

## Effect of Bt-Maize on Epigeic Spiders (Araneae) and Harvestmen (Opiliones)

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### Abstract

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The effect of two strategies, used to control *Ostrinia nubilalis*, on the abundance and diversity of epigeic spiders and harvestmen in maize was monitored. The two strategies were (1) transgenic insect-resistant maize, and (2) biological control by *Trichogramma* wasps on an isogenic maize hybrid. They were compared with a conventional system (isogenic maize hybrid), which was taken as the control. The investigation was performed at two localities (Ivanovice na Hané and Prague) in the Czech Republic from 2002 to 2004. Spiders (Araneae) and harvestmen (Opiliones) were collected by means of pitfall traps. We found that the annual abundance and diversity of arachnids on plots with the two strategies were not significantly different from a conventional system. The overall abundance of spiders decreased over three years in all systems. There was no difference in the family and guild (hunters *versus* web-builders) composition between strategies and the conventional system. A displacement of linyphiid spiders with lycosids on all plots over time was observed. This change is attributed to the existence of a sparse weed cover of maize fields as a result of consecutive monocultural planting.

**Keywords:** Bt-maize; diversity; *Trichogramma*

The European corn borer, *Ostrinia nubilalis* Hubner, is one of the most important pests of maize in Central Europe (SZOKE *et al.* 2002). Besides direct damage to plants caused by boring larvae, their injuries facilitate infection by *Fusarium* species (GATCH & MUNKVOLD 2002). Several methods have been used to control *O. nubilalis*: pesticide applications (CAGAN *et al.* 1995), ploughing (BIGLER *et al.* 1995), and very recently development of insect resistant maize (SMITH 1997).

The use of pesticides led to dramatic changes in the composition of non-target organisms (STEVENSON *et al.* 2002). In this respect, the use of transgenic insect-resistant crops that carry a gene from *Bacillus thuringiensis* responsible for intracellular production of the endotoxin Cry1A, appear to be “safer”. But as the endotoxin can be released to the environment (SAXENA & STOTZKY 2000) it can affect non-target organisms. Therefore, considerable attention has been paid to the introduction

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of genetically modified crops into agroecosystems (OBRYCKI *et al.* 2001). Large scale studies found no or only negligible effect of Bt maize on a complex of natural enemies (CANDOLFI *et al.* 2004). But laboratory investigations of a particular predator/prey trophic system showed a detrimental effect on the predator's fitness characteristics (PRUTZ *et al.* 2004).

Spiders are one of the most important natural enemies in various crops, including maize (NYFFELER & BENZ 1987). In Central Europe, 30 to 60 species and belonging to several families are frequently occurring in agrobiocenoses (PEKÁR 1997). Among these, particularly the Linyphiidae and Lycosidae are the most abundant on the ground surface. As the great majority of spiders are polyphagous, i.e. feeding on a variety of invertebrates, they are not able of prey population tracking that is typical of specialised predators (RIECHERT 1992). Nevertheless, they contribute to the suppression of pests and have a potential to limit lepidopterans, including *O. nubilalis* (NYFFELER & SUNDERLAND 2003).

Unlike pesticide management systems, the use of transgenic insect-resistant crops had no negative effect either on the diversity or abundance of beneficial arthropods (REED *et al.* 2001). Similarly, no adverse effects on arachnafauna were found in the use of Bt maize investigated in several countries in Europe: the Czech Republic (SEHNAL *et al.* 2004), Hungary (ARPAS *et al.* 2004), Germany (VOLKMAR *et al.* 2004) and Italy (LOZZIA 1999).

In this study we aimed to monitor the three-year effects of two different strategies, used to control *O. nubilalis*, on epigeic spiders and harvestmen in maize. The two strategies were (1) transgenic insect-resistant maize (Bt maize), and (2) biological control by *Trichogramma* wasps on an isogenic maize hybrid or a local hybrid. These were compared with a conventional system based on the use of isogenic maize hybrid (or local hybrid). We expected that the two strategies will have a negligible effect on the epigeic fauna of arachnids.

## MATERIALS AND METHODS

Due to space restrictions the experimental study could not be done in a randomised block design with true replications in a single locality. Instead, two distant localities were used: Prague-Ruzyně (central Bohemia) and Ivanovice na Hané (central Moravia). The locality in central Moravia is

permanently infested with *O. nubilalis*, whereas in central Bohemia *O. nubilalis* has spread only recently due to global warming and the use of early maize varieties. The study was conducted for three years, from 2002 until 2004. At each locality a 1 ha plot was selected. The whole plot was divided into three smaller plots (each of 0.3 ha), situated side by side. On one plot Bt maize hybrid MON 810 (MEB307Bt-Monumental in 2002 and 2003, DKC 3421YG in 2004, both Monsanto) was planted. Varieties susceptible to *O. nubilalis* were planted on the remaining two plots: on one the isogenic hybrid Monumental (in 2002 and 2003) and DKC 3420 (in 2004) for the *Trichogramma* strategy, on the second the local hybrid Raissa for the conventional system. The plots remained at the same place for the three years of study. All around the plots an 8 m wide strip of the hybrid Raissa was planted. The maize was sown in May and harvested at the end of September each year, except for 2004 when it was harvested in early December. On all plots the straw was crushed and deep ploughed after harvest.

All plots were treated with a pre-emergent (Guardian, dose 2.5 l/ha) and a post-emergent (Grid, dose 20 g/ha) herbicide. On the plots with the Bt maize and the conventional system no other application was made. On the plot with biological control strategy, *Trichogramma* wasps (Trichocarp, Biocont Laboratory) were released. Release dates were based on the reproductive activity of *O. nubilalis*.

The epigeic communities of arthropods were monitored using pitfall traps. A plastic cup (opening 8 cm, 300 ml volume) was half filled with 4% formaldehyde and made flush with the soil surface. The trap was covered with a steel roof. In each plot three traps were placed in the diagonal so that the distance between traps was 8 m. From May to September the traps were, in fortnightly cycles, open for 7 days, then emptied, and closed with a lid for another 7 days. In total there were 8 to 11 collections each year. Spiders and harvestmen were identified according to NENTWIG *et al.* (2005) and ŠILHAVÝ (1971), respectively.

Data on the annual abundance of each species were first subjected to a multivariate analysis (TER BRAAK & ŠMILAUER 2002), detrended correspondence analysis (DCA). Short gradient (2.3) suggested a linear response between abundance and the gradient of factors, therefore redundancy analysis (RDA) was used. Each locality was ana-

lysed separately as the composition of arachnids differed. Significance of two factors (treatment, year of study) was assessed by the Monte Carlo permutation test (MCPT) with 499 simulations. In order to evaluate the effect of treatments, the localities were considered as replications and traps within each plot as pseudoreplications. Data on annual abundance of all arachnids, the five most abundant families (Linyphiidae, Lycosidae, Phalangiidae, Theridiidae, Tetragnathiidae), two guilds (hunters, web-builders) and annual diversities (Shannon-Weaver index (LUDWIG & REYNOLDS 1988)), were analysed separately using split-plot ANOVA within R (R Development Core Team 2004), with a treatment as a factor and year of study as a covariate. Split-plot ANOVA was applied to take account of spatial pseudoreplications in the design (traps nested within plots, and plots nested within localities). As the errors in the data were not normally distributed and the variance was not homogeneous, log transformation was applied to the data prior to each analysis (CRAWLEY 2002).

## RESULTS

In total, 2686 individuals of spiders (belonging to 60 species) and 615 individuals of harvestmen (belonging to 5 species) were captured during three years (Table 1). Multivariate analysis showed

that there is no significant effect of the treatment (MCPT,  $P > 0.14$ ) on the community of arachnids in both localities. But there was a significant effect of the year of study (MCPT,  $P = 0.002$ ). Linyphiids species, e.g. *E. atra*, *E. dentipalpis* and *O. apicatus*, were most abundant in 2002; harvestmen species, *O. saxatilis*, *P. opilio* and *N. semproni*, and a lycosid, *P. palustris*, were most abundant in 2003; and a theridiid, *R. arundineti*, was most abundant in 2004 (Figure 1).

The total annual abundance of spiders decreased significantly during the study (ANOVA,  $P = 0.0004$ ), with no difference between treatments (ANOVA,  $P = 0.84$ ). In 2002 there were on average 79.8 (SE = 10.4) individuals, in 2003 there were 51.0 (SE = 3.9) individuals and in 2004 there were 47.2 (SE = 3.6) individuals. The diversity was similar for both strategies and the conventional system (ANOVA,  $P = 0.12$ ) and did not change during the study (ANOVA,  $P = 0.90$ ). The Shannon-Weaver index of diversity was on average 1.76 (SE = 0.04).

The annual abundance of linyphiid spiders and phalangiid harvestmen changed significantly during the study (ANOVA,  $P < 0.008$ ), with no difference between treatments (ANOVA,  $P = 0.84$ ). The abundance of linyphiids decreased gradually, whereas that of phalangiids increased in the second year, and then dropped. For lycosid and

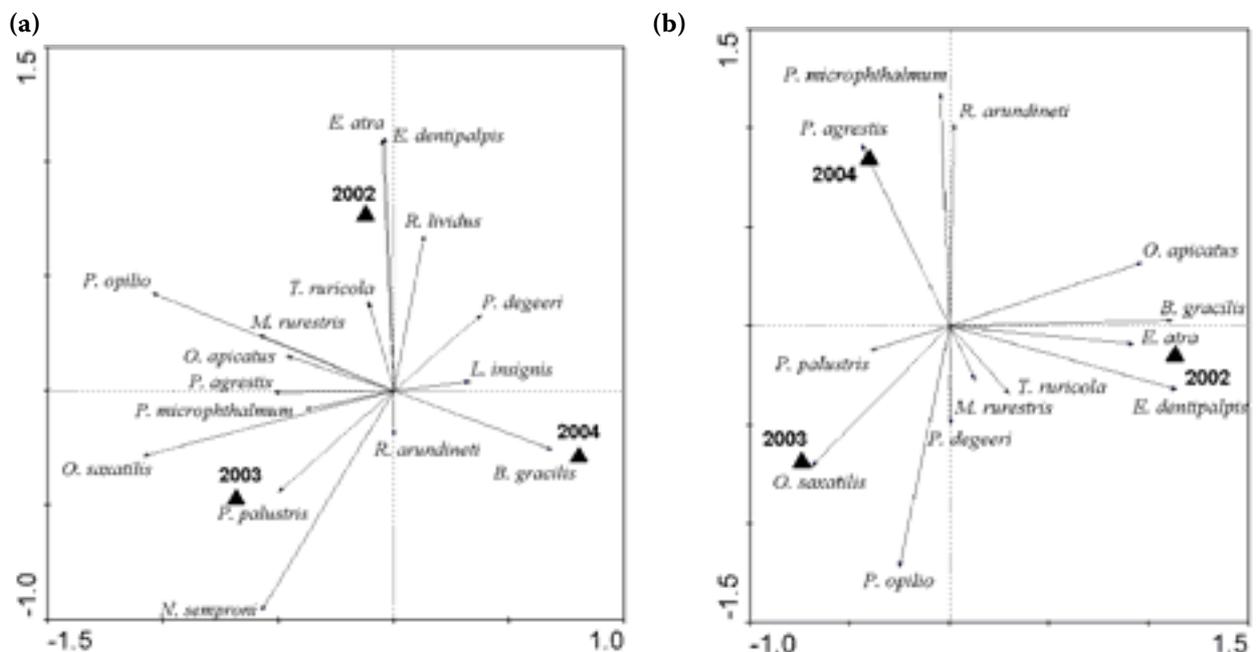


Figure 1. Ordination diagrams of RDA showing the effect of year of study on the abundance of spiders and harvestmen (underlined) in Ivanovice (a) and Prague (b). The eigenvalues being  $\lambda_1 = 0.127$  and  $\lambda_2 = 0.081$  for Ivanovice;  $\lambda_1 = 0.374$  and  $\lambda_2 = 0.139$  for Prague. Species with abundance lower than 1% of the total capture are not displayed

Table 1. List of harvestmen (Opiliones) and spiders (Araneae) captured at two localities (plots pooled) during three years of study. Numbers are total annual captures. The percentages represent dominance of species or families within the respective order

	Ivanovice			Prague			(%)
	2002	2003	2004	2002	2003	2004	
Opiliones							
Trogulidae							0.52
<i>Trogulus tricarinatus</i> (Linnaeus)			2	1			0.52
Leiobunidae							6.09
<i>Nelima semproni</i> Szalay				5	21	9	6.09
Phalangiidae							93.4
<i>Opilio saxatilis</i> C. Koch		28	3	18	46	6	16.8
<i>Phalangium opilio</i> Linnaeus	78	196	36	66	70	24	75.5
<i>Rilaena triangularis</i> (Herbst)	1	1				4	1.04
Araneae							
Pholcidae							0.04
<i>Pholcus opilionoides</i> (Schrank)			1				0.04
Dysderidae							0.19
<i>Dysdera erythrina</i> (Walckenaer)	1	1					0.08
<i>Harpactea rubicunda</i> (C. L. Koch)		2	2				0.11
Mimetidae							0.11
<i>Ero aphana</i> (Walckenaer)		1	2				0.11
Theridiidae							5.06
<i>Achaearanea riparia</i> (Blackwall)					2	2	0.15
<i>Enoplognatha thoracica</i> (Hahn)	1						0.04
<i>Neottiura bimaculata</i> (Linnaeus)	1						0.04
<i>Robertus arundineti</i> (O. P. Cambridge)	7	3	18	21	23	32	3.95
<i>R. lividus</i> (Blackwall)				13	3	6	0.84
<i>Steatoda phalerata</i> (Panzer)			1				0.04
Linyphiidae							77.3
<i>Bathyphantes gracilis</i> (Blackwall)	90	2	9	14	13	37	6.32
<i>Ceratinella brevipes</i> (Westring)					1		0.04
<i>Diplocephalus cristatus</i> (Blackwall)				1			0.04
<i>D. latifrons</i> (O. P. Cambridge)						1	0.04
<i>Diplostyla concolor</i> (Wider)	7		3	1		2	0.50
<i>Erigone atra</i> (Blackwall)	123	4	1	16	2	3	5.67
<i>E. dentipalpis</i> (Wider)	30	1	3	19	3	3	2.26
<i>Lepthyphantes insignis</i> O. P. Cambridge				10	7	12	1.11
<i>L. pallidus</i> (O. P. Cambridge)	1					2	0.11
<i>L. tenuis</i> (Blackwall)	5		2		2	2	0.42
<i>Maso sundevalli</i> (Westring)	1						0.04
<i>Meioneta fuscipalpis</i> (C. L. Koch)	1						0.04
<i>M. rurestris</i> (C. L. Koch)	32	33	21	17	16	6	4.56
<i>Micrargus herbigradus</i> (Blackwall)					1		0.04
<i>M. subaequalis</i> (Westring)	2		1	1			0.19
<i>Microlinyphia pusilla</i> (Sundevall)	1						0.04
<i>Oedothorax apicatus</i> (Blackwall)	453	76	114	237	266	189	50.2

Table 1 to be continued

	Ivanovice			Prague			(%)
	2002	2003	2004	2002	2003	2004	
<i>Ostearius melanopygius</i> (O. P. Cambridge)						1	0.04
<i>Porrhomma errans</i> (Blackwall)				4	4	1	0.34
<i>P. microphthalmum</i> (O. P. Cambridge)	21	16	83	6	8	4	5.06
<i>Silometopus reussi</i> (Thorell)	1				1		0.08
<i>Trematocephalus cristatus</i> (Wider)				1			0.04
<i>Walckenaeria vigilax</i> (Blackwall)				2	1		0.11
Tetragnathiidae							1.76
<i>Pachygnatha clercki</i> Sundevall			1				0.04
<i>P. degeeri</i> Sundevall	14	26	9	2		2	1.72
Lycosidae							13.8
<i>Aulonia albimana</i> (Walckenaer)		2	1				0.08
<i>Alopecosa cuneata</i> (Clerck)	1						0.04
<i>A. pulverulenta</i> (Clerck)	3		1				0.15
<i>Pardosa agrestis</i> (Westring)	44	78	127	9	13	7	9.89
<i>P. amentata</i> (Clerck)						1	0.04
<i>P. palustris</i> (Linnaeus)	7	18	15		3		1.69
<i>P. prativaga</i> (L. Koch)		2	2				0.11
<i>Trochosa ruricola</i> (De Geer)	3	2		13	10	7	1.34
<i>Xerolycosa miniata</i> (C. L. Koch)	1	4	3			1	0.31
<i>X. nemoralis</i> (Westring)		2	1			1	0.11
Agelenidae							0.34
<i>Agelena gracilens</i> C. L. Koch		2					0.04
<i>Tegenaria agrestis</i> (Walckenaer)		4	3		2		0.31
Hahniidae							0.04
<i>Hahnia nava</i> (Blackwall)		2					0.04
Dictynidae							0.19
<i>Argenna subnigra</i> (O. P. Cambridge)		2	3				0.15
<i>Lathys humilis</i> (Blackwall)				1			0.04
Liocranidae							0.08
<i>Phrurolithus festivus</i> (C. L. Koch)				1		1	0.08
Zodariidae							0.04
<i>Zodarion rubidum</i> Simon				1			0.04
Gnaphosidae							0.38
<i>Drassodes</i> sp.		1					0.04
<i>Drassyllus praeficus</i> (L. Koch)	3		3				0.23
<i>D. pusillus</i> (C. L. Koch)			2				0.08
<i>Trachyzelotes pedestris</i> (C. L. Koch)			1				0.04
Thomisidae							0.65
<i>Ozyptila simplex</i> (O. P. Cambridge)		3					0.08
<i>Xysticus cristatus</i> (Clerck)			1				0.04
<i>X. kochi</i> Thorell	4	1	6		2	1	0.54
Salticidae							0.08
<i>Euophrys frontalis</i> (Walckenaer)		2	1				0.08
<b>Sum</b>	<b>937</b>	<b>515</b>	<b>482</b>	<b>480</b>	<b>520</b>	<b>367</b>	

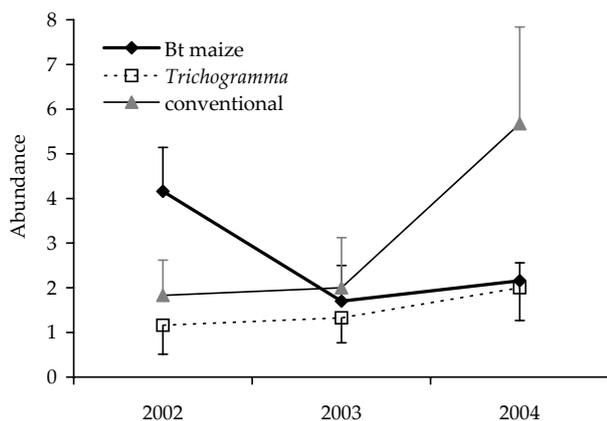


Figure 2. Change in the abundance (mean  $\pm$  SE) of theridiid spiders in two control strategies and the conventional system over three years of study. Data from two plots for each strategy were pooled

tetragnathid spiders there was neither a difference between treatments (ANOVA,  $P > 0.81$ ) nor during years (ANOVA,  $P > 0.18$ ). For theridiid spiders the abundance differed between treatments and years (ANOVA,  $P = 0.019$ ); on the conventional plot their abundance increased with years, whereas in Bt maize it decreased and on the *Trichogramma* plot it did not change (Figure 2).

A comparison of abundances of the two guilds, hunters and web-builders, showed no difference between treatments (ANOVA,  $P = 0.88$ ). But there was a marginally significant decrease of web-building spiders and slight increase of hunting spiders (ANOVA,  $P = 0.07$ ) over the course of the study. At the beginning of the study, web-builders were almost seven times more abundant than hunters (ANOVA,  $P = 0.003$ ), while in the last year web-builders were less than twice more abundant (Figure 3).

## DISCUSSION

We had hypothesised that there would be no difference between the two strategies and the conventional system in their effect on the abundance and diversity of epigeic arachnids as a result from the direct use of Bt maize or parasitoid release. The results support this prediction. The two strategies had a similar effect. No difference in the abundance or diversity of epigeic arachnids was observed despite the fact that the abundance of *O. nubilalis* differed markedly between plots (KOCOUREK & ŘÍHA unpublished).

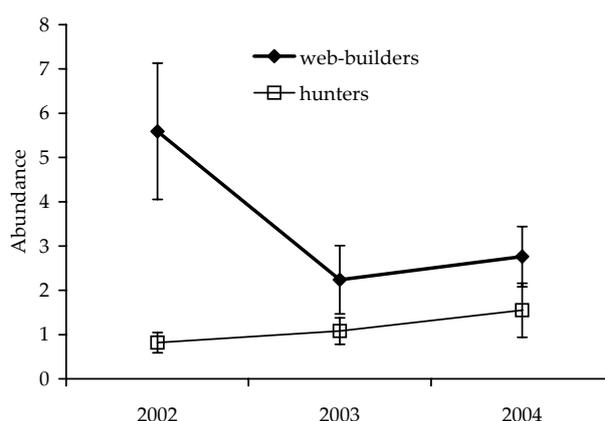


Figure 3. Change in the abundance (mean  $\pm$  SE) of hunting and web-building spiders during three years. Data from all plots were pooled

Epigeic spiders will presumably play only a limited role in the control of *O. nubilalis*. Although some species (e.g. *Erigone* or *Pardosa*) were observed to hunt on wheat plants (MANSOUR & HEIMBACH 1993), individuals of *O. nubilalis* are their potential prey only during hatching of adults from straw or when young larvae are occurring on the upper surface of leaves and stalks. Therefore, *O. nubilalis* should not dramatically affect the spiders' population dynamics.

Certain effects, such as a change in the family or guild composition of arachnids, could result from a different prey spectrum available on plots of the study (PEKÁR & KOCOUREK 2004). At present we do not have data on the abundance of other prey groups, therefore we can not infer any effects on spiders. ARPAS *et al.* (2004) found a higher abundance of prey on the isogenic non-Bt than on Bt maize. SEHNAL *et al.* (2004), on the contrary, did not observe any significant difference in the abundance of thrips, aphids, and heteropterans between Bt and non-Bt maize plots.

It has been suspected that the Cry1 protein can be transferred in the food chain to the predators and decrease their fitness. Evidence came both from laboratory and field observations. Densities of *Chrysoperla* larvae that fed on caterpillars on Bt maize were reduced in comparison with those on non-Bt maize (WOLD *et al.* 2001). A study of JIANG *et al.* (2004) found that *Pirata subpiraticus* (Boesenberg et Strand) spiders fed with preys (planthopper or aphid) that had ingested Bt rice, had this toxin in their body. The effect of the toxin on the fitness of the spider, however, has not been assessed yet.

In agreement with other studies, the main differences observed in this study can be explained by geographic location and temporal variation (e.g. SEHNAL *et al.* 2004; VOLKMAR *et al.* 2004). The epigeic fauna of arachnids is, however, quite similar, dominated by linyphiid spiders (e.g. ALDERWEIRELDT 1989), disregarding the geographic position. The overall decrease of abundance during the study is presumably a result of a population fluctuation. For example, LEDERGERBER and BAUR (1998) observed temporal fluctuation of *Agelena* spider density during four years of study. Similar temporal changes in the abundance of arachnids were documented in other studies (e.g. PEKÁR & KOCOUREK 2004).

However, one interesting aspect remains: the decrease of web-building (particularly linyphiid) spiders was compensated by an increase of hunting spiders. As this was similar for all plots, this displacement may result from different habitat preferences. Web-builders build their webs in ground crevices and prefer denser vegetation cover, presumably due to higher moisture (HOLLAND *et al.* 2004). On the other hand, hunting spiders prefer open habitats of bare ground to sparse vegetation (BOGYA & MOLS 1996). Maize plots in this study were sparsely covered with weeds, thus offering more favourable conditions for hunters. Further, two species of the genus *Robertus*, *R. arundineti* and *R. lividus* (Theridiidae), increased their abundance on the plot with the conventional system. Little information is available on the ecology of these species (WIEHLE 1937), therefore the increase is difficult to explain. It is likely a result of a temporal change in the population dynamic.

It can be concluded that the Bt maize strategy had no adverse effect on epigeic arachnids, which is in agreement with previous investigations of Bt maize in Europe (LOZZIA 1999; SEHNAL *et al.* 2004; VOLKMAR *et al.* 2004).

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