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# SZENT ISTVÁN UNIVERSITY

# Environmental risk assessment of *Diabrotica*-resistant (Cry34/35Ab1, Cry34/35Ab1 x Cry1F protein producing) maize hybrids for certain arthropods

PhD thesis

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## 1. Objectives

In 2014, more than 180 million hectares of biotech crops were grown worldwide (James, 2014). There is a continuous increase in the global hectarage of biotech crops, whereas the magnitude of increase in Europe is less; however the import – therefore the use - of hybrids with gene-modified (GM) Bt and herbicide tolerant (HT) events, for the purpose of food-, fodder and processing, is significant.

Maize is grown in large areas in the European Union; one of its significant economic pest (presently apart from the Spanish and Portuguese maize growing regions), is the western corn rootworm (*Diabrotica virgifera virgifera* LeConte). Management of this pest (as one of the main non-chemical elements of the integrated pest management of maize) is crop rotation, since females lay their eggs mainly into the soil of maize fields, where, after overwintering, hatching larvae feed on maize roots. However, crop rotation is not applicable everywhere (due to technological, economic etc. reasons) (Kiss et al., 2005; Fall és Wesseler, 2008). Too high percentage of crop rotation (>80 %), which would mean the exclusive use of a single management tool, is not in harmony with the principles of integrated pest management and might foster the development of the so-called "rotation resistant" population (Onstad et al., 2001). Therefore the use of crop rotation in every single year and field is not expedient; it is reasonable to have continuous maize in some part of the arable land. In those continuous maize fields, various insecticide application, such as seed-dressing or insecticide application in rows might be valid. Spectrum of possible insecticidal management options has been broadened by the cultivation of *Diabrotica*-resistant (Cry3A, Cry3Bb1, Cry34/35Ab1 protein producing) maize hybrids (USA and Canada).

The European Union and its member states face new challenges due to the cultivation of GM crops and *Diabrotica*-resistant maize hybdrids, within. It is reasonable to assess the possible advantages or environmental risks of these new management tools, furthermore, to broaden existing risk assessment results- and databases. Moreover, it is essential to assess the interaction between each potential receptive environment (EFSA, 2010a,b) and the hybdrids, or rather analyze the application in wider agricultural-, economic systems (Szénási et al., 2009), which can provide scientific information for other regions and give feedback for risk management. One of the key fields of risk assessment of GM plants is analyzing the effects on non-target organisms (primarily on arthropods) (Wolt et al., 2010), in which the principal of so-called "tiered approach" is crucial (USEPA, 1998; Romeis et al., 2006, Romeis et al., 2008), which is based, considering insecticidal

protein producing plants, on tests under laboratory, glass-house, semi-field and field conditions (Poppy, 2000; Wilkinson et al., 2003; Garcia-Alonso et al., 2006; Rose, 2007; Romeis et al., 2008).

Target organisms of the Cry34/35Ab1 binary protein producing hybrids are species belonging to the *Diabrotica* species complex, however, among these; the western corn rootworm (*Diabrotica virgifera virgifera*, Coleoptera) is relevant presently. Hybrids, containing events that provides resistance against this pest, and the protein produced by them may have undesired effects on non-target organisms, such as species which are taxonomically "close" to Coleoptera (Carabidae, Coccinellidae, Staphylinidae and certain Chrysomelids feeding on maize) through consuming plant parts (herbivores, or certain ladybird species consuming pollen due to their mixed feeding strategy), and through consuming herbivor preys feeding on maize plants (flea beetles, certain true bugs, thrips, cicadas consumed by certain ladybird, ground beetle and rove beetle species).

Laboratory results of tested species at the lower levels (Tier I-II.) did not result in undesired side effects at multi-level (usually ten times of the plant level) protein concentration. However, due to the specialities of the "host environment" (predator and herbivore arthropods in field crops, especially in maize fields, in the Pannon Biogeographical Region, such as Hungary, Czech Republic, Slovakia, Romania, Serbia, Croatia, and certain parts of Ukraine), evaluation of the relationship between plants exposed to field stress and arthropods is an important scientific goal (e.g. validation or refutation of former study results).

Plant Protection Institute of the Szent István University, Hungary has been conducting environmental risk assessment of GM Bt maize hybrids under field conditions near Sóskút village (30km SW from Budapest, Hungary) since 2001. First, the impact of European corn borer resistant hybrid (MON 810) on diversity of NT (non-target) arthropods (pollinators, herbivores and predators) was studied (EU-5 R&D project: Bt-BioNoTa). Then, Bt (against lepidopteran and coleopteran pests) and HT hybrids were studied 2006–2010 to assess their impact on NT arthropods. Considering the above written facts, my objectives were as follows:

- Analyzing the impact of different, genetically modified (GM) (Cry34/35Ab1 and Cry34/35Ab1 x Cry1F protein producing) maize hybrids on non-target arthropods as well as quantitative comparison of arthropod assemblages in maize hybrids containing the above events to the arthropod assemblages of closely related non-GM (isogenic) maize hybrids.
- Mapping the relationships within arthropod assemblages and analyzing their stability in different, genetically modified (Cry34/35Ab1 and Cry34/35Ab1 x Cry1F protein producing) maize hybrids and in closely related non-GM (isogenic) maize hybrids.
- Enrichment of the risk assessment of the maize hybrids containing the above mentioned events.

Evaluating GM plants may happen in various fields of interests (such as economic, coexistence, environmental, animal feeding, food security, ethical, personal, emotional etc). The aim of my work was exclusively to contribute to the scientific risk assessment.

## 2. Material and methods

#### 2.1. Location, date and set-up of the experiment

Release for the field sampling happened in Sóskút region (30kms northwest from Budapest) in accordance with the order of the assigned authority (Ministry of Agriculture, at the time). Studies were carried out in a 5,7 hectare rectangle shaped field, surrounded by a stone-fruit orchard and several arable fields of different sizes, between 2006 and 2008. The study location was owned by Sóskút Fruct Ltd.

In all three years of the study, uniformly, 40 plots (25m x 25m each) with four replicates per treatment were formed in random block arrangement. One meter wide operation roads between plots and 3 meter wide operation roads between blocks were made. In all three years of the study, the arrangement of plots and exact locations did not change. In harmony with the authorization document, the study field was protected by fence, guarded by security personnel 24hours a day and the study field was surrounded by buffering maize strip (pollen trap) in order to avoid pollen drifting.

Sowing was done with the use of sowing-gun, later the exact number of seedlings per plot (65.000/hectare) was adjusted by hand. Technological and pest management operations that are

typical of the region were followed, except when the treatment itself did not make this possible (Table 1.). Maize was the forecrop in all three years, to reduce the impact of external factors; after harvesting, autumn deep ploughing was conducted.

In all three years (4 replicates) x 6 Bt maize plots (treatments) were formed (Coleoptera (Cry34/35Ab1); Lepidoptera (Cry1F) resistant) and/or herbicide (CP4 EPSPS) tolerant), where, instead of the conventional weed control practice, glyphosate was applied (treatment 4 and 6) in two treatmens. Besides, 4 (replicates) x 4 control maize plots (two different maize hybrids, Control A and Control B) were formed where soil insecticide was applied in two treatments (treatment 72 and 74). Hybrid A, which was present in treatment number 1,2,5 and 6, was genetically closely related to the two isogenic control A hybrids (treatment 71 and 72), whereas hybrid B, which was present in treatments number 3 and 4, was genetically closely related to the two isogenic control B hybrids (treatment 73 and 74) (**Table 1.**).

Treatment	OECD identifier	Hybrid	Protein	Resistance/Tolerance	Pesticide treatment
1	DAS-59122-7	А	Cry34/35Ab1	Coleoptera	"conventional" weed control
2	DAS-01507-1 x DAS-59122-7	А	Cry34/35Ab1 x Cry1F	Coleoptera x Lepidoptera	"conventional" weed control
3	DAS-01507-1 x MON-00603-6	-00603-6 B Cry1F x C4 EPSPS Lepid		Lepidoptera x Herbicid	"conventional" weed control
4	DAS-01507-1 x MON-00603-6	В	Cry1F x C4 EPSPS	Lepidoptera x Herbicid	glyphosate
5	DAS-59122-7 x DAS-01507-1 x MON-00603-6	А	Cry34/35Ab1 x Cry1F x C4 EPSPS	Coleoptera x Lepidoptera x Herbicid	"conventional" weed control
6	DAS-59122-7 x DAS-01507-1 x MON-00603-6	А	Cry34/35Ab1 x Cry1F x C4 EPSPS	Coleoptera x Lepidoptera x Herbicid	glyphosate
71	Control A (closely related isogenic) (PR-36B08 hybrid)	А	-	-	"conventional" weed control
72	Control A (closely related isogenic) (PR-36B08 hybrid)	А	-	-	"conventional" weed control + teflutrine
73	Control B (closely related isogenic) (PR-35Y65 hybrid)	В	-	-	"conventional" weed control
74	Control B (closely related isogenic) (PR-35Y65 hybrid)	В	-	-	"conventional" weed control + teflutrine

**Table 1.:** Treatments in the study field (Sóskút, 2006-2008).

Soil insecticide application was done concurrently with sowing (treatment 72 and 74), teflutrin, Force 1.5G was applied. In year 2007, due to technological contstraints, treatment 72, 73 and 74 was missing. Apart from the two treatments, no insecticidal application was conducted in the study fields, in any of the study years. In treatment number 4 and 6 (in 4-leaf (V4) and 8-leaf (V8) phenological stages of maize) glyphosate was applied two times in order to control weeds. Additionally, in other parts of the study location, "conventional" weed control measurements were applied, i.e. mesotrione (Callisto 4SC) and atrazine (Gesaprim 500FW) in the 4-leaf stage of maize.

#### 2.2. Arthropod sampling methods

In all three years of the study (2006-2008) sampling was done based on those sampling methods (soil trap, Pherocon AM sticky trap, individual plant examination) that are routinely applied in European risk assessment studies. (Sampling was a team work and assistance fluctuated yearly; in the thesis I will refer to the team as "we".)

Sampling was carried out in vegetation period, in four different phenological stages of maize (8-leaf stage (V8), before pollination (VT), during pollination (R1) and after pollination (Ritchie et al., 1992)). Besides arthropod sampling, additional botanical surveys were conducted in years 2007 and 2008 in the plots (four times a year), however, I did not include the detailed results of these surveys in my thesis.

Soil trapping, as an effective, simple, cheap and standard method, is a widely-used tool to sample beetles moving on the ground (Southwood, 1978; Merret and Snazell, 1983; Dinter, 1995; Kádár and Samu, 2006). Recently, new designs of soil traps have been routinely used in order to study the undesired impact of Bt-plants on non-target arthropods (Riddick et al., 1998; French et al., 2004; de la Poza, 2005; Szekeres et al., 2006; Prasifka et al., 2007).

Modified version of the Barber-trap (Kádár and Samu, 2006) was used in all three years of our study. In the beginning of the vegetation period, 120 and 84 cups (3 cups/plot, 9 meters apart from each other) were digged into the soil in years 2006/2008 and in year 2007, respectively. According to the study of Prasifka et al. (2007), as activator, killing and conservation agent, 70% ethylene-glicol solution (non-evaporating, odourless) was applied. Traps were collected one week after activation, filtered material was labelled and transferred to the SZIE Plant Protection Institute laboratory; material was stored in 60% ethanol solution and taxons were determined under stereo-microscope.

Pherocon AM yellow sticky trap (Trécé Inc., Adair, OK USA), due to the attraction of the yellow colour (visual stimulus) is capable of sampling flying and jumping insects (http1). In order to reduce the edge-effect, similarly to the soil traps, Pherocon AM yellow sticky traps were placed

in the middle of the plots in a 9mx9m area. In the beginning of the vegetation period, 120 and 84 robinia wooden sticks (3/plot) were digged into the soil in years 2006/2008 and in year 2007, respectively, therefore three Pherocon AM yellow sticky traps were placed in a triangle shape into each plot during the sampling period. Sticky traps were collected one week after placement, traps were labelled (number of the plot and the trap), were stored in refrigerator and the material was analyzed in the SZIE-NVI laboratory under stereo-microscope.

One of the simplest and most widely-used method to sample herbivore and predator arthropods in maize is individual plant examination. Sampling was carried out in the middle of the plots in a 9mx9m area, in order to avoid edge-efects. Within this area, three sampling locations were determined. 5 randomly selected plants per location, so 15 plants per plot were fully examined (stalk, leaf, silk, cob, husk) from the bottom to the top; all kinds and quantities of arthropods were recorded.

#### **2.3. Data clearing and statistical analysis**

Raw data was handled in Excel sheets. A separate excel sheet was assigned for recorded arthropods for each year, treatment and sampling period (4 times a year). Only those arthropod groups were included in the statistical analysis which contained at least 100 individuals per plot per year per sampling method. Sampled arthropods were divided into trophical (herbivore vs. predator) groups. Those species that consume nearly identical food are considered a trophical group. This method is frequently applied in the analysis of food-chains (Gagic et al., 2011; Jordán et al., 2012). Considering these facts, 14 predator- and 8 herbivore arthropod groups were included in the analysis. 5 groups from soil traps, 12 taxons from Pherocon Am sticky traps and 12 taxons from individual plant examination were analysed (**Table 2**.).

**Table 2.:** Arthropod taxons that are included in the statistical analysis, sampled by different sampling methods. (Note: For those arthropod taxons, where no developmental stage is indicated, adult stage is relevant.)

	Tana	Sampling method					
Arthropod taxons	laxonomic	C all t	Pherocon AM	Individual plant			
	level	Solitrap	sticky trap	examination			
Pi	redators						
Arachnida							
Araneae	order	х		Х			
Insecta							
True bugs (Heteroptera)							
Orius spp. adult	genus		Х	x			
Orius spp. larvae	genus			X			
Nabis spp. adult	genus			X			
Nabis spp. larvae	genus			X			
Beetles (Coleoptera)							
Ground beetles (Carabidae)	family	Х					
Rove beetles (Staphylinidae)	family	х	Х				
Aphidophagous ladybirds (Coccinellidae)	family		X	X			
Stethorus pusillus adult	species			х			
Stethorus pusillus pupae	species			Х			
Stethorus pusillus larvae	species			Х			
Planipennia							
Green lacewing adults (Chrysopidae)	family		Х	х			
Green lacewing eggs (Chrysopidae)	family			х			
Diptera							
Hoverflies (Syrphidae)	family		Х				
He	rbivores						
Insecta							
Springtails (Collembola)*	order	х					
Thrips (Thysanoptera)	order		Х				
Hemiptera							
Cicadas (Auchenorrhyncha)	suborder		Х				
True bugs (Miridae)	family		X				
Aphids (Aphididae)	family		Х	х			
Coleoptera							
Click beetles (Elateridae)	family		Х				
Flea beetles (Alticinae)	subfamily	х	Х	X			
Western corn rootworm (Diabrotica virgifera virgifera)	species		X				

\* Those species which belong to the Collembola order are active in the soil as decomposers. For simplification, a distinct trophical group (i.e. predator) was assigned to those species, whose certain developmental stages show up mixed feeding strategy (some ground beetles, ladybirds) or whose different developmental stages follow different feeding strategies (i.e. adult hoverflies feed on nectare versus larvae consume aphids).

This was followed by the setup of a RANK order based on the percentage share of arthropod groups for each treatment, as follows: species or group that was the most abundant was assigned the number 1, the second most abundant was assigned number 2, and so on. Using this method, the

dominant arthropod groups within samples were determined both for GM and for isogenic control maize plots.

Analysis of variance considering multiple factors, i.e. year, sampling period, treatment and their interactions, was conducted in order to analyse the impacts on the adundance of the sampled arthropod groups. Homogenity of variances were tested using Levene-test. In further statistical analysis, in order to compare arthropod groups paired per treatment, and conducting normality test (using histograms) prior to that, ANOVA (Tukey test for paired comparison) was carried out using the SPSS 24.0 software. In those cases, where data did not follow normal distribution, Kruskal-Wallis test was applied (Mann-Whitney test in case of paired comparison). Significance level was determined at 5% (Baráth et al., 1996).

In the second part of the thesis, after standardization of data and summarizing the three different sampling methods, Pearson correlation was calculated among arthropod those groups included in the statistical analysis. Only significant (p<0,05) positive and negative correlations were taken into consideration. Concretely, if in a given sampling period high aphid abundance was recorded parallel with high numbers of aphidophagous ladybirds and the correlation between them proved to be significant, then a clear and direct relationship was assumed between these two groups. Similarly, for all other groups and for each treatment (GM and control maize) correlation analysis was carried out. This method is routinely applied in the analysis of trophical relationships within food-chains (arthropod networks) (Martinez et al., 1999; Szénási et al., 2014).

The following parameters, describing the stability of arthropod relationships were analysed for each treatment (Martinez, 1999): S - "number of trophic groups/species, L - number of links between trophic groups, B - trophic links = L/S, D - link density =  $2/N(N-1)\sum d_{ij}$ ), C - connectance =  $L/S^2$ .

Stability parameters of arthropod relationships in each treatments were analysed by multivariate ANOVA, with a considered significance level of p<0,05.

## 3. Results

### **3.1. Adundance of sampled arthropod taxons**

Alltogether 520 285 individuals were collected during the 4 sampling periods per year and in the three vegetation periods, between 2006 and 2008. Abundance of the sampled arthropods fluctuated through the three years of the study, the most individuals were collected in 2008 (206 835), whereas the least in 2006 (143 486). Alltogether 85 782 individuals were collected using soil traps through the study period. The highest number of individuals were collected by Pherocon

AM sticky traps (319 438 individuals, 12 arthropod taxons). 115 065 individuals were recorded during whole plant examinations in the total of the three years (**Table 3.**).

Compling mothed		Total		
Samping method	2006	2007	2008	number of
Soil trap	37 265	18 624	29 893	85 782
Pherocon AM sticky trap	67 247	145 752	106 439	319 438
Idividual plant examination	38 974	5 588	70 503	115 065
Total number of individuals	143 486	169 964	206 835	520 285

**Tables 3.:** Total number of individuals sampled by three different sampling methods (Sóskút,2006-2008).

## 3.2. RANK order of the arthropod taxons in each treatment

In case of soil trap captures, RANK order clearly shows that ground beetles, springtails and spiders are dominant in each treatment (low RANK value) (**Table 4.**), in most cases individuals captured by soil traps added up to more than 90% of the total captures.

Table 4.: RANK	order of the soil	trap captured arthro	pods per treatment	(Sóskút, 2006–2008).
	order of the som	aup cuptured urant	pous per treatment	(DOBRUL, 2000 2000).

		Treatments (RANK order)									Moon (9/.)	
Arthropod groups (soil trap)	1	2	3	4	5	6	71	72	73	74	Mean	Wiean (70)
Carabidae	1	1	1	1	1	1	1	1	1	1	1	56,61
Collembola	2	2	2	2	2	2	2	2	2	2	2	30,93
Araneae	3	3	3	3	3	3	3	3	3	3	3	7,93
Alticinae	4	4	4	4	4	4	4	4	5	4	4,1	2,81
Staphylinidae	5	5	5	5	5	5	5	5	4	5	4,9	1,22

Relatively stable RANK order was detectable in each treatment in case of the Pherocon AM sticky trap captures as well. Thrips, cicadas, flea beetles, aphids and the western corn rootworm were dominant in each treatment (**Table 5.**), in most cases adding up to more than 90% of the total captures.

Arthropod groups (Pherocon				Trea	atmen	ts (RA	NK o	rder)				Moon (94)
AM sticky trap)	1	2	3	4	5	6	71	72	73	74	Mean	Mean (%)
Thysanoptera	1	1	1	1	1	1	1	1	1	1	1	52,13
Auchenorrhyncha	2	2	2	2	2	2	3	4	4	3	2,6	13,15
Alticinae	3	3	3	3	3	4	2	2	3	4	3	12,59
Aphididae	4	5	5	4	4	3	4	3	2	2	3,6	10,96
Diabrotica virgifera virgifera	5	4	4	5	5	5	5	5	5	5	4,8	6,49
Orius spp. (adult)	6	6	6	6	6	7	6	7	7	6	6,3	1,57
Coccinellidae (aphidophagous)	7	7	7	7	7	6	7	6	6	7	6,7	1,29
Syrphidae	10	8	8	8	8	8	8	8	8	8	8,2	0,52
Chrysopidae (adult)	8	11	9	10	9	10	9	9	9	10	9,4	0,38
Staphylinidae	11	9	10	9	11	9	10	11	11	11	10,2	0,35
Miridae	9	10	11	11	10	11	11	10	10	9	10,2	0,30
Elateridae	12	12	12	12	12	12	12	12	12	12	12	0,09

**Table 5.:** RANK order of the Pharocon AM sticky trap captured arthropods per treatment (Sóskút,2006–2008).

In case of the individual plant examination, aphids, lacewing eggs, *Orius* spp. adults and flea beetles were abundant (**Table 6.**), adding up to nearly 90% of the total records among the visually counted arthropods.

**Table 6.:** RANK order of the sampled arthropods per treatment based on individual plantexamination (Sóskút, 2006–2008).

Arthropod groups (individual	Treatments (RANK order)											
plant examination)	1	2	3	4	5	6	71	72	73	74	Mean	Wiean (76)
Aphididae	1	1	2	1	1	1	1	1	1	2	1,2	47,22
Chrysopidae (egg)	2	2	1	2	2	2	2	2	2	1	1,8	26,48
Orius spp. (adult)	3	3	4	4	3	3	4	3	3	3	3,3	7,97
Alticinae	4	4	3	3	4	4	3	4	4	4	3,7	7,76
Araneae	5	6	5	5	6	5	5	5	5	6	5,3	2,92
Orius spp. (larvae)	6	5	6	6	5	6	6	6	6	5	5,7	2,67
Stethorus pusillus (larvae)	7	9	7	9	7	7	7	7	7	8	7,5	1,16
Stethorus pusillus (adult)	8	7	8	7	9	8	8	8	8	7	7,8	1,05
Stethorus pusillus (pupae)	9	11	11	8	8	12	11	9	9	9	9,7	0,86
Coccinellidae (aphidophagous)	10	8	9	10	11	9	9	10	10	10	9,6	0,72
Nabis spp. (adult)	11	10	10	11	10	10	10	11	12	11	10,6	0,54
Nabis spp. (larvae)	12	12	12	13	12	11	12	13	11	12	12	0,38
Chrysopidae (adult)	13	13	13	12	13	13	13	12	13	13	12,8	0,28

#### **3.3.** Analysis of the impacts on predator arthropod taxons

Based on the results of the multifactorial analysis of variances, the year, sampling period and the interaction between the two had an impact on the abundance of all studied predator arthropods. Treatments had an impact on all three predator arthropod groups, such as spiders, ground beetles and rove beetles, captured by soil traps. In case of the predators captured by Pherocon AM sticky traps, treatment had a significants impact only on Orius spp adults, whereas in case of individual plant examination, treatments had significant effect on *Stethorus pusillus* adults and larvae.

#### 3.3.1. Pairwise comparison of average abundance of predator arthropods per treatment

Based on the average abundance of predator arthropods, analyzing significant differences among treatments, it has been determined that in 31 out of the 57 possible cases (data separated by arthropod groups and years), there were a significant differences among treatments (at least in one case); these differences were visualized by separate diagrams in the dissertation.

Analysis of differences among treatments is demonstrated based on the example of the most abundant, sampled predator arthropods, namely the ground beetles.

There was no significant difference in terms of the abundance of ground beetles captured by soil traps, among treatments, in year 2007. In 2006, there was no significant difference detected between control plots and the Cry34/35Ab1 (1) protein producing-, as well as the control plots and Cry34/35Ab1 x Cry1F (2) protein producing *Diabrotica* resistant maize hybrids (**Graph 1./A**). In 2008, the average abundance of ground beetles was significantly lower in the Cry34/35Ab1 x Cry1F (2) protein producing (Hybrid A) maize plots compared to the control B (73) plots with no soil insecticide application (**Graph 1./B**). In 2006, among those maize hybrids that produce CP4 EPSPS protein as well (treatmens 3–6) there were no significant differences detactable in the average abundance of ground beetles compared to the control plots (**Graph 1./A**). In both 2006 and 2008, the lowest numbers of captured ground beetles were recorded in treatment 4 and 6, where maize plots were treated with glyphosate; in 2008, both in the control plots where soil insecticide was applied and in that control plot (control B, 73 and 74) where there was no such application, the abundance of ground beetles were significantly higher than in those plots treated with glyphosate (**Graph 1.**).



**Graph 1.:** Average number of ground beetles in each treatment (Sóskút, 2006, 2008). Note: letters indicate significant differences, lines represent standard deviation.

#### 3.4. Analysis of the impacts on the abundance of herbivore arthropod taxons

Based on the results of multifactorial analysis of variances, the year, sampling period and the interaction between the two had an impact on the abundances of all sampled herbivore/decomposer arthropod groups; with the only exception of true bugs since the year had no effect on their abundance. Treatments had an impact on both herbivore/decomposer arthropod groups, i.e. abundance of springtails and flea beetles, captured by soil traps. Significant effect of treatment was detectable in case of flea beetles and the western corn rootworm among herbivores captured by Pherocon AM sticky traps; in case of individual plant examination, the impact of treatment was significant only for flea beetles.

#### 3.4.1. Pairwise comparison of average abundance of herbivore arthropods per treatment

Based on the average abundance of herbivore arthropods and analyzing the significant differences among treatments, it has been determined that in 20 out of the 33 possible cases per arthropod groups and per years) there were a significant differences among treatments, which were described in details using diagrams in the thesis.

Analysis of differences among treatments is demonstrated based on the example of the most abundant, sampled herbivore arthropods, namely the thrips.

There were significant differences among treatments in terms of the numbers of the Pherocon AM sticky trap captured thrips, in years 2006 and 2008 (Appendix, Tables 73.-73). In 2006, in case of four treatments (treatment 2, 6, 73 and 74) there were significantly fewer thrips present compared to the control A plot (treatment 71), which was not treated with soil insecticide (**Graph 2/A.**). In 2008, in case of the Cry34/35Ab1 (1) and Cry34/35Ab1 x Cry1F (2) protein producing maize hybrid (Hybrid A) the average number of thrips was significantly lower compared

to the control B plot (treatment 74), which was treated with teflutrine active ingredient. In 2008, there were no significant differences in terms of average number of thrips, in any of the CP4 EPSPS protein producing maize plots compared to control plots (treatment 71-74) (**Graph 2/B.**).



**Graph 2.:** Average number of individuals of thrips per treatment (Sóskút, 2006, 2008). Note: leters indicate significant differences, line represent standard deviation.

#### 3.5. Relationships among arthropods and analysis of their parameters

In the paragraph below, relationships among arthropod groups in the GM (treatment 1-6) and control maize plots (treatment 71-74) have been analysed based on the cumulative data of the three sampling methods. Total of 14 predator and 8 herbivore/decomposer groups were included in the analysis, so that in each treatment relationships among 22 arthropod groups were investigated per treatment. Out of the 231 possibilities, the number of relationships among arthropod groups varied between 16 and 23 in the GM and control isogenic plots. Comparing the stability parameters of arthropod relationships in the different treatments (trophic links (B), link density (D), connectance (C)), significant difference occured only in case of the plot with the Cry1F x CP4 EPSP protein producing maize and the plot treated with glyphosate (treatment 4) in terms of trophic links (B) and link density (D). None of the other cases proved to be significant, in all of the other treatments connectances were the same (**Table 7.**).

Treatment	S	L	B	D	С
1	22	20	0,909 <sup>ns</sup>	0,173 <sup>ns</sup>	0,041 <sup>ns</sup>
2	22	21	0,955 <sup>ns</sup>	0,181 <sup>ns</sup>	0,043 <sup>ns</sup>
3	22	17	0,772 <sup>ns</sup>	0,147 <sup>ns</sup>	0,035 <sup>ns</sup>
4	22	23	1,045*	0,199*	0,047 <sup>ns</sup>
5	22	17	0,773 <sup>ns</sup>	0,147 <sup>ns</sup>	0,035 <sup>ns</sup>
6	22	21	0,954 <sup>ns</sup>	0,181 <sup>ns</sup>	0,043 <sup>ns</sup>
71	22	17	0,772 <sup>ns</sup>	0,147 <sup>ns</sup>	0,035 <sup>ns</sup>
72	22	19	0,863 <sup>ns</sup>	0,164 <sup>ns</sup>	0,039 <sup>ns</sup>
73	22	19	0,863 <sup>ns</sup>	0,164 <sup>ns</sup>	0,039 <sup>ns</sup>
74	22	16	0,727 <sup>ns</sup>	0,138 <sup>ns</sup>	0,033 <sup>ns</sup>

**Table 7.:** Comparison of the stability parameters of arthropod relationshipsin GM and control maize plots. Note: \* – p<0,05; ns – not significant

## 4. Discussion, conclusions

#### 4.1. Structural characteristics of the samples arthropod groups

In the three vegetation periods (4 sampling periods per year) different arthropod groups and many individuals were sampled, in total, more than 520 000 individuals were recorded during the individual plant examinations or captured by soil traps and Pherocon AM sticky traps. The highest number of sampled arthropods was recorded in 2008, probably due to the higher than average precipitation between June and Spetember, whereas the lowest number of sampled individuals was recorded in 2007, which was the hottest and driest year during the study period.

Analysing the RANK order of the arthropods captured by different sampling tools, similarly to other studies (Kiss et al., 2002; Bhatti et al., 2005a; 2005b) it can be concluded that uniformly both in the GM and control plots, the same arthropods groups were dominant. In terms of the soil trap captures ground beetles, springtails and spiders, in terms of the Pherocon AM sticky traps thrips, cicadas, flea beetles, aphids and the western corn rootworm were dominant. In case of the individual plant examinations, aphids, eggs of green lacewings, *Orius* spp. adults and flea beetles were abundant in each treatment.

### 4.2. Comparison of the sampled predator arthropods per treatment

Similarly to other studies that were done in maize (Bhatti et al., 2005a; 2005b; Higgins et al., 2009), the year and sampling period had an impact on captured predator arthropods.

When comparing the GM and isogenic maize plots (pairwise comparison), there was a significant difference in the soil trap captured and the individual plant examination recorded

number of spiders in three cases; however, these differences were not consistent and did not show any tendency during the three years of the study, therefore the Cry34/35Ab1 protein producing maize hybrids did not have any impact on the abundance of spiders. Using different sampling methods, in most cases studies did not find any difference in terms of abundance (Pilcher et al., 1997; Lozzia and Rigamonti, 1998; Jasinski et al., 2003; Delrio et al., 2004; Daly and Buntin, 2005; de la Poza et al., 2005; Eckert et al., 2006; Fernandes et al., 2007) and diversity (Volkmar and Freier, 2003; Sehnal et al., 2004; Meissle and Lang 2005; Farinos et al., 2008) of spiders comparing the Cry1Ab protein producing and isogenic (not treated with insecticide) maize. In some of the cases, where a difference was found, this difference was not consistent, such as in the study of Lang et al. (2005); in their three-year study in one particular year a lower number of spiders was observed in the Cry1Ab protein producing maize than in the isogenic. Árpás et al. (2004a; 2004b; 2005) in their field study in Hungary found no significant difference between the content of the spiderweb of Theridion impressum L. Koch. (Therididae) when comparing the ones sampled in a Cry1Ab protein producing (MON 810 (DK-440 BTY)) maize field with the ones sampled in the closely related isogenic maize. In other European studies no significant difference was detectable in the abundance of spiders in Cry3Bb1 and CP4 EPSPS protein producing maize (Svobodova et al., 2012b). My results are in harmony with studies that were done in other regions prior to mine and show that the Cry3Bb1 proteinproducing maize, which is closely related to the Cry34/35Ab1 protein, has no effect on the abundance of spiders (Bhatti et al., 2005a; Al-Deeb and Wilde, 2003).

In my study there was only one case found in which a significant difference was repeated, that of the ground beetles in 2006 and 2008. In both years there were fewer gorund beetles sampled by soil trap in GM maize plots treated with glyphosate compared to other plots. Similar results have been published, Szekeres et al. (2008) and Pálinkás et al. (2012), in their studies in Hungary, recorded lower number of ground beetles in maize treated with glyphosate, therefore with less weed cover. Cárcamo et al. (1995) found positive correlation between abundance of ground beetles and weed cover. Other studies have shown as well that ground beetles are more active in fields that are covered by dicotyledonous weeds compared to those ones where grasses are dominant or are completely free of weeds (Pavuk et al., 1997). In a Hungarian field study, there was no difference found in the number of individuals of ground beetles between Cry1Ab protein producing and isogenic maize (Szekeres et al., 2006). Testing the Cry3Bb1 protein producing maize, similar results were obtained in the Check Republic, Bt maize did not have an impact on the abundance of ground beetles (Svobodova et al., 2012a). In the United States, in a Cry3Bb1 protein producing maize field no undesired side-effect was detectable on the abundance of ground beetles in a field study between 200-2001 (Al-Deeb and Wilde, 2003). In harmony with these results, Ahmad et al.

(2005) in their two-year study (2002–2003) did not found significant differences in the number of ground beetles in Cry3Bb1 protein producing (seedcovered with clothianidine) and isogenic maize.

In our study, neither of the Cry34/35Ab1 protein producing maize had an impact on the number of rove beetles. Similar results were obtained in the Check Republic with the Cry3Bb1 protein producing maize, Bt maize did have imact on the abundance of rove beetles (Svobodova et al. 2012a). Farinós et al. (2008) found that in their field study, maize with the MON810 event did not have any impact on the activity and density of rove beetles, however, in contrast to that, the effect of the year had a significant influence on rove beetles. Besides, maize treated with imidacloprid active ingredient significantly reduced species richness of rove beetles but had no negative impact on the abundance of most of the species. In a 2001 study in Spain, near Leida, rove beetles were present in higher numbers on the Cry1Ab protein producing maize, whereas in 2000, near Madrid higher numbers of rove beetles were recorded in the isogenic maize (de la Poza et al., 2005). In a two-year (2002-2003) US field study, using soil traps, Ahmad et al. (2005) did not find any significant difference in the numbers of rove beetles between the Cry3Bb1 (seedcovered with clothiadine) and isogenic maize. Similarly, in the US, in case of the Cry3Bb1 protein (Al-Deeb and Wilde, 2003) and Cry1Ab protein (Wolfenbarger et al., 2008) no negative side-effect on the abundance of rove beetles was found.

Similarly to other studies where the *Diabrotica* resistant Cry3Bb1 protein producing maize was tested on several species belonging to the Coccinellidae family (Al-Deeb and Wilde, 2003; Ahmad et al., 2006; Rosca and Cagan, 2012a), I could not prove any significant reduction in the number of the aphidophagous ladybird species in any of the Cry34/35Ab1 protein producing maize plots during our three year study. McManus et al. (2005), in their field study, tested the potential undesired side-effect of the Cry3Bb1 protein producing maize on the abundance of the aphidophagous ladybird species, *Coleomegilla maculata*. Aphidophagous ladybirds were observes in three different developmental stages of the maize (adults, pupae and larvae were sampled) in Bt maize, maize treated with soil insecticide (teflutrine) and untreated control (isogenic) maize. In many cases, significantly higher number of ladybirds (in different developmental stages) were recorded (using Pherocon AM sticky traps and individual plant examinations) in Bt maize compared to the other two treatments, however, in case of *Coleomegilla maculate*, no abundance reducing impact of the Cry3Bb1 protein producing Bt maize was proved.

There were significant differences detected in more than one occasion in terms of the abundance of adults, larvae and pupae of *Stethorus pusillus*. The abundance differences among treatments did not show any tendency, except the year 2008, when all three developmental stages of *Stethorus pusillus* were present in highest number in the maize plots treated with soil insecticide. Li

and Romeis (2010) chose Stethorus pusillus (as test organism) from ladybird species for their laboratory studies because this species is frequently found is maize fields and is special predator of the red spider mite (Putman, 1955; Rott and Ponsonby, 2000; Biddinger et al., 2009), a given Bt protein can be present at high quantities in this prey. Former laboratory tests proved that those red spider mites collected in different Bt maize fields (events Bt176 and MON88017) had the same level of toxin in their body as the green leaves of the maize itself (Obrist et al 2006; Meissle and Romeis, 2009a). Obrist et al. (2006) found that among sampled arthropods, the highest level of toxin was detected in the larvae of Stethorus psusillus. Li and Romeis (2010) in their climate chamber study, did not find any difference in the fertility and development of red spider mite reared on Bt and isogenic maize; similar results were obtained by Dutton et al. (2002) in case of the Cry1Ab protein. As a follow up of their study, Stethorus pusillus were fed with red spider mites that either containe or did not contain Bt toxin; no differences were detected in their development or fertility. Interestingly, the pre-oviposition period of the females became shorter, their tendency to mate and their egg-productivity increased in Bt maize compared to the isogenic maize. According to the authors, the reason beyond this phenomenon might be such changes in the characteristics of the maize plant, which are yet to be discovered. The level of toxin was 6-fold higher in the red spider mites than in the Stethorus larvae and 20-fold higher than in the adults (Li and Romeis, 2010). Álvarez-Alfageme et al. (2008) came to similar results, the level of Cry1Ab protein was 7-fold higher in red spider mites than in *Stethorus* adults.

Similarly to other field studies (in case of the Cry3Bb1 protein producing maize) the number of individuals of hoverflies (Bhatti et al., 2005b; Svobodova et al., 2012a) and adults&eggs of green lacewings (Rosca and Cagan, 2012b) did not decrease in our three-year field study in the Cry34/35Ab1 protein producing maize plots.

In terms of the predator true bugs captured with Phrocon AM sticky traps or recorded by individual plant examination, number of adults and larvae of *Orius* spp. was not significantly lower in any of the three years in the Cry34/35Ab1 protein producing maize plots compared to the control (isogenic) maize, which result is in harmony with the study of Al-Deeb and Wilde (2003) and Rauschen (2008). According to the results of Ahmad et al. (2006) the Cry3Bb1 protein producing maize did not have any negative impact on the number of *Orius insidiosus* adults and larvae recorded by individual plant examination, in their two-year field study, however, in one year adults were present in less numbers in the isogenic maize compared to the maize treated with insecticide (clothiadine).

In the Cry34/35Ab1 protein producing maize, the abundance of *Nabis* spp, recorded by individual plant examination, did not decrease significantly compared to the isogenic maize,

similarly to other studies where the possible undesired side-effects of the Cry3Bb1 protein producing maize was tested (Bhatti et al, 2005b). In 2007 the average number of *Nabis* spp. larvae was significantly lower in all herbicide tolerant maize than in the Cry34/35Ab1 x Cry1F protein producing maize, however, this difference did not occur in the other two years of the study.

#### **4.3.** Comparison of the sampled herbivore/decomposer arthropods per treatment

Similarly to other studies conducted in maize, the year and the sampling period had an impact on the number of herbivore/decomposer arthropods (with the only exception of true bugs) (Bhatti et al., 2005a; 2005b; Higgins et al., 2009).

Number of springtails, captured in isogenic maize plots by soil trap during the three years of the study (2006-2008), did not show significant difference to those ones captured in the Cry34/35Ab1 protein producing maize plots, which result is in harmony with the findings of US field studies (Al-Deeb et al, 2003; Ahmad et al., 2005). In 2006 higher number of captured springtails was recorded in isogenic, soil insecticide treated plots compared to other treatments. Bitzer et al. (2005) and Ahmad et al. (2005) came to similar results sampling higher number of springtails in insecticide treated maize; the authors claimed, citing Christiansen (1964), the cause of this phenomenon is that springtails can resist insecticides more effectively than their predators.

Meissle and Romeis (2009b) in their laboratory study found great differences among the Cry3Bb1 toxin levels of sampled herbivore preys, lower concentration of protein was detected at those ones feeding from phloem (such as aphids) and higher at those ones feeding from cytoplasm (such as thrips, true bugs, flea beetles and most of the cicadas). Tested mize leaves had high concentration (160–220  $\mu$ g/g), whereas pollen had lower concentration (27  $\mu$ g/g) of the Cry3Bb1 protein. Among herbivore arthropods, aphids did not contain detectable level of Cry3Bb1 protein (with the only exception of *Rhopalosiphum padi* (<0,1  $\mu$ g/g) captured after flowering), thrips and true bugs had (5–10  $\mu$ g/g), flea beetles had (7–33  $\mu$ g/g) concentration of the protein. Most of the cicada species contained less than 1  $\mu$ g/g quantity.

There was no significant consistent difference in the average number of aphids between the treated and control maize plots during the three vegetation periods. Since aphids feed from the phloem, they get on hrdly any Cry protein from the maize plant (Head et al., 2001).Comparing the control maize plots to the Cry34/35Ab1 (1) and Cry34/35Ab1 x Cry1F (2) protein producing plots, significant difference was detected only in 2008 when there was a lower number of aphids, captured by Pherocon AM sticky traps, in the Coleoptera and Lepidoptera resistant maize (treatment 2) than in the soil insecticide treated control plot (treatment 74). In the CP4 EPSPS protein producing plot (treatment 3) with "conventional weed control" (no glyphosate application) the number of aphids

captured by Pherocon AM sticky trap was significantly lower in 2008 than in the soil insecticide treated (treatment 72 and 74) plots. In contrast to that, in 2006, the number of aphids, recorded by individual plant examination, was significantly lower in treatment number 3 whith no glyphosate application, among the CP4 EPSP protein producing (treatment 3-6) mize plots, compared to the control plots with no soil insecticide treatment. Therefore, weed control did not consistently influence the number of aphids; there was no clear trend detectable among hybrids and treatments. Similarly to my results, Rauschen (2008) did not find adverse effect of the Cry3Bb1 protein producing maize on aphids, which is closely related to the Cry34/35Ab1 protein.

Among the herbivores feeding from the cytoplasm, in most cases there was no difference between the Cry34/35Ab1 protein producing and isogenic maize plots in terms of the number of thrips, cicadas, true bugs captured by Pherocon AM sticky trap. Some differences that occurred during the study did not follow any trend. Similarly to my results, in Slovakian field studies (in case of the Cry3Bb1 protein producing maize), there was no differenc detectable between Bt and isogenic maize in terms of the number of thrips (Svobodova et al., 2012a). The MON88017 event (Cry3Bb1 and CP4 EPSP protein producing) maize did not have negative effect on the number of cicadas (Zyginidia scutellaris) (Rauschen, 2008; Rauschen et al., 2010b) and true bugs (Trygonotylus caelestialium) (Rauschen, 2008; Rauschen et al., 2009). Rauschen et al. (2009) sampled the number of *Trygonotylus caelestialium* with the so-called "sweep netting" method in a Cry3Bb1 and CP4 EPSPS protein producing (MON88017 event), in the closely related isogenic (DKC5143) and in two conventional (Benicia és DK315) maize in three vegetation periods (three sampling periods per year). In more than one case, in 2005 and 2006, significant difference was found in the number of true bugs in the two conventional maize plots however, this difference was not detectable in 2007. There was significant difference between the Bt and isogenic maize plots only in one occasion (in August of 2006), which the authors ascribe to the various characteristics of different hybrids (such as differences in odours, in leaf surface, microclimatic conditions) (Niiyama et al., 2007).

During the three years of the study three herbivore arthropod groups (click beetles, flea beetles and the western corn rootworm) were sampled within the Coleoptera order. Similarly to other field studies where Coleoptera resistant (Cry3Bb1 protein producing) maize was involved (Al-Deeb és Wilde, 2003; Ahmad et al., 2005), my results showed no negative effect of the Cry34/35Ab1 protein producing maize on the abundance of click beetles. Similar results were obtained for western corn rootworm, since in most cases there was no significant difference among treatments in terms of the number of individuals. Difference was detected only in 2008, showing no tendency referring to the whole study period. Considering the three different sampling methods, there were

significant differences found in terms of the number of flea beetles among treatments, nevertheless, just like in the above mentioned cases, no clear tendency was revealed during the years, therefore the tested Cry34/35Ab1 protein producing maize did not have any adverse effect on the number of flea beetles. In terms of the flea beetles, neither in the USA (Dively, 2005), nor in Hungary (Szénási and Markó, 2015) was any diference found in the number of individuals between Bt (Cry1Ab protein producing) and isogenic maize. In field trials in Germany (Rauschen et al., 2010a) Diabrotica resistant (Cry3Bb1 protein producing) and isogenic maize hybrids were compared, and no difference was found between the number of flea beetles in Bt and non-Bt maize.

#### 4.4. Relationships among arthropods and comparison of their parameters

14 among predator- and 8 among herbivore arthropod groups made it possible to analyse the stability of relationships in different treatments.

Out of the 231 possibilities, the number of relationships among arthropod groups varied between 16 and 23 in the GM and control isogenic plots. On the whole it can be concluded that parameters expressing the stability of arthropod relationships (Martinez et al., 1999) were nearly identical in all cases. Comparing the various stability parameters per treatments, there was a significant difference only in case of the Cry1F x CP4 EPSPS protein producing and the glyphosate treated (treatment 4) maize plots in terms of trophic links (B) and link desity (D). Since trophic link (B) does not inevitably increase with the increase of number of species and groups involved (Barabási and Albert, 1999; Albert and Barabási, 2002; Dunne et al., 2002; Antoniou and Tsompa, 2008; Jordán et al., 2012), therefore the difference in this case can be explained by the high L value (23). Link density (D) is the shortest relation or path between two trophical groups. High D values indicate linear structure of the food-chain, while low D values are signs of a stable and compact food-chain structure (Barabási and Albert, 1999; Albert and Barabási, 2002; Dunne et al., 2002; Antoniou and Tsompa, 2008). In all cases, the 0,1 and 0,2 values indicate compact and stable food-chain, which is also proven by the fact that predator arthropods were steadily present in all treatments. There are no literature data available for the comparison of my results, since very few studies applied the technique of stability parameter comparison for agricultural (arable) environment and especially for GM plant within (Szénási et al., 2014). Nevertheless it can be concluded that in case vegetation (maize and weeds) were involved in the analysis as well, differences became slurred and uniformly stable food-chains were observed in each treatment and control maize plots (Szénási et al., 2014).

## 5. New scientific results

During my three year study that was conducted in the vegetation period of maize, using diferent capturing/sampling techniques (soil trap, Pherocon AM sticky trap and individual plant examination), in the quantitative comparison of arthropode assemblages in GM Bt (Cry34/35Ab1 x Cry1F protein producing) and closely related isogenic maize, the following results were determined:

- 1. The Cry34/35Ab1 and Cry34/35Ab1 x Cry1F protein producing hybrids did not have adverse effect on the abundance of dominant non-target herbivore- (thrips, cicadas, true bugs, click beetles, chrysomelids, aphids) and decomposer (springtails) arthropods.
- The Cry34/35Ab1 and Cry34/35Ab1 x Cry1F protein producing hybrids did not have adverse effect on the abundance of dominant non-target predator arthropods (spiders, *Orius* spp., *Nabis* spp., ground beetles, rove beetles, aphidophagous ladybirds, spider-mite destroyers, green lacewings, and hoverflies).
- 3. Year and sampling period had greater effect on the abundance of herbivore-, predator- and decomposer arthropods than any of the treatments.
- 4. In all of the cases and sampling methods, both in GM and control maize plots, the same arthropod groups were dominant, adding up to nearly 90% of the total captures.
- 5. Ground beetles, springtails and spiders were dominant among soil trap captured arthropods, whereas thrips, cicadas, flea beetles and the western corn rootworm were dominant among Pherocon AM sticky trap captured arthropods. Aphids, eggs of green lacewings, *Orius* spp. adults and flea beetles were dominant among arthropods recorded by individual plant examination.
- 6. Uniformly stable food-chains were present in both GM and isogenic maize plots, parameters describing the stability of food-chains were identical or nearly identical.

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## **7.** Publications from this work

## **Book chapter:**

Szénási Á., Kiss J., Pálinkás Z., Szekeres D. és Kádár F. (2009): Az amerikai kukoricabogár Európában: rezisztens kukoricahibridek környezeti hatásvizsgálata Európában és Magyarországon. In: Dudits D. (szerk.): Zöld géntechnológia és agrárinnováció. Barabás Zoltán Biotechnológiai Egyesület. Szeged. 101-108.

## Papers:

- Meissle M., P. Mouron, T. Musa, F. Bigler, X. Pons, V.P. Vasileiadis, S. Otto, D. Antichi, J. Kiss,
  Z. Pálinkás, Z. Dorner, R. van der Weide, J. Groten, E. Czembor, J. Adamczyk, J.B Thibord, B. Melander, G. Cordsen Nielsen; R. Thostrup Poulsen; O. Zimmermann, A. Verschwele and E. Oldenburg (2009): Pests, pesticide use and alternative options in European maize production: Current status and future prospects. *Journal of Applied Entomology* 134 (5): 357-375. IF: 1,11
- Kiss J., Szénási Á., **Pálinkás Z.,** Dorner Z., Zalai M., Balog A. és Kádár F. (2010): Környezeti kockázat-vizsgálatok GM kukoricákkal. *Zöld biotechnológia*, 6, (11), 4-10.
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- Szénási Á., **Z. Pálinkás**, M Zalai, O. J. Schmitz, A. Balog (2014): Short-term effects of different genetically modified maize varieties on arthropod food web properties: an experimental field assessment. *Scientific Reports*, 4: 5315. **IF: 5.58**
- Pálinkás Z., M. Zalai, Á. Szénási, F. Kádár, Z. Dorner, A. Balog (2015): Rove Beetles (Coleoptera Staphylinidae) their abundance and competition with other predatory groups in Bt maize expressing Cry34Ab1, Cry35Ab1, Cry1F and CP4 EPSPS proteins. *Crop Protection*, 80: 87-93. IF: 1,493
- Pálinkás Z., M. Zalai, Á. Szénási, Z. Dorner, A. Balog (2016): Who eats who? Bt maize events expressing Cry34Ab1, Cry35Ab1, Cry1F and CP4 EPSPS proteins do not affect the arthropod food web. PLoS ONE. (benyújtva)

## **Conference abstracts:**

- Pálinkás Z., Á. Szénási, J. Kiss, Dorner Z. and F. Kádár (2009): Methods for assessing the impact of transgenic maize hybrids on non-target arthropods. 8<sup>th</sup> International Symposium in the Series Recent Advances in Plant Biotechnology. Szeged, 1-4 September 2009. p. 70-71.
- Kiss J., Szénási Á., **Pálinkás Z**. (2009): GM növények: Tudunk-e élni a lehetőségeinkkel? II. Országos Agráripari Konferencia. Összefoglaló, MTA Budapest, 2009. május 21.
- **Pálinkás Z.,** Szénási Á. és Dorner Z. (2010): "Litter bag" módszer alkalmazása környezeti hatásvizsgálatra GM-kukoricában. 56. Növényvédelmi Tudományos Napok, Budapest, p. 79.
- Szénási Á., **Pálinkás Z**. és Szekeres D. (2010): Kukoricabogár- (DAS-59122) és Lepidopterarezisztens (DAS-1507 x NK603), továbbá Glifozát-toleráns (DAS-1507 x NK603) és DAS-

59122 x NK603) kukoricák környezeti kockázatelemzése: kockázati hipotézis, kitettség és szabadföldi tesztek. 56. Növényvédelmi Tudományos Napok, Budapest, p. 80.

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