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THE RISKS OF SWEET CORN AND POPCORN CONTAMINATION BY FUMONISIN FB_1 PRODUCED DUE TO *Fusarium verticillioides* INFECTION

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ABSTRACT

Based on two-year experiments on inoculated corn, including genotypes of sweet corn (*Zea mays* var. *saccharata*) and popcorn (*Zea mays* var. *everta*), the analysis of fumonisin FB_1 content in kernels was performed. Infection degree of sweet corn was 2.00 and 2.13, which was distinctly stronger than infection of popcorn cobs (0.52 and 1.05). Despite of higher disease rating of *Zea mays* var. *saccharata*, the most dynamic increase in fumonisin FB_1 biosythesis was observed in kernels of *Zea mays* var. *everta*. During the two cropping seasons, the mean level of FB_1 in sweet corn ranged from 0.52 to 6.94 μg g^{-1}, while in popcorn kernels from 0.96 to 28.49 μg g^{-1} in the 1st and 8th week after inoculation. Botanical varieties of maize as well as physiological state of kernels, determined by the water, amylose and starch contents, influenced on infection degree by *Fusarium verticillioides* and level of ear contamination by fumonisin FB_1. Efficiency of biosynthesis of mentioned metabolites was inversely proportional to kernel water content.

Key words: *Fusarium verticillioides*, fumonisin FB_1, *Zea mays*, amylose content

INTRODUCTION

Zea mays var. *saccharata* is a type of maize grown primarily for processing, as well as for direct consumption. It can be consumed raw, boiled or grilled. Through cultivation of varieties of the type of *Zea mays* var. *saccharata* in different vegetation periods, various sowing terms, under cover flat and high crops, it is available on the market since mid-June [Warzecha 2013]. Nutritional and dietary value of sweet maize mainly stems from the high content of easily digestible sugars and high amount of fiber that regulates activity of human gastrointestinal tract. In addition to macro- and microelements in sweet maize kernels, it has high selenium content [Warzecha 2013, Alan et al. 2014].

World sowing area of sweet corn is estimated for over 1 million ha, including about 17 thousand ha in Poland. In Europe, the main producer of this crop is France and Hungary where sweet hybrids are cultivated on 32 and 25 thousand ha, respectively. According to sugar content, maize cultivars are classified as: standard, sugary enhanced and super sweet. Standard sweet corn genotypes hold mutated sugary1 (*Su1*) gene, which determines higher sucrose concentration, decreased concentration of amylopectin and

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accumulation of glucopolysaccharide phytoglycogen [James et al. 1995]. At immature milky stage, the level of sucrose reaches 10.2% of sucrose and 22.8% of water soluble polysaccharides.

Trait of sugary enhanced genotypes is governed by the „*se*" gene. It is recessive modifier of *Su1* endosperm, located on long arm of chromosome 4 or „*se*+". The „*se*" genotypes characterize total sugar level comparable to *Sh2* without reduction of phytoglicogen [Ferguson et al. 1979].

Among *Zea mays* var. *saccharata* plants, at least two recessive genes *Su1* and *Sh2* determine the sweetness in maize through inhibition of starch biosynthesis. The *Su1* genotypes are characterized by higher levels of reducing sugars, sucrose and water-soluble polysaccharides, whereas mature kernels are wrinkled. The presence of *Su1* gene results also in the occurrence of range of side effects in thin pericarp forms, less dry weight and higher kernels water content during the growing season. Even a higher content of reducing sugars is found in supersweet plant varieties with *Sh2* [Wakuliński et al. 2012].

Kernels of that variety are small, wrinkled, characterized by a thin seed coat and low germination energy [Adetimirin et al. 2006, Manicacci et al. 2007]. Extending germination time may contribute to increased susceptibility of seedling to soil pathogens [Baird et al. 1994]. Significantly higher susceptibility of *Zea mays* var. *saccharata*, especially *Sh2* genotypes to infection by *Fusarium verticillioides* (Sacc.) Nirenberg is an individual consequence of the interaction of pathogen – host at the species level. Similar dependence of higher susceptibility of *Sh2* genotypes on the infection by *Erwinia stewartii* (Smith) Dye, *Puccinia sorghi Schwein*, *Exserohilum turcicum* (Pass.) K.J. Leonard and Suggs or *Bipolaris maydis* (Y. Nisik. and C. Miyake) Shoemaker [Pataky et al. 1998], was not noticed.

However, *Zea mays* var. *everta* is a type of maize commonly known as popcorn; it is popular and due to antioxidants, also valuable kind of snack, which contains more antioxidants than fruits and vegetables, to eliminate free radicals from the body, which have positive impact on an organism defense mechanisms. The content of polyphenols in kernels plays a very important role in the prevention of human cancer [Karababa 2006]. The kernels popping is a result of rapid rupture of kernel pericarp by superheated water generated during grain heating. Among popcorn varieties, the candidate gene GRMZM2G060579 encodes protein involved in pectin biosynthesis and shows an intermediate level of expression of pericarp embryo and endosperm [Paes et al. 2016].

The common species of genus *Fusarium*, lowering the quality of grain, both of sweet corn and popcorn, is *F. verticillioides.* The fungus is a producer of fumonisins, secondary metabolites causing encephalomalacia in horses, edema, arrhythmia, atherosclerotic lesions and liver, kidneys and gastrointestinal cancer in warm-blooded organisms [Desjardins 2006]. *F. verticillioides* is the endophyte and hermaphroditic, heterothallic species [Xu and Leslie 1996, Desjardins 2006, Wakuliński et al. 2012].

Due to high susceptibility of maize cob varieties of *Zea mays* var. *saccharata* and *Zea mays* var. *everta,* to infection by *Fusarium* spp. [Bottalico and Perrone 2002, Leonard and Bushnell 2003, Logrieco et al. 2003, Chełkowski et al. 2006, Desjardins 2006, Wit et al. 2007, Czembor et al. 2015], the studies were undertaken to determine the *F. verticillioides* infection degree of cobs of selected varietes cultivated in Poland and fumonisin FB_1 contamination, depending on the physiological state of kernels, starch, amylose and amylopectin content.

MATERIAL AND METHODS

Plant material. The study included materials of two botanical varieties of maize: *Zea mays* var. *everta*, the group of popcorn: Dobosz and Nana, and *Zea mays* var. *saccharata* from the group of sweet corn varieties containing *Su1* gene: Harvest Gold, Jubile and *Sh2* gene: Candle, Sheba, Trophy. Seed material of plants used for the research was obtained from the Plant Breeding and Acclimatization Institute (IHAR) – National Research Institute in Radzików, Poland. The experiment was carried out on IHAR experimental fields in Radzików during two growing seasons in 2008–2009. In each of the vegetation seasons, experiments were conducted in a three-factorial (isolate, variety, date of harvest) type split plot, two blocks using 10 plants as individual experimental plot.

Susceptibility of *Zea mays* to infection by *F. verticillioides.* In the inoculation experiment, two mating types *MAT1-1* and *MAT1-2* isolates KFI-269 and KFI-270 of *F. verticillioides* were used, respectively. The isolates KFI-269 and KFI-270 were grown on potato dextrose agar (PDA, Difco) at 20–22°C temperature for 10 days. Mycelium of fungus was harvested from the surface of media and resuspended in sterile distilled water Milli-Q quality (0.054 μS, temperature 25°C), and the mixture was homogenized. The obtained suspension was filtered throuogh sterile gauze (mesh size 2 × 2 mm), and the obtained filtrate was adjusted to desired concentration of spores 10^6 1 ml^{-1}. The concentration of the spore suspension was determined using a hemacytometer (Thoma cell counting chamber). The inoculation of plants was carried on the 7^{th} day from the beginning of plant flowering, while the stigmas were green in the R2 phase of plant maturity [Zadoks et al. 1974]. The spore suspension of the volume 5 ml, was introduced into the channel of cob using a syringe. Assessment of infection degree of cobs was conducted using a 6-degree infection scale, described in detail by Zajkowski and Kwaśna [1987] and adopted by Wit et al. [2007]. The basis for a single assessment was plant material derived from the individual plot covering 10 plants.

Starch, amylose and fumonisin content. The analysis of starch and amylose content was conducted in healthy and symptomatic kernels obtained from the cobs, inoculated with *F. verticillioides*, as described by Waśkiewicz et al. [2012]. The analysis of water content in grains was performed by evaporation method. For this purpose, randomly collected sample of kernels, obtained from the corn cobs, was weighed, and then the kernels were placed in a dryer at 60°C. Drying of the material was performed until the moment, when weight loss of grain between successive measurements did not exceed 2%. The water content was determined according to the formula:

$$x = \frac{(m - m_0)}{m} 100\ (\%)$$

where:
x – water content (%),
m – weight of kernels before drying (g),
m_o – weight of kernels after drying (g).

The analysis was performed for the material in each separate plot. The fumonisin FB_1 contamination level of kernels was carried out according to the method described by Waśkiewicz et al. [2012]. Fumonisins were extracted using methanol and analyzed by means of HPLC (high performance liquid chromatography). Samples for analysis of starch, amylose and FB_1 contents were collected at seven-day intervals, for eight consecutive weeks after inoculation.

Statistical analysis. The obtained results were processed by analysis of variance (ANOVA). Comparison of means was performed using Fisher's test; relationships between estimated parameters (amylose, starch, water and fumonisin FB_1 content) were analyzed using Pearson's correlation coefficient. The tested hypotheses were verified at significance level of $\alpha = 0.05$. Statistical calculations were performed using Statgraphics 4.1 for Windows (Statistical Graphics Corp. 1999, Statpoint Technologies, Inc. Warrenton, Virginia, USA).

RESULTS

The obtained results of two-year study show the dynamics of changes of infection degree of cobs, fumonisin FB_1, water, starch and amylose contents, in analyzed material. During 2008 and 2009 growing seasons, infection degree of *Zea mays* var. *everta* measured from the first to the last harvest date ranged from 0.13 to 1.00 in 2008 and 0.75 to 1.31 in 2009, according to the used scale (from 0 to 5). In the case of *Zea mays* var. *saccharata*, value of this parameter ranged from 1.39 to 2.64 in 2008 and from 1.04 to 2.82 in 2009 (Tab. 1).

On average, in 2008 and 2009, the lowest susceptibility characterized *Zea mays* var. *everta* (0.52 and 1.05). Infection degree of *Zea mays* var. *saccharata* cobs in mentioned period was 2.00 and 2.13 (Fig. 1). Among analyzed genotypes of *Z. mays* var. *saccharata*, significaly stronger were infectected genotypes including *Sh2* gene (2.64 and 2.66) than *Su1* (1.78 and 1.83), in 2008 and 2009, respectively (Tab. 2).

Table 1. Mean value of infection degree and fumonisin FB_1 content in ears of maize varieties in relation to term after *F. verticillioides* inoculation, kernel stage development

Maize variety	Number of week after inoculation	Mean value of infection degree		Fumonisin FB_1 level (μg g^{-1})	
		2008	2009	2008	2009
Zea mays var. *saccharata*	1	1.43	1.04	0.14 a	0.90 a
	2	1.39	1.38	0.67 ab	1.37 a
	3	1.71	1.86	2.11 ab	2.33 ab
	4	1.82	1.88	2.38 ab	3.12 ab
	5	2.43	2.61	4.13 bc	2.13 ab
	6	2.43	2.71	4.17 bc	3.21 ab
	7	2.18	2.50	6.44 c	4.18 b
	8	2.64	2.82	6.55 c	7.32 c
Regression equation		$y = –0.009x^2 + 0.267x + 1.048$	$y = –0.031x^2 + 0.536x + 0.481$	$y = 0.233e^{0.486x}$	$y = 0.840e^{0.248x}$
Coefficient of determination		$R^2 = 0.855$	$R^2 = 0.940$	$R^2 = 0.818$	$R^2 = 0.872$
Zea mays var. *everta*	1	0.13	0.81	1.04 a	0.87 a
	2	0.63	0.75	1.21 a	1.21 a
	3	0.38	1.06	2.99 a	0.93 a
	4	0.31	1.00	3.59 a	2.11 ab
	5	0.31	1.19	4.02 a	1.36 a
	6	0.50	1.31	5.99 a	1.38 a
	7	0.81	1.19	19.56 a	6.02 ab
	8	1.00	1.13	52.66 b	4.32 ab
Regression equation		$y = 0.021x^2 – 0.102x + 0.427$	$y = –0.015x^2 + 0.200x + 0.541$	$y = 0.660e^{0.557x}$	$y = 0.610e^{0.238x}$
Coefficient of determination		$R^2 = 0.687$	$R^2 = 0.793$	$R^2 = 0.905$	$R^2 = 0.675$

Numbers followed by the same letter, for each year, are not significantly different at $\alpha = 0.05$

Table 2. Assessment of mean infection degree of genotypes of *Z. mays* var. *saccharata* in 2008 and 2009

Maize variety	Genotype	Infection degree	
		2008	2009
Harvest Gold	*Su1*	1.27	1.60
Jubile	*Su1*	2.28	2.06
Mean for *Su1* genotypes		1.78 a	1.83 a
Trophy	*Sh2*	2.90	2.59
Sheba	*Sh2*	2.25	2.75
Candle	*Sh2*	2.78	2.65
Mean for *Sh2* genotypes		2.64 b	2.66 b

Numbers followed by the same letter, for each year, are not significantly different at $\alpha = 0.05$

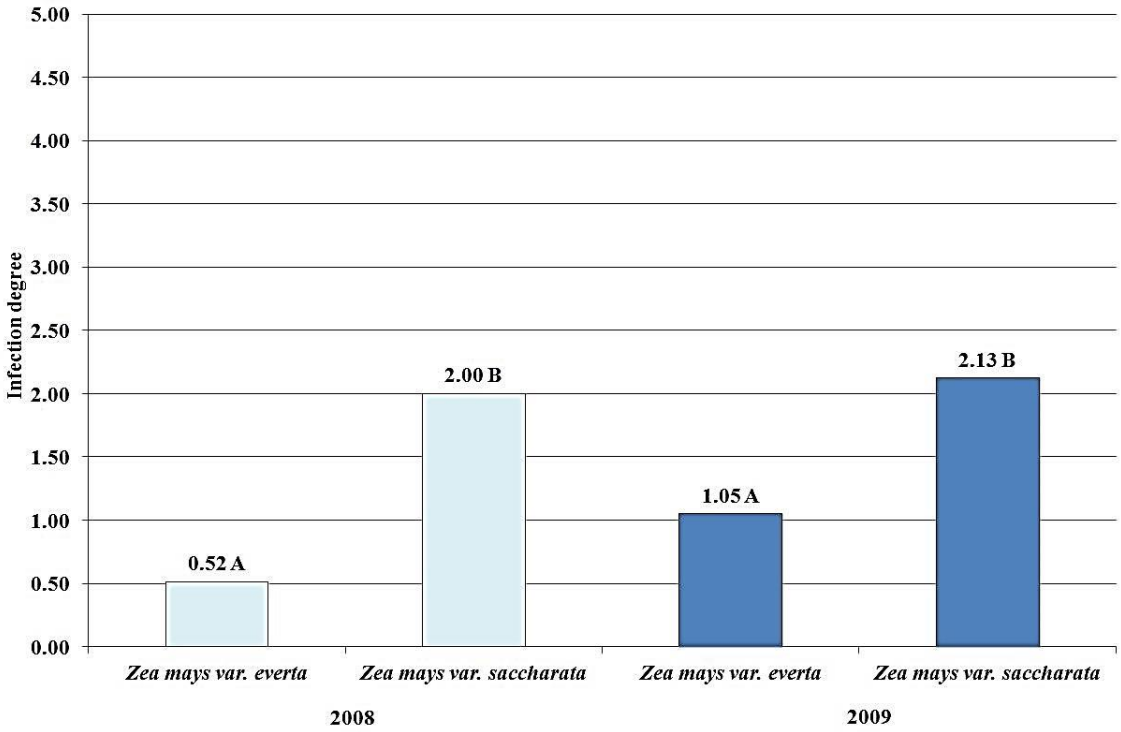

Fig. 1. Mean infection degree of cobs of maize varieties after ears inoculation with *F. verticillioides* in cropping seasons 2008 and 2009. Numbers followed by the same letter, for each year, are not significantly different at $\alpha = 0.05$

In this study, physiological condition of kernels was determined by a) the water content, b) starch content and c) amylose content. Values of these parameters were the reference for further analysis to determine the infection degree of each cultivar and the occurrence of fumonisin FB_1 in kernels. Regardless of the growing stage, the kernels of *Z. mays* var. *saccharata* were characterized by the highest average water content. In two successive growing seasons, kernels of this variety contained 82.31% and 88.16% of water in 1st week respectively, whereas 68.80% and 58.72% of water in 8th week, respectively (Tab. 3). The starch in kernels was also observed approximately 10 days after flowering (as early as 7 days after the inoculation) of cobs, and the process of biosynthesis and deposition proceeded very rapidly regardless of variety. The increase in the polysaccharide content during the time is described by polynomial function (Tab. 3). Regardless of the growing season, significantly least of starch was contained by sweet corn varieties. In 8th week after infection in 2008 and 2009, kernels of *Z. mays* var. *saccharata* had 47.12% and 30.18% of starch, respectively, whereas in the case of *Z. mays* var. *everta*, the value was twice as high and varied in between 63.12% and 70.19% (Tab. 3).

The process of creating the components of starch (amylose and amylopectin) during the growing season among studied botanical varieties proceeded quite similar. Amylopectin was the dominant fraction in the first week after flowering. Its content coincided substantially with the starch level, as the amylose content was low during initial period of kernel organization, regardless of the variety and

Table 3. Water, starch and amylose content in ears of maize varieties in relation to term after *F. verticillioides* inoculation, kernel stage development

Maize variety	Number of week after inoculation	Water content (%)		Starch content (%)		Amylose content (%)	
		2008	2009	2008	2009	2008	2009
Zea mays var. *saccharata*	1	82.31 ±0.01	88.16 ±0.01	7.53 ±1.97	2.49 ±1.14	7.26 ±0.41	4.79 ±0.36
	2	81.41 ±0.01	87.62 ±0.01	11.45 ±1.97	4.27 ±1.14	7.80 ±0.37	6.47 ±0.36
	3	77.76 ±0.01	80.26 ±0.01	15.51 ±1.97	9.59 ±1.16	9.10 ±0.37	8.36 ±0.37
	4	72.63 ±0.01	74.62 ±0.01	19.96 ±1.97	14.75 ±1.14	9.59 ±0.37	8.27 ±0.36
	5	73.18 ±0.01	75.28 ±0.01	26.18 ±1.97	19.07 ±1.14	10.79 ±0.37	9.70 ±0.36
	6	66.44 ±0.01	64.63 ±0.01	26.25 ±1.97	23.89 ±1.14	12.19 ±0.37	10.65 ±0.36
	7	69.22 ±0.01	65.05 ±0.01	30.11 ±1.99	25.00 ±1.14	13.84 ±0.37	10.58 ±0.36
	8	68.80 ±0.01	58.72 ±0.01	47.12 ±2.20	30.18 ±1.14	13.29 ±0.41	13.91 ±0.36
Regression equation		$y = 0.8887e^{-0.0424}$	$y = 0.9737e^{-0.0624}$	$y = 0.4054x^2 + 1.2179x + 7.1947$	$y = -0.0687x^2 + 4.7226x - 3.342$	$y = -0.0015x^2 + 1.0001x + 6.0215$	$y = 0.0062x^2 + 1.0478x + 4.2162$
Coefficient of determination		$R^2 = 0.9616$	$R^2 = 0.9828$	$R^2 = 0.9373$	$R^2 = 0.9885$	$R^2 = 0.9631$	$R^2 = 0.9330$
Zea mays var. *everta*	1	96.19 ±0.02	83.31 ±0.01	3.52 ±3.11	7.82 ±1.80	2.35 ±0.59	3.92 ±0.57
	2	80.92 ±0.02	76.50 ±0.01	5.57 ±3.11	19.60 ±1.80	6.27 ±0.59	7.26 ±0.57
	3	81.91 ±0.02	59.58 ±0.01	27.00 ±3.11	34.39 ±1.80	8.54 ±0.59	12.69 ±0.57
	4	71.89 ±0.02	42.59 ±0.01	26.60 ±3.11	49.79 ±1.80	10.52 ±0.59	15.81 ±0.57
	5	70.26 ±0.02	40.31 ±0.01	28.47 ±3.11	47.89 ±1.80	13.00 ±0.59	18.51 ±0.57
	6	58.24 ±0.02	30.46 ±0.01	62.77 ±3.11	59.78 ±1.80	16.85 ±0.59	21.67 ±0.57
	7	38.96 ±0.02	25.19 ±0.01	36.33 ±3.11	57.90 ±1.80	23.32 ±0.59	27.42 ±0.57
	8	27.21 ±0.02	28.35 ±0.01	63.12 ±3.11	70.19 ±1.80	29.19 ±0.59	26.96 ±0.57
Regression equation		$y = 1.0932e^{-1300}$	$y = 0.9927e^{-0.1806}$	$y = -0.216x^2 + 10.041x - 8.0046$	$y = -0.8768x^2 + 16.253x - 7.3589$	$y = 0.3372x^2 + 0.5431x + 2.7125$	$y = -0.142x^2 + 4.7508x - 0.9774$
Coefficient of determination		$R^2 = 0.8004$	$R^2 = 0.9420$	$R^2 = 0.7869$	$R^2 = 0.9668$	$R^2 = 0.9877$	$R^2 = 0.9855$

Table 4. Correlation between infection degree of corn cobs expressed in 6-point scale, and water, starch and amylose content

Content	Year of studies	1st week	2nd week	3rd week	4th week	5th week	6th week	7th week	8th week
Water	2008	–0.0878	–0.2356	0.1972	–0.2165	**0.4716**	0.1966	**0.8703**	**0.7916**
	2009	–0.1119	0.6605	0.4453	**0.7041**	**0.7600**	**0.5608**	**0.6413**	**0.7783**
Starch	2008	–0.0514	0.1077	0.0521	0.1469	–0.2760	**–0.5199**	**–0.4222**	**–0.8717**
	2009	–0.2849	–0.5397	**–0.6217**	**–0.8059**	**–0.6758**	**–0.6668**	**–0.6389**	**–0.8493**
Amylose	2008	0.1762	–0.0446	0.1303	0.2932	–0.0872	–0.2710	**–0.6709**	**–0.7409**
	2009	–0.5670	–0.4703	–0.1425	–0.4442	–0.6274	**–0.4813**	**–0.5637**	**–0.6740**

Bold numbers indicate statistically significant correlation at $\alpha = 0.05$

growing season. The maximum amylose content, which was found in grains in the first week after flowering phase of the plants, was 7.26% and 4.79% (*Z. mays* var. *saccharata*), for two seasons of analysis, respectively (Tab. 3). This also means that the minimum amylopectin content in the group of studied varieties, during two growing seasons was 92.74% and 95.21% of the total starch content, respectively. In the last period of studies (8th harvest period of analysed cobs), amylose content that was observed for tested varieties of *Z. mays* var. *everta*, was 29.19% (first testing season) and 26.96% (second season of tests) (Tab. 3). The process of amylose formation in grains was described by polynomial function (Tab. 3). Regardless of the growing season, indeed the lowest average starch and amylose content characterized kernels of *Z. mays* var. *saccharata*.

Correlation analysis between the infection degree corn cobs and the water, starch and amylose contents indicates that susceptibility is related to the water content of kernels and also inversely proportional to the starch and amylose contents. For a direct proportional relationship between water content and infection degree, a positive correlation coefficients between infection degree and water content can be seen. Values of these factors were variable for different terms, in which the determination was carried out, but they highlight the close relationship between these values. Negative value of the correlation coefficients between the infection degree and starch and amylose contents indicates that a high concentration of polysaccharides in kernels is associated with low susceptibility of cobs to infection (Tab. 4).

In 2008 and 2009, a clear regularity connected with the dynamics of the fumonisin FB_1 biosynthesis was observed (Tab. 1). In the first weeks (3–4) after inoculation of cobs, biosynthesis of the metabolite proceeded with very low yields, without significant differences between weeks of observation. A significant increase in the level of FB_1 was observed from 4–5 weeks from the time of inoculation. The dynamics of changes in levels of this metabolite was consistent with the distribution of the exponential function. The analysis of these functions shows that in all seasons, the most dynamic increase of the biosynthesis of FB_1, understood as the change in the concentration of this metabolite in time, was observed in grains of *Z. mays* var. *everta*, (in 2008: 1.04–52.66 and in 2009: 0.87–6.02, respectively), whereas the least dynamic process occurred in kernels of *Z. mays* var. *saccharata* (in 2008: 0.14–6.55 and in 2009: 0.90–7.32, respectively) (Tab. 1).

The correlation analysis among fumonisin FB_1, water and starch content revealed occurrence of significant relationship between these parameters. The water content in grains was negatively correlated with the level of fumonisin FB_1 (Fig. 2 and 3).

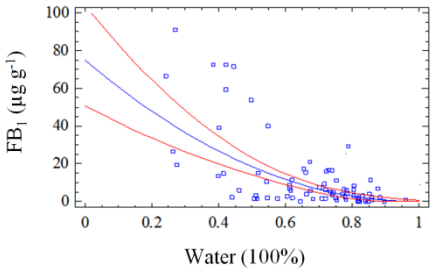

Fig. 2. Fumonisin FB_1 content in kernels in relation to water content in 2008

$FB_1 = (8.66875 – 8.76574\ water)^2$, r = –0.66, r^2 = 45
$FB_1(A) = (159.77 – 182.406\ water)^2$, r = –0.91
$FB_1(B) = (4.58 – 3.43\ water)^2$, r = –0.26
A = *Zea mays* var. *everta*, B = *Zea mays* var. *saccharata*

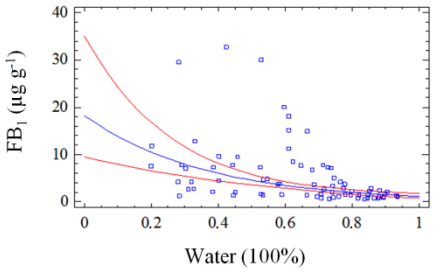

Fig. 3. Fumonisin FB_1 content in kernels in relation to water content in 2009

$FB_1 = \exp(2.9045 – 2.78842\ water)$, r = –0.55, r^2 = 30
$FB_1(A) = \exp(2.82 – 3.43\ water)$, r = –0.68
$FB_1(B) = \exp(5.19 – 5.33\ water)$, r = –0.67
A = *Zea mays* var. *everta*, B = *Zea mays* var. *saccharata*

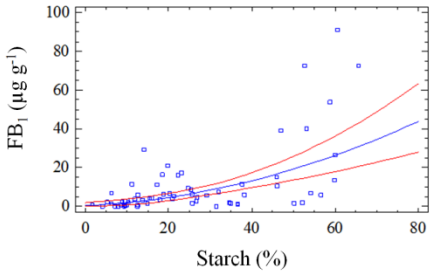

Fig. 4. Fumonisin FB_1 content in kernels in relation to starch content in 2008

$FB_1 = (0.640648 + 0.0747501\ starch)^2$, r = 0.62, r^2 = 39
$FB_1(A) = (–17.99 + 7.19\ starch)^2$, r = 0.81
$FB_1(B) = (2.30 + 0.86\ starch)^2$, r = 0.17
A = *Zea mays* var. *everta*, B = *Zea mays* var. *saccharata*

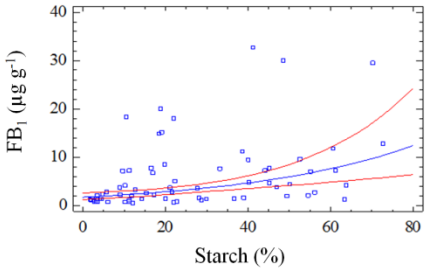

Fig. 5. Fumonisin FB_1 content in kernels in relation to starch content in 2009

$FB_1 = \exp(0.552331 + 0.0246144\ starch)$, r = 0.45, r^2 = 21
$FB_1(A) = \exp(–0.26 + 0.032\ starch)$, r = 0.62
$FB_1(B) = \exp(–0.74 + 0.03\ starch)$, r = 0.41
A = *Zea mays* var. *everta*, B = *Zea mays* var. *saccharata*

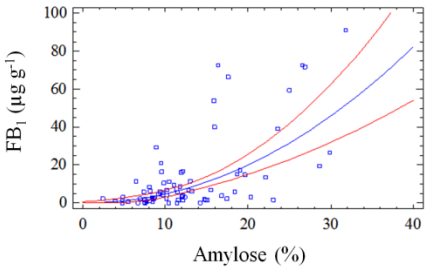

Fig. 6. Fumonisin FB_1 content in kernels in relation to amylose content in 2008

$FB_1 = (–0.143021 + 0.230423\ amylose)^2$, r = 0.65, r^2 = 45
$FB_1(A) = (–0.50 + 0.25\ amylose)^2$, r = 0.77
$FB_1(B) = (0.88 + 0.11\ amylose)^2$, r = 0.27
A = *Zea mays* var. *everta*, B = *Zea mays* var. *saccharata*

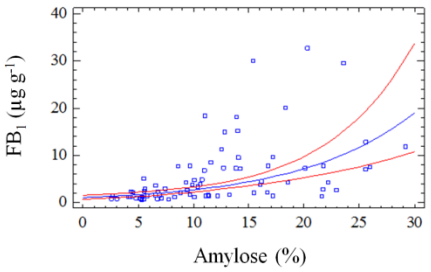

Fig. 7. Fumonisin FB_1 content in kernels in relation to amylose content in 2009

$FB_1 = \exp(–0.00571144 + 0.0982872\ amylose)$, r = 0.60, r^2 = 36
$FB_1(A) = \exp(–0.45 + 0.09\ amylose)$, r = 0.74
$FB_1(B) = \exp(–0.50 + 0.18\ amylose)$, r = 0.72
A = *Zea mays* var. *everta*, B = *Zea mays* var. *saccharata*

Figures 2–7. Regression curve between fumonisin FB_1 level (µg g^{-1}) in maize kernels inoculated with *Fusarium verticillioides* and water, starch and amylose content

The average value of the correlation coefficient (r) between fumonisin FB_1 and the water content in period 2008–2009 was negative and ranged from –0.66 to –0.55; the value of this parameter was significantly different in the case of individual varieties (Fig. 2 and 3). The lowest value of the correlation coefficient for these features was found in the analyzed material of *Z. mays* var. *saccharata*. In subsequent growing seasons, its value fluctuated significantly reaching –0.26 in 2008 (Fig. 2) and –0.67 in 2009 (Fig. 3). In addition, the fumonisin FB_1 level was significantly correlated with starch content (Fig. 4 and 5), including amylose (Fig. 6 and 7).

Value of the correlation coefficient was dependent on the maize variety, and detected range of this parameter between the fumonisin FB_1 level and the starch content, the two-year period amounted to *Z. mays* var. *everta* (0.81 and 0.62) and 0.17 to 0.41 for *Z. mays* var. *saccharata* (Fig. 4 and 5). Significantly higher values of the correlation coefficient were found between the fumonisin FB_1 level and the amylose content. In the same period, it amounted to *Z. mays* var. *everta* (0.77 and 0.74) and *Z. mays* var. *saccharata* (0.27 and 0.72) (Fig. 6 and 7).

DISCUSSION

Among the two botanical varieties of corn (*Z. mays* var. *saccharata* and *Z. mays* var. *everta*) included in the studies, sweetcorn was the highest infected, whereby regardless of the growing season super sweet genotypes of corn, with *Sh2* gene were significantly higher infected. Significaly stronger infection degree of plants with *Sh2* gene was caused by *F. verticillioides*, but also *F. graminearum* was reported by Ledenčan et al. [2008].

High susceptibility of kernels and seedling to diseases caused by *Fusarium* spp. was already indicated just after introduction of sugar varieties of maize to cultivation [Berger and Wolf 1974]. Their strong infection is associated with higher sugar content [Styler et al. 1980], even 2–3 times in the case of the *Sh2* hybrids [Szymanek et al. 2015]. In addition, the grains have much thinner pericarp [Baird et al. 1996].

Infection level of *Zea mays* var. *everta* by *F. verticillioides* was generaly low. The low susceptibility of popcorn to *F. verticillioides* infection, in relation to flint or semi-dent forms was mentioned also by Presello et al. [2007]. According to the authors, it is a consequence of significantly higher endosperm hardness of kernels as a result of the occurrence of hard and horny endosperm and greater protein than carbohydrate content, as compared to other botanical varieties of corn [Wu and Schwartzberg 1992].

Chemical analysis of fumonisin content in *Zea mays* var. *saccharata* and *Zea mays* var. *everta* samples showed the presence of fumonisin FB_1. In the plant material infected by *F. verticillioides*, it is the most common and most abundant occurring fumonisin derivative, in addition to fumonisin FB_2 and FB_3 [Marasas 1995, Mallmann et al. 2001, Pietri et al. 2004, Butrón et al. 2006, Desjardins 2006]. Occasionally, the occurrence of greater amount of FB_2 than FB_1 is noted, however these results are considered as uncertain [Ramirez et al. 1996]. Besides fumonisin B-series (FBs) in the corn kernels, the presence of analogues of A, C and P fumonisin groups were recognized [Gałązka et al. 2011, Shephard et al. 2011]. The metabolite share is estimated at less than 5% of the total pool, unless there are present [Bartók et al. 2006].

Although cobs of sweet corn were infected in significantly highest degree, fumonisin level match the content of this metabolite was less than in kernels of *Z.mays* var *everta*, which is significantly less infected botanical variety. This indicates that the infection degree and the ability to accumulate FB_1 are independent features.

Different content of metabolites in kernels of each variety may be the consequence of different capacity of fumonisin biosynthesis in an environment of individual grains. The problem of capacity of metabolites biosynthesis in infected plant tissues is analyzed extremely rare. According to Wit [2012], this parameter is negatively correlated with the value of osmotic potential. This means that the high water content of kernels of *Z. mays* var. *saccharata*, at 40% level and more, during the whole growing season slows down thus weakening the FB_1 biosynthesis. Therefore, even high infection of sweet varieties of corn not necessarily means a high general level of FB_1.

CONCLUSIONS

1. Water and starch content including amylose and amylopectin is dynamically changed during kernel development and it is inter-individual feature of selected botanical varieries of *Zea mays.*

2. The infection degree of corn cobs is a trait peculiar of varieties, with the clear trends of stronger infection of form, the kernels of which are characterized by slowly extending process of dehydration and low amylose content during development.

3. The starch and water content are factors that significantly affect the infection degree of corn cobs by *Fusarium verticillioides*.

4. Efficiency of fumonisin FB_1 biosynthesis is inversely proportional to kernel water content.

5. The level of fumonisin FB_1 synthesized by *Fusarium verticillioides* in infected cobs of *Zea mays* is a variety dependent feature.

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REFERENCES

Adetimirin, V.O., Kim, S.-K., Szczech, M. (2006). Factors associated with emergence of *Shrunken-2* maize in Korea. J. Agric. Sci., 144(1), 63–68. DOI: https://doi.org/10.1017/S002185960500571X.

Alan, O., Kinaci, G., Kinaci, E., Budak Basciftci, Z., Sonmez, K., Evrenosoglu, Y., Kutlu, I. (2014). Kernel quality of some sweet corn varieties in relation to processing. Not. Bot. Horti Agrobot., 42(2), 414–419. DOI: 10.1583/nbha4229425.

Baird, R.E., Huber, D.M., Mullinix, B.G. (1996). The mycobiota from seeds of *shrunken-2* (*sh2*) sweet corn. Mycopathologia, 132(3), 147–154. DOI: 10.1007/BF01103980.

Baird, R.E., Nankam, C., Fallah Moghaddam, P., Pataky, J.K. (1994). Evaluation of seed treatments on *shrunken-2* sweet corn. Plant Dis., 78, 817–821. DOI: 10.1094/PD-78-0817.

Bartók, T., Szécsi, Á., Szekeres, A., Mesterházy, Á., Bartók, M. (2006). Detection of new fumonisin mycotoxins and fumonisin-like compounds by reversed-phase high-performance liquid chromatography/electrospray ionization ion trap mass spectrometry. Rapid Commun. Mass Spectrom., 20(16), 2447–2462. DOI: 10.1002/rcm.2607.

Berger, R.D., Wolf, E.A. (1974). Control of seedborne and soilborne mycoses of 'Florida Sweet' corn by seed treatment. Plant Dis. Rep., 58, 922–923.

Bottalico, A., Perrone, G. (2002). Toxigenic *Fusarium* species and mycotoxins associated with head blight in small-grain cereals in Europe. Eur. J. Plant Pathol., 108(7), 611–624, https://doi.org/10.1023/A:1020635214971.

Butrón, A., Santiago, R., Mansilla, P., Pintos-Varela, C., Ordas, A., Malvar, R.A. (2006). Maize (*Zea mays* L.) genetic factors for preventing fumonisin contamination. J. Agric. Food Chem., 54(16), 6113–6117. DOI: 10.1021/jf0611163.

Chełkowski, J., Ritieni, A., Wiśniewska, H., Mulè, G., Logrieco, A. (2006). Occurrence of toxic hexadepsipeptides in preharvest maize ear rot infected by *Fusarium poae* in Poland. J. Phytopathol., 155(1), 8–12. DOI: 10.1111/j.1439-0434.2006.01173.x.

Czembor, E., Stępień, Ł., Waśkiewicz, A. (2015). Effect of environmental factors on *Fusarium* species and associated mycotoxins in maize grain grown in Poland. PLoS ONE, 10(7), e0133644. DOI: 10.1371/journal.pone.0133644.

Desjardins, A.E. (ed.). (2006). *Fusarium* mycotoxins. Chemistry, genetics, and biology. APS Press, St. Paul. DOI: 10.1111/j.1365-3059.2006.01505.x.

Ferguson, J.E., Dickinson, D.B., Rhodes, A.M. (1979). Analysis of endosperm sugars in a sweet corn inbred (Illinois 677a) which contains the sugary enhancer (se) gene and comparison of se with other corn genotypes. Plant Physiol., 63(3), 416–420.

Gałązka, A., Wit, M., Jabłońska, E., Wakuliński, W. (2011). Molekularne podstawy biosyntezy fumonizyn. Post. Nauk Roln., 4, 21–33.

James, M.G., Robertson, D.S., Myers, A.M. (1995). Characterization of the maize gene sugary1, a determinant of starch composition in kernels. Plant Cell., 7(4), 417–429. DOI: https://doi.org/10.1105/tpc.7.4.417.

Karababa, E. (2006). Physical properties of popcorn kernels. J Food Eng., 72(1), 100–107. DOI: https://doi.org/10.1016/ j.jfoodeng.2004.11.028.

Ledenčan, T., Sudar, R., Šimić, D., Zdunić, Z., Brkić, A. (2008). Sweet corn resistance to the ear rots caused by *Fusarium gramiearum* and *Fusarium verticillioides.* Cer. Res. Comm., 36(Suppl. 6), *119–120.*

Leonard, K.J., Bushnell, W.R. (eds). (2003). Fusarium head blight of wheat and barley. APS Press, St Paul.

Logrieco, A., Bottalico, A., Mulé, G., Moretti, A., Perrone, G. (2003). Epidemiology of toxigenic fungi and their associated mycotoxins for some Mediterranean crops. Eur. J. Plant Pathol., 109(7), 645–667. DOI: https://doi.org/ 10.1023/A:1026033021542.

Mallmann, C.A., Santurio, J.M., Almeida, C.A.A., Dilkin, P. (2001). Fumonisin B_1 levels in cereals and feeds from southern Brazil. Arq. Inst. Biol., 68(1), 41–45.

Manicacci, D., Falque, M., Le Guillou, S., Piégu, B., Henry, A.-M., Le Guilloux, M., Damerval, C., De Vienne, D. (2007). Maize *Sh2* gene is constrained by natural selection but escaped domestication. J. Evol. Biol., 20(2), 503–516. DOI: 10.1111/j.1420-9101.2006.01264.x.

Marasas, W.F.O. (1995). Fumonisins: Their implications for human and animal health. Nat. Toxins, 3(4), 193–198. DOI: 10.1002/nt.2620030405.

Paes, G.P., Viana, J.M., Silva, F.F., Mundim, G.B. (2016). Linkage disequilibrium, SNP frequency change due to selection, and association mapping in popcorn chromosome regions containing QTLs for quality traits. Genet. Mol. Biol., 39(1), 97–110. DOI: 10.1590/1678-4685--GMB-2015-0126.

Pataky, J.K., du Toit, L.J., Revilla, P., Tracy, W.F. (1998). Reactions of open-pollinated sweet corn cultivars to Stewart's wilt, common rust, northern leaf blight, and southern leaf blight. Plant Dis., 82(8), 939–944, https://doi.org/10.1094/PDIS.1998.82.8.939.

Pietri, A., Bertuzzi, T., Pallaroni, L., Piva, G. (2004). Occurrence of mycotoxins and ergosterol in maize harvested over 5 years in Northern Italy. Food Addit. Contam., 21(5), 479–487. DOI: 10.1080/02652030410001662020.

Presello, D.A., Iglesias, J., Botta, G., Eyhérabide, G.H. (2007). Severity of *Fusarium* ear rot and concentration of fumonisins in grain of Argentinian maize hybrids. Crop Prot., 26(6), 852–855. DOI: https://doi.org/ 10.1016/j.cropro.2006.08.004.

Ramirez, M.L., Pascale, M., Chulze, S., Reynoso, M.M., March, G., Visconti, A. (1996). Natural occurrence of fumonisins and their correlation to *Fusarium* contamination in commercial corn hybrids growth in Argentina. Mycopathologia, 135(1), 29–34. DOI: 10.1007/BF00436572.

Shephard, G.S., van der Westhuizen, L., Sewram, V., van Zyl, J., Rheeder, J.P. (2011). Occurrence of the C-series fumonisins in maize from the former Transkei region of South Africa. Food Addit. Contam. Part A., 28(12), 1712–1716. DOI: 10.1080/19440049.2011.605773.

Styler, R.C., Cantliffe, D.J., Hannah, L.C. (1980). Differential seed and seedling vigor in shrunken-2 compared to three other genotypes of corn at various stages of development. J. Am. Soc. Hortic. Sci., 105(3), 329–332.

Szymanek, M., Tanaś, W., Kassar, F. (2015). Kernel carbohydrates concentration in sugary-1, sugary enhanced and shrunken sweet corn kernels. Agric. Agric. Sci. Proc., 7, 260–264, https://doi.org/10.1016/j.aaspro.2015.12.044.

Wakuliński, W., Wit, M., Warzecha, R., Ochodzki, P., Waśkiewicz, A., Jabłońska, E. (2012). Susceptibility of maize varieties to opposite mating type strains of *Fusarium verticillioides*. Maydica, 57(1), 19–25.

Warzecha, R. (2013). Coraz większe znaczenie kukurydzy cukrowej w Polsce. In: Kukurydza – roślina przyszłości spełnia oczekiwania. Poradnik dla producentów. 7th ed. Agro Serwis, Warszawa, 33–36.

Waśkiewicz, A., Wit, M., Goliński, P., Chełkowski, J., Warzecha, R., Ochodzki, P., Wakuliński, W. (2012). Kinetics of fumonisin B1 formation in maize ears inoculated with *Fusarium verticillioides*. Food Addit. Contam. Part A., 29(11), 1752–1761, http://dx.doi.org/ 10.1080/19440049.2012.712061.

Wit, M. (2012). Znaczenie *Fusarium verticillioides* (Saccardo) Nirenberg w etiologii fuzariozy kolb kukurydzy Doctoral thesis, Warsaw University of Life Sciences – SGGW, Władysław Grabski Main Library, 208 pp.

Wit, M., Ochodzki, P., Warzecha, R., Wakuliński, W. (2007). Znaczenie *Fusarium verticillioides* (Sacc.) Nirenberg w etiologii fuzariozy kolb kukurydzy. Biul. IHAR, 245, 171–180.

Wu, P.J., Schwartzberg, H.G. (1992). Popping behavior and zein coating of popcorn. Cereal Chem., 69(5), 567–573.

Xu, J.R., Leslie, J.F. (1996). A genetic map of *Gibberella fujikuroi* mating population A (*Fusarium moniliforme*). Genetics, 143(1), 175–189.

Zadoks, J.C., Chang, T.T., Konzak, C.F. (1974). A decimal code for the growth stages of cerals. Weed Res., 14(6), 415–421. DOI: 10.1111/j.1365-3180.1974.tb01084.x.

Zajkowski, P., Kwaśna, H. (1987). Corn cob fusariosis – natural infection of various genotypes in 1985 and 1986. European Seminar "*Fusarium*, Mycotoxins, Taxonomy, Pathogenicity" Warsaw, 8–10 September 1987, Mycotoxin Res., 3, Suppl. 1, 95–98. DOI: 10.1007/BF03192038.