

A Quantitative Assessment of the Unintended Effects of Bt-Maize (MON 810) on Rove Beetle (Col., Staphylinidae) Assemblages

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Abstract

The aim of this study was to determine the impact of a genetically modified (GM) maize cultivar MON 810 containing Cry1Ab protein in comparison to conventional plants on rove beetle assemblages (Coleoptera: Staphylinidae) as non-target arthropods. This is the first large-scale Bt-maize experiment in Poland. A Bt transgenic maize cultivar (DKC 3421 Yield Gard[®]) and the respective isogenic maize DKC 3420 were cultivated at two locations: Budziszów, near Wrocław in southwestern Poland, and in Głuchów, near Rzeszów in the southeastern region, in the 2008-2010 growing seasons. For comparative analysis two additional non-Bt cultivars sprayed with a lambda-cyhalotrine insecticide also were included. To monitor the population density of soil surface-active invertebrates of the Staphylinidae family, 80 pitfall traps were used at each location. The average number of rove beetle populations in the Bt-maize habitat did not differ significantly from the number of beetles in the conventional ones. Significant differences in the number of beetles occurred only on individual dates. The variation in the number of beetles was probably caused by environmental factors, and therefore it cannot be related to the cultivar effect.

Keywords: Bt-maize, conventional, non-target organisms, rove beetles, staphylinids

Introduction

Substantial discrepancies in the risk assessment of transgenic maize cultivars indicate that there is an urgent need to study this problem. Up to the present time, there has been insufficient data concerning non-target arthropod occurrence on Bt-maize habitats in Poland. One of the reliable groups of bioindicators often used for the assessment of environmental hazard risks are rove beetles (Coleoptera: Staphylinidae) [1]. In agroecosystems rove beetles play an important role in many ecological processes. They are a substantial part of the community of polyphagous predatory organisms of arable

fields. However, within multitrophic interactions few staphylinids can also act as saprophagous or parasitic organisms [2-4]. Their diet allows them to be treated as omnivorous, except for those species consuming the living tissues of higher plants. Rove beetles' contribution to biodiversity also results from the relatively high biomass and usually high number of species collected in a particular area [5]. Thus, these insects, despite difficulties in identifying their species, can be used as good-bioindicators of changes caused by human activity [1]. This group of bioindicators constitutes measurable components of the environment that provide a rapid, cost-effective and simplified system to gather complex information about the ecosystems in which they occur, and about human-environment interactions [6].

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The effect of genetically modified (GM) plants on non-target organisms including Bt-maize still appears to be unclear when considering rove beetles [7, 8]. Staphylinids, as with mainly epigeic insects, could be indirectly exposed to the δ -endotoxin Cry1Ab toxin produced by transgenic plants containing the *Bacillus thuringiensis* Berliner var. *kurstaki* gene. Involved in the breakdown and recycling of crop residues, these insects could be in contact with decaying GM-plant material since they are scavengers and detritivores. They can also act as natural enemies of pests feeding on Bt-maize, which is another possible way of being in contact with the GM-plant material ingested by the herbivore. Quantitative studies of arthropod predators have been recommended to test the hypothesis that the abundance of staphylinid populations is correlated with GM maize in agroecosystems.

The aim of our study was to determine the impact of the Bt-maize cultivar MON 810, expressing the Cry1Ab protein, aimed at controlling the European corn borer (*Ostrinia nubilalis* Hübner) on non-target rove beetle (Coleoptera: Staphylinidae) assemblages in comparison to conventional maize plants. Evaluations of the effects of Bt toxin on staphylinids have been performed as part of broader field studies aimed at determining the effects of GM plants on the non-target arthropods within the maize ecosystem.

Materials and Methods

The environmental effects of the Bt gene were tested through studies conducted in maize fields at two locations in southern Poland: in Budziszów (51°06' N, 17°02' E), near Wrocław, and in Głuchów (50°01' N, 22°17' E), near Rzeszów (distance ca. 400 km), from 2008 to 2010. An experiment was allocated in the area where infestation by the European corn borer is a substantial problem [9]. The following treatments were used in the experiment:

- (1) Bt transgenic maize MON 810 (DKC 3421 Yield Gard®) (Monsanto Company)
- (2) isogenic, non-GM cultivar without insecticide application (DKC 3420)
- (3) isogenic (DKC 3420) with insecticide application and for comparative analysis two non-Bt conventional cultivars:
- (4) Bosman and
- (5) Wigo, both sprayed with insecticide, were also included (as reference control Ref. 1 and Ref. 2).

The reference cultivars had similar FAO numbers but were otherwise unrelated to MON 810 and DKC group. Each year, the insecticide (active ingredient: lambda-cyhalothrin in Karate Zeon 050 CS) was once applied in select treatments in the second half of July at maize stage BBCH 55-59, at a dose of 0.2 ml per ha. Weed control was done with nikosulfuron (Milagro Extra 6 OD), shortly after maize emergence (in the second half of May), in the entire area of the experiment. This herbicide has low potential for bioaccumulation and is not persistent in soil. Neither fungicides nor other pesticides were applied. All the agrotechnology applied in the maize field, including fertil-

Table 1. Design of the field experiment.

*DKC 3420 Prot.	4.5 m	DKC 3421 YG	4.5	DKC 3420 Prot.	4.5	Ref. 1 Prot.	40	218 m
40 m		40		40		40	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	
							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. – insecticide treated

Ref. 2 Prot. – insecticide treated

izers, were identical throughout the entire experiment area. The design of this experiment consisted of randomized complete blocks with five treatments and four replications (Table 1). For the experimental design a large plot was set up (1,600 m²). An alley distance of 4.5 m was used between 40×40 m plots. Experiments were conducted on the same plots for three consecutive years, hence the growing amount of Bt endotoxin we could expect on the same plots.

A total of 80 circular, plastic pitfall traps (diameter 9 cm, 14 cm in height) 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. They were filled with 50:50 water, with ethylene glycol used as a preservative. To prevent rain from filling the cup and to keep flying insects from being caught in the trap, a cover made of a (20×20 cm) transparent plastic square was installed. The traps were emptied weekly from the beginning of June (plants with 4-6 leaves) until maize maturation (end of September). In each of three growing seasons 14-16 sample sets were collected (datasets). The arrangement of the traps within plots was performed so as to avoid any side-effect.

Data were analyzed by using the GLM (Generalized Linear Model) as a repeated measure 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).

Table 2. Differences between average temperatures and rainfalls in two locations through the three-year experiment.

Year	Month	Głuchów		Budziszów	
		Temperature [°C]	Total rainfall [mm]	Temperature [°C]	Total rainfall [mm]
2008	VI	19.5	33.7	19.7	45.3
	VII	20.2*	55.4	18.7	117.6
	VIII	18.6	94.7	18.9	55.3
	IX	13.1	22.2	13.0	103.2
2009	VI	11.2	142.9	16.6	146.4
	VII	19.7	118.1	20.0	98.0
	VIII	19.2	59.6	18.7	21.8
	IX	15.2	7.7	15.2	25.5
2010	VI	18.0	33.0	17.9	126.1
	VII	21.5	77.5	20.7	200.2
	VIII	18.9	105.1	19.4	98.6
	IX	12.3	116.7	12.1	97.5

*bold marked a considerable difference, i.e. in precipitation two times high and in temperatures more than 1.5°C

Homogeneity subsets of maize cultivars were checked by Tukey's HSD (post-hoc) test (the data were normally distributed). Statistical significance was evaluated at $P \leq 0.05$ level. To avoid the influence of seasonal trends statistical analyses were calculated separately for each date. For a summary of ANOVA analyses logarithmic standardization was applied to minimize skewness in two cases.

Results

There was a strong site effect in general rove beetle activity-density, as demonstrated by the number of caught individuals, due to considerable climatic differences between the two locations (Table 2). This was clearly visible, especially in the two first years of research (Figs. 1 a and b). However, despite climatic differences and insecticidal treatment (ca. 20 July), a general trend of rove beetle activity was observable. The increase in the number of caught individuals occurred in the second half of June, and also from July to the middle of August. The number of caught specimens usually decreased after the first ten days of September.

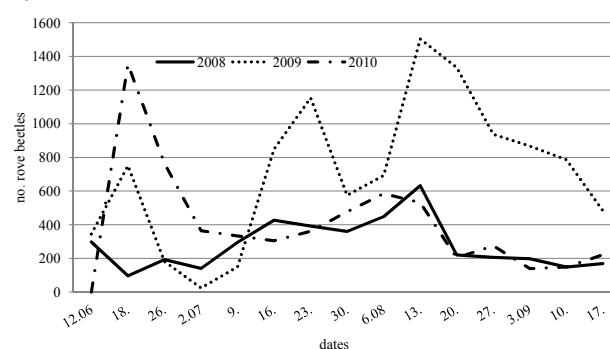
In our experiment, during the whole period of 2008-10, over 35 thousand individuals were collected in 14-16 datasets/year, most of them in Budziszów (21,033 beetles) (Table 3). Significant differences between treatments were confirmed in Budziszów (2009) and Głuchów (2009-10). However, the proportion of caught beetles in any cultivar did not differ between treatments, except in the case of DKC 3421 YG with non protected DKC 3420 in Głuchów (2009 and 2010), and non protected DKC 3420 with Ref. 1

in Budziszów (Table 3). Also, the low level of rove beetle activity requires certain interpretative caution. Despite the at least double quantitative differences between locations, the range of proportion within objects shows clear stability from year to year. The range of changeability in the period of three years for DKC 3421 YG varies from 6% in the less numerous sample in Głuchów to 1.8% in the more constant Budziszów. In additional analysis there were also no significant differences found between the number of staphylinids collected within the blocks in the whole experiment in the two localities.

Detailed analysis at each date shows that 24 out of 89 object cases presented significant differences, and three cases showed considerable difference for DKC 3421 YG to all other objects (Table 4). In each of the 24 cases where significant differences were found staphylinids were included in one of three homogeneous groups but not as a sole extreme value. At only two other dates was the number of collected specimens in DKC 3421 YG higher than in the remaining objects. In that cultivar significantly fewer beetles were recorded on only one occasion during the three-season research. All these three data sets were found to be significantly different, with the highest and the lowest rove beetle mean number in DKC 3421 YG in Głuchów (Tables 4 and 5). The case of a small number of beetles appeared at the time when the general abundance level of rove beetles was low. Differences between neighbouring lowest mean values are smaller than one specimen. So, because of the strong influence of randomness, deduction requires great caution (Table 5).

In the remaining cases similar status occurred when the number of collected beetles was low, on 8 September 2010

a) Budziszów



b) Głuchów

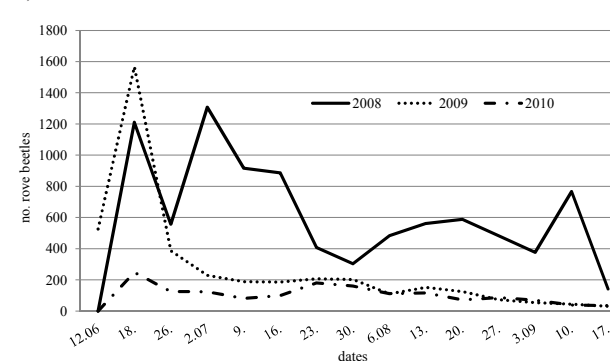


Fig. 1. Total seasonal activity of rove beetles in two locations (a) Budziszów and (b) Głuchów in 2008-10.

Table 3. Total number of rove beetles collected on each treatment in 2008-10.

Site	Treatment	2008	Tukey test*	2009	Tukey test*	2010	Tukey test*	Total per object	Proportion range for 3 years [%]
Budziszów	Ref. 1 Prot.	1052	a	2,856	a	1,160	a	5,068	19.1-26.8
	Ref. 2 Prot.	828	a	2,478	bc	1,378	a	4,684	19.1-23.3
	DKC 3420 Prot.	900	a	2,036	abc	1,387	a	4,323	19.1-22.9
	DKC 3420 Non-Prot.	833	a	1,439	c	1,001	a	3,273	13.5-19.2
	DKC 3421 YG	721	a	1,825	ab	1,139	a	3,685	16.6-18.8
	Total per year	4334		10,634		6,065		21,033	100.0
Głuchów	Ref. 1 Prot.	1960	a	786	ab	272	ab	3,018	17.6-21.7
	Ref. 2 Prot.	1731	a	807	ab	328	ab	2,866	19.2-21.2
	DKC 3420 Prot.	1703	a	895	ab	315	ab	2,913	18.8-21.9
	DKC 3420 Non-Prot.	1771	a	717	a	391	a	2,879	17.6-25.3
	DKC 3421 YG	1872	a	875	b	240	b	2,987	15.5-21.4
	Total per year	9037		4,080		1,546		14,663	100.0

*different letters within columns, separately for each location and year of the study indicate a significant difference between treatments (ANOVA, Tukey HSD test, $p \leq 0.05$)

Table 4. Summary results of GLM analyses for each data set (date) in both locations in 2008-10.

Site	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 objects	
								Higher than other	Lower than other
Budziszów	2008	9 Jun./22 Sep.	16	4,334	5	10	2	0	0
	2009	12 Jun./17 Sep.	15	10,634	1	14	5	0	0
	2010	17 Jun./16 Sep.	14	6,065	7	7	5	0	0
Głuchów	2008	17 Jun./25 sep.	15	9,037	1	8	4	0	0
	2009	10 Jun./15 Sep.	15	4,080	3	7	3	1	0
	2010	16 Jun./15 Sep.	14	1,546	2	14	5	1	1
Total			89	35,696	19	60	24	2	1

*Sphericity correction (Mauchly's test)

**based on Levene's test of Equality of Error Variance

Table 5. Mean number of rove beetles (per plot) caught in treatments within homogeneous subgroup (ANOVA, Tukey's HSD test) for 25 July 2010, ($F = 4.44$, $p = 0.014$) in Głuchów.

Treatment	N	Subset	
		1	2
DKC 3421YG	4	0.63	
Ref. 1 Prot	4	1.13	
DKC 3420 Non-Prot.	4	1.38	
Ref. 2 Prot.	4	1.69	1.69
DKC 3420 Prot	4		3

Table 6. Mean number of rove beetles (per plot) caught in treatments within homogeneous subgroups (ANOVA, Tukey's HSD test) for two data sets – Głuchów 2010.

Treatment	N	23 June 2009 (F=7.86, p=0.001)		8 September 2010 (F=3.87, p=0.24)	
		Subset		Subset	
		1	2	1	2
Ref. 2 Prot	4	3.4		0.12	
Ref. 1 Prot	4	3.5		0.3	
DKC 3420 Non-Prot.	4	4.5		0.4	
DKC 3420 Prot	4	4.9		0.5	
DKC 3421YG	4		7.94		1.0

(Table 6). A clear difference in the number of caught insects was found in the samples of 23 June 2009 only. However, at both dates the number of rove beetles caught in the DKC group came just after the DKC 3421YG number in a descending order. The mean number of recorded rove beetles was highest in DKC 3421 YG of the whole group of DKC studied objects.

Discussion

Rove beetles, as with ground beetles, are highly abundant in maize crops [9], and many studies have been done to evaluate the ecological risk assessment on this epigeal group of insects. Nevertheless, there is still a need to study the possible effects of Bt toxin under field conditions. In general, the performed studies show variable responses that do not reach any reliable conclusion on the effects of Bt-maize against staphylinids [7, 8, 11-13.]. Balog et al. [14] studied the potential exposure of non-targeted adult rove beetles and their larvae to Bt toxins (Cry34Ab1, Cry35Ab1, Cry1F) designed to target the western corn rootworm and the European corn borer. They also confirmed that the overall assemblage of staphylinid was not significantly affected by the production of stacked proteins.

Among epigeal arthropods occurring within the Bt-maize ecosystem the most studied group seems to be the ground beetle community [9, 15-21]. Rove beetles have been used rather rarely as bioindicators of ecological changes potentially caused by the Cry1Ab toxin. The majority of studies regarding rove beetle assemblages conducted in other countries showed no significant differences between Bt and isogenic maize [12]. The variability in activity-density patterns of the aboveground fauna was mainly year-specific, or numerous other factors played a role, but no detrimental effects could be attributed to Bt-maize [8, 10, 17]. Epigeal arthropod communities such as ground or rove beetles may also be affected by crop type, and possibly crop rotation, rather than by the genotype of the GM plants [22].

In conclusion, also in our trials no significant differences were recorded in the abundance of the total rove beetle assemblages in Bt-maize with Cry1Ab endotoxin in comparison to conventional cultivars in both research areas. Cases when DKC 3421YG differ significantly from all other objects were confirmed in single date measurements only. They related most often to a considerably smaller number of beetles. Our findings suggest that environmental conditions had the greatest impact on staphylinid assemblages, rather than the crop itself (Bt or isoline).

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References

1. BOHAC J. Staphylinid beetles as bioindicators. *Agric. Ecos. Environ.* **74**, 357, **1999**.
2. POTTS G.R., VICKERMAN G.P. Studies on the cereal ecosystem. *Adv. Ecol. Res.* **8**, 107, **1974**.
3. ANDERSEN A. Predation by selected carabid and staphylinid species on the aphid *Rhopalosiphum padi* in laboratory and semifield experiments. *Norwegian J. Agric. Sci.* **6**, 265, **1992**.
4. DENNIS P., WRATTEN S.D., SOTHERTON N.W. Feeding behaviour of the staphylinid beetle *Tachyporus hypnorum* in relation to its potential for reducing aphid numbers in wheat. *Ann. Appl. Biol.* **117**, 267, **1990**.
5. BALOG A., MARKO V., SZARVAS P. Dominance, Activity Density and Prey Preferences of Rove Beetles (Coleoptera: Staphylinidae) in Conventionally Treated Agro-Ecosystems. *Bull. Entomol. Res.* **98**, 259, **2008**.
6. ANDERSEN A.N. My bioindicator or yours? Making the selection. *J. Insect Conserv.* **3**, 61, **1999**.
7. HIGGINS L.S., BABCOCK J., NEESE P., LAYTON R.J., MOELLENBECK D.J., STORER N. Three-year field monitoring of Cry1F, event DAS-01507-1, maize hybrids for non-target arthropod effects. *Environ. Entomol.* **38**, 281, **2009**.
8. JASINSKI J.R., EISLEY J.B., YOUNG C.E., KOVACH J., WILLSON H. Select nontarget arthropod abundance in transgenic and nontransgenic field crops in Ohio. *Environ. Entomol.* **32**, 407, **2003**.
9. BEREŚ P.K. Harmfulness of *Ostrinia nubilalis* HBN. on some non-Bt versus genetically modified Bt maize (*Zea mays* L.) cultivars in Poland in 2006-2007. *J. Plant Prot. Res.* **50**, 110, **2010**.
10. FARINÓS G.P., DE LA POZA M., HERNÁNDEZ-CRESPO P., ORTEGO F., CASTAÑERA P. Diversity and seasonal phenology of aboveground arthropods in conventional and transgenic maize crops in Central Spain. *Biol. Control* **44**, 362, **2008**.
11. DIVELY G.P. Impact of transgenic VIP3A × Cry1Ab Lepidopteran-resistant field corn on the nontarget arthropod community. *Environ. Entomol.* **34**, 1267, **2005**.

12. BALOG A., KISS J., SZEKERES D., SZENASI A., MARKO V. Rove beetles (Coleoptera: Staphylinidae) communities in transgenic Bt (MON 810) and near isogenic maize. *Crop Prot.* **29**, 567, **2010**.
13. GARCIA M., ORTEGO F., CASTANERA P., FARINOS G.P. Effects of exposure to the toxin Cry1Ab through Bt maize fed-prey on the performance and digestive physiology of the predatory rove beetle *Atheta coriaria*. *Biol. Control* **55**, 225, **2010**.
14. BALOG A., SZÉNÁSI A., SZEKERES D., PÁLINKÁS Z. Analysis of soil dwelling rove beetles (Coleoptera: Staphylinidae) in cultivated maize fields containing the Bt toxins, Cry34/35Ab1 and Cry1F x Cry34/35Ab1. *Biocontrol Sci. Tech.* **21**, (4), 293, **2011**.
15. LOZZIA G.C. Biodiversity and structure of ground beetle assemblages (Coleoptera Carabidae) in Bt corn and its effects on non target insects. *Boll. Zool. Agr. Bachic.* **31**, 37, **1999**.
16. MANACHINI B. Ground beetle assemblages (Coleoptera, Carabidae) and plant dwelling non-target arthropods in isogenic and transgenic corn crops. *Boll. Zool. Agr. Bachic.* **32**, 181, **2000**.
17. DE LA POZA M., PONS X., FARINOS G.P., LOPEZ C., ORTEGO F., EIZAGUIRRE M., CASTAÑERA P., ALBA-JES R. Impact of farm-scale Bt maize on abundance of predatory arthropods in Spain. *Crop Prot.* **24**, 677, **2005**.
18. LOPEZ M.D., PRASIFKA J.R., BRUCK D.J., LEWIS L.C. Utility of ground beetle species in field pests of potential nontarget effects of Bt crops. *Environ. Entomol.* **34**, 1317, **2005**.
19. SZEKERES D., KÁDÁR F., KISS J. Activity density, diversity and seasonal dynamics of ground beetles (Coleoptera: Carabidae) in Bt-(MON810) and in isogenic maize stands. *Entomol. Fennica* **17**, 269, **2006**.
20. TWARDOWSKI J.P., BEREŚ P., HUREJ M., KLUKOWSKI Z. Ground beetles (Col., Carabidae) in Bt maize-first field large scale experiment in Poland. In: *GMO's in Integrated Plant Production, IOBC/wprs Bull.* **52**, 97, **2010**.
21. TWARDOWSKI J.P., BEREŚ P., HUREJ M., KLUKOWSKI Z., DĄBROWSKI Z.T., SOWA S., WARZECHA R. The quantitative changes of ground beetles (Col., Carabidae) in Bt and conventional maize crop in southern Poland. *J Plant Prot. Res.* **52**, (4), 404, **2012**.
22. LESLIE T.W., HOHEISEL G.A., BIDDINGER D.J., ROHR J.R., FLEISHER S.J. Transgenes sustain epigeal insect biodiversity in diversified vegetable farm systems. *Environ. Entomol.* **36**, 234, **2007**.