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EXPRESSION OF A *BACILLUS THURINGIENSIS* TOXIN IN MAIZE DOES NOT AFFECT EPIGEIC COMMUNITIES OF CARABID BEETLES AND SPIDERS

EXPRESIA TOXÍNU *BACILLUS THURINGIENSIS* Z KUKURICE NEMÁ NEGATÍVNY VPLYV NA SPOLOČENSTVÁ EPIGEICKÝCH CHROBÁKOV Z ČEĽADE BYSTRUŠKOVITÉ A PAVÚKOV

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Transgenic maize cultivar MON810 (Monsanto, USA), which carries the gene for the Cry1Ab toxin, was grown on five plots, each of 0.5 ha, which alternated with five equally large plots seeded with the non-transgenic parental cultivar. Epigeic animals were collected in five pitfall traps on each plot once before maize sowing, four times during the growing season, and once after the harvest. Ground beetles (Coleoptera, Carabidae) and spiders (Araneae), which were represented by 40 and 46 species, respectively, were highly abundant in all plots. There was no significant difference either in species diversity or in the number of individuals of carabids and spiders caught in the Bt and Non-Bt plots. A few species dominated in all cases. Approximately one third of carabids were *Pterostichus melanarius,* and three other species represented about 15% each. A single species, *Oedothorax apicatus,* made up 90% of all spiders. The distribution of some of the highly abundant species was uneven but local differences depended on other factors than toxin expression in maize.

Key words: GMO, Bt maize, agroecosystems, Carabidae, spiders, sustainable agriculture

Bacillus thuringiensis is the oldest and most successful microbial agent used in the control of insect pests. This soil bacterium produces several toxins, of which the crystallic proteins (Cry) are most important. Differences in the type of Cry specify the activity of each strain of *B. thuringiensis* on certain taxa of Lepidoptera, Diptera, and Coleoptera (MacIntosh et al. 1990). The toxin Cry1Ab is highly active on the stem borers such as the European corn borer, *Ostrinia nubialis* (Hübner), which causes great economic losses in maize cultivation throughout Europe and North America. Introduction of the *Cry1Ab* gene into maize genome provides protection against various stem borers (Schuler et al., 1998). The plantation of such Bt-maize is economically advantageous and reduces soil and water contamination with synthetic pesticides. The commercial use of Bt-maize in Europe, however, is limited to Spain. One of the major obstacles to a broader use is the fear of unwanted side effects on the environment (Wolfenbarger and Phifer, 2000). To probe justification of this concern, we examined a possible impact of Bt-maize on the communities of epigeic insects and spiders in a field experiment.

Material and methods

The experiment was performed in a standard farm field 7.6 ha large in South Bohemia (300 m a.s.l.) in 2002. Two rows of five squares, each of 0.5 ha, were picketed in the central part of the field. On May 15, five plots (Bt plots) were sown with transgenic maize cultivar MON810 that expresses a truncated version of the *Bacillus thuringiensis* toxin *Cry1Ab,* and five alternating plots with a parental non-transgenic cultivar (non-Bt plots). The non-transgenic cultivar was also grown in the field margins. The whole field was treated with the herbicide Guardian EC once before sowing and once after maize germination.

Five (eight in early spring before maize sowing) plastic pitfall traps (Work et al., 2000) 10 cm in diameter and with a layer of 3–4 % formaldehyde at the bottom (Pekar, 2002) were installed in each plot, always for about a fortnight period. One collection was done before maize sowing, four collections during the period, and one after harvest. Most collected animals were identified to the species level. The catches were analysed separately for each trap and collection time but eventually all data concerning a plot were summed up. When some traps were lost (19% in Bt and 13% in Non-Bt plots), the missing values were compensated for with average data from the remaining collections in the respective plot and time. The incidence of ground beetles (Coleoptera, Carabidae) and spiders (Araneae) in different plots was evaluated by χ^2 analysis.

Results and discussion

The abundance of ground beetles and spiders greatly varied over the season and also spatially (Table 1). In most times it was significantly different in the four most upper (two Bt and two non-Bt) and the four most lower plots (two Bt and two non-Bt). When summed for the whole season, the probability of equal distribution of ground beetles was about 0.1% and that of spiders even less. Somewhat smaller but also significant differences were found between the five Bt and five non-Bt plots, including some collections before and just after sowing when the presence of Bt toxin could not exert any effect. We conclude that the total abundance of ground beetles and spiders on individual plots was determined by plot position and not by the type of maize. The field was locatedon a lightly gradual slope and a gradient in soil moisture was a likely source of plot diversity.

Table 1 Changes in the distribution of ground beetles and spiders over the season in relation to the plot position and maize type. Four plots in the upper part of the field are compared with four plots in the lower part, and five Bt plots with five Non-Bt plots. First collection was done before sowing and the last after the harvest. The dates of trap exposure are given in month/day format. Probability (P) values were calculated by the χ^2 method

Dates (1)	30. 3–14. 4	18. 5.–2. 6.	10. 6.–26. 6.	12. 7.–27. 7.	3. 9.–16. 9	23. 9.–6. 10.	Total
Ground beetles (Carabidae) (2)							
Upper plot (3)	262	257	286	1292	635	93	2825
Lower plot (4)	336	607	380	1043	729	109	3204
P values (5)	0.032		0.010	0.005	0.072	0.426	0.001
Bt maize (6)	334	487	327	1404	932	130	3614
Non-Bt maize (7)	435	596	414	1448	920	174	3987
P values (5)	0.010	0.019	0.024	0.560	0.844	0.074	0.020
Spiders (Araneae)							
Upper plot (3)	251	110	3088	695	118	88	4350
Lower plot (4)	407	106	1575	792	79	72	3031
P values (5)	0.000	0.847	0.000	0.075	0.048	0.370	0.000
Bt maize (6)	423	116	2715	1032	126	91	4503
Non-Bt maize (7)	398	170	3745	822	121	125	5381
P values (5)	0.537	0.023	0.000	0.001	0.822	0.101	0.000

Tabuľka 1 Zmeny v rozšírení pôdnych chrobákov a pavúkov v priebehu celej sezóny v závislosti od polohy pokusnej plochy a typu kukurice. Štyri horné pokusné plochy sú porovnané so štyrmi dolnými pokusnými plochami, a päť Bt plôch s päť Non Bt plochami. Prvý zber bol uskutočnený pred sejbou a posledný po zbere kukurice. Doba trvania rozmiestnenia pascí na pokusnej ploche je daná vo formáte mesiac.deň. Pravdepodobnosť P bola hodnotená χ^2 metódou
(1) dátum, (2) bystruškovité (Carabidae); (3) horná pokusná plocha, (4) dolná pokusná plocha, (5) hodnoty P, (6) kukurica s Bt, (7) kukurica bez Bt (rodičovský kultivar)

In the course of the year we collected 40 species and 7601 specimens of carabid beetles. *Pterostichus melanarius, Poecilus cupreus, Bembidion quadrimaculatum* and *Calathus fuscipes* clearly dominated, and with four other species they accounted for over 92% of specimens (Table 2). *Pterostichus melanarius* and *Bembidion quadrimaculatum* were significantly more abundant on the non-Bt plots but a comparison of the 4 most upper and 4 most lower plots (Table 4) suggests that it was an effect of plot position. The occurrence of *P. melanarius* was significantly higher in the lower part of the field. The numbers of species on the Bt and Non-Bt plots were similar and their distribution over the field was independent of the type of

Table 2 Comparison of the abundance of carabid beetles caught on five plots of the Bt maize and five of the Non-Bt maize

Species (1)	Bt- maize (2)	Non-Bt maize (3)	χ^2
Pterostichus melanarius (Illiger, 1798)	878	1221	p = 0.000
Calathus fuscipes (Goeze, 1777)	537	540	p = 0.948
Poecilus cupreus (Linneus, 1758)	527	501	p = 0.566
Bembidion quadrimaculatum (Linneus, 1761)	525	425	p = 0.022
Trechus quadristriatus (Schrank, 1781)	194	185	p = 0.744
Bembidion lampros (Herbst, 1784)	154	128	p = 0.273
Pseudoophonus rufipes (De Geer, 1774)	124	167	p = 0.074
Clivina fossor (Linneus, 1758)	94	109	p = 0.456
Remaining 32 carabid species (4)	248	276	p = 0.710

Tabuľka 2 Porovnanie výskytu pôdnych chrobákov na piatich Bt a piatich Non Bt pokusných plochách
(1) druh, (2) kukurica s Bt, (3) kukurica bez Bt (rodičovský kultivar), (4) zvyšných 32 druhov z čelade bystruškovité

Table 3 Comparison of the abundance of spiders caught on five plots of the Bt and five of the non-Bt maize

Species (1)	Bt- maize (2)	Non-Bt maize (3)	χ^2
Oedothorax apicatus (Blackwall, 1850)	3 765	4 626	p = 0.000
Erigone dentipalpis (Wider, 1834)	87	100	p = 0.502
Pardosa agrestis (Westring, 1861)	68	35	p = 0.020
Erigone atra (Blackwall, 1833)	40	56	p = 0.247
Porrhomma microphthalmum (O. P-Cbr., 1871)	37	32	p = 0.671
Trochosa ruricola (De Geer, 1778)	14	21	p = 0.397
Pardosa palustris (Linné, 1758)	14	14	p = 1.000
Meioneta rurestris (C. L. Koch, 1836)	11	23	p = 0.134
Remaining 38 spider species (4)	47	70	p = 0.130

Tabuľka 3 Porovnanie výskytu pavúkov na piatich Bt a piatich Non Bt pokusných plochách
(1) druh, (2) kukurica s Bt, (3) kukurica bez Bt (rodičovský kultivar), (4) zvyšných 38 druhov z čelade bystruškovité

Table 4 Distribution of selected ground beetle and spider species in the four most upper and four most lower plots

Species (1)	Upper plot(2)	Lower plot(3)	χ^2
Pterostichus melanarius (Illiger, 1798)	783	919	0.022
Bembidion quadrimaculatum (Linné, 1761)	415	484	0.103
Oedothorax apicatus (Blackwall, 1850)	2 691	3 119	0.000
Pardosa agrestis (Westring, 1861)	41	39	0.874

Tabuľka 3 Distribúcia vybraných druhov pôdnych chrobákov a pavúkov na štyroch horných a štyroch dolných pokusných plochách
(1) druh, (2) horná pokusná plocha, (3) dolná pokusná plocha

maize. About half of all species was represented just by one or a few specimens with random occurrence.

The analysis of spider catches has yielded similar results, with even greater difference between the dominating and the rare (Table 3). From the 9884 collected specimens of 46 species, *Oedothorax apicatus* represented 90%, while the second most common species, *Erigone dentipalpis*, only about 2.6%. Abundance of the former species was much higher on the non-Bt plots but this was due to plot position rather than the maize type (Table 4).

Taken as a whole, our data show that Bt maize exerted no adverse effect on the ground beetles and spiders that dominated epigeic animal communities in the experimental field. This result indicates that the environmental impact of the insect-resistant transgenic crops is negligible in comparison to the insecticide treatments that reduce numbers of beneficial insects such as carabid beetles (Lee et al., 2001). It seems that environmental concerns about the commercial use of Bt maize in Europe and the complexity of GMO legislation in the European Union (Saeglitz and Bartsch, 2003) are unjustified.

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Súhrn

Transgénna kukurica kultivaru MON810 (Monsanto, USA), nesúca gén pre toxín *Cry 1Ab* sa pestovala na piatich 0,5 ha parcelkách, ktoré sa striedali s piatimi rovnako veľkými parcelkami s Non Bt, t. j. rodičovským kultivarom. Na zber epigeického hmyzu sa použili pôdne pasce, na každú parcelku päť kusov. Pasce boli rozmiestnené jedenkrát pred sejbou, štyrikrát v priebehu sezóny a jedenkrát po zbere kukurice. Chrobáky z čeľade bystruškovité (Coleoptera, Carabidae) reprezentované 40 druhmi a pavúky (*Araneae*), ktoré boli reprezentované 46 druhmi, boli vo veľkej miere zastúpené na všetkých pokusných plochách. Nepotvrdil sa žiadny preukazný rozdiel v druhovej skladbe ani v počte jedincov chrobákov a pavúkov chytených na Bt a Non Bt plochách. Niekoľko druhov prevládalo vo všetkých prípadoch. Takmer jedna tretina chrobákov patrila do druhu *Pterostichus melanarius* a ďalšie tri druhy predstavovali každý okolo 15%. Druh *Oedothorax apicatus* tvoril 90% z celej skupiny pavúkov. Bohaté rozšírenie niektorých druhov bolo nerovnomerné ale lokálne rozdiely záviseli od iných faktorov ako je expresia Bt toxínu z kukurice.

Kľúčové slová: GMO, agroekosystém, Carabidae, pavúky, poľnohospodárstvo

References

LEE, J. C. – MENALLED, F. B. – LANDIS, D. A. 2001. Refuge habitats modify impact of insecticide disturbance on carabid beetle communities. In: Journal of Applied Ecology, vol. 38, no. 2, p. 472–483.

MACINTOSH, S. C. – STONE, T. B. – SIMS, S. R. – HUNST, P. – GREENPLATE, J. T. – MARRONE, P. G. – PERLAK, F. J. – FISCHHOFF, D. A. – FUCHS, R. L. 1990. Specificity and efficacy of purified *Bacillus thuringiensis* proteins against agronomically important insects. In: Journal of Insect Pathology, vol. 56, p. 258–266.

PEKAR, S. 2002. Differential effects of formaldehyde concentration and detergent on the catching efficiency of surface active arthropods by pitfall traps. In: Pedobiologia, vol. 46, no. 6, p. 539–547.

SAEGLITZ, C. – BARTSCH, D. 2003. Regulatory and associated political issues with respect to Bt transgenic maize in the European Union. In: Journal of Invertebrate Pathology, vol. 83, no. 2, p. 107–109.

SCHULER, T. H. – POPPY, G. M. – KERRY, B. R. – DENHOLM, I. 1998. Insect-resistant transgenic plants. In: Trends in Biotechnology, vol. 16, p. 168–175.

WOLFENBARGER, L. L. – PHIFER, P. R. 2000. The ecological risks and benefits of genetically engineered plants. In: Science 290, p. 2088–2093.

WORK, T. T. – BUDDLE, C. M. – KORINUS, L. M. – SPENCE, J. R. 2002. Pitfall trap size and capture of three taxa of litter-dwelling arthropods: Implications for biodiversity studies. In: Environmental Entomology, vol. 31, no. 3, p. 438–448.

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