Effect of MON 810 Cultivation and Prevention to Adventitious Presence in Non-GM Fields: A Case Study in Slovakia

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Abstract

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The efficiency of border rows to prevent the adventitious presence of GM maize in non-GM maize plots was evaluated as well as the effect of the MON 810 maize of the yield and self-protection against the European corn borer. The GM maize MON 810 event was drilled at 3 locations over the Slovakia and the grain samples were collected from the neighbouring conventional maize fields. The data obtained by Real-Time PCR indicate that coexistence between GM and conventional maize is feasible and the isolation distance of 200 m (respectively 100 border rows of conventional maize) separates GM maize from the conventional one more than sufficiently. The effective isolation distance is 3–4 times greater than the actually needed. The MON 810 revealed also the highest yield and the best self-protection against European corn borer in both growing seasons with different climatic conditions.

Keywords: GM crop; maize; MON 810; coexistence; isolation distance; yield; resistance

Commercial cultivation of genetically modified (GM) plants officially started in 1996 with genetically engineered tomato modified for the fruit quality. Since that time, GM crops cultivation was expanded over the world and reached globally 160 mil. ha in 2011, representing an annual increase of 8% (James 2011). The Slovak agriculture started with the cultivation of GM maize in 2006 and it continuous planting it to the present time. The only one GM crop deliberated for growing in the European Union (EU) until the year 2010 was the maize MON 810 (YieldGard®) resistant against the European corn borer (*Ostrinia nubilalis*, ECB). The second one approved was the GM potato cv. Amflora with a modified starch composition (2010).

The maize MON 810 expresses in all its cells the insecticidal protein Cry1Ab, the product of the gene *cry1Ab* inserted into maize from the soil bacteria *Bacillus thuringiensis* subsp. *kurstaki*. The MON 810 was approved for releasing into environment in the year 1995 and for food or feed production in 1996 (both in the USA). The cultivation of MON 810 in the EU is concentrated in Spain where approximately 80% of the total MON 810 of the EU was planted, and representing more than one quarter of total Spanish maize in 2011. The MON 810 is cultivated also in Slovakia even though the growing area has been very limited since 2006 (761 ha in 2011). The relevant legislation and regulation for commercial growing of GM plants in Slovakia have been created (Act No. 184/2006 Z. z. and Regulation No. 69/2007 Z. z.), nevertheless they do not encourage increasing GM crops cultivation. The above mentioned regulation includes also the rules for coexistent cultivation of GM, conventional, and organic plants. The out-crossing in maize is also one of the factors seriously considered in the coexistence rules because cross-fertilisation through pollen grain flow from GM to neighbouring maize fields contributes significantly to biologically based adventitious mixing on-farm. Nevertheless pollen grains of maize are large and heavy in comparison with those of other wind-pollinated species and its flight distance is limited to a short range. Taking into account also this characteristic, different isolation distances between the conventional and GM maize and organic and GM maize, respectively, have been adapted in the EU member states (Devos *et al.* 2009). The isolation distances 200 m between the conventional and GM maize and 300 m between the organic and GM maize were adopted in Slovakia and farmers are allowed to replace the mandatory isolation distance by border rows of conventional maize according to the principle: 1 border row can replace 2 m of the isolation distance. If GM maize is planted close to the conventional one, then, according to the rules, at least 100 border rows are needed to separate both types of the maize production. Sanvido (2008) reviewed the existing cross-fertilisation studies on maize and, to keep GM-inputs in the final product well below the 0.9% threshold, they proposed the isolation distances of 20 m for silage and 50 m for grain maize, respectively.

The objectives of this study were to: (*i*) analyse the level of out-crossing and adequacy of the isolation distances between GM and conventional maize under regional conditions and (*ii*) evaluate the basic parameters of MON 810 cultivated in two climatically different years.

MATERIAL AND METHODS

Out-crossing analyses. The commercial maize hybrid DKC4442YG containing the MON 810 event was drilled at three different locations – two in Southern Slovakia (Bajč and Lipové), the third one in Eastern Slovakia (Nacina Ves). The field of GM maize in Lipové was completely surrounded by 36 rows of the non-GM near-isogenic conventional maize DK 440, in Nacina Ves it bordered on three sides 36 rows of DK 440 and 6 rows on the fourth side, in Bajč it was bordered only on the downwind side by 126 rows of DK 440 (Figure 1). The GM field areas comprised 10 ha at all locations and the nicking between GM and near-isogenic conventional maize provoked the worst case scenario for out-crossing.

Grain samples were taken during the harvest from conventional maize in strips, defined by the width of the 6-row harvester. Samples at locations Lipové and Nacina Ves were taken from strips consisting of rows: 1–6 (distance 0.7–4.2 m from the GM plot), 7–12 (4.9–8.4 m), 13–18 (9.1–12.6 m), 19–24 (13.3–16.8 m), 25-30 (17.5–21.0 m), and 31–36 (21.7–25.2 m). An increased number of downwind samples were taken at Bajč, up to the strip consisting of rows 121–126 and representing 84.7–88.2 m distance from the GM field. The influence of the wind direction was taken into account by sampling all four sides of two fields (Lipové, Nacina Ves), the third field Bajč with single neighbouring near isogenic field having been sampled only in the downwind direction. For each of these strips, one individual sample of 10 kg was taken (10 times ~ 2 kg during unloading, homogenised to final 10 kg sample). The trailers containing the harvested strips were sampled with a specific grain sampling probe (20 times ~ 0.5 kg from different parts of the trailer, homogenised to final 10 kg sample). The samples were dried before the Real-Time PCR analysis.

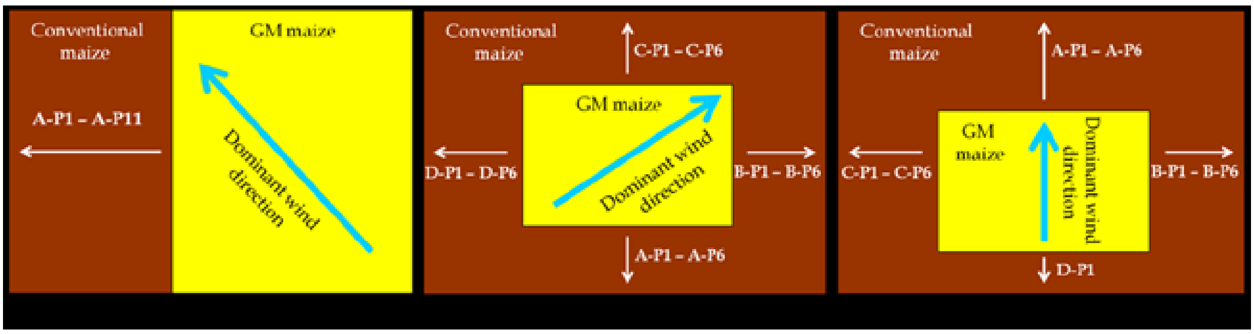

Figure 1. Plot design for maize out-crossing in testing locations

Subsamples of 3000 grains were ground in a laboratory blender (Robotcoupe). DNA was extracted from 2 samples of 0.1 mg of the ground material with the DNeasy kit (Qiagen, Hamburg, Germany). Two replicates of the validated MON 810 event-specific Real-Time Quantitative TaqMan® PCR were performed on 62 ng of DNA using the ABI Prism 7900 with each DNA extraction. The specific 66-bp fragment of MON 810 was amplified using specific primers (5'-agccaccacttctccttgga-3', 5'-aggctaccgaaagtcctcg-3'). PCR products were measured in real-time by means of target-specific TaqMan® oligonucleotide probe labelled with two fluorescent dyes: FAM as a reporter at 5'-end and TAMRA as a quencher at 3'-end (6-FAM-atcgatgtgggctgcaccgacct-TAMRA). For the relative quantification of MON 810 DNA was used a corn-specific reference system amplifying 70-bp fragment of the corn endogenous gene *adh1* using the *adh1* gene-specific primers (5'-ccagcctcatggccaaag-3', 5'-ccttcttggcggcttatctg-3') and *adh1* gene-specific probe labelled with FAM and TAMRA (FAM-cttaggggcagactcccgtgttcc-TAMRA) as described above. PCR reaction mix (25 µl) contained 62 ng of DNA template, 150nM of both primers, 50nM of TaqMan® probe, TaqMan® Universal PCR Master Mix, and nuclease-free water. The cycling conditions of PCR were: initial 2 min at 50°C and 10 min at 95°C, followed by 45 cycles of 15 s at 95°C and 1 min at 60°C.

The standard material used for calibration was prepared by mixing MON 810 hybrid seeds with conventional corn (0.1–40% of transgenic seeds per standard sample). The results of the adventitious presence were expressed as a seed percentage. The translation of these results into percent (%) of DNA (defined as "the percentage of GM-DNA copy numbers in relation to target taxon specific DNA copy numbers calculated in terms of haploid genomes"), which is the unit of measurement considered in the European regulation for food and feed (including grain), became mandatory. The percentage grain result was recalculated using a conversion factor of 2, accepting that the impurities detected are expected to be hemizygous for the MON 810 event. The average value of the 4 replicates (2 DNA × 2 PCR) of each sample is presented here.

Yield and resistance against ECB. The growing seasons of 2006 and 2007 were climatically different (Figure 2). The parameters of the MON 810 were compared with those of the conventional hybrids at the locality Borovce. The field trial included six hybrids with the FAO 310-420 and MON 810 hybrid DKC 4442YG (FAO 350). The hybrid DK 440 has identical genetic background with the DKC 4442YG. All hybrids were cultivated in the same conditions, by conventional operation, after winter wheat, and without irrigation. The herbicide Guardian EC (2.5 l/ha) was applied after sowing. Other herbicide or insecticide applications were not used.

RESULTS AND DISCUSSION

Impact of distance on out-crossing frequency

All three field plots simulated practical on-farm maize cultivation considering the scale of the cultivation area (10 ha). The results from all locations, regardless of the prevalent wind direction, revealed that the level of the adventitious presence of MON 810, measured as percentage of

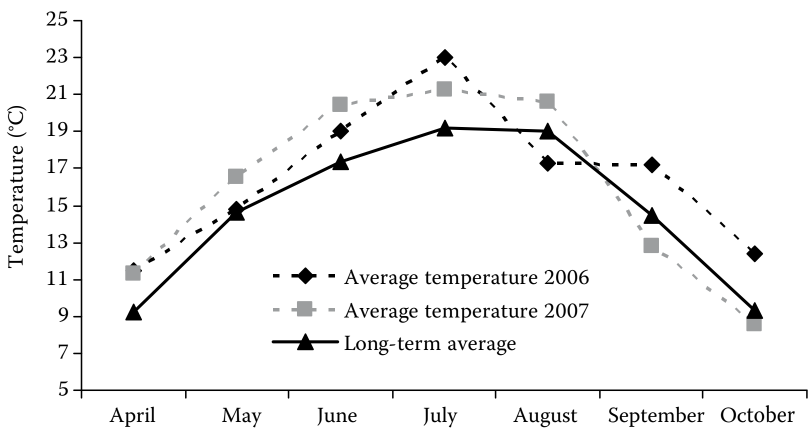

Figure 2. Temperatures and precipitations during maize growing seasons 2006 and 2007

Table 1. Out-crossing in neighbouring conventional maize fields by MON 810

Location								
Lipové			Nacina Ves			Bajč		
Code	rows	% DNA	code	rows	% DNA	code	rows	% DNA
A-P1	1–6	N.A.	A-P1	1–6	1.6	A-P1	1–6	1.0
A-P2	7–12	0.3	A-P2	7–12	0.4	A-P2	7–12	0.2
A-P3	13–18	0.1	A-P3	13–18	0.3	A-P3	13–18	0.1
A-P4	19–24	0.1	A-P4	19–24	0.2	A-P4	19–24	0.1
A-P5	25–30	< 0.1	A-P5	25–30	0.1	A-P5	25–30	0.1
A-P6	31–36	0.2	A-P6	31–36	0.1	A-P6	31–36	0.1
T_A calculated	**1–36**	**0.2**	**T_A calculated**	**1–36**	**0.5**	A-P7	37–42	< 0.1
			T_A measured	**1–36**	**0.2**	A-P8	49–54	< 0.1
B-P1	1–6	1.8	B-P1	1–6	1.5	A-P9	67–72	< 0.1
B-P2	7–12	0.7	B-P2	7–12	0.2	A-P10	91–96	< 0.1
B-P3	13–18	0.4	B-P3	13–18	0.2	A-P11	121–126	< 0.1
B-P4	19–24	0.2	B-P4	19–24	0.1	**T_A calculated**	**1–126**	< 0.2
B-P5	25–30	0.1	B-P5	25–30	< 0.1	**T_A measured**	**1–126**	< 0.1
B-P6	31–36	0.1	B-P6	31–36	0.1			
T_B calculated	**1–36**	**0.6**	**T_B calculated**	**1–36**	**0.4**			
			T_B measured	**1–36**	**0.5**			
C-P1	1–6	N.A.	C-P1	1–6	1.2			
C-P2	7–12	1	C-P2	7–12	0.4			
C-P3	13–18	0.3	C-P3	13–18	0.3			
C-P4	19–24	0.2	C-P4	19–24	0.1			
C-P5	25–30	0.2	C-P5	25–30	0.1			
C-P6	31–36	0.1	C-P6	31–36	< 0.1			
T_C calculated	**1–36**	**0.4**	**T_C calculated**	**1–36**	**0.4**			
			T_C measured	**1–36**	**0.3**			
D-P1	1–6	0.8	D-P1	1–6	1.0			
D-P2	7–12	0.2	D-P2	7–12	N.A.			
D-P3	13–18	< 0.1	D-P3	13–18	N.A.			
D-P4	19–24	< 0.1	D-P4	19–24	N.A.			
D-P5	25–30	0.1	D-P5	25–30	N.A.			
D-P6	31–36	< 0.1	D-P6	31–36	N.A.			
T_D calculated	**1–36**	**0.2**						
$T_{A,B,C,D}$ calculated	**1–36**	**0.4**						
$T_{A,B,C,D}$ measured	**1–36**	**0.3**						

A, B, C, D – codes for the neighbouring conventional maize field sections; P1–11 – harvest passage; T_A – total section A; T_B – total section B; T_C = total section C; T_D – total section D; %DNA – percent of adventitious presence of MON 810; N.A. – not analysed; N.A. location Lipové – not analysed samples due to mixing of two maize types during planting alien DNA in the surrounded maize plants, did not exceed 0.25% at a distance greater than 19–24 maize rows (i.e. 13.3–16.8 m) from the MON 810 plot (Table 1). The impact of the downwind pollen grains transport was evident at all locations but the presence of MON 810 in the first six rows of the non-GM maize, regardless of the prevalent wind direction, was 0.8% or higher. The values for the entire field section were calculated as average values of the individually obtained analytical results per strip in the respective field section (i.e. T_A is an average of A-P1, A-P2, A-P3, A-P4, A-P5, A-P6; Table 1). The results obtained on the bulk grain samples collected from the entire 36 rows (25.2 m) of the conventional maize field sections neighbouring the MON 810 plot did not exceed the legal labelling threshold for food and feed, i.e. 0.9% of alien DNA. The average adventitious presence of MON 810 DNA in the bulk sample coming from all neighbouring fields of conventional maize around the MON 810 plot was 0.3% (Table 1). We observed that the mandatory isolation distance replaced by 100 border rows of conventional maize was effective and surpassed the actual need at least 3–4 times (in the sense of the 0.9% DNA labelling threshold). The adventitious presence in harvested conventional grains which is further bulked up for the total fields (and which often can be larger but includes the first 25 m) will automatically further decrease because of simple dilution effects (grain collection points). Chilcutt and Tabashnik (2004) reported that the pollen grains flow in Bt-maize was up to 31 meters. The results of Messeguer *et al.* (2006) were more similar to ours, the authors concluded that the reliable distance between GM and conventional maize fields should be about 20 m to maintain the adventitious presence of GM maize as a result of pollen flow below the 0.9% threshold in the total yield of the field. Van de Wiel *et al.* (2009) in the field trials in the Netherlands concluded that the grain admixtures as a consequence of pollen mediated gene transfer from GM maize were much lower: 0.080–0.084% at 25 m and 0.005–0.007% at 250 m, respectively. Our results contributed to and confirmed several previous conclusions as published by Gustafson *et al.* (2006), Messean *et al.* (2006), Devos *et al.* (2007), and Sandivo *et al.* (2008) who observed that the maize pollen grains flow and pollen mediated gene transfer decline rapidly with increased distance. These studies do also draw the attention to the impact of the isolation distances for the implementation, as well as to the negative impact on the feasibility and production cost if disproportionate measures should be implemented. Importantly, it needs to be realised that larger isolation distances are not operative especially with maize cultivated by farmers in smaller areas. The reduction of the isolation distances to obtain appropriate measures could allow the access to MON 810 technology to more farmers and to exploit the benefits from the co-existence principles in the plant production. Such European example is the adoption of Bt-maize cultivation in Spain where during 6-year period (1998–2003) was the gain 15.5 mil. € (Demont & Tollens 2004). Park *et al.* (2011) estimated that, if the area of transgenic maize increased there where agronomic need exists then farmers in EU12 countries would increase the benefit by at least 157 M€/year (Slovak farmers by 3.6–5.9 M€/year).

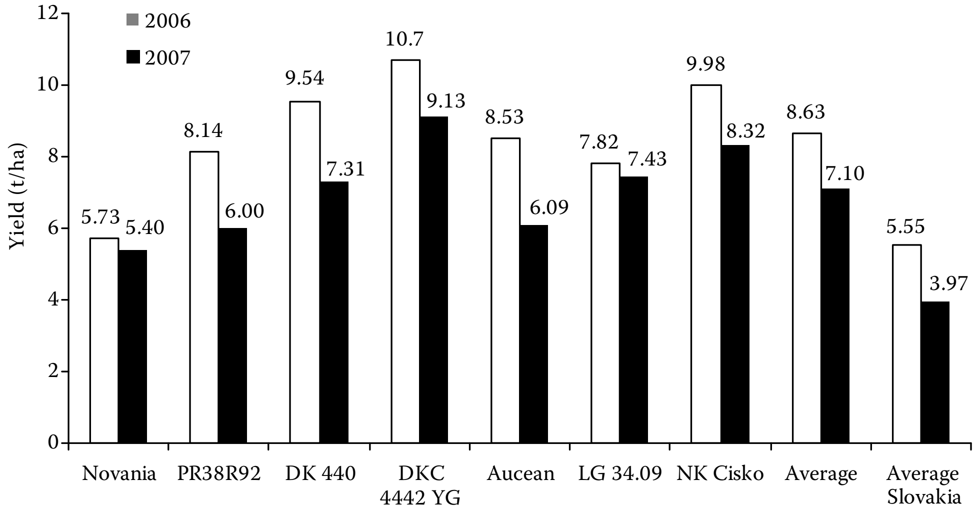

Figure 3. Maize yields in growing seasons 2006 and 2007

Yield and resistance against ECB

The best way to evaluate the parameters of MON 810 maize in practice is field testing in climatically different years (growing seasons). An average yield of 6 maize hybrids tested in this study was 8.63 t/ha (average maize yield in Slovakia was 5.55 t/ha) in the year 2006, the highest yield (10.70 t/ha) was obtained with the GM hybrid DKC 4442YG (Figure 3). The second vegetation season (2007) was worse for maize due to the higher average annual temperature (+0.8°C), especially the higher temperature in comparison to long-term average from May to the end of August (+0.9°C +3.0°C, +1.1°C, +1.6°C) and the lower precipitation in comparison to the long-term average in June and July (12.5 mm, –23.2 mm) and higher in August and September (+39.6 mm, +55.4 mm) (Figure 2). All maize hybrids declined in yield in comparison to the previous year while the highest yield (9.13 t/ha) was again achieved with DKC 4442YG (Figure 3). The two years of field testing in different climatic conditions revealed a statistically significant (P = 0.007) impact of the hybrid on the yield. The best proved to be the hybrid DKC 4442YG, also in comparison with the non-GM isogenic line DK 440. All maize hybrids were attacked by ECB but to a different extent. Significantly, the highest proportion of attacked plants was in the early hybrid Novania (76.19%) and significant differences were found between the DKC 4442YG and the other conventional hybrids (Figure 4). The effect of genetic modification aimed to provide resistance against the ECB was evident especially with DKC 4442YG (3% of attacked plants) and its isogenic line DK 440 (33% of attacked plants), as measured before the harvest. The yield of grain was statistically significantly (P = 0.044) dependent on the frequency of ECB infestation.

The GM maize hybrid DKC 4442YG was evaluated as the best one of all those tested in the two climatically very different growing seasons. The genetic event inserted into GM hybrid increased the average yield in years 2006 and 2007 by 17.7% (i.e. by 1.49 t/ha), and it also resulted in almost complete resistance against the European corn borer. The yields of Bt maize MON 810 evaluated in different studies were similar to that of conventional maize even in different soil treatment systems (conventional, reduced tillage), nevertheless, in environments with a higher corn borer infestation it usually given higher yields more even than conventional non-Bt cultivars sprayed with insecticide or allows a significant reduction in pesticide use (Andersen *et al.* 2007).

Both types of the presented results confirmed that the GM maize with the inserted event MON 810 is advanced from the agronomical and economical points of view. MON 810 achieved the best results in climatically different growing seasons and demonstrated an effective self-protection against the ECB. The border rows of conventional maize as required by the relevant regulation effectively eliminated the pollen flow from the GM plot and prevented the adventitious presence of GM material in non-GM grain production.

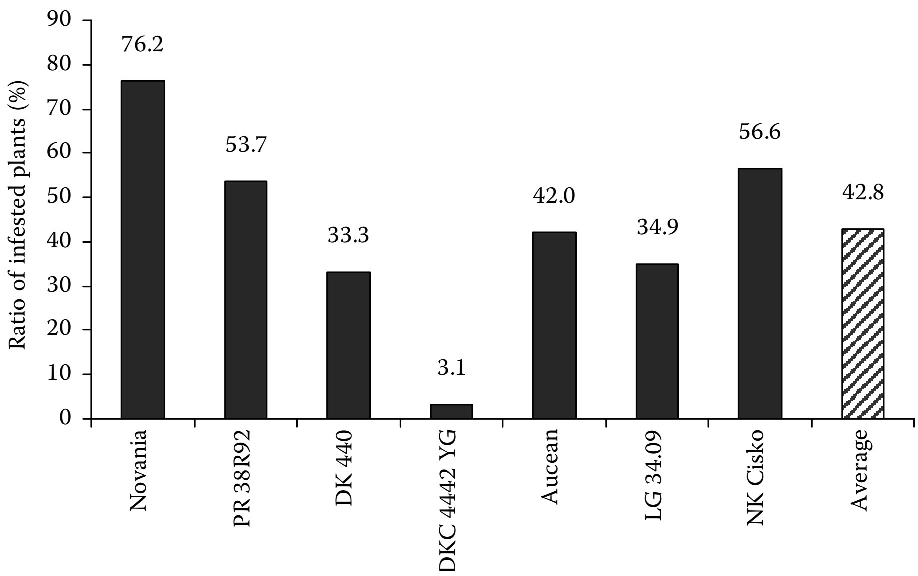

Figure 4. Frequency of maize plants infested by ECB during growing seasons 2006 and 2007

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