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POPULATION DEVELOPMENT OF BIRD CHERRY-OAT APHID *RHOPALOSIPHUM PADI* (L.) (HEMIPTERA, APHIDIDAE) ON CONVENTIONAL AND BT-MAIZE EXPRESSING THE INSECTICIDAL PROTEIN CRY1AB

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Abstract

HUREJ, M., J. P. TWARDOWSKI, P. BERES and Z. KLUKOWSKI, 2014. Population development of bird cherry-oat aphid *Rhopalosiphum padi* (L.) (Hemiptera, Aphididae) on conventional and BT-maize expressing the insecticidal protein Cry1Ab. *Bulg. J. Agric. Sci.,* 20: 658-665

The aim of the study was to determine the long-term effect of transgenic maize expressing toxin Cry1Ab derived from *Bacillus thuringiensis* (Bt-maize) on bird cherry-oat aphid *Rhopalosiphum padi* (L.) population development in comparison to conventional, non-transgenic variety. According to this analysis it is possible to achieve a step-wise approach to assess potential non-target effects of GM plants. Such large open-field trials concerning impact of Bt-maize on aphids are the first of this kind under Polish environmental conditions. Studies were conducted in maize fields at two locations in South part of Poland (distance ca. 400 km from each other), in 2008-2010. In the experiment, five treatments were tested: Bt-maize (DKC 3421 Yield Gard®, Monsanto Company), near-isogenic lines sprayed or unsprayed with lambda-cyhalotrine (DKC 3420, Monsanto Company), and for comparative analysis two conventional varieties (Bosman and Wigo). Aphids were monitored directly on plants. In three years of this study, the population dynamics of *R. padi* was very similar in each treatment. The aphid number on Bt-maize did not differ significantly in comparison to reference varieties. Significant differences between abundance of bird cherry-oat aphid and numbers of their colonies in the studied varieties were confirmed only occasionally, at single date measurements and cannot be related to Cry1Ab toxin present in plant tissue.

Key words: Rhopalosiphum padi, bird cherry-oat aphid, aphids, Bt-maize, Cry1Ab

Introduction

Maize can be attacked by a wide range of arthropods. The main pests of this plant worldwide are lepidopteran species among which European corn borer (*Ostrinia nubilalis* Hübner) (Lepidoptera: Crambidae) seems to be the most injurious and regular in central Europe and elsewhere or *Sesamia nonagrioides* (Lefèbvre) (Lepidoptera: Noctuidae) in southern part of the continent (Meissle et al., 2009). One of the strategies to control maize borers is cultivate genetically modified plants (GM). Bt-maize expressing the insecticidal protein Cry1Ab controls very effectively the primary target *Ostrinia nubilalis* (Dively and Rose, 2002; Venditti and Steffey, 2002; Twardowski et al., 2008; Bereś, 2010). In plant protection this prevents from causing economic damage throughout the season, thus eliminating the need for extensive scouting and application of a corn borer insecticide.

Another important group feeding on maize are aphids (Hemiptera: Aphididae). However they occur irregularly and are not a problem every season, aphids are one of the predominant herbivores feeding on maize (Dicke and Guthrie, 1988). Adults and nymphs suck sap and produce honeydew. High densities can cause plants to turn yellow and appear unthrifty. Yield loss may occur on water stressed plants (Honek, 1994). Till now, four species were identified on maize in Poland, i.e. *Rhopalosiphum padi* (Linnaeus, 1758), *Metopolophium*

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dirhodum (Walker, 1849), *Sitobion avenae* (Fabricius, 1775) and *Rhopalosiphum maidis* (Fitch, 1856) (Kania and Sobota, 1992, Pieńkosz et al., 2005; Krawczyk et al., 2006; Krawczyk et al., 2008; Strażyński, 2008; Bereś, 2011). A similar finding prevails in most of the European countries, eventually with the changes of dominant species (Honek, 1994). In the Lower Silesia region in Poland, *R. padi* is the dominant species (Kania and Sobota, 1992; Krawczyk et al., 2006), while in the Warsaw and Wielkopolska *M. dirhodum* is the most abundant (Pieńkosz et al., 2005; Strażyński, 2008). Aphids have recently become a steadily increasing threat for maize crops (Krawczyk et al., 2006; Strażyński, 2008; Bereś, 2011), therefore in many farms, they are controlled with chemical aphicides. Bird cherry-oat aphid is the polyphagous species with a nearly worldwide distribution (Blackman and Eastop, 1984). In Europe this is the one of the most important species on cereal plantations which severely affects maize production. On growing monocotyledonous plants they suck sap from leaf tissue, but usually not associated with grain. This species is also considered as a serious vector of viruses such a barley yellow dwarf luteovirus (Lucio-Zavaleta, 2001).

The protein toxin from GM maize is considered very selective in their action and should not affect non-target arthropods associated with maize. However, some unintended effects may occur. Resistance to Bt and its toxins has been reported from selection studies in laboratory populations of several insect species (Pereira et al., 2008; Crespo et al., 2009). The potential effects of Bt-maize on non-target phytophagous insects may lead for example to changes in their rate of population increase (Lumbierres et al., 2004). In recent years a lot of attention has been paid to investigate potential adverse effects of Bt-plants on beneficial insects and other non-target organisms (Lővei and Arpaia, 2005; O'Callaghan et al., 2005; Pons et al., 2005; Romeis et al., 2006). These organisms are exposed to the insecticidal protein if they feed on transgenic plant tissue or consume organisms that have eaten the toxin. Aphids feed predominantly on phloem sap (Douglas, 2003), but they also provide food for many entomophagous arthropods. Worth knowing examples are larvae of lacewings and hoverflies as well as larvae and adults of ladybird beetles (Völkl et al., 2007). The fact that the toxin is not transported in the phloem proposed by Raps et al. (2001); Dutton et al. (2002) and Head et al. (2001), should make aphids very unlikely targets of direct effect of the poison. Despite this, newest data comes from ELISA, indicate possibilities of Bt-proteins flow from the plant to predatory via the aphids (Stephens et al., 2012). Therefore there is a still need to examine possible changes of *R. padi* population in different part of the world.

The aim of the study was to determine the long-term effect of Bt-maize on *Rhopalosiphum padi* (Linnaeus, 1758) in comparison to conventional, non-transgenic varieties. Such large field trials concerning impact of Bt-maize on aphids are the first of this kind under Polish environmental conditions.

Material and Methods

Studies were conducted in maize fields at two locations in South 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), during three consecutive maize growing seasons 2008-2010. The following treatments were used in the experiment: (1) Bt-transgenic maize (event DKC 3421 Yield Gard®) (Monsanto Company), (2) isogenic, non-Bt variety without insecticide application (DKC 3420, Monsanto Company), (3) isogenic non-Bt variety with lambda-cyhalotrine treatment (DKC 3420, Monsanto Company), and for comparative analysis also two non-Bt conventional, national varieties (4) Bosman and (5) Wigo sprayed with lambda-cyhalotrine were included (as reference control Ref. 1 and Ref. 2). Each year, the insecticides in chosen treatments 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 (Figure 1). For each experimental design, a large size plot was set up (40 x 40 m, 1600 m^2). Experiments were conducted on the same plots for three consecutive years.

Aphids were counted on 18 plants per plot. Plants were chosen at random at 3 points along the diagonal of the plot (6 plants at each point). In both locations, the total number of aphids per plant was counted every two weeks. In Budziszów, additionally the number of wingless and winged aphids and the number of colonies per sample were also taken into account.

Data were analyzed by ANOVA at repeated measures procedure (with partial eta-squared value) (Tabachnick and Fidell, 1989). Sphericity was tested by Mauchly's procedure. In the case that the error covariance matrix of the orthonormalized transformed dependent variables is not proportional to an identity matrix, lower-bound adjustment was applied (conservative approach). Homogeneity subsets of maize treatment was done by Tukey`s HSD (post-hoc) test. To avoid seasonal trends influence also statistical analysis was calculated, separately by one-way ANOVA ($P < 0.05$), for each data set from Budziszów 2008-2010.

Results

In both locations, number of aphids feeding on maize plants was not considerably large. In Budziszów distincly more *Rhopalosiphum padi* individuals was observed than in Głuchów, therefore in Figure 2 only for this location the population dynamics on DKC 3421 YG (Bt) and DKC 3420 (isogenic) varieties are presented. In each year, migrating aphids started to colonize maize leaves in the first decade of June. The highest number of bird cherry-oat aphid in 2008 as well as in 2009 was observed in the middle of second or beginning of third decade of July, while in 2010 at the end of June. In 2008, at the maximum aphid's population plants were in the BBCH stage 63, in 2009 BBCH 55-59 and in 2010 BBCH 34. In 2008, high number of bird cherry-oat aphid was maintained to the end of monitoring season, similarly in both treatments. In case of third year of the study the second peak of *R. padi* occurrence in maize plantations was noticed (16th September). In each year, the population dynamics of this aphid was very similar on DKC 3421 YG (Bt) and DKC 3420 isogenic varieties. Despite the clearly visible higher abundance of aphids in isogenic line it was inappreciable because no significant differences were calculated presumably due to a small number of analysed herbivore species.

Within the studied period, no statistical differences were found between treatments in Głuchów (Table 1). Number of

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								

Fig. 1. Design of the field experiment (duplicated in the two locations)

*DKC 3421 YG (Bt) - no insecticide treated; DKC 3420 Prot. - insecticide treated; DKC 3420 Non-Prot. - no insecticide treated; Ref. 1 Prot. - Bosman - insecticide treated; Ref. 2 Prot. - Wigo - insecticide treated

Table 1
Experiment design data and ANOVA analysis of *Rhopalosiphum padi* on maize plants in Głuchów

Year	First and Last date of analysis	Total no. of data sets	ANOVA analysis		Mean no. aphids/samples					
			F-test/sign.	Partial Eta Squared	DKC 3420 N-Prot	DKC 3421 YG	DKC 3420 Prot	Ref. 1 Prot.	Ref. 2 Prot.	Stand. error
2008	11 Jun./20 Sep.	8	0.93/(0.46) ns	0.10	18.50	13.78	11.31	17.25	17.66	3.15
2009	6 Jun./5 Sep.	7	1.18/(0.34) ns	0.14	17.36	13.82	10.14	12.39	10.79	2.65
2010	12 Jun./2 Oct.	9	0.37/(0.83) ns	0.04	4.28	3.56	2.86	3.50	2.81	1.0

ns. - not significant

aphids infesting transgenic variety was neither significantly lower nor higher than in the other treatments. However, aphids on the Bt-maize were clearly less abundant than on isogenic one, but the trend for lower numbers was not significant from the reference cultivars Bosman and Wigo. Thus these results are very similar to those found in Budziszów.

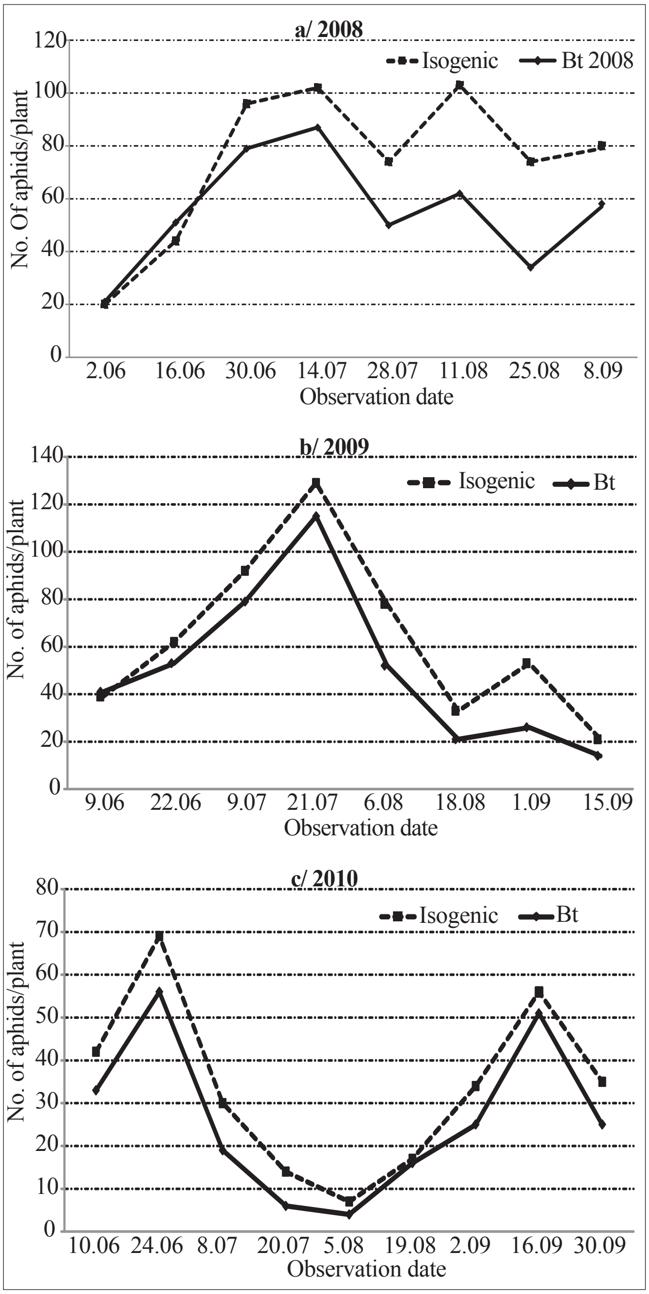

Fig. 2. Seasonal dynamics of *Rhopalosiphum padi* inhabiting Bt and isogenic maize in Budziszów in 2008-2010

The number of aphid colonies on tested treatments in whole research period differed significantly only in 4 cases in Budziszów (Table 2). Twice these differences were calculated in 2009 (1st and 15th of September) as well as twice in 2010 (20th of July and 20th of September). However, these short-term differences did not relate to DKC 3431 YG. They mainly resulted from differences in plants architecture of two national cultivars and group of DKC. These clearly differ (especially Ref. 1) in height of plants, density of foliage that created diverse microclimate for aphids' development.

Winged aphid number also did not significantly differ within treatments in the period June-July of the research seasons in Budziszów (Table 3). However, each September there appeared a case with obvious difference in number of *R. padi* infesting the studied varieties. Conventional cultivars were more attractive also during this period than plants of DKC group. Within that group, on DKC 3421 YG plants *R. padi* population was less numerous. Winged bird cherry-oat aphids were numerous on maize only in June and partly in July in Budziszów. No significant differences were found between treatments.

Discussion

For the production of genetically modified crops it is advantageous that single toxins are extremely toxic to specific pest insects. Bt crops offer farmers a new tool for pest control, thus reducing their reliance on hazardous conventional pesticides. There is a lot of evidence for a precise performance of Bt-maize for target lepidopteran species. However, never enough is known about different non-target species living within GM ecosystem. Till now, numerous studies have addressed the potential impact of Bt-maize on these organisms. The vast majority of them indicate no effect of such varieties on non-target arthropods including aphids and their natural enemies. The general results of our studies are consistent with these findings. The strengths of this paper is that it was repeated on the same fields for three years, which show, that Bt effects do not accumulate over years. The toxin coming from *Bacillus thuringensis* var. *kurstaki* is thought as selective in action against European corn borer larvae. However, when other phytophagous organisms feed on such a plants, the direct or indirect effect could be also expected. Several studies have also assessed the risk for aphids (Lozzia et al., 1998; Raps et al., 2001; Lumbierres et al., 2004; Ramirez-Romero et al., 2008). In the case of these arthropods, more studies were done under laboratory conditions where the direct effect could be easily measured (Head et al., 2001; Raps et al., 2001; Czapla et al., 2011). In most cases, laboratory investigations concluded that aphids cannot be

Table 2
Population parameters of wingless *Rhopalosiphum padi* inhabiting maize plants in Budziszów (mean no. aphids/18 plants/replicate; significance in colony numbers between treatments)

Treatments - 2008	2.06	16.0	30.06	14.07	28.07	11.08	25.08	8.09	22.09
Ref. 1 Prot	148.7 a	96.8 a	209.1 a	6.8 a	11.0 a	0.5 a	0.5 na.	407.5 a	2630 a
Ref. 2 Prot.	127.9 a	79.9 a	89.8 a	0.4 a	0.3 a	22.0 a	0.0 na.	32.0 b	540.3 a
DKC 3420 Prot	126.6 a	117.6 a	250.9 a	6.4 a	0.0 a	0.0 a	0.3 na.	3.5 b	44.8 a
DKC 3420 N-Prot.	159.0 a	222.9 a	252.9 a	10.1 a	0.5 a	0.0 a	0.3 na.	31.5 b	26.3 a
DKC 3421YG	89.1 a	174.8 a	191.3 a	6.2 a	1.3 a	0.0 a	0.3 na.	0.3 b	256.5 a
Difference between colony numbers	na.	ns.	ns.	ns.	na.	na.	na.	na.	na.
Treatments - 2009	9.06	22.06	9.07	21.07	6.08	18.08	1.09	15.09	
Ref. 1 Prot	0.5 a	291.0 a	61.8 a	3.0 a	7.0 a	12.5 a	221.8 a	8066.5 a	
Ref. 2 Prot.	0.1 a	202.8 a	54.5 a	1.0 a	3.3 a	12.5 a	435.3 a	13799.0 a	
DKC 3420 Prot	0.3 a	170.5 a	50.8 a	0.0 a	9.8 a	7.5 a	33.8 a	617.3 b	
DKC 3420 N-Prot.	4.5 a	211.5 a	45.3 a	1.0 a	1.0 a	3.3 a	1.3 a	443.0 b	
DKC 3421YG	0.5 a	165.5 a	42.3 a	1.3 a	1.3 a	1.0 a	11.5 a	311.8 b	
Difference between colony numbers	ns.	ns.	ns.	ns.	ns.	ns.	Ref. 2/ DKC3420N-Prot	Ref. 1 and 2 /all DKC var.	
Treatments - 2010	10.06	24.06	8.07	20.07	5.08	19.08	2.09	20.09	
Ref. 1 Prot	213.3 a	327.3 a	215.3 a	6.3 a	4.8 na.	0.5 na.	188.8 na.	368.3 a	
Ref. 2 Prot.	247.3 a	252.0 a	82.5 a	1.0 a	1.0 na.	0.0 na.	0.5 na.	50.0 a	
DKC 3420 Prot	117.5 a	243.5 a	187.3 a	12.0 a	1.8 na.	0.0 na.	0.0 na.	0.0 b	
DKC 3420 N-Prot.	172.5 a	230.8 a	169.5 a	3.8 a	0.0 na.	0.0 na.	0.0 na.	1.5 b	
DKC 3421YG	254.3 a	215.8 a	118.8 a	6.8 a	0.0 na.	0.0 na.	0.0 na.	5.5 b	
Difference between colony numbers	ns.	ns.	ns.	DKC3420 Prot. /Ref. 2 and DKC 3420 N-Prot	na.	na.	na.	Ref. 1/all var.	

na. - not applicable; ns. - not significant

affected because the toxin is not transported in the phloem sap on which these insects feed (Head et al., 2001; Raps et al., 2001; Dutton et al., 2002; Pons et al., 2005). These type of studies are being sometimes criticized as ecologically unrealistic and useless for the prediction of large-scale and long-term effects (Andow and Hilback, 2004; Lövei and Arpaia, 2005; Romeis et al., 2006). On the other hand, seasonal population dynamics of aphids can only be studied in a large field experiment. Open field studies are crucial for the evaluation of potential side effects of GM crops, and outcomes on aphids could cascade up to higher trophic levels thus affecting the whole food web. We carried it out at two locations in south part of Poland. In our trials, no effect of Bt-maize on bird cherry-oat aphid population development was recorded. The similar results in the open area were achieved by Bourguet et al. (2002); Eckert et al. (2006) and Ramirez-Romero et al. (2008). Also Delrio et al. (2004) and Habustova et al. (2005), showed in field studies that the infestation of aphids, mainly *Rhopalosiphum padi*, was similar in both Bt and non-Bt plots. The latter authors reached the same results in case of *Metopolophium dirhodum*. However, Lumbierres et al. (2004) as well as Pons et al. (2005), in their study found a higher density of migrants and young nymphs of *R. padi* and other cereal species colonizing Bt-transgenic maize, especially when the plants started to grow in a commercial field. These authors simultaneously indicated that this fact also could not be closely linked with the expression of the Bt-toxin in the phloem. Górecka et al. (2007) had similar observations in greenhouse experiments on transgenic MON 810 maize in comparison with its isogenic line Monumental. The duration of development of each life stages of pest was shorter on GM maize. Differences were less significant on older plants. Recently a different finding was done by some researchers, but in case of Cry3Bb toxin produced to control western corn rootworm (*Diabrotica virgifera virgifera* LeConte) (Stephens et al., 2012). ELISA test these authors

Table 3
Population parameters of winged *Rhopalosiphum padi* inhabiting maize plants in Budziszów

Treatments - 2008	2.06	16.06	30.06	14.07
Ref. 1 Prot.	20.06 a	88.69 a	30.56 a	0.75 a
Ref. 2 Prot.	16.50 a	80.06 a	20.63 a	0.75 a
DKC 3420 Prot.	17.25 a	65.81 a	18.75 a	1.50 a
DKC 3420 Non-Prot.	13.31 a	79.31 a	23.63 a	0.75 a
DKC 3421YG	11.25 a	89.63 a	24.0 a	1.69 a
Difference between colony numbers	na.	ns.	ns.	ns.
Treatments - 2009	9.06	22.06	9.07	21.07
Ref. 1 Prot.	4.0 a	73.5 a	53.0 a	1.25 a
Ref. 2 Prot.	3.0 a	71.25 a	40.0 a	1.0 a
DKC 3420 Prot	2.25 a	73.5 a	36.0 a	1.25 a
DKC 3420 Non-Prot.	3.0 a	65.0 a	22.5 a	1.25 a
DKC 3421YG	2.75 a	70.75 a	46.75 a	2.50 a
Difference between colony numbers	ns.	ns.	ns.	ns.
Treatments – 2010	10.06	24.06	8.07	20.07
Ref. 1 Prot.	33.75 a	25.50 a	30.50 a	8.50 a
Ref. 2 Prot.	33.75 a	21.50 a	21.75 a	2.25 a
DKC 3420 Prot.	16.25 a	23.50 a	15.75 a	6.50 a
DKC 3420 Non-Prot.	20.25 a	16.25 a	15.0 a	3.50 a
DKC 3421YG	26.0 a	14.25 a	18.75 a	3.75 a
Difference between colony numbers	ns.	ns.	ns.	ns.

na. - not applicable; ns. - not significant

did showed the presence of Cry3Bb proteins in the *Rhopalosiphum maidis*, species closely related to *R. padi*. They also highlighted that the proteins are mediated to the next, predaceous trophic level namely *Harmonia axyridis* Pallas. Together these data suggest that *H. axyridis* can be affected indirectly by Cry3Bb Bt-maize via a secondary pest, maize leaf aphids. According Stephens et al. (2012), ladybird feeding upon *R. maidis* on Cry3Bb plants in the field may experience greater mortality and shorter life spans. *H. axyridis* is in the order of insects known to be affected by protein Cry3Bb, so a negative effect was not totally unexpected. Nonetheless in data taken from Faria et al. (2007) in which all transgenic plants used express the *B. thuringiensis* gene which codes for the Cry1Ab toxin, it is showed increased susceptibility of Bt-maize to corn leaf aphid *R. maidis*. Simultaneously, mentioned authors considered if this situation has a positive (aphid produced honeydew attractive for parasitoids) or undesirable side effect. However, authors rather think that the higher colony densities on Bt-maize were caused by differences in chemical constituents that rendered the plants less well defended and/or more nutritious for the aphids.

Conclusions

- In each year, the population dynamics of *Rhopalosiphum padi* was very similar on DKC 3421 YG (Bt) and DKC 3420 (isogenic) varieties. The studied bird cherry-oat aphid did not occur in significantly smaller or bigger number on Bt-maize than on the conventional reference varieties Bosman and Wigo.
- Significant differences between numbers of bird cherry-oat aphid and numbers of their colonies in the studied varieties were confirmed only at single date measurements. The differences were related most often to differences in plant architecture of studied cultivars (e.g. height of plants, density and compactness of foliage) that created diverse microclimate for aphids' development.

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