SZENT ISTVÁN UNIVERSITY

ASSESSMENT OF THE IMPACT OF BT (MON 810, CRY1Ab) MAIZE ON SELECTED NON-TARGET COLEOPTERA (CARABIDAE, COCCINELLIDAE)

Thesis of the doctoral (PhD) dissertation

MRS ÁCS DÓRA SZEKERES

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Name of the PhD
School: **Doctoral School of Plant Sciences**

Scientific Branch: **Plant Production and Horticulture**

Head: **Dr. László Heszky**
Professor, member of Hungarian Academy of Sciences
Szent István University
Faculty of Agriculture and Environmental Sciences
Institute of Genetics and Biotechnology

Consultant: **Dr. József Kiss**
Professor
Szent István University
Faculty of Agriculture and Environmental Sciences
Institute of Plant Protection

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signature of the head of the school **signature of the supervisor**

1 Preliminary research and the objectives set

One of the features of today's global agriculture is that the production of genetically modified (GM) plants is gaining more and more ground especially in certain regions. Since their introduction (1996) their cultivation area has been continuously growing and by 2009 it reached 134 million hectares (James, 2010) of which more than 35 million hectares are insect resistant (Cry toxin producing) GM maize (James, 2009). The gene of *Bacillus thuringiensis* soil bacterium strain is incorporated into the genome of the insect resistant plants encoding the expression of protein that is more or less specific to certain insect taxons and target insect pest species. The *cry1Ab* gene of the *Bacillus thuringiensis* bacterium species responsible for the production of Cry1Ab toxin is implanted in the Bt maize hybrids that are the result of MON 810 transformation. This toxin is very effective against the larvae of some Lepidoptera species including the larva of the European Corn Borer, (ECB) *Ostrinia nubilalis*. In all the cells of the Bt maize plants toxin is produced so though the feeding of the different herbivor insects it can be transmitted to the higher level of the food chain such as to the natural enemies of the insects like predators and parasitoids.

In 2001 I joined one of the EU-5 projects at the Institute of Plant Protection of Sent István University entitled "Effects and mechanisms of Bt transgenes on biodiversity of non target insects: pollinators, herbivors and their natural enemies" (QLK3-CT-2000-00547) the main objective of which was to analyse the impacts of ECB resistant Bt-maize (MON 810) on the biodiversity of arthropods. The analysis of the impacts of the Bt transgene and the Cry1Ab toxin produced by it in the GM (Bt) maize on some phytophagous, predator and parasitoid insects, certain parts of the food chain and biodiversity (Kiss, 2000; Kiss et al., 2002) was one of the objectives of the project. When I started my research there was only limited European experience on the environmental risk assessment of GM plants especially Bt plants. At the same time, our research work and risk assessment approach was based on the well known principle of "hazard x exposure = risk" (Wilkinson et al., 2003) and was in accordance with the environmental risk assessment of GM plants published in significant scientific publications later (Hilbeck, et al. 2006, Andow et al. 2006, Romeis et al. 2008 and Hilbeck et al., 2008).

During the three-years field experiment I analysied the impacts of Bt maize on two predator insects associated with to two different vertical levels of Bt maize within the agricultural ecosystem, i.e. ground beetles (Carabidea) that are active on the surface of the ground and ladybirds (Coccinellidae) that are active on the level of the foliage. The trophic role of these two groups in the agricultural ecosystem is significant. Most ground beetles are opportunists regarding their feeding so they can act as a biological regulator as they can survive in the given area if preys are reduced (Sunderland and Vickerman, 1980; Luff, 1987; Lövei and Sunderland, 1996; Kádár, 1999). The coccinellid predators can contribute to reducing the density of aphids and mites, and therefore delay their gradation (Merkl, 1982; Radwan and Lövei, 1983; Hodek and Honek, 1996).

My present analysis focussed to the ground beetle and ladybird assemblages of maize are justified by a few national and international researches on this topic and the important role these insects play in the food chain in maize ecosystem. Till the start of my research the possible direct or indirect impacts of Cry1Ab toxin produced by Bt (MON 810) maize hybrid on ground beetle and ladybird assemblages were not well investigated under field conditions. Although in the case of ground beetles the opportunist way of feeding can reduce the indirect impacts of the toxin (the effect of the toxin through prey) as the predator switches to another prey species if the former one's abundance declines or its quality is weakened. However, these predators are exposed to the toxin (direct impact by the toxin taken up by the prey) to a greater extent. According to the results of Meissle et al. (2005) the increased mortality of the *P. cupreus* larvae fed by *S. littoralis* reared on Bt maize and refers to the indirect impact of the toxin but they could not exclude the possibility of the direct impact, either (the ELISA test proved that *P. cupreus* was exposed to toxin). The species of the ladybird assemblages in maize stands are specialists such as aphidophagous ladybird species that mainly consume aphids (such as *Coccinella septempunctata* L., *Hippodamia variegata* Goeze) and *Stethorus puctillum* that preys mites Weise (Merkl, 1982). When starting our research our general hypothesis was that no toxin can be taken up by aphids as they are phloem feeders. Based on this, in theory Cry1Ab toxin was not expected to have a direct impact on the ladybird species that mainly consume aphids. However, toxin can affect these non-target species through its impact on preys (density, change in quality) not only directly but also indirectly. In addition, ladybirds can also consume pollens as alternative food so the direct effect of the toxin can also be supposed. Toxin was detected in limited amounts in aphids (*Aphis gossypii*) that were fed on cotton producing Cry1Ab and Cry1Ac toxin (Zhang et al., 2006). The open field impact analysis in the case of *Stethorus punctillum* that preys mites is also justified as the mite *Tetranychus urticae* sucks the content of the cell while feeding so the Cry1Ab toxin content in their body also proved to be high (Dutton et al., 2002). In this way these tests also justified the need for carrying out the open field ecological impact analysis of Bt maize that produces Cry1Ab toxin (Cry1Ab) on these two predator groups in our country, too.

My objectives were the following:

- the comparative analysis of the structural characteristics of the predacious ground beetle and ladybird assemblages in Bt (MON 810) and isogenic maize stands;
- the characteristics and comparison of the seasonal changes of the predacious ground beetle and ladybird assemblages in Bt (MON 810) and in isogenic maize stands;
- through aboves contribution to the environmental risk assessment of Bt (MON 810) maize.

2 Material and method

2.1. Experimental layout, location and sampling periods

The three-year long (2001-2003) field experiment was established in Sóskút lying 30 km northwest of Budapest in compliance with the FVM approval of experimental release. The isogenic hybrid: (DK 440) and Bt (hybrid: the transgenic version of isogenic maize hybrid produced by MON810 transformation, DK 440 BTY) maize plots were arranged in alterations in six repetitions (Figure 1). In compliance with the release approval the experimental field part (containing 12 plots, 28 x 28m each) was surrounded by 6 m wide pollen retention maize rows of isogenic hybrid (DK 440) to minimise the risk of the pollen shift. The field of the experiments was bordered by orchard with stone-fruits and field crops.

Sowing took place on 2 May 2001, 25 April 2002 and 22 April 2003. Harvesting was carried out on 17 October in the first, 3 November in the second and on 14 October in the third year. Cultivation practices including nutrient supply in all three years followed the general practice applied in the region. Insecticide treatment was not used with the exception of the first year when diasinon insecticide was used as soil application at sowing. The preemergent herbicide treatment and mechanical weeding in the four-six leaf phenologic stage of the maize if necessary were applied as a form of herbicide treatment.

Figure 1: The aerial photo of the arrangement of Bt and isogenic maize plots (Sóskút, Hungary, 2003, photo: J. Kiss)

2.2. Sampling methods

The sampling of ground beetles was carried out by two Barber-type traps that were emptied on a weekly basis. Four percent formaldehyde was used as a killing-conservating agent. No trap cover or baits were used. Traps were placed on 18 July 2001, 23 May 2002 and 26 March 2003 and the first emptying took place on 25 July 2001, 30 May 2002 and 02 April 2003. We removed the traps at the time of the last emptying, i.e. on 17 October 2001, 25 October 2002 and 13 October 2003. The collected material was transported to the Zoology Department of MTA NKI where it was stored in formaldehyde till identification. The imagos were identified with the help of the works of Hurka (1996) and, for some species, Müller-Motzfeld (2004) and Freude et al. (1976).

The individual plant check (visual survey and sampling) was conducted for the ladybirds. The survey took place from 04 July 2001 to 17 October 2001, 21 June 2002 to 02 October 2002 and 25 June 2003 to 17 September 2003 on a weekly basis.

All the developmental stages of ladybirds (egg, larva, pupae, and imago) were collected from the total of 10 randomly chosen maize plants per plot, which were transported to the Zoology Department of MTA NKI to be reared and identified. The phenologic status of the maize plants and also the potential preys of ladybirds (aphids, mites) were recorded. The identification of the ladybird imagos was carried out with the help of O. Merkl (Hungarian Natural History Museum) and based on his PhD dissertation (Merkl, 1982). Larvae were identified on the basis of the work of Hodek (1973).

2.3. Data analysis

After the examination of the normality of data (Kolmogorov-Smirnoff test) the Mann-Withney (Wilcoxon's rank sum) test was used to evaluate and compare the data as well as to explore the correlations between them. Normality was not achieved in the case of most samples so non-parametric tests were used. The standard deviations were checked by Levene-test. In some cases the striking values were omitted or ln(x+1) transformation was carried out. The 5% threshold was considered regarding the significance level of differences. The well known Shannon diversity, α diversity and (Magurran, 1988) were applied to characterise the population structure as species diversity while in the case of diversity profiles the Rényi diversity (Tóthmérész, 1994), and regarding the hierarchical classification of quantitative similarities, the Ward method (Manhattan metric) were applied (Podani 1997, Statsoft 2000). To point out the qualitative overlap of the assemblages the Jaccard index (Magurran, 1988, Podani 1997) while concerning quantitative similarities the Renkonen value (see, e.g. Lövei, 1982) was used.

To compare the synchronicity of the seasonal abundance changes of certain groups (e.g. ladybird-aphid) the cross correlation function was used (Szentkirályi, 1997; Statsoft, 2000) which is known from time series analysis. The date of catching the dominant ground beetle and ladybird species from the point of view of the interaction of treatments and dates was analysed by means of the generalised linear method (GLM, Repeated measures ANOVA (Sokal and Rohlf, 1995). The active species density and the correlation system of treatment and dates (both of them as variables of the environment) were explored by means of canonical correspondence analysis that was carried out by the Principal Response Curves module (CANOCO, PRC) of CANOCO programme (ter Braak and Šmilauer, 2002). The analyses, different calculations and figures were made and drawn by means of STATISTICA 6.0 (Statsoft, 2000), NuCoSa (Tóthmérész, 1993), Excel and CANOCO for Windows 4.5 (ter Braak and Šmilauer, 2002) programmes.

3 Results

3.1. Ground beetles (Carabidae)

3.1.1. Comparison of the structural characteristics of ground beetle assemblages in Bt- and isogenic maize

During the three years of the experiment altogether 44103 individuals of 58 carabid species were collected. In all the three years the same six species, *Pseudoophonus* (*Harpalus*) *rufipes* (De Geer), *Harpalus distinguendus* (Duftschmid), *Poecilus sericeus* Fischer von Waldheim, *Dolichus halensis* (Schaller), *Calathus ambiguus* (Paykull), and *Trechus quadristriatus* (Schrank) (in the ranking of decreasing dominance) were dominant both on Bt and isogenic plots. The dominant species amount to approximately 93% of the trapped individuals.

Neither the number of ground beetle individuals nor the number of their species differed significantly within years on Bt and isogenic maize (Table 1). The species' diversity were at medium value and of medium evenness. Within the same years the diversity indices of the ground beetles of the two maize types (Shannon, α diversity) do not differ significantly. Based on diversity patterns, Bt maize had no detectable adverse effect on the ground beetle assemblages although the species diversity of the ground beetle assemblages of the isogenic maize was slightly higher than that of the Bt maize plot in the first year, the difference is rather small (Figure 2).

When comparing samples in Bt and isogenic maize in the given year both the qualitative (Jaccard index: 68-74%) and the quantitative (Renkonen index: 92-97%) similarity between the assemblages showed high values (Table 1). In all the three years within the given years the ground beetles resembled to a great extent both on Bt and isogenic maize. When comparing the two final years, the ground beetle assemblages of Bt and isogenic maize plots were very similar but stood apart from 2001. In all the three years the ground beetles on the isogenic maize plots showed a higher diversity regarding most scale parameters although to a small extent.

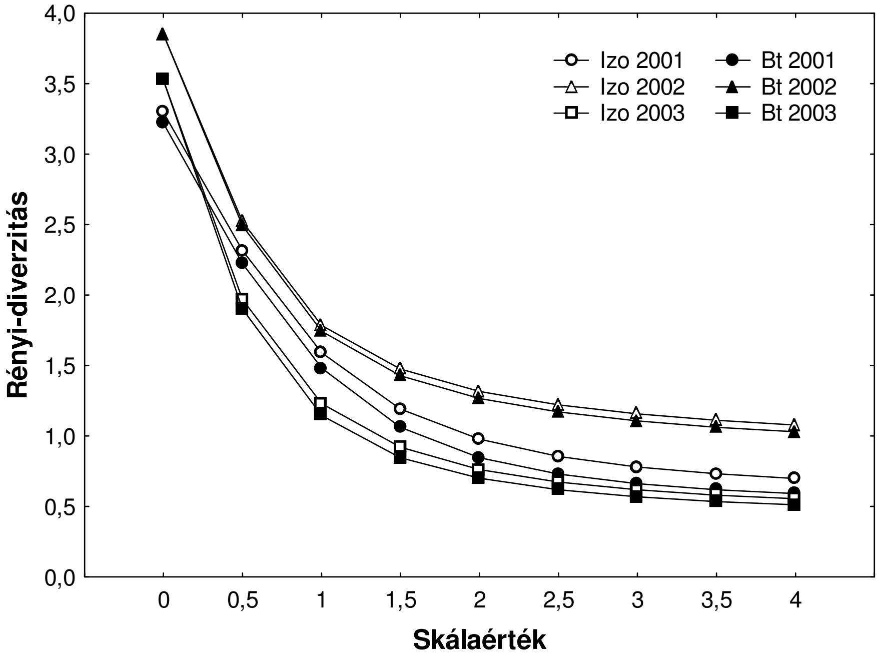

Figure 2: The Rényi diversity profiles of the ground beetle assemblages in Bt and in isogenic maize plots (Sóskút, Hungary, 2001-2003)

Table 1: The structural characteristics of ground beetle assemblages on Bt and isogenic maize plots (Sóskút, Hungary, 2001-2003). When making annual comparisons there was no significant difference. The values marked by the same letters do not differ significantly (Mann-Withney Wilcoxon's rank sum test). Significance level: p<0,05, (plot average±SE).

Structural features		**Annual species**	**Total species/ plot/year**	**Annual total species**	**Total species/ plot/year**	**Shannon diversity**	**Evenness**	**α-diversity**
2001	**Bt**	25	12.33±0.43a	2027	168.92±7.55a	1.36±0.05a	0.54±0.02a	3.08±0.13a
	Iso	27	12.92±0.54a	1847	153.92±13.04a	1.47±0.04a	0.58±0.02a	3.45±0.24a
2002	**Bt**	47	21.25±0.97a	7109	592.42±43.8a	1.64±0.04a	0.54±0.01a	4.33±0.19a
	Iso	46	22.67±0.93a	7372	614.33±39.6a	1.72±0.05a	0.55±0.01a	4.66±0.21a
2003	**Iso**	33	15.0±0.66a	12758	1063.17±71.95a	1.13±0.04a	0.42±0.01a	2.50±0.14a
	Bt	34	16.08±0.67a	12990	1082.5±34.67a	1.20±0.03a	0.44±0.01a	2.68±0.12a

There was no significant difference in the average number of individual per trap of the ground beetles collected during the trapping period within a year on Bt and isogenic plots regarding either the total number of ground beetle groups (Mann-Withney test; Z=-1.761 –

0.462, in all cases $p>0.05$; Figure 3/A) or dominant species (Mann-Withney test; Z=-1.905 – 1.790, in all cases $p>0.05$; *P. rufipes*: Figure 3/B).

A **B**

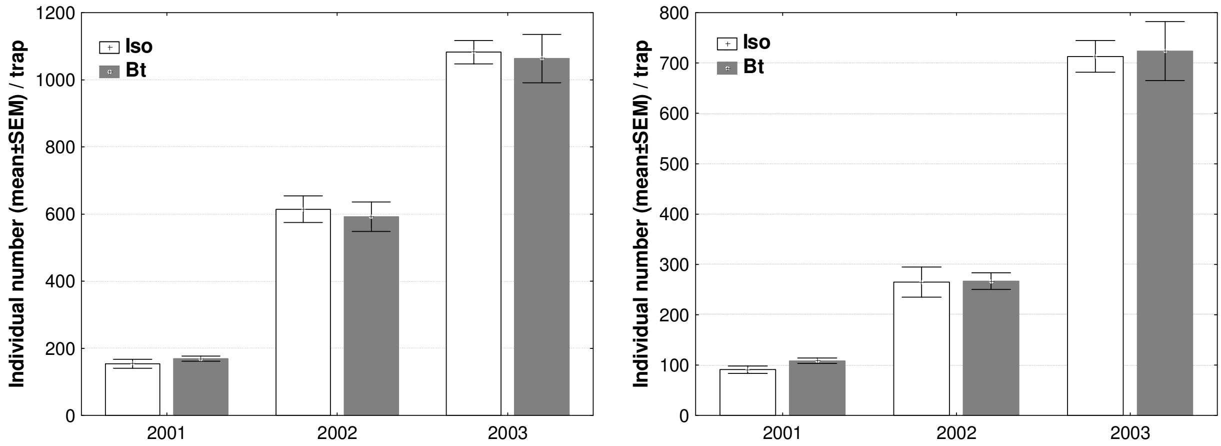

Figure 3: A: The changes in the abundance of the total ground beetle species collected in three years on Bt and isogenic plots (Sóskút, Hungary, 2001-2003). **B:** The changes in the abundance of *Pseudoophonus rufipes* collected in the three years on Bt and isogenic plots, (Sóskút, 2001-2003). Marking: the same letters mean there is no significant difference.

On the basis of the structural analyses within the seasons, the ground beetle assemblages of Bt and isogenic maize plots do not differ significantly regarding the species composition, the dominance rank or the number of species.

3.1.2. Comparison of the seasonal abundance dynamics of ground beetles on Bt and isogenic maize

The activity peak of ground beetle assemblages were different in time each year but was similar when comparing values from Bt and isogenic maize plots. On 8 August, 17 July 2002 and 2 July 2003 the number of ground beetle individuals was the highest both on Bt and isogenic maize plots. Regarding average abundance within one year, GLM did not show significant difference between Bt and isogenic maize plots between two treatments with the exception of some cases (GLM; total round beetle assemblage 2001: F=2.308, p=0.013; 2003: F=0.255, p=0.998; *P. rufipes* 2001: F=2.578, p=0.006; 2003: F=0.156, p=0.999; *C. ambiguus* 2002: F=2.683, p=0.015 and *P. sericeus* 2003: F=2.009, p=0.033). The comparison of the average individual number of carabids at the same timeperiod on Bt and isogenic plots showed a very mixed picture and not even in the case of the total ground beetle assemblages and the dominant ground beetle species did it show an obvious tendency. At certain times the average number on Bt and at other times that of the isogenic plots was higher.

Of the dominant species *C. ambiguus, D. halensis, P. rufipes,* and *P. sericeus* showed one activity peak while *H. distinguendus* and *T. quadristriatus* two. In the case of *C. ambiguus* in addition to the high activity peak there were some lower activity peaks, e.g. in May in the last two years. The time of the activity peak of *D. halensis* was changing year by year. With the exception of 2001 *H. distinguendus* had an activity peak at the beginning of summer and another at the end of autumn. The fact that in 2001 the first activity peak could not be observed may have been due to the late beginning of setting traps. The surface activity of *P. rufipes* showed a very varied picture although it was characterised by one activity peak in all the three years. In 2002, however, there was a second smaller activity peak at the end of summer and the beginning of autumn. *P. sericeus* showed one activity peak in all the three years except 2002 when an early summer and mid-summer activity peak could be observed. *T. quadristriatus* generally showed two activity peaks although the first early summer peak was missing in 2001 due to the late setting of traps and also the second autumn peak was missing in the final year. Although there were differences in the activity of the sampled dominant ground beetle species year by year, they were similar when comparing Bt and isogenic maize plots. PRC did not show a significant difference in any of the years on the first canonical axle (in all the cases $p>0.05$). The cumulative variance of the species-environment correlation in percentages varied from 34.2% to 46.1% on the first canonical axle.

3.2. Ladybirds (Coccinellidae)

3.2.1. Comparison of the structural characteristics of ladybird assemblages on Bt and isogenic maize

During the three years samplings altogether 11 ladybird species of which 8 aphidophagous, a scale insect preying (*Exochomus quadripustulatus* L.) predator, one that mainly preys mites and aphids (*Stethorus punctillum* Weise) and one that even consumes fungi tissues and insect eggs (*Psyllobora vigintiduopunctata* L.) were collected on Bt and isogenic maize plots. In all the three years the same three aphidophagous ladybird species (*C. septempunctata* L., *Hippodamia variegata* Goeze, *Propylea quatuordecimpunctata* L.) were proved to be dominant in the assemblage although the ranking of dominance varied annually, but within the given year it was similar when comparing Bt and isogenic maize assemblages.

The values of the diversity indices (H', *Q*) are between 1 and 2 relative to the number of species, i.e. there were ladybird assemblages of low species diversity both on Bt and isogenic maize plots. The evenness of the assemblages can be stated high in 2002 and 2003 both on Bt and isogenic maize plots while in the first year it was average. In all the three years when comparing the ladybird assemblages of Bt and isogenic maize plots the similarity indices of both the binary (Jaccard index) and the quantitative (Renkonen index) index were high, which suggests that the species composition and dominance ranking of the ladybird assemblages formed in the given year on Bt and isogenic maize plots practically did not differ (Table 2).

Within the given years in all the three years there were very similar ladybird assemblages on both Bt and isogenic maize. When comparing the last two years the ladybird assemblages of Bt and isogenic maize plots were similar and stood apart from 2001.

Table 2: The structural characteristics of aphidophagous ladybird assemblages on Bt and isogenic maize plots (Sóskút, Hungary, 2001-2003). When making annual comparisons there was no significant difference. The values marked by the same letters do not differ significantly (Mann-Withney Wilcoxon's rank sum test). Significance level: $p<0.05$, (plot average±SE).

Structural features		**Annual species**	**Total species/ plot/year**	**Annual total species**	**Total species/ plot/year**	**Shannon diversity**	**Evenness**	**α-diversity**
2001	**Iso**	7	4.67±0.21a	395	65.83±4.53a	0.98±0.03a	0.64±0.02a	1.15±0.06a
	Bt	9	5.50±0.22b	331	55.16±4.37a	1.14±0.02b	0.67±0.02a	1.54±0.09b
2002	**Iso**	6	4.17±0.17a	122	20.33±3.01a	1.09±0.05a	0.77±0.04a	1.72±0.19a
	Bt	6	4.50±0.43a	133	22.17±3.04a	1.25±0.07a	0.85±0.02a	1.76±0.20a
2003	**Iso**	6	3.83±0.40a	118	19.67±3.29a	1.11±0.06a	0.85±0.03a	1.56±0.27a
	Bt	5	3.83±0.17a	78	13.0±1.83a	1.13±0.05a	0.85±0.02a	1.97±0.18a

In the first year the diversity curve that belongs to the ladybird assemblages of Bt maize plots runs above the isogenic one regarding the total scale parameter values so that is why the species diversity of the ladybird assemblage sampled on Bt maize plots was a bit higher. Regarding the dominant species in 2002 Bt maize while in the third year regarding the ladybird species of the highest dominance isogenic maize was more diverse (Figure 4). Of the acariphagous ladybirds only the *Stethorus punctillum* Weise species was collected during the three years. This species occurred with an approximately 1.8-2.3 times higher abundance on the plots in 2001 than in 2002. This difference was characteristic of all the examined developmental stages. All of them could be observed with the slightest abundance in the final year both on Bt and isogenic plots. This ladybird species was exclusively collected in the colonies of *Tetranychus urticae* Koch, which refers to its strong relation with its prey.

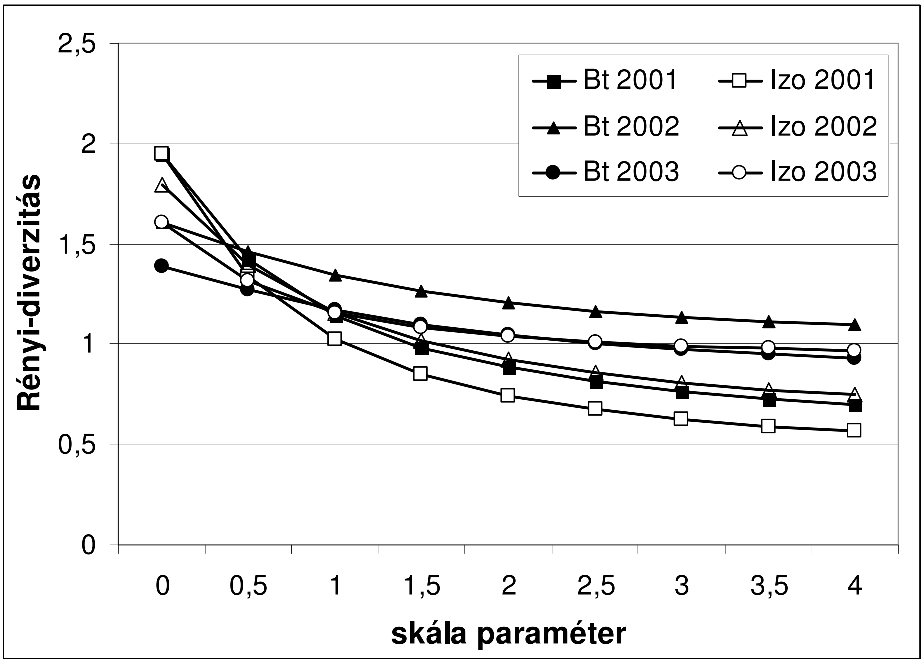

Figure 4: The Rényi diversity profiles of the ladybird assemblages in Bt and in isogenic maize plots (Sóskút, Hungary, 2001-2003)

There were no significant differences in the average individual number of the ladybirds recorded during the three years within the given year on either Bt and isogenic maize plots in any of the years regarding either all the ladybird species (Mann-Withney test; Z=-0.722 – 1.648, in all the cases p>0.05; Figure 5/A) or the dominant species (Mann-Withney test, Z=-1.949 – 1.338, in all cases p>0.05; Table 2). Only in the case of *H. variegata* was there a significantly higher average individual number per plot on isogenic plots than on Bt maize plots in the first and the last year (the Z value of the Mann-Whitney test; 2001: Z=2.330, p=0.019; 2002: Z=-1.234, p=0.217; 2003: Z=2.119, p=0.034, Figure 5/B). At the same time, there was no significant difference in the average individual number of *S. punctillum* per plot within the given season (Mann-Whitney test; 2001: Z=-0.320, p=0.749; 2002: Z=-0.643, p=0.520; 2003: Z=-0.895, p=0.371). On the basis of the results of the structural analyses within the years the ladybird assemblages of both Bt and isogenic maize did not significantly differ regarding species composition, dominance ranking or species number. According to diversity patterns Bt maize did not have an adverse impact on the ladybird assemblage as on Bt plots a bit more diverse assemblage could be observed than on the isogenic maize plots.

A **B**

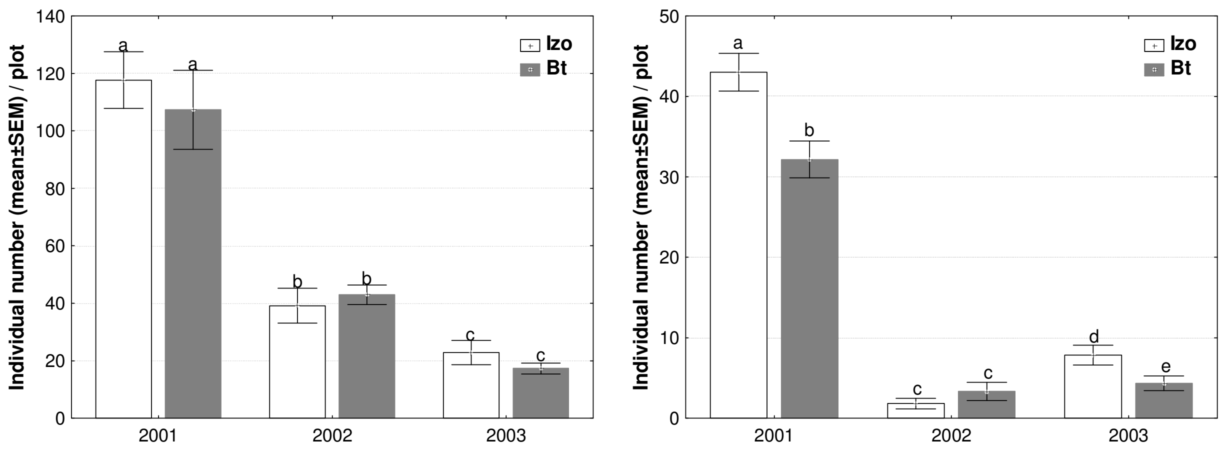

Figure 5: A: The changes of the average abundance of aphidophagous ladybird imagos in the sample Bt and isogenic plots (Sóskút, Hungary, 2001-2003). **B:** The changes of the average abundance of *Hippodamia variegata* Goeze imagos in the samples from Bt and isogenic plots (Sóskút, 2001-2003). The values marked by the same letters do not differ significantly.

3.2.2. Comparison of the seasonal abundance of ladybird imagos on Bt and isogenic maize plots

The statistical analysis did not show a significant difference in any of the years regarding the abundance level of aphids, and this was changed in time simultaneously on Bt and isogenic maize plots (Mann-Withney test; Z= -0.160 – 0.480, in all the cases p>0.05). The seasonal population dynamics of aphids could be characterised by a two-peaked bimodal curve in all the three years. The first lower peak took place in the first half of July in 2001 and 2003 while in 2002 it was at the end of June.

In the first year the aphidophagous ladybird populations followed the increase of aphids with a week's delay while in the second and third years a slighter abundance increase of ladybirds could be observed in parallel with *R. padi* second smaller aphid increasing period. However, the result of the cross correlation does not support this. In general it can be stated that there were no discernible correlations in the seasonal abundance changes of ladybirds and aphids with the exception of two cases. In 2001 in the case of *H. variegata* a negative correlation could be shown at the one week's delay period while in 2003 *P. quatuordecempunctata* followed the peak in the population growth of the aphids with a week's delay. GLM did not show any significant differences in any of the years regarding the total aphidophagous ladybird assemblage recorded by individual plant sampling and the species number of the dominant ladybird species within the year in the relations of treatment and date (GLM; F=0.123 – 5.565, in all the cases p>0.05) on Bt and isogenic maize plots. *C. septempunctata* (GLM; F=2.297, p=0.026) is an exception in the first year although of the eleven dates only at two and on Bt plots was the number of the given species significantly higher when comparing the pairs afterwards.

In all the three years the abundance changes of the *Stethorus punctillum* imagos followed the dynamics of infections by mites despite the fact that the periods of the mite population increase greatly differ in the three years. In 2001 the growth of mite population could be characterised by three activity periods (mid-August, the beginning and the end of September). In the second year the setting and growth of mites started earlier, i.e. in the middle of June and the period of maximum infection lasted throughout July although infection by mites was swept away by the end of August. No significant differences could be observed in the individual number of *S. punctillum* recorded by individual plant sampling within the year in relation to treatment and date in any of the years (GLM; F=0.445 – 0.607, in all the cases p>0.05) between Bt and isogenic maize plots.

PRC did not show a significant difference in any of the years on the first canonical axle (in all the cases p>0.05). The cumulative variance of the species-environment correlation in percentages was 46.6% in the first year, 48.8% in 2002 and 53.9% in the final year on the first canonical axle.

4 Conclusions and recommendations

4.1. Ground beetle assemblages

4.1.1. Comparison of the structural characteristics of ground beetle assemblages in maize

During the three years survey we found ground beetle population rich in species. The number of species (58) exceeds the upper limit given on the Hungarian agricultural areas in this respect (Lövei 1984; Kádár and Lövei 1989) and also other numbers of species of

European maize fields are even lower (Sekulić, 1976; Andriescu et al., 1984). The six most frequent species trapped in Sóskút belong to the one typical of agricultural areas (Thiele, 1977; Lövei and Sárospataki, 1990).

Our medium diversity values could be explained with the fact that there are several species in the assemblages that reduce its maximum value by their outstanding abundance. Moreover, several other species have a low relative frequency in the population of which some could not be linked to maize. Based on our results Bt maize did not have an impact on reducing the diversity of species on the ground beetle assemblages in accordance with the literature (Lozzia, 1999). The high similarity values indicate that stable assemblage(s) were formed in the maize plots. The ground beetle assemblages show a higher diversity in the case of crop rotation than on monocultural plots but their number of species and stability in time can be a lot smaller than on the monocultural plots. Monoculture is more favourable for the species that reproduce in autumn as agritechnics applied in crop rotation has unfavourable impacts on the species that reproduce in autumn (Lövei, 1984). Although our maize plots were a bit similar to a rotated maize (as according to the output license Bt maize cannot return to the same area), the traps placed in the area mainly caught species that reproduce in autumn, which is in contrast with the previous examination. One of the possible reasons for it can be the different sizes of the plots/fields surveyed (our study was carried out on small plots while that of Lövei on larger fields) and the significant distance between the two areas.

During our three-year study I could not point out a significant difference in the abundance of the ground beetle assemblages between transgenic and isogenic maize plots similarly to others (Volkmar et al., 1998; Lozzia, 1999; Manachini, 2000; Dively and Rose, 2002; Sehnal et al.; 2004; Lopez et al., 2005; Leslie et al., 2007; Farinós et al., 2008; Priestley and Brownbridge, 2009). In general it can be stated that in most studies regarding the ground beetle population as a whole there was no significant difference between Bt and isogenic maize regarding abundance. However, in certain cases some minor differences might have occurred. On the basis of our results there was no significant difference in the abundance of either the total ground beetle assemblage or the dominant ground beetles. In contrast, in the study of Floate et al. (2007) a significant difference was shown in the case of 3 of the 39 species of ground beetles while in the case of two an almost significant difference ($p<0,08$) was shown between Bt (Cry1Ab toxin) and isogenic maize although this difference was not regarded consequent. Toschki et al. (2007) in the first year of their three-year long study showed a significant difference in the abundance of several ground beetle species between Bt

(MON 810) and isogenic maize plots although this difference must have been caused by the more unfavourable micro climate (less biomass) due to the damage by *O. nubilalis* and not Bt.

The relatively small size of the plots (28 m x 28 m) can raise the question whether the results of the present study can be evaluated or whether it was possible to draw conclusions on bigger areas, too. The release permit limited our plot size. The possible migration of ground beetles between Bt and isogenic plots was reduced by creating bare alleys between plots. The literature extensively deals with the impact of different cultivation methods on the activity of the ground beetles. For example, the different cultivation practices can influence the activity of the ground beetles, i.e. soil cover (intercropping) obviously increases the population of ground beetles while the single species can differently react to agronomic practices, the use of pesticides and crop rotation (Hummel, et al., 2002). The results of field study with a smaller plot size (10 m x 25 m) than ours show that it can also be applied in the case of this spatial scale due to the adapting ability of the ground beetles to habitat (Cárcamo and Spence, 1994). Zwahlen and Andow (2005) pointed out the presence of Cry1Ab toxin in the body of seven ground beetles in the open field. The presence of toxin in the body of the ground beetles that were collected from Bt maize residue/isogenic maize plot suggests that toxin derives from maize residues so Bt maize can affect ground beetle assemblages not only in the year of its production. Our result subsequently justifies our hypothesis according to which ground beetle species are exposed to the possible impact of Bt toxin even in the open field. Our results could not be influenced by the fact that Bt maize could not repeatedly be sown exactly in the same place in two consecutive years as it was stipulated by the release permit. Based on above results, Bt toxin can both have direct and indirect impact on ground beetle assemblages although in contrast with this fact, we could not detect significant difference in the structural characteristics of ground beetle assemblages between Bt (MON 810) and isogonics maize.

4.1.2. The comparison of the seasonal dynamics of ground beetle assemblages on maize plots

On the basis of the activity results I have concluded that the seasonal patterns of the trapped population on isogenic and Bt maize plots are similar in total and on the level of frequent species, too, i.e. Bt maize (MON 810) did not have an impact on the seasonal dynamics of ground beetles. The differences in the activity peaks of certain species can partly derive from competition (Müller, 1987). This difference ensures that one or more ground beetle species are represented in the area with high abundance level.

The activity pattern of the dominant ground beetle species in our study showed a complex picture. The temporal differences of peaks can be explained by the factors of weather on the one hand and it can also be due to their possible density dependent reproduction on the other hand. The traps principally caught species that reproduce in autumn in greater numbers as the high season of those reproducing in spring is well before maize comes up. The literature also shows a very varied picture in comparison with our patterns and these deviations in activity tendencies may be caused by reproducing periods and the temporal restructuring of the appearance of tenerals.

4.2. Ladybird assemblages

4.2.1. Comparison of the seasonal dynamics of ladybird assemblages on maize plots

During the three years study aphidophagous ladybird fauna consisting of altogether rnine species were found on Bt and isogenic maize plots. On the basis of literature reviews six species of the annually forming ladybird assemblages were considered typical of maize, which are the common, mostly dominant features of ladybird assemblages in the maize fields of the European region including the Carpathian Basin. *C. septempunctata*, a *P. quatuordecimpunctata*, and *H. variegate* were dominant of them. These three species reproduced on maize as their eggs and larvae were also found on the plots surveyed. The settlement of the six characteristic ladybird species is an important fact as one of the objectives of our study was to examine the possible effects of Bt (MON 810) maize on predators exclusively linked to maize. The fact that the assemblages consist of ladybird species typical of maize was less probable on the isolated place by orchards.

Based on our results and regarding the diversity characteristics annual assemblages of low diversity and with a relatively few species (6-8 spp.) were formed on maize plots similarly to the results of other studies on maize fields (Radwan and Lövei, 1983). The assemblages showed asimilarity of a high degree (71-92%) both during the years and among the maize hybrids, which suggests the local stability of species compound and dominance structure. Radwan and Lövei (1983) also found strong species similarity (65-85%) amoung the ladybird imago assemblages of typically large maize fields disregarding the method of cultivation. Based on these results, the formation of aphidophagous ladybird assemblages consisting of a relatively low number but constant species including three dominant species and three with lower abundance level can be expected on the domestic maize plots and those situated in the temperate zone.

4.2.2. Comparison of the structural characteristics of ladybird assemblages on Bt and isogenic maize plots

We have sampled 9 ladybird species in our Bt x isogenic maize hybrid comparison, which number was the highest number for such comparison (possible direct or indirect effect of Cry1Ab toxin, Hilbeck, 2001). During the three years it was only in the case of *H. variegata* that significantly fewer ladybird species were present on Bt plots compared to isogenic ones although this difference was too small due to the low average individual number, which cannot be contributed to the impact of Bt for sure. As this did not occur in other cases and there was no considerable difference either in diversity, the values of structural characteristics or seasonal dynamics of coccinellids on Bt and isogenic maize plots it can be stated that Bt maize did not have an adverse effect on the ladybird assemblage (using the individual plant sampling method). Such a difference was shown in the case of *C. maculata* similarly to us (Wold et al,. 2001, Pilcher et al., 2005) although their results reflect that primarily it was the phenological stage of the maize plant that affected the examined species and the density of preys did not influence the abundance of ladybirds.

However, most of those carrying out the impact analysis of Bt maize (e.g. Pilcher et al., 1997; Manachini et al., 1999; Wold et al., 2001; Hilbeck, 2001; Bourguet et al., 2002) emphasised that despite they found no differences in the population level of beneficial insects between the two maize type of hybrids, the non-detectable possibly adverse direct or indirect impacts of Cry1Ab toxin could not be excluded (larger field and longer term studies were missing). Nevertheless, according to the results of laboratory analyses Bt maize is not expected to have a negative (increased mortality, reduced productivity and life time) impact on aphidophagous insects such as ladybirds. According to these studies Cry1Ab toxin produced by maize is strongly species (ECB) and taxon (Lepidoptera) specific regarding its impact on the one hand (Koziel et al., 1993) and also the Cry1Ab protein produced in the cells is not present in phloem so the phloem feeding herbivores (e.g. aphids) do not take up toxic protein and therefore do not transmit it to their natural enemies through trophic interactions (Fearing et al., 1997; Dutton et al., 2002).

However, new laboratory studies have proved that in the body of aphids feeding on Bt cotton and also in the body of their ladybirds predator Bt toxin can be detected. Based on these results Bt toxin (Cry1Ab, Cry1Ac) might impact the reproduction of ladybirds (Zhang et al., 2006). The transmission of Bt toxin between the trophic levels in the food web is not entirely known. In addition, ladybird species can get in touch with toxin not only by consuming the prey, i.e. aphids but also by consuming plant tissues and alternative foods. Such alternative food can be pollens and the eggs of *Ostrinia nubilalis* (Hübner) in which toxin might be present. However, if pollens and aphids are at the disposal of ladybirds in vast numbers, the consumption of ECB eggs is significantly reduced (Musser and Shelton, 2003), which can further decrease the prevalence of the possible negative impacts of Bt maize. Although ladybirds can get in touch with Bt toxin through their feeding (aphids, *Tetranychus urticae,* pollen, eggs of ECB), during our three-years field study there was no significant difference shown in the abundance of ladybirds between Bt and isogenic maize plots by means of the samplings we used disregard one exception.

During our study I could not find a significant difference in the average population level of the mite preying *S. punctillum* between the two types of maize plots, either. Despite the fact that *Tetranychus urticae* sucks the content of the cells and the Cry1Ab toxin concentration is provably high in their body (Dutton et al., 2002), a negative effect was not experienced either on the tested *T. urticae* mite (Lozzia et al., 2000) or *C. carnea* larvae consuming them (Dutton et al., 2002). Toxin was presumed to transform somehow in the bowel system of mites and thus can lose its toxic effect on lacewing larvae (Dutton et al., 2002). However, a recent study has shown that *T. urticae* is incapable of degreading Cry1Ab toxin due to the lack of serin protease so toxin is transmitted to the third trophic level. As the survival rate and the lengths of development did not change, they came to the conclusion that the epithelial cells of *S. punctillum* did not contain the receptor cells that are responsible for tying toxin (Álvarez-Alfageme et al., 2008).

4.2.3. Comparison of the seasonal dynamics of ladybird assemblages on maize plots

The pattern of the seasonal dynamics of the examined ladybirds was characterised by some delay in all the three years, they followed the abundance changes of aphids. The members of the aphidophagous ladybird assemblages were settled in the maize plots at the time of the first aphid spread where their egg laying and larvae activity were also started.

Similarly to the other studies (Radwan and Lövei, 1983; Park and Obrycki, 2004) the first, higher abundance peak of the imago population in all the three years occurred simultaneously with the period of pollen shed so the probability of pollen consumption was high. A smaller population increase in ladybird imagos could be observed at the time of aphid increase at the beginning of September-October. The imagos of the new generation were fed on the available aphids during this period before winter comes but they did no longer reproduce (Hodek and Honek, 1996). Similarly to them we could not experience egg-laying when taking samples in September.October, either.

During the weekly comparison there was no significant difference in the abundance of ladybirds except the fist year in the case of *C. septempunctata* when the number of ladybirds was significantly higher on Bt maize plots than on isogenic ones for two weeks. Although on the basis of season dynamic patterns ladybird populations follow the increase of aphids with some delay, a positive correlation could not be shown between aphids and the abundance of ladybirds.

The abundance level of *S. punctillum* adaptively changed in parallel with the dynamics of mite populations that serve as prey. Imagos and larvae showed a higher abundance level early in July in one of the years, late in September in another while in the third year it was mid-August. The population dynamics of mites in the three years differed significantly. According to the study of Kozma (1980) it frequently happens in maize fields in Hungary.

On the basis of our results Bt maize does not influence the seasonal abundance dynamics of aphidophagous and acariphagous ladybird assemblages.

5 New scientific results

I have studied the potential direct or indirect impacts of Bt (MON 810, Cry1Ab) maize on ground beetle assemblages that are active on the soil surface and ladybird assemblages being mainly active in the foliage (structural characteristics, compound, diversity, abundance and seasonal activity as parameters). This study was the first of field studies with Bt maize in Hungary.

In our study on the basis of the comparative analysis of ground beetle assemblages of Bt (MON 810, Cry1Ab) and isogenic maize I have concluded that

1. ground beetle assemblages that are rich in species were formed both on Bt (MON 810, Cry1Ab) and on isogenic maize plots. The ground beetle assemblages of Bt (MON

810, Cry1Ab) and isogenic maize do not statistically differ either in the number of species or in their composition;
2. Bt (MON 810, Cry1Ab) maize did not cause a difference in diversity;
3. high similarity values suggest the formation of stable assemblages both on Bt (MON 810, Cry1Ab) and isogenic maize plots;
4. Bt (MON 810, Cry1Ab) maize did not have an impact on the individual number of ground beetle. There was not a significant difference between Bt (MON 810, Cry1Ab) and isogenic maize regarding the abundance of either the total ground beetle assemblages or that of the dominant species;
5. Bt (MON 810, Cry1Ab) maize did not have an impact on the seasonal dynamics of ground beetles as the seasonal samples of ground beetle populations on isogenic and Bt maize were similar in total and also on the level of frequent species.

During our three-year field study, based on the comparative analysis of the ladybird assemblages of Bt (MON 810, Cry1Ab) and isogenic maize I have concluded that:

6. Ladybird assemblages both on Bt (MON 810, Cry1Ab) and isogenic maize plots are composed of species that are typical in Hungarian maize fields. The ladybird assemblages of Bt (MON 810, Cry1Ab) and isogenic maize plots did not significantly differ either regarding the species number or species composition;
7. Bt (MON 810, Cry1Ab) maize did not cause difference in diversity of assemblages on Bt and on isogenic plots;
8. Bt (MON 810, Cry1Ab) maize did not have an impact on the abundance level of ladybird assemblages;
9. Bt (MON 810) maize did not have an impact on the seasonal dynamics of ladybirds. During the weekly comparison of the abundance of ladybirds there was no significant difference observed. The abundance fluctuations of Bt (MON 810, Cry1Ab) plots were in line with the isogenic ones.

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7 List of publications

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