

## Rove beetle (Coleoptera: Staphylinidae) communities in transgenic Bt (MON810) and near isogenic maize

Adalbert Balog<sup>a,b,\*</sup>, József Kiss<sup>c</sup>, Dóra Szekeres<sup>c</sup>, Ágnes Szénási<sup>c</sup>, Viktor Markó<sup>d</sup>

<sup>a</sup> Department of Horticulture, Faculty of Technical Science, Sapientia Hungarian University of Transylvania, 1/C Sighisoara str., Tg. Mures, Romania

<sup>b</sup> Institute of Ecology, Friedrich-Schiller University, 159 Dornburger str., 07743 Jena, Germany

<sup>c</sup> Institute of Plant Protection, Faculty of Agriculture and Environmental Sciences, Szent István University, 1 Páter Károly str., Gödöllő, Hungary

<sup>d</sup> Department of Entomology, Faculty of Horticultural Science, Corvinus University Budapest, A/II., 29-43 Villányi str., H-1052 Budapest, Hungary

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### ABSTRACT

Field experiments were conducted to investigate the mechanism of the underlying patterns (abundance, species richness, diversity and similarity) of rove beetles in transgenic Bt (MON810) and in near isogenic maize stands in Hungary. During the three-year (2001–2003) survey, 1538 individuals and 21 species were sampled with pitfall traps. The *Cry1Ab* protein expressed by the MON810 maize hybrid did not influence the overall community structure. After grouping staphylinids into guilds we found no significant differences for non-aphidophagous predators and parasitoids, whereas there were significantly and marginally significantly higher abundances for predators with aphids in their diet in isogenic maize stands in 2002 and 2003 respectively. The abundance of the prey *Rhopalosiphum padi* (L.) showed a high fluctuation between stands and years and was numerically higher only in isogenic stands in the second half of the maize-growing season. The abundance of predatory guilds including aphids in their diet did not correlate with the total annual number of *R. padi* in the same year, but there was a linear correlation in successive years.

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### 1. Introduction

The adoption of genetically modified insect-resistant plants in agriculture has become a powerful tool for controlling key pests (Farinós et al., 2008). The planting of transgenic maize MON810 with the *Cry1Ab* gene from *Bacillus thuringiensis* Berliner var. *kurstaki* expressing *Cry1Ab* insecticidal protein, specific to certain lepidopteran species, was first approved for cultivation in Europe in 1998 to control the European corn borer (*Ostrinia nubilalis* Hübner) (Kiss et al., 2003). In 2009, transgenic varieties of cotton and maize that express Bt proteins were grown on 32.1 million hectares worldwide. Numerous crops expressing novel insecticidal proteins are also under development, and these are expected to soon be commercialised (Romeis et al., 2008). In spite of a broad range of available scientific information on the impact of Bt maize on non-target arthropods, certain relevant taxa have not been studied in detail in Europe (Pons et al., 2005; Eizaguirre et al., 2006). This issue

is crucial to the soil biodiversity considerations of insect-resistant GM crops (Saxena et al., 2004). Representative non-target insects that have been tested include Diptera, Neuroptera, Odonata, Trichoptera, Hymenoptera and Coleoptera. These groups include such species as the major predators and parasitoids that attack insect pests as well as the disease vectors that are the targets of Bt applications (Kiss et al., 2003; Metz, 2003; Farinós et al., 2008).

Staphylinidae is one of the largest beetle families with more than 47,000 species and is distributed worldwide in almost all types of ecosystems (Bohac, 1999; Markgraf and Basedow, 2002). However, rove beetles have rarely been used in integrated pest management largely because of taxonomic constraints and a lack of information on species ecology and prey preferences (Balog et al., 2008a,b,c; Balog and Markó, 2008). Studies in maize have demonstrated that natural enemies consist primarily of ants, spiders, rove beetles, predaceous mites and ground beetles. These predator groups comprise 24% of total individuals, staphylinids being the most abundant coleopteran group, averaging 13.6 beetles per trap per week (Rose and Dively, 2007). The variability in activity–density patterns of the rove beetle fauna in maize is mainly influenced by the year, but no detrimental effects have been attributed to its management (Farinós et al., 2008). In Spanish studies, the transgenic variety MON810 had more rove beetles at Lleida in

\* Corresponding author. Department of Horticulture, Faculty of Technical Science, Sapientia Hungarian University of Transylvania, 1/C Sighisoara str., Tg. Mures, Romania. Tel.: +40 740 562240; fax: +40 265 206211.

E-mail address: [adalbert.balog@gmail.com](mailto:adalbert.balog@gmail.com) (A. Balog).

2001, whereas the non-transgenic one exhibited a significantly higher abundance in the Madrid region in 2000 (Poza et al., 2005). Wolfenbarger collaborators (2008) found that Bt crops showed no significant effects on the detritivores on any of the five collembolan families and their carabid and staphylinid predators, or on the non-collembolan detritivore families Lathridiidae and Japygidae.

Rove beetles are rarely tested under laboratory conditions (with Cry proteins and *in planta* tests) because of difficulties in rearing and maintenance (Stacey et al., 2006; Raybould et al., 2007). Moreover, there is a lack of field data on arthropod communities in Bt crops from Europe, especially on rove beetles. Although the exposure of rove beetles to Cry proteins is likely to be low, the additive and unanticipated effects on the transgene can only be assessed in field trials. In this study, we evaluated the activity-density of non-target rove beetles in Bt MON810 and near isogenic maize. We estimated the effect of the prey *Rhopalosiphum padi* on rove beetle populations.

## 2. Materials and methods

A three-year (2001, 2002 and 2003) field experiment was carried out in an experimental maize stand surrounded by large peach and apricot orchards west of Budapest (47° 25' N, 18° 47' E) in Hungary. Plots (30 m × 30 m each) with Bt maize (DK 440 BTY) transformation event MON810 and its near isogenic line (DK 440) were established on Chernozem soil and arranged alternatively, with six replications each. An alley distance of 3 m was used between plots. A maize hybrid of similar maturity to the test hybrid was planted in the retention zone (a pollen capture crop surrounded the entire test field) in accordance with the requirements of the release permit. Maize was planted between late April and early May at a seed rate of 65,000 seeds/ha, which was reduced to 50,000 plants/ha after emergence, and harvested between mid-October and early November, depending on the year. No insecticides were applied during the experiment.

Rove beetles were collected using pitfall traps (300 cm<sup>3</sup> in size, 8 cm in diameter, half-filled with 4% formaldehyde solution as killing and preservative). Two pitfall traps were placed in the central part (15th row) of each plot, 10 m from each other and the left and right borders of the plot. Sampling lasted from late July in 2001 and from late May in 2002 and 2003 until harvest. Samples were collected weekly. Rove beetles were sorted and identified into species using the works of Freude et al. (1964, 1974). The abundance of the most common and almost exclusively occurring aphid species *R. padi* was assessed in each block weekly by the washing off method using leaves from 10 randomly selected maize plants (Jenser et al., 2010). The aphids were collected in plastic bags and counted in the laboratory.

Analyses of variance were performed and similarities were compared using the O'Brien and Levene tests to determine the differences in abundance, species richness and diversity of rove beetles in Bt and isogenic crops. Values of *F* and *p* were computed using SPSS software and confidence limits of  $p \leq 95\%$  were considered significant. To avoid pseudoreplication analyses of variance were performed using the means of abundance and species richness of the cumulative data of each treatment. The Fisher alpha diversity index was calculated as a measure of biodiversity for each treatment. Metric ordination, principal coordinate analysis (PCoA) and the Horn index were all computed to study the similarities of rove beetle communities in maize stands. Because of the low abundances in 2001 and 2002 the cumulative data from the three years of collection were pooled for PCoA analysis.

Rove beetle species were classified into three guilds according to their prey preference: parasitoids; predatory guilds with aphids in their diet (PredAph+); and predatory guilds without aphids in their

**Table 1**

The classification on the guilds of the rove beetle species. Significantly high activity-density was observed for PredAph+ guild ( $df = 2$ ,  $F = 5.2$ ,  $p < 0.001$ ).

Guilds	Species	Individuals	
PredAph+ guild (Vickerman et al., 1986; Good and Giller, 1986)	<i>Anotylus inustus</i> (Gravenhorst)	44	
	<i>Philonthus cognatus</i> (Stephens)	5	
	<i>Platystethus spinosus</i> Erichson	1143	
	<i>Tachyporus chrysomelinus</i> (L.)	6	
	<i>Tachyporus hypnorum</i> (F.)	68	
	<i>Tachinus signatus</i> Gravenhorst	14	
	<b>Total PredAph+ guild</b>	<b>1280***</b>	
	Parasitoids guild (Good and Giller, 1986)	<i>Aleochara bilineata</i> Gyllenhal	65
		<i>Aleochara bipustulata</i> (L.)	47
		<b>Total parasitoids</b>	<b>112</b>
PredAph- guild (Good and Giller, 1986)	<i>Amisha analis</i> (Gravenhorst)	2	
	<i>Aloconota gregaria</i> (Erichson)	12	
	<i>Drusilla canaliculata</i> (Fabricius)	25	
	<i>Heterothops dissimilis</i> (Gravenhorst)	13	
	<i>Lordithon trinotatus</i> (Erichson)	1	
	<i>Ocypus olens</i> Müller	2	
	<i>Omalium caesum</i> Gravenhorst	18	
	<i>Quedius cinctus</i> (Paykull)	2	
	<i>Paederus litoralis</i> Gravenhorst	2	
	<i>Platydracus stercorarius</i> (Olivier)	1	
	<i>Stenus</i> sp.	4	
	<i>Xantholinus linearis</i> (Olivier)	51	
	<i>Xantholinus longiventris</i> (Heer)	13	
<b>Total PredAph- guild</b>	<b>136</b>		

Notation: \*\*\*:  $p < 0.001$ .

**Table 2**

Species of rove beetles, their distribution by year and relative abundance (R) in Bt MON810 and near isogenic line.

Species/year	2001		2002		2003		R (%)
	Bt	Iso	Bt	Iso	Bt	Iso	
1 <i>Aleochara bilineata</i>	2	2	21	12	25	3	6.9
2 <i>Aleochara bipustulata</i>	2		7	14	13	11	4.5
3 <i>Aloconota gregaria</i>	1		4	5	1	1	2.1
4 <i>Amisha analis</i>						2	0.1
5 <i>Anotylus inustus</i>	6	4	7	7	12	8	2.6
6 <i>Drusilla canaliculata</i>			7	4	7	7	1.6
7 <i>Heterothops dissimilis</i>			3	2	1	7	1.3
8 <i>Lordithon trinotatus</i>					1		0.1
9 <i>Ocypus olens</i>					1	1	0.6
10 <i>Omalium caesum</i>	1	1	1	6	6	3	2.3
11 <i>Paederus litoralis</i>	1		1				0.1
12 <i>Philonthus cognatus</i>					1	1	0.3
13 <i>Platydracus stercorarius</i>						1	0.1
14 <i>Platystethus spinosus</i>	24	12	1	4	456	646	65.6
15 <i>Quedius cinctus</i>						2	0.1
16 <i>Stenus</i> sp.			3			1	0.2
17 <i>Tachinus signatus</i>	4		2	7		1	1.2
18 <i>Tachyporus chrysomelinus</i>			1	4		1	0.3
19 <i>Tachyporus hypnorum</i>	1	11	17	29	5	5	5.4
20 <i>Xantholinus linearis</i>			14	6	13	18	3.1
21 <i>Xantholinus longiventris</i>			2	1	6	4	0.8
<b>No. of individuals</b>	<b>42</b>	<b>30</b>	<b>91</b>	<b>103</b>	<b>548</b>	<b>724</b>	<b>1538</b>
<b>No. of species</b>	<b>9</b>	<b>5</b>	<b>15</b>	<b>15</b>	<b>14</b>	<b>18</b>	<b>21</b>

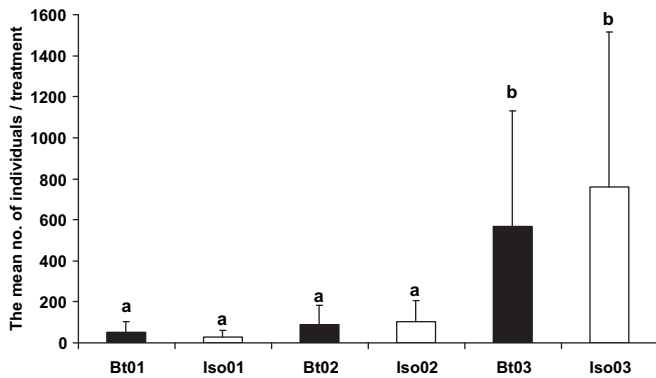


Fig. 1. The variation of the individual number of rove beetles by year of collection, white columns isogenic, black columns Bt. Different letters  $p < 0.001$  (ANOVA).

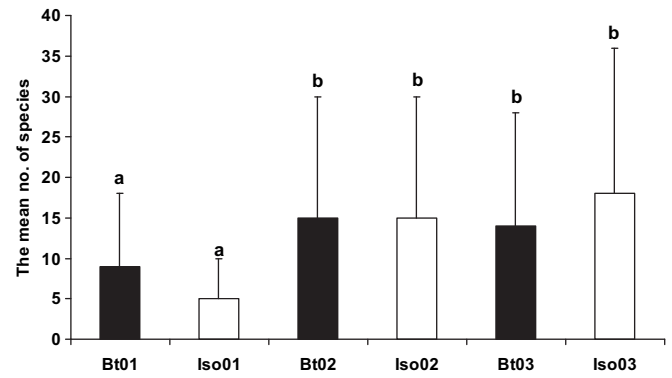


Fig. 2. The variation of the species number by year of collection. Different letters  $p < 0.01$  (ANOVA).

diet (PredAph–) (Vickerman et al., 1986; Good and Giller, 1986) (Table 1). The activity–density of rove beetle guilds and *R. padi* were tested using Welch’s modified *t* test, which assumes an inequality of population variances. Pearson’s linear correlation was also computed between the abundance of aphids and rove beetle guilds (weekly data and total annual abundance for both groups).

### 3. Results

During the three-year survey 1538 individuals and 21 species were recorded in six Bt and six isogenic maize plots. Altogether, 681 individuals and 18 species were captured in Bt and 857 individuals and 18 species in isogenic stands (Table 2). The overall community was not significantly affected by the expression of *Cry1Ab* protein or genetic modification as a whole. The activity–density pattern was similar within each year but varied from one year to another. Significantly higher values were observed in both Bt and isogenic stands in 2003 (Fig. 1). Five species were dominant, accounting for 88.36% of the total individuals (Table 3). In the minority groups (with a relative abundance lower than 3%), the temporal activity–density patterns were unclear because numbers of captures were low, but the cumulative data showed significantly higher values in Bt stands (Table 3). The mean species richness ranged from 9 to 15 in Bt and 5 to 18 in isogenic plots with significantly lower numbers in 2001 (Fig. 2). The diversity was slightly higher in Bt (0.526) than in isogenic stands (0.504) but no significant differences from one treatment to another were measured ( $df = 2, F = 0.320, p = 0.801$ ). The forming (Horn log) and dominance structure (Horn) of the community were relatively similar for both stands; however, the similarity pattern presented a high fluctuation even between the same plots of treatment (Fig. 3).

The overall activity–density pattern of the PredAph+ guild was higher than the PredAph– and parasitoids ( $df = 2, F = 1.07, p = 0.001$ ) (Table 1). No significant differences between Bt and

isogenic stands were observed for PredAph–, parasitoids and PredAph+ guilds in 2001, but there was a significant difference for PredAph+ in 2002 and a marginally significant difference in 2003 (Table 4). The dominant and almost exclusively occurring aphid species was *R. padi*, most likely the main prey for the PredAph+ guild. The overall activity–density of *R. padi* showed a high fluctuation among the stands and no clear differences were observed between Bt and isogenic stands in a first part of the growing season (June and July). In a second part of the growing season the abundance of aphids was numerically higher in isogenic stands, especially in 2001 and 2002 which showed a high aphid infestation (favourable weather conditions for aphid population build-up) (Table 5).

Linear relationships were observed between the abundance of *R. padi* in 2002 and abundance of the PredAph+ guild in 2003 (cumulative data of both Bt and non-Bt, Pearson’s product–moment correlation:  $r = 0.801, p = 0.01$ ) (Fig. 4). No linear correlation was observed between aphid density and the parasitoids ( $r = 0.502, p = 0.30$ ) or PredAph+ guilds ( $r = 0.024, p = 0.98$ ) respectively.

### 4. Discussion

According to our results, no differences in the activity–density pattern of staphylinids can be attributed to the Bt *Cry1Ab* protein-expressing maize hybrid compared with its isogenic comparator. Most rove beetle species identified in our survey have been recorded by other authors as being frequent in maize fields in Europe (Farinós et al., 2008). In similar studies, the community level analyses of the non-target arthropod abundance including rove

Table 3  
The relative abundance (%) and (SE) of the five most common rove beetle species in Bt and isogenic maize stands in 2001–2003 and its variation by treatment (ANOVA).

Species	Relative abundance (%)		F and p values
	Bt	Iso	
<i>P. spinosus</i>	66.8 (8.3)	75.5 (4.8)	2.2 (0.05) <sup>+</sup>
<i>A. bilineata</i>	6.4 (3.2)	4.5 (2.0)	0.9 (0.389)
<i>A. bipustulata</i>	3.6 (3.0)	2.7 (1.9)	0.4 (0.663)
<i>T. hypnorum</i>	4.8 (3.0)	5.0 (2.8)	0.3 (1.000)
<i>X. linearis</i>	3.7 (1.9)	2.6 (1.4)	8.1 (0.209)
Minority groups ( $R < 3\%$ )	14.4 (4.0)	9.4 (2.0)	3.9 (0.001)**

Notation: <sup>+</sup>:  $p < 0.05$ , \*\*:  $p < 0.01$ , SE: standard error.

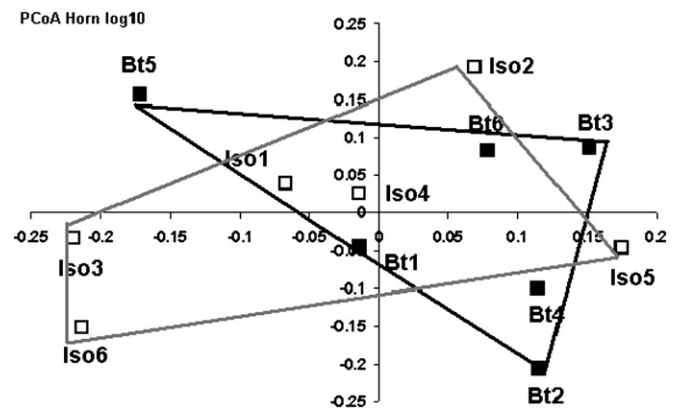


Fig. 3. The forming structure (Horn<sub>log10</sub>) of the rove beetle communities in Bt and isogenic (Iso) maize stands, pooled values 2001–2003.

**Table 4**

The effects of treatments on the abundance and (SE) of the most common rove beetle guilds using Welch's modified *t* test.

Guilds	Year	Bt	Iso	Welch <i>t</i> and <i>P</i> values
PredAph+	2001	7.8 (5.6)	4.0 (1.7)	1.0 (0.342)
	2002	3.7 (1.6)	8.5 (3.6)	−3.4 (0.01)**
	2003	78.0 (23.4)	125.5 (49.7)	−2.0 (0.052)+
PredAph−	2001	1.3 (1.5)	0.7 (1.2)	–
	2002	6.8 (4.5)	4.3 (1.9)	0.8 (0.413)
	2003	9.8 (4.9)	9.7 (3.6)	−0.1 (0.954)
Parasitoids	2001	0.7 (1.0)	0.3 (0.5)	–
	2002	4.7 (5.8)	4.3 (4.1)	−0.3 (0.729)
	2003	6.3 (6.6)	6.8 (2.8)	−0.8 (0.415)

Notation: +:  $p < 0.05$ , \*\*:  $p < 0.01$ .

beetles showed no significant impact on community abundance when comparing Bt *Cry1Ab* with non-Bt maize fields. Analyses of the individual taxa also showed no significant differences in abundance between Bt and non-Bt fields (Higgins et al., 2009). In general, herbivores contain lower concentrations of Bt protein than the plants on which they feed. Phloem-feeding insects, such as aphids, contain only trace amounts of *Cry1Ab* when feeding on Bt maize (Head et al., 2001; Raps et al., 2001; Dutton et al., 2002; Meissle and Romeis, 2009). Therefore, it is expected that aphidophagous rove beetles are influenced by this protein. This is also confirmed by large-scale field studies that have revealed only subtle shifts in the beneficial arthropod community caused by the effective control of the target pest (Sanvido et al., 2009).

Analysing the effects of Bt MON810 maize on staphylinid guilds we found a significant difference for the PredAph+ guild in 2002 and a marginally significant difference in 2003 in both years with a higher abundance in isogenic than Bt stands. These results, however, could be erroneous for two main reasons. First, the Bt hybrid and its near isolines might have different susceptibilities to aphids because of different background genetics. Finding proper isolines is difficult and continues to be an important issue for such research. The best way to control this problem is to include multiple strains of Bt hybrids each with their own isoline. This helps eliminate such confounding that cannot be deciphered with one strain of maize. Second, aphid infestations tend to be patchy, which could greatly influence and skew beetle distributions within a field.

The density of the parasitoids guild (*Aleochara* species) was low in both Bt and isogenic stands. The functional role of the larvae of these species developing inside the *Delia* fly puparium in maize fields has not been fully clarified (Balog et al., 2008b). They probably developed inside the puparium of *Delia florilega* Zetterstedt present in maize. *Aleochara bilineata* Gyllenhal is an IOBC test organism for investigating the side effects of toxins and pesticides. The species was used to assess the toxicity of mCry3A to non-target organisms by other authors. There was no observable adverse effect on *A. bilineata* when exposed to a 50 µg mCry3A/g diet (Grimm

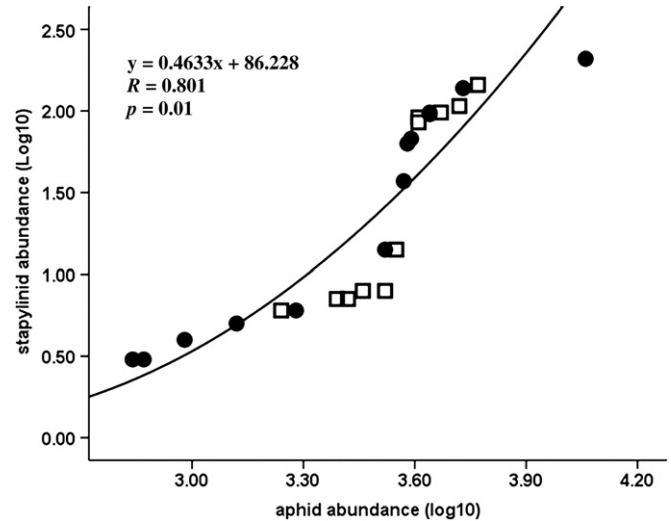


Fig. 4. Correlation between the total abundance of *R. padi* in 2002 and PredAph+ guild in 2003 in Bt (black dots) and isogenic (white squares) maize.

et al., 2000). A similar result might be expected for other parasitoid Aleocharinae groups inhabiting GM maize crops.

Few species of the PredAph− guild (*Ocyopus olens* Müller, *Platydracus stercorarius* (Olivier) and *Xantholinus longiventris* (Heer)) are frequent in orchards and as a result they can occur accidentally in maize fields, although no direct connection can be attributed between Bt protein and their activity–density patterns in maize fields. Other predator species are widely polyphagous and consume the eggs and larvae of other arthropods including predators (carabids) (Balog et al., 2008c). Thus, no direct correlation can be attributed between their activity–density patterns and Bt protein.

Some rove beetle species have been reported as having a wide prey spectrum in cereals including aphids, especially the bird cherry-oats aphid, and this means they are capable of suppressing their numbers (Kollat-Palenga and Basedow, 2000). According to our results, the abundance of predatory guilds that consumes aphids (PredAph+) did not correlate with the total annual and monthly *R. padi* density in the same year, but we did find a linear correlation between the aphid abundance in 2002 and PredAph+ in 2003. Functional responses by many staphylinid predators that consume aphids have been demonstrated (Good and Giller, 1986; Collins et al., 2002). Aggregative and reproductive numerical responses have also been confirmed in this group (Bryan and Wratten, 1984; Kollat-Palenga and Basedow, 2000), which have showed that a reproductive numerical response by *Philonthus* sp. and *Tachyporus hypnorum* (F.) resulted in density-dependent predation. However, we cannot exclude the importance of other potential prey (fly larvae, Collembola, Nematoda). The evaluation of the importance of staphylinid predation clearly depends on the

**Table 5**

Total abundance of *R. padi*/10 plants (SD). Similar letters within a month and year represents no significant differences; different letters (a and b)  $p = 0.028$ .

Year	Bt/Isogenic	June	July	August	September	October
2001	Bt	–	545 (334) a	365 (242) a	941 (580) a	2245 (941) a <sup>c</sup>
	Is	–	325 (74) a	916 (440) b	1550 (1429) a	2585 (1550) a
2002	Bt	657 (182) a	26 (29) a	29 (38) a <sup>c</sup>	1536 (1161) a	–
	Is	561 (134) a	35 (28) a	200 (167) a	2552 (1886) a	–
2003	Bt	5 (2) a	45 (14) a	33 (48) a <sup>c</sup>	4 (4) a <sup>c</sup>	–
	Is	5 (2) a	61 (34) a	25 (18) a	8 (13) a	–

Sample dates within month were pooled.

<sup>c</sup> Abundances were  $\ln(x+1)$  transformed before Welch's *t* test to provide a normal distribution. SD: standard deviation.

major pest in each given field (Good and Giller, 1991). In central Hungary, the cereal aphid *R. padi* is the major pest of maize. They occur in epigeal and plant zones; therefore, many species of predatory rove beetles active only at a soil level are unlikely to be important (PredAph–). Species with a pronounced climbing ability (*Tachyporus*, *Platystethus*, *Philonthus*), which are frequently captured in yellow sticky traps, can instead play an important role in aphid population dynamics. The importance of winter epigeal species in relation to aphid survival is unknown, so they cannot be eliminated as potential predators (Good and Giller, 1986). Further research is needed in this issue. Furthermore, although this study enhances our understanding of the possible interactions between rove beetles and *R. padi* in maize, further research is required to quantify the significance of specific trophic linkages in the field.

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