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Article

Resistance to Fusarium head blight, kernel damage and concentration of *Fusarium* mycotoxins in grain of winter triticale (*x Triticosecale* Wittmack) lines

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Abstract: Fusarium head blight (FHB) can cause contamination of cereal grain with mycotoxins. Triticale is also infected with FHB; however, it is more resistant than wheat to head infection. The aim of this study was to identify triticale lines that combine low head infection with low toxin contamination. Resistance to FHB of 15 winter triticale and three winter wheat lines was evaluated over a three-year experiment established in two locations. At the anthesis stage, heads were inoculated with *Fusarium culmorum* isolates. The FHB index was scored and the percentage of *Fusarium*-damaged kernels (FDKs) assessed. The grain was analysed for type B trichothecenes (deoxynivalenol and derivatives, nivalenol) and zearalenone content. The average FHB index was 10.7%. The proportion of FDK was 18.1% (weight) and 21.6% (number). An average content of deoxynivalenol for wheat amounted to 7.258 mg/kg and nivalenol to 5.267 mg/kg. In total, it was 12.788 m/kg of type B trichothecenes. The zearalenone content in the grain was 0.805 mg/kg. Relationships between FHB index, FDK and mycotoxin contents were statistically significant for triticale lines; however, they were stronger for FDK versus mycotoxins. Lines combing all types of FHB resistance were found, and two of them had resistance similar to that of wheat lines with the *Fhb1* gene.

Keywords: deoxynivalenol, *Fusarium culmorum*, Fusarium head blight, nivalenol, triticale, trichothecenes, zearalenone

1. Introduction

Triticale (x *Triticosecale* Wittmack) is a fully artificial and the first obtained amphiploid cereal, which originated in 1874, from hybridization of hexaploid wheat (*Triticum aestivum* L.) and rye (*Secale cereale* L.) [1]. The intergeneric synthetic hybrids combined the complementary traits of both parental species—the high yielding capacity of wheat and the stress tolerance of rye. Because triticale compromises the beneficial agronomic traits of wheat and the resistance to environmental stresses of rye, at the end of 20th century, the production of this cereal had significantly grown [2,3]. Triticale cultivation has been raised twofold, from 6 M tons in 1995 to almost 13 M tons in 2018 worldwide. Similarly, in Poland, cultivation increased from 2 M tons to four M tons over 23 years. Although the arable land for wheat (2,417,227 ha) is twice as large than for the triticale species (1,287,969 ha), Poland leads in the production of this crop worldwide. In Poland, triticale is cultivated twice as much as in Germany and four times more than in Belarus, France, Spain and China (FAOSTAT, 2020). Nowadays, triticale plants are used in a variety of ways, mostly as grain intended for feed and food production. Additionally, during spring, the land cultivated for triticale is used for pasture, as fresh feed for livestock or for hay and silage. Recently triticale has also been cultivated for biofuels [3] and bioethanol [4]. Moreover, humans and farm animals consume triticale grain; thus, it is important to maintain good quality grains, especially in the case of detrimental toxins content.

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Recently, a decrease in triticale resistance to pathogens of the *Fusarium* genus has been observed. Fusarium head blight (FHB) is a destructive disease of wheat and triticale, which causes significant loss of yield and quality as well as the accumulation of hazardous mycotoxins in the grain. Numerous species of *Fusarium* have been associated FHB in triticale and wheat, especially *Fusarium culmorum* (W.G. Smith) Sacc. [5,6]. Suitable, rainy weather during the flowering and soft dough stages of kernel development plays a crucial role for the establishment and severity of the disease [7–11]. *Fusarium* develops in the infected flower, then overgrows to the next ones and afterwards spreads through the rachis along the whole head. *Fusarium* colonise chaff and kernels in the ear, damaging them at different levels. It reduces grain yield and grain quality by contaminating grain with mycotoxins [12,13]. FHB resistance consists of several mechanisms (types): resistance to initial infection (type I), resistance to *Fusarium* spread within the spike (type II), resistance to kernel infection (type III), tolerance to accumulated toxins (type IV), resistance to accumulation of *Fusarium* toxins in the grain (chemical modification/synthesis inhibition = type V) [14–16].

Resistance to FHB is a quantitative feature [17]. The presence of several quantitative trait loci (QTL) associated with FHB resistance has been reported. Loci associated with FHB resistance originate from various types of Asian spring wheats, e.g., "Sumai 3", "Wuhan 1", "Nyubai", "Wangshuibai", "Nobeokbozukomugi" [18].

Breeding for improved FHB resistance is laborious task as this trait is quantitative in nature. It is greatly affected by the genetic characteristics of the host plant and fungal pathogens. Environmental conditions, mostly temperature and rainfall, from anthesis to the soft dough stage also have a substantial influence on FHB's development and make efficient selection a difficult task [7–9,12,14,19]. Breeding cultivars' resistant to FHB plays a key role in disease control and the prevention of mycotoxin contamination [20–22].

Fusarium species produce numerous toxic secondary metabolites (mycotoxins) belonging to different chemical groups. Those most often found in cereal grains are type B trichothecenes: deoxynivalenol (DON) and nivalenol (NIV); type A trichothecenes: T-2 and HT-2 toxins; zearalenone (ZEN) and moniliformin [23]. They are extremely stable, non-metabolizable compounds with great harm to humans and animals [24]. Grain contamination with mycotoxins is found even when no reduction in yield is observed. Recent research shows that triticale, like wheat, is significantly threatened by FHB and the critical accumulation of mycotoxins in grain.

A large issue with modern cereal cultivation is the presence of *Fusarium* mycotoxins, such as NIV, DON and ZEN in grain [25]. In 2007, the European Commission set the maximum level of deoxynivalenol in common wheat, triticale and rye grains at 1.250 mg/kg and zearalenone at 0.100 mg/kg. The maximum level of DON was also established in flour at 0.750 mg/kg and in bread at 0.500 mg/kg level. The maximum ZEN content in flour was set at 0.075 mg/kg and in bread at 0.050 mg/kg (Commission Regulation (EC) no. 1126/2007). Whereas, in feed production, the lowest level of DON is required in pig feed production at 0.9 mg/kg and ZEN in piglet and sow feed production at 0.1 mg/kg (Commission Regulation (EC) no. 2006/576). WHO, together with the Food and Agriculture Organization (FAO), in 2000, settled the daily maximum intake of ZEN at 0.5 μg/kg per body weight (Joint FAO/WHO Expert Committee on Food Additives, 1999) and in 2001 the DON level at 1 μg/kg per body weight (Joint FAO/WHO Expert Committee on Food Additives, 2001). In 2011, the European Food Safety Authority decreased the maximum ZEN daily intake to 0.25 μg/kg per body weight (EFSA, 2019). Small grain cereals differ considerably in their resistance to FHB and accumulation of mycotoxins. Research which compared triticale and its parental forms in terms of FHB and deoxynivalenol content revealed that triticale and rye had the lowest FHB severity and kernel damage, whereas the lowest deoxynivalenol concentration was obtained in rye followed by triticale and wheat species [25]. However, in an earlier by paper Langevin et al. [26], they found that triticale responded similarly to wheat to the point inoculation with *F. graminearum*. In our study, we observed that triticale lines were more resistant to FHB and kernel damage than wheat but were similar in regard to *Fusarium* toxins accumulation [27]. Depending on environmental conditions and genotype, triticale could accumulate high amounts of trichothecene toxins [13,28].

The aim of this study was to compare the susceptibility of winter triticale lines to Fusarium head blight and accumulation of mycotoxins in grain. Experiments were established under different

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condition in two locations in Poland. Plants were inoculated with *Fusarium culmorum* isolates. We studied different types and mechanisms of resistance: resistance to head infection, resistance to kernel damage, tolerance to accumulated toxins, and resistance to accumulation of *Fusarium* toxins in the grain.

2. Materials and Methods

5.1 Materials for experiments

Plant material comprised 18 triticale and wheat lines of winter type:

- Polish triticale cultivar "Meloman" and 14 breeding lines: "BOH 1025-2", "BOH 1062", "BOH 534-4", "BOH 537-2", "BOH 898-1", "DANKO 6 (2014)", "DANKO 9(2013)", "DL 446", "DL 593/07", "DS.1238", "DS.9", "LD 121/08", "MAH 33544-4", "MAH 33881-1/3";
- Polish susceptible wheat breeding line "DL 325/11/3" [29];
- Lines of wheat resistant to FHB with *Fhb1* gene: "S10" and "S32" [30].

Polish breeding companies developed the above triticale lines. They were selected based on low *Fusarium* head infection investigated in two environments (data not shown) [13].

5.2 Fungal material for inoculation

The material for inoculum production consisted of three isolates of *Fusarium culmorum* (W.G.Sacc.). KF 846 (DON chemotype) and KF 350 (NIV chemotype) originated from the collection of the Institute of Plant Genetics Polish Academy of Sciences (Poznań, Poland). The ZFR 112 (DON chemotype, producing high amounts of ZEN in vitro) originated from the collection of Plant Breeding and Acclimatization Institute, National Research Institute (NRI) (Radzików, Poland) [31].

Isolates were incubated on autoclaved wheat grain in glass Erlenmeyer flasks (300 ml) for one week at 20 °C in darkness and then exposed to near UV light (360 nm) under a 16 h photoperiod for 3 weeks at 15 °C. Flask were manually shaken daily to avoid the grain sticking together and to break-p the mycelial clumps. The mycelium-colonised grain with visible spore masses was air dried and stored in a refrigerator at 2–5 °C. Prior to the inoculation, the grain with *F. culmorum* spores was soaked in distilled water for approximately 2 h. Next, the suspension was filtered through a double cheesecloth layer to harvest spores and remove grains and mycelium. The conidial suspensions from three *F. culmorum* isolates were adjusted to 500,000 spores/ml using a haemocytometer. Equal volumes of suspension from the three isolates were mixed.

5.3 Description of the field experiment

A three-year field experiment (2016, 2017 and 2018) was established in two locations. The first experimental field location was the Institute of Plant Genetics Polish Academy of Sciences in Cerekwica (30 km north–west from Poznań, Poland; 82 m above sea level; GPS coordinates 52.522579, 16.688624). The second experimental field location was the Plant Breeding and Acclimatization Institute, NRI in Radzików (central Poland; 87 m above sea level; GPS coordinates 52.212612, 20.633111).

Experiments were established as randomized block designs. Triticale lines were sown in 1 m^2 (Radzików) or 0.5 m^2 (Cerekwica) plots in four replicates/blocks.

5.4 Inoculation procedure

At full anthesis (65 BBCH scale), at the end of May to a five-day period in June, triticale lines were inoculated by spraying heads with a spore suspension [32]. Three blocks of plots were inoculated and a fourth served as a control. Inoculation was repeated three days later. Two days after inoculation, micro-irrigation was applied to maintain high moisture levels [31,33].

Three weeks after inoculation, disease progress was visually evaluated as the Fusarium head blight index (FHBi):

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$$FHBi = \frac{\%\ of\ head\ infection\ \times \%\ of\ heads\ infected\ per\ plot}{100} \quad (1)$$

At harvest, 20 randomly selected heads from each plot (one control and three inoculated plots) in each location were collected and threshed with a laboratory thresher.

The percentage of *Fusarium*-damaged kernels (FDK) was scored visually according to the methods described earlier [34,35]. The FDK weight in relation to the weight of the whole sample was marked as FDKw, and the FDK number in relation to the total sample size was marked as FDK#.

Reductions in the yield components caused by FHB in relation to the non-inoculated control were calculated. The components were as follow: grain yield per head, kernel number in head, and thousand kernels weight (TKW).

5.5 Toxins analysis

The concentration of *Fusarium* toxins in triticale grain was analysed using the technique of gas chromatography. The type B trichothecenes (DON, 3-acetyldeoxynivalenol (3AcDON), 15-acetyldeoxynivalenol (15AcDON) and NIV) were detected. The methodology used for the extraction and detection of the samples with use of gas chromatography is described in detail by Góral et al. [27,36].

The content of ZEN was determined using a quantitative direct, competitive enzyme-linked immunosorbent assay (ELISA) AgraQuant® ZON 40/1000 (LOD 10 ppb) (Romer Labs Inc., Newark, DE, USA). The detailed methodology used for the quantitative analysis of ZEN is described by Góral et al. [27].

Ergosterol was chromatographically analysed via high-performance liquid chromatography (HPLC) on a silica column using methanol. A detailed evaluation of the method is given in a paper by Perkowski et al. [37]. Samples containing 100 mg of ground grains were placed into 17 ml culture tubes, suspended in 2 ml of methanol, treated with 0.5 ml of 2 M aqueous sodium hydroxide and tightly sealed. The culture tubes were then placed within 250 ml plastic bottles, tightly sealed and placed inside a microwave oven operating at 2450 MHz and 900 W maximum output. Samples were irradiated (370 W) for 20 s, and following approximately 5 min, for an additional 20 s. After 15 min, the contents of the culture tubes were neutralized with 1 M aqueous hydrochloric acid, 2 ml MeOH were added and extraction with pentane (3 × 4 ml) was carried out within the culture tubes. The combined pentane extracts were evaporated to dryness in a nitrogen stream. Before analysis samples were dissolved in 4 ml of MeOH, they were filtered through 13 mm syringe filters with a 0.5 mm pore diameter (Fluoropore Membrane Filters, Millipore, Ireland) and evaporated to dryness in a N_2 stream. The sample extract was dissolved in 1 ml of MeOH and 50 µl were analysed by HPLC. Separation was performed on a 150 × 3.9 mm Nova Pak C-18, 4 mm column and eluted with methanol/acetonitrile (90:10) at a flow rate of 0.6 ml/min. Ergosterol was detected with a Waters 486 Tunable Absorbance Detector (Milford, MA, USA) set at 282 nm. The presence of ergosterol (ERG) was confirmed by a comparison of retention times and by co-injection of every tenth sample with an ergosterol standard.

5.6 Statistical analysis

Statistical analysis was made using Microsoft® Excel 2016/XLSTAT© (Version 2020.4.1.1027, Addinsoft, Paris, France) statistical software.

FHB and FDK ratings, reduction of yield components and concentration of ERG and toxins data were analysed by means of analysis of variance (ANOVA) using the XLSTAT procedure: Modelling data–ANOVA. Year effect was considered random and location and line were considered fixed. Normality of data distribution was tested with the Shapiro–Wilk test (XLSTAT procedure: Normality test). All variables were not normally distributed; hence, they were transformed with the square root (FHBi, FDKw, FDK#, yield reduction, kernel# reduction, TKW reduction) or log10 (ERG, DON, 3ADON, 15AcDON, NIV, trichothecenes (TCT B), ZEN) transformations.

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The relationships between FHBi, FDK, reduction in the yield components and the ERG and mycotoxins concentrations were analysed using Pearson's correlation tests (XLSTAT procedure: Correlation tests). Prior to analysis, variables (means for 18 lines) that were not normally distributed were square root (FHBi, FDKw, FDK#, yield reduction, kernel# reduction, TKW reduction) or log10 transformed (ERG).

The data on FHB resistance (FHBi, FDKw, yield reduction, TKW reduction, ERG, DON, NIV, ZEN) measured with different units were analysed together using multivariate statistical analysis. Principal component analysis (XLSTAT procedure: Principal Component Analysis PCA) was used to show how triticale (and wheat) lines are distributed with respect to the variation described by the first two principal components and how FHB resistance variables influenced the two components. PCA results also show relations among variables measured by the angle among variable vectors.

3. Results

The average severity of FHB was FHBi = 10.7%. It was similar in both locations and amounted to 11.3% in Radzików and 9.7% in Cerekwica (Figure 1). The range of reactions was from 0% to 64.0% in Radzików and from 2.7% to 40.0% in Cerekwica. The proportion of *Fusarium*-damaged kernels was higher in Cerekwica (FDKw = 34.9%; FDK# = 27.9%) than in Radzików (FDKw = 12.7%; FDK# = 11.4%). The range of reaction was from 0.2% to 47.3% in Radzików and from 0.3% to 84.5% in Cerekwica for FDKw and from 0.6% to 51.4% in Radzików and from 0.2% to 88.6% in Cerekwica for FDK#.

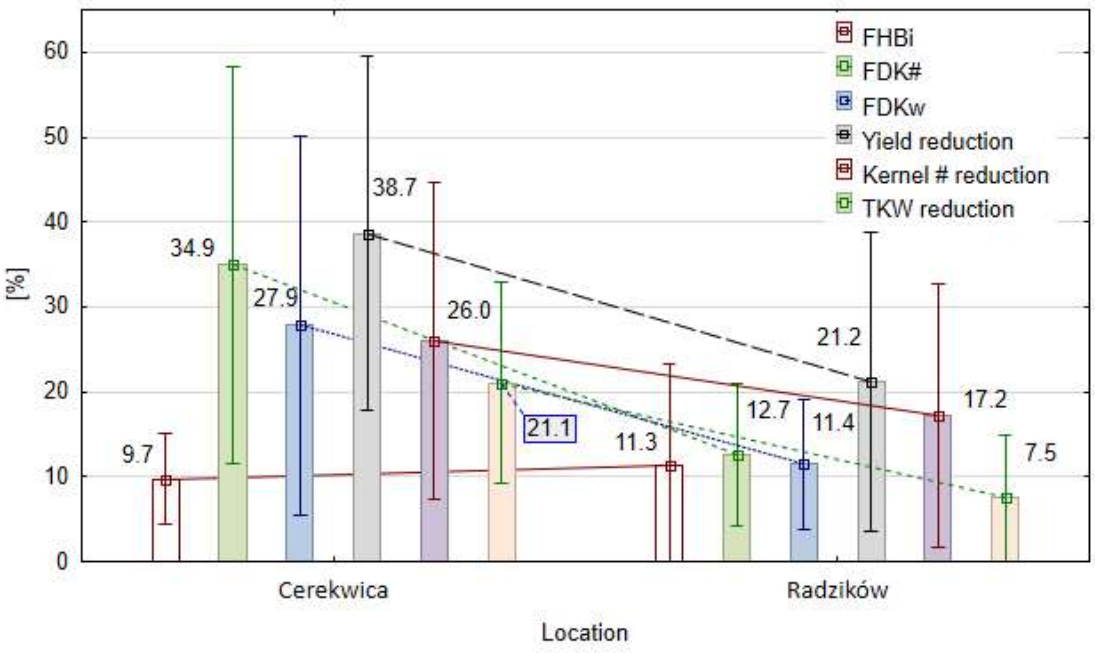

Figure 1. Average Fusarium head blight index (FHBi), *Fusarium*-damaged kernels proportion (FDK#—number, FDKw—weight) and reductions in the yield components (grain yield, kernel number, and 1000 kernels weight (TKW)) in two experimental locations. Boxes show mean +/− standard error, whiskers show mean +/− standard deviation.

Over three experimental years, the FHB index amounted to 14.4% in 2016, 13.6% in 2017 and 4.0% in 2018. As regards the FDK proportion, it was 21.2% and 26.2% in 2016, 22.8% and 25.2% in 2017 and 10.0% and 13.4% in 2018 for FDKw and FDK#, respectively.

Reductions in the three studied yield components were, on average, grain yield 28.3%, kernel number 20.8% and TKW 13.0%. In the experiment in the Cerekwica, the reductions were higher than in Radzików (Figure 1). Over the three experimental years, the reductions in yield, kernel number

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and TKW were as follows: 26.3%, 16.2% and 14.9% in 2016; 27.8%, 21.8% and 13.0% in 2017; 30.7%, 24.3% and 11.0% in 2018.

Concentration of ERG in grain was, on average, 8.3 mg/kg. It was similar for both locations (Figure 2). The concentration range was 1.5–38.7 mg/kg in Cerekwica and 0.6–35.2 mg/kg in Radzików. In 2016, the average ERG content in grain was 5.6 mg/kg, in 2017 8.8 mg/kg and in 2018 11.0 mg/kg.

The amount of DON in grain was, on average, 7.258 mg/kg at a range 0.028–50.330 mg/kg and the amount of NIV in grain was 5.267 mg/kg in the range 0–44.628 mg/kg. In Radzików, the concentration of DON was twice as high than in Cerekwica (Figure 2). On the contrary, the concentration of NIV in Radzików was low (0.855 mg/kg) and 10 times lower than in Cerekwica (9.679 mg/kg). Over the three experimental years, the amounts of DON and NIV were as follows: 2016—5.991 and 4.402 mg/kg, 2017—13.248 and 7.974 mg/kg and 2018—2.536 and 3.426 mg/kg.

Acetylated derivatives of DON (e.g., 3AcDON, 15AcDON) were detected in low amounts. On average, it was 0.228 mg/kg of 3AcDON (0–1.352 mg/kg) and 0.035 mg/kg of 15AcDON (0–0.445 mg/kg). In Cerekwica, the concentration of 3AcDON and 15AcDON was 0.284 and 0.001 mg/kg, respectively, and in Radzików 0.172 and 0.068 mg/kg. Over the three experimental years, the amounts of 3AcDON and 15AcDON were as follows: 2016—0.236 and 0.019 mg/kg, 2017—0.444 and 0.078 mg/kg and 2018—0.004 and 0.007 mg/kg.

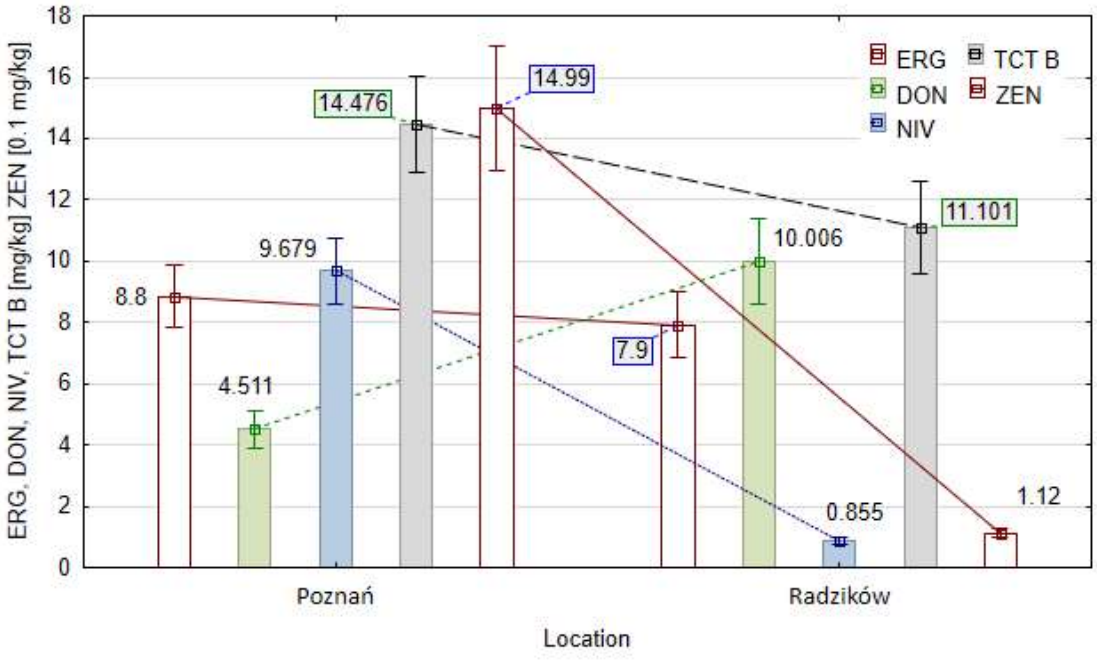

Figure 2. Average concentration of ergosterol (ERG), deoxynivalenol (DON), nivalenol (NIV), type B trichothecenes (TCT B) and zearalenone (ZEN) in grain of 15 triticale and 3 wheat lines in two experimental locations. Neither 3-acetyldeoxynivalenol (3AcDON) or 15-acetyldeoxynivalenol (15AcDON) are shown. Boxes show mean +/− standard error, whiskers show mean +/− standard deviation.

The total amount of analysed type B trichothecenes was 12.788 mg/kg at a range of 0.100–65.565 mg/kg. In Cerekwica, the amount of TCT B was 14.476 mg/kg at a range 1.453–65.565 mg/kg and in Radzików 11.101 mg/kg at a range 0.100–53.595 mg/kg. Over three years, the average amount of TCT B was as follows: 2016–10.648 mg/kg, 2017–21.744 mg/kg and 2018–5.973 mg/kg.

Zearalenone was detected in grain at an average amount of 0.805 mg/kg. The amount ranged from 0 to 5.055 mg/kg. ZEN was present mainly in samples from Cerekwica at amount of 1.500 mg/kg. In samples from Radzików, the concentration was 10 times lower and amounted to 0.112 mg/kg. The

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highest concentration of ZEN was detected in 2017 at 1.446 mg/kg, followed by 2016 at 0.900 mg/kg. In 2018, it was very low at 0.071 mg/kg.

Table 1. Analysis of variance of Fusarium head blight index (FBI) and *Fusarium*-damaged kernels percentage (weight, number)

Source	DF	FHBi		FDKw		FDK#	
		Mean squares	F	Mean squares	F	Mean squares	F
Year	2	91.803	6.178	73.178	1.001	54.475	1.155
Location	1	1.830	0.122	170.582	2.306	298.949	6.259 *
Line	17	7.071	16.109 ***	12.880	6.355 ***	15.348	7.110 ***
Year × Location	2	15.026	24.799 ***	73.981	25.486 ***	47.765	17.377 ***
Year × Line	34	0.439	0.724	2.027	0.698	2.159	0.785
Location × Line	17	4.087	6.744 ***	3.482	1.200	4.565	1.661
Year x Location × Line	34	0.606	1.449	2.903	6.762 ***	2.749	5.223 ***
Error	162	0.418		0.429		0.526	

***, * significant at $p < 0.001$ and 0.05, respectively.

Analysis of variance of the FHB index showed a very high effect of the triticale/wheat line and no effect of year (random) and location (Table 1). No interaction year × line was observed. Highly significant interactions for year × location and location × line were found. Similarly, for FDKw and FDK#, the effect of line was highly significant as well year × location interaction. Interactions for location × line were not significant for FDK. For reductions in yield component, the effects of line were significant for grain yield and TKW but not for kernel number. Year × location interactions were significant for all components.

Table 2. Analysis of variance of reductions in yield per head, kernel number in head, and 1000 kernel weight.

Source	DF	Yield		Kernel number		TKW	
		Mean squares	F	Mean squares	F	Mean squares	F
Year	2	7.919	0.038	25.939	0.175	9.829	0.179
Location	1	263.319	1.285	94.387	0.641	317.916	5.845 *
Line	17	16.187	3.579 ***	11.723	1.856	8.286	2.545 **
Year × Location	2	204.966	56.587 ***	147.266	28.703 ***	54.392	20.617 ***
Year × Line	34	4.522	1.249	6.316	1.231	3.256	1.234
Location × Line	17	6.666	1.840	10.997	2.143 *	3.795	1.438
Year × Location × Line	34	3.622	2.924 ***	5.131	3.929 ***	2.638	5.634 ***
Error	162	1.239		1.306		0.468	

***, **, * significant at $p < 0.001$, 0.01 and 0.05, respectively.

We found highly significant effects of year on concentration of all analysed toxins (Tables 3, 4). Location had a highly significant effect on concentration of trichothecenes and ZEN but only weak one on ERG concentration.

Table 3. Analysis of variance of concentration of ergosterol, DON, 3ACDON, and 15AcDON in grain.

Source	DF	ERG		DON		3AcDON		15AcDON	
		MS	F	MS	F	MS	F	MS	F
Year	2	1.154	25.786 ***	3.634	87.057 ***	0.200	70.513 ***	0.008	10.253 ***
Location	1	0.193	4.311 *	2.087	49.989 ***	0.031	11.008 ***	0.018	24.035 ***
Line	17	0.187	4.190 ***	0.259	6.203 ***	0.008	2.876 ***	0.000	0.635
Error	87	0.045		0.042		0.003		0.001	

***, ** significant at $p < 0.001$ and 0.01, respectively.

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Location mean squares for 15AcDON, NIV and ZEN were higher than year mean squares. These toxins were mainly detected in samples from Radzików (15AcDON) or Cerekwica (NIV, ZEN) (Figure 2). The effect of the triticale line was highly significant for ERG and trichothecenes (except 15AcDON) and low significant for ZEN.

Table 4. Analysis of variance of concentration of nivalenol, trichothecenes B (sum of DON, 3AcDON, 15AcDON and NIV) and zearalenone in grain.

Source	DF	NIV		TCT B		ZEN	
		MS	F	MS	F	MS	F
Year	2	0.825	31.975 ***	2.526	74.300 ***	13.499	18.648 ***
Location	1	13.156	509.917 ***	0.719	21.139 ***	26.304	36.337 ***
Line	17	0.147	5.707 ***	0.352	10.356 ***	1.293	1.787 *
Error	87	0.026		0.034		0.724	

***, * significant at $p < 0.001$ and 0.05, respectively.

Winter wheat lines showed the highest ("DL 325/11/3") and the lowest (lines carrying *Fhb1* resistance gene) values of FHB index (Table 5). Among triticale lines, the lowest FHBi was observed for six lines: "DANKO 9 2013", "LD 121/08", "BOHD 1025-2", "BOH 534-4", "MAH 33881-1/3", "DS.9". The most infected heads had two lines "DANKO 6 (2014)", "DL 446/08" and cultivar "*Meloman*". The lowest FDK proportions (weight, number) were observed for two low FHB infected wheat lines. FHB-susceptible wheat lines showed only medium FDK values. Low FDK's were found only for two low FHB-infected triticale: "DS.9" and "BOHD 1025-2". The highest kernel damage was observed in line "DANKO 9 2013" that showed only weak FHB symptoms. FDK was also high in three lines which showed the highest FHB index among triticale lines "DANKO 6 (2014)", "DL 446/08" and "BOH 1062-2".

Reduction of grain yield per head was the highest in triticale line "DANKO 6 (2014)" as well as wheat line "DL 325/11/3". These lines showed high levels of head infection and kernel damage. This was also noted in lines "DANKO 9 2013" and "LD 121/08". The second line showed low head and kernel infection. Low grain yield reduction was found in resistant wheat lines and low-infected triticale "BOH 534-4" and "DS.9" as well as in medium-infected line "BOH 537-2". Reduction of 1000 kernel weight was the highest in the susceptible wheat line "DL 325/11/3" and the lowest in resistant wheat lines and resistant triticale line "DS.9".

Table 5. Fusarium head blight index (%), *Fusarium*-damaged kernels (weight. number) (%) and reduction of yield, kernel number and TKW (%) for 15 winter triticale and three winter wheat lines.

Line	FHBi	FDKw	FDK#	Yield reduction	Kernel number reduction	TKW reduction
DL 325/11/3 [a]	32.6 a	25.7 abcd	22.2 abcd	41.9 ab	27.1	22.7 a
Meloman	14.0 bcd	22.8 bcde	18.5 bcde	33.3 abcde	25.5	10.8 bcde
DANKO 6 (2014)	13.9 b	31.0 ab	25.2 ab	44.4 a	36.4	18.7 abc
DL 446/08	13.5 bc	30.8 ab	26.5 ab	29.7 cde	23.3	13.8 bcd
BOH 1062-2	11.6 bcde	28.0 abc	24.8 abc	27.0 cde	20.8	14.7 bcd
BOHD 898-1	10.7 bcdef	23.1 abcde	18.1 abcde	24.3 cde	17.4	10.5 bcde
BOH 537-2	10.2 bcdef	20.8 abcde	18.1 bcde	23.5 ef	16.1	11.4 bcde
DL 593/07	9.5 cdef	23.6 abcde	19.6 abcde	29.0 bcde	22.0	11.8 cdef
MAH 33544-4	9.4 bcdef	18.0 cde	13.9 cde	28.0 abcde	19.1	14.3 abc
DS.1238	9.4 bcdef	24.7 abcde	21.2 abcde	26.9 cde	19.8	12.3 bcd
DANKO 9 2013	9.1 ef	33.6 a	28.6 a	35.6 abc	22.6	16.3 abc
LD 121/08	9.0 ef	18.6 bcde	15.6 cde	35.8 abcd	27.9	13.8 bcd
BOHD 1025-2	8.6 def	14.4 e	11.8 e	26.0 cde	17.5	13.2 bcd
BOH 534-4	8.4 ef	21.3 abcde	17.6 bcde	23.9 ef	15.8	12.0 bcd

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MAH 33881-1/3	7.1 f	22.7 abcde	19.1 abcde	25.6 cde	15.1	16.7 ab
DS.9	7.1 f	16.7 de	13.0 de	23.4 def	21.0	8.0 ef
S 10 [a]	4.0 g	4.0 f	3.4 f	14.4 g	13.1	4.5 f
S 32 [a]	3.9 g	8.7 f	7.1 f	16.0 fg	13.2	7.6 def
Means	10.7	18.1	21.6	28.3	20.8	12.9

[a] wheat; those marked with the same letter are not significantly different at $p < 0.05$ according to Fisher's least significant difference (LSD) test performed on transformed variables; means ranked by FHBi values.

Ergosterol concentration was the highest in grain of susceptible wheat line "DL 325/11/3" (Table 6). It was twice as high than in two triticale lines that had the highest concentrations of this metabolite ("DANKO 6 (2014)", "BOH 1062-2"). These lines had also high kernel damage. Line "DANKO 9 2013" exhibiting highest kernel damage had medium concentration of ERG in grain. The lowest ERG content was found in grain of five triticale lines "DS. 9", "BOHD 898-1", "BOHD 1025-2", "BOH 534-4" and "BOH 537-2". FHB-resistant wheat lines had medium concentration of ERG in grain.

Deoxynivalenol accumulated the highest amount in grain of the triticale line "DANKO 6 (2014)" and in the wheat line "DL 325/11/3". Four triticale lines, "BOH 1062-2", "DL 446/08", "DANKO 9 2013" and "DS.1238", also had high amounts of DON in grain. The lowest concentration of DON was detected in the grain of resistant wheat lines and two triticale lines "BOH 534-4" and "BOHD 1025-2". Nivalenol was present mainly in the grain of four triticale lines: "DANKO 6 (2014)", "BOH 1062-2", "DL 446/08", "DANKO 9 2013" and "MAH 33881-1/3". The lowest concentration of NIV was detected in the grain of resistant wheat lines and two triticale lines "DS.9" and "BOHD 1025-2". 3AcDON was detected mainly in susceptible wheat line and triticale line "DANKO 6 (2014)", that accumulated high amount of trichothecene toxins. Of the total amount of the four trichothecenes, the highest was in the grain of three triticale lines "DANKO 6 (2014)", "BOH 1062-2" and "DL 446/08", and the lowest was in the grain of resistant wheat lines. The wheat line "DL 325/11/3" had a medium amount of type B trichothecenes in grain. Four triticale lines, "BOHD 898-1", "DS. 9", "BOH 534-4" and "BOHD 1025-2", accumulated the lowest amounts of type B trichothecenes.

Differences in ZEN concentrations among triticale lines had low significance. The lowest amount of ZEN was found in the grain of the "DS.9" line. Considerably lower amounts of ZEN accumulated only in the grain of resistant wheat lines.

Table 6. Concentration of ergosterol (mg/kg), type B trichothecenes (DON, 3AcDON, 15AcDON, NIV, TCT B) (mg/kg) and zearalenone (mg/kg) in grain of 15 winter triticale and three winter wheat lines.

Line	ERG	DON	3Ac DON	15Ac DON	NIV	TCT B[b]	ZEN
DANKO 6 (2014)	13.0 b	14.327 a	0.426 ab	0.081	7.653 ab	22.487 a	0.966 ab
BOH 1062-2	12.1 bc	10.207 ab	0.327 abcd	0.012	10.966 a	21.511 ab	1.443 abc
DL 446/08	9.4 bcde	10.882 abcd	0.374 abc	0.050	10.197 a	21.503 ab	0.956 ab
DANKO 9 2013	8.6 bcde	10.944 abc	0.321 abcd	0.043	7.838 a	19.146 abc	1.169 a
MAH 33881-1/3	6.4 b-g	9.380 abcd	0.251 abcde	0.046	7.974 a	17.652 abc	0.791 abcd
DL 325/11/3 [a]	28.3 a	11.887 ab	0.461 a	0.021	4.930 abcd	17.298 abc	0.833 ab
DS.1238	7.5 b-g	10.549 abcde	0.335 abcde	0.072	4.981 abcd	15.937 abcd	1.238 a
Meloman	9.5 bcd	9.089 abcde	0.214 b-g	0.064	5.733 abc	15.101 abcd	0.999 abcd
DL 593/07	6.2 b-g	7.278 bcdef	0.224 bcdef	0.070	5.856 abcd	13.429 bcde	0.921 abc
MAH 33544-4	5.8 b-g	5.204 cdef	0.161 c-g	0.015	6.301 abc	11.681 cde	0.635 abc
BOH 537-2	5.4 defg	6.447 bcdef	0.139 defg	0.031	3.657 bcde	10.273 def	0.757 ab
LD 121/08	5.7 b-g	4.338 f	0.165 c-g	0.026	4.611 bcde	9.139 ef	0.763 bcd
BOHD 898-1	3.7 fg	5.387 ef	0.182 c-g	0.026	2.958 def	8.553 ef	0.720 abc
DS.9	4.9 efg	5.111 def	0.137 defg	0.019	2.888 efg	8.156 ef	0.529 bcd
BOH 534-4	4.9 cdefg	3.977 f	0.173 c-g	0.019	3.440 cde	7.609 ef	0.846 abc
BOHD 1025-2	4.2 g	3.086 fg	0.116 efg	0.008	2.492 efg	5.701 f	0.775 abcd

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S 32 [a]	7.5 b-g	1.161 h	0.045 g	0	1.362 fg	2.567 g	0.061 d
S 10 [a]	7.7 b-f	1.398 gh	0.053 fg	0.021	0.976 g	2.448 g	0.092 cd
Means	8.4	7.258	0.228	0.035	5.267	12.788	0.805

[a] wheat; [b] sum of DON, 3AcDON, 15AcDON and NIV; means marked with the same letter are not significantly different at $p < 0.05$ according to Fisher's LSD test performed on transformed variables; means ranked by TCT B concentration.

The Fusarium head blight index correlated significantly with other variables except 15AcDON and NIV (Table 7). Coefficients had high values (>0.600) but were low for the sum of type B trichothecenes and ZEN concentrations. *Fusarium*-damaged kernel proportions (i.e., weight, number) correlated highly significantly with reductions in yield components and concentration of mycotoxins. The highest values had coefficients of correlations with TCT B and ZEN. The FDKs did not correlate with the ERG amount in grain. This resulted from higher than expected FDK value concentrations of ERG in grain of the wheat lines. For sole triticale lines, FDKs correlated significantly with ERG (0.768 and 0.759). Reductions in the yield components correlated significantly with concentrations of ERG and mycotoxins. The highest were coefficients of correlation with DON and 3AcDON, the lowest with ERG and NIV.

Ergosterol concentration in grain correlated significantly with DON and 3AcDON and did not correlate with the amounts NIV and ZEN. Trichothecene toxins correlated significantly with each other and with ZEN. The highest values had coefficients of correlation DON versus 3AcDOn and DON versus NIV.

Table 7. Coefficients of correlation between Fusarium head blight index, *Fusarium*-damaged kernels proportion (weight, number), reductions in yield components and concentration of ergosterol and mycotoxins in grain of 15 winter triticale and three winter wheat lines.

Variables	FHBi	FDK w	FDK #	Yield red.	Kernel # red.	TKW red.	ERG	DON	3Ac DON	15Ac DON	NIV	TCT B
FDKw	0.611 **											
FDK#	0.606 **	0.996										
Yield red.	0.763	0.753	0.766									
Kernel# red.	0.645 **	0.609 **	0.628 **	0.915								
TKW red.	0.740	0.774	0.770	0.844	0.612 **							
ERG	0.698	0.362 ns	0.326 ns	0.532*	0.526 *	0.526 *						
DON	0.673 **	0.865	0.855	0.773	0.699	0.750	0.656* *					
3AcDON	0.769	0.837	0.822	0.787	0.700	0.806	0.730	0.949				
15AcDON	0.227 ns	0.531 *	0.540 *	0.501 *	0.543 *	0.269 ns	0.216 ns	0.674 **	0.540 *			
NIV	0.376 ns	0.789	0.773	0.549 *	0.479 *	0.632 **	0.432 ns	0.773	0.724	0.413 ns		
TCT B	0.584 *	0.884	0.870	0.721	0.644 **	0.744	0.598 **	0.960	0.908	0.599 **	0.920	
ZEN	0.484 *	0.879	0.864	0.627 **	0.506 *	0.635 **	0.276 ns	0.753	0.717	0.483 *	0.750	0.797

Coefficients significant at $p < 0.001$, except when marked with *, **, ns—significant at $p < 0.05$, 0.01 or non-significant, respectively.

Multivariate PCA analysis showed that the highest FHB resistance explained by eight variables (FHBi, FDKw, yield, TKW, ERG, DON, NIV and ZEN) was found in resistant wheat lines carrying the *Fhb1* gene and in two triticale lines ("DS.9" and "BOHD 1025-5") (Figure 3). Five other lines had also considerable FHB resistance ("BOHD 898-1", "BOH 534-4", "BOH 537-2", "MAH 33544-1" and "LD 121/08"). Susceptible wheat line "DL 325/11/3" could be characterised by high Fusarium head blight severity (FHBi) and high ERG concentration. More susceptible triticale lines could be characterized by high kernel damage and toxins accumulation.

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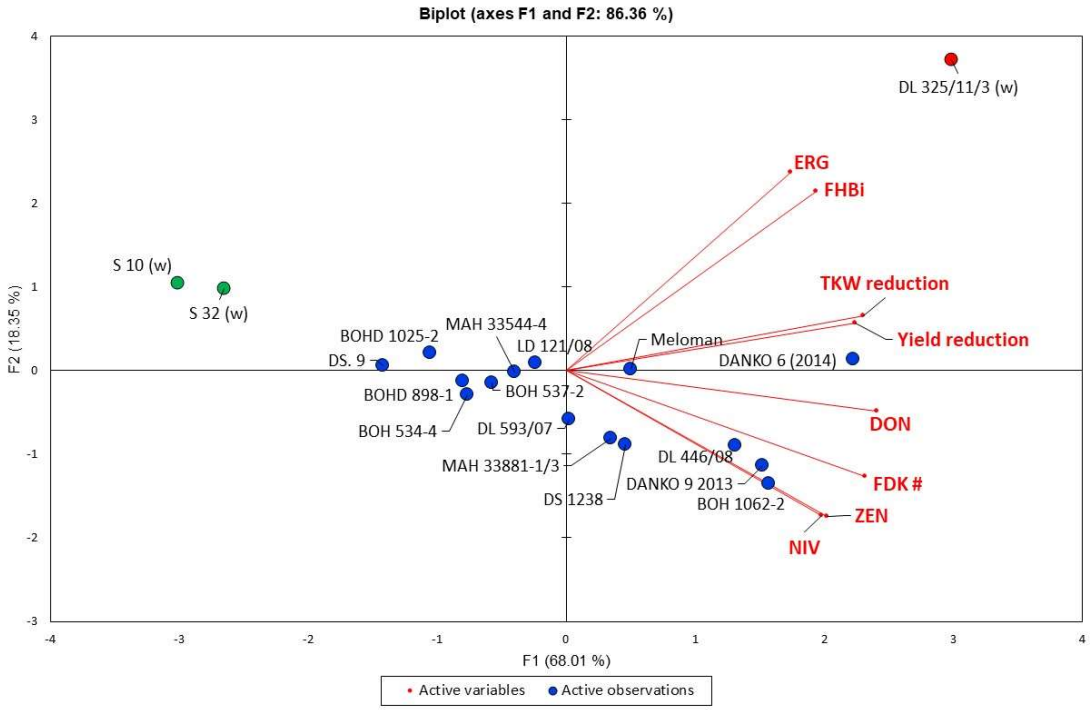

Figure 3. Biplot of principal component analysis (PCA) analysis of FHBi, FDK#, yield reduction, TKW reduction, ERG, DON, NIV and ZEN for 15 winter triticale and 3 winter wheat lines (w).

4. Discussion

Due to the increase in crop area and exposure to a variety of pathogens in triticale, there has been a breakdown in their resistance against fungal diseases [38,39]. The most remarkable examples have been powdery mildew and yellow rust [40,41]. Recently, a decrease in triticale resistance to pathogens of the *Fusarium* genus have been observed. FHB outbreaks in wheat have become more serious and frequent in recent decades, possibly due to the changes in climate and agronomic practices [42]. Globally, FHB causes approximately 10–70% yield loss in epidemic years [43,44]. Because triticale is consumed mainly by farm animals, it is important to maintain good quality grains, especially in case of detrimental toxins content [45,46].

We identified triticale lines highly resistant to FHB and, in particular, to the accumulation of *Fusarium* toxins. However, some lines despite their low head infection accumulated considerable amounts of trichothecenes in grain, e.g., "DANKO 6 (2014)", "BOH 1062-2", "DL 446/08", "DANKO 9 2013" and "MAH 33881-1/3". All these lines (except "MAH 33881-1/3") had high *Fusarium* kernel damage. We observed this problem previously when comparing wheat and triticale under the same conditions in other inoculation experiments [27]. According to conference presentations by Randhawa et al. (2013) and Langevin (2009) (cited by Randhawa et al. [3]), screening of a large number of triticale accessions resulted in only a few lines with a good level of FHB resistance. Some lines showed higher DON accumulation than expected from low head infection. Langevin et al. (2009) speculated that the higher DON content in triticale grain may be caused by a more fragile pericarp during the initial development of the triticale seed.

Research has shown that environmental conditions significantly affect the development of FHB and the accumulation of toxins in the grain [9,47]. Fusarium head blight severity, kernel damage and concentration of *Fusarium* metabolites were significantly affected by the experimental year and the location. This study on resistance to FHB and accumulation of *Fusarium* toxins was conducted over three years in two locations: Cerekwica near Poznań and Radzików near Warsaw. To maintain humidity during inoculation and after inoculation, mist irrigation was used in Cerekwica. The infection of heads was similar in Cerekwica and Radzików, but the other parameters examined—

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percentage of FDK, number and weight of grains per head and the reduction in the yield structure parameters—were much higher in Poznań than in Radzików. Similarly, the amount of toxins: type B trichothecenes and zearalenone was higher in Cerekwica.

Weather conditions in 2016 were similar in Cerekwica and Radzików (Table S1). Rainfall before anthesis (May) was low and higher (approximately 50%) during and after anthesis (June). This resulted in similar head infections in both locations. Next, in July, rainfall in Cerekwica was much higher than in Radzików which (accompanied by higher temperature) resulted in higher kernel damage in the first location. In addition, toxin accumulation in grain in Cerekwica was higher. In 2017, the weather in May was similar in both locations, and in June, rainfall in Radzików was twice as high than in Cerekwica. This led to a higher head infection in Radzików. Similar to 2016 in July, rainfall in Cerekwica was double that of Radzików. This caused very high kernel damage in Cerekwica. However, the amounts of trichothecenes in grain in both locations were similar and higher than in 2016. We observed differences in the accumulation of DON and NIV in both locations. DON was mainly found in grain from Radzików and NIV mainly in samples from Cerekwica. Weather in 2018 was unfavourable for FHB development, particularly in Cerekwica. Rainfall in June was low compared to previous years. In both locations, head infection was low, even despite application of mist irrigation in Cerekwica. Kernel damage was lower than in previous years and similar in locations. The same was found for trichothecenes and ZEN. As in 2017, we observed the opposite results for DON and NIV concentrations.

Under the above variable conditions, some triticale lines showed stable reactions for most variables describing FHB resistance. The most stable were the resistant lines "BOHD 1025-5", "DS.9" and "MAH 33544-4". Line "BOH 534-4" had a stable reaction to head infection and accumulation of ERG and trichothecenes (low infection and low accumulation); however, less it was stable as regards kernel damage. Similarly, the "BOH 898-1" line was less stable for head infection and kernel damage but stable for low ERG and trichothecenes accumulation.

Selection of FHB-resistant genotypes is more complicated in triticale than in wheat. Frequently *Fusarium* head infection is lower, but other FHB components are comparable to wheat which is more susceptible. Low visible head infection can result in significant amounts of infected kernels and accumulation of toxins in grain [48,49]. Studies conducted on triticale lines showed that the assessment of resistance to FHB based only on head infection symptoms was only ineffective for the selection of resistant genotypes [48]. The results may differ in the locations of experiments as can be seen from the significance of the interaction between location × line. Reliable assessment of FHB resistance has to be associated with the evaluation of kernel damage and the amount of toxins accumulated in the grain. Ollier et al. [50] showed that in triticale, FHB severity symptoms on grain measured digitally as "whitened kernel surface" [51] had higher heritability coefficients than FHB symptoms on heads. This variable highly correlated with the mycotoxin content. We also observed much higher values of coefficients for correlations FDK versus mycotoxins than for FHB index versus mycotoxins.

The introduction into triticale of genes associated with resistance to FHB is very desirable, although it is difficult due to the complex genome of this cereal. Only a few papers have been published on the genetics of FHB resistance in triticale [24,52,53]. In papers by Kalih et al. [24,53], 17 FHB-resistance QTLs were presented including six on the rye chromosomes. The most effective (34%) was the QTL on chromosome 4R. Dhariwal et al. [52] obtained similar results. Using single nucleotide polymorphism (SNP) genotyping, they identified 17 QTLs explaining more than 10% of the variability in FHB resistance. Seven of them were located on rye chromosomes (4R, 5R). The highest effect had four QTLs on chromosomes 1A, 2B, 4R and 5R. Association mapping, carried out on varieties and breeding lines differentiated in origin, allowed the identification of QTLs on chromosomes 2A, 2B, 5B, and for the first time on 3R, with individual QTLs explaining variability in the range of 0.28–30.23% [54]. The authors highlight the possibility of increasing triticale resistance to FHB in the early generations using tools proposed by genomics.

Recently, an attempt was made to introduce FHB resistance from the spring wheat line "CM-82036" into the triticale varieties [50,55]. The *Fhb1* gene was detected and validated in triticale background. Nine triticale lines with very high levels of FHB resistance were identified. QTL analysis

of FHB resistance showed the presence of additional loci of resistance on chromosomes 2B, 5R and 7A. QTL on chromosome 5R coincided with the location of the dwarfing gene *Ddw1*. Similar research on the introduction of the *Fhb1* gene into triticale was also carried out by the Institute of Plant Genetics group [56].

Supplementary Materials: The following are available online at www.mdpi.com/xxx/s1, Table S1: Air temperature and rainfall in May, June and July of 2016, 2107 and 2018 in two experimental locations.

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