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ORIGINAL PAPER

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THE INFLUENCE OF SPRING BARLEY GRAIN (*Hordeum vulgare* L.) INFECTION BY *Bipolaris sorokiniana* (Sacc.) Shoem. ON THE LEAF INFECTION AND GRAIN CONTAMINATION BY STERIGMATOCYSTIN

Małgorzata Cegiełko[1]✉, Irena Kiecana[1], Elżbieta Mielniczuk[1], Agnieszka Waśkiewicz[2], Jan Bocianowski[2]

[1] University of Life Sciences in Lublin, Poland

[2] University of Life Sciences in Poznań, Poland

ABSTRACT

Bipolaris sorokiniana is a dangerous pathogen of plants from *Poaceae* family. A secondary metabolite with carcinogenic properties produced by this fungus is sterigmatocystin. A field experiment with inoculation of the grain of 9 spring barley genotypes with *B. sorokiniana* No. 36 was carried out in the years 2012–2014 in the Zamość region. Field observations revealed leaf spot caused by *B. sorokiniana* in all studied genotypes. In 2012, values of the leaf infection index varied from 21.88 (Oberek) to 48.12 (Hajduczek), in 2013 from 25.31 (Skald, Oberek) to 50.00 (STH 7910) and in 2014 from 21.88 (Oberek) to 50.00 (Hajduczek). In the experimental combination with *B. sorokiniana*, colonies of this fungus in the years 2012–2014 accounted for: 81.22%, 93.11% and 71.78%, respectively, and in control combination: 40.06%, 32.26% and 33.72%, respectively. The chemical analysis of grain of 9 barley genotypes obtained from plants in an experimental combination with *B. sorokiniana* in 2014, revealed the presence of sterigmatocystin in the genotypes: Hajduczek, Kormoran, Stratus and STH 7910. The sterigmatocystin concentration ranged from 5.39 ng·g^{-1} (STH 7910) to 67.05 ng·g^{-1} (Hajduczek). A statistically significant correlation was found between the value of the leaf infection index and the concentration of sterigmatocystin in the grain.

Key words: *Bipolaris sorokiniana*, spot blotch, spring barley, sterigmatocystin

INTRODUCTION

Bipolaris sorokiniana (Sacc.) Shoem. Teleomorph *Cochliobolus sativus* (Ito et Kurib.) Drechsler ex Dastur causes seedling blight, foliar spot blotch, root rot, and black points on the grain [Manamgoda et al. 2014]. It is one of the most important pathogens of barley cultivated in different regions of the world and Poland [Łacicowa and Pięta 1991, Kumar et al. 2001, 2002, Baturo 2005, Wiewióra 2009]. It is a serious pathogen of barley in the warm, humid climate of the world, such as the Upper Midwest region of the United States and the Prairie Provinces of Canada [Mathre 1982]. *Bipolaris sorokiniana* is the cause of seedling damping-off, and leaf spot disease of turfgrasses [Prończuk 2000, Kiecana et al. 2012, 2015].

The role of the seed material and the soil is significant as the source of *B. sorokiniana* infection for

✉ malgorzata.cegielko@up.lublin.pl

the plants obtained [Pandley et al. 2008, Wiewióra 2010, Chodwdhury et al. 2013, Kumar et al. 2015]. The fungus is a facultative pathogen that can live saprotrophically in soil. At the soil temperature of 20°C. *B. sorokiniana* can survive longer than 16 weeks [Subramanian 1983, Kwaśna 1995]. *B. sorokiniana* is passed through the grain through the mycelium located between chaffs and seed coat and by conidia contaminating the surface of the kernels. In infected grain it can survive up to 4 years [Barba et al. 2002]. The prepenetration phase of a fungal pathogen is important for its establishment on the host surface and its successful infection. Thus, spore attachment to the plant surface, germination, germ tube elongation and appressorium development are essential for penetration and further invasion. Spores of *B. sorokiniana* germinated on barley leaves between four and six hours [Yadav 1982; Dehne and Oerke 1985, Han et al. 2010] and after one hour on water agar at 22°C [Jansson et al. 1988, Han et al. 2010]. Appressoria develop more frequently over grooves formed by the juncture of anticlinal walls of epidermis cells [Clay et al. 1994,Han et al. 2010].

The leaf infection process of susceptible barley cultivars included biotrophic and a subsequent necrotrophic growth phase [Kumar et al. 2002]. The biotrophic phase includes cuticle and cell wall penetration, followed by the development of hyphae within the invaded, living epidermal host cell while the necrotrophic phase includes hyphae invasion into the mesophyll layer, accompanied by epidermal and mesophyll cell death [Kumar et al. 2002]. The increase of electrolyte leakage coincides with the host cell damage by *B. sorokiniana* in the necrotrophic phase of its growth [Wiśniewska et al. 1998].

Mycotoxins are produced by a wide array of diverse fungal species. Most of the mycotoxins that are considered to be important are produced primarily by three genera of fungi, namely *Aspergillus*, *Penicillium* and *Fusarium* [Richard and Payne 2003]. *Bipolaris sorokiniana* can produce secondary metabolites with phytotoxic effects [Nakajima et al. 1998, Kumar et al. 2002, Jahani et al. 2014]. These include: sorokinianin, helminthosporol, prehelminthosporol, victoxinin and bipolaroxin. They can play an important role in the etiology of spot blotch disease, affect the permeability of cell membranes, or the germination of cereal grains [Kachlicki 1995, Nakajima et al. 1998, Kumar et al. 2002, Jahani et al. 2014]. This species also produces sterigmatocistin possessing zootoxic properties [Aucamp and Holzapfel 1970, Baertschi et al. 1989]. Sterigmatocystin, a precursor of aflatoxins, is a toxic and carcinogenic secondary metabolite produced mainly by *Aspergillus nidulans* [Klich and Pitt 1988, Keller et al. 1994, 1997], *A. tamarii*, *A. ochraceoroseus* [Klich et al. 2000], and some *Bipolaris* and *Chaetomium* species [Aucamp and Holzapfel 1970, Baertschi et al. 1989]. Sterigmatocystin caused liver cell carcinoma in laboratory rats and was mutagenic in several in vitro tests [Berry 1988]. Tumorigenicity of sterigmatocystin was smaller than aflatoxin B1 to rats [Berry 1988]. Sterigmatocystin covalently bonds to DNA at approximately 20 to 30% of the level observed with aflatoxin B1 [Berry 1988].

Taking into consideration the great harmfulness of *B. sorokiniana* as the pathogen of leaves and grains of barley cultivated in the world and in Poland [Kumar et al. 2002, Arabi and Jawhar 2004, Wiewióra 2009, 2010, Al-Daoude et al. 2013] and little information on the ability of this fungus to produce sterigmatocystin in infected grains, it was considered appropriate to carry out such studies.

MATERIAL AND METHODS

The experiment was conducted in the years 2012–2014. Eight cultivars (Hajduczek, Kormoran, Oberek, Promyk, Serwal, Skald, Skarb, Stratus) and one line (STH 7910) of spring barley were double inoculated – under laboratory and field conditions by *B. sorokiniana* strain No. 36 at the experimental plots in the Zamość region (South-Eastern part of Poland) on a leached brown soil, formed on loess deposits [FAO 1998] where root crops had been grown previously. Every year the recommended rates of NPK fertilization [Korbas and Mrówczyński 2010] and manual weeding were applied. All inoculated lines were of Polish origin and were obtained from Plant Breeding Strzelce Ltd., Co. – IHAR-PIB Group The isolate of *B. sorokiniana* No. 36 was obtained from the culture collection at the Department of Phytopathology and

Mycology, the University of Life Sciences in Lublin (Poland). Pathogenicity of *B. sorokiniana* No. 36 was tested using the method of Mishra and Behr [1976] by the germination potential of Promyk cultivar grains following inoculation with the isolate. Isolate *B. sorokiniana* No. 36 reduced seed germination ability up to 5%.

In each year of the study the experiment included a block of plots sown with grain inoculated with *B. sorokiniana* No. 36 and a block of control plots sown with uninoculated grain. The methods of inoculation, mixture preparation and grain inoculation before sowing were the same as in the studies of triticale cultivars susceptibility on *Microdochium nivale* [Łacicowa and Kiecana 1986]. The experiment was set up using the fully randomized block method. In each year the experiment included a block with plots sown with grains artificially infected by *B. sorokiniana* and a block with control plots without grain and artificial infection. 100 grains of each genotypes were sown in four reps on plots with the area of 1.25 m^2 (2.5 × 0.5 m) (25 grains were sown in a row at a 10-cm-long distance).

In the flowering phase on each experimental plot the disease symptoms of 40 flag leaves (4 × 10) of each investigated spring barley genotype in the experimental combination with grain inoculated with *B. sorokiniana* No. 36 and in the control combination were determined according to the graphical scale [Łacicowa et al. 1991]. This assessment of leaf health was used to calculate the disease index according to the McKinney's formulae [Łacicowa 1969]. In the laboratory during three years of studies the mycological analysis of grain collected from plants developed from artificially inoculated grain and from the control grain was made in order to isolate fungi by means of the method described earlier by Kiecana and Mielniczuk [2010] using the mineral medium [Mielniczuk et al. 2010]. For each genotype in each year of study 50 grains were analyzed in both experimental combinations. The cultures of fungi were identified to the species using monographs and keys referenced in the paper by Kiecana et al. [2012].

Information on weather conditions during this study was obtained from the Department of Agrometeorology and Environmental Protection and Management, the University of Life Sciences in Lublin.

The chemical analysis on the presence of sterigmatocystin in samples of grain of 9 spring barley genotypes collected from plants developed from artificially inoculated grain with *B. sorokiniana* No. 36 was made at the Department of Chemistry the University of Life Sciences in Poznań. Each sample of the plant material (about 10 g) was collected in three biological replicates per genotype. Sterigmatocystin (STC) from barley samples was extracted with acetonitrile-water (9 : 1, v/v). The extracts after filtration were collected for chromatographic analysis. Quantitative and qualitative analysis of sterigmatocistin concentration for barley cultivars was performed using UPLC/TQD technique (Waters, Milford, PA, USA). An electrospray ionization (ESI) probe in the positive mode was used in the analysis of STC. The separation of sterigmatocistin was achieved using the analytical column (150 mm × 2.1 mm i.d., 3 µm particle size) with gradient elution. The mobile phase consisted of H_2O (A) and acetonitrile (B), both containing 0.1% (v/v) formic acid. The injection volume was 3 µL with the flow rate of the mobile phase 0.3 $mL \cdot min^{-1}$ and the column temperature of 30°C. For the structural identification in multiple reaction monitoring (MRM) mode, the molecular ion $[M+H]^+$ (m/z = 325) was fragmented within the MS to its product ions (325 > 310 and 325 > 281). Argon at the pressure of 3.5 bar was used as collision gas. The product ion with m/z = 281 was used for the quantification of STC. The limit of detection (LOD) and the limit of quantification (LOQ) were 0.03 $ng \cdot g^{-1}$ and 0.10 $ng \cdot g^{-1}$. Empower™ 1 software (Waters, Milford, PA, USA) was used for data processing.

The normality of distribution of the trait was tested using Shapiro&Wilk's normality test. Two-way analysis of variance (ANOVA) was carried out to determine the effects of year and cultivar as well as year-by-cultivar interaction on the variability of observed trait. When critical differences were noted, multiple comparisons were carried out, using Tukey's least significant differences (LSDs). Based on this, homogeneous groups (not significantly different from each other) were determined for analyzed trait in

each year of observation. The means and standard deviation for each cultivar calculated. Comparisons of isolates number for *B. sorokiniana* and control for individual genotypes were made by F-Snedecor test. The relationship between the leaf spot index and sterigmatocystin content was analyzed by the Pearson correlation coefficient. Analysis of the data was performed using the statistical package GenStat v. 17.

RESULTS

Field observations made at the flowering stage in 2012–2014, revealed leaf spot caused by *B. sorokiniana* in all the studied spring barley genotypes and in all growing seasons. However, differences were noticed in susceptibility of the barley cultivars and breeding line to leaf infection by this pathogen (tab. 1). On the infected leaf blades, round or slightly elongated spots were formed (up to 5 mm of diameter). Most of the spots were 2–3 mm of diameter, dark brown with a yellow halo. In the case of infection by *B. sorokiniana*, conidia were formed all over the surface of necrotic spots. In 2012, values of the leaf infection index of the analyzed genotypes varied from 21.88 (Oberek) to 48.12 (Hajduczek), in 2013 from 25.31 (Skald, Oberek) to 50.00 (STH 7910), while in 2014, from 21.88 (Oberek) to 50.00 (Hajduczek) and they differed significantly (tab. 1). Mean values of the leaf infection index in the studied barley cultivars and breeding line for the 3 years varied from 21.02 to 47.60 (fig. 1). After three years of experiments the mean values of leaf infection for plants obtained in the control combination ranged from 7.29 to 17.19 (fig. 1).

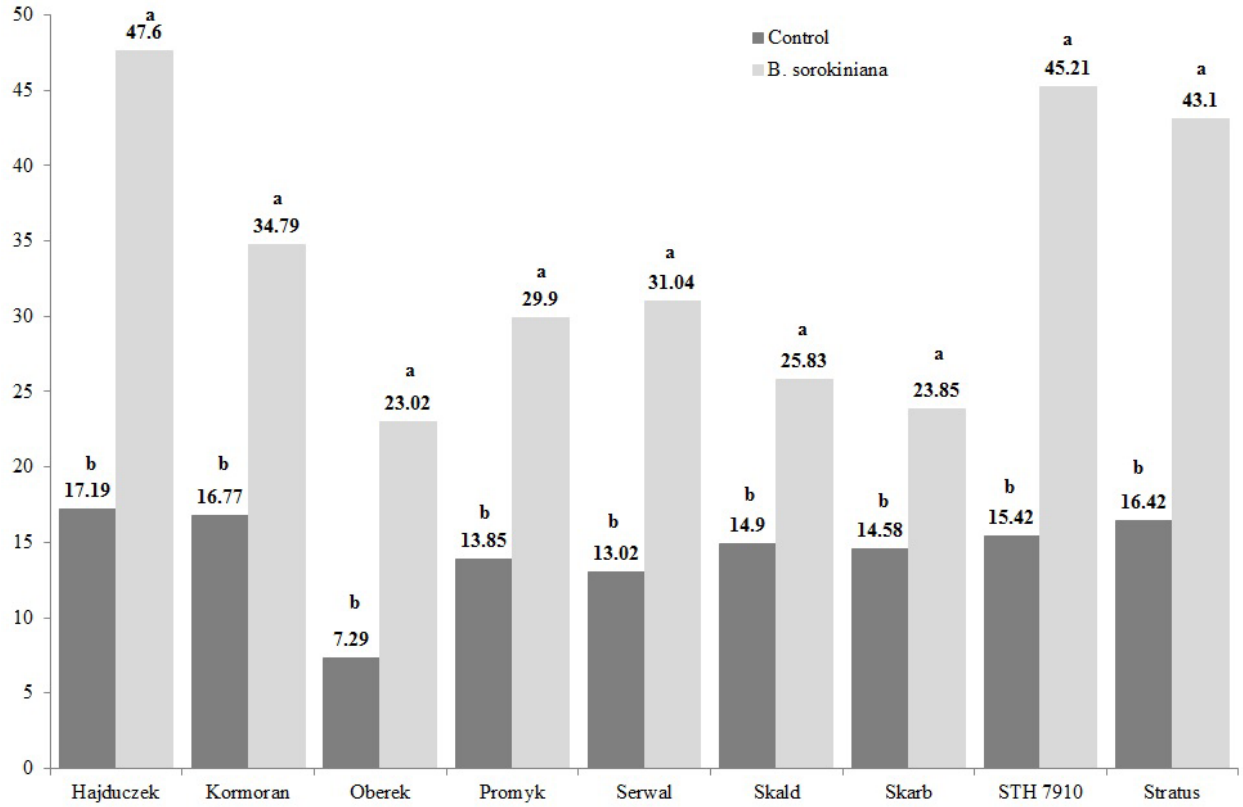

Fig. 1. Mean values of the disease index of the leaves of investigated spring barley genotypes obtained from plants grown from grain inoculated with *B. sorokiniana* No. 36 compared to the control in 2014

Table 1. Mean values of the disease index for the leaves of the investigated spring barley genotypes obtained in field conditions from plants in the experimental combination with artificial infection of grain with *B. sorokiniana* No. 36 and in control in years 2012–2014

Year	2012				2013				2014			
Combination	control		*B. sorokiniana*		control		*B. sorokiniana*		control		*B. sorokiniana*	
Genotype	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.
Hajduczek	18.12 bc	2.976	48.12 a	0.722	16.56 a-c	0.625	44.69 a	9.862	16.88 ab	0.722	50 a	2.7
Kormoran	16.25 cd	0	32.81 d	1.875	16.56 a-c	1.573	36.88 b	3.75	17.5 a	1.768	34.69 c	2.135
Oberek	7.19 f	0.625	21.88 e	1.25	9.38 de	2.394	25.31 c	2.366	5.31 e	0.625	21.88 e	1.25
Promyk	15.31 de	0.625	32.5 d	5.303	13.12 b-d	1.25	35 b	3.953	13.12 c	1.25	22.19 e	1.197
Serwal	18.75 ab	1.021	33.12 cd	5.543	8.44 e	1.197	36.88 b	6.495	11.88 c	0.722	23.12 e	1.25
Skald	14.38 de	0.722	23.75 e	1.443	15 a-c	1.021	25.31 c	1.875	15.31 b	1.197	28.44 d	1.197
Skarb	14.06 e	0.625	22.19 e	1.573	17.19 ab	2.135	25.62 c	1.614	12.5 c	0	23.75 e	1.021
STH 7910	19.38 ab	0.722	37.5 c	1.021	17.5 a	1.021	50 a	1.021	9.38 d	0.722	48.12 a	0.722
Stratus	20.62 a	2.165	43.44 b	4.002	13 cd	7.427	45.56 a	3.325	15.62 b	0.722	40.31 b	4.719
$LSD_{0.05}$	1.98		4.49		4.14		6.72		1.42		3.12	
Mean	16.01 B	4.029	32.81 A	9.25	14.08 B	4.08	36.14 A	9.833	13.06 B	3.82	32.5 A	10.942

Table 2. Fungi isolated from grains of investigated spring barley genotypes obtained from plants developed from the grain inoculated with the strain *B. sorokiniana* No. 36 in 2012

	Hajduczek		Kormoran		Oberek		Promyk		Serwal		Skald		Skarb		Stratus		STH 7910		Total		Total g+ng (%)
	n. isolates		n. isolates		n. isolates		n. isolates		n. isolates		n. isolates		n. isolates		n. isolates		n. isolates				
	g	ng	g	ng	g	ng	g	ng	g	ng	g	ng	g	ng	g	ng	g	ng	g	ng	
Alternaria alternata (Fr.) Keissler	0	0	2	0	0	0	0	0	0	0	7	0	2	0	0	0	2	0	13	0	13 (2.94)
Bipolaris sorokiniana (Sacc.) Shoem.	40	3	39	5	35	5	45	0	44	0	24	4	29	4	43	3	35	1	334	25	359 (81.22)
Cladosporium cladosporioides (Fresen.) G.A. de Vries	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	3 (0.68)
Drechslera teres (Sacc.) Shoem.	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	2	0	2 (0.45)
Epicoccum nigrum Link ex Link	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	2	0	2 (0.45)
Fusarium avenaceum (Fr.) Sacc.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	1(0.23)
Fusarium culmorum (W. G. Sm.) Sacc.	2	0	2	0	0	0	3	0	3	0	4	4	3	0	1	0	0	0	18	4	22 (4.98)
Fusarium poae (Peck.) Wollenw.	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	2	0	2 (0.45)
Fusarium sporotrichioides Sherb.	0	0	0	0	0	0	2	0	0	0	0	0	9	0	0	0	0	0	11	0	11 (2.49)
Mucor hiemalis Wehmer	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	1 (0.23)
Penicillium aurantiogriseum Dierckx	0	0	0	0	1	0	0	0	0	0	0	0	2	0	1	0	7	0	11	0	11 (2.49)
Trichoderma polysporum (Link.) Rifai	0	0	0	0	8	0	0	0	0	0	0	0	0	0	0	0	0	0	8	0	8 (1.81)
Trichothecium roseum Link	0	0	0	0	0	0	0	0	0	0	3	2	1	0	1	0	0	0	5	2	7 (1.58)
Total	45	3	44	5	45	5	50	0	50	0	40	10	46	4	47	3	44	1	411	31	442 (100)
Total g+ng	48		49		50		50		50		50		50		50		45		442		

g – germinating grains
ng – non-germinating grains
n. isolates – number of isolates

Table 3. Fungi isolated from grains of investigated spring barley genotypes obtained from plants developed from the grain inoculated with the strain *B. sorokiniana* No. 36 in 2013

Fungi species	Hajduczek		Kormoran		Oberek		Promyk		Serwal		Skald		Skarb		Stratus		STH 7910		Total		Total g+ng (%)
	n. isolates		n. isolates		n. isolates		n. isolates		n. isolates		n. isolates		n. isolates		n. isolates		n. isolates				
	g	ng	g	ng	g	ng	g	ng	g	ng	g	ng	g	ng	g	ng	g	ng	g	ng	
Alternaria alternata (Fr.) Keissler	0	0	0	0	0	0	0	0	0	0	0	0	1		2	1	6	4	9	5	14 (3.11)
Bipolaris sorokiniana (Sacc.) Shoem.	44	4	40	10	40	9	38	12	40	6	45	4	41	8	39	1	33	5	360	59	419 (93.11)
Epicoccum nigrum Link ex Link	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	1	0		2	1	3 (0.67)
Fusarium culmorum (W. G. Sm.) Sacc.	1	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	1	1	4	1	5 (1.11)
Fusarium poae (Peck.) Wollenw.	1	0	0	0	0	1	0	0	3	1	0	1	0	0	0	0	0	0	4	3	7 (1.56)
Fusarium sporotrichioides Sherb.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	2	0	2 (0.44)
Total	46	4	40	10	40	10	38	12	43	7	45	5	42	8	47	3	40	10	381	69	450 (100)
Total g+ng	50		50		50		50		50		50		50		50		50		450		

g – germinating grains
ng – non-germinating grains
n. isolates – number of isolates

Table 4. Fungi isolated from grains of investigated spring barley genotypes obtained from plants developed from the grain inoculated by the strain *B. sorokiniana* No. 36 in 2014

Fungi species	Hajduczek		Kormoran		Oberek		Promyk		Serwal		Skald		Skarb		Stratus		STH 7910		Total		Total g+ng (%)
	n. isolates		n. isolates		n. isolates		n. isolates		n. isolates		n. isolates		n. isolates		n. isolates		n. isolates				
	g	ng	g	ng	g	ng	g	ng	g	ng	g	ng	g	ng	g	ng	g	ng	g	ng	
Alternaria alternata (Fr.) Keissler	1	0	5	0	4	1	7	2	4	4	2	1	6	1	2	0	4	0	35	9	44 (9.78)
Bipolaris sorokiniana (Sacc.) Shoem.	44	6	35	6	23	9	30	0	28	9	26	4	25	4	35	6	27	7	273	50	323 (71.78)
Epicoccum nigrum Link ex Link	0	1	0	0	2	1	1	0	2	0	2	0	4	1		0	1	0	12	3	15 (3.33)
Fusarium culmorum (W. G. Sm.) Sacc.	0	0	1	0	4	0	1	2	0	0	2	3	5	1	2	4	3	1	18	11	29 (6.44)
Fusarium equiseti (Corda) Sacc.	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0			2	3	2	5 (1.11)
Fusarium oxysporum Schltdl.	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1 (0.22)
Fusarium poae (Peck.) Wollenw.	0	0	1	2	0	0	0	1	0	1	5	1	0	0	0	1	0	0	6	6	12 (2.67)
Fusarium sporotrichioides Sherb.	0	0	0	0	2	1	4	1	2	0	1	1	1	0	0	0	2	1	12	4	16 (3.56)
Mucor hiemalis Wehmer	1	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	3	0	3 (0.67)
Non sporulating forms	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1	2 (0.44)
Total	46	6	42	8	36	14	44	6	36	14	38	10	43	7	39	11	39	11	363	87	450 (100)
Total g+ng	52		50		50		50		50		48		50		50		50		450		

g – germinating grains
ng – non-germinating grains
n. isolates – number of isolates

Table 5. Fungi isolated from grains of investigated spring barley genotypes obtained from plants developed from the grain in control combination in 2012

Fungi species	Hajduczek		Kormoran		Oberek		Promyk		Serwal		Skald		Skarb		Stratus		STH 7910		Total		Total g+ng (%)
	n. isolates		n. isolates		n. isolates		n. isolates		n. isolates		n. isolates		n. isolates		n. isolates		n. isolates				
	g	ng	g	ng	g	ng	g	ng	g	ng	g	ng	g	ng	g	ng	g	ng	g	ng	
Alternaria alternata (Fr.) Keissler	8	0	3	0	25	0	8	1	0	0	15	0	18	0	14	0	2	0	93	1	94 (27.89)
Bipolaris sorokiniana (Sacc.) Shoem.	7	1	23	1	0	0	16	0	28	0	14	0	8	0	18	0	15	4	129	6	135 (4006)
Cladosporium cladosporioides (Fresen.) G.A. de Vries	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	2 (0.59)
Drechslera teres (Sacc.) Shoem.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	2	0	2 (0.59)
Epicoccum nigrum Link ex Link	0	0	0	0	0	0	0	0	0	0	1	0	6	0	0	0	2	0	9	0	9 (2.67)
Fusarium culmorum (W. G. Sm.) Sacc.	0	0	0	0	0	0	9	0	1	0	0	0	5	3	0	0	0	1	15	4	19 (5.64)
Fusarium graminearum Schwabe	0	0	8	0	1	0	0	0	9	0	4	0	0	0	0	0	0	0	22	0	22 (6.53)
Fusarium oxysporum Schltdl.	5	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	8	0	8 (2.37)
Fusarium sporotrichioides Sherb.	0	0	0	0	2	0	3	0	3	0	6	0	0	0	0	0	0	0	14	0	14 (4.16)
Penicillium aurantiogriseum Dierckx	0	0	3	0	2	0	4	0	0	0	0	0	3	0	0	0	1	0	13	0	13 (3.86)
Trichoderma viride Pers.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8	0	8	0	8 (2.37)
Trichothecium roseum (Pers.) Link	5	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	7	0	7 (2.08)
Non sporulating forms	1	1	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	3	1	4 (1.19)
Total	28	2	37	1	32	0	40	1	41	0	40	0	40	3	39	0	28	5	325	12	337 (100)
Total g+ng	30		38		32		41		41		40		43		39		33		337		

g – germinating grains
ng – non-germinating grains
n. isolates – number of isolates

Table 6. Fungi isolated from grains of investigated spring barley genotypes obtained from plants developed from the grain in control combination in 2013

Fungi species	Hajduczek		Kormoran		Oberek		Promyk		Serwal		Skald		Skarb		Stratus		STH 7910		Total		Total g+ng (%)
	n. isolates		n. isolates		n. isolates		n. isolates		n. isolates		n. isolates		n. isolates		n. isolates		n. isolates				
	g	ng	g	ng	g	ng	g	ng	g	ng	g	ng	g	ng	g	ng	g	ng	g	ng	
Alternaria alternata (Fr.) Keissler	19	7	8	5	0	0	25	11	16	5	13	5	0	0	0	0	0	0	81	33	114 (28.29)
Aureobasidium pullulans (de Bary & Löwenthal) G. Arnaud	0	0	0	0	8	0	0	0	0	0	0	0	0	0	0	0	0	0	8	0	8 (1.98)
Bipolaris sorokiniana (Sacc.) Shoem.	12	7	8	10	2	14	1	1	0	3	15	1	29	8	8	1	7	3	82	48	130 (32.26)
Drechslera teres (Sacc.) Shoem.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	3	0	0	6	3	9 (2.23)
Epicoccum nigrum Link ex Link	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	1	0	4	0	4 (0.99)
Exserohilum pedicellatum (A.W. Henry) K.J. Leonard & Suggs	0	0	4	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	3	7 (1.74)
Fusarium avenaceum (Fr.) Sacc.	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	2	2 (0.50)
Fusarium culmorum (W. G. Sm.) Sacc.	0	0	0	0	16	4	0	0	8	0	0	0	6	3	0	0	10	10	40	17	57 (14.14)
Fusarium crookwellense Burgess, Nelson, Toussoun	0	0	0	0	0	0	7	0	1	0	0	0	0	0	0	0	5	5	13	5	18 (4.47)
Fusarium equiseti (Corda) Sacc.	0	0	0	0	0	0	0	0	2	0	1	1	0	0	0	0	3	2	6	3	9 (2.23)
Fusarium poae (Peck.) Wollenw.	3	0	7	5	3	0	2	0	2	2	2	0	4	0	9	5	0	0	32	12	44 (10.92)
Fusarium sporotrichioides Sherb.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	1 (0.25)
Total	34	14	27	23	29	18	35	12	32	10	31	8	39	11	24	10	26	20	277	126	403 (100)
Total g+ng	48		50		47		47		42		39		50		34		46		403		

g – germinating grains
ng – non-germinating grains
n. isolates – number of isolates

Table 7. Fungi isolated from grains of investigated spring barley genotypes obtained from plants developed from the grain in control combination in 2014

Fungi species	Hajduczek		Kormoran		Oberek		Promyk		Serwal		Skald		Skarb		Stratus		STH 7910		Total		Total g+ng (%)
	n. isolates		n. isolates		n. isolates		n. isolates		n. isolates		n. isolates		n. isolates		n. isolates		n. isolates				
	g	ng	g	ng	g	ng	g	ng	g	ng	g	ng	g	ng	g	ng	g	ng	g	ng	
Alternaria alternata (Fr.) Keissler	4	1	0	0	6	0	1	1	4	0	2	0	0	0	6	0	2	1	25	3	28 (6.56)
Bipolaris sorokiniana (Sacc.) Shoem.	17	3	27	2	0	0	16	1	26	2	20	5	10	5	9	1	0	0	125	19	144 (33.72)
Epicoccum nigrum Link ex Link	1	0	6	3	2	0	0	0	2	0	2	0	0	0		1	2	0	15	4	19 (4.45)
Fusarium culmorum (W. G. Sm.) Sacc.	3	1	8	0	15	1	9	2	6	0	0	0	8	6	1	1	19	7	69	18	87 (20.37)
Fusarium crookwellense Burgess, Nelson, Toussoun	0	0	0	0	0	0	0	0	1	2	9	2	2	0	16	5	0	0	28	9	37 (8.66)
Fusarium equiseti (Corda) Sacc.	0	1	1	0	4	0	4	2	0	0	0	0	0	0	0	0	0	2	9	5	14(3.28)
Fusarium oxysporum Schltdl.	0	0	0	0	3	2	0	0	1	0	0	0	0	0	0	0	0	0	4	2	6 (1.41)
Fusarium poae (Peck.) Wollenw.	5	0	0	0	2	0	0	0	4	0	3	0	0	2	1	2	0	0	15	4	19 (4.45)
Fusarium sporotrichioides Sherb.	0	1	1	0	1	0	9	2	0	0	7	0	6	2	7	0	0	0	31	5	36 (8.43)
Rhizopus stolonifer (Ehrenb.) Vuill	5	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	9	1	18	1	19 (4.45)
Non sporulating forms	8	0	0	0	0	0	2	1	0	0	0	0	0	0	0	0	7	0	17	1	18 (4.22)
Total	43	7	43	5	37	3	41	9	44	4	43	7	26	15	40	10	39	11	356	71	427 (100)
Total g+ng	50		48		40		50		48		50		41		50		50		427		

g – germinating grains
ng – non-germinating grains
n. isolates – number of isolates

As a result of the statistical analysis of the disease index for leaves of spring barley genotypes grown from grains artificially infected with *B. sorokiniana* No. 36, including three years of studies 2012–2014, significant differences were found compared to control for each year of the study and for the means of the three years of the study (tab. 1, fig. 1).

In the analyzed years of studies 2012–2014 significant differences were also noted in the values of the disease index for leaves between the studied spring barley genotypes. In 2012, in the combination experiment with *B. sorokiniana*, there were no significant differences in the values of the disease index for leaves – between cv. Oberek, Skald and Skarb, and between Kormoran and Promyk. In 2013, there were no significant differences in the disease index between genotypes Hajduczek, Stratus and STH 7910, between the Kormoran, Serwal and Promyk cv. as well as Oberek, Skald and Skarb cv., while in 2014 significant differences in the values of the disease index for leaves were not found between genotypes STH 7910 and Hajduczek, and between cultivars Oberek, Promyk, Serwal and Skarb (tab. 1).

As a result of the mycological analysis of grain collected from plants of the studied spring barley genotypes, developed from the grain inoculated by the strain *B. sorokiniana* No. 36 in years 2012–2014: 442, 450 and 450 fungal colonies were obtained, respectively (tab. 2–4). In 2012 out of this number 411 colonies were derived from germinating grains and 31 from non-germinating grains (tab. 2), in 2013–381 and 69 isolates, respectively (tab. 3) and in 2014 – 363 and 87 fungal colonies, respectively (tab. 4).

In 2012 isolates of *B. sorokiniana* accounted for 81.22%, in 2013 – 93.11%, and in 2014 – 71,78% of the total number of fungi isolated (tab. 2–4). Isolates of other fungi in the experimental combination with grain inoculation with *B. sorokiniana*, belonged to: *Alternaria alternata*, *Epicoccum nigrum*, *Mucor hiemalis*, *Cladosporium cladosporioides*, *Penicillium aurantiogriseum*, *Trichoderma polysporum* and *Drechslera teres*. Fungi of the genus *Fusarium* were represented by: *F. avenaceum*, *F. culmorum*, *Fusarium equiseti*, *F. poae* and *F. sporotrichioides* (tab. 2–4).

As a result of the mycological analysis of grain collected from control plants of the studied spring barley genotypes in the years 2012–2014, 337 fungal colonies were isolated in 2012 (325 colonies from germinating grain and 12 from non-germinating grain), 404 in 2013 (277 from germinating grain and 126 from non-germinating grain) and 427 in 2014 (356 colonies from germinating grain and 71 from non-germinating grain) (tab. 5–7). From grain of 9 spring barley genotypes collected in the control combination, colonies of B. sorokiniana were isolated the most frequently and in the years 2012–2014 they accounted for 40.06%, 32.26% and 33.72%, respectively, of the total number of fungi (tab. 5–7).

After three years of studies the number of *B. sorokiniana* colonies obtained from grain of each of the analyzed spring barley genotypes in a combination with artificial grain inoculation with *B. sorokiniana* No. 36 differed significantly compared to the number of isolates of this fungus obtained from the grain in the control combination in the case of six cultivars (tab. 8), whereas in the case of the number of *B. sorokiniana* isolates obtained from the cultivars Serwal, Skald and Skarb, there were no significant differences (tab. 8). The species frequently isolated from the control grain was *A. alternata*. Colonies of other fungi belonged to: *Aureobasidium pullulans*, *C. cladosporioides*, *D. teres*, *E. nigrum*, *P. aurantiogriseum*, *R. stolonifer*, *Trichoderma viride*, *Trichothecium roseum* and non-sporulating forms. Fungi of genera *Fusarium* were also isolated from the control grain and they were represented by: *F. avenaceum*, *F. culmorum*, *F. equiseti*, *F. graminearum*, *F. poae* and *F. sporotrichioides* (tab. 5–7).

In Zamość the growing season in 2012 was characterized by higher mean monthly temperatures in April, July and August, as compared to long-term monthly means for those months by (0.6–3.1°C). Monthly rainfall, compared with long-term monthly means, was higher only in August (by 164.5%), but much lower mainly in May and June. During the growing season in 2013, monthly mean air temperatures were generally a little higher than long-term monthly means (by 0.1–1.2°C), except in July, which was 0.4°C colder than average. In 2013 in May and

June the monthly rainfall exceeded long-term monthly means, by 52.7% and 115.5%, respectively. In the growing season 2014 monthly mean temperatures were higher than long-term means in April, May, July and August (by 0.3–2.5). Monthly rainfall was generally higher than long-term monthly means in April, May, July and in August, by: 15%, 180%, 11% and 63%, respectively. The lowest precipitation was recorded in June of that year (tab. 9).

The chemical analysis of grain of 8 cultivars and one line of spring barley obtained from plants developed from the grain inoculated by the strain *B. sorokiniana* No. 36 in the year 2014 revealed the presence of sterigmatocystin in the case of four genotypes: Hajduczek, Kormoran, Stratus and STH 7910. The detected sterigmatocystin concentration ranged from 5.39 $ng \cdot g^{-1}$ (STH 7910) to 67.05 $ng \cdot g^{-1}$ (Hajduczek) (tab. 10).

The statistical analysis revealed a statistically significant correlation (r = 0.6796 at p = 0.044), between the leaf spot index and sterigmatocystin content in the grain obtained from the plants tested for spring barley genotypes, grown under strict field experiment.

Table 8. Correlation between the number of *Bipolaris sorokiniana* isolates obtained from the grain of investigated spring barley genotypes in the experimental combination with grain artificial infection with *B. sorokiniana* No. 36 and in control from three years of the studies

Genotype		*B. sorokiniana*	Control	ANOVA *F*	$LSD_{0.05}$
Hajduczek	mean	47 a	15.67 b	0.002	12.14
	s.d.	3.606	6.658		
Kormoran	mean	45 a	23.67 b	0.007	11.48
	s.d.	4.583	5.508		
Oberek	mean	40.33 a	5.33 b	0.008	20.13
	s.d.	8.505	9.238		
Promyk	mean	41.67 a	11.67 b	0.018	21.43
	s.d.	10.408	8.386		
Serwal	mean	42.33 a	19.67 a	0.061	24.34
	s.d.	4.726	14.434		
Skald	mean	35.67 a	18.33 a	0.082	20.82
	s.d.	11.59	5.859		
Skarb	mean	37 a	20 a	0.186	29.6
	s.d.	10.58	15.13		
Stratus	mean	42.33 a	12.33 b	<0.001	9.44
	s.d.	3.215	4.933		
STH 7910	mean	37.33 a	9.67 b	0.009	16
	s.d.	3.055	9.504		
Total	mean	367 a	136.3 b	0.001	78.6
	s.d.	48.5	7.09		

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

Table 9. Air temperature and rainfall in Zamość during the growing seasons of spring barley 2012–2014

Month	Long-term monthly means (1971–1995)		Deviations of temperatures			Percentage of long-term monthly mean rainfall (%)		
	Temperature (°C)	Rainfall (mm)	2012	2013	2014	2012	2013	2014
April	8	40	+1.9	+0.1	+0.4	87	90.7	115
May	15	100	0	+0.9	+1.2	55.3	152.7	280
June	18	170	–0.1	+1.2	–1.0	48.82	215.5	77
July	18	70	+3.1	–0.4	+2.5	75.57	71.1	111
August	18	20	+0.6	+0.2	+0.3	264.5	36.8	163

Table 10. Sterigmatocystin content ($ng \cdot g^{-1}$) in the grain of spring barley genotypes developed from the grain artificially infected with *B. sorokiniana* No. 36 in 2014

Barley genotypes	Sterigmatocystin concentration ($ng \cdot g^{-1}$)
Hajduczek	67.05
Kormoran	13.81
Oberek	Nd
Promyk	Nd
Serwal	Nd
Skald	Nd
Skarb	Nd
Stratus	6.20
STH 7910	5.39

DISCUSSION

To our knowledge, the present paper is one of the first reports on agronomic performance, genotype resistance on leaf infection by *B. sorokiniana* and the formation of sterigmatocystin metabolite in spring barley grain obtained from the grain of selected genotypes inoculated by the strain *B. sorokiniana* No. 36. The created conditions of artificial barley's grain inoculation by *B. sorokiniana*, caused the appearance of spot blotch on the leaves of plants obtained from it.

Disease symptoms on barley leaves caused by *B. sorokiniana* in the form of light brown lesions with whitish gray centers and chlorotic margins were similar to the symptoms caused by this species on the leaves of oat [Cegiełko 2006] and wheat [Prates and Fernandes 2001] and the same as on barley leaves [Kumar et al. 2002].

In the autumn of 2007 this pathogen caused root rot and leaf spot of turfgrasses on the lawns in Lublin [Kiecana et al. 2015]. Infection of host tissues by *B. sorokiniana* includes several phases – from germination of conidia on the plant surface and the formation of an appressorium from the germ tube that supports direct penetration of the host surface by an infection hypha and colonization of the host plant tissue [Dehne and Oerke 1985, Yadar 1981, Han et al. 2010].

According to Chaurasia et al. [1999], the risk of spot blotch epidemics is high in areas characterized by average temperature above 17°C during the crop-

ping season with high relative humidity. In the three analyzed growing seasons, during which the experiments were carried out, weather conditions were favorable for spring barley infection by *B. sorokiniana*, mainly in 2013, because May and June in that year were characterized by humid and warm weather. It was confirmed by the highest values of the disease index for leaves in the case of most spring barley genotypes. Similar weather conditions in June 2007 were also favorable for the infection of barley leaves by *B. sorokiniana* in natural infection conditions [Wiewióra 2009]. Precipitation and high temperatures in June, during the heading, have a significant influence on the development of *B. sorokiniana* and seed infection by this fungus [Agarwal and Sinclair 1997, Cegiełko 2006, Wiewióra 2009]. The frequency of barley grain infection by *B. sorokiniana* also depends on the amount of the infectious material, most commonly airborne conidia transferred from infected leaves, which are in the vicinity of plants during flowering and grain formation [Łacicowa 1970].

A large numbers of colonies of *B. sorokiniana* isolated from the experimental combination with grain artificially inoculated with this species, shows that the grain of cereals is an important source of infection for the plants developed from it, seedling blight, spot blotch and black points on grain surface [Kiecana and Cegiełko 2007, Kumar et al. 2015].

Field trials showed that leaf infection affects weaker barley grain formation and is the cause of black points on the grain surface, like it is in the case of oats [Clear et al. 2000]. The results confirmed previous national reports that barley grain, like other plants of the *Poaceae* family, is infected by the fungi of the genus *Fusarium* including toxinogenic species: *F. culmorum*, *F. sporotrichioides* and *F. poae* [Perkowski et al., 1996, 2003, Perkowski and Kiecana 1997, Mielniczuk et al. 2010, Kiecana et al. 2012, 2014]. In Poland it was established that *F. culmorum* has a significant share in the infection of the stem base of barley [Łacicowa et al. 1990, Łacicowa and Kiecana 1991] as well as the roots and lower internodes of ornamental grasses [Kiecana et al. 2014] or the roots and leaves of turfgrasses [Kiecana et al. 2015]. With the common spike and grain infection by *F. culmorum* and *B. sorokiniana*, the second pathogen is attributed to the foreground [Eng-Chong Pua and Pelletier 1985]. During the occurrence at the same time, these fungi probably do not harm each other. As with other plants of the *Poaceae* family, the species *Alternaria alternata* is often found on barley grain [Kiecana 1994, Muthomi et al. 2008, Wiewióra 2009, Kiecana et al. 2012, 2014].

The species isolated from barley grains proved to be *Epicoccum nigrum*. This fungus has antagonistic effects on fungi transmitted with the seed material such as *Botrytis cinerea* and *Colletotrichum gleosporioides* [Pascual et al. 2002]. *Epicoccum nigrum* is an antagonist of many pathogens, including *B. sorokiniana* [Zhou and Reeleder 1989].

By analyzing the presence of sterigmatocystin, it was shown that this toxin is produced in barley grains infected with *B. sorokiniana.* The content of sterigmatocystin in the grains of the examined spring barley genotypes makes it possible to recognize *B. sorokiniana* as a species that is responsible for the large accumulation of this toxin in barley grains along with *Aspergillus tamarii*, *A. ochraceus*, *A. flavus*, *A. nidulans* [Keller et al. 1994, 1997, Klich et al. 2000]. The optimum temperature for sterigmatocystin production by *B. sorokiniana* is 23°C [Rabie et al. 1976]. In the case of the studies conducted, July temperatures above 20°C in the growing season of 2014 favored the formation of this metabolite in the contaminated grain of the analyzed spring barley genotypes.

CONCLUSIONS

1. Climate conditions have a modifying influence on the occurrence of spot blotch and the infection of spring barley grain by *B. sorokiniana*.

2. Bipolaris sorokiniana, as a species infected barley grain, deserves on consideration for its ability to produce significant amounts of sterigmatocystin in infected barley grains.

3. Barley grains infection caused by *B. sorokiniana* depends on the degree of leaf infection.

4. Cultivar Hajduczek should not be cultivated under Lublin conditions as it is susceptible to leaf infection and grain contamination by sterigmatocystin.

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