Diversity of Carabid Beetles (Coleoptera: Carabidae) under Three Different Control Strategies against European Corn Borer in Maize

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Abstract

Kocourek F., Saska P., Řezáč M. (2013): **Diversity of carabid beetles (Coleoptera: Carabidae) under three different control strategies against European corn borer in maize**. Plant Protect. Sci., **49**: 146–153.

We compared three control strategies against European corn borer (*Ostrinia nubilalis* Hubner) in maize with respect to carabid beetles, beneficial epigeal arthropods. The impact of the focal treatment (insect resistant Bt maize MON 810) was compared with conventionally farmed and *Trichogramma*-treated plots at two sites (Prague-Ruzyně and Ivanovice na Hané) in the Czech Republic, replicated in three cropping seasons (2002–2004). The sampled assemblages were species-poor. The species were unevenly distributed in terms of their catch size – the communities were dominated by 7 (Ruzyně) or 3 (Ivanovice) species. No differences were found in species richness or species composition between treatments, seasons or sites, suggesting no effect of planting transgenic insect resistant Bt maize MON 810 on the assemblages of carabid beetles in the study fields.

Keywords: risk assessment; transgenic crops; Bt maize; Cry endotoxins; ground beetles

Various cultivars of insect resistant transgenic maize have become intensively grown during recent years all over the world (Devos *et al.* 2009). In the European Union, MON 810 is the only insect resistant maize cultivar authorised for commercial production (Gomez-Barbero *et al.* 2008). It contains genes from the soil bacterium *Bacillus thuringiensis* encoding Cry1Ab delta-endotoxin, which has detrimental effect on herbivores feeding on modified plants (Smith 1997). However, these endotoxins may potentially be released to the environment via pollen dispersal and subsequent crossbreeding with non-modified cultivars, leaking from plant residues or through food chain to different trophic levels (Saxena & Stotzky 2001; Messeguer *et al.* 2006; Obrist *et al.* 2006). Although empirical studies have shown that Cry endotoxin did not persist in the soil after 3 years (Dubelman *et al.* 2005), the assessment of potential environmental risks of planting Bt maize is of prime importance (Romeis *et al.* 2008).

It has been shown in the laboratory that in a particular trophic system the use of Bt crops had negative effect on fitness of a hyperparasitoid (Prutz *et al.* 2004), but this result has not been supported by other studies. Although Jiang *et al.* (2004) detected Bt-toxin in the body of a spider *Pirata subpiraticus*, this result was not confirmed for other groups of invertebrate predators or parasitoids. Feeding on herbivore prey originating from Bt maize did not affect the generalist predator *Poecilus cupreus* (Meissle *et al.* 2005), spider mite predator *Stethorus punctillum* (Alvarez-Alfageme *et al.* 2008) or parasitoid *Campoletis sonorensis* (Sanders *et al.* 2007). Similarly, feeding on Bt maize residues had no negative impact on the detritophagous millipede *Allajulus latestriatus* (Weber & Nentwig 2006).

Several studies investigated the effects of cropping Bt maize on the communities of non-target organisms such as soil natural enemies of pests. In most

Supported by the Ministry of Agriculture of the Czech Republic (Projects No. 1B53043 (FK) and No. QH91093 (PS, MŘ)).

cases, assemblages of epigeal arthropods seemed not to be affected by presence of transgenic Bt maize (Volkmar & Freier 2003; Candolfi *et al.* 2004; Sehnal *et al.* 2004; Habuštová *et al.* 2005, 2006; Pons *et al.* 2005; Eizaguirre *et al.* 2006; Farinos *et al.* 2008; Balog *et al.* 2011; but see Wold *et al.* 2001), and these results were repeated also for Bt cotton and vegetables (Leslie *et al.* 2007; Torres & Ruberson 2007). Indeed, some authors conclude that compared to conventional farming, the use of transgenic plants enhances arthropod biodiversity in crop fields via reduced input of insecticides to the system (Ferry *et al.* 2006; Leslie *et al.* 2007).

We conducted a field study in which three control strategies against European corn borer (*Ostrinia nubilalis*), economically the most important pest on maize (Szoke *et al.* 2002), were compared in relation to their effects on non-target beneficial arthropods. The impact of Bt maize MON 810 was compared with the isogenic cultivar in conventionally farmed and in *Trichogramma*-treated plots. The results on spider and harvestmen assemblages were published elsewhere (Řezáč *et al.* 2006). In this paper we report on how the three control strategies against the European corn borer affected diversity and species richness of the assemblages of carabid beetles in the study fields.

MATERIAL AND METHODS

Experimental design. The experiment was conducted at two localities: Prague-Ruzyně (Central Bohemia) and Ivanovice na Hané (Central Moravia), which slightly differ in their climatic conditions. The mean annual temperature in Ivanovice na Hané and Prague-Ruzyně was 9.5 and 9.4°C, respectively, and the mean precipitations were 565 mm in Ivanovice na Hané and 519 mm in Prague-Ruzyně, respectively. All values represent mean seasonal values of over 20 years of observation. The vegetative season in Ivanovice na Hané is a bit longer and conditions for growing maize for corn are more favourable than in Prague-Ruzyně, which provides better conditions for development and damage caused by the European corn borer in Ivanovice na Hané compared to Prague-Ruzyně. The study was replicated during three consecutive years (2002–2004). At each locality, a 1 ha plot was selected and divided into three smaller plots (each 0.3 ha large), situated side by side. Bt maize hybrid MON 810 (MEB307Bt Monumental in 2002 and 2003, DKC 3421YG in 2004; both Monsanto, St. Louis, USA) was planted on one plot. The remaining plots were planted with varieties susceptible to *O. nubilalis*: an isogenic hybrid (Monumental in 2002 and 2003, DKC 3420 in 2004) and a local hybrid Raissa. At each locality, one of the non-transgenic plots was treated with the *Trichogramma* wasps (Trichocarp; Biocont Laboratory, Brno, Czech Republic) (release dates based on the phenology of *O. nubialis*); the remaining plot was treated conventionally.

The arrangement of the study plots remained the same for the three years of study. All around the plots, an 8 m wide strip of Raissa hybrid was planted as a buffer zone. The maize was sown in May and harvested by the end of September each year, except for 2004 when it was harvested in early December. The straw was crushed and deep ploughed after harvest on all plots. All plots were treated with a pre-emergent (Guardian, dose 2.5 l/ha; DuPont, Wilmington, USA) and a post-emergent (Grid, dose 20g/ha; DuPont, Wilmington, USA) herbicide.

Sampling. Epigeal arthropods were sampled using pitfall traps. A plastic cup (orifice 8 cm, volume 300 ml) was set to ground (flush with soil surface), half filled with 4% formaldehyde and covered with a steel roof. In each plot three traps were spaced 8 m apart in a line. The traps were open for 7 days in a fortnight intervals from May to September, so after each 7 days of service the traps were emptied and covered with a lid for another 7 days. There were 8 to 11 sample dates per season in total. Prior to analysis, the catch from the same plot was pooled. Carabid beetles were identified to species after Hůrka (1996).

Data analysis. Observed species richness (S_{obs}) was calculated for each plot, sampling date, and year. Because the observed species richness is a function of sample size (Gotelli & Colwell 2001), Chao 1 index (± SD) was used to estimate the true species richness (Chao 1987), which is the predicted value that is taking into account number of unrecorded but present species, based on the number of species found in one or two specimens (Colwell 2005). Species accumulation curves and sample-based rarefaction curves were plotted for each plot and year separately using data for each sampling week as samples. The rarefaction curves were produced by repeated re-sampling the pool of *N* individuals and *Q* samples at random for 50 times (Colwell 2005). To be able to compare the species

Table 1. Activity-density (AD; No. of specimens) and relative activity density (RAD; in %) of carabid beetles collected at Prague-Ruzyně site, all three seasons combined

Species	Control strategy						Total	
	Bt		*Trichogramma*		conventional			
	AD	RAD	AD	RAD	AD	RAD	AD	RAD
Pterostichus melanarius	696	32.39	690	30.34	628	30.84	2014	31.18
Pseudoophonus rufipes	355	16.52	571	25.11	571	28.05	1497	23.18
Poecilus cupreus	503	23.41	451	19.83	369	18.12	1323	20.48
Bembidion lampros	236	10.98	147	6.46	205	10.07	588	9.1
Anchomenus dorsalis	209	9.73	246	10.82	108	5.3	563	8.72
Harpalus affinis	25	1.16	27	1.19	32	1.57	84	1.3
Microlestes minutulus	17	0.79	25	1.1	21	1.03	63	0.98
Carabus cancellatus	17	0.79	17	0.75	24	1.18	58	0.9
Calathus fuscipes	18	0.84	23	1.01	12	0.59	53	0.82
Trechus quadristriatus	17	0.79	20	0.88	15	0.74	52	0.81
Bembidion quadrimaculatum	12	0.56	14	0.62	10	0.49	36	0.56
Microlestes maurus	4	0.19	6	0.26	8	0.39	18	0.28
Loricera pilicornis	7	0.33	5	0.22	1	0.05	13	0.2
Stomis pumicatus	6	0.28	5	0.22	2	0.1	13	0.2
Brachinus explodens	4	0.19	3	0.13	3	0.15	10	0.15
Notiophilus palustris	3	0.14	3	0.13	4	0.2	10	0.15
Brachinus crepitans	4	0.19	3	0.13	2	0.1	9	0.14
Ophonus azureus	1	0.05	3	0.13	4	0.2	8	0.12
Pseudoophonus griseus	2	0.09	0	0	5	0.25	7	0.11
Bembidion obtusum	2	0.09	1	0.04	2	0.1	5	0.08
Bradycellus csikii	2	0.09	1	0.04	2	0.1	5	0.08
Acupalpus meridionalis	2	0.09	0	0	2	0.1	4	0.06
Amara consularis	0	0	1	0.04	2	0.1	3	0.05
Harpalus distinguendus	1	0.05	1	0.04	1	0.05	3	0.05
Leistus ferrugineus	0	0	3	0.13	0	0	3	0.05
Poecilus versicolor	0	0	3	0.13	0	0	3	0.05
Amara aulica	2	0.09	0	0	0	0	2	0.03
Amara convexiuscula	0	0	1	0.04	0	0	1	0.02
Amara equestris	0	0	0	0	1	0.05	1	0.02
Amara montivaga	1	0.05	0	0	0	0	1	0.02
Amara ovata	0	0	1	0.04	0	0	1	0.02
Anisodactylus signatus	0	0	0	0	1	0.05	1	0.02
Calathus ambiguus	0	0	1	0.04	0	0	1	0.02
Carabus granulatus	0	0	0	0	1	0.05	1	0.02
Carabus intricatus	0	0	1	0.04	0	0	1	0.02
Dolichus halensis	0	0	1	0.04	0	0	1	0.02
Harpalus rubripes	1	0.05	0	0	0	0	1	0.02
Harpalus tardus	1	0.05	0	0	0	0	1	0.02
Ophonus (*Methophonus*) sp.	1	0.05	0	0	0	0	1	0.02

richness between plots/years, a correction must be made for the number of the specimens collected (Gotelli & Colwell 2001, for further discussion). Therefore, rarefied species richness was plotted ("re-scaled") against rarefied number of specimens collected; this standardization eliminates the effect of sample sizes on observed species richness.

The evenness of the assemblages that indicates dominance structure within an assemblage was visually tested on plots of log-abundance on species ranked according to their activity-density for both fields (Southwood & Henderson 2000). In order to assess the dominance of the assemblages, relative activity-density, i.e. proportion of total individuals accounted, was determined for each species. The species together constituting 95% of relative activity-density were considered dominant (Luff 2002).

The composition of carabid assemblages was compared between fields using the estimated abundance-based Chao-Jaccard similarity index (Chao *et al.* 2005), which takes into account the

Table 2. Activity-density (AD; No. of specimens) and relative activity density (RAD; in %) of carabid beetles collected at Ivanovice na Hané site, all three seasons combined

Species	Control strategy						Total	
	Bt		*Trichogramma*		conventional			
	AD	RAD	AD	RAD	AD	RAD	AD	RAD
Pseudoophonus rufipes	634	62.4	455	48.1	862	63.34	1951	58.71
Pterostichus melanarius	265	26.08	281	29.7	297	21.82	843	25.37
Calathus fuscipes	79	7.78	150	15.86	141	10.36	370	11.13
Poecilus cupreus	8	0.79	7	0.74	8	0.59	23	0.69
Harpalus distinguendus	2	0.2	3	0.32	14	1.03	19	0.57
Anchomenus dorsalis	7	0.69	8	0.85	3	0.22	18	0.54
Trechus quadristriatus	2	0.2	10	1.06	2	0.15	14	0.42
Harpalus affinis	5	0.49	2	0.21	6	0.44	13	0.39
Carabus scheidleri	1	0.1	2	0.21	5	0.37	8	0.24
Leistus ferrugineus	2	0.2	3	0.32	2	0.15	7	0.21
Bembidion lampros	2	0.2	1	0.11	3	0.22	6	0.18
Calathus ambiguus	1	0.1	2	0.21	3	0.22	6	0.18
Dolichus halensis	1	0.1	3	0.32	2	0.15	6	0.18
Stomis pumicatus	3	0.3	3	0.32	0	0	6	0.18
Amara bifrons	2	0.2	3	0.32	0	0	5	0.15
Pseudoophonus griseus	0	0	1	0.11	3	0.22	4	0.12
Amara aulica	0	0	2	0.21	1	0.07	3	0.09
Bembidion quadrimaculatum	1	0.1	2	0.21	0	0	3	0.09
Acupalpus meridionalis	0	0	0	0	2	0.15	2	0.06
Carabus ullrichi	0	0	2	0.21	0	0	2	0.06
Harpalus tardus	0	0	0	0	2	0.15	2	0.06
Ophonus azureus	1	0.1	0	0	1	0.07	2	0.06
Ophonus rufibarbis	0	0	1	0.11	1	0.07	2	0.06
Synuchus vivalis	0	0	0	0	2	0.15	2	0.06
Anisodactylus signatus	0	0	1	0.11	0	0	1	0.03
Bembidion obtusum	0	0	1	0.11	0	0	1	0.03
Bembidion properans	0	0	1	0.11	0	0	1	0.03
Brachinus explodens	0	0	1	0.11	0	0	1	0.03
Ophonus (*Methophonus*) sp.	0	0	1	0.11	0	0	1	0.03
Zabrus tenebrioides	0	0	0	0	1	0.07	1	0.03

contribution made per species estimated to be present (but not detected) at both sites (Colwell 2005). This approach substantially reduces the weakness of traditional similarity indices when assemblages are incompletely sampled (Colwell 2005). All rarefaction curves, species richness estimators, and indexes were computed using the free software program EstimateS 7.50 (Colwell 2005).

RESULTS

In total, 48 carabid species in 9782 individuals were collected over the three seasons (Tables 1 and 2). In Prague-Ruzyně, 39 species were recorded compared to 30 species in Ivanovice na Hané. However, the values of the Chao 1 index suggest that the real species richness was much higher in most study plots and years (Table 3), due to a number of species that were found as singletons or doubletons. Twenty-three carabid species were shared by both localities, giving Chao-Jaccard similarity = 0.98; the assemblages in the study fields were thus predicted to be highly similar. Pooled across treatments and years, relative contribution of carabid species in Prague-Ruzyně was more even according to log-ranked "abundance" of individual species (Figure 1) and dominated by seven species (Table 1). The assemblage in Ivanovice na Hané was highly uneven (Figure 1) and dominated by three carabid species only (Table 2).

Although the species richness and number of collected individuals differed between the study plots at both localities and in all studied years (Table 3), the rarefaction curves revealed that the type of control strategy against *O. nubilalis* did not affect carabid species richness significantly (Figure 2) since the confidence intervals of particular curves broadly overlapped (not shown in the figures). Similarly, the values of the Chao-Jaccard similarity index were also very high (0.93–1.00) for all pairs of plots within each year and locality.

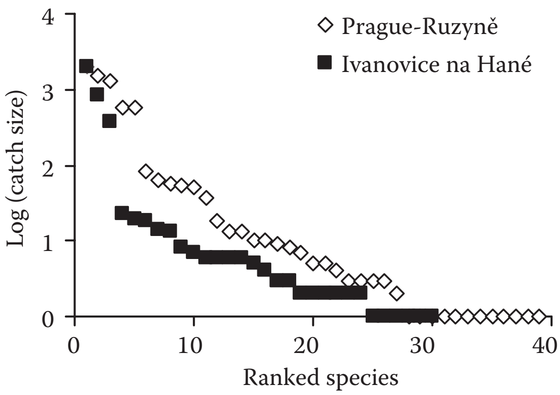

Figure 1. Evenness of the carabid assemblages as ranked log(catch size) plot.

The use of different control strategies against *O. nubilalis* did not affect populations of carabid beetles in the study plots between the years. Pooled across treatments, the species richness remained unchanged at both localities (Figure 3), so did the composition of carabid assemblages (Chao-Jaccard similarity index = 0.93–0.99).

DISCUSSION

Before the GM-crops can be widely approved for commercial cropping, an extensive risk assess-

Table 3. Species richness estimators for communities of carabid beetles

	Bt			*Trichogramma*			Control		
	2002	2003	2004	2002	2003	2004	2002	2003	2004
Prague-Ruzyně									
N_{ind}	749	967	433	822	991	461	760	809	467
S_{obs}	23	16	18	19	19	18	24	24	16
S_{est} (Chao 1)	34 ± 10	22 ± 8	34 ± 17	44 ± 31	25 ± 7	24 ± 7	32 ± 8	27 ± 17	18 ± 2
Ivanovice na Hané									
N_{ind}	535	349	12	366	388	225	466	673	222
S_{obs}	14	10	5	13	18	9	12	15	12
S_{est} (Chao 1)	15 ± 2	12 ± 4	5 ± 1	14 ± 1	48 ± 29	14 ± 7	22 ± 10	18 ± 3	21 ± 10

N_{ind} – number of collected individuals; S_{obs} – number of observed species; S_{est} (Chao 1) – estimated species richness using Chao 1 index ± SD

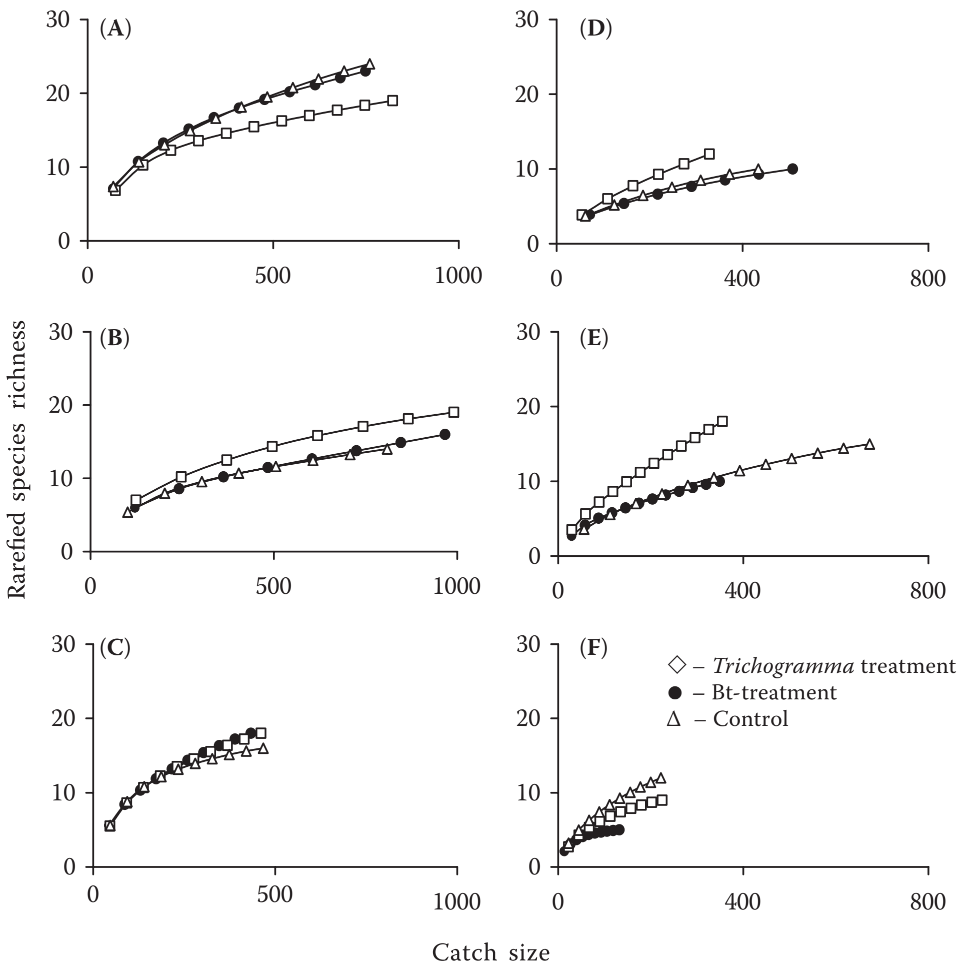

Figure 2. Comparison of the rarefaction curves within season and site. **A–C**: Prague-Ruzyně, **D–F**: Ivanovice na Hané; **A**, **D**: 2002, **B**, **E**: 2003, **C**, **F**: 2004

ment must be conducted (Romeis *et al.* 2008). As a part of the risk assessment of insect resistant Bt maize in the Czech Republic, this study investigated indirect effects of different control strategies against *O. nubilalis*, including planting Bt maize, on carabid beetles. Carabids are natural enemies of many insect pests and weeds (Holland 2002). Similarly to spiders and harvestmen (Řezáč *et al.* 2006), we could not detect any treatment effect on the assemblage of carabid beetles in any of the study fields. Our results are thus congruent with previous findings (Volkmar & Freier 2003; Candolfi *et al.* 2004; Pons *et al.* 2005; Eizaguirre *et al.* 2006; Farinos *et al.* 2008; Balog *et al.* 2011), including those from the Czech Republic. No significant negative effect of Bt maize on carabid beetles, rove beetles, and spiders was found in the study from south-easten part of the Czech Republic (Habuštová *et al.* 2006) nor significant differences between Bt maize and izogenic cultivar were found in the occurrence of aphids, thrips, and predatory bugs (Sehnal *et al.* 2004).

Additionally, this study provides relevant information on carabid assemblages in maize. In general, sampled carabid assemblages were rather poor in species richness, dominated by only a few species. The most common species were *Pterostichus melanarius* and *Pseudoophonus rufipes*, which altogether constituted 65% of the catch. In similar experiment with Bt-maize in south-easten part of the Czech Republic, *Pterostichus melanarius* and *Poecilus cupreus* were the most abundant (Habuštová *et al.* 2006). In general, maize crop is regarded as a very hostile habitat for arthropod diversity (Thiele 1977), probably due to unfavourable microclimate, and this was confirmed in our study regardless of the control strategy used. The carabid fauna was almost identical in Prague-Ruzyně and Ivanovice na Hané, despite the geographical distance and climatic difference. Our data thus indicate that intensive use of arable land by agricultural production may deplete epigeal fauna to only few eurytopic species capable of withstanding drastic disturbance such

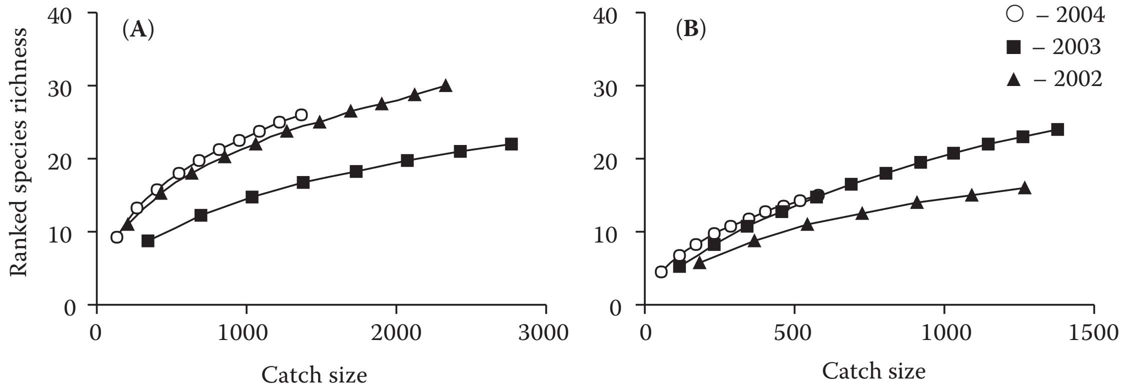

Figure 3. Comparison of rarefaction curves from one site between seasons. (A) Prague-Ruzyně, (B) Ivanovice na Hané

as deep tillage and frequent pesticide application and change in microclimate, regardless of the initial species diversity. Regarding to non-target epigeal fauna, soil cultivation or pesticide use might thus be more detrimental to diversity than the use of transgenic crops (Leslie *et al.* 2007).

Acknowledgements. We would like to thank M. Bárnet, P. Hledík, K. Říha, K. Pepperný and L. Slámová for their help with field work and sorting the material.

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Received for publication November 1, 2012
Accepted after correction February 24, 2013

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