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ORIGINAL CONTRIBUTION

Impact of Cry1Ab toxin expression on the non-target insects dwelling on maize plants

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Abstract

The effect of transgenic maize MON810 (Bt maize) on the diversity and abundance of plant-dwelling insects was tested under field conditions in southern Bohemia (coordinates 48°N, 14ºE, 384 m a.s.l.) for three successive years. The experiment was carried out on 10 0.5-ha plots of which five were seeded with the Bt maize and five with the non–Bt parental cultivar. The content of Bt toxin (Cry1Ab) was measured in plant tissues with a commercial ELISA kit. Randomly chosen plants (10 per plot) were taken from the field during the vegetation period in about 2-week intervals and thoroughly examined in the laboratory. Collected insects were identified and their counts were statistically analysed with CANOCO with respect to the Bt toxin, developmental stage of maize and the year of cultivation. No significant effect of Bt maize on the plant-dwelling non-target insects was detected. Correlation between the number of plants and detected insect diversity revealed that inspection of 20 plants (four per each of five plots) provided data reliable at 95% probability level; six plants per plot were sufficient for the analysis of aphids, thrips and *Orius* bugs.

Introduction

Insect pests are often the major cause of yield losses in agricultural crops. The most important pest of maize in Europe, the European corn borer, *Ostrinia nubilalis* (Hbn.) (Lepidoptera: Crambicidae), causes yield losses of 5–30% (Meissle et al. 2010). Pest management currently relies on the applications of insecticides but their use often brings about ecological damage owing to lethality to the non-target species, insecticide accumulation in the soil, contamination of waters and sometimes the presence of insecticide residues in the harvested crops. Excessive use of insecticides may cause outbreaks of secondary pests by eliminating their natural enemies and altering trophic relationships in the ecosystem. In spite of this potential danger, European corn borer is often controlled with broad-spectrum insecticides including pyrethroids and organophosphates that are cheap, suppress several arthropod pests simultaneously, and their use has a long tradition.

The deployment of genetically modified (GM) crops, which express a toxin targeted to specific herbivores, is regarded as a suitable alternative to the pesticide use (Sharma et al. 2004; Romeis et al. 2006; Meissle et al. 2011). Evaluation of the risks and benefits of the GM crops has been in the focus of attention since their commercialization (Ferber 1999) but some concerns remain, particularly about possible environmental side effects (Firbank et al. 2005). In response to the fear of possible unwanted effect, EU authorities will demand post-market environmental monitoring (PMEM). We examined environmental impact of the GM maize event MON810 that was cultivated on the same plots for 3 years. Our data will be used in the development of PMEM methodology.

MON810 maize has been genetically engineered to express the insecticidal protein Cry1Ab, one of the toxins produced by the bacterium *Bacillus thuringiensis*. Different strains of *B. thuringiensis* produce different kinds of the Cry proteins, called Bt toxins, that act specifically against certain insect groups. Thus, it is possible to develop GM crops lethal to a narrow range of insect pests and not to other organisms. The maize expressing Cry1Ab is fully resistant to the European corn borer (Gill et al. 1992; Orr and Landis 1997; Clerk et al. 2000; Burkness et al. 2001). Environmental impact of GM maize expressing this or other Cry toxins has been addressed in several studies that generally disclosed no effect on the non-target plant-dwelling arthropods (Candolfi et al. 2004; Novillo et al. 2004; De la Poza et al. 2005; Dively 2005; Romeis et al. 2006; Marvier et al. 2007; Rauschen et al. 2008, 2010; Wolfenbarger et al. 2008; Naranjo 2009; Meissle et al. 2011). In spite of this evidence, many EU countries imposed a ban on the commercial deployment of 'Bt maize' cultivars.

We examined impact of Cry1Ab expression on insect community dwelling on the MON810 maize plants. The abundance of insects collected on the plants was correlated with the toxin presence/absence (i.e. plot type), year and date of collection within the year. We also estimated the minimal number of plants needed for a reliable assessment of the impact of a crop treatment on arthropod communities on the plants.

Materials and Methods

Study site

Experiments were performed in 2003–2005 on ten plots of 0.5 ha each that were set with the help of GPS in two rows in a field of 14 ha near the town České Budějovice, Czech Republic (48ºN, 14ºE, elevation 384 m a.s.l.). The rows were separated by a 10 m wide strip of bare land and the plots within each row by 2 m wide unseeded walkways. Bt maize cultivar YieldGard® Monumental (MONSANTO Technology LLC, St. Louis, MO), which contained modified *Cry1Ab* gene (transgenic event MON810, Armstrong et al. 1995), and the parental non-transgenic cultivar Monumental were each planted on five alternating plots (fig. 1). The area surrounding the plots (30–70 m wide) was seeded with the non-transgenic cultivar. Seeds of both maize types were kindly supplied by Monsanto CR s.r.o., Londýnské nám. 856/2, 639 00 Brno. The dates of sowing (24 April 2003; 27 May 2004; 11 May 2005) varied in dependence on weather and availability of machinery. Temperature and precipitation (fig. 2) were measured ca. 5 km away from the field in the Hydrometeorological Institute in České Budějovice. One side of the field was flanked by forest, the other sides by fields planted with cereals (wheat and barley). The field was yearly treated with Guardian EC herbicide (3 l/ha) and fertilized with DAM (225 l/ha) before sowing and with AMOFOS (88 kg/ha) simultaneously with maize sowing. The maize was cut in the waxy stage of kernel ripening (stage BBCH 89 according to Lancashire et al. 1991). In 2003, the

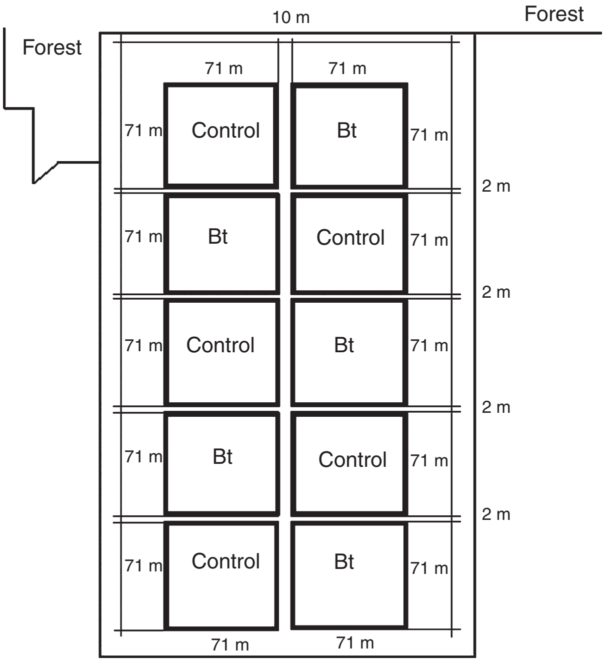

Fig. 1 Distribution of plots with the Bt and non-Bt maize cultivars in the experimental field in 2003, 2004 and 2005).

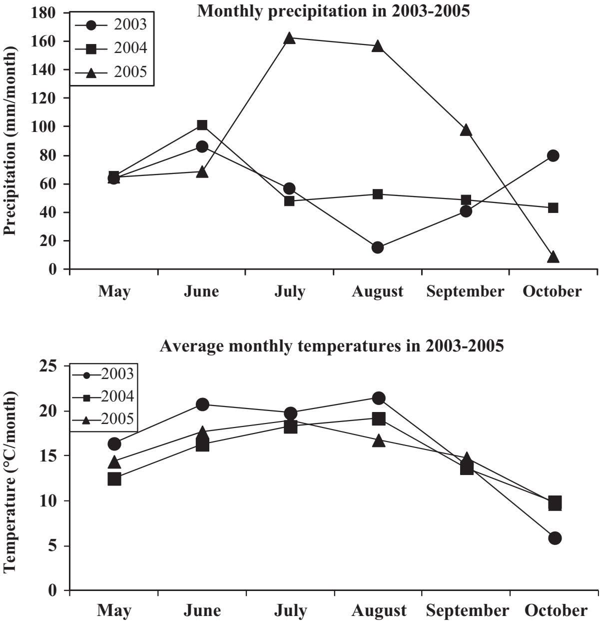

Fig. 2 Average monthly precipitations (top) and temperatures (bottom) during the vegetation periods of 2003, 2004 and 2005. Data from a meteorological station located about 5 km from the field.

plants were then shred to pieces smaller than 1 cm and ploughed about 25 cm deep into the soil. Plant debris was left on the same plot where the respective maize cultivar had been grown. Each plot was planted with the same cultivar in all 3 years.

Sampling

Ten randomly selected plants were taken from each plot in ca. 2-week intervals between the stages BBCH14-16 and BBCH 89. Plants were transported in closed plastic bags to the laboratory where they were taken apart and thoroughly examined for the presence of arthropods. This method was probably unsuitable for collecting spiders and leafhoppers because very few could be collected (very few were also detected by visual inspection in the field). Collected insects were identified to the genus/species level with the aid of determination keys described by Balthasar et al. (1957) and Balát et al. (1959) and counted. Multivariate analysis (Canoco for windows 4.5) was used to evaluate species composition and abundance in relation to the *Plot type* (Bt vs. non-Bt maize), *Date* (number of days as the first sampling taken as number 1) and *Year* (2003, 2004 and 2005). Based on the results of preliminary detrended correspondence analysis (DCA), canonical correspondence analysis (CCA) with Monte-Carlo significance testing (999 permutations, full model) was used for the final evaluation of interspecies differences in log transformation. Because these permutations do not allow empty cells, a fictional species with abundance = 1 was added to each sample (cf. Lepš and Šmilauer 2003). The abundance of European corn borer was assessed indirectly as a percentage of damaged plants at the time of harvest (15 plants per plot were inspected).

EstimateS 7.5.0 software (R. K. Colwell: http://viceroy.eeb.uconn.edu/estimates) was employed to assess species richness based on the species abundance data and to estimate how many plants must be analysed to obtain reliable data. The species richness/plant number estimator is based in these calculations on the fitting of an asymptotic function (the Michaelis–Menten equation) to the sample-based rarefaction curve.

The amount of Cry1Ab was measured in 1 g samples of root, stem, leaves, flower and grain of six plants per plot with enzyme-linked immunosorbent assay (ELISA) using Agdia commercial kit Bt-Cry1Ab ELISA, Catalogue No. PSB 05500 (purchased from Linaris, Germany, http://www.agdia.com). Protocol provided with the kit was meticulously followed and provided Cry1Ab was used as quantification standard. Sensitivity threshold of the assay was 0.25 ng Cry1Ab per 1 g of fresh plant tissue.

Results

Toxin expression

Toxin expression in plant tissues changed in the course of season in similar fashion every year (table 1). Highest concentrations were found in the leaves, in which the toxin was measured at 0.85 ± 0.46 μg/g of fresh biomass in the young plants (3–4 leaves stage, BBCH 13), peaked to 1.36 ± 0.97 μg/g at the time of flowering (BBCH 65) and decreased to <0.70 μg/g at the time of seed ripening (BBCH 89). A nearly continuous decline from 0.31 to 0.10 μg/g was recorded during the year in the stem. The roots harboured moderate toxin concentrations, and the flowers low. Very low amounts of 0.05 to 0.06 μg/g were found in kernels taken from the plants; seeds obtained from the Monsanto ČR and used for sowing contained in average 0.08 ± 0.004 μg Cry1Ab per g.

Aphids, thrips and predatory insects

Aphids and thrips were the most abundant insects found on the maize plants (table 2). Two aphid

Table 1 The content of Cry1Ab in microgram per gram fresh maize tissue. Measurements performed in 2003–2005 were combined to calculate average ± SD values*

Developmental stage of maize	Leaves	Stem	Root	Tassel	Bloom	Seed†
4–6 leaves	0.85 ± 0.46	0.31 ± 0.08	0.24 ± 0.05	–	–	–
Close to flowering	0.99 ± 0.47	0.12 ± 0.04	0.39 ± 0.17	0.17 ± 0.07	–	–
Flowering	1.36 ± 0.97	0.16 ± 0.05	0.19 ± 0.11	0.22 ± 0.18	0.014 ± 0.004	–
Milk stage	0.68 ± 0.34	0.09 ± 0.02	0.23 ± 0.06	–	–	0.05 ± 0.03
Waxy stage	0.70 ± 0.18	0.09 ± 0.02	0.19 ± 0.21	–	–	0.06 ± 0.002

*Samples were collected six times every year (2003–2005), and five plants (one per plot) were analysed each time. Every value in the Table is therefore based on 90 independent measurements.

†Cry1Ab content in the planted seeds was 0.08 ± 0.004 μg/g.

species, *Rhopalosiphum padi* and *Metopolophium dirhodum*, dominated in June and July in all 3 years. *R. padi* outnumbered *M. dirhodum* in 2003 and 2004, whereas in 2005, the abundance of these species was reversed. Another aphid species, *Sipha maydis*, occurred in low numbers only in June 2003, and *Sitobione avenae* was found at the beginning of July 2004 and 2005. The herbivorous thrips *Franklinella occidentalis* was abundant every year, with a population maximum in July. The predatory thrips *Aeolotrips fasciatum* was rare and occurred only in July and August 2004 and 2005. Its absence in 2003 could be due to low temperature and high precipitation in the spring of that year. Spidermites were rare and therefore excluded from statistical evaluation. The natural enemies of aphids and thrips, the predatory pirate bug *Orius* spp., several species of ladybirds and the lacewing *Chrysopa carnea* were encountered regularly in all experimental years.

Statistical analysis revealed independence of insect abundance on the *Plot type*, that is, on the Bt vs. non-Bt maize plants (fig. 3). According to the results of DCA analysis (the 1st axes length of gradient = 3.680), the unimodal CCA method proved suitable for evaluating the effects of three variables (*Plot type*, *Date* and *Year*). In the first test, all data on the non-target species (listed in caption to fig. 3) were combined (table 3). Common rating of such data showed no significant effect of the *Plot type* (responsible only for 0.1% of established variation; P = 0.109; F = 1. 7) but significant influence of the *Year* (6.1% explained variation; P = 0.001; F = 115.2). The occurrence and abundance of insects were obviously dependent on the collection time during the season (*Date* explained 8.9% variation; P = 0.001; F = 173.0). Similar correlations were found when only aphids were analysed (table 4). Ordination of individual taxa in relation to the three variables confirmed general independence of the Bt toxin (*Plot type*) and species-specific dependence on the *Date* and *Year* (fig. 3).

Lepidoptera

The European corn borer, *O. nubilalis*, was the only moth infesting the maize every year. Eggs were deposited before and during flowering on both Bt and non-Bt maize, but larvae were found only on the non-Bt cultivar (Table 2). As most of the eggs collected on both types of maize had been after larval hatching, the hatchability was obviously also independent of the *Plot* type. However, caterpillars occurred only on the non-Bt plants, apparently because those hatching on the Bt plants had died as neonates. The inspection of plants before harvest revealed that 8% of the non-Bt plants were infected in 2003, 16% in 2004 and more than 47% in 2005. The data confirm great interannual fluctuations in the rate of infestation. The Bt maize was fully resistant against the European corn borer in all years, and no damage was seen. In 2004, both Bt and non-Bt plants were occasionally attacked by caterpillars of the Cotton bollworm, *Helicoverpa armigera.*

Analysis of sample size

In this study, we analysed 50 Bt and 50 non-Bt plants at each sampling time (10 plants per plot). To verify that this number was sufficient and in hope that such a tedious analysis could be simplified in the future, we used our data to estimate the minimal number of plants that would yield reliable comparison between

Table 2 The most abundant insects found on maize plants during the growing period in three consecutive years. Numbers are average ± SD per one plot (n = 10 plants per plot)

	2003		2004		2005	
Species/genus	Non-Bt	Bt	Non-Bt	Bt	Non-Bt	Bt
Metopolophium dirhodum	4.10 ± 1.39	2.10 ± 0.44	12.97 ± 3.90	5.71 ± 1.59	136.70 ± 47.50	211 ± 74.70
Rhopalosiphum padi	52.30 ± 6.99	21.77 ± 2.84	25.50 ± 6.70	17.21 ± 5.40	66.80 ± 18.30	70.80 ± 19.20
Sipha maydis	1.57 ± 0.47	0.37 ± 0.14	0	0	0	0
Sitobione avenae	0	0	6.30 ± 1.88	2.80 ± 0.84	0.11 ± 0.04	0
Franklinella occidentalis	184.40 ± 26.70	185 ± 30.46	132 ± 11.40	112.42 ± 10.10	102.50 ± 7.26	98.20 ± 6.80
Aeolotrips fasciatum	0	0	1.30 ± 0.24	2.21 ± 0.56	0.60 ± 0.16	0.53 ± 0.09
Orius spp.	11.20 ± 1.64	10.17 ± 1.43	15.8 ± 1.17	9.47 ± 1.03	6.70 ± 1.27	9.26 ± 1.38
Coccinellidae spp.	0.1 ± 0.02	0.23 ± 0.05	0.47 ± 0.05	0.73 ± 0.07	0.70 ± 0.12	0.73 ± 0.12
Chrysopa carnea eggs	8 ± 1.17	10.50 ± 1.50	4.50 ± 0.91	2.30 ± 0.25	1.60 ± 0.35	1.17 ± 0.23
Ostrinia nubilalis	3.30 ± 0.34	0	0.47 ± 0.05	0	4.20 ± 0.68	0
Helicoverpa armigera	0	0	0.13 ± 0.03	0.07 ± 0.02	0	0

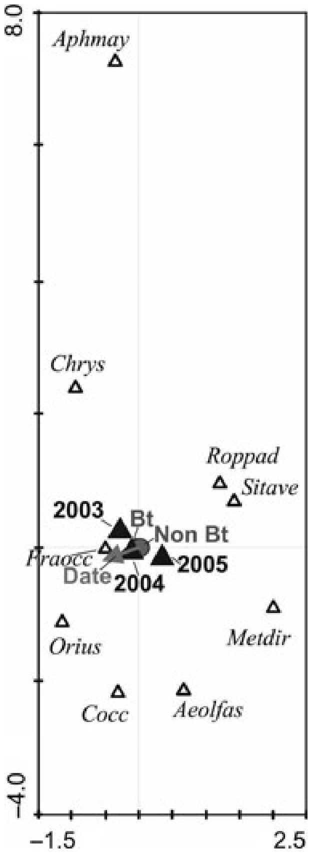

Fig. 3 Statistical analysis (unimodal CCA method) of the effects of *Plot* type (Bt and non-Bt), *Date* (days as the first sampling in given year = 1) and *Year* (2003, 2004 and 2005) on the occurrence of insects on the maize plants. Superposition of the Bt and non-Bt centroids (grey circles) reveals that these two variables had negligible effect on biodiversity and species abundance. The influence of *Year* was only slightly more pronounced (black triangles are relatively close to one another). *Date* vector (grey arrow indicating continuity of this variable) shows that species on the right appeared in the field earlier in the season than those in the left half of the diagram. Species points (empty triangles) are estimated optima, that is, conditions in which respective species reached its highest abundance with respect to the *Plot* type, *Year* and *Date* gradients. For example, the position of Aphmay at the top and of Metdir at the bottom right (i.e. with no proximity to any of defined variables) indicates very low correlation of these species to the *Plot* type (Bt and non-Bt), *Year* and *Date* but great influence of unknown environmental variables (for example, plot position close to the forest). The significance of known variables is shown in tables 3 and 4. Aeolfas, *Aeolotropism fasciatus*; Chrys, *Chrysopa carnea*; Cocc, *Coccinellidae*; Fraocc, *Franklinella occidentalis*; Metdir, *Metopolophium dirhodum*; Orius, *Orius* spp.; Roppad, *Rhopalosiphum padi*; Sitave, *Sitobione avenae*; Aphmay, *Sipha maydis*. CCA, canonical correspondence analysis.

Table 3 Results of canonical correspondence analysis analysis of the impact of environmental variables Bt (Cry1Ab presence), D (date of collection), Y (year of collection) and Bt, D and Y combined on the counts of non-target insects*. Column EXP% shows the impact of respective variable in per cent of total variability

Variable	P	F	EXP%
Bt	0.109	2.56	0.1
D	0.001	172.97	8.9
Y	0.001	115.15	6.1
Bt, D, Y	0.001	312.44	15.0

*Aphids, thrips, lacewing, ladybirds, pirate bug.

Table 4 Results of canonical correspondence analysis analysis of the impact of environmental variables Bt (Cry1Ab presence), D (date of collection), Y (year of collection), and Bt, D and Y combined, on the counts of aphids. Column EXP% shows the impact of respective variable in per cent of total variability

Variable	P	F	EXP%
Bt	0.131	1.90	0.3
D	0.001	16.52	2.2
Y	0.001	84.51	10.2
Bt, D, Y	0.001	88.26	10.6

the Bt vs. non-Bt treatments. Data on all frequently encountered taxa and separately on the aphids and on the thrips plus their major predator *Orius* spp. were used to construct 'species-area' curves with the EstimateS 7.5 program, using one plant as area unit. The program compares combinations of all data from all sampled plants and calculates expected species richness established when different numbers of plants were analysed. The curve in fig. 4a indicates that analysis of 20 plants ensured more than 95% reliability of the data and analysis of 36 plants approached 100% reliability. Computation of the abundance and incidence-based species richness indicated that 14 plants were needed for these levels of variability. When analysed separately for the main insect groups (aphids, trips and pirate bugs), Estimates results suggested that inspection of six plants on each plot (no matter Bt or non-Bt) provided sufficient data for statistical analysis (fig. 4b).

Discussion

The content of Cry1Ab toxin in maize depended on the tissue and plant growth phase. The highest content was recorded in the leaves at the flowering stage; consistently lowest content was in the seeds (table 1). Values established in the rainy year 2005 (fig. 2) were somewhat (not significantly) lower than in two preceding years. It is possible that the enhanced water content diluted the toxin concentration in plants or that weather conditions reduced toxin expression.

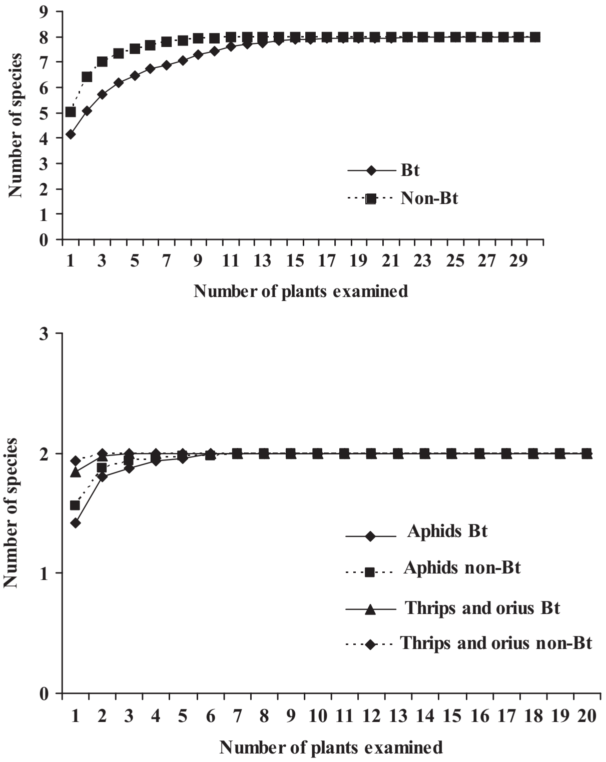

Fig. 4 Correlation curves (constructed with the Estimates 7.5 software) between the number of detected insect taxa and the number of Bt or non-Bt plants examined. Top: curves for taxa listed in caption to fig. 3. Bottom: curves for the aphids *Rhopalosiphum padi* and *Metopolophium dirhodum* and separately for the combination *Franklinella occidentalis* and *Orius* spp.

Great variability in the amount of Cry1Ab in MON810 was reported by Nguyen and Jehle (2007) who found significant differences in the toxin content among various plant tissues and developmental stages. The conditions of maize cultivation were important; values established by Nguyen and Jehle (2007) were generally about ten times higher than our data and those reported by Ramirez-Romero et al. (2008). Nguyen et al. (2008) compared quantification of Cry3Bb1 in six laboratories in Germany and Switzerland; all used identical PathoScreen kit (Agdia) to test four standardized solutions of Cry3Bb1 and two standardized samples of maize MON88017 expressing Cry3Bb1. Considerable variation of data established in different laboratories was largely owing to differences in the extraction method and, to lesser extent, to the rate of sample dilution. Unexplained variability in the toxin content was found in GM lines derived from diverse transgenic events. For example, Cry1Ab toxin levels found by Raps et al. (2001) in maize event Bt11 were 2–7 times higher then those reported by Lynch et al. (1999). We must admit that current quantification methods provide reliable relative data but probably do not reveal absolute toxin amounts.

Toxin presence prevented maize infestation with *O. nubilalis*. Relatively high and growing incidence of this pest in our field reflected its rise in Central Europe. While maize infestation in 2003 and 2004 was relatively stable, the year 2005 was characterized by a rapid increase in damaged plants. This expansion may be attributed to suitable weather conditions in the autumn of 2004 and spring 2005. Compared with the previous years, the autumn of 2004 was considerably warmer (average October temperature 9.9°C in comparison with 5.9°C in 2003) and drier (October precipitation 42.7 mm in 2004 and 79.5 mm in 2003). These conditions apparently also favoured temporal establishment of *H. armigera* that had previously been recorded only from warmer regions of the Czech Republic (Březíková 2007). *H. armigera* larvae seemed to be not affected by the Cry1Ab toxin. Similar lack of effect was observed in Bt cotton and was explained by a reduction in Bt toxin expression during the growing season (Fitt 1998; Gunning et al. 2005; Whitehouse et al. 2005). On the other hand, Novillo et al. (2004) mentioned in their article that the MON810 maize provided excellent protection against this pest.

Taken as a whole, our study confirms and extends results of similar field trials (Candolfi et al. 2004; Novillo et al. 2004; Dively 2005) demonstrating that Cry1Ab expression in maize does not affect plant-dwelling non-target insects. The lack of such effect on aphids is consistent with the Cry1Ab absence in the phloemic sap that the aphids consume (Head et al. 2001; Raps et al. 2001; Dutton et al. 2002; Romeis and Meissle 2011). Lozzia et al. (1998) demonstrated that the feeding on Bt maize did not influence the performance of the aphid *R. padi* and also did not affect its predator *C. carnea*. Pons et al. (1995) described that the expression of Bt toxin had no effect on the attack by aphids migrating in spring from the winter cereals, and neither it influenced the autumn migration of aphids from the dry maize plants back to the winter cereals. Surprisingly, more aphids were consistently found on the Bt than on the non-Bt maize in some field trials (Pons et al. 2005). The cause of this phenomenon has not been elucidated. Differences in the alluring volatiles (Lumbierres et al. 2004) or in the amino acid content in the plants (Faria et al. 2007) were considered, but neither of these factors is directly affected by the Bt protein (Lumbierres et al. 2011).

Relatively high (above 5°C) and stable temperatures in the autumns and cool and humid springs in the 3 years of study favoured aphid development. The summer decline of population densities was apparently because of relatively high temperature, low precipitation, ageing of maize and possibly also action of aphid parasitoids and predators. The availability of primary host plants is often the major factor determining the population densities of different aphid species on the cereals (Honěk et al. 2002). Chokecherry (*Prunus padus*), which is the primary host of *R. padi*, was very abundant at the edge of the forest adjoining our field, and the rose bushes (*Rosa* sp.), on which *M. dirhodum* overwinters, grew in hedges on two other field margins. Chokecherry and grasses, which are primary hosts of *S. maydis* and *S. avenae*, respectively, also grew in the vicinity of the field but the populations of these two aphid species were low.

The population dynamics of thrips identified as *F. occidentalis* depended on the growth phase of maize plants. Their abundance culminated in 2003 and 2005 in July; the peak was shifted to the beginning of August 2004 owing to later planting. The predatory thrips *A. fasciatum* was rare and occurred only in July and August 2004 and 2005. Its absence in 2003 can be linked to low temperature and high precipitation in the spring of that year. Although thrips and planthoppers ingest Cry toxins by consuming the cell sap (Eckert et al. 2006), we found no difference in the populations of *F. occidentalis* on the Bt and non-Bt maize. This independence from the Cry1Ab presence is consistent with the data of Obrist et al. (2005), who compared life tables of *F. tenuicornis* on a Bt and non-Bt maize. The tolerance of planthoppers and leafhoppers to maize MON810 was shown by Rauschen et al. (2008).

Changes in the population density of *Orius* spp. roughly followed population fluctuations in *F. occidentalis* with a delay of 20–28 days, similarly to an earlier report (Coll and Ridgway 1995). Most collected bugs were non-flying nymphs confined to a certain plot and possibly to a single plant. Their abundances on the Bt and non-Bt plants were similar. Similar observation was made by De la Poza et al. (2005) who reported that *Orius* bugs on Bt maize possibly consumed some Cry1Ab with their prey but their abundance was not affected. Jasinski et al. (2003) and Musser and Shelton (2003) described occasional population density increases in *Orius* spp. in the Bt maize and suggested that this could be related to their preference for ears and silks free from lepidopteran feeding. The consumption of thrips grown on Bt maize had no effect on *Orius majusculus* (Zwahlen et al. 2000). Similarly, Al Deeb et al. (2001) detected no effect in *Orius insidiosus* consuming caterpillars that had ingested Cry1Ab. These authors, together with Orr and Landis (1997), also found no difference in bug abundance between the Bt and non-Bt maize fields.

Large predatory insects occurred in the field in low numbers, consistently with other reports (Bourguet et al. 2002). Statistical analysis showed that Cry1Ab did not affect the abundance of Coccinellidae and the lacewing *C. carnea*, which were major predators of aphids and other small insects in our maize field.

Our findings are consistent with earlier reports, for example by Orr and Landis (1997) Pilcher et al. (1997) and many later investigations, and confirm that Cry1Ab acts on *O. nubilalis* without detectable side effects on other components of insect assemblage on the maize plants. This is in contrast to the insecticide applications. For example, in a study of arthropods dwelling in maize ears Eckert et al. (2006) found no differences between MON 810 maize and the non-Bt maize but the abundance of arthropods was reduced after the insecticide treatment in two of three study years.

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