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Original Research

Profile of *Fusarium* Mycotoxins in Various Maize (*Zea mays* L.) Hybrids

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Abstract

Fungal development in fodder substrates including maize has noxious effects: changes in technological properties, reduction of nutritive value, the development of mycosis and allergy agents, and the biosynthesis of refuse factors and zootoxic compounds called mycotoxins. The aim of this study was to evaluate the variation in concentrations of major *Fusarium* toxins along with the analysis of ergosterol in the grains of 16 maize cultivars of flint type (F), dent type (D), and combined flint/dent (F/D) and dent/flint type (D/F), respectively. The results showed that type of maize grain was a factor in determining the level of concentration of fumonisins (B_1, B_2, B_3), zearalenone, and moniliformin. However, there was no difference among grain type due to deoxynivalenol and ergosterol.

Keywords: deoxynivalenol, ergosterol, *Fusarium* spp, fumonisins, HPLC, maize, moniliformin, zearalenone

Introduction

Maize (*Zea mays* L.) is one of the main cereals as a source of food, forage, and processed products for industry [1]. Cosmopolite fungi from the genus *Fusarium* belong to the group of most frequently isolated crop pathogens worldwide [2-5]. The structure and size of kernels as well as the long period of ear ripening result in a situation when maize is infected by microscopic fungi with a much higher frequency than small grain cereals. Sources of *Fusarium* spp. infection include sowing material, soil, and the so-called reservoir plants, i.e. weeds, on which the fungi develop showing no symptoms, with no loss of pathogenicity [6]. The difficulty with the control of these fungi results from the fact that they are ubiquistic organisms, well-adapted to variable atmospheric and soil conditions, exhibiting high tolerance toward environmental conditions and developing within a wide range of temperatures from 0 to 30ºC. They have also developed numerous mechanisms facilitating effective competition for ecological niches.

Fusarium species not only cause yield reduction and its poor quality, but also release mycotoxins in infected grain [7-8]. In small-grain cereals they cause *Fusarium* head blight (FHB) and in maize atalk and ear rot. Several factors affect mycotoxin occurrence, the most important being plant genotype, fungus strain, agricultural practices, and environmental conditions [9-10].

The most frequent species identified in Poland and its neighboring countries on maize kernels were *F. verticillioides*, *F. proliferatum*, *F. graminearum,* and

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F. temperatum [11-14]. The great diversity of *Fusarium* species on maize suggests interactions between species. Additionally, maize grain is often contaminated by *Fusarium* mycotoxins such as fumonisins (FBs), zearalenone (ZON), deoxynivalenol (DON), and moniliformin (MON) [15, 16]. These compounds exhibit a broad spectrum of toxic effects, including nephrotoxicity, hepatotoxicity, teratogenicity, cancirogenicity, or hiperestrogenicity [7, 17]. Because of the significant health hazard posed by *Fusarium* mycotoxins to both humans and animals, many countries have implemented legal regulations specifying maximum acceptable levels of these compounds in maize and small-grain cereals [18].

The aim of our study was to assess the variation in concentrations of major *Fusarium* mycotoxins along with the analysis of ergosterol as an indicator of fungal biomass, in grain of 16 cultivars of fodder maize.

Materials and Methods

Experimental Field

A field experiment was carried out in the Department of Agronomy of Poznań University of Life Sciences at the Research and Education Unit in Swadzim in two vegetation periods, 2009 and 2010, with four replications. Research material included the grain of 16 fodder maize cultivars of flint type (F), dent type (D) and combined flint/dent (F/D) and dent/flint types (D/F): PR 39 D 60 (F, FAO 210), PR 39 K13 (F, FAO 220), PR 39 G 12 (F, FAO 230), P-8000 (D, 240), PR 39 T 45 (F/D, FAO 240), PR 39 D 23 (F/D, FAO 260), PR 39 F 58 (D/F, FAO 260), PR 38 N 86 (D, FAO 270), PR 38 H 20 (D, FAO 290), Clarica (D, FAO 280), LG 3215 (F, FAO 220), LG 3216 (F/D, FAO 250), Absolut (F/D, FAO 250), LG 3232 (F/D, FAO 240), LG 3233 (F/D, FAO 240), and LG 3252 (F/D, FAO 250). In the whole experimental field, in each year of research the same NPK mineral fertilization was used prior to maize sowing in the amount of: 100 kg N ha^{-1} ha in the form of urea, 80 kg P_2O_5 ha^{-1} in the form of Polifoska 6, and 120 kg K_2O ha^{-1} in the form of potassium salt 60%. Sowing rate was 79,000 plants·ha^{-1}. Cultivation measures and other elements of cultivation technology were implemented according to recommendations in maize cultivation for grain. Harvest was carried out with the use of a wintersteiger plot combine. Directly after maize harvest, samples of threshed mass were collected for laboratory analyses. The grain was not stored, so no biochemical changes took place in the plant material. Therefore the obtained results correspond solely to the occurrence of mycotoxins in the plant material under field conditions. According to the FAO international soil classification, the soils should be classified as Phaeozems, or as Mollisols – according to the US Soil Taxonomy [19]. Soil content of essential macroelements and soil pH in the individual years of research is presented in Table 1. Estimation of Mg in soil was performed using the Schachtschabel method, while K and P were determined using the method of Egner-Riehm.

Table 1. Soil condition at Swadzim.

Specification	Years	
	2009	2010
P [mg P kg^{-1} of soil]	36.1	39.2
K [mg K kg^{-1} of soil]	97.1	95.4
Mg [mg Mg kg^{-1} of soil]	69.0	64.0
pH [in 1 mol dm^{-3} KCl]	6.5	6.3

Temperature and Humidity Conditions

Temperature and humidity conditions in the years of research were favourable to growth and development of maize (Table 2). Influence of air temperature as well as rainfall are comprehensively best described with hydrothermal coefficient of water supply [S] according to Sielianinov [20]. The optimal value of the coefficient is 1. Values below 1 represent drought, while values over 1 represent a period of relative humidity. It should be noted that in two research periods the value of this coefficient was identical for the whole growing season.

Mycotoxin Analysis

Standards and Chemical Reagents

Fumonisins B_1, B_2, B_3, zearalenone, deoxynivalenol, moniliformin, and ergosterol standards were purchased with a standard grade certificate from Sigma-Aldrich (Steinheim, Germany). Sodium dihydrophosphate, potassium hydroxide, sodium hydroxide, potassium chloride, acetic acid, hydrochloric acid, and *o*-phosphoric acid, and *n*-hexane *t*-butyl-ammonium hydroxide were purchased from POCh (Gliwice, Poland). Organic solvents (HPLC grade), disodium tetraborate, *n*-pentane, 2-mercaptoethanol, and sodium acetate and all the other chemicals were

Table 2. Meteorological conditions during the research period.

Months	Years					
	2009			2010		
	T	R	S	T	R	S
IV	12.9	19.2	0.49	9.3	26.8	0.96
V	14.0	109.9	2.53	12.2	110.5	2.92
VI	16.0	113.8	2.37	18.4	43.4	0.78
VII	20.3	75.4	1.19	22.6	97.5	1.39
VIII	20.1	26.2	0.42	19.2	143.5	2.41
IX	15.8	48.6	1.02	13.0	69.9	1.79
X	7.6	59.2	2.51	7.0	9.1	0.42
Growing season	15.2	452.3	1.50	14.5	500.7	1.52

T – average monthly air temperature (ºC)
R – monthly amount of rainfall (mm)
S – hydrothermal coefficient of water supply according to Sielianinov [32]

also purchased from Sigma-Aldrich (Steinheim, Germany). Water for the HPLC mobile phase was purified using a Milli-Q system (Millipore, Bedford, MA, USA).

Extraction and Purification Procedure

10 g of homogenized ground samples of maize kernels were prepared for analysis. All mycotoxins (FBs, ZON, DON, MON) and ergosterol were extracted and purified according to the detailed procedure described earlier [4, 15]. The elute was evaporated to dryness at 40ºC under a stream of nitrogen. Dry residue was stored at -20ºC until HPLC analyses.

HPLC Analysis

The chromatographic system consisted of a Waters 2695 high-performance liquid chromatograph (Waters, Milford, USA) with detectors:

- Waters 2475 Multi λ Fluorescence Detector (λ_{ex}= 335 nm, λ_{em}= 440 nm) with an XBridge column (3.0 × 100 mm) for FBs analysis
- Waters 2996 Photodiode Array Detector with Nova Pak C-18 column (150 × 3.9 mm) for ERG (λ_{max}= 282 nm) analysis and with Nova Pak C-18 column (300 × 3.9 mm) for DON (λ_{max}= 224 nm) and MON (λ_{max}= 229 nm) analysis
- Waters 2475 Multi λ Fluorescence Detector (λ_{ex}= 274 nm, λ_{em}= 440 nm) and Waters 2996 Photodiode Array Detector with Nova Pak C-18 column (150 × 3.9 mm) for ZON analysis

Quantification of mycotoxins was performed by measuring the peak areas at the retention time according to relevant calibration curve. Limits of detection were: 0.001 $\mu g \cdot g^{-1}$ for ZON, 0.01 $\mu g\ g^{-1}$ for DON, FBs, ERG, and 0.1 $\mu g \cdot g^{-1}$ for MON.

Statistical Analysis

The normal distributions of the traits were established using the Shapiro-Wilk normality test [21]. A two-way analysis of variance (ANOVA) was carried out to determine the effects of cultivars, years and cultivars × years interaction on the variability in the concentrations of FB_1, FB_2, FB_3, ZON, DON, MON, and ERG. Because the years and cultivars × years interaction were not significant, further analyses were performed on years' mean. The one-way ANOVA was carried out to determine the effects of grain type on the variability of studied traits. Least significant differences (LSDs) for each trait were calculated. Homogeneous groups for the analyzed traits were determined on the basis of least significant differences. Dependence of observed traits on FAO number were analyzed using linear regression, the analysis being done for each trait independently. Percentage variance accounted (coefficient of determination, $R^2 \cdot 100$) was calculated by the formula $R^2 = 1 - SS_E/SS_T$, where SS_E denotes the sum of squares of residuals (also called the residual sum of squares), and SS_T – the total sum of squares (proportional to the sample variance). The relationships between concentrations of FB_1, FB_2, FB_3, ZON, DON, MON, and ERG were estimates using correlation coefficients [22]. Additionally, the obtained results were processed by multivariate statistical analysis. Firstly, multivariate analysis of variance (MANOVA) was used along with testing the hypotheses of lack of differences between cultivars, grain type and FAO number, independently. Mahalanobis' distance [23] was suggested as a measure of "polytrait" cultivars similarity, whose significance was verified by means of critical value D_α called "the least significant distance". A possibility of graphic distribution of maize cultivars described by all studied traits was obtained with the use of the analysis of canonical varieties [24].

Results and Discussion

Mycotoxins are secondary metabolites of filamentous fungi with significant variation, these molecules do not all have the same mechanism of toxic action, however, showing common characteristics, including complex chemical structure and resistance to thermal degradation during the processing of commodities [25].

Maize is a very good substrate for fungal growth and toxinogenesis. Many surveys conducted worldwide showed that this food can be contaminated by mycotoxins such as zearalenone, deoxynivalenol, and fumonisins.

The influence of years and cultivars × years interaction were not observed on the values of all traits ($P>0.05$ for all studied traits). Hence, the next analysis was carried out on the mean values across years. The results of the analysis of variance indicated that the main effects of cultivars were significant for all the studied traits ($P\leq0.05$). Type of maize grain was a factor in determining the level of concentration FB_1 ($P<0.001$), FB_2 ($P<0.001$), FB_3 ($P<0.001$), ZON ($P = 0.040$), and MON ($P<0.001$) (Table 3). However, there was no difference among grain type due to DON ($P = 0.086$) and ERG ($P = 0.073$) (Table 3). On the other hand, a study by Szymańska et al. [26] showed that genetic variability of the cultivars examined in terms of resistance to *Fusarium* spp. was low. Type of maize grain was a factor determining concentration of FB_1 ($P<0.001$), FB_2 ($P<0.001$), FB_3 ($P<0.001$), ZON ($P = 0.040$), and MON ($P<0.001$) (Table 3). However, no differences were observed among grain type in DON ($P = 0.086$) and ERG ($P = 0.073$) (Table 3). Czembor and Ochodzki [27] showed that the flint kernels were more resistant than the dent ones to pre- or post-harvest attack by *Fusarium* spp.; however, after tooth-pick inoculation the flint forms turned out to be more susceptible to *F. verticillioides* than the dent forms. These authors obtained significant influence of kernel type on FB_1, FB_2, and FB_3, and not significantly on DON [27]. The symptoms of the disease were more visible and DON content was significantly higher in the dent forms than in the flint ones (Table 3). Similar results were obtained in other studies [28, 29] where the disease caused by *F. verticillioides* was milder

Table 3. Mean values of mycotoxins [µg·g^{-1}] and ergosterol [µg·g^{-1}] with standard deviations (s.d.) for studied traits.

Cultivar	FB_1		FB_2		FB_3		ZON		DON		MON		ERG	
	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.
Absolut	0.885cde	0.82	0.0975c	0.07	0.085c	0.04	0.065b	0.01	3.45cdef	0.43	0.2925bcd	0.19	13.715abc	6.26
Clarica	0.265e	0.12	0.0775c	0.02	0.0675c	0.02	0.0225b	0.01	4.008cdef	0.30	0.4025bc	0.12	7.803def	1.00
LG 3215	0.962bcde	0.87	0.160bc	0.08	0.130bc	0.09	0.0325b	0.02	3.138ef	1.10	0.255bcd	0.10	4.272f	1.10
LG 3216	0.260e	0.13	0.085c	0.03	0.0625c	0.02	0.0175b	0.01	2.353f	0.29	0.1125cd	0.05	6.09ef	2.86
LG 3232	0.460de	0.27	0.100c	0.03	0.0725c	0.02	0.030b	0.02	3.738cdef	0.57	0.0275d	0.03	9.465cde	1.99
LG 3233	0.265e	0.06	0.080c	0.01	0.055c	0.01	0.065b	0.05	3.277def	0.20	0.0275d	0.02	8.312def	2.77
LG 3252	0.272e	0.16	0.090c	0.02	0.0625c	0.02	0.0185b	0.00	4.312cdef	0.45	0.2125bcd	0.04	7.423def	2.26
P-8000	0.785cde	0.47	0.180bc	0.07	0.0975bc	0.03	0.1775b	0.16	5.948bcd	1.57	0.1275bcd	0.07	10.503bcd	1.96
PR 38 H 20	1.448bcd	0.48	0.1625bc	0.07	0.115bc	0.02	0.205b	0.02	5.308bcde	2.86	0.3225bc	0.16	9.6cde	1.82
PR 38 N 86	2.040ab	2.20	0.255b	0.26	0.095bc	0.13	0.0575b	0.05	4.805bcdef	2.08	0.2975bcd	0.06	8.25def	6.38
PR 39 D 23	0.302e	0.03	0.0875c	0.01	0.075c	0.01	0.065b	0.06	9.293a	3.24	0.19bcd	0.17	5.83ef	0.64
PR 39 D 60	1.645bc	0.54	0.2475b	0.05	0.140bc	0.02	0.2275b	0.05	5.945bcd	1.20	0.9275a	0.65	15.842a	2.75
PR 39 F 58	0.488de	0.23	0.1075c	0.03	0.0725c	0.02	0.0925b	0.04	6.13bc	4.06	0.1225bcd	0.13	5.62ef	0.29
PR 39 G 12	3.535a	1.42	0.585a	0.12	0.3025a	0.14	0.1525b	0.13	7.385ab	3.36	0.405b	0.23	8.888de	3.29
PR 39 K 13	1.652bc	0.66	0.240b	0.09	0.180b	0.09	0.490a	0.54	9.312a	1.89	0.335bc	0.12	14.637ab	2.14
PR 39 T 45	0.988bcde	0.59	0.2475b	0.03	0.0925c	0.03	0.215b	0.14	4.555cdef	0.75	0.24bcd	0.16	7.132def	2.39
$LSD_{0.05}$	1.123		0.123		0.087		0.214		2.776		0.29		4.254	
Grain type	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.
D	1.134b	1.24	0.1687b	0.14	0.0937b	0.06	0.1156b	0.11	5.017a	1.88	0.2875b	0.14	9.039a	3.32
D/F	0.488c	0.23	0.1075b	0.03	0.0725b	0.02	0.0925b	0.04	6.130a	4.06	0.1225c	0.13	5.620a	0.29
F	1.949a	1.30	0.3081a	0.19	0.1881a	0.11	0.2256a	0.30	6.445a	2.99	0.4806a	0.42	10.910a	5.28
F/D	0.490c	0.46	0.1125b	0.06	0.0721b	0.03	0.0680b	0.08	4.425a	2.42	0.1575bc	0.14	8.281a	3.78
$LSD_{0.05}$	0.508		0.067		0.035		0.091		2.744		0.13		5.684	
P-values	<0.001		<0.001		<0.001		0.040		0.086		<0.001		0.073	

In columns, means followed by the same letters are not significantly different.

Table 4. Mean values, range (minimal and maximal values), and coefficient of variation (CV) observed traits as well as results of regression analysis for influence FAO number on observed traits.

Trait	Characteristics			Results of regression analysis			
	Mean	Min-Max	CV [%]	Estimates of regression coefficients	P-value	The proportion of total phenotypic variance explained by trait	Standard error of observations
FB_1	1.016	0.09 - 4.95	109.00	-0.01044	0.111	2.5	1.090
FB_2	0.1752	0.04 - 0.71	83.61	-0.002106*	0.014	7.9	0.141
FB_3	0.1066	0.00 - 0.48	75.74	-0.001231**	0.009	9.1	0.077
ZON	0.1208	0.01 - 1.03	147.40	-0.00211*	0.045	4.8	0.174
DON	5.185	2.09 -12.47	51.02	-0.0129	0.414	x	2.650
MON	0.2686	0.00 - 1.61	101.50	-0.00275	0.088	3.1	0.268
ERG	8.961	2.19 - 20.00	46.36	-0.0542*	0.026	6.2	4.020

* P<0.05; ** P<0.01
x - residual variance exceeds variance of response variable

and the toxin content was lower when compared to the disease caused by *F. graminearum*. Visual screening is cheaper than mycotoxin analyses, and there is a correlation between ear rot rating and the level of mycotoxins. However, mycotoxin analysis in the preliminary breeding program is also required. The various responses of flint and dent genotypes to *Fusarium* spp. under field and laboratory conditions could indicate a kind of coadaptation between the host and the pathogen, related to the site of origin [27]. Löffler et al. [30] reported higher disease severity on maize with flint than dent endosperm type: opposite results were presented by Doko et al. [31].

Hallauer et al. [32] state that maize *Zea mays* ssp. *indentata* and *Zea mays* ssp. *indurata* are one of the most common maize subspecies. The main criterion for division of species *Zea mays* into subspecies, apart from typically morphological traits, is the morphological and anatomical structure of kernels. Kernels of flint type ssp. *indurata* are characterized by a hard outer layer of the endosperm covering the floury endosperm, while kernels of dent type ssp. *indentata* do not have the above-mentioned layer on the top of kernels [33]. In flint kernels (ssp. *indurata*), the front part of the kernel becomes harder and thereby less permeable to water. As a result, the plant has to pump assimilates to the kernel through its outer layer having even greater resistance. For this reason they give away less water from the kernel at later stages of maturation, which does not have a favourable effect on starch accumulation. Therefore hybrids with flint grain are suitable for earlier harvest time. On the other hand, in dent grain, together with subsequent maturation stages, side edges of the kernel become harder and less permeable to water, while its front part remains soft and highly permeable to water. As a result, subsequent maturation stages are followed by water loss from grain. Long-lasting water loss takes place especially in the situation of low air humidity and open dried cover leaves of the ear. Such conditions indicate that starch is still being accumulated in the kernel, which may result in grain yield increase. Thus dent hybrids are able to accumulate assimilates for a longer period, even until the late autumn, so they are suitable for later harvesting. Also Daynard [34] proposed a hypothesis that the forms with vitreous grain (flint), despite their earlier tasseling, release water from grain significantly slower than dent

Table 5. Correlation matrix for studied traits of maize.

Trait	FB_1	FB_2	FB_3	ZON	DON	MON	ERG
FB_1	1						
FB_2	0.8982***	1					
FB_3	0.8782***	0.8889***	1				
ZON	0.2119	0.2197	0.1712	1			
DON	0.2324	0.2441	0.3015*	0.5244***	1		
MON	0.2842*	0.2727*	0.2026	0.0577	0.037	1	
ERG	0.317*	0.2742*	0.2655*	0.4728***	0.243	0.3728**	1

* P < 0.05, ** P < 0.01, *** P < 0.001

Table 6. Mahalanobis distances between maize cultivars.

Cultivar		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	PR 39 D 60	0															
2	PR 39 K 13	2.80	0														
3	PR 39 G 12	4.31	2.16	0													
4	P-8000	3.29	2.00	2.07	0												
5	PR 39 T 45	2.50	2.34	3.13	1.51	0											
6	PR 39 D 23	2.78	2.27	2.86	1.03	0.64	0										
7	PR 39 F 58	3.10	1.31	2.20	1.35	1.51	1.43	0									
8	PR 38 N 86	3.63	2.48	2.89	2.52	2.2	2.25	1.72	0								
9	PR 38 H 20	3.11	2.10	2.49	2.88	2.95	2.91	2.33	2.29	0							
10	Clarica	5.87	4.94	4.75	4.75	4.73	4.74	4.24	3.76	4.05	0						
11	LG 3215	5.35	3.45	3.77	4.14	4.07	4.14	3.00	2.95	3.59	4.49	0					
12	LG 3216	4.72	4.19	5.20	5.80	5.72	5.82	5.00	4.67	3.72	6.01	5.56	0				
13	Absolut	4.37	2.41	2.30	2.38	2.65	2.53	1.57	1.76	2.50	3.67	1.98	5.39	0			
14	LG 3232	9.43	7.87	6.90	8.04	8.30	8.30	7.50	6.47	7.04	5.51	6.68	7.42	6.61	0		
15	LG 3233	4.90	4.31	4.72	5.44	5.26	5.32	4.77	3.96	3.3	6.40	4.78	3.40	4.67	7.28	0	
16	LG 3252	5.90	4.16	3.46	3.87	4.36	4.14	3.55	2.73	3.73	3.12	4.04	5.57	2.87	4.81	5.30	0

$D_\alpha = 5.98$

forms under field conditions. This is of great significance in maize cultivation for grain, and especially in seed production where fast ear drying during additional drying is important [35]. The obtained results show the necessity of continuing research on maize evaluation for mycotoxin content. It is possible that different times of harvest will be taken into account.

According to Commission Regulation No 1126/2007 [18], which sets out the maximum level of DON in unprocessed maize at the level of 1.75 μg/g, all obtained mean values of the toxin exceed determined threshold. For ZON and FB_s (sum of B_1 and B_2) the maximum level are defined at the concentration of 0.35 and 4.00 μg/g, respectively. Among all analyzed samples, only PR 39

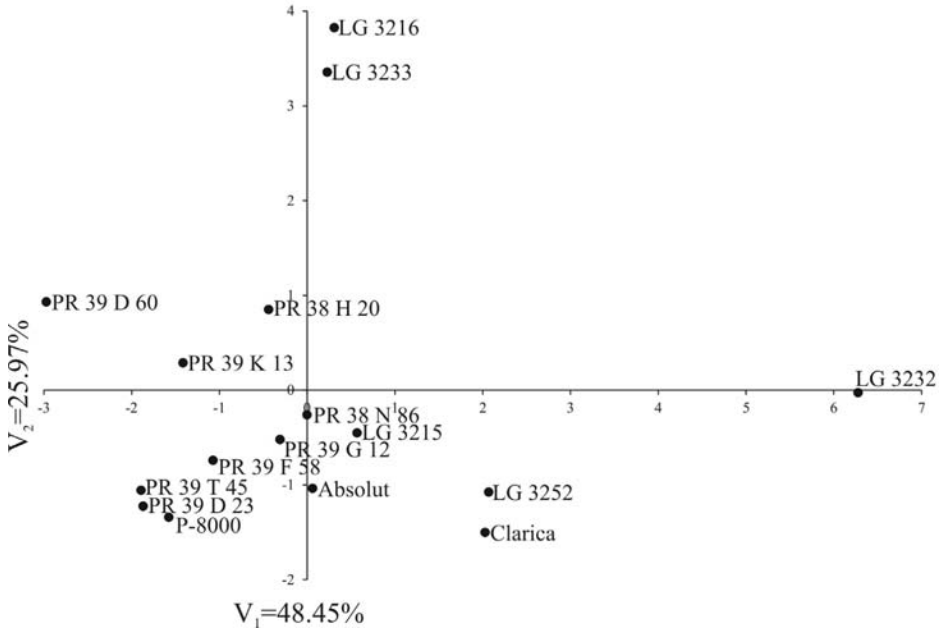

Fig. 1. Configuration of maize cultivars in the space of two first canonical variates calculated for all studied traits.

K 13 exceeded the acceptable maximum content of the ZON (0.49 μg/g), and PR 39 G 12 with FB_s content about 4.12 μg/g.

FAO number statistically significantly differentiated FB_2, FB_3, ZON, and ERG (Table 4). The percentage of variability of these traits explained by variability of FAO number amounted to 7.9%, 9.1%, 4.8% and 6.2%, respectively (Table 4). All the obtained regression coefficients were negative (Table 4), which means that the content of all the observed mycotoxins was lower in the cultivars with greater FAO number. Lack of association between earliness of cultivars and their infection by *Fusarium* spp. was demonstrated by Michalski et al. [36]. According to the author, desease resistance and morphological structure of corn and weather conditions during the growing season period influenced infection of the plants by *Fusarium* spp.

Harvest time is of great importance to maize health because maize that stands in the field is very prone to lodging, infection with *Fusarium* ssp. and other pathogens, causing stalk rot and ear rot. A study by Doerge [37] demonstrated that stalk rot is the most common maize disease resulting in plant lodging. Influence of the disease on lodging is particularly noticeable under the conditions of delayed harvesting. According to the author it hinders mechanical harvest and leads to reduction in yield and grain quality. As stated by Butzen [38], ear rot may develop on lodged plants, especially on those that are in contact with soil. Hence the delay of harvest, especially if the autumn is rainy – increases the risk of ear infection by *Fusarium* ssp. Therefore appropriate (optimal) time of maize harvest should limit yield losses and ensure high grain quality. According to Aldrich et al. [39], the process of maize maturation includes several stages with initial physiological transformations and then physical changes after reaching the black layer stage, which is an indicator of maize plant maturity. The area at the base of the kernel becomes dark due to breaking and compression of several cell layers near the base, which results in closing of channels delivering assimilates and arrests of their transport to the kernel [39]. Sulewska et al. [40] state that the optimal time of harvest is two weeks after kernel achieving the black layer stage. According to the authors, both harvest acceleration and delay resulted in grain yield losses ranging from 7.3 to 15.5%. Such a response was observed irrespective of maturity class of a given variety (FAO number). In our research, maize harvest, irrespective of maturity class (FAO number), was made at the same time. Hence hybrids with lower FAO number had greater mycotoxin content in grain in comparison with late-maturing varieties. Therefore it can be concluded that desease severity in early-maturing varieties was related to their faster maturation and therefore longer infection time time and development of pathogens that develop the most intensively on drying plants. Fungi of the genus *Fusarium* ssp. play a considerable role in the process because they inhabit diseased plants, which finally results in a greater mycotoxin content in the grain.

Table 5 shows a correlation matrix for the observed traits. Significant positive correlations were observed between: FB_1 and FB_2 (r = 0.8982), FB_1 and FB_3 (r = 0.8782), FB_1 and MON (r = 0.2842), FB_1 and ERG (r = 0.317), FB_2 and FB_3 (r = 0.8889), FB_2 and MON (r = 0.2727), FB_2 and ERG (r = 0.2742), FB_3 and DON (r = 0.3015), FB_3 and ERG (r = 0.2655), ZON and DON (r = 0.5244), ZON and ERG (r = 0.4728), and MON and ERG (r = 0.3728) (Table 5). The relationships between resistance and mycotoxin contamination were reported by a number of researchers [41-43].

The results of the multivariate analysis of variance (MANOVA) indicated that the main effects of cultivars, grain type, and FAO number were significant ($P < 0.001$). The first and second canonical variaties elucidated 48.45% and 25.97%, respectively, of multivariate variability of cultivars (Fig. 1). The highest Mahalanobis distance (equal to 9.43) was observed between cultivars LG 3232 and PR 39 D 60, whereas the lowest Mahalanobis distance (equal to 0.64) was found for PR 39 T 45 and PR 39 d 23 (Table 6).

Fusarium control in the field is not very effective. Weather conditions play a crucial role in fungal growth and mycotoxin formation [44]. Mycotoxins in maize pose a costly and challenging problem, but available agricultural practices and genetic approaches for mycotoxin management can be a solution [45]. Agricultural practices used in the field have limited capabilities, but they can be changed in order to reduce the risk of grain contamination with zearalenone, deoxynivalenol, and fumonisins. One of the most effective methods of limitation of maize infection by pathogens is the choice of less susceptible hybrids for cultivation [46]. According to Adamczewski et al. [47], such varieties are from twice to three times less damaged than the susceptible varieties. Mesterházy et al. [48] states that the degree of plant infection depends not only on the type of variety but also on plant water supply in the time before and after flowering. There are no registered fungicides preparations for fighting against the disease in Poland, and the only way is prevention with the use of integrated farming methods [49, 50].

Conclusions

- In all analyzed samples of maize the DON concentration level exceeded value of 1.75 μg/g – maximum acceptable level specified by Commission Regulation No. 1126/2007.
- Only two cultivars (PR 39 K 12, PR 39 G 12) reported exceeding maximum levels of ZON (0.35 μg/g) and FB_s (4.00 μg/g).
- Food and feed safety requires more healthy grain (also silage); the investment in this sector of breeding and science may be expected to increase.
- Emphasis should be laid on prevention against pathogen attacks during the growing phase of the plants, as removal or inactivation of the toxins often proved to be quite difficult or not economical.

– Apart from hybrid genotype, mycotoxin content in maize grain is determined also by optimal time of harvest.

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