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ORIGINAL CONTRIBUTION

Communities of ground-dwelling arthropods in conventional and transgenic maize: background data for the post-market environmental monitoring

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Abstract

To verify the validity of concerns about environmental safety of maize expressing insecticidal Cry toxins (referred to as Bt maize), we compared communities of ground beetles (Carabidae), rove beetles (Staphylinidae) and spiders (Araneae) in plots planted either with Bt maize cultivar YieldGard® or with the non-transgenic parental cultivar Monumental. Each cultivar was grown on 5 plots of 0.5 ha for three consecutive years. To increase the field load of Cry toxin, the fully grown maize of the first study year was shredded to small pieces that were ploughed into the soil. Arthropods were collected in pitfall traps and determined to the species level. The abundance and species richness of all studied groups greatly varied over the season and between the seasons but without statistically significant differences between the Bt and non-Bt plots. A single spider species and three ground beetle species dominated in the catches every year, whereas a set of 1–4 most abundant rove beetle species changed every year. Frequently occurring species were typical for most of Europe. The total counts of ground beetles, rove beetles and spiders collected once or twice per season are proposed to serve as bioindicators in the post-market environmental monitoring (PMEM).

Introduction

Maize is one of the most important cereal crops in the world. It is used as fodder and food and is an important industrial crop. The European corn borer (ECB, *Ostrinia nubilalis* Hübner, Lepidoptera: Pyralidae) is the major maize pest in many parts of the world. ECB larvae inflict severe damage to the maize plants, resulting in major yield losses and lower grain quality (Bourguet et al. 2002). ECB control by insecticides includes one and in some cases two sprays per one crop cycle, in some regions in addition to the seed and soil treatments (Meissle et al. 2010). Chemical plant protection usually not only reduces the pest population but also influences, in a direct or indirect way, the beneficial arthropods such as pollinators, decomposers and pest enemies (Meissle and Lang 2005). Furthermore, insecticides often impose high selection pressure on the pest population that becomes resistant and more difficult to control (Pimentel et al. 2005).

The deployment of genetically modified (GM) maize is a modern alternative to chemical insecticides. The insect-resistant GM cultivars contain one or more genes encoding Cry proteins that kill a narrow range of insect pests, for example ECB. As the Cry genes are derived from *Bacillus thuringiensis*, the insect-resistant maize is often referred to as Bt maize. The first Bt maize cultivar was commercially released in the USA in 1996 (Roush 1997). The area cropped with Bt maize has been increasing rapidly since then. A variety of GM crops, mainly soybean, maize, cotton and oilseed rape, have spread rapidly worldwide: a 100-fold increase in hectarage from 1.7 million hectares in 1996 to 175 million hectares in 2013 makes GM crops the fastest adopted crop technology in the history of modern agriculture (James 2013). The adoption of genetically modified insect-resistant plants in agriculture has become a powerful tool for the control of crucial pests. The highly specific toxicity of Bt crops and the reduction of insecticides render environment more favourable for natural enemies of the pests (Farinós et al. 2008).

A number of laboratory feeding studies as well as field trials demonstrated innoxiousness of the Cry1Ab expression in maize for the non-target organisms (Naranjo 2009). However, the acceptance of Bt maize and other transgenic crops in Europe is still hindered by fears of their possible environmental side effects. The Bt maize YieldGard® (MON810) expressing Cry1Ab protein is the only GM maize that has been approved for cultivation in the EU since 1998. It is now grown on large scale in Spain and marginally in a few other EU countries. In the Czech Republic, it occupied 150 ha in 2005, first year of growing, and reached a maximum of 8380 ha in 2008 (Křstková 2009).

The purpose of our study was to examine the safety of MON810 on a range of species that could be considered as bioindicators for the post-market environmental monitoring (PMEM) defined in Directive 2001/18/EC. Results of our 3-year monitoring of insects dwelling on the plants have been published (Habuštová et al. 2014); results of parallel monitoring of ground-dwelling ground beetles, rove beetles and spiders are summarized in this report. Examined taxa included predators, omnivores and saprophages that may consume Cry toxins in diverse ways. The prey of predatory beetles and spiders includes invertebrates that feed on the aerial and/or subterranean parts of the maize plants and potentially transmit the toxin to the next trophic level. For example, Zurbrügg and Nentwig (2009) detected Cry1Ab in the bodies of two slugs, the preferred prey of some ground beetles (Larochelle 1990). García et al. (2010) found toxin in the spider mites, and Knecht and Nentwig (2010) in Diptera larvae, common preys of various ground-dwelling predators. Some ground beetles may be impacted by Cry toxins through the prey and also through direct consumption of the maize or its residues (Zwahlen et al. 2003; Szekeres et al. 2006). This dietary diversity and the abundances of the ground beetles, rove beetles and spiders render them suitable for PMEM. In this article, we examine variations in their abundance and species richness associated with the maize type (Bt and non-Bt) and year of sampling.

Material and Methods

Experimental site and trial design

The field trial was performed in the vicinity of České Budějovice (Czech Republic, district of South Bohemia) in a field at 409 m a.s.l. in 2003–2005 (see Habuštová et al. 2014 for details). The field had slight south-western inclination; the soil was classified as cambisol and sandy loam brown soil, with a pH from acidic to slightly acidic, and with medium to high concentrations of phosphorus, potassium and magnesium. The weather conditions differed year to year (Habuštová et al. 2014). The July and August 2005 were extremely wet (precipitation 162 and 158 mm, respectively) in comparison with 2003 and 2004 (15–52 mm), and the autumn of 2004 and 2005 was exceptionally warm (average October temperature 9.9 and 9.7°C in comparison with 5.9°C in 2003) and dry (October precipitation 8.4 mm in 2005; 42.7 mm in 2004; 79.5 mm in 2003).

Bt maize (*Zea mays* L.) cultivar YieldGard® (MONSANTO Technology LLC, St. Louis, MO, USA), which contained modified Cry1Ab gene (transgenic event MON810, Armstrong et al. 1995), and its parental non-transgenic cultivar Monumental were each planted on five 0.5-ha plots that were distributed checker-wise in a 14-ha field (Habuštová et al. 2014). Two rows of 5 plots were separated from one another by a 10-m-wide stripe of bare land and the plots within each row by 2-m-wide unsown walkways. Field margins (30–70 m) were seeded with the non-transgenic hybrid. Seeds of both maize types were kindly provided by Monsanto ČR s.r.o. (Londýnské nám. 856/2, 639 00 Brno, Czech Republic). The neighbouring fields were seeded with wheat and barley or oilseed rape.

The herbicide Guardian EC (3 l/ha) and the fertilizer DAM (225 l/ha) were applied before sowing, and the fertilizer AMOFOS (88 kg/ha) at the time of sowing. No treatments were given during the vegetation period. The trials ended when maize reached the waxy ripening stage (Table S1). In 2003, plants were shredded to small pieces and ploughed into the soil about 25 cm deep. Each plot was planted with the same type of maize hybrid (Bt or non-Bt) in all three years.

Insects sampling and data analyses

The ground-dwelling insects were collected once before maize sowing, four times during the growing season and once after the harvest (Table S1). The first two collections (before sowing and at the time of maize sprouting) were omitted in 2003 because of bad weather conditions. Five pitfall traps (plastic cups 9 cm in diameter, volume 0.5 l, filled with ca 300 ml 10% NaCl solution with 2–3 drops of a detergent and covered with an aluminium coping against rain) were placed on each plot and exposed for about 14 days (total exposure time was 38, 85 and 82 days in 2003, 2004 and 2005, respectively). One trap was placed in the plot centre and four others in the middle of diagonals connecting centre with the plot corners.

Entrapped species of ground beetles (Coleoptera: Carabidae), rove beetles (Coleoptera: Staphylinidae) and spiders (Arachnida: Araneae) were separated and stored in 70% ethanol. They were identified to the species level: ground beetles according to Hůrka (1996), rove beetles according to Lohse (1964) and Benick (1974) and spiders according to Miller (1971) and with the aid of the Internet identification key of Nentwig et al. (2010). The lists of all collected species and their abundances are available in Table S2.

Repeated-measures ANOVA (StatSoft Statistica 8, Statsoft, Inc., Tulsa, OK, USA) was employed to evaluate the abundance and species richness (number of species) of collected arthropods in dependence on the maize type (Bt and non-Bt) and year. Subsequent comparisons were done by the post hoc Tukey HSD test. The dependence on maize type was examined for each year separately and for all 3 years together. For RM ANOVA testing effect of Bt maize in separate years and in all years together, data were summed per plot and divided according to samplings. For RM ANOVA testing effect of year, data were summed per year (only the samplings that were available in all 3 years; separation to Bt and non-Bt plots maintained). Interaction was tested, but it was not significant in any case. Result of F-test was accompanied by degrees of freedom and degrees of freedom of the error (within-groups degrees of freedom). GraphPad Prism 5 (GraphPad Software Inc., San Diego, CA, USA) was used for the construction of graphs. The numbers of collected specimens and species were expressed per trap and day.

Results

Impacts of maize type and year on the examined arthropod groups

Ground beetles, rove beetles and spiders dominated in pitfall captures. Occasionally, we found considerable numbers of springtails (Collembola) and adult Diptera that apparently emerged from the soil in the vicinity of the respective trap. These and other occasionally caught arthropods were not evaluated. The impact of maize type (Bt versus non-Bt) on the overall abundance and species richness of ground beetles, rove beetles and spiders was examined separately for each year (Table 1) to minimize interference with variable weather conditions. Slightly higher number of rove beetle species in the Bt plots in the second year of study was statistically insignificant ($F_{1,8} = 4.70$, $P = 0.06$) and reflected coincidental fluctuations of the number of rare rove beetle species (Table S2). This conclusion was supported by statistical analysis of the 3-year sums of the examined arthropods. The comparison of ground beetles in the Bt and non-Bt plots revealed no impact of the maize type on either abundance ($F_{1,8} = 2.42$, $P = 0.16$) or species richness ($F_{1,8} = 0.98$, $P = 0.35$). Corresponding values for the abundance and species richness of the rove beetles were $F_{1,8} < 10^{-2}$, $P = 0.94$ and $F_{1,8} < 10^{-2}$, $P > 0.99$, respectively. Similarly, neither the abundance ($F_{1,8} = 0.20$, $P = 0.67$) nor the species richness ($F_{1,8} = 1.06$, $P = 0.33$) of spiders were influenced by the maize type.

The magnitude of seasonal variations and the extent of inter-annual differences in both abundance and species richness (fig. 1) indicates great impact of weather conditions, field management (e.g., the term of sowing), kind of crops grown in adjacent fields and the initial composition and size of arthropod communities in the monitored field. It was impossible to identify specific effects of these factors, but their collective impact was proved by data analysis with RM ANOVA (Table 2). Subsequent post hoc Tukey HSD test revealed that arthropod communities of 2003 and 2004 did not differ from one another except for spider abundance. On the other hand, spider abundance was the only feature that did not prove different in comparisons of 2003 with 2005 and 2004 with 2005. No difference was also found in spider richness between 2004 and 2005. The low inter-annual differences in spider abundance and partly also in species richness reflect the fact that a few spider species occurred in high numbers every year. The ground beetles and rove beetles were more sensitive to environmental changes than the spiders.

Species composition and abundance of the examined arthropod groups and impact of maize type on dominant species

We detected no impact of the maize type on the ground beetles, rove beetles and spiders when analysed as groups. However, such cumulative data do not

Table 1 Abundance and species richness (average number per one pitfall trap and day ± SE) of collected ground-dwelling arthropods sampled in Bt maize and non-Bt maize in 2003–2005 and F and P values of statistical comparison (RM ANOVA)

		2003				2004				2005			
Taxon	Total number	Bt	non-Bt	$F_{1,8}$	P	Bt	non-Bt	$F_{1,8}$	P	Bt	non-Bt	$F_{1,8}$	P
Ground beetles	Species richness	0.44 ± 0.03	0.45 ± 0.03	*0.07*	*0.80*	0.31 ± 0.01	0.31 ± 0.02	*0.01*	*0.91*	0.33 ± 0.02	0.33 ± 0.02	*0.63*	*0.45*
	Abundance	1.83 ± 0.18	1.81 ± 0.18	*0.48*	*0.51*	2.64 ± 0.23	2.94 ± 0.26	*2.14*	*0.18*	2.72 ± 0.23	3.14 ± 0.34	*0.61*	*0.46*
Rove beetles	Species richness	0.14 ± 0.01	0.16 ± 0.01	*0.01*	*0.93*	0.10 ± 0.01	0.09 ± 0.01	*4.70*	*0.06*	0.05 ± 0.01	0.06 ± 0.01	*1.45*	*0.26*
	Abundance	0.27 ± 0.03	0.30 ± 0.03	*0.23*	*0.65*	0.22 ± 0.02	0.21 ± 0.02	*0.16*	*0.70*	0.08 ± 0.01	0.09 ± 0.01	*0.16*	*0.70*
Spiders	Species richness	0.23 ± 0.02	0.24 ± 0.02	*3.07*	*0.12*	0.18 ± 0.01	0.19 ± 0.01	*0.04*	*0.85*	0.20 ± 0.01	0.20 ± 0.01	*0.31*	*0.59*
	Abundance	2.61 ± 0.20	2.51 ± 0.17	*0.17*	*0.69*	2.30 ± 0.20	2.33 ± 0.19	*0.01*	*0.91*	1.56 ± 0.15	1.77 ± 0.20	*0.58*	*0.47*

exclude that some species are suppressed by Bt maize and are replaced by other, less sensitive species. The overall numbers of species and specimens in the Bt and non-Bt maize may remain similar. To verify this possibility, we examined the impact of maize type on the most frequently occurring species.

With an average of 2.65 ± 0.11 specimens per trap and day, ground beetles were the most abundant arthropods we collected. Their species composition was similar in the Bt and non-Bt plots, in contrast to differences between the years (fig. 2). For example, the counts of ground beetles collected in 2003 included 1629 specimens of 21 species in the Bt and 1735 specimens of 25 species in the non-Bt plots. Highest numbers of ground beetles were collected in 2004: 5704 specimens belonging to 37 species in the Bt and 6387 specimens of 40 species in the non-Bt plots. Bt and non-Bt plots did not differ in the abundance of the individually monitored species that included *Bembidion quadrimaculatum* (Linneaus), *Calanthus fuscipes* (Goeze), *Poecilus cupreus* (Linnaeus), *Poecilus versicolor* (Sturm), *Pseudoophonus rufipes* (De Geer) and *Pterostichus melanarius* (Illiger) (Table S3). Seasonal changes in their abundance (*P. versiocolor* was not found in 2003) differed somewhat between the years, but their profiles and absolute abundance values in the Bt and non-Bt plots were practically identical (fig. 3).

The community of rove beetles was small compared to the ground beetles and spiders but was species rich (Table S2). The abundance in the Bt plots was statistically identical as in the non-Bt plots (Table 1). Altogether 1833 specimens representing 60 identified species were captured in pitfall traps during the study, with only 8 species occurring in all years. The counts of specimens included *Aleochara* spp. and five tentative genera that could not be determined with certainty and were therefore not considered in the species counts. They represented 19–21% of the catches. Eleven species were chosen for the analysis of Bt impact at the species level. They included *Aleochara bipustulata* (Linnaeus), the most abundant rove beetle in 2003 and second most abundant in 2005. It was the only species that was common in all years. *Philonthus atratus* (Gravenhorst) dominated rove beetle community in 2004 and 2005, being followed in 2004 by *Lesteva longoelytrata* (Goeze). No maize-dependent differences in the abundances of monitored species were detected (Table S3).

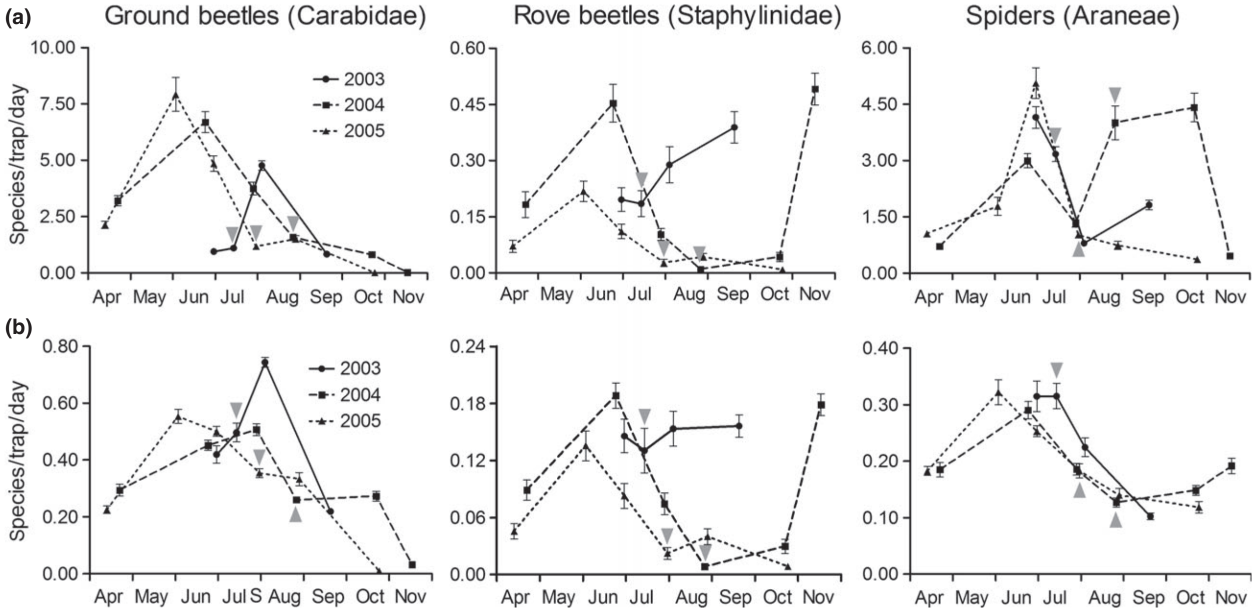

Fig. 1 Average abundances (a) and species numbers (b) of the ground beetles, rove beetles and spiders in the first (solid lines), second (broken lines) and third study year (dotted lines) where data from the Bt and non-Bt plots are combined. X-axis: Months, the medians of the dates of trap exposure were set on this scale. Y-axis: Mean ± SE of the counts of specimens and species per trap and day. The grey arrows in the graphics indicate the full flowering (BBCH 65). BBCH stage of maize determined according to Lancashire (1991).

Table 2 Results of statistical analysis of the numbers of species (species richness) and specimens (abundance) captured in the 3rd through 6th samplings in the years of study. Data from the Bt and non-Bt plots were tested together. Simultaneous analysis of all years was carried out with RM ANOVA and of year doublets by the post hoc Tukey HSD test (remarks in parentheses specify which of the compared years was richer).

Taxon	Feature	Comparison of 3 years	2003 × 2004	2003 × 2005	2004 × 2005
Ground beetles	Species richness	$F_{2,16} = 13.22, P < 10^{-3}$	$P = 0.10$	$P < 10^{-3}$ (more in 2005)	$P = 0.03$ (more in 2005)
	Abundance	$F_{2,16} = 17.77, P < 10^{-4}$	$P = 0.17$	$P < 10^{-3}$ (more in 2005)	$P < 10^{-2}$ *(more in 2005)*
Rove beetles	Species richness	$F_{2,16} = 13.89, P < 10^{-3}$	$P = 0.20$	$P < 10^{-3}$ (more in 2003)	$P = 0.01$ (more in 2004)
	Abundance	$F_{2,16} = 21.07, P < 10^{-4}$	$P = 0.40$	$P < 10^{-3}$ (more in 2003)	$P < 10^{-3}$ (more in 2004)
Spiders	Species richness	$F_{2,16} = 8.28, P < 10^{-2}$	$P = 0.12$	$P < 10^{-2}$ (more in 2005)	$P = 0.15$
	Abundance	$F_{2,16} = 6.61, P = 0.01$	$P = 0.01$ (more in 2004)	$P = 0.07$	$P = 0.51$

In total, 20 786 spiders of 67 species were captured during the field trial. Only 19 species were found in all years. The differences in abundance between the dominating species and the remainder were enormous. The prevalent species *Oedothorax apicatus* (Blackwall) constituted 81.5–93.7% of all spiders every year, while the second most common spiders, i.e. *Trochosa ruricola* (De Geer) in 2003 and *Pardosa agrestis* (Westring) in 2004 and 2005 represented only 2.3–8.9% spider species. Possible effect of the maize type was examined in these and 3 additional species; the group of 6 species represented more than 95% of all spiders every year (Table S3). Thirteen independent analyses of the abundance data by RM ANOVA demonstrated absence of Bt maize influence on any species.

Inter-annual and seasonal differences in the occurrence of monitored species

In contrast to the futile search for an effect of Bt maize on arthropod communities, the effects of year on the abundance and species richness of the ground beetles, rove beetles and spiders were obvious (fig. 2, Table 2). Altogether 26 420 ground beetles of 63 species were caught in the 3 years of study, but only 19 species were present in all three years. Six omnipresent species accounted for more than 90% of all ground beetle specimens (Table S3). In an extreme case, *Poecilus cupreus* (Linnaeus) alone represented 70% of the ground beetles. *Poecilus versicolor* was absent in 2003 but accounted for 18.6% of ground beetles in 2005 (Table S3). *Carabus granulatus* (Linnaeus) and *Harpalus affinis* (Schrank) were also

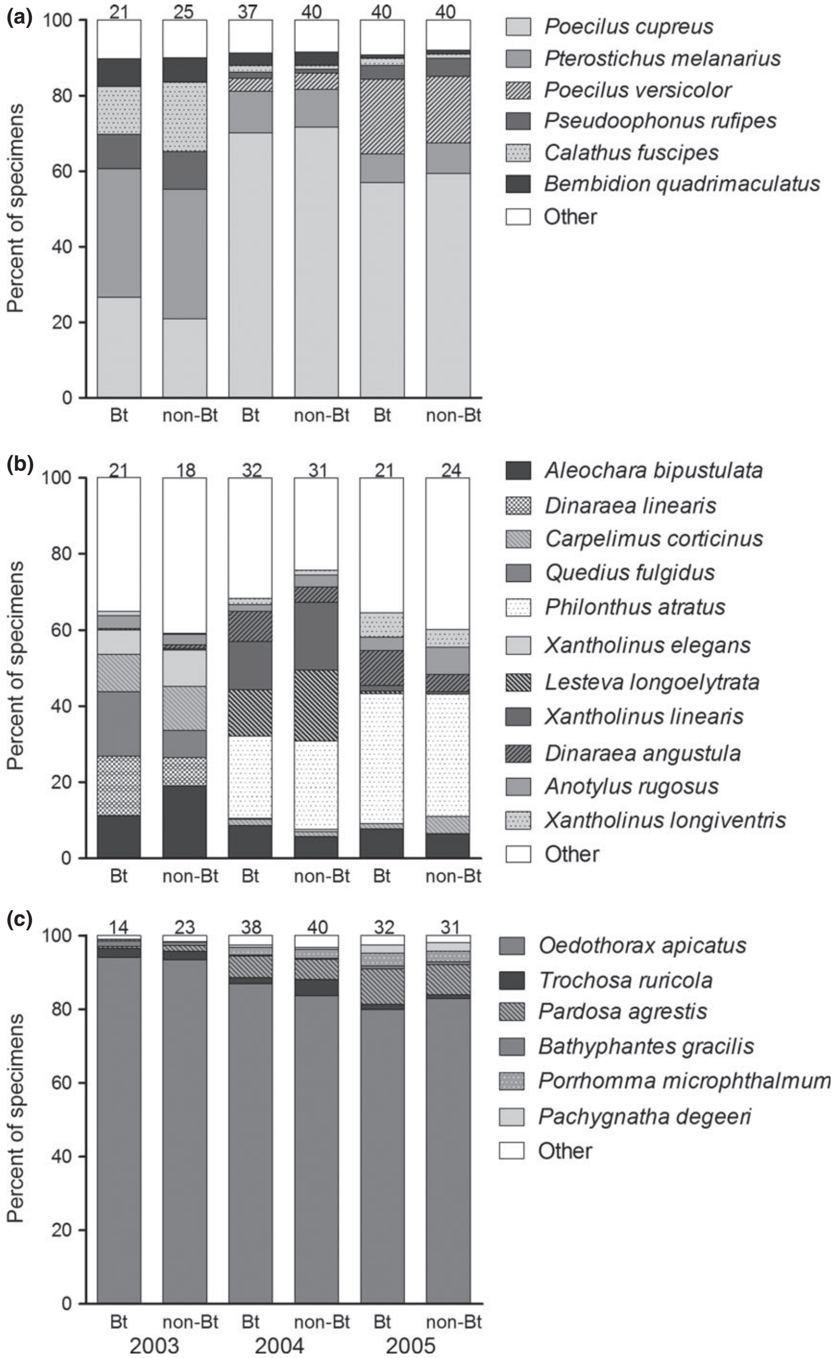

Fig. 2 Proportional representation of the most abundant species of the ground beetles (a), rove beetles (b) and spiders (c) caught in the Bt and non-Bt maize plots in 2003–2005. Numbers above the columns show the total numbers of species (undetermined ones are not included).

rare in 2003 and relatively common in 2004 and 2005 (Table S2). By contrast, *Pterostichus melanarius* (Illiger) made up about one-third (34.1%) of all ground beetles captured in 2003, but its abundance decreased markedly in the following years (fig. 2a; Table S3). Many ground beetles were present in all years but in varying population densities. In 2003, 11 species were caught in single specimens and four species in two specimens. Nineteen species were represented by one or two specimens in 2004; six of these species were absent in all other years.

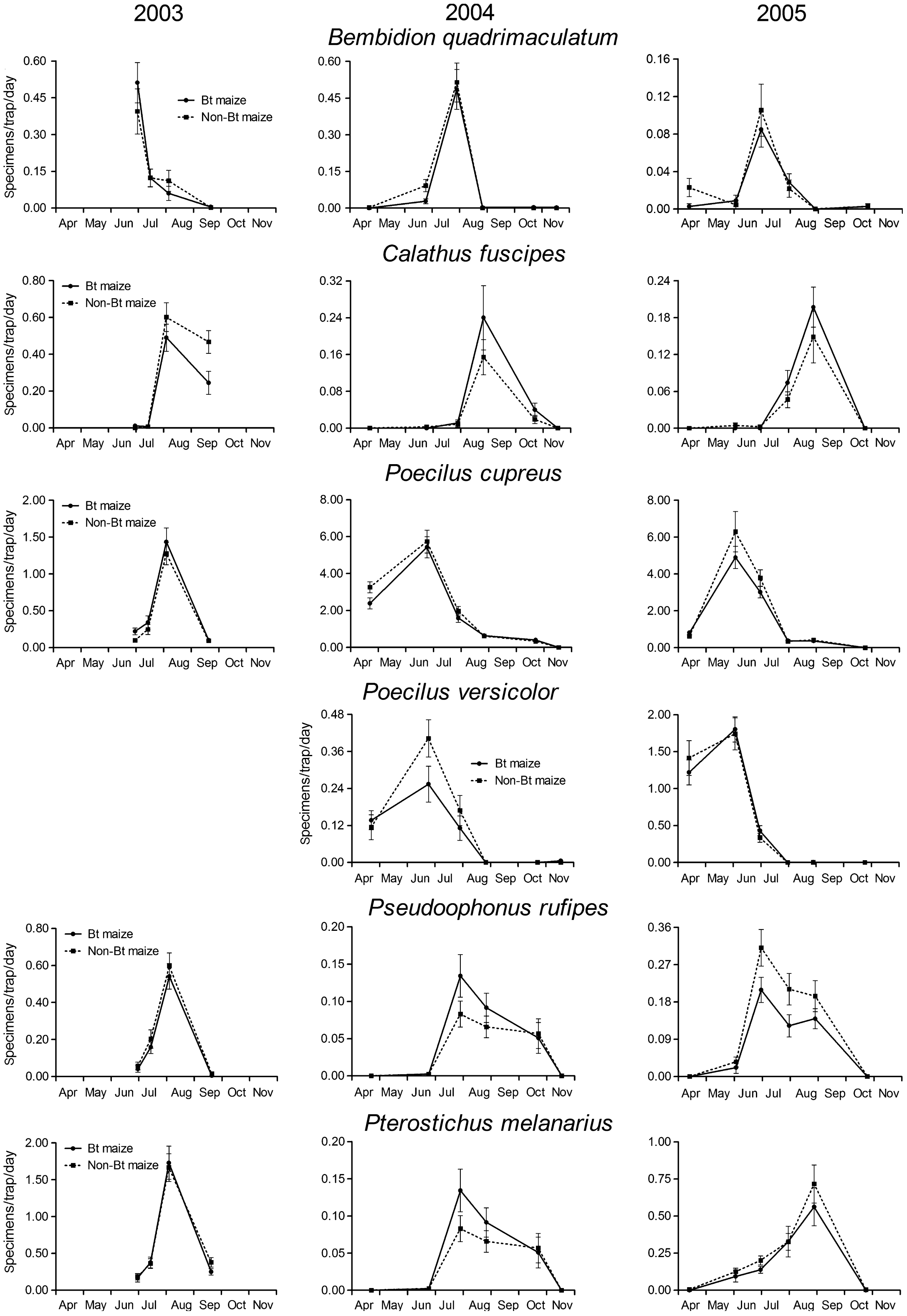

Fig. 3 Mean ± SE of the numbers of most abundant ground beetle species collected in plots planted with the Bt (solid line) and non-Bt maize (dotted line). Y-axis: number of specimens expressed per one trap and day. X-axis: 1st sampling: before sowing, 2nd sampling: BBCH 09, 3rd sampling: BBCH 16, 4th sampling: BBCH 65, 5th sampling: BBCH 87, 6th sampling: after harvest (see Table S1 for dates).

The representation of monitored species in the ground beetle community changed considerably between the years of study (fig. 2a). *P. melanarius* was the only stabile component (Table S4). Differences in the occurrence of *B. quadromaculatum* between 2003 and either 2004 or 2005 were nearly significant, and all other comparisons of inter-annual differences proved highly significant. Seasonal changes in the abundance and species richness were also year specific. Species richness and abundance were moderate in April (before maize sowing) and increased about three times at the time of rapid maize growth (fig. 1). Maximal values were reached at different times in the compared years, but in all years, they dropped to nearly zero in late October and November. Only 7 beetles were captured after the harvest in the first half of October in 2005, and 25 specimens were caught in late November in 2004.

Seasonal changes in the abundance of 6 frequently occurring ground beetle species followed similar profiles in the Bt and non-Bt plots but differed between years (fig. 3). *P. cupreus* reached maximal abundance at the time of maize sprouting in 2004 and 2005 but at the time of maize flowering in 2003, possibly due to rainy and cold spring. The population density of *B. quadromaculatum* was highest at the time of maize flowering in 2004 and at full maize maturity in both 2003 and 2005. The abundance of *P. rufipes* and *P. melanarius* rose to a sharp peak at the time of maize maturity in 2003, but in two following years, both species occurred in high population density since the maize stage of 6 unfolded leaves. The data on ground beetles show that the total counts of specimens depend on the species composition and representation and on the sensitivity of dominating species to the environmental conditions.

The community of rove beetles was characterized by very dramatic changes in species composition and abundance between years (fig. 2b). For example, *Philonthus atratus* (Gravenhorst) and *Lesteva longoelytrata* (Goeze) were absent in 2003 but abundant in 2004 (Table S3). By contrast, *Dinaraea linearis* (Gravenhorst) and *Quedius fulgidus* (Fabricius) were abundant in 2003 but were not detected in 2004 and 2005 (fig. 4). Every year, there were 4–5 species that were distinctly more common than the remaining species. One species accounted at most for 33% of total rove beetle counts; species domination was small by comparison with the ground beetles and spiders. Representation of a species by one (singleton) or two specimens was common. The collections of 2003 included 11 singleton species, 9 of which were not detected in other years. Similarly, 17 species were found only in 2004 and 8 only in 2005. Collections of these years included 15 and 12 singletons, respectively.

Inter-annual differences in the abundance of chosen rove beetles proved insignificant in *A. rugosus* and *X. longiventris* but significant in *A. bipustulata, C. cortisimus* and *X. linearis* (Table S4). As some species were not present in all collections, some tests were limited to comparisons between two years. These tests revealed significant differences between 2003 and 2004 in the abundance of *X. elegans*. Differences between 2004 and 2005 were significant in case of *L. longoerytrata*. Rove beetle abundance and species richness rose in the period of rapid maize growth (shifted to a later phase in 2003) and declined after flowering (fig. 1). A second rise of abundance was found in the autumn 2004. The profiles of seasonal occurrence of the rove beetles demonstrated great differences in the phenology of the seven examined species. Similar changes in population densities during season were detected in *A. bipustulata* and *P. atratus* in 2004 and 2005, in all other cases, they were unique with respect to the species and year (fig. 4).

Inter-annual differences in the abundance of spider species were also considerable. In 2003, 1943 specimens of 14 species were collected in the Bt and 2069 specimens of 23 species in the non-Bt plots. Six species were singletons, including 3 found exclusively in 2003. Spider captures in 2004 yielded 4685 specimens of 38 species in the Bt, and 4799 specimens of 40 species in the non-Bt plots. Eighteen species were singletons and 19 occurred only in this year. In 2005, 3413 specimens of 32 species were collected in the Bt plots, and 3797 specimens of 31 species in the non-Bt plots. Detected species included 17 singletons and 12 species which were not found in previous years. Significant impact of year was found in all 6 analysed species (Table S4).

General spider abundance was low before sowing, increased at the time of maize sprouting, peaked at the maize stage of six unfolded leaves in June and declined during maize maturation in July and August (fig. 1). Lowest numbers of specimens, most of them *O. apicatus* and *T. ruricola*, were trapped in the stand of fully matured maize and after the harvest. In 2004, there was a second peak of spider abundance at the time of maize ripening in August through October. Spider catches became very low only in November (fig. 1). The species richness of spiders was high in all collections, and its seasonal changes were similar in all years: a peak at the time of maize sprouting was followed by a slow decline, but considerable species richness was maintained even after the harvest

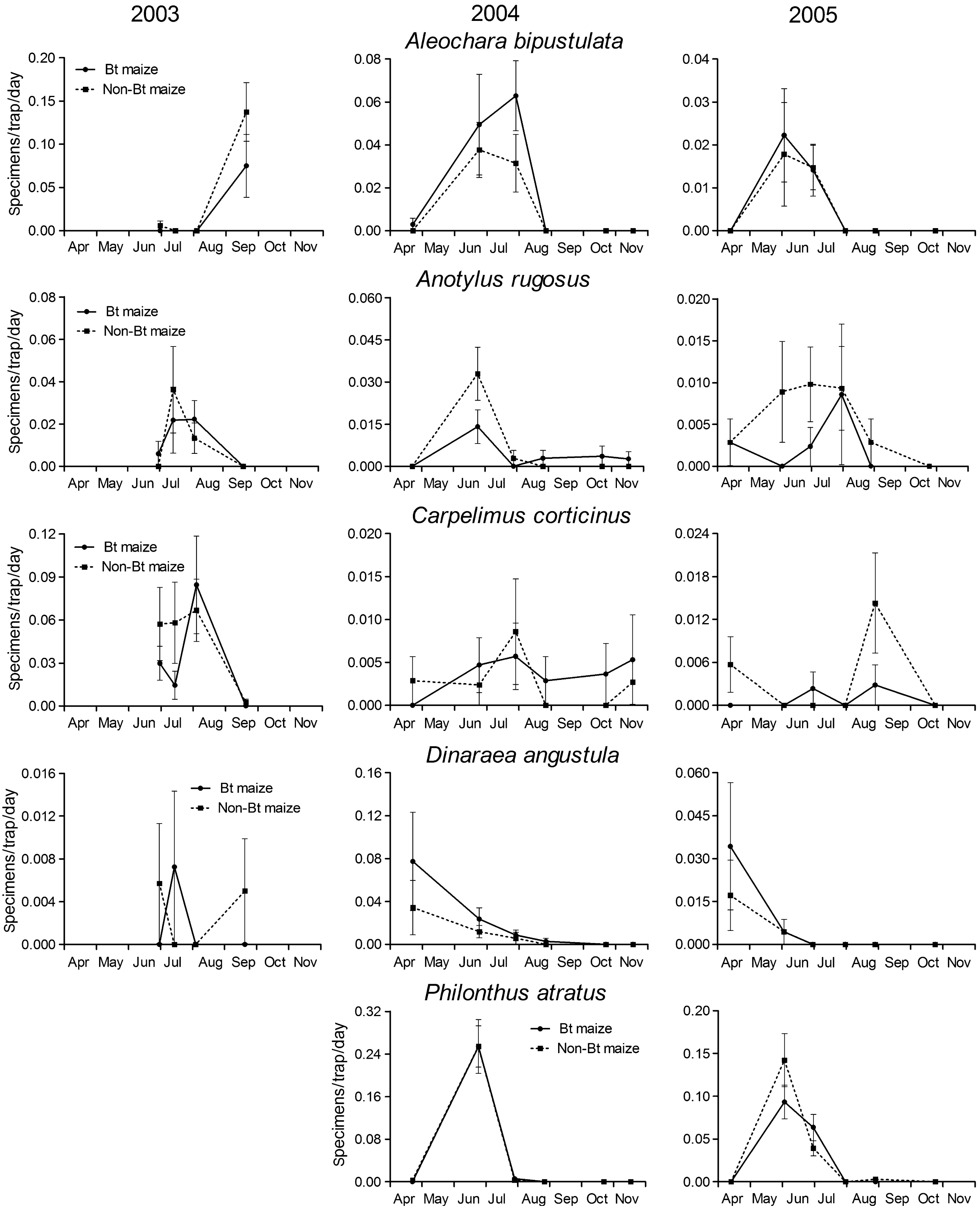

Fig. 4 Mean ± SE of the numbers of most abundant rove beetle species collected in all years in plots planted with the Bt (solid line) and non-Bt maize (dotted line). Description of the graph is the same as fig. 3.

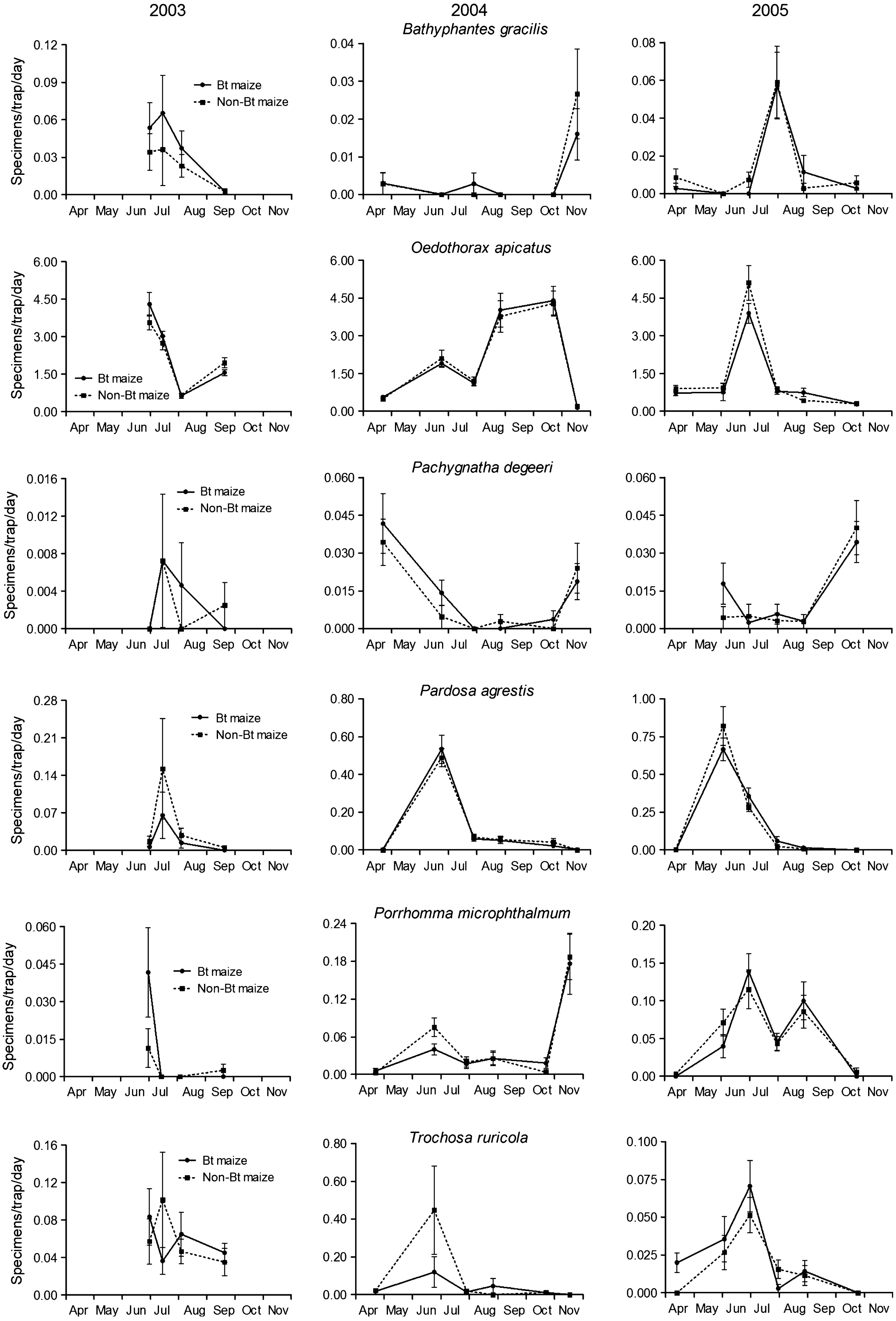

Fig. 5 Mean ± SE of the numbers of most abundant spiders species collected in plots planted with the Bt (solid line) and non-Bt maize (dotted line). Description of the graph is the same as Fig. 3.

(fig. 1). However, seasonal changes in the abundance of six common spider species were diversified (fig. 5). Every species was different, and profiles detected in 2003 were not repeated in the following years. As a matter of fact, similar profiles in two years were found only in *P. agrestis* in 2004 and 2005.

Discussion

Bt maize has no adverse effect on arthropod communities

The assemblages of the plant-dwelling (Habuštová et al. 2014) and ground-dwelling arthropods (present study) in plots planted with the Bt maize YieldGard® or with the non-transgenic parental cultivar Monumental were examined for three consecutive years. The expression of Cry1Ab protein in the Bt maize was verified with ELISA and confirmed by the observations of ECB mortality (Habuštová et al. 2014). Statistical analysis did not reveal significant differences in the total abundance and species richness of the examined ground-dwelling arthropods. The lack of differences between the Bt and non-Bt maize treatments was confirmed for the abundance of the frequently occurring species (Table S3). Seasonal changes of species abundances in the Bt and non-Bt plots exhibited virtually identical profiles (figs 3–5). All our results demonstrate that the examined arthropod communities are not affected by the Bt maize. This conclusion is consistent with the results of other studies on the effect of maize expressing Cry1Ab on the ground-dwelling arthropods (e.g., Leslie et al. 2007; Rose and Dively 2007; Farinós et al. 2008; Comas et al. 2014). In Central Europe, no significant differences in the abundance of ground beetles were found in Poland (Twardowski et al. 2012), of rove beetles in Hungary (Balog et al. 2010) and of spiders in the Czech Republic (Řezáč et al. 2006) and Germany (Toschki et al. 2007).

Variations in the composition and size of arthropod communities

Both the abundances and species compositions of the examined ground-dwelling arthropods differed between years (Table 2). Inter-annual differences were most likely caused by diverse weather patterns, changes in adjacent habitats and variability of agrotechnical operations in the field. Only a few species, referred to as agrobionts, dominate in agricultural habitats (Luczak 1979). This was clearly visible in our collections of spiders and ground beetles (fig. 2). The assemblages of all three examined groups in 2003 differed from the collections of 2004 and 2005. High abundances of the rove beetles and spiders in 2004 may be a consequence of biomass deposition in the field in autumn 2003. Ground beetle species and especially the rove beetle species dominating in 2003 were replaced by other species in the subsequent years, while the prevalence of *O. apicatus* among the spiders was persistent. Some species, namely the ground beetle *P. versicolor* and the rove beetles *Q. fulgidus*, *D. linearis*, *C. corticinus*, *X. elegans*, *P. atratus* and *L. longoelytrata,* were abundant in some years and absent in others.

The monitoring of individual species disclosed significant inter-annual differences in their abundances (Table S4) and revealed great variations in the profiles of their occurrence during season (figs 3–5).

Species occurring in our samples frequently were typical for the fields of European regions with moderate climate. The ground beetles *P. cupreus*, *P. rufipes, B. quadrimaculatum* and *C. fuscipes* common in our field were also prevalent in Bulgaria, along with *P. versicolor* (Kalushkov et al. 2009). *P. melanarius* and *P. cupreus* dominated ground beetle catches in the fields of the south-eastern part of Russia (Timraleev et al. 2002), and together with *P. rufipes* also in a region south of Moscow (Wolfenbarger and Phifer 2000) and in southern Poland (Olbrycht and Czerniakowski 2002). Our results are in accordance with those of Luka (2000), who examined seasonal population dynamics of ground beetles in Switzerland and concluded that *P. cupreus* was most common in spring, being replaced by *P. melanarius* at the beginning of summer. According to Irmler (2003), the domination of *P. melanarius* and *Bembidion lampros* (Herbst) in northern Germany was associated with high abundances of *P. versicolor* and *Anchomenus dorsalis* (Pontoppidan); the latter species was trapped rarely in our field.

The rove beetle *A. bipustulata* with parasitic ontogenesis in the puparia of Diptera attacking *Brassicaceae* plants dominated in 2003 and was abundant also in subsequent years, possibly because some neighbouring fields were seeded with oilseed rape. The other rove beetles common in our material, such as the saprophagous *A. rugosus*, algae feeders of the genus *Carpelimus* and predatory *Q. fulgidus*, *D. linearis*, *L. longoelytrata* and species of the genera *Xantholinus* and *Philonthus* feeding on aphids and insect larvae, are regular components of the ground-dwelling field fauna of Central Europe (Boháč 1999). Most of them are ubiquitous species occurring abundantly in the deforested agricultural landscape or preferring intermediate habitats affected by human activities. The species composition of our rove beetle collection was very similar to that reported from a field trial with Bt maize in Hungary (Balog et al. 2010). Some species obviously occupy large geographic regions; *C. corticinus* was found in a field in central Spain (Farinós et al. 2008).

Spider species *O. apicatus*, *P. degeeri*, *Meioneta rurestris* (C.L. Koch) (Linyphiidae) and *Erigone dentipalpis* (Wider) (Linyphiidae), which were identified as dominating agrobionts of Central Europe (Hänggi et al. 1995), ranked first, fifth, ninth and fourteenth, respectively, in the relative abundance of the species we collected. The second most common species in our material, *P. agrestis*, was identified as a dominating spider in the fields of Hungary (Samu and Szinetár 2002). Relatively high abundance of *T. ruricola* (third in abundance in our field), *P. microphthalmum* (fourth), *B. gracilis* (sixth) and *Erigone atra* (Blackwall) (Linyphiidae, thirteenth) is consistent with the ecology of these species: *O. apicatus* colonizes the fields preferentially, *T. ruricola* and *P. agrestis* are also recognized agrobionts, and *E. dentipalpis*, *E. atra*, *P. microphthalmum* and *M. rurestris* are typical inhabitants of open habitats, including cultivated fields (Luczak 1979; Buchar and Růžička 2002). A few specimens of *Arctosa leopardus* (Sundevall) and *Pardosa prativaga* (L. Koch) (Lycosidae), which are typical for wet habitats, probably invaded our plots from the moist stripes of land adjacent to our field. Species composition in our study was similar to that reported by Řezáč et al. (2006); 28 species reported by these authors were also present in our collections.

Post-market environmental monitoring

Directive 2001/18/EC and related legislation concerning PMEM receive considerable attention (EFSA 2011). To be applied on a large scale, PMEM should be (i) inexpensive, (ii) based on reliable bioindicators easy to monitor, (iii) applicable within a long enough time during season to allow adjustment to weather conditions and (iv) suitable for setting a threshold between 'no environmental impact' and 'environmental risk'. It has been proposed that arthropod species occurring in agrarian ecosystems in high abundances could be used as bioindicators (Meissle and Lang 2005; Sanvido et al. 2009). We suggest that the counts of ground beetles, rove beetles and spiders meet the first two requirements and are therefore suitable for a general surveillance of agrarian ecosystems. The three taxa in question can be recognized easily, are always present and their counts are similar across large fields – this facilitates selection of the sampling sites. On the other hand, the counts undergo unpredictable changes between years and during each season (fig. 1), obviously in response to weather conditions and other changing environmental factors (Sanvido et al. 2005, 2011).

We have initially considered the developmental stage of maize for setting up the time of sampling. We recognized, however, that the developmental stage of maize may be suitable as a reference for the seasonal fluctuations of species that feed directly on the maize plants but not for ground-dwelling predators whose prey and life cycle do not directly depend on maize development (fig. 1). The ground beetles, rove beetles and spiders are obviously more affected by other factors. One of them, the annual changes of the day length versus night length, is fully predictable and known to control developmental cycles of most organisms, including arthropods. We therefore regard calendar dates as most suitable for defining the time of PMEM based on ground-dwelling arthropods. June would be most suitable in respect to the high counts of all monitored groups. It may be objected that the exposure to the Cry toxin is low at this time and can be exceeded by toxin residues remaining in the plant biomass left in the field after a Bt crop cultivation in the previous year. This argument was invalidated by our monitoring of arthropods after deposition of all maize biomass in the soil in the autumn of 2003. General arthropod abundance increased in 2004, obviously in response to the food surplus, but no specific effect of Bt maize residues was detected.

Considerable changes in the profile of arthropod catches in the same field in successive years (fig. 1) and differences between the fields due to various factors, including the maize cultivar (Rauschen et al. 2010), impede the use of isolated arthropod counts for assessing environmental impact of any variable factor, including the cultivation of Bt maize. Collecting parallel samples in a nearby non-Bt maize field subjected to standard pest management would be the best solution, but the cost of PMEM would double. Use of historical data from the region is a less expensive but also less reliable alternative.

All available information shows that there are no or only negligible differences in arthropod abundances between the Bt and non-Bt maize. The effect of conventional insecticide applications is more evident (Bhatti et al. 2005) and could be used for setting the threshold for unwanted environmental impact. Until such data are available, a dramatic drop of counts in comparison with historical data in the region could be used as a signal calling for a thorough investigation. We are afraid that a more accurate PMEM is impossible.

The method we propose does not specifically address species diversity. Based on available data, it can be assumed that the collections will always include one to several agrobionts and a blend of less frequently occurring and also rare species. All species can be determined in case of justified concern. The proportions between the numbers of collected ground beetles, rove beetles and spiders, respectively, could be used as an indicator of general biodiversity. Most species of these taxa are predatory but differ in the type of prey and the site and method of hunting, and this may be correlated with species diversity in each of these group.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Times of sample collections in relation to maize development and calendar dates in years 2003, 2004 and 2005.

Table S2. Abundance of collected ground-dwelling species in Bt maize and non-Bt maize in 2003–2005 in the vicinity of České Budějovice, South Bohemia.

Table S3. Total numbers and per cent representations (Bt and non-Bt plots together) of the frequently captured ground beetles, rove beetles and spiders. The highest occurrence is highlighted grey and the second highest light grey. The abundance of individual species on the Bt and non-Bt plots was analyzed with RM ANOVA (F and P values).

Table S4. Results of statistical analysis of the numbers of specimens of the most common ground-dwelling arthropod species captured in the 3rd through 6th samplings in the years of study. Comparison of all years was done with RM ANOVA; in case of significant differences ($P \leq 0.05$) year pairs were analyzed by the post-hoc test Tukey HSD (remarks in parentheses specify the year with higher abundance). RM ANOVA was applied [data in brackets] to the analysis of *P. versicolor* (more in 2004), *Dinarea angustula* (no difference between 2003 and 2004), *Lesteva longoelytrata* (more in 2004) and *Xantholinus elegans* (more in 2003). Some analyses could not be performed because the species was absent in some year(s).