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Maize Plants Infestation by *Fusarium* spp. and Deoxynivalenol in Genetically Modified Corn Hybrid and Traditional Maize Cultivars

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Abstract

The objective of the performed investigations was to isolate pathogenic fungi from contaminated maize cobs, to assess the appearance of maize cob fusariosis and to determine grain contamination with deoxynivalenol in the cultivation of genetically modified maize containing a gene resistance against European corn borer (*Ostrinia nubilalis* Hbn) as well as selected non-modified cultivars. The plant material comprised the following genetically modified maize cultivar: DKC 3421 YG (MON 810) and non-modified cultivars obtained from Smolice Plant Breeding Ltd., IHAR Group: Junak (FAO 210–220), Prosna (FAO 220), SMH (FAO 230), Baca (FAO 220). Prior to harvesting, the occurrence of maize cob fusariosis was determined in the 89 (BBCH) developmental ripening stage. Microbiological assessment was carried out on grains selected from cobs characterized by various pathological symptoms. In 2008, a total of 133 isolates was obtained from the examined samples of infected maize plants, of which 51 isolates were species-identified, while in 2009, the total of 123 isolates were determined, of which 63 were species-identified. In both experimental years, the majority of isolates contained fungi from the *Fusarium* genus. The performed analysis of mean levels of cob contamination by fusarioses revealed that DKC 3421 YG (MON 810) and SMH (FAO 230) cultivars showed the smallest levels of contamination as well as the lowest percent of cob contamination per plant, while Junak (FAO 210–220) and Baca (FAO 220) cultivars were characterized by the highest degree of contamination. The lowest deoxynivalenol concentrations were determined in years 2008 and 2009 in the case of the DKC 3421 YG (MON 810) cultivar, whereas Prosna (FAO 220) cultivar was characterized by the highest deoxynivalenol concentration.

Key words: *Fusarium* spp., deoxynivalenol, maize, GMO

Introduction

Pathogenic fungi from the *Fusarium* genus causing foot-rot and cob fusariosis pose a very serious threat to maize cultivations in Poland (Tekiela and Gabarkiewicz, 2007; Selwet, 2009). Fusarioses are caused, primarily, by *Fusarium culmorum*, *Fusarium avenaceum*, *Fusarium graminearum*, *Fusarium oxysporum* (Fiedorow *et al.*, 2001). Cob fusariosis causes mild crop losses; nevertheless, it results in deterioration of grain quality due to contamination with mycotoxins, *e.g.* deoxynivalenol (Lisowicz and Tekiela, 2004). Mycotoxins may cause a number of diseases both in people and animals, including allergies, hormonal disorders, and neoplasms (Tekiela, 2008). Mean yield losses due to the foot rot vary from 10 to 35% (Sulewska *et al.*, 2006). Among important factors leading to the infection of maize with *Fusarium* is feeding of various pests, primarily, European corn borer (*Ostrinia nubilalis* Hbn) as this facilitates tissue penetration by the pathogen and damages plants (Tekiela *et al.*, 2005; Selwet, 2009). One of the methods reducing the infestation of maize by pathogens could be cultivation of resistant varieties genetically modified by a gene resistant to the European corn borer (Tekiela and Gabarkiewicz, 2007; Selwet, 2009). However, there are serious reservations concerning cultivation of genetically modified (GM) cultivars due to their possible negative influence on the environment. Many researchers reported increased mortality of butterflies and ladybirds following ingestion of toxic protein from GM maize (Hilbeck and Schmidt, 2006) or impairment of spatial orientation in bees (Ramirez-Romero *et al.*, 2008). Transfer of traits resulting from the applied genetic modification between totally alien organisms may also turn out to be a serious threat (Duggan *et al.*, 2002). Although for the above-mentioned reasons, cultivation of genetically modified cultivars remains controversial, the area under MON 810 maize cultivation in Poland increased from approximately 300 ha in 2007 to 3000 ha in 2008.

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The presented paper is a continuation of a research project conducted in 2006–2007. It involved isolation of pathogen fungi from infected cobs, assessment of the appearance of maize cob fusariosis and determination of grain infection by deoxynivalenol in cultivations of GM maize containing the gene of resistance against the European corn borer (*Ostrinia nubilalis* Hbn) as well as selected non-modified cultivars.

The experiment was established in Nowa Wieś Kącka in Kąty Wrocławskie commune, of Wrocław District in the Lower Silesia Voivodeship (51°02' N, 16°46' E). The village is situated in the Silesian Lowland, in the eastern part of the Wrocław Plain (132 m a.s.l). It is situated centrally between Środa Upland and Grodkowo Plain. Glacial and glacial-fluvial deposits are covered by loess on which brown soils and chernozems were formed.

Experimental

Material and Methods

Plant material. The experimental plant material comprised a GM maize cultivar: DKC 3421 YG (MON 810) (containing a Bt gene of resistance against the European corn borer *Ostrinia nubilalis* Hbn) as well as non-modified cultivars from the Smolice Plant Breeding Ltd, IHAR Group: Junak (FAO 210–220), Prosna (FAO 220), SMH (FAO 230), and Baca (FAO 220). Investigations were carried out in 2008–2009.

Prior to the harvest of maize conducted at the developmental phase 89 (BBCH), the occurrence of maize cob fusariosis was determined in accordance with the methodology given by Tekiela and Gabarkiewicz (2007). One hundred plants (20 plants from 5 replicates) were analysed using for this purpose a five-score scale (Kwaśna *et al.*, 1991), (Table I).

Table I
Scale used for evaluation of cobs infestation by *Fusarium* genus

Degree	Description
1	Very small (1–6 grains, 2%)
2	Small (7–30 grains, 3–10%)
3	Medium (1/3 of cob, 11–30%)
4	Large (1/2 of cob, 31–50%)
5	Very large (>1/2 of cob, 51–100%)

Microbiological evaluation was performed on kernels obtained from cobs exhibiting different types of pathogenic symptoms. The employed procedures followed methodology developed by Rataj-Guranowaska and Frąckowiak (2006).

Media and cultivation conditions. Tissue fragments (collected from the border between healthy and sick tissues situated in different places of the overground shoot) were surface disinfected in 15% sodium hypochlorite for the period of 45 seconds. Next, samples were rinsed three times in sterile distilled water. Disinfected slices were placed on Petri dishes with the Potato Dextrose Agar (PDA, DIFCO) of pH 4.5–5.0 and incubated for 5–7 days at the temperature of 24°C. The developed fungal colonies were inoculated onto the Potato Dextrose Agar (PDA, DIFCO) and, in the case of *Fusarium*, also on Saltwater Nutrient Agar (SNA). Following incubation, mycelium was subjected to macroscopic analysis (colour and structure of mycelium). Microscopic descriptions of fungi were prepared 5 and 10 days from the moment of isolate inoculation. The performed microscopic analysis involved cultures grown on the Saltwater Nutrient Agar (SNA) and comprised determination of the size of conidial spores, conidiogenesis and the type of fialide as well as the presence of microconides and chlamidospores. Identification was carried out on the basis of Both's (1971) and Burgess's *et al.* (1988) keys.

Deoxynivalenol content. Deoxynivalenol was determined according to methodology given by Wiśniewska-Dmytrow and Kozak (2006). Deoxynivalenol was extracted from the experimental plant material with water in the presence of polyethylene glycol. The extract was then purified on the immunological affinity column (DONtest™HPLC, of VICAM Company) containing antibodies specific for this mycotoxin. Deoxynivalenol was eluted from the column with 1.5 ml methyl alcohol of HPLC purity with the speed of 1 drop min^{-1} into a test tube of 5 ml volume. The eluate was evaporated dry in a stream of nitrogen at the temperature of 40°C. The residue was dissolved in 0.5 ml of standard mobile phase (0.2 μg ml^{-1}) and mixed on a Vortex. After thickening, the eluent was determined qualitatively and quantitatively with the assistance of liquid chromatography (LC) method using the UV-VIS detector.

Meteorological conditions. Values of air temperature and rainfall were obtained from the Institute of Meteorology and Water Management (IM&WM) in Wrocław.

Statistics. The obtained results were subjected to statistical analysis employing the glm procedure of the SAS package (1998) and the significance of differences was verified by the Duncan method.

Results

Meteorological conditions prevailing during the experimental years as well as the extent of cob damage by the European corn borer could have favoured the development of fungi from the *Fusarium* genus (Tables II–III).

Table II
Fusarium infestation on corn cobs in Nowa Wieś Kącka

No	Cultivars	Average degree of cob infestation in years		% of cob infestation in years	
		1st year	2nd year	1st year	2nd year
1	DKC 3421 YG (MON 810)	0.07a	0.10a	1.60a	1.73a
2	Junak (FAO 210–220)	0.49b	0.52b	20.01b	25.61b
3	Prosna (FAO 220)	0.40b	0.41b	6.10c	6.21c
4	SMH (FAO 230)	0.21c	0.23c	2.91d	2.22d
5	Baca (FAO 220)	0.66d	0.72d	17.21b	18.02b

a, b, c, d – means in columns designated with the same letters do not differ significantly at the level of $P < 0.05$

Table III
Monthly average air temperatures and atmospheric precipitation

Years	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
Air temperatures in °C												
1971–2000	–0.9	0.2	3.9	8.2	13.5	16.3	18,1	17.8	13.6	8.9	3.6	0.7
2008	2.2	3.1	4.2	8.5	14.3	18.2	19.4	18.0	13.1	9.0	6.2	1.5
2009	–3.0	0.1	4.1	12.1	14.4	15.2	19.2	19.0	15.1	7.3	6.1	–1.1
Total precipitation in mm												
1971–2000	28	24	30	37	57	79	91	64	51	38	37	34
2008	50	20	43	75	42	27	54	76	25	52	25	23
2009	32	51	53	11	65	153	154	53	20	61	31	43

In 2008, a total of 133 isolates were obtained from all the examined samples of infected plants, of which 51 were identified with regard to species, whereas in 2009, a total of 123 isolates were determined, of which 63 were identified with regard to species. In both experimental years, fungi from the *Fusarium* sp. genus constituted the majority of the obtained isolates.

In addition, high proportions of black fungi from the Alernaria alternata species were also determined in our investigations. Moreover, fungi from the *Trichoderma, Phoma Mucor* genus and *Cladosporium cladosporoides* were also identified (Tabele IV).

Table IV
Number of fungal isolates obtained from maize

Species	No isolates 1st year	No isolates 2nd year
Fusarium oxysporum	10	12
Fusarium graminearum	17	19
Fusarium culmorum	5	7
Fusarium avenaceum	6	8
Alternaria alternata	10	15
Cladosporium cladosporoides	*3*	*3*
Trichoderma sp.	5	3
Phoma sp.	3	3
Mucor sp.	3	2
Other fungi, nonsporulating fungi	20	52

When analysing the mean degree of cob infection with fusarioses, it was found that DKC 3421 YG (MON 810) and SMH (FAO 230) cultivars exhibited the lowest degree of infestation as well as the lowest percent of infected cobs on a plant. On the other hand, Junak (FAO 210–220) and Baca (FAO 220) cultivars were found to have been infected most severely (Table II).

Due to strong infestation of maize by pathogenic fungi from the *Fusarium* genus, the content of deoxynivalenol in the examined samples was determined. The lowest deoxynivalenol concentrations were determined in years 2008 and 2009 in the case of the DKC 3421 YG (MON 810) cultivar and they amounted to 52 ppb and 56 ppb, respectively, whereas the highest ones (244 ppb and 261 ppb, respectively) were found in the Prosna (FAO 220) cultivar. Deoxynivalenol concentrations in individual maize cultivars are shown in Table V.

Table V
Deoxynivalenol content in corn grain (ppb)

Cultivars	1st year	2nd year
DKC 3421 YG (MON 810)	41a	32a
Junak (FAO 210–220)	213b	201b
Prosna (FAO 220)	254c	243c
SMH (FAO 230)	198b	186b
Baca (FAO 220)	201b	199b

a, b, c, – means in columns designated with the same letters do not differ significantly at the level of $P < 0.05$

Discussion

The obtained results indicated the most severe maize infestation with fungi from the *Fusarium* genus. In 2008, *Fusarium graminearum* species constituted the highest quantity of isolates (17 isolates). According to Boreski (2001), this species is characteristic for this period of vegetation (89 BBCH) and infects, primarily, towards the end of maize vegetation. Also *Fusarium oxysporum* (a species rarely determined on maize plants at the phase of full kernel maturity) with its considerable number of isolates (10) deserves attention as its high intensity of development according to Kwaśna *et al.* (1991) and Fiedorow *et al.* (2001), coincides with maize emergence and may lead to seedling root-rot in plants. The smallest numbers of isolates were determined for *Fusarium culmorum* (5) and *Fusarium avenaceum* (6) species which, according to Rataj-Guranowska (2006), infect maize plants mainly during 33–39 (BBCH) phase. In 2009, the greatest numbers of determined isolates were as follows: *Fusarium graminearum* (19 isolates), *Fusarium oxysporum* (12), *Fusarium avenaceum* (8) and *Fusarium culmorum* (7). High quantities of *Fusarium graminearum* isolates observed during the 89 BBCH phase of maize development corroborates literature data (Borecki, 2001). In years 2008 and 2009 high numbers of *Alternaria alternata* isolates were determined. High quantities of this species in maize were also reported by Rataj-Guranowska and Frąckowiak (2006) as well as Selwet (2009).

Concentrations of deoxynivalenol in the examined cultivars varied. In the case of the GMO DKC 3421 YG (MON 810) cultivar, low concentrations of mycotoxins were found in comparison with the non-modified cultivars which corroborated findings by Tekiela and Gabarkiewicz (2007) as well as Selwet (2009) who also reported in the GMO DKC 3421 YG maize cultivar the lowest concentrations of deoxynivalenol in comparison with non-modified cultivars. These results confirm lower sensitivity of the GM cultivar to the presence of secondary metabolites of mould fungi in comparison with non-modified cultivars (Munkvold and Desjardins 1997).

Tekiela and Gabarkiewicz (2007) maintain that in Poland no monitoring of the content of mycotoxins in maize grain is conducted. It can only be assumed on the basis of partial research that, depending on the intensity of occurrence of the European corn borer, the degree of plant infestation with *Fusarium* genus and meteorological conditions, up to 30% of harvested grain may contain mycotoxins in quantities exceeding acceptable standards. Higher levels of maize infestation with *Fusarium* genus may also be due to delayed harvest (Sulewska *et al.,* 2006).

At the present time, the content of mycotoxins in maize grain is regulated by the EU instruction No. 1126/2007 of the 28th September 2007 which changed the earlier EU regulation No. 1881/2006. The above regulation allows the highest allowable level of deoxynivalenol as of the 1st October 2007 at 1750 ppb (Tekiela 2008).

Recapitulating the obtained results, the following conclusions can be drawn. Pathogenic fungi from the *Fusarium* genus turned out to be the most serious threat for maize cultivated in years 2008 and 2009 in the area of Kąty Wrocławskie commune. The most frequently isolated species were *Fusarium graminearum* and *Fusarium oxysporum*. Maize cob fusariosis was more intense in non-modified cultivars. The obtained results confirmed literature data that in order to limit the content of mycotoxins in maize grain, cultivation of genetically modified cultivars may be a feasible alternative.

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