The Response of Selected *Triticum* spp. Genotypes with Different Ploidy Levels to Head Blight Caused by *Fusarium culmorum* (W.G. Smith) Sacc.

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**Abstract:** Several cultivars and pure lines of *Triticum monococcum*, *T. dicoccon*, *T. polonicum*, *T. spelta* and *T. aestivum* were inoculated with *Fusarium culmorum*, the causal agent of *Fusarium* head blight in wheat. During the three-year study, the infection decreased the values of the analyzed yield components: spike weight (by 5.6% to 15.8%), number of kernels per spike (by 2.8% to 11.8%) and one kernel weight (by 8.4% to 10.7%). *T. spelta* was characterized by the weakest average response to infection. The grain from inoculated spikes contained significantly higher concentrations of deoxynivalenol (DON) and its 3-β-D-glucoside (D3G) than control grain. The D3G/DON ratio ranged from 11.4% to 21.4% in control grain and from 8.1% to 11.6% in inoculated grain. The lowest levels of mycotoxins were found in spelt, and the highest in *T. polonicum* lines and Kamut. PCA revealed that the grain of *T. polonicum* was characterized by an entirely different mycotoxin profile. The weakest response to *F. culmorum* infections was noted in *T. spelta*, and the strongest response in *T. polonicum* breeding lines and Kamut.

**Keywords:** wheat; ploidy level; mycotoxins; principal component analysis; *Fusarium* head blight

1. Introduction

Consumers have a growing interest in foods that offer high nutritional value and deliver health benefits. Breeders search for new, high-quality cereal cultivars that meet those requirements. The grain of such cultivars should be characterized by satisfactory processing suitability, high nutrient concentrations and low levels of antinutritional factors [1]. The progress in the cultivation of common wheat was achieved mainly in response to farmers’ demand for high-yielding varieties. Breeding for high yield in cereals is difficult because most of the yield-forming traits are inherited polygenically. Despite those problems, many wheat breeding programs have been successful around the world [2]. The grain of modern high-yielding wheat varieties is much less abundant in protein [3] and micronutrients in comparison with wheat cultivars grown decades ago [4]. Mycotoxins, in particular those produced by pathogens of the genus *Fusarium*, also pose a significant problem for breeders and food producers [5]. Although significant progress has been made in protecting wheat plants against
Fusarium infections through chemical control [6,7], integrated plant protection methods [8] and the use of antagonistic organisms [9], the cultivation of resistant cultivars does not deliver satisfactory results. Grain infected by Fusarium pathogens is characterized by lower processing suitability [10] because starch endosperm is severely damaged by infections [11]. The rheological properties of flour are less desirable [12]. Wheat’s resistance to Fusarium head blight (FHB) is determined polygenically, which implies that wholly resistant (immune) varieties cannot be obtained [13]. The sources of resistance to FHB that are most commonly used in breeding, including cultivars Sumai-3 and Frontana [14,15], and the recently discovered genotypes of Asian origin [16], are characterized by several undesirable traits and have poor agronomical characteristics.

The limited number of effective sources of resistance against FHB pathogens in T. aestivum has prompted researchers to analyze other species that are closely related to common wheat. Diploid species such as Triticum monococcum L., tetraploid taxa of Triticum dicoccon Schrank, Triticum dicoccoides (Körn. ex Asch. et Graebner) Schweinf, Triticum polonicum L. and Triticum turanicum Jakubz., and the hexaploid Triticum spelta L. are generally characterized by high processing suitability and satisfactory nutritional value, and they constitute valuable initial material for quality [17,18] and resistance breeding [19]. Selected breeding lines of tetraploid wheat species are highly resistant to Fusarium pathogens and accumulate relatively low amounts of mycotoxins in grain [19–21]. The toxic effects of Fusarium pathogens, which can be attributed to their metabolites that belong to different chemical groups (mainly type A and B trichothecenes, fumonisins, zearalenone and its derivatives), have been widely investigated and described in literature [22,23].

The resistance of Triticum species other than common wheat to spike infections caused by Fusarium pathogens has been explored by relatively few studies [19,24–26], and only Triticum durum Desf. has been researched quite extensively [27–30]. A comprehensive analysis of a variety’s response to pathogens should involve evaluations of yield components and quality parameters of grain. The presence of mycotoxins should also be investigated.

The objective of this study was to evaluate the responses of 14 genotypes of five Triticum species characterized by different degrees of ploidy to spike infections caused by Fusarium culmorum (W.G.Smith) Sacc.

2. Results

2.1. Weather Conditions

Field experiments were carried out during three successive years of 2010, 2011 and 2012. Weather conditions (Figure 1) were of critical importance during spike inoculation and in early stages of kernel development (from late June to mid-July). In this respect, significant differences were noted between experimental years. The highest precipitation levels in the first half of July were noted in 2010 and 2012, in late June for 2010, and in mid-July for 2012. In 2012, the highest temperatures were reported between early July and the end of the growing season. Weather conditions were most favorable for spike infections in 2011 and 2012, and least favorable in 2010. Relatively low precipitation levels and high temperatures between late April and early July 2012 created unsupportive growth conditions for the drought-sensitive species T. aestivum and T. monococcum.
Figure 1. Precipitation and average temperatures in growing seasons of 2010, 2011, and 2012 in the experimental station in Bałcyny.

2.2. Yield Components

The average spike weight, number of kernels per spike and one kernel weight (OKW) of spikes inoculated with *F. culmorum* are presented in Table 1. *F. culmorum* infections decreased the values of the analyzed yield components, mostly OKW and spike weight. Both *T. aestivum* cultivars were characterized by a similar average decrease in spike weight and number of kernels per spike, which did not exceed 20% throughout the experimental period. The noted decrease in OKW values in inoculated spikes of cv. Sumai-3 (2.0%) was more than 12-fold lower than in cv. Torka (25.3%). Both breeding lines of *T. polonicum* and Kamut wheat responded to inoculation with a relatively high drop in the values of the analyzed traits (from 9.5% in OKW to 13.6% in spike weight). The lowest values of the above traits were noted in *T. monococcum* (32.6 mg and 0.9 g, respectively). The F-values produced by the analysis of variance (Table 2) point not only to significant variations (genotype—G, inoculation with pathogen—I, year of study—Y), but also to interactions between experimental factors. All interactions involving the number of kernels per spike and one kernel weight proved to be significant. The above indicates that the applied genotypes not only responded differently to inoculation (*G × I*) and were characterized by different values of both traits in successive years (*G × Y*), but that those responses differed in successive years (*G × I × Y*). Interestingly, *G × I* and *G × Y* interactions were not significant for spike weight. The average values of yield components were analyzed by PCA (Figure 2). The results indicate that the examined genotypes were characterized by significant intraspecific similarities in both control and inoculated treatments. The most distant clusters were observed for *T. monococcum* and *T. polonicum*. Significant similarities were observed between *T. dicocon* lines and two cultivars of *T. aestivum*. 
Table 1. The values of three yield components of five *Triticum* sp. taxa inoculated with *F. culmorum* in each year of the experiment (2010–2012).

| Species      | Treatment | Weight of One Spike (g) | Number of Kernels per Spike | One Kernel Weight (mg) |
|--------------|-----------|-------------------------|----------------------------|------------------------|
| *T. monococcum* | C         | 0.9                     | 0.9                        | 19.7                   |
|              | I         | 0.9                     | 0.8                        | 17.2                   |
|              | 1 – (I/C) (%) | 0                     | 11.1                      | 12.7                   |
| *T. dicoccon*  | C         | 1.7                     | 1.9                        | 32.9                   |
|              | I         | 1.6                     | 1.8                        | 30.1                   |
|              | 1 – (I/C) (%) | 5.9                    | 5.3                       | 21.1                   |
| *T. spelta*    | C         | 2.3                     | 1.9                        | 31.4                   |
|              | I         | 2.1                     | 1.7                        | 28.5                   |
|              | 1 – (I/C) (%) | 8.7                    | 0                         | 9.2                   |
| *T. polonicum* | C         | 1.8                     | 2.3                        | 21.2                   |
|              | I         | 1.5                     | 2.1                        | 15.4                   |
|              | 1 – (I/C) (%) | 16.7                   | 8.7                       | 27.4                   |
| *T. aestivum*  | C         | 1.6                     | 2.0                        | 34.3                   |
|              | I         | 1.3                     | 1.6                        | 31.6                   |
|              | 1 – (I/C) (%) | 18.8                   | 20.0                      | 17.4                   |

C—control; I—inoculation.
Table 2. The F-values (with two degrees of freedom) and the relevant probability values (p) for the weight of one spike, number of kernels per spike, and one kernel weight in three years of the experiment (2010–2012) under head inoculation with F. culmorum.

| Source of Variation | Weight of One Spike (g) | Number of Kernels per Spike | One Kernel Weight (mg) |
|---------------------|-------------------------|-----------------------------|------------------------|
| Genotype (G)        | F(13, 168) = 30.4, p < 0.00001 | F(13, 168) = 2.085,2, p < 0.00001 | F(13, 168) = 4.497, p < 0.00001 |
| Inoculation (I)     | F(1, 168) = 3.8, p = 0.04925 | F(1, 168) = 785,7, p < 0.00001 | F(1, 168) = 307, p < 0.00001 |
| Year (Y)            | F(2, 168) = 1.1, NS      | F(2, 168) = 6,389,4, p < 0.00001 | F(2, 168) = 1,893, p < 0.00001 |
| G × I               | F(13, 168) = 0.29, NS    | F(13, 168) = 38,7, p < 0.00001 | F(13, 168) = 24, p < 0.00001 |
| G × Y               | F(26, 168) = 0.79, NS    | F(26, 168) = 183,2, p < 0.00001 | F(26, 168) = 78, p < 0.00001 |
| I × Y               | F(26, 168) = 9.2, p = 0.0002 | F(26, 168) = 164,8, p < 0.00001 | F(26, 168) = 17,085, p < 0.00001 |
| G × I × Y           | F(26, 168) = 3.4, p < 0.00001 | F(26, 168) = 49,0, p < 0.00001 | F(26, 168) = 292, p < 0.00001 |

NS—not significant.

2.3. Mycotoxin Concentrations in Grain

The mycotoxin profile was more complex in 2011 (26 metabolites) than in 2010 (21 metabolites), but inoculation with F. culmorum led to a dramatic increase in the concentrations of type B trichothecenes, in particular deoxynivalenol (DON), deoxynivalenol-3-β-D-glucopyranoside (D3G) and 3-acetyl-deoxynivalenol (3-Ac DON), in both years (Tables 3 and 4). In all wheat species except T. polonicum, the average DON concentrations in the grain of inoculated spikes were higher in the second than in the first year of the experiment, and more than a three-fold difference was observed in T. monococcum. A similar but weaker trend was noted in the concentrations of 3-AcDON. A very strong correlation was observed between DON and D3G levels, and the value of the correlation coefficient r reached 0.990 in the first year and 0.953 in the second year of the experiment. In both years, the highest mycotoxin levels were observed in both control and inoculated grain of T. polonicum. Interestingly, in addition to very high concentrations of type B trichothecenes and zearalenone (ZEA) produced by F. culmorum (52.6 × 10³ and 56.1 × 10³ µg kg⁻¹ in inoculated grain, respectively, and 266 and 1451 µg kg⁻¹ in the first and second year of the study, respectively), the grain of T. polonicum harvested from control spikes contained considerable amounts of moniliformin (MON) and hexadepsipeptides (3.7 × 10³ µg kg⁻¹ and 12.9 × 10³ µg kg⁻¹ in 2010, respectively). The grain of both T. polonicum breeding lines and Kamut wheat was also characterized by very high levels of aurofusarin AUF at 41.4 × 10³ and 2.0 × 10³ µg kg⁻¹ in each year of the study, respectively, which were higher than in the grain harvested from the inoculated spikes of the remaining wheat genotypes. The average content of type B trichothecenes in total mycotoxin concentrations was similar in both years of the study in both inoculated grain (75.1% and 77.1%) and control grain (28.9% and 34.7%) (Table 5). The above results suggest that weather conditions significantly influenced mycotoxin concentrations in grain, but had a far less pronounced effect on the relationships between fungal metabolites. Precipitation levels and air temperature differed considerably between 2010 and 2011, in particular in the flowering stage, which affected not only the intensity of F. culmorum infections, but also the rate of grain colonization by fungi other than Fusarium species. In the group of the examined wheat species, the highest mycotoxin concentrations were noted in the grain of T. polonicum. In 2010, fungal metabolite levels in the control grain of Polish wheat were higher than in the grain of the remaining species harvested from inoculated spikes. In 2011, mycotoxin concentrations in T. polonicum and the remaining wheat species differed by two orders of magnitude. A comparison of T. polonicum and other wheat genotypes indicates that the content of type B trichothecenes in total concentrations of identified toxins was lowest in both control grain (0.6% and 14.8%) and inoculated grain (14.8% and 70%) of T. polonicum in both years of the study. A Principal Component Analysis (PCA) examining the concentrations of all identified mycotoxins revealed completely different fungal metabolite profiles in both T. polonicum breeding lines and Kamut wheat and a relatively strong response in cv. Torka, in particular in the first year of the experiment (Figures 3 and 4). In both years of the study, the combined effect of PC1 and PC2 explained more than 79% of variance, which points to high discriminative power. Most importantly, PCA results were similar and comparable in both years.
Toxins 2016, 8, 112

**Figure 3.** PCA results for mycotoxin concentrations in the grain of *Triticum* genotypes analyzed in 2010. Factor loading values based on the correlation between the Principal Component (PC) and variable (mycotoxin) and contribution of variables are given in Table S1.

**Figure 4.** PCA results for mycotoxin concentrations in the grain of *Triticum* genotypes analyzed in 2011. Factor loading values based on the correlation between the Principal Component (PC) and variable (mycotoxin) and contribution of variables are given in Table S1.
Table 3. Mean toxin concentrations (µg·kg⁻¹) in the grain of five examined *Triticum* species in 2010. Limits of detection (LODs) are given in brackets. If the toxin concentration in a sample was below the LOD, then half of the LOD was used for calculation; †—sum of enniatins (A, A1, A2, B, B1, B2, B3) and beauvericin; #>LOD—number of samples > LOD.

| Species      | Treatment | Measure | DON (8) | D-3G (1) | 3-Ac DON (2) | Deepoxy-DON (13) | NIV (5) | ZEA (2) | ZEA-4 Sulfate (1) | MON (20) | Apicidin (0.04) | Tentoxin (0.2) | AOH (30) | AME (0.8) | AUF (24) | Hexadepsipeptides † |
|--------------|-----------|---------|---------|----------|--------------|-------------------|---------|---------|-------------------|-----------|----------------|----------------|---------|----------|---------|-------------------|
| *T. monococcum* | C         | mean    | 9       | 3        | <LOD         | <LOD              | <LOD    | <LOD    | <LOD              | 10         | 59             | <LOD           | <LOD    | <LOD    | <LOD   | 184           |
|              |           | RSD (%) | 6.7     | 0.9      | 3.1          | 1.0               | 1.0     | 0.7     | 1.5               | 1.10       | 6            | <LOD           | <LOD    | <LOD    | <LOD   | 1.10 × 10⁷   |
|              |           | †        | 1.0     | 0.9      | 1.0          | 1.0               | <LOD    | <LOD    | <LOD              | 1.0        | 59            | <LOD           | <LOD    | <LOD    | <LOD   | 1.10 × 10⁷   |
|              |           | I        | 2.0     | 0.9      | 3.1          | 1.0               | 1.0     | 0.7     | 1.5               | 1.10       | 6            | <LOD           | <LOD    | <LOD    | <LOD   | 1.10 × 10⁷   |
|              |           | #>LOD    | 1.0     | 1.0      | 3.1          | 1.0               | <LOD    | <LOD    | <LOD              | 1.10       | 6            | <LOD           | <LOD    | <LOD    | <LOD   | 1.10 × 10⁷   |
| *T. dicoccum* | C         | mean    | 10.8    | 0.9      | 3.1          | 1.0               | 1.0     | 0.7     | 1.5               | 1.10       | 6            | <LOD           | <LOD    | <LOD    | <LOD   | 1.10 × 10⁷   |
|              |           | RSD (%) | 85.0    | 96.0     | 3.1          | 1.0               | 1.0     | 0.7     | 1.5               | 1.10       | 6            | <LOD           | <LOD    | <LOD    | <LOD   | 1.10 × 10⁷   |
|              |           | †        | 1.0     | 0.9      | 3.1          | 1.0               | <LOD    | <LOD    | <LOD              | 1.10       | 6            | <LOD           | <LOD    | <LOD    | <LOD   | 1.10 × 10⁷   |
|              |           | I        | 2.0     | 0.9      | 3.1          | 1.0               | 1.0     | 0.7     | 1.5               | 1.10       | 6            | <LOD           | <LOD    | <LOD    | <LOD   | 1.10 × 10⁷   |
|              |           | #>LOD    | 1.0     | 1.0      | 3.1          | 1.0               | <LOD    | <LOD    | <LOD              | 1.10       | 6            | <LOD           | <LOD    | <LOD    | <LOD   | 1.10 × 10⁷   |
| *T. spelta*  | C         | mean    | 135     | 31.0     | 3.1          | 1.0               | <LOD    | <LOD    | <LOD              | 10         | 59            | <LOD           | <LOD    | <LOD    | <LOD   | 184           |
|              |           | RSD (%) | 118.0   | 170.0    | 3.1          | 1.0               | <LOD    | <LOD    | <LOD              | 10         | 59            | <LOD           | <LOD    | <LOD    | <LOD   | 184           |
|              |           | †        | 1.0     | 0.9      | 3.1          | 1.0               | <LOD    | <LOD    | <LOD              | 1.10       | 6            | <LOD           | <LOD    | <LOD    | <LOD   | 1.10 × 10⁷   |
|              |           | I        | 2.0     | 0.9      | 3.1          | 1.0               | 1.0     | 0.7     | 1.5               | 1.10       | 6            | <LOD           | <LOD    | <LOD    | <LOD   | 1.10 × 10⁷   |
|              |           | #>LOD    | 1.0     | 1.0      | 3.1          | 1.0               | <LOD    | <LOD    | <LOD              | 1.10       | 6            | <LOD           | <LOD    | <LOD    | <LOD   | 1.10 × 10⁷   |
| *T. polonicum* | C         | mean    | 225     | 64.0     | 3.1          | 1.0               | 1.0     | 0.7     | 1.5               | 1.10       | 6            | <LOD           | <LOD    | <LOD    | <LOD   | 184           |
|              |           | RSD (%) | 128.0   | 158.0    | 3.1          | 1.0               | 1.0     | 0.7     | 1.5               | 1.10       | 6            | <LOD           | <LOD    | <LOD    | <LOD   | 184           |
|              |           | †        | 1.0     | 0.9      | 3.1          | 1.0               | <LOD    | <LOD    | <LOD              | 1.10       | 6            | <LOD           | <LOD    | <LOD    | <LOD   | 1.10 × 10⁷   |
|              |           | I        | 2.0     | 0.9      | 3.1          | 1.0               | 1.0     | 0.7     | 1.5               | 1.10       | 6            | <LOD           | <LOD    | <LOD    | <LOD   | 184           |
|              |           | #>LOD    | 1.0     | 1.0      | 3.1          | 1.0               | <LOD    | <LOD    | <LOD              | 1.10       | 6            | <LOD           | <LOD    | <LOD    | <LOD   | 184           |
| *T. aestivum* | C         | mean    | 3.5     | 0.3      | 3.1          | 1.0               | 1.0     | 0.7     | 1.5               | 1.10       | 6            | <LOD           | <LOD    | <LOD    | <LOD   | 184           |
|              |           | RSD (%) | 128.0   | 158.0    | 3.1          | 1.0               | 1.0     | 0.7     | 1.5               | 1.10       | 6            | <LOD           | <LOD    | <LOD    | <LOD   | 184           |
|              |           | †        | 1.0     | 0.9      | 3.1          | 1.0               | <LOD    | <LOD    | <LOD              | 1.10       | 6            | <LOD           | <LOD    | <LOD    | <LOD   | 184           |
|              |           | I        | 2.0     | 0.9      | 3.1          | 1.0               | 1.0     | 0.7     | 1.5               | 1.10       | 6            | <LOD           | <LOD    | <LOD    | <LOD   | 184           |
|              |           | #>LOD    | 1.0     | 1.0      | 3.1          | 1.0               | <LOD    | <LOD    | <LOD              | 1.10       | 6            | <LOD           | <LOD    | <LOD    | <LOD   | 184           |

RSD—relative standard deviation; C, I—control and inoculated objects; toxin abbreviations—refer to the “Abbreviations” section.
| Species     | Treatment | Measure | DON | D-3G | 3-Ac | DON | BUT | NIV | ZEA | ZEA-4 | Sulfate | MON | Apicidin | Equisetin | Emodin | Tentoxin | Calmorin | 1E-Hydropyr- | 5-Hydropyr- | AUF | Chlamydo- | Avenacin Y | Hexadepsipeptides |
|-------------|-----------|---------|-----|------|------|-----|-----|-----|-----|-------|---------|-----|----------|-----------|--------|----------|----------|-------------|-------------|-----|----------|-----------|------------------|
|             |           |         | (8) | (1)  | (2)  | (25) | (5) | (2) | (1) | (10)  | (0.04) | (3) | (0.50)   | (0.2)     | (10)  | (10)     | (24)     | (1.5)        | (135)      |     |          |            |                  |
| T. monococcum | C         | Mean    | 0.26×10³ | 10   | 36   | 11   | <LOD | <LOD | <LOD | <LOD | 67    | 37   | 77      | 124      | 152   | 73       | 50       | 30           | 37          | 9   | 37       | 80        | 164              |
|             |           | RSD (%) |      |      |      |      |      |      |      | 3     | 3     | 2      | 1      | 2       | 2        | 2     | 3         | 3         | 3            |             |
|             | I         | mean    | 30.1×10³ | 1.3×10³ | 245  | 2469 | 33   | 121 | 17 | 122   | 6.9   | 33    | 14      | 7.1      | 1,409 | 2,056   | 538      | 57×10³       | <LOD        | 12  | <LOD     | <LOD       | 245             |
|             |           | RSD (%) |      |      |      |      |      |      |      | 14    | 11    | 9      | 4      | 11      | 4        | 23    | 25        | 24        | 31           | 39         | 163 | <LOD     | <LOD       | 95              |
| T. dicoccum  | C         | mean    | 0.52×10³ | 9     | 94   | 135  | <LOD | <LOD | <LOD | <LOD | 67    | 64   | 6<LOD   | 165      | 168   | 57       | 100      | <LOD         | <LOD        | 64  | <LOD     | <LOD       | 65              |
|             |           | RSD (%) |      |      |      |      |      |      |      | 3     | 3     | 3      | 3      | 3       | 3        | 3     | 3         | 3         | 3            | 1          | 3   |          |            |                 |
| T. spelta    | C         | mean    | 76    | 9    | 132  | 90   | <LOD | <LOD | <LOD | <LOD | 30    | 71   | 76      | 134      | 88    | 73       | <LOD     | <LOD         | 71          | 3   | 108      | <LOD       | 39              |
|             |           | RSD (%) |      |      |      |      |      |      |      | 3     | 3     | 3      | 3      | 3       | 3        | 3     | 3         | 3         | 3            | 3          | 3   |          |            |                 |
| T. polonicum | C         | mean    | 1.5×10³ | 0.1×10³ | 233  | 42   | 32   | 1   | 1507 | 14.3 | 275  | 82    | 1.6    | 37       | <LOD     | <LOD     | <LOD     | <LOD      | 148         | 1,563 | 3,570    | <LOD     | 20×10³        |
|             |           | RSD (%) |      |      |      |      |      |      |      | 3     | 3     | 2      | 3      | 2       | 3        | 2     | 3         | 3         | 3            | 3          | 3   |          |            |                 |
| T. aestivum  | C         | mean    | 56.1×10³ | 4.8×10³ | 342  | 4,168 | 121 | 1,451 | 103 | 853  | 37.3  | 499   | 20      | 1.2     | 1,875    | 4,675    | 909      | 90×10³   | 110         | 479        | 2,085 | <LOD     | <LOD       | 176              |
|             |           | RSD (%) |      |      |      |      |      |      |      | 3     | 3     | 2      | 3      | 2       | 3        | 3     | 3         | 3         | 3            | 3          | 3   |          |            |                 |
| T. aestivum  | C         | mean    | 10.5×10³ | 1.1×10³ | 189  | 888   | 14   | 104  | 5    | 35    | 4.2   | 21     | 10      | 0.5     | 309      | 994      | 346      | 97×10³   | 7           | 263        | 183   | <LOD     | <LOD       | 33              |
|             |           | RSD (%) |      |      |      |      |      |      |      | 3     | 3     | 5      | 3      | 5       | 3        | 2     | 2         | 2         | 2            | 2          | 2   |          |            |                 |

Table 4. Mean toxin concentrations (µg·kg⁻¹) in the grain of five examined *Triticum* species in 2011. All explanations and designations as in Table 3.
Table 5. Mean toxin concentration (µg kg⁻¹) in the grain of the examined *Triticum* species in 2010 and 2011.

| Species     | Year | Treatment | 2010                      |            | 2011                      |            |
|-------------|------|-----------|---------------------------|------------|---------------------------|------------|
|             |      |           | Σ Tox                     | Including  | Σ Tox                     | Including  |
|             |      |           | Trich. B                  | other      | Trich. B                  | other      |
|             |      |           | 2010                      |            | 2011                      |            |
|             |      |           | Σ Tox                     | Including  | Σ Tox                     | Including  |
|             |      |           | Trich. B                  | other      | Trich. B                  | other      |
|             |      |           | 2010                      |            | 2011                      |            |
| T. monococcum | C    | 275       | 14.1 x 10³                 | 25         | 9.1                       | 659        |
|             |      |           | 12.8 x 10³                 |            | 42.4 x 10³                |            |
|             |      |           | 1.2 x 10³                  |            | 31.7 x 10³                |            |
|             |      |           |                           |            |                          |            |
|             | I    | 14.1 x 10³ | 12.8 x 10³                 | 25         | 9.1                       | 659        |
|             |      |           | 1.2 x 10³                  |            | 42.4 x 10³                |            |
|             |      |           |                           |            |                          |            |
| T. dicoccon  | C    | 407       | 13.9 x 10³                 | 300        | 73.7                      | 785        |
|             |      |           | 12.0 x 10³                 |            | 25.1 x 10³                |            |
|             |      |           | 1.9 x 10³                  |            | 21.9 x 10³                |            |
|             | I    | 13.9 x 10³ | 12.0 x 10³                 | 300        | 73.7                      | 785        |
|             |      |           | 1.9 x 10³                  |            | 25.1 x 10³                |            |
|             |      |           |                           |            |                          |            |
| T. spelta    | C    | 368       | 10.5 x 10³                 | 182        | 49.5                      | 366        |
|             |      |           | 8.7 x 10³                  |            | 13.1 x 10³                |            |
|             |      |           | 1.8 x 10³                  |            | 10.3 x 10³                |            |
|             | I    | 10.5 x 10³ | 8.7 x 10³                  | 182        | 49.5                      | 366        |
|             |      |           | 1.8 x 10³                  |            | 13.1 x 10³                |            |
|             |      |           |                           |            |                          |            |
| T. polonicum | C    | 58.4 x 10³ | 13.8 x 10⁴                 | 357        | 87.6 x 10³                | 1,660      |
|             |      |           | 59.6 x 10³                 |            | 87.6 x 10³                |            |
|             |      |           | 78.8 x 10³                 |            | 61.4 x 10³                |            |
|             | I    | 13.8 x 10⁴ | 59.6 x 10³                 | 357        | 87.6 x 10³                | 1,660      |
|             |      |           | 78.8 x 10³                 |            | 61.4 x 10³                |            |
|             |      |           |                           |            |                          |            |
| T. aestivum  | C    | 210       | 57.5 x 10³                 | 25         | 11.9                      | 778        |
|             |      |           | 41.8 x 10³                 |            | 15.9 x 10³                |            |
|             |      |           | 15.6 x 10³                 |            | 12.0 x 10³                |            |
|             | I    | 57.5 x 10³ | 41.8 x 10³                 | 25         | 11.9                      | 778        |
|             |      |           | 15.6 x 10³                 |            | 15.9 x 10³                |            |
|             |      |           |                           |            |                          |            |
| Mean        | C    | 210       | 57.3 x 10³                 | 25         | 11.9                      | 778        |
|             |      |           | 41.8 x 10³                 |            | 15.9 x 10³                |            |
|             |      |           | 15.6 x 10³                 |            | 12.0 x 10³                |            |
|             | I    | 57.3 x 10³ | 41.8 x 10³                 | 25         | 11.9                      | 778        |
|             |      |           | 15.6 x 10³                 |            | 15.9 x 10³                |            |

Σ Tox—sum of all detected mycotoxins; Trich. B—group B trichothecenes.
3. Discussion

The responses of 14 diploid, tetraploid and hexaploid Triticum genotypes to inoculation with the F. culmorum pathogen were presented in this study. The analyzed cultivars (Torka, Nexon, Kamut, Bondka, Lamela, Terzino) are characterized by satisfactory distinctness, uniformity and stability as well as high variety value for cultivation and use, and Sumai-3 is used extensively as a major source of resistance to Fusarium head blight FHB.

The responses of wheat species other than T. aestivum and T. durum to inoculation with pathogens responsible for FHB have been insufficiently studied. The results noted in this study cannot be discussed in depth due to the lack of published data relating to other wheat genotypes. As expected, inoculation contributed to a significant decrease in the values of the three analyzed yield components (in particular OKW), and the variations noted in successive years of the experiment can be attributed to differences in weather conditions that determined the severity of spike infections and the progression of FHB [31]. In 2011, a minor increase in the number of kernels per spike and a simultaneous drop in OKW values were noted in hulled wheat, which can be attributed to low precipitation levels during inoculation and less supportive conditions for the development of infection. In the group of tested cultivars, T. spelta was characterized by the weakest average response to inoculation, which is consistent with the results of our previous studies [20]. Spelt’s relatively low susceptibility to infections caused by FHB pathogens could result from long culms and loose spikes which contribute to the lower moisture content of spikelets in comparison with varieties characterized by shorter culms and denser spikes [32]. The above hypothesis was indirectly validated by Mao et al. [33] who performed a meta-analysis of quantitative trait locus (QTL) and observed that the presence of Rht-B1, Rht-D1 and Rht8 dwarfing genes significantly increased susceptibility to FHB, although the above mechanism has not been fully explained.

Wild emmer (T. dicoccoides), which is closely related to emmer wheat, is an interesting genetic source that can be potentially used for breeding FHB-resistant cultivars [34]. Due to its genetic similarity to T. durum, wild emmer could be a good candidate for breeding research aiming to develop disease-resistant cultivars of durum wheat [35]. Our results indicate that the interactions between experimental factors were significant in many cases. The significance of $G \times I$ interactions could be attributed to differences in the resistance of the analyzed genotypes to the applied pathogen, which indicates that not all tested genotypes are highly suitable for resistance breeding. The significance of $G \times Y$ interactions for the number of grains per spike and one kernel weight is undesirable for breeding because it indicates that both traits are highly influenced by environmental factors. The discussed interaction was not significant for spike weight. The above observations could point to an absence of considerable fluctuations in the yield of the analyzed genotypes under various environmental conditions. However, yield can be influenced by differences in the number of kernels per spike and kernel plumpness.

Although mycotoxin concentrations in grain differed between experimental years, PCA results demonstrated that the analyzed Triticum species responded similarly to infection. Very high metabolite levels, in particular AUF and MON, were observed in the grain of T. polonicum, which suggests that T. polonicum responded completely differently to inoculation with F. culmorum or that the analyzed period was characterized by highly supportive conditions for the development of other Fusarium pathogens. Aurofusarin, a pigment belonging to the group of dimeric naphthoquinones, is produced by major FHB pathogens, including F. avenaceum, F. culmorum, F. graminearum, F. sporotrichioides and F. tricinctum [36]. This metabolite is toxic for poultry, in particular turkeys. Moniliformin, produced mainly by F. avenaceum, F. tricinctum, F. proliferatum and F. subglutinans, is an inhibitor of several thiamine pyrophosphate-dependent enzymes, glutathione peroxidase and reductase [37]. The lowest content of type B trichothecenes in total mycotoxin concentrations was noted in both control and inoculated grain of T. polonicum. The above results could indicate that T. polonicum is particularly susceptible to Fusarium pathogens that produce MON. T. polonicum is a threshable species (with naked grain, similar to common wheat) with long and broad spikelet glumes that can exceed 4 cm.
This structural arrangement preserves the high moisture content of spikes and contributes to the development of filamentous fungi, including toxin-producing species. In both years of the study, the maximum level of DON in unprocessed cereals ($1,250 \, \mu g \, kg^{-1}$) [38] was not exceeded in any of the control samples. The presence of D3G, a metabolite of DON, is associated with wheat’s response to FHB pathogens. According to Lemmens et al. [39], the ability of wheat lines to convert DON to D3G is linked to quantitative trait locus (QTL) Qfhs.ndsu-3BS that had been previously associated with FHB resistance. The presence of the Fhb1 gene linked to QTL Qfhs.ndsu-3BS decreases the severity of FHB symptoms and increases the D3G/DON ratio. D3G is characterized by significantly lower toxicity and metabolic activity than DON, but it is partially metabolized by enzymes in the mammalian digestive tract to release DON [40]. A similar process is observed with various intensity during grain fermentation in beer and bread production. The cited authors also noted that the D3G/DON ratio could be a measure of the immune response and the host’s ability to inactivate DON. According to their observations, the D3G/DON ratio can range from 46% to even 70%, although values approximating 20% have been reported in other studies. In our experiment, the average D3G/DON ratio ranged from 11.4% (2011) to 21.4% (2010) in control grain and from 8.1% (2011) to 11.6% (2010) in grain inoculated with F. culmorum. Interestingly, the lowest values (up to 9%) were observed in T. monococcum that responded strongly to inoculation. The relatively highest values (9.8% to 23%) were reported in T. spelta, which was characterized by the weakest average response to F. culmorum, and in T. polonicum (10.8% to 28.4%) which produced the strongest response to the pathogen. Our findings indicate that the host plant’s ability to produce D3G could be an important, but not the only, defense mechanism for fighting off infections caused by DON-producing pathogens of the genus Fusarium.

4. Conclusions

The results of this study demonstrate that spelt grain accumulates the lowest amounts of mycotoxins, in particular type B trichothecenes. The analyses of yield-forming traits revealed that spelt was characterized by the weakest response to infection. The strongest responses were noted in T. polonicum breeding lines and Kamut wheat, in particular with regard to mycotoxin concentrations in grain, and the above findings were validated by PCA results. PCA supported discrimination between the evaluated genotypes based on their yield-forming traits. Spelt constitutes valuable initial material for breeding new wheat lines characterized by increased resistance to spike infections caused by Fusarium pathogens.

5. Material and Methods

5.1. Material

The experimental material comprised 14 Triticum genotypes: two breeding lines and one cultivar of T. monococcum, two cultivars and one breeding line of T. dicoccon, two breeding lines and one cultivar of T. spelta, two breeding lines of T. polonicum, and Kamut wheat (Table 6). Two cultivars of T. aestivum were also evaluated: Torka, characterized by the highest processing suitability (classified in the group of elite wheat cultivars [41]), and Sumai-3, a widely recognized source of resistance to FHB and a reference cultivar (material obtained from the Nanjing Agricultural University, Nanjing, China). Breeding lines were selected from the extensive collection of the Department of Plant Breeding and Seed Production of the University of Warmia and Mazury in Olsztyn based on at least satisfactory agricultural suitability (yield, grain plumpness, earliness, low susceptibility to lodging). The isolate of F. culmorum I1 [42] was derived from naturally infected grain of spring wheat grown in northeastern Poland. Pathogenicity and toxin production were checked before the experiment. Species identity and chemotype were additionally confirmed by PCR assays in the Department of Entomology, Phytopathology and Molecular Diagnostics of the University of Warmia and Mazury in Olsztyn.
Table 6. Genotypes of *Triticum* sp. examined in the experiment.

| Species       | Line/Cultivar       | Origin                                                                 |
|---------------|---------------------|------------------------------------------------------------------------|
| *T. monococcum* | K-1 K-6 cv. Terzino | National Centre for Plant Genetic Resources, Radzików, Poland          |
| *T. dicoccon*  | cv. Lamela          | Department of Plant Breeding and Seed Production, University of Warmia and Mazury in Olsztyn, Poland |
|               | cv. Bondka          | Department of Plant Breeding and Seed Production, University of Warmia and Mazury in Olsztyn, Poland |
| *T. spelta*    | K-20 UWM-13 cv. Nexon | Leibniz-Institut für Pflanzen genetik und Kulturpflanzenforschung, Gatersleben, Germany Breed ing strain from the Department of Plant Breeding and Seed Production, University of Warmia and Mazury in Olsztyn, Poland |
| *T. polonicum* | Pol-11 Pol-19 cv. Kamut* | National Germplasm Resources Laboratory, USA National Germplasm Resources Laboratory, USA Kamut International Ltd., USA |
| *T. aestivum*  | cv. Torka cv. Sumai-3 | Plant Breeding Strzelce Ltd., Poland Nanjing Agricultural University, China |

*Kamut is classified as *T. turgidum* ssp. *turanicum*, a close relative of *T. turgidum* ssp. *polonicum*. Sowing material supplied by Kamut Int. Ltd., was purchased in a supermarket in Austria.

Genomic DNA was isolated with the use of hexadecyltrimethylammonium bromide (CTAB) [9]. The pathogen was identified to species level under a light microscope (Nikon E 200) based on their sporulation characteristics and the sequence of the ITS 1–5.8SrDNA–ITS 2 region. This fragment was amplified with the use of specific primers ITS5 (F) GTATCG GA CGGAGATCCAGC and ITS4 (R) TTGCTCAGTGCATTG TCGG [43] and the FailSafe PCR system (Epicentre). Chemotypes were differentiated by two primer pairs of TRI13DON F 5'-CATC ATGAGACTACTTGTAGTTTGG-3' and TRI13DONR 5'-GCTAGATCGATTGTTGCATTGAG-3' as well as TRI13NIVF 5'-CCAAATCCGAAAACCGCA-3' and TRI13NIVR 5'-TTGA AAGCTCAAATCCGAAAACCGCA-3' [44]. The applied isolate belongs to a DON-producing chemotype (synthesizes deoxynivalenol).

5.2. Field Experiment

The field experiment was established at the Experimental Station in Bałcyny near Ostróda, Poland (53°36' N; 19°51' E) in a slightly undulating area, on Luvisols formed from silty light loam. Local soils are characterized by suitable physicochemical parameters and organic matter content for wheat production. The experiment had a randomized block design with three replications. Plot area was 6 m². Sowing density was 250 germinating kernels per m² for hulled wheats, *T. polonicum* and Kamut, and 360 kernels per m² for common wheat. NPK fertilizer was applied at the pre-sowing rate of 30–25–80 kg ha⁻¹. Seeds were not dressed, and Chwastox Extra 300 SL (Organika-Sarzyna S.A., Nowa Sarzyna, Poland, active substance: MCPA, 300 g in 1 L of the product) was used for chemical weed control at 3 L ha⁻¹.

Spikes were inoculated by spraying the experimental plots twice with an inoculum suspension of 500,000 conidia per mL in the full flowering stage (BBCH65) [45] at two-day intervals. The suspension was used at a dose of 100 mL per m². Plants from untreated plots served as the control. In the fully ripe stage (BBCH 89), 30 randomly chosen spikes were harvested manually from each inoculated (I) and control (C) plot to measure the average weight of one spike, number of grains per spike and one kernel weight (OKW). Spikelets and grain were harvested with a plot harvester.
5.3. Determination of Mycotoxins in Grain

Mycotoxin concentrations in grain were determined according to the method described by Suchowilska et al. [20]. Each year, grain samples weighing approx. 1 kg harvested with a plot harvester were collected from each of the field replicates before being pooled into one bulk sample weighing approx. 500 g, which was further subdivided into subsamples. The subsamples of 10 g were ground to fine powder in a laboratory mill (Cullati MFC, Zürich, Switzerland). An amount of 0.5 g of ground sub-samples was extracted with 2 mL of the solvent mixture (acetonitrile/water/acetic acid, 79:20:1, v/v/v). Acetonitrile (LC gradient grade) was supplied by J.T. Baker (Deventer, The Netherlands) and glacial acetic acid (p.a.)—by Sigma-Aldrich (Vienna, Austria). Water was purified successively by reverse osmosis using the Milli-Q plus system from Millipore (Molsheim, France). The samples were extracted for 90 min using the GFL 3017 rotary shaker (GFL, Burgwedel, Germany) and centrifuged for 2 min at 3,000 rpm (15 cm radius) in the GS-6 centrifuge (Beckman Coulter Inc., Fullerton, CA, USA). After centrifugation, the extracts were transferred to glass vials using Pasteur pipettes. Following this, 350 µL aliquots were diluted with the same volume of the dilution mixture (acetonitrile/water/acetic acid, 20:79:1, v/v/v) and directly injected into the LC-MS/MS instrument. For the determination of DON concentrations higher than 500 µg L⁻¹ (corresponding to 4 mg kg⁻¹ in samples), the raw extract was diluted 1 + 49 (v + v).

Chromatographic separation was performed at 25 °C in the 1100 Series HPLC System (Agilent, Waldbronn, Germany) equipped with a C₁₈ 4 × 3 mm-i.d. security guard cartridge and the Gemini® C₁₈ column, 150 × 4.6 mm i.d., 5 µm particle size (Phenomenex, Torrance, CA, USA). Detection and quantification were performed in the Selected Reaction Monitoring (SRM) mode using the QTrap 4000 LC-MS/MS System (Applied Biosystems, Foster City, CA, USA) equipped with a TurboIonSpray electrospray ionization (ESI) source.

For quantification, external calibration was performed using multi-analyte standards prepared and diluted in a 1:1 mixture of extraction and dilution solvent. The results were adjusted for apparent recoveries, which were determined by analyzing a blank wheat sample that had been spiked at one concentration level in triplicate. The relevant values of analytes in the investigated Triticum samples were generally in the range of 100% ± 10%, with the exception of D3G (47%), NIV (82%), AOH (69%), AME (69%) β–ZOL (76%).

5.4. Statistical Analysis

The results of all measurements and analyses were processed statistically using STATISTICA software (V. 10, StatSoft, Tulsa, OK, USA, 2011) [46]. Normal distribution was checked in the Kolmogorov-Smirnov test, the significance of differences between means was estimated by analysis of variance, and mean values were compared by the Student-Newman-Keuls (SNK) test at p < 0.01 or p < 0.05. The PCA was performed for two sets of traits: yield components and mycotoxin concentrations.

Supplementary Materials: The following are available online at www.mdpi.com/2072-6651/8/4/112/s1; Table S1: Factor loading values based on the correlation between the PC and variable and contribution of variables (in brackets, in percent) for the mycotoxin concentration in grain of examined wheat genotypes in two years of experiment.

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Conflicts of Interest: The authors declare no conflict of interest.
Abbreviations

The following abbreviations are used in this manuscript:

- FHB: Fusarium head blight
- OKW: one kernel weight
- PCA: principal component analysis
- d.m.: dry matter
- DON: deoxynivalenol
- D3G: deoxynivalenol-3-β-D-glucopyranoside
- NIV: nivalenol
- 3-AcDON: 3-acetyl-deoxynivalenol
- Deepoxy-DON: de-epoxy-deoxynivalenol
- ZEA: zearalenone
- ZEA-4: sulfate-zearalenone 4-sulfate
- MON: moniliformin
- AOH: alternariol
- AME: alternariol monomethyl ether
- AUF: aurofusarin
- BUT: butenolide

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