

## Activity density, diversity and seasonal dynamics of ground beetles (Coleoptera: Carabidae) in *Bt*- (MON810) and in isogenic maize stands

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To compare carabid assemblages from transgenic *Bt*- and isogenic maize in Hungary, we used pitfall traps in an experiment (30×30 m plots, arranged alternately in 6 replications), during the growing season of maize during 2001–2003. We captured altogether 44,103 individuals of 58 species. The most common species in our sampling were *Calathus ambiguus*, *Dolichus halensis*, *Harpalus distinguendus*, *H. rufipes*, *Poecilus sericeus* and *Trechus quadristriatus*. The same species dominated both in the *Bt*- and in the isogenic maize plots. Under our test conditions (plot size and 3 years of sampling), no significant differences were found either in the structural characteristics (number of individuals, number of species and diversity), activity density or seasonal activity patterns of the dominant species.

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### 1. Introduction

Maize is one of the major crops in Hungary, covering about 1.2 million ha (<http://faostat.fao.org/faostat/>). Lepidopteran pests of maize, the European Corn Borer (*Ostrinia nubilalis* Hübner; ECB) and the Cotton Bollworm (*Helicoverpa armigera* Hübner; CB) can cause yield losses in maize though their actual damage varies largely by location and year (Pálffy 1983, Szeőke 1994). Ground beetles are common predators in agricultural fields playing an important role in reducing pest populations in many crop ecosystems (Lövei & Sunderland 1996). Carabids are widely used

indicators for measuring ecological impacts because the family is rich in species, taxonomically well known, they are very abundant in arable crops and seem highly sensitive to habitat changes (Lövei & Sunderland 1996, Rainio & Niemelä 2003).

One of the control options for managing ECB population level is using transgenic maize with *cry1Ab* gene from *Bacillus thuringiensis* Berliner var. *kurstaki* that expresses Cry1Ab toxin (*Bt* maize) which is effective against ECB larvae. The coexistence potential of these two types of pest control needs careful assessment.

The Cry1Ab toxin is effective against larvae

of Lepidoptera, the predatory arthropods in maize stands may be affected by the altered maize plant and through various trophic interactions. Many species of carabids are generalist predators consuming arthropods on foliage or on the ground, larvae and pupae of Lepidoptera on the plant or in the soil (Thiele 1977). Therefore carabids may be impacted by Cry1Ab toxin through preyed arthropods. Carabids may also consume maize plant tissues or residues. The presence of Cry1Ab toxin in seven carabid adults sampled from fields with *Bt* maize residues was recently demonstrated by Zwahlen and Andow (2005). Larvae of ground beetles can also be exposed to Cry1Ab toxin in the soil by root exudates in the rhizosphere of *Bt* maize plants (Saxena et al. 2002).

Some laboratory experiments indicated that *Bt*-toxin had negative impact on the development of *Chrysoperla carnea* larvae as non-target predator (Hilbeck et al. 1998, Dutton et al. 2002). Transgene products can adversely affect also ground beetles (Jørgensen & Lövei 1999, Meissle et al. 2005). Field studies indicate that the seasonal activity in Europe of non-target arthropods (Lozzia & Rigamonti 1998, Bourguet et al. 2002, Sehnal et al. 2004, Daly & Buntin 2005, De la Poza et al. 2005) and among them that of carabid beetles (Manachini et al. 1999, Volkmar et al. 1999, Sehnal et al. 2004) does not differ between the transgenic and isogenic maize.

No studies have been performed on the potential impact of GM maize in the Carpathian Basin, which forms a special biogeographical area (Anon. 2004). In a three-year (2001–2003) study under the Bt-BioNoTa project “Effects and mechanism of *Bt* transgenes on biodiversity of non-target insects: pollinators, herbivores and their natural enemies” (No. QLK3-CT-2000-00547), we have surveyed the carabid assemblages in *Bt*- and in isogenic maize plots. Our aim was to assess the possible impact of *Bt*-maize on carabid assemblages measured by their activity density, the structural characteristics and seasonal activity patterns of the carabid assemblages. Our hypothesis was that these parameters will reflect any adverse effect of transgenes on carabid individuals either through direct or indirect way.

## 2. Material & Methods

### 2.1. Experimental area and sampling method

The three-year field experiment was carried out in an isolated field surrounded by large peach and apricot orchards near Budapest (47°25' N, 18°47' E), Hungary. Plots (30 m × 30 m) with *Bt*-maize (DK 440 BTY-transformation event MON 810) and with its isogenic line (DK 440) were established on chernozem soil and arranged alternately, with 6 replications (Fig. 1). An alley distance of 3 m was used between replications. A maize hybrid of similar maturity ground to the test hybrid was planted in the retention zone (pollen capture crop surrounding the entire test field) in accordance with the requirements of the release permit.

Maize was planted at a seed rate of 65,000 seeds/ha reduced to 50,000 plants/ha after emergence. Planting was between late April and early May, and maize was harvested between mid-October and early November, depending on the year. Regulations did not allow replanting GM plants in exactly the same place; hence the plots, even though close, were physically different from year to year: the maximum distance between two areas was some meters. The previous crop was tomato for the first year and sunflower for the second and third years. There was no insecticide application in the maize plots (except a soil application of diazinon at planting in the first year).

Carabid adults were collected using pitfall traps. Traps were made from two 300 ml plastic

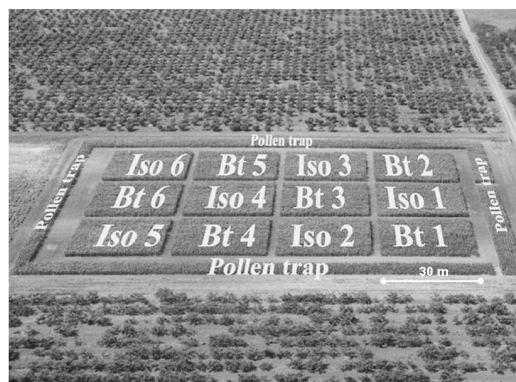


Fig. 1. Aerial view of the experimental block design with plots of transgenic (*Bt*) and isogenic (Iso) maize in 2001 in Sósokút, Hungary.

Table 1. Percentage of carabid individuals captured in 2001–2003, Sósokút, Hungary (+ indicates &lt;1%).

Species	2001		2002		2003	
	Iso	<i>Bt</i>	Iso	<i>Bt</i>	Iso	<i>Bt</i>
<i>Amara ingenua</i> (Duftschmid)	–	–	1.90	2.14	+	+
<i>Anchomenus dorsalis</i> (Pontoppidan)	+	2.07	–	+	+	+
<i>Anisodactylus signatus</i> (Panzer)	+	+	+	+	2.07	1.55
<i>Brachinus explodens</i> Duftschmid	+	–	6.20	5.95	+	+
<i>Brosicus cephalotes cephalotes</i> (Linnaeus)	1.08	+	+	+	+	+
<i>Calathus ambiguus</i> (Paykull)	13.05	8.78	6.14	4.45	2.31	1.73
<i>Calathus melanocephalus</i> (Linnaeus)	2.11	2.02	+	+	+	+
<i>Dolichus halensis</i> (Schaller)	5.04	5.77	6.58	6.86	2.99	2.12
<i>Harpalus distinguendus distinguendus</i> (Duftschmid)	3.41	3.16	26.03	25.83	16.22	16.78
<i>Poecilus sericeus</i> Fischer von Waldheim	2.60	3.01	3.04	3.26	5.92	5.09
<i>Pseudoophonus calceatus</i> (Duftschmid)	3.63	3.75	+	+	+	+
<i>Pseudoophonus (Harpalus) rufipes</i> (De Geer)	59.12	64.18	43.14	45.01	65.87	68.07
<i>Trechus quadristriatus</i> (Schrank)	5.85	3.95	2.70	2.08	2.65	2.80
Other species	3.47	2.61	3.00	3.04	+	+
Total number of species	27	25	47	46	33	34
Total number of individuals	2,027	1,847	7,109	7,372	12,758	12,990

cups containing 4% formaldehyde solution as killing agent and preservative. Two pitfall traps were placed in the middle (15<sup>th</sup>) row of the plot, at 10m distance from each other and from the border of the plot. Samplings lasted from late July in 2001 and from late May in 2002 and 2003 until harvest. Traps were emptied weekly. The content of traps was taken to the laboratory for identification of carabid adults to species level, using keys by Hürka (1996).

## 2.2. Statistical analyses

The structural characteristics of the assemblages: the number of species, Shannon diversity index (Magurran 2003) and the activity density of dominant species on *Bt*- and isogenic plots were compared using Kruskal-Wallis test and ANOVA with repeated measures, respectively (Sokal & Rohlf 1995). The level of significance was set at  $p=0.05$ . The Rényi diversity (Tóthmérész 1995) was used for comparing the diversity of assemblages in *Bt*- vs. isogenic plots. Analyses were made using STATISTICA (Statsoft 2000) and the DivOrd (Tóthmérész 1993) program packages.

## 3. Results

A total of 44,103 individuals of 58 ground beetle species were collected in maize plots during the three sampling periods (Table 1). *Harpalus rufipes* De Geer, 1774, *Harpalus distinguendus* Duftschmid, 1812, *Poecilus sericeus* Fischer von Waldheim, 1823, *Dolichus halensis* Schaller, 1783, *Calathus ambiguus* Paykull, 1790, and *Trechus quadristriatus* Schrank, 1781 (in order of decreasing catch) were the most common species. These six species made up 93% of the total number of individuals captured. The same species dominated both in *Bt*- and in isogenic maize plots.

No significant differences were found in structural characteristics in terms of ground beetles captured between the two treatments in the given year, using Kruskal-Wallis test (Table 2). For the mean number of species/trap, the H values were (1) in 2001:  $H_{1,24} = 0.223$ ; (2) in 2002:  $H_{1,24} = 0.986$ ; and (3) in 2003:  $H_{1,24} = 1.055$ . Likewise, for the mean number of individuals/trap, the values were (1) in 2001:  $H_{1,24} = 3.102$ ; (2) in 2002:  $H_{1,24} = 0.213$ ; and (3) in 2003:  $H_{1,24} = 0.013$ . The mean diversity/trap did not show significant differences either; the H values were as follows: (1)

Table 2. Structural characteristics of carabid assemblages in *Bt*- and in isogenic maize plots (2001, 2002 and 2003, Sós-kút, Hungary). Values are means  $\pm$  SE. None of the comparisons within year were significantly different (Kruskal-Wallis test).

Characteristic	2001		2002		2003	
	<i>Bt</i>	Iso	<i>Bt</i>	Iso	<i>Bt</i>	Iso
Total no. species	25	27	47	46	33	34
No. species/trap	12.33 $\pm$ 0.43	12.92 $\pm$ 0.54	21.25 $\pm$ 0.97	22.67 $\pm$ 0.93	15.0 $\pm$ 0.66	16.08 $\pm$ 0.67
Total no. individuals	2,027	1,847	7,109	7,372	12,758	12,990
No. indiv/trap.	168.92 $\pm$ 7.55	153.92 $\pm$ 13.04	592.42 $\pm$ 43.8	614.33 $\pm$ 39.6	1,063.17 $\pm$ 71.95	1,082.5 $\pm$ 34.67
Shannon diversity	1.36 $\pm$ 0.05	1.47 $\pm$ 0.04	1.64 $\pm$ 0.04	1.72 $\pm$ 0.05	1.13 $\pm$ 0.04	1.2 $\pm$ 0.03

Table 3. Mean activity density of the most common carabid species/trap in *Bt*- and in isogenic maize plots (2001, 2002 and 2003, Sós-kút, Hungary). Values are means  $\pm$  SE. None of the comparisons within year were significantly different (Kruskal-Wallis test).

Treatment	2001		2002		2003	
	<i>Bt</i>	Iso	<i>Bt</i>	Iso	<i>Bt</i>	Iso
<i>C. ambiguus</i>	14.83 $\pm$ 1.72	20.08 $\pm$ 3.81	26.33 $\pm$ 5.15	37.75 $\pm$ 6.54	18.42 $\pm$ 2.58	25.0 $\pm$ 2.93
<i>D. halensis</i>	9.75 $\pm$ 1.34	7.75 $\pm$ 2.1	40.67 $\pm$ 7.14	40.42 $\pm$ 3.56	22.5 $\pm$ 2.34	32.33 $\pm$ 6.32
<i>H. distinguendus</i>	5.33 $\pm$ 0.87	5.25 $\pm$ 0.91	153.0 $\pm$ 19.63	159.92 $\pm$ 15.73	178.42 $\pm$ 15.53	175.58 $\pm$ 11.77
<i>H. rufipes</i>	108.42 $\pm$ 5.49	91.0 $\pm$ 7.18	266.67 $\pm$ 16.45	265.0 $\pm$ 29.89	723.67 $\pm$ 58.36	713.0 $\pm$ 31.57
<i>P. sericeus</i>	5.08 $\pm$ 0.92	4.0 $\pm$ 0.84	19.33 $\pm$ 2.55	18.67 $\pm$ 3.07	54.17 $\pm$ 5.37	64.08 $\pm$ 8.69
<i>T. quadristriatus</i>	6.67 $\pm$ 1.7	9.0 $\pm$ 1.4	12.33 $\pm$ 1.34	16.58 $\pm$ 2.61	29.75 $\pm$ 5.24	28.67 $\pm$ 4.88

in 2001:  $H_{1,24} = 3.630$ ; (2) in 2002:  $H_{1,24} = 1.333$ ; and (3) in 2003:  $H_{1,24} = 1.921$ ;  $p > 0.05$  in all cases). The evenness of the assemblages was not different either (Table 2).

There were no significant differences in the mean total activity density of carabids/year/trap between the *Bt*- and isogenic maize plots regarding all carabids for three years (Kruskal-Wallis test; 2001:  $H_{1,24} = 3.102$ ; 2002:  $H_{1,24} = 0.213$ ; 2003:  $H_{1,24} = 0.013$ ;  $p > 0.05$  in all cases) or among the dominant species (Table 3, Kruskal-Wallis test; (1) *C. ambiguus*, in 2001:  $H_{1,24} = 0.482$ , in 2002:  $H_{1,24} = 2.343$ , in 2003:  $H_{1,24} = 3.234$ ; (2) *D. halensis*, in 2001:  $H_{1,24} = 1.480$ , in 2002:  $H_{1,24} < 0.001$ , in 2003:  $H_{1,24} = 1.849$ ; (3) *H. distinguendus*, in 2001:  $H_{1,24} = 0.014$ , in 2002:  $H_{1,24} < 0.001$ , in 2003:  $H_{1,24} = 0.021$ ; (4) *H. rufipes*, in 2001:  $H_{1,24} = 3.635$ , in 2002:  $H_{1,24} = 0.608$ , in 2003:  $H_{1,24} = 0.053$ ; (5) *P. sericeus*, in 2001:  $H_{1,24} = 1.159$ , in 2002:  $H_{1,24} = 0.303$ , in 2003:  $H_{1,24} = 0.522$ ; (6) *T. quadristriatus*, in 2001:  $H_{1,24} = 1.864$ , in 2002:  $H_{1,24} = 1.289$ , in 2003:  $H_{1,24} =$

0.141;  $p > 0.05$  in all cases).

The Rényi diversity profiles ran very close to each other (Fig. 2), and while the profile from the *Bt* plots usually indicates a lower diversity, the difference was minimal.

Within-season comparisons of activity densities showed generally no significant differences either in total activity density (Fig. 3; GLM, in 2002:  $F = 0.56$  and in 2003:  $F = 0.58$ ; d.f. = 16,  $p > 0.05$  in all cases, except in 2001; see later) or in the activity density of the most common species (*H. rufipes* in 2003, Fig. 4; GLM, *C. ambiguus*: in 2001:  $F = 0.94$ , d.f. = 6; in 2002:  $F = 0.98$ , d.f. = 4, *D. halensis*: in 2001:  $F = 1.79$ , d.f. = 4; in 2002:  $F = 1.08$ , d.f. = 5; in 2003:  $F = 0.43$ , d.f. = 6, *H. distinguendus*: in 2001:  $F = 0.004$ , d.f. = 2; in 2002:  $F = 1.71$ , d.f. = 13; in 2003:  $F = 0.57$ , d.f. = 15, *H. rufipes*: in 2002:  $F = 0.22$ , d.f. = 13; in 2003:  $F = 0.51$ , d.f. = 12, *P. sericeus*: in 2001:  $F = 0.46$ , d.f. = 4; in 2002:  $F = 0.91$ , d.f. = 8, *T. quadristriatus*: in 2001:  $F = 0.69$ , d.f. = 4; in 2003:  $F = 0.25$ , d.f. = 1;  $p > 0.05$ , in all cases; ex-

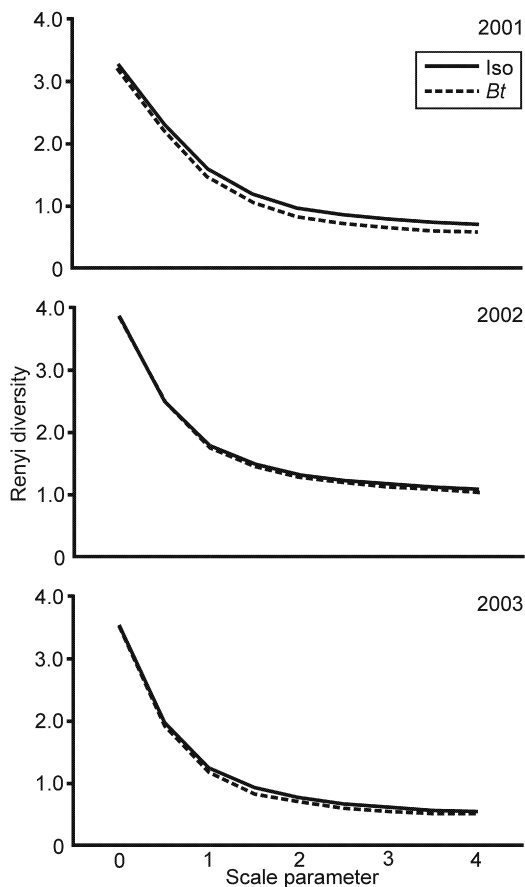


Fig. 2. Rényi diversity profiles of the ground beetle assemblages in *Bt*- and in isogenic maize plots for 2001 (up), 2002 (middle) and 2003 (bottom); Sós-kút, Hungary.

cept for given species; see below) in the *Bt*- and in isogenic maize in any year.

The activity peaks of adult carabids varied from year to year (in 2001, late July; in 2002, mid-July; and in 2003, early July) both in the *Bt*- and the isogenic plots. Several species showed bimodal activity (e.g., *H. distinguendus*: May and October, *T. quadristriatus*: May–June and October), while other species showed unimodal activity (e.g., *D. halensis*: July, *H. rufipes*: early July). No significant differences were found in the seasonal activity patterns of above species in *Bt*- and in isogenic plots with the exception for total species in 2001 (GLM;  $F = 2.57$ , d.f. = 11,  $p = 0.006$ ), due to the different activity pattern of *H. rufipes* (GLM;  $F = 2.53$ , d.f. = 11,  $p = 0.009$ ) in 2001, *C. ambiguus* (GLM;  $F = 3.5$ , d.f. = 7,  $p = 0.003$ ), *T.*

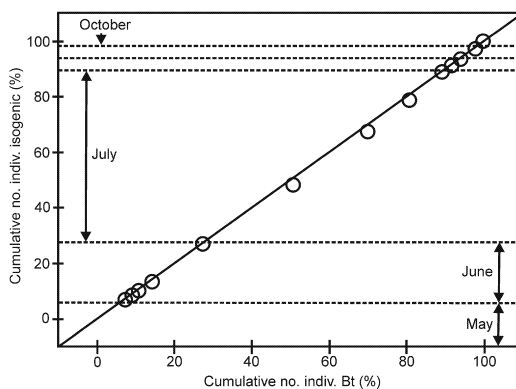


Fig. 3. A comparison of the seasonal activity pattern of adult carabids captured by pitfall traps in *Bt*- and in isogenic maize plots in 2003, at Sós-kút, Hungary. The two activity curves did not deviate from each other (GLM;  $F = 0.58$ , d.f. = 16,  $p > 0.05$ ).

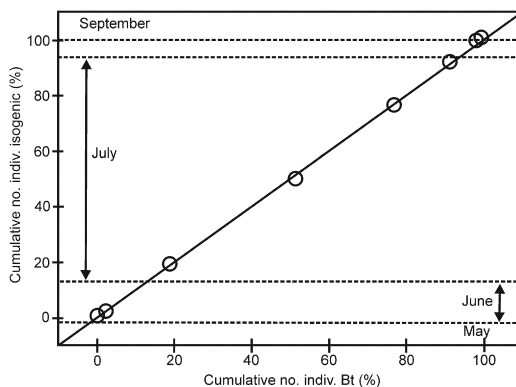


Fig. 4. A comparison of seasonal activity patterns of *Harpalus rufipes* adults captured by pitfall traps in *Bt*- and isogenic in maize plots in 2003, at Sós-kút, Hungary. The two curves were not different from each others (GLM;  $F = 0.51$ , d.f. = 12,  $p > 0.05$ ).

*quadristriatus* (GLM;  $F = 0.02$ , d.f. = 4,  $p = 0.021$ ) in 2002 and *P. sericeus* (GLM;  $F = 2.09$ , d.f. = 11,  $p = 0.025$ ) in 2003. The activity density values of these three species alternated in time either for *Bt*- or for isogenic plots without any constant difference between treatments.

#### 4. Discussion

We found a species-rich carabid assemblage with high individual numbers both in the *Bt*- (Cry1Ab) and in the isogenic maize plots. The number of collected species (58) exceeded the one found in

maize stands in various regions in Central Europe (Sekulić 1976, Andriescu *et al.* 1984, Lövei 1984). A possible reason for this is the immigration of some species from the neighbouring orchard to the maize plots. The most common species in our sampling are typical in agricultural fields in Europe (Thiele 1977, Lövei & Sáros-patakai 1990).

The relatively small plot size (30 m × 30 m) raises the question of the validity of our data and the potential to draw conclusions regarding larger fields. This was a logistical constraint as the release permit did not allow larger plots sizes. Bare ground between plots probably decreased the movement of carabid adults between plots (Frampton *et al.* 1995, García *et al.* 2000). Field tests on even smaller plots [10 m × 25 m; Cárcamo & Spence (1994)] showed that carabid assemblages adjust habitat use in relation to variation at this spatial scale.

During our three-year field investigation, we found no difference in assemblage characteristics in *Bt*- vs. non-*Bt* maize, similarly to other studies (Manachini *et al.* 1999, Lozzia 1999, Manachini 2000, Dively & Rose 2002, Sehnal *et al.* 2004, Daly & Buntin 2005, De la Poza *et al.* 2005, Lopez *et al.* 2005). Our results showed that the activity patterns of the most common species were also mostly similar in *Bt*- and in isogenic maize plots, thus the treatment had minor influence on their seasonal dynamics. Shifts of capture peaks between years were probably caused by weather factors. Although the seasonal activity patterns of sampled ground beetle assemblages in *Bt*- and in isogenic maize plots were synchronised, the activity peaks of abundant species were sometimes different. Autumn breeding species (*C. ambiguus*, *D. halensis*, *H. rufipes*) dominated the assemblage, because at the time of peak activity by spring breeders (*H. distinguendus*, *Poecilus sericeus*, *Amara* spp.), the maize has not yet emerged (Lövei 1984).

We found some differences in species activities with respect to published data. These are probably caused by differences in local conditions or changes in climate. *H. rufipes* had one activity peak in July, not two as in Serbia (Sekulić 1976) and not in August–September as in Slovakia (Štepanovičová & Beláková 1960). In our case, the timing of activity peak of *H. rufipes* fits

between the two peaks observed by Sekulić (1976). *D. halensis* is common in August in Central Hungary (Gergely & Lövei 1987) while we found a longer activity period. *T. quadristriatus* showed a similar activity pattern to that described by Desender and Pollet (1987). These patterns were probably caused by their reproductive periods and the appearance of teneral specimens.

In conclusion, we did not detect drastic differences between carabid assemblages of *Bt*- and isogenic maize plots, considering activity density, and seasonal activity density at our plot size. The impact of planting *Bt*-maize on larger field and over a longer period should be tested.

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