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THE QUANTITATIVE CHANGES OF GROUND BEETLES (COL., CARABIDAE) IN BT AND CONVENTIONAL MAIZE CROP IN SOUTHERN POLAND

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Abstract: In the southern part of Poland, ground beetle fauna was studied in the first large-scale Bt maize experiment. The aim of this study was to determine the long term impact of the Bt maize cultivar in comparison to conventional plants, on selected non-target arthropods. The DKC 3421 YG cultivar (Bt maize) and the respective isogenic non-Bt varieties (DKC 3420) were cultivated at two locations: (a) Budziszów near Wrocław and in Głuchów near Rzeszów in the south-eastern region of Poland, in the 2008–2010 growing seasons. For comparative analysis, two additional non-Bt cultivars sprayed with a lambda-cyhalotrine insecticide were also included. To monitor population density of surface-active invertebrates of the Carabidae family, eighty pitfall traps were used at each location. The average number of ground beetle populations in the Bt-maize cultivar DKC 3421 YG did not significantly differ from the number of beetles in the conventional ones. Significant differences between the number of beetles occurred on individual dates only. Usually, these differences related to the considerably smaller total number of beetles in the whole replication. Probably, the variation in the number of beetles was caused by climatic factors or the terrain layout, therefore it cannot be related to the cultivar effect.

Key words: Bt-maize, conventional plants, ground beetles, quantitative changes

INTRODUCTION

An ecological risk assessment evaluating potential adverse effects on non-target arthropods affecting the biodiversity of agrocenoses is necessary when releasing a novel transgenic crop (Naranjo 2009; Hui-Lin 2011). Ground beetles are one of the key organisms in the maize field ecosystem often acting as generalist predators of many crop pests (Holland 2002; Lopez *et al.* 2005). The gene expressing the δ-endotoxin Cry 1Ab from *Bacillus thuringensis* Berliner var. *kurstaki* is usually used only against well-defined caterpillars that feed on maize, especially on European corn borer (*Ostrinia nubilalis* Hübner, Lepidoptera: Crambidae). Ground beetles are exposed to Bt proteins directly through ingesting maize litter and maize pollen, and indirectly through eating prey that feeds on the maize plants. The ecological exigencies of Carabidae are known and well defined for many species that form communities in cultivated fields, making them excellent bioindicators (Thiele 1977; den Boer 1979). Moreover, some species can be phytophagous, detrivorous and predatory; even controlling maize pests. Such species can be appropriate groups for describing the trophic relationships and degree of biodiversity in the soil (Lozzia 1999). Till now, there has not been sufficient information concerning non-target organisms on Bt maize in Poland and such a large experiment has never been conducted.

The aim of the study was to determine the long term impact of the Bt-maize cultivar on non-target organisms, in comparison to conventional plants. The study was done under environmental conditions in the southern part of Poland. In this study only the effect of Bt-maize on the number of ground beetles (Coleoptera, Carabidae) was taken into account.

MATERIALS AND METHODS

The direct environmental effects of the Bt gene were tested through the studies conducted in maize fields

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at two locations in the southern part of Poland, *i.e.* in Budziszów (51°06′N, 17°02′E), near Wrocław and in Głuchów (50°01″N, 22°17″E), near Rzeszów (distance between locations ca. 400 km), in the time period from 2008 to 2010. A large-scale experiment was allocated in the area where infestation by the European corn borer is a substantial problem (Bereś 2010). The following treatments were used in the experiment: (1) Bt transgenic maize (DKC 3421 Yield Gard®) (Monsanto Company), (2) isogenic non-Bt varieties without insecticide application (DKC 3420), (3) isogenic non-Bt varieties (DKC 3420) with lambda-cyhalotrine treatment, and for comparative analysis two non-Bt conventional cultivars (4) Bosman and (5) Wigo sprayed with lambda-cyhalotrine were also included (as reference control Ref. 1 and Ref. 2). Each year, the insecticides were applied in the second half of July at the maize stage BBCH 55–59. The design of this experiment consisted of randomized complete blocks with five treatments and four replications (Fig. 1). For each experimental design, a large size plot was set up (160 m^2). Experiments were conducted on the same plots for three consecutive years.

A total of 80 plastic pitfall traps (diameter 6 cm) were used in each location to collect the epigeal arthropods (four on each plot). The traps were dug into the soil with the opening at the soil surface. Traps were filled with 50:50 water, with ethylene glycol used as a preservative. The traps were emptied weekly from June (plants with 4–6 leaves) until maize maturation.

Block 1		Block 2		Block 3		Block 4		
*DKC 3420 Prot.		DKC 3421 YG		DKC 3420 Prot.		Ref. 1 Prot.	40	218 m
							4,5	
Ref. 1 Prot.		Ref. 2 Prot.		DKC 3420 Non-Prot.		DKC 3421 YG	40	
							4,5	
DKC 3421 YG		DKC 3420 Non-Prot.		Ref. 1 Prot.		Ref. 2 Prot.	40	
							4,5	
DKC 3420 Non-Prot.		Ref. 1 Prot.		Ref. 2 Prot.		DKC 3420 Prot.	40	
40		40		40		40	4,5	
Ref. 2 Prot.		DKC 3420 Prot.		DKC 3421 YG		DKC 3420 Non-Prot.	40	
178 m								

*DKC 3420 Prot. – insecticide treated
DKC 3420 Non-Prot. – not insecticide treated
DKC 3421 YG (Bt) – not insecticide treated
Ref. 1 Prot. – Bosman – insecticide treated
Ref. 2 Prot. – Wigo – insecticide treated

Fig. 1. Design of the field experiment

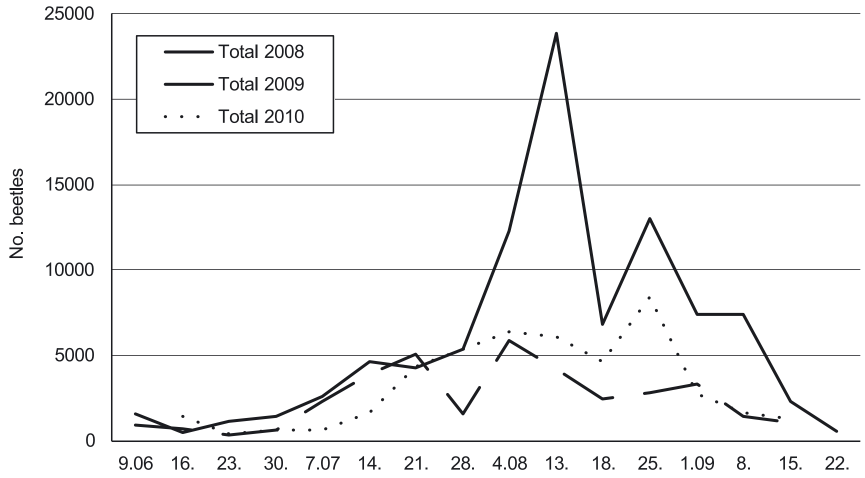
Data were analyzed by using GLM (Generalized Linear Model) at a repeated measures procedure. Mauchly's sphericity test was used. When the error covariance matrix of the orthonormalized transformed dependent variables was not proportional to an identity matrix, lower-bound adjustment was applied (conservative approach). Homogenity subsets of maize cultivars were checked by Tukey's HSD (post-hoc) test. To avoid the influence of seasonal trends, statistical analyses were calculated separately for each date. For a summary ANOVA analyses, logarithmic standardisation was applied to minimise skewness in two cases.

RESULTS AND DISCUSSION

Seasonal changes in beetle assemblages

Considerable differences in the weather conditions between Budziszów and Głuchów were observed during the whole research period. Without a doubt, this factor influenced quantitative differences in the number of ground beetles caught at both locations, especially in 2008. These differences did not occur in the next research seasons or were not so pronounced. However, even in the summary graph for the three years of the experimental period, beetle activity was similar at both locations (Fig. 2a, b). The highest number of beetles was caught in July-August and the peak number occurred approximately at the same time in Budziszów and Głuchów.

a) Budziszów



b) Głuchów

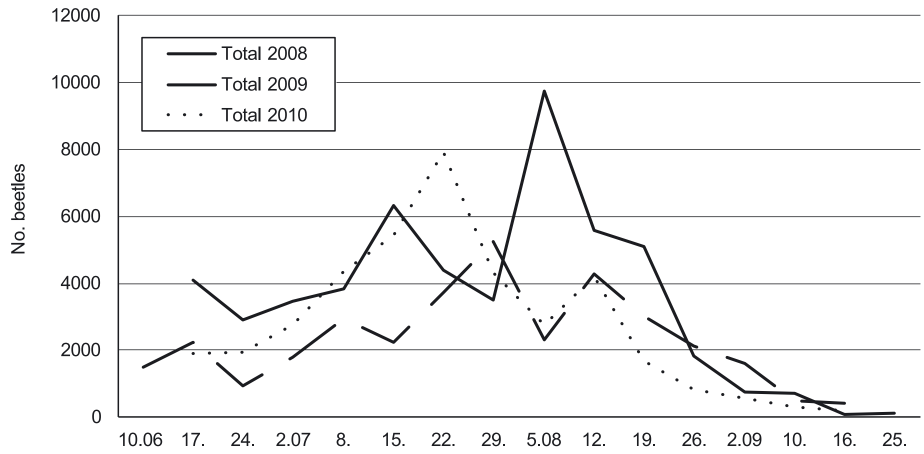


Fig. 2. Total seasonal activity of ground beetles in (a) Budziszów and (b) Głuchów in the 2008–2010

Quantitative differences in ground beetles assemblages

Ground beetles are a polyphagous group of epigeal insects highly abundant in maize crops (Lang *et al.* 1999). Also our data confirm that, during the whole period of 2008–2010, a huge number of ground beetles was collected in 14–16 data-sets/year. During the three years of the study, more than 177 thousands beetles were collected in Budziszów and more than 126 thousands in Głuchów (Table 1). Significant differences between the numbers of the caught beetles in different treatments occurred only in Budziszów. However, quantitative proportions of collected beetles in the respective experiment objects did not differ significantly for DKC 3421 YG. In 2008 and 2009, significantly fewer beetles were caught in the DKC 3420 non-protected treatment in comparison to protected Bosman. In 2010, fewer beetles were caught in the DKC 3421 YG treatment than in protected Bosman. It is worth emphasizing, that in Budziszów, 25.2–26.6% of all beetles in each season were recorded in the Bosman cultivar (Table 1).

Despite considerable quantitative differences between locations, the range of proportions in the number of the beetles caught between treatments at each location showed considerable stability from year to year. The variation in the number of the caught beetles in the period of three years for DKC 3421 YG and the isogenic non-protected cultivar DKC 3420, only reached 3%. The detailed analysis of each trapping date, confirmed that significant differences only occurred in 7 object cases of the total 89

Table 1. Total number of ground beetles collected on each treatment in the 2008–2010

Location	Treatment	2008	Tukey test sign.*	2009	Tukey test sign*.	2010	Tukey test sign.*	Total per object	Proportion range for 3 years [%]
Budziszów	Ref. 1 (Bosman) Prot.	25,332	b	9,218	b	11,599	b	46,149	25.2–26.6
	Ref. 2 (Wigo) Prot.	20,837	ab	7,376	ab	10,380	ab	38,593	20.2–22.7
	DKC 3420 Prot.	17,289	ab	7,858	ab	9,162	ab	34,309	18.2–24.5
	DKC 3420 Non–Prot.	14,590	a	5,377	a	7,641	ab	27,608	14.7–16.7
	DKC 3421 YG	17,110	ab	6,744	ab	6,923	a	30,777	15.1–18.4
	Total per year	95,158		36,573		45,705		17,7436	100%
Głuchów	Ref. 1 (Bosman) Prot.	10,212	ns	6,367	ns	7,846	ns	24,425	18.3–20.1
	Ref. 2 (Wigo) Prot.	10,943	ns	6,776	ns	6,780	ns	24,499	17.3–20.8
	DKC 3420 Prot.	10,052	ns	7,167	ns	8,340	ns	25,559	19.2–21.3
	DKC 3420 Non–Prot.	9,846	ns	7,372	ns	7,875	ns	25,093	18.8–21.2
	DKC 3421 YG	11,429	ns	7,096	ns	8,282	ns	26,807	20.4–21.8
	Total per year	52,482		34,778		39,123		126,383	100%

*(ANOVA, post-hoc Tukey test separate for each year and location), different letters in the columns denote significant differences between treatments, ns – not significant

Table 2. Summary results of GLM analyses for each data set (date) in both locations in the 2008–2010

Location	Year	First and last date of analysis	Total No. of data sets	Total No. of beetles	Statistical analysis – Number of data sets with: Lower bound correction*	significant difference** between replicates	significant difference between any objects	significant difference between DKC 3421 YG and all other treatments (higher or lower than other treatments)
Budziszów	2008	9 Jun./22 Sep.	16	95, 158	3	12	1	0
	2009	12 Jun./17 Sep.	15	36, 573	4	7	1	0
	2010	17 Jun./16 Sep.	14	45, 705	3	6	2	0
Głuchów	2008	17 Jun./25 Sep.	15	52, 482	3	9	2	0
	2009	10 Jun./15 Sep.	15	34, 778	3	8	1	0
	2010	16 Jun./15 Sep.	14	39, 123	1	12	0	0
		Total	89	303, 819	17	54	7	0

*sphericity correction (Mauchly's test)
**based on Levene's test of Equality of Error Variance

Table 3. Mean number of ground beetles (per plot) collected in treatments within homogeneous subgroup (ANOVA, Tukey's HSD test)

Budziszów, 18th June 2009 (f = 4.44, p = 0.014)				Głuchów, 28th July 2008 (f = 5.35, p = 0.007)		
Object	N	subset 1	subset 2	object	subset 1	subset 2
DKC 3421YG	4	6.62		Ref. 2 (Wigo) Prot.	28.44	
DKC 3420 Non-Prot.	4	7.12	7.12	Ref. 1 (Bosman) Prot.	30.25	
Ref. 2 (Wigo) Prot.	4	7.12	7.12	DKC 3420 Prot.	31.94	
DKC 3420 Prot.	4	11.94	11.94	DKC 3420 Non-Prot.	56.31	56.31
Ref. 1 (Bosman) Prot.	4		13.06	DKC 3421YG		71.44

Table 4. Mean number of ground beetles (per plot) collected in treatments within homogeneous subgroup (ANOVA, Tukey's HSD test)

Budziszów, 13rd August 2008 (f = 5.37, p = 0.007)			Budziszów, 29th July 2010 (f = 4.13, p = 0.014)		
Object	subset 1	subset 2	object	subset 1	subset 2
DKC 3420 Prot	227.5		DKC 3420 Prot	37.1	
DKC 3420 Non-Prot.	240.8		DKC 3420 Non-Prot.	38.8	
DKC 3421YG	288.1	288.12	DKC 3421YG	55.7	55.7
Ref. 2 (Wigo) Prot.	317.88	317.9	Ref. 2 (Wigo) Prot.		99.1
Ref. 1 (Bosman) Prot.		418.4	Ref. 1 (Bosman) Prot.		102.1

(Table 2). In each of these 7 cases, cultivar DKC 3421 YG was included in one of two homogeneous groups. However, no case was confirmed in which that cultivar significantly differed from all the remaining objects. Original data sets calculated by GLM using the repeated measures procedure, delivered a sufficiently large number of the degrees of freedom to assume the experimental design was well-balanced. Thus, the considerable skew data or the replications with non-equality of variance can be surveyed in the statistical procedure. The number of cases with the lower bound correction and significant difference between replicates are only important as being informative.

As a case study, two incidents (data sets) in which the highest and the lowest ground beetle mean numbers were confirmed in the DKC 3421 YG plots, were presented (Table 3). In the first case, only the difference of the DKC 3421 YG related to the protected variety Bosman was important. This cultivar clearly differs in the features of canopy architecture (better leaved and higher plants), from cultivars DKC 3420 and DKC 3421 YG.

In contrast, Bt maize cultivar did not differ from the not protected isogenic one. Moreover, it was not hard to notice, that separate homogeneous subgroups concerned the protected and not protected objects. Such a status appeared most often in Budziszów. This mainly proves the strong insecticidal influence on non-target fauna. An average number of ground beetles was caught in the DKC 3421 YG plots. However, the range order of the tested objects was sometimes identical (Table 4). The mean number of recorded insects in those cases was the highest in DKC 3421 YG, of all the groups of the DKC studied objects.

Ground beetles are considered the dominant group of arthropods, as the adults mainly inhabit the surface of the soil. Their presence could potentially demonstrate whether the Cry proteins in the soil affect the community structure and diversity of soil organisms (Grabowski *et al*. 2010; Twardowski *et al.* 2010). Beetles can be exposed in different ways to toxins produced by transgenic plants. They can feed directly on plants or indirectly on non-target phytophagous organisms. Beetle development is closely connected to the soil where the toxins persist, so a response is possible. The majority of ground beetle assemblage studies conducted in other countries, show no significant difference between Bt and isogenic maize (Lozzia 1999; Manachini 2000; Sehnal *et al.* 2004; de la Poza *et al.* 2005; Lopez *et al.* 2005; Szekeres *et al*. 2006; Leslie *et al.* 2007; Farinós *et al*. 2008; Priestley and Brownbridge 2009). In a six-year monitoring of non-target organisms in Germany, researchers Schorling and Freiser (2006) did not find general tendencies in differences in the population densities of Carabidae as well as of other arthropods. A few reports suggest some minor differences. A change in the ground beetle community structure was found by Toschki *et al.* (2007) only in the first year of their three-year study. These authors concluded the more unfavourable microclimate and massive European corn borer infestation caused that differences rather than the direct toxic effects linked with Bt maize.

In our trials, we did not find any significant differences in the abundance of the total ground beetle assemblage in Bt maize in comparison to conventional cultivars in both of the research areas. Our findings suggest that crop management practices and/or environmental conditions had the greatest impact on ground beetle assemblages, rather than the crop itself (Bt or isoline) affecting ground beetle assemblages.

CONCLUSIONS

1. On Bt-maize cultivar DKC 3421 YG, ground beetles did not occur in significantly smaller numbers than on conventional ones.
2. Significant differences in the number of beetles in the studied cultivars occurred only at individual date measurements. Differences related most often to the considerably smaller total number of beetles in the whole replication at those dates. Such a type of variation is most often caused by climatic factors or the terrain slope, and cannot be bound with the substantial features of the studied cultivars.

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