193	157	<i>Lycosidae juvenile</i>	Lycosidae	128	129	155
<i>Calathus melanocephalus</i> L.	24	1	1	<i>Meioneta rurestris</i> C. L. Koch	Linyphiidae	182	177	66
<i>Calathus rotundicollis</i> Dejean	8	5	0	<i>Micrargus herbigradus</i> Blackwall	Linyphiidae	0	1	0
<i>Carabus auratus</i> L.	0	1	0	<i>Micrargus subaequalis</i> Westring	Linyphiidae	17	13	5
<i>Carabus monilis</i> F.	8	104	16	<i>Microlinyphia pusilla</i> Sundevall	Linyphiidae	1	1	0
<i>Carabus nemoralis</i> O.F. Mller	3	4	2	<i>Oedothorax apicatus</i> Blackwall	Linyphiidae	1,931	1,080	229
<i>Chivina fossor</i> L.	0	0	1	<i>Oedothorax fuscus</i> Blackwall	Linyphiidae	14	48	7
<i>Demetrius atricapillus</i> L.	0	1	1	<i>Oedothorax retusus</i> Westring	Linyphiidae	44	15	9
<i>Harpalus affinis</i> Schrank	77	123	68	<i>Ostearius melanopygius</i> O. P.-Cambridge	Linyphiidae	6	5	0
<i>Harpalus distinguendus</i> Duftschmid	0	5	0	<i>Pachygnatha clercki</i> Sundevall	Tetragnathidae	6	5	2
<i>Harpalus latus</i> L.	0	2	0	<i>Pachygnatha degeeri</i> Sundevall	Tetragnathidae	15	5	17
<i>Harpalus rubripes</i> Duftschmid	2	0	1	<i>Pachygnatha spec.</i>	Pachygnathidae	0	0	4
<i>Leistus ferrugineus</i> L.	0	1	0	<i>Pardosa agrestis</i> Westring	Lycosidae	4	10	0
<i>Limodromus assimilis</i> Paykull	3	1	0	<i>Pardosa amentata</i> Clerck	Lycosidae	2	0	0
<i>Loricera pilicornis</i> F.	92	103	57	<i>Pardosa palustris</i> L.	Lycosidae	6	13	25
<i>Microlestes minutulus</i> Goeze	2	15	0	<i>Pardosa prativaga</i> L. Koch	Lycosidae	0	3	3
<i>Nebria brevicollis</i> F.	53	33	14	<i>Pardosa pullata</i> Clerck	Lycosidae	2	0	0
<i>Notiophilus aesthuans</i> Dejean	1	0	0	<i>Pisaura mirabilis</i> Clerck	Pisauridae	1	0	0
<i>Notiophilus biguttatus</i> F.	2	3	8	<i>Porrothomma microphthalmum</i> O. P.-Cambridge	Linyphiidae	145	120	72
<i>Notiophilus palustris</i> Duftschmid	0	2	1	<i>Robertus neglectus</i> O. P.-Cambridge	Theridiidae	1	0	0
<i>Ophonus ardosiacus</i> Lutshnik	0	2	2	<i>Tegenaria atrica</i> C. L. Koch	Agelenidae	2	1	0
<i>Ophonus nitidulus</i> Stephens	1	2	0	<i>Tetragnathidae juvenile</i>	Tetragnathidae	1	6	0
<i>Ophonus puncticeps</i> Stephens	1	0	0	<i>Thomisidae juvenile</i>	Thomisidae	6	6	10
<i>Ophonus rufibarbis</i> F.	1	4	1	<i>Tiso vagans</i> Blackwall	Linyphiidae	4	0	1
<i>Ophonus schaubergerianus</i> Puel	0	1	0	<i>Trochosa ruricola</i> De Geer	Lycosidae	57	50	42
<i>Poecilus cupreus</i> L.	74	236	156	<i>Trochosa spec.</i>	Lycodidae	0	0	42
<i>Poecilus versicolor</i> Sturm	0	1	0	<i>Trochosa terricola</i> Thorell	Lycosidae	0	0	7
<i>Pseudophonus rufipes</i> De Geer	1,178	1,523	2,557	<i>Walckenaeria dysderoides</i> Wider	Linyphiidae	2	14	1
<i>Pterostichus madidus</i> F.	1	15	0	<i>Walckenaeria unicornis</i> O. P.-Cambridge	Linyphiidae	0	0	1
<i>Pterostichus melanarius</i> Illiger	60,023	31,119	34,827	<i>Walckenaeria vigilax</i> Blackwall	Linyphiidae	8	8	2
				<i>Xysticus bifasciatus</i> C. L. Koch	Thomisidae	1	0	0
<i>Pterostichus vernalis</i> Panzer	0	5	0	<i>Xysticus cristatus</i> Clerck	Thomisidae	2	0	0
<i>Stomis pumicatus</i> Panzer	0	1	1	<i>Xysticus kochi</i> Thorell	Thomisidae	0	2	0
<i>Synuchus vitalis</i> Illiger	9	5	7	<i>Zelotes pusillus</i> C. L. Koch	Gnaphosidae	0	1	0
<i>Trechus quadristriatus</i> Schrank	580	968	142	<i>Zelotes subterraneus</i> C. L. Koch	Gnaphosidae	0	0	1
<i>Zabrus tenebrioides</i> Goeze	0	1	0					
Total no. of individuals	64,664	38,448	39,008	Total no. of individuals	10600	5,952	3,509	1,139
Number of species	38	46	32	Number of species	50	37	35	25

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Table 3. Species activity abundances of 2001 based on exact nonparametric two-sided (1-2 α , 90%) confidence intervals

Species	Sex	ISO-INS			INS-Bt			ISO-Bt		
		Low	Est	Up	Low	Est	Up	Low	Est	Up
<i>Bathypantes gracilis</i>	Total				1.33	1.72 ^a	2.43	1.14	1.49	1.98
	♂♂				1.14	1.48	2.29			
	♀♀				1.65	1.96 ^a	2.94	1.11	1.93	2.46
<i>Bembidion quadrimaculatum</i> ^b	Total				0.15	0.21 ^a	0.30	0.21	0.27 ^a	0.51
	♂♂							0.22	0.42 ^a	0.77
	♀♀				0.24	0.44	0.93	0.12	0.27 ^a	0.51
<i>Calathus fuscipes</i>	Total							0.31	0.53	0.92
	♂♂									
	♀♀									
<i>Erigone atra</i>	Total				1.25	1.54 ^a	1.90			
	♂♂	0.54	0.67 ^a	0.83	1.46	1.88 ^a	2.36			
	♀♀	1.48	2.36 ^a	4.64						
<i>Lepthyphantes tenuis</i>	Total				1.13	1.30 ^a	1.48	1.26	1.45 ^a	1.69
	♂♂				1.12	1.38	1.62	1.21	1.43	1.74
	♀♀				1.11	1.30	1.55	1.27	1.55 ^a	1.78
<i>Loricera pilicornis</i>	Total				1.48	1.93	2.91			
	♂♂							0.36	0.57	0.84
	♀♀							0.27	0.52	0.76
<i>Meioneta rurestris</i> ^b	Total							0.18	0.28 ^a	0.46
	♂♂							0.12	0.31	0.86
	♀♀				0.22	0.32 ^a	0.58	0.15	0.26 ^a	0.47
<i>Pseudophonus rufipes</i>	Total							0.19	0.29 ^a	0.46
	♂♂							0.72	0.82	0.98
	♀♀							0.68	0.81	0.95
<i>Pterostichus melanarius</i>	Total									
	♂♂									
	♀♀									
<i>Trechus quadristriatus</i> ^b	Total				1.33	1.59 ^a	1.99	1.08	1.36	1.76
	♂♂									
	♀♀									

Lower value (low), point of estimation (est), upper value (up) for the maize variety comparison INS versus ISO, Bt versus INS, and Bt versus ISO. Only those significant values whose upper and lower confidence intervals are both <1 (decrease) or both >1 (increase) are presented.

^a Significant values based on exact nonparametric two-sided (1- α , 95%) confidence intervals.

^b Species not considered to be representative regarding its phenology, because it was detected only at the very beginning or very end of its population's growth.

sum of the individuals of one species per year of capture with two traps per plot for 2001 and 2002 (pseudo-replicates) and one trap per plot for 2003 (2001, 2002, and 2003; $n = 8$). Only those species with a significant difference by the 95% CI method ($P < 0.05$) were included, except *Pterostichus melanarius* (Carab.), which was included because of its eudominant status.

Phenology. The results of the pitfall trap counts were further represented with phenology curves, which showed the variation in the activity abundance over the course of a year. The sum of the spiders and beetles captured on the plots of each maize variety was recorded weekly. Phenology curves displayed activity abundances over a period of time and thus suggested further criteria for possible differences at the population level. The curves revealed whether the difference was caused by a steady increase or decrease in the activity abundances over time or only to a short period of divergence. As in the boxplots, only those species with significant differences by the 95% CI method ($P < 0.05$) were included in the analysis.

Results

During the study, 142,120 carabid beetles and 10,600 spiders were caught, separated, and examined. There were 57 species of carabid beetles and 50 species of spiders. The highest number of carabid species (46) was found in 2002 and the lowest number (32) in 2003. The highest number of spider species (37) was observed in 2001 and the lowest number (25) in 2003 (Table 2).

The dominant species of carabid beetle were *P. melanarius* (eudominant) and *Pseudophonus rufipes* (subdominant). *P. melanarius* had by far the highest activity abundance, as evidenced by the 125,969 individuals caught in the traps. The dominant species of spiders, all of which belonged to the family Linyphiidae, consisted of *Oedothorax apicatus* (Blackwall), *Lepthyphantes tenuis* (Blackwall), *Erigone atra* Blackwall (dominant), *Bathypantes gracilis* (Blackwall), and *Meioneta rurestris* C. L. Koch (subdominant). The 3,240 *O. apicatus* individuals had the highest activity abundance (Table 2).

Year 2001. In 2001, 7 and 10 taxa showed significant differences in activity abundance with respect to maize variety using the 95% and 90% CI methods, respectively (Table 3). Except for *Erigone atra* (Aran.), all effects were Bt-specific, in that four species showed an increase in activity abundance in Bt plots, [*Bathypantes gracilis* (Aran.), *Lepthyphantes tenuis* (Aran.), *Loricera pilicornis* F. (Carab.), *Trechus quadristriatus* Schrank (Carab.)], and five species decreased in activity abundance in Bt plots [*Bembidion quadrimaculatum* L. (Carab.), *Calathus fuscipes* Goeze (Carab.), *Meioneta rurestris* (Aran.), *Pseudophonus rufipes* (Carab.), and *Pterostichus melanarius* (Carab.)]. The strongest effect could be seen for *P. rufipes*, whose mean activity abundance decreased in Bt plots to 72% of the ISO control (Table 3).

More *B. gracilis* (Aran.) individuals were caught in traps in the Bt plots than in the plots of either of the controls. The counts for this species increased by 72% compared with the INS treatment and by 49% compared with the ISO variety (Table 3). The differences

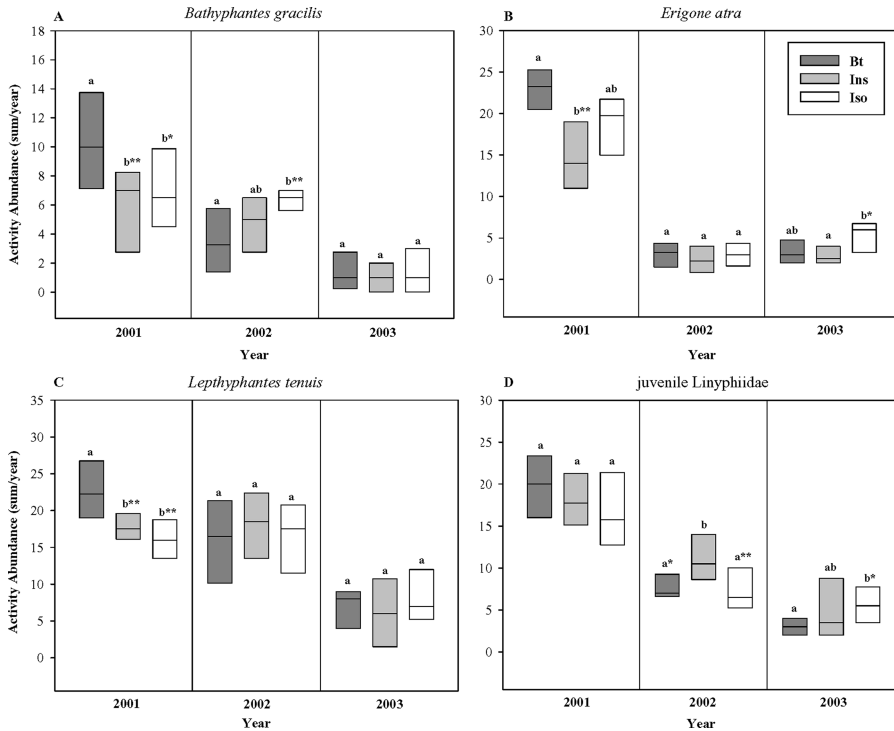


Fig. 3. Variance within the dataset ($n = 8$) of the spider species (A) *B. gracilis*, (B) *E. atra*, (C) *L. tenuis*, and (D) juvenile Linyphiidae (Aran.). The activity abundances (sum per year) for 2001, 2002, and 2003 are shown. In a boxplot, the median value (horizontal line) is between the 25th and 75th percentiles (box). Different letters above the columns indicate significant differences between respective treatments: (1) *Bt*-maize, (2) isogenic variety + insecticide (control), and (3) isogenic variety (control). **Significant difference according to the 95% CI approach; *significant difference according to the 90% CI approach.

are visualized in the corresponding boxplot (Fig. 3). The phenological course of this species also showed clear differences with respect to the three maize varieties (Fig. 4). Although fluctuations were comparable for all varieties, the size of the abundance peak for the *Bt* plot was clearly larger. For this species, the results of all methods used to evaluate the data, i.e., confidence intervals, boxplots, and phenology curves, were in agreement.

On average, 42% fewer *C. fuscipes* (Carab.) were captured in the *Bt* plots than in the ISO control. The average number of males decreased to 27% of the control and the average number of females to 53%. However, this considerable effect was not confirmed by the corresponding boxplot (Fig. 2), and the phenological method showed similar trends for the *Bt* and ISO varieties (Fig. 4). The peak number of *C. fuscipes* (Carab.) individuals captured occurred earlier and was larger in ISO plots than in *Bt* plots.

There was a significant decrease in the number of *E. atra* (Aran.) in the INS plots, in that the counts for this species in *Bt* plots were 54% higher than those in INS plots (Table 3). The median value was lower for INS than for either *Bt* or ISO (Fig. 3). The phenology curves from the three treatments all showed the same behavior, with a single peak between the 27th and 32nd week (Fig. 4). There were slight differences in the heights of the peaks.

The capture of *L. tenuis* (Aran.) was 44 and 30% higher in *Bt* plots than in ISO and INS plots, respectively (Table 3). The boxplots for the variance of this species showed the same result (Fig. 3). The phenology curves from the three maize varieties showed similar behaviors, except that on two dates, the abundance of this species was generally higher for *Bt* than ISO (Fig. 4).

The most prominent effect of the three maize varieties was on the carabid beetle species *P. rufipes*. The number of captures in *Bt* plots was 28% of that in the ISO control plot and 35% of that in the INS control plot (Table 3). A clear difference also was seen in the phenology curves from the three varieties. The plots of the two controls were characterized by increasing activity abundances of *P. rufipes* between the 31st and 37th weeks, whereas there was no such increase in *Bt* plots during that time (Fig. 4). This finding must be considered in light of the high variance within the datasets of the control varieties (Fig. 2). The low number of captures together with the small variance within the dataset from the *Bt* plots support the observed difference.

In 2001, significant differences in capture of *P. melanarius* (Carab.) in the *Bt* variety was indicated only by the 90% CI method. The individuals captured in that year reflected a reduction to 82% that of the controls, on average (Table 3); however, there was a high de-

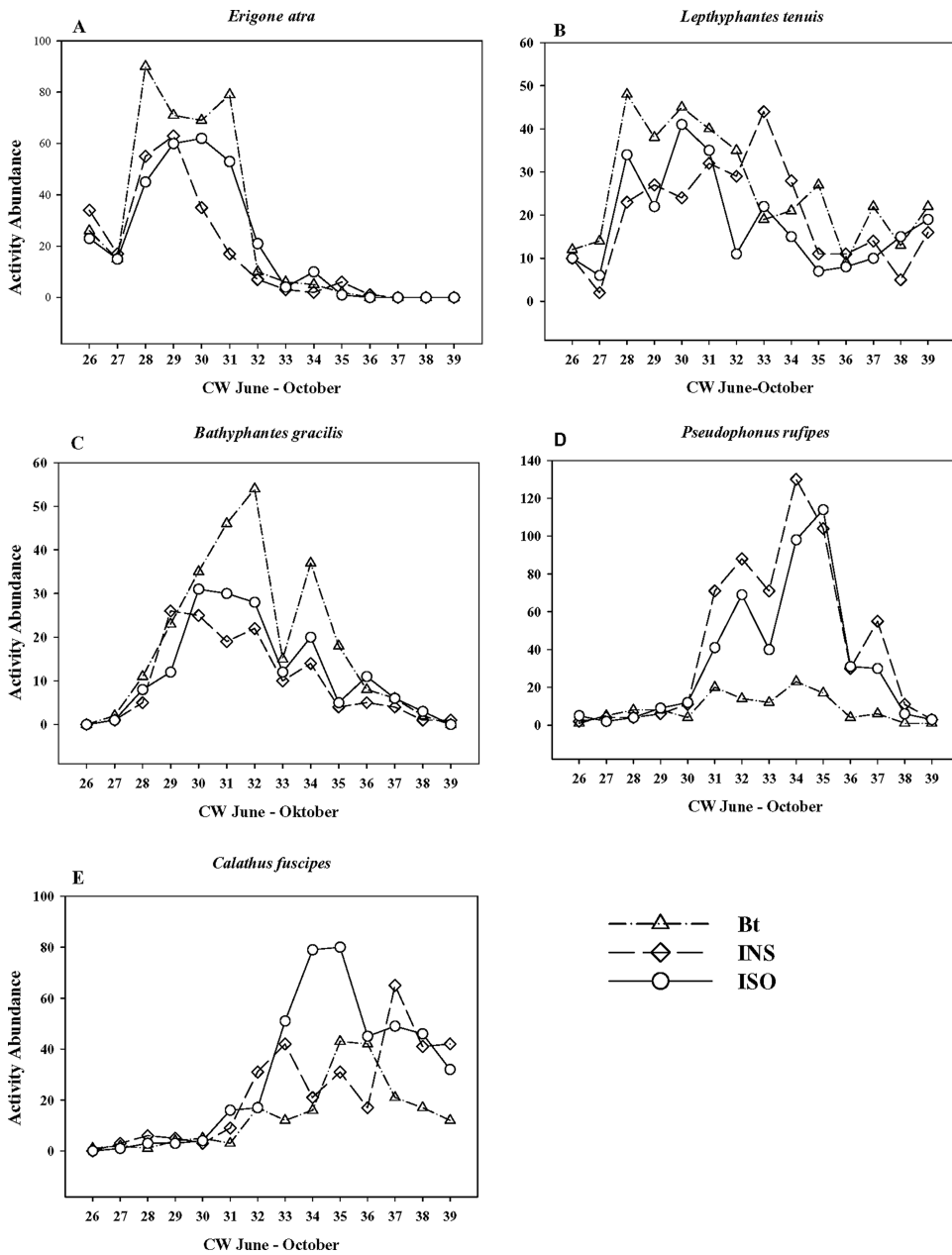


Fig. 4. Phenology of activity abundances in 2001 for the spider species (A) *E. atra*, (B) *L. tenuis*, (C) *B. gracilis*, and for the carabid species (D) *P. rufipes* and (E) *C. fuscipes*. The sum of individuals captured in the 16 pitfall traps for every calendar week (CW) and for each treatment, (1) *Bt*-maize, (2) isogenic variety + insecticide (control), and (3) isogenic variety (control), is shown.

gree of overlap between the boxplots of the three varieties (Fig. 2).

Except for the spider species *E. atra* (Aran.), whose numbers decreased in INS plots, all other species that showed a significant change in number occurred in *Bt* plots relative to the ISO plots. This result was confirmed by multivariate analysis of the species community, which also revealed a difference for *Bt* plots in 2001; that is, the INS and ISO vectors pointed in one

direction and the *Bt* vector in the other (Fig. 5). At the same time, the variance of the *Bt* sites within the dataset was lower than that of the control sites. The longest gradient within the dataset is represented by the axis of the abscissa. Because the *Bt* variety vector and the vectors of the control varieties corresponded to this gradient, it can be concluded that the main differences in the data resulted from the difference between the *Bt* and the control varieties.

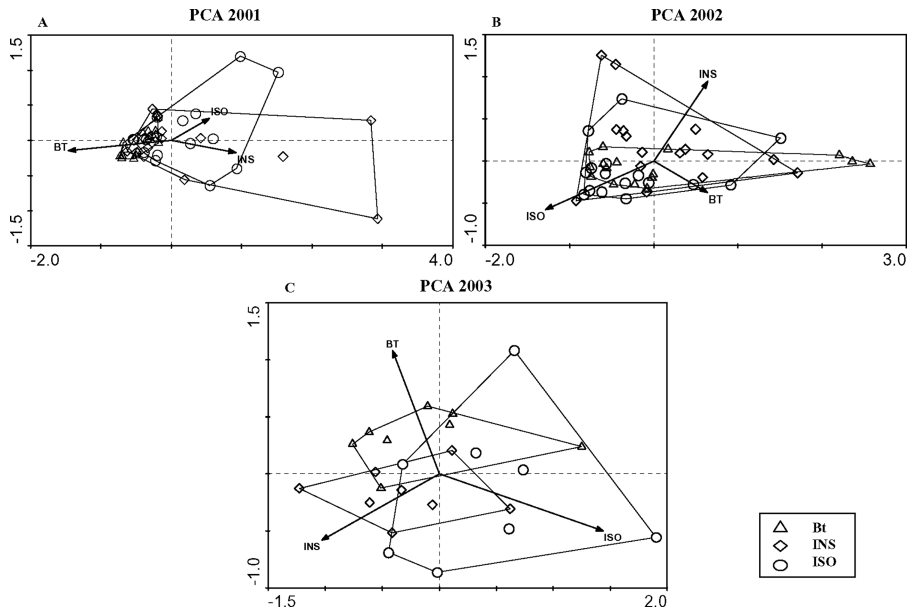


Fig. 5. PCA of all species. Significant differences in activity abundances according to the 90% CI approach were observed for all species and each treatment (*Bt*, *INS*, and *ISO*), except *P. melanarius*. Each point represents the sum of the spiders and carabid beetles captured in one pitfall trap. The vectors show the main direction of the distribution for each treatment. The classified sites (*Bt*, *ISO*, *INS*) are gathered within the envelopes. The units of the axes are relative to the heterogeneity within the dataset. (A) 2001, with nine species of carabid beetles and spiders from 16 pitfall traps. The eigenvalue of the two axes is 76.6%. Precondition: length of gradient DCA: 0.59. (B) 2002 with six species of carabid beetles and spiders from 16 pitfall traps. The eigenvalue of the two axes is 88.0%. Precondition: length of gradient DCA: 0.96. (C) 2003 with four species of carabid beetles and spiders from eight pitfall traps. The eigenvalue of the two axes is 66.6%. Precondition: length of gradient DCA: 0.62.

Year 2002. In 2002, four species were identified that differed significantly with respect to activity abundance on the three maize varieties using the 95% CI method, and seven with the 90% CI method (Table 4). Only *B. gracilis* (Aran.) and *P. melanarius* (Carab.) showed an effect for *Bt* versus *ISO*—and only according to the 90% CI method. All other effects were relative to the *INS* treatment.

The counts for *B. gracilis* (Aran.) were lower in *Bt* maize plots than in the isogenic control plots. On average, the number captured for this species decreased to 52% of that in the isogenic control plots (Table 4); for females, the decrease was even greater, to 38%. The difference was validated with the boxplot (Fig. 3). The phenology of *B. gracilis* on each of the three types of maize was very similar, although the peak between the 29th and 31st weeks was more prominent for the *ISO* and, to a lesser extent, the *INS* controls (Fig. 6).

None of the juveniles belonging to the family *Linyphiidae* could be identified to the species level, and thus were classified as “juvenile *Linyphiidae*.” For this group of individuals, there was a significant (63%) increase in the number of captures in the *INS* plot compared with captures in the *ISO* control, and there was a reduction in the number of captures in the *Bt* plots, down to 70% of the *INS* control (Table 4). The difference for the *INS* treatment also is evident in the boxplot (Fig. 3), where the median value was significantly different from that in the *ISO* or *Bt* boxplots.

In the phenology curve for juvenile *Linyphiidae* *INS* plots, there was one high peak of individuals in the 30th week (Fig. 6). This could be because of capture of individuals from a nearby spider nest.

No uniform difference in the captures of *P. rufipes* (Carab.) was detected for the three maize varieties. Females increased by 76% in the *INS* plots compared with the *ISO* control (Table 4). Based on the 90% CI approach, the number of females in *Bt* plots was 28% lower than in the *INS* control plots. The boxplots showed a slight difference in the *INS* treatment compared with the other two treatments (Fig. 2). The phenology curves were similar for all treatments except for *Bt* during the 36th week, and the counts for the *ISO* control were generally lower, especially in the 33rd and 35th weeks (Fig. 6).

The 90% CI approach indicated only slight differences in the number of *P. melanarius* (Carab.) captured in the plots of the three maize treatments (Table 4). The differences were not supported by either the boxplots (Fig. 2) or the phenology curves.

As shown by PCA for 2002 (Fig. 5), there were no significant differences in the community structures for the three treatments. The vectors for the main distributions of the treatments pointed in opposite directions, indicating that there were only small differences between the controls. However, no specific or significant changes occurred because the envelopes of the three treatments overlapped widely.

Table 4. Species activity abundances of 2002 based on exact nonparametric two-sided (1-2 α , 90%) confidence intervals

Species	Sex	ISO-INS			INS-Bt			ISO-Bt		
		Low	Est	Up	Low	Est	Up	Low	Est	Up
<i>Bathyphantes gracilis</i>	Total							0.38	0.52 ^a	0.76
	♀ ♀							0.22	0.38 ^a	0.67
<i>Bembidion tetracolum</i> ^b	Total	1.25	1.92	3.38						
<i>Linyphiidae juvenil</i>	Total	1.25	1.63 ^a	2.10	0.57	0.70	0.86			
	♂ ♂				0.18	0.38	0.76			
	♀ ♀	2.82	4.16 ^a	31.00	0.05	0.31	0.61			
<i>Loricera pilicornis</i>	Total				0.24	0.52	0.72			
<i>Meioneta rurestris</i> ^b	Total	0.34	0.51	0.68	1.91	2.40 ^a	3.90			
	♀ ♀				1.91	8.43	21.00			
<i>Pseudophonus rufipes</i>	Total	1.22	1.72	2.21						
	♂ ♂	1.12	1.60	2.43						
	♀ ♀	1.28	1.76 ^a	2.13	0.53	0.72	0.96			
<i>Pterostichus melanarius</i>	Total							1.00	1.25	1.58
	♂ ♂							1.17	1.40	1.65

Lower value (low), point of estimation (est), upper value (up) for the maize variety comparison INS versus ISO, Bt versus INS, and Bt versus ISO. Only those significant values whose upper and lower confidence intervals are both <1 (decrease) or both >1 (increase) are presented.

^a Significant values based on exact nonparametric two-sided (1- α , 95%) confidence intervals.

^b Species not considered to be representative regarding its phenology, because it was detected only at the very beginning or very end of its population's growth.

Year 2003. In 2003, the 95% CI approach showed significant differences in only one species with respect to treatment, but differences in four species were observed using the 90% CI approach (Table 5). *O.*

apicatus (Aran.) was the only species that changed significantly, by the 95% CI approach; however, its phenological behavior was not representative with respect to population growth during the year.

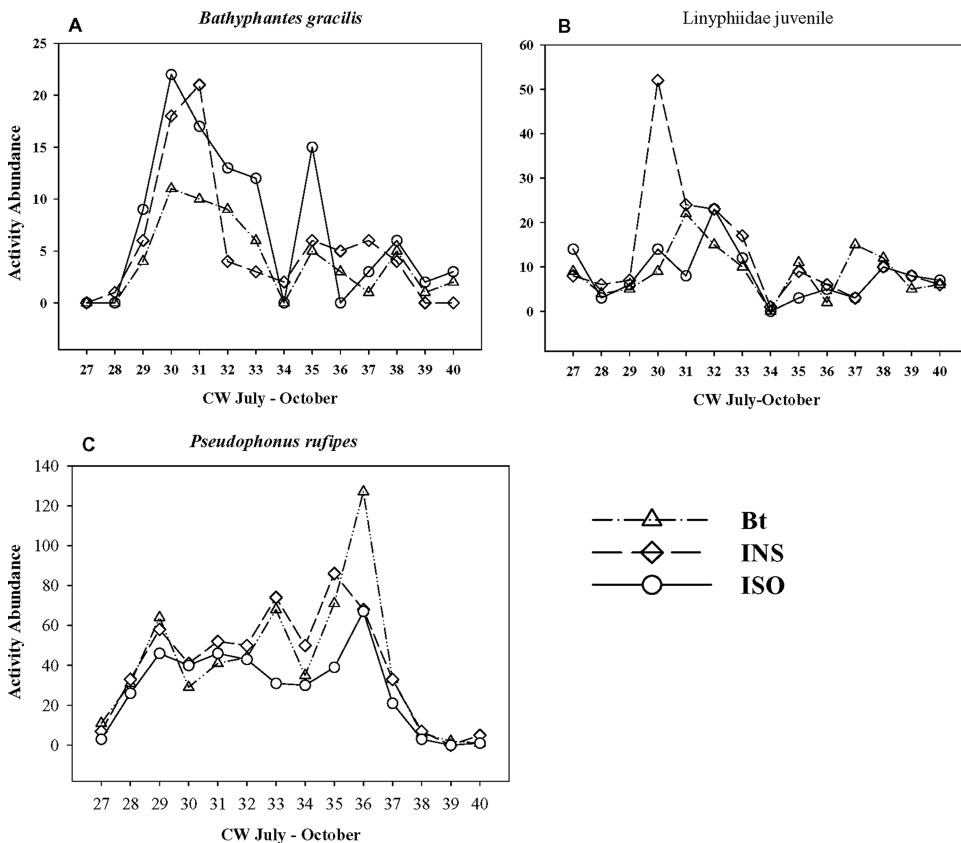


Fig. 6. Phenology of activity abundances in 2002 for (A) *B. gracilis* (Aran.), (B) juvenile *Linyphiidae* (Aran.), and (C) *P. rufipes* (Carab.). The sum of individuals captured in the 16 pitfall traps for every calendar week (CW), and each treatment, (1) Bt-maize, (2) isogenic variety + insecticide (control), and (3) isogenic variety (control), is shown.

Table 5. Species activity abundances of 2003 based on exact nonparametric two-sided (1-2 α , 90%) confidence intervals

Species	Sex	ISO-INS			INS- <i>Bt</i>			ISO- <i>Bt</i>		
		Low	Est	Up	Low	Est	Up	Low	Est	Up
<i>Erigone atra</i>	Total	0.31	0.54	0.76						
	♂♂	0.11	0.35	0.70						
<i>Linyphiidae juvenile</i>	Total							0.41	0.54	0.80
<i>Oedothorax apicatus</i> ^b	Total							0.28	0.47	0.70
	♂♂				0.38	0.58	0.84	0.26	0.39 ^a	0.67
	♀♀							0.34	0.59	0.80
<i>Trechus quadristriatus</i>	Total	0.22	0.47	0.75						

Lower value (low), point of estimation (est), upper value (up) for the maize variety comparison INS versus ISO, *Bt* versus INS, and *Bt* versus ISO.

Only those significant values whose upper and lower confidence intervals are both <1 (decrease) or both >1 (increase) are presented.

^a Significant values based on exact nonparametric two-sided (1- α , 95%) confidence intervals.

^b Species not considered to be representative regarding its phenology, because it was detected only at the very beginning or very end of its population's growth.

The PCA results for 2003 showed no significant differences in community structure because the envelopes of the three treatments overlapped widely. The vectors for the three treatments pointed in different directions, i.e., no grouping of any treatment was observed (Fig. 5).

Discussion

Clear changes in the activity abundances of carabid and spider coenoses were found in 2001. The counts of several species (four species in the 95% CI approach and six in the 90% CI approach) of carabids and spiders captured in *Bt* maize plots differed significantly from the counts of the same species captured in the control plots. Only one species was affected by the INS treatment in that year. Multivariate analysis also showed that, in 2001, changes occurred in the community of epigeic predators in *Bt* plots. Although changes in the activity abundances of single species in 2002 were also detected, these were mostly differences between the counts in the INS versus the ISO plots, whereas changes in the counts in the *Bt* plots versus the isogenic control plots were detected only for *B. gracilis* (Aran.). In contrast to 2001, multivariate analysis showed no significant changes in coenosis for 2002.

2003 was characterized by a homogeneous distribution of most species. No collected species underwent significant changes, as indicated by the 95% CI approach, for any of the maize treatments. In the more sensitive 90% CI approach, only juvenile *Linyphiidae* showed differences for *Bt*, whereas community analysis with multivariate statistics showed no effect for any variety.

Both of the fields used in the 3-yr field trial underwent a different type of crop cultivation at the start of the study. Whereas before the study, wheat and maize were cultivated, during the study only maize was grown. Furthermore, in contrast to usual crop management practice, the maize fields were not deeply plowed after harvest; instead, the litter was mulched and superficially grubbed. Although such changes could have affected species abundances over the course of the study, the advantages of this approach were the accumulation of plant residues and thus of *Bt*

protein in the soil, with potential reinforcement of any *Bt* protein effect.

The maize plants were highly infested by cornborers in 2001. During August and September of that year, invasion coincided with a period of high temperatures. Whereas *Bt* maize was green and grew well (average plant height >2 m, greening effect) until harvest, plants of the ISO plots, and to a lesser extent the INS plots, were brown, dry, and often damaged by cornborer activity. In 2002 and 2003, there were no drastic differences in the growth of maize plants in the three treatment plots that could be ascribed to the cornborer. Nonetheless, differences in the site conditions during the 3 study yr must be considered when interpreting the results.

The *Bt* protein content of the plants was higher in 2001 and 2003 (Nguyen 2004). Thus, the influence of direct or indirect toxicity on predator populations should have been detectable mainly during these 2 yr.

The activity abundances in the plots of the three maize treatments could be differentiated by community analysis only in 2001, whereas in 2002 and 2003, they were more or less indistinguishable. This result was most likely caused by the different properties (moisture, temperature, etc.) of the sites resulting from differences in the amount of cornborer damage in 2001 versus 2002 and 2003. Species that showed significant changes in numbers among the three maize treatments, i.e., *B. gracilis* (Aran.) and *E. atra* (Aran.), are hygrophilic regarding their ecological needs (Martin 1991, Maurer and Hänggi 1990), as is *L. pilicornis* (Carab.) (Koch 1989), which only showed a small but significant difference in activity abundance as detected by the 90% CI method (Table 3). In 2001, all these species showed significant increases in abundance in the *Bt* plots compared with the control plots. *P. rufipes* (Carab.), *B. quadrimaculatum* (Carab.), and *C. fuscipes* (Carab.), as well as *M. rurestris* (Aran.), can be characterized as xerophilic species (Koch 1989, Martin 1991), and their numbers in the *Bt* plots decreased in 2001. The lower number of xerophilic species and the higher number of hygrophilic species in plots of densely growing *Bt* maize could explain these significant changes in abundance. Carabid distribution within fields is known to be as associated with micro-

climate properties, especially moisture (Thomas et al. 2002). However, during the study, there was no correlation between species abundance and the higher concentration of *Bt* protein in 2001 and 2003 as mentioned above (Nguyen 2004).

The changes in field management implemented at the beginning of the study led to obvious changes in abundances during this 3-yr period. Whereas species such as *P. rufipes* (Carab.) continuously increased in number, contrary changes occurred in the abundance of some spider species, e.g., *L. tenuis* (Aran.) and *O. apicatus* (Aran.) (Table 2). However, these species showed no significant changes with respect to maize variety.

In this study, no effect of *Bt* maize cultivation could be detected, i.e., no harm of epigeic predators was evident. Although increases and decreases in abundances were detected for single species in the studies of Lozzia (1999), Hawes et al. (2003), and Brooks et al. (2003), no differences in the transgenic maize coenoses of carabid beetles and spiders were found by Hassel and Shepard (2002), Volkmar and Freier (2003), Meissle and Lang (2005), and Ludy and Lang (2006) (spiders), Lozzia (1999) and French et al. (2004) (carabid beetles), or Candolfi et al. 2004, de la Poza et al. 2005, and Daly and Buntin 2005 (spiders and carabid beetles). The results of Lozzia (1999) showed no difference of coenosis based on diversity, but rather changes in single species, including *P. rufipes* and *B. quadrimaculatum*. For *P. melanarius*, Dammer and Heyer (1997) found that the abundance of species can change from year to year. Similar to the results of this study, Lozzia (1999), French et al. (2004), Candolfi et al. (2004), and Bhatti et al. (2005) found big differences between years, but no differences between GM maize and the control. However, it should be kept in mind that, in each of these studies, different methods were used—i.e., field design, plot size, number of replicates, statistical evaluation, taxonomic resolution; therefore, comparability is limited.

Many carabid species can move around an area of several hectares, whereas some species do not move in this range (Wallin and Ekbohm 1994, Thomas et al. 1998). Additionally, ballooning provides spider species in the families Linyphiidae and Theridiidae with high mobility (Bishop and Riechert 1990, Frank and Nentwig 1995). To minimize the effects of adjacent plots, Prasifka et al. (2005) recommended using large plot sizes (>18 by 18 m). In this study, the plot size was in the recommended range, but because a sufficient number of replicates was deemed of similar importance, the plot size and design were adjusted to accommodate both.

Brooks et al. (2003) emphasized the importance of species level resolution, because averaging over families or orders tended to mask opposing effects (see also Holland et al. 2002). This conclusion was confirmed for both spiders and carabid beetles in this study.

Statistical power is limited by the activity abundance of the species. Because ≈60% of the spider and carabid species had only low activity abundances (sum per year = 1–5 individuals), it may be difficult to detect a changed population. However, these rare species are

typical in predator communities on arable land (Luff 2002, French et al. 2004) and could have been also affected by the cultivation of GM maize. To detect an impact on these species, one should compare their degree of presence through long-term monitoring.

The results of the statistical approaches in which 95 and 90% CIs were calculated showed that it is both useful and necessary to test for differences between varieties using sensitive statistical techniques. In 2001, which was characterized by clear changes in biocoenoses in *Bt* maize compared with either of the isogenic controls, three additional affected species were detected with the more sensitive, 90% CI approach (*P. melanarius* (eudominant Carab.), *L. pilicornis* (Carab.), and *M. rurestris* (Aran.). In field studies aimed at identifying potential effects of GM crops on the environment and the consumer, the number of replicates ($n = 8$) may be high for a field evaluation but low for statistical needs. This further underlines the importance of using sensitive analytical methods, such as those applied in this study.

In summary, no detrimental effect of the cultivation of *Bt* maize was observed for either spiders or carabid beetles in a 3-yr study. Differences that were detected for some species in the first year were caused by a high cornborer infestation followed by microclimate changes.

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