The Effect of Foliar Fertilization with Micronutrients on Disease Severity and Mycotoxin Concentrations in the Grain of Winter Spelt (*Triticum aestivum* spp. *spelta* L.): A Case Study

Bożena Cwalina-Ambroziak 1, Arkadiusz Stępień 2,* , Agnieszka Waśkiewicz 3 and Małgorzata Grzywińska-Rąpca 4

1 Department of Entomology, Phytopathology and Molecular Diagnostics, Faculty of Agriculture and Forestry, University of Warmia and Mazury, 10-719 Olsztyn, Poland; bambr@uwm.edu.pl
2 Department of Agroecosystems and Horticulture, Faculty of Agriculture and Forestry, University of Warmia and Mazury, 10-719 Olsztyn, Poland
3 Department of Chemistry, Faculty of Forestry and Wood Technology, Poznań University of Life Sciences, 60-625 Poznań, Poland; agnieszka.waskiewicz@up.poznan.pl
4 Department of Market and Consumption, Faculty of Economic Sciences, University of Warmia and Mazury, 10-719 Olsztyn, Poland; malgo@uwm.edu.pl

* Correspondence: arkadiusz.stepien@uwm.edu.pl; Tel.: +48-895-233-266

Abstract: The effect of mineral fertilization (NPK), foliar fertilization with micronutrients (Cu, Zn and Mn) and the NanoGro biostimulant on the severity of leaf, spike, stem base and root diseases in winter spelt cv. Schwabenkorn was evaluated in a field-plot experiment. A mycological analysis was performed and the content of Fusarium mycotoxins in grain was determined. Mineral fertilization (NPK), foliar fertilization with micronutrients and the NanoGro biostimulant exerted varied effects on the severity of Septoria leaf blotch and Septoria glume blotch, they promoted the spread of brown rust (excluding the NPK + NanoGro treatment) and inhibited the spread of black head mold (excluding the NPK treatment), eyespot and Fusarium foot and root rot (excluding the NPK + Mn treatment). Fertilization had no influence on grain yield or the content of Fusarium mycotoxins in grain. The concentrations of deoxynivalenol (DON), nivalenol (NIV), zearalenone (ZEA) and fumonisins FB1 + FB2 did not exceed the maximum permissible levels (except for DON in NPK + Cu + Zn + Mn and NPK + NanoGro treatments). Throughout the experiment, ergosterol (ERG) concentrations were highest in the grain of unfertilized spelt plants.

Keywords: Fusarium mycotoxins; winter spelt; disease; fertilization

1. Introduction

Spelt (*Triticum aestivum* spp. *spelta* L.) is an ancient wheat species which is being increasingly cultivated around the world. Spelt grain is widely used in the food processing industry on account of its high content of nutrients, including protein and high-quality gluten [1,2]. Spelt has lower environmental requirements than winter wheat, it is more resistant to adverse climatic conditions and utilizes soil nutrients more efficiently [3]. In high-input production systems, fertilization with macronutrients and micronutrients is the agronomic factor that exerts the greatest influence on grain yield, the chemical composition of grain and crop quality [4–7]. Sustainable mineral fertilization also increases plant resistance to pathogens [8,9].

Powdery mildew is a dangerous disease of cereals around the world [10,11]. Special forms of *Blumeria graminis* identified by Menardo et al. [12] infect tetraploid wheat (*Triticum durum*) and hexaploid wheat (*Triticum aestivum*). According to Cyrlker-Degulis and Bulińska-Radomska [3], winter spelt is characterized by relatively high resistance to Septoria tritici blotch, Septoria glume blotch, powdery mildew and brown rust, but it is less resistant to yellow rust. Contemporary wheat cultivars are susceptible to a new strain...
of *Puccinia striiformis* f. *tritici* fungus that has been spreading rapidly in Europe [13]. Eyespot is a dangerous disease of wheat [14] and rye [15]. Fusarium head blight (FHB), another devastating disease of cereals, is caused by fungal species of the genus *Fusarium*, including *F. graminearum*, *F. culmorum* and *F. avenaceum* [16]. Micronutrient fertilizers inhibit the spread of fungal pathogens of cereals [17] such as *Puccinia recondita*, *Fusarium* spp. and *Cladosporium herbarum* [18].

Fungi of the genus *Fusarium* (whose development is promoted by moderate and high temperatures and high humidity during and after flowering) infect the spikes of, e.g., small-grain cereals, and produce mycotoxins such as zearalenone (ZEA), type B trichothecenes (deoxynivalenol—DON and nivalenol—NIV) and fumonisins (FB₁ + FB₂) [19–21]. Szulc [22] observed that unbalanced fertilization involving very high rates of nitrogen and insufficient rates of potassium (a macronutrient that stimulates the formation of mycelial plugs in the infection site) increased crops’ susceptibility to infections caused by *Fusarium* spp. Maize supplied with two-component NP fertilizer was less susceptible to pathogens and mycotoxin contamination [23]. Phosphorus is also an important nutrient that decreases the accumulation of mycotoxins. Selected micronutrients (Zn, Cu, Fe), important for crops, inhibit the growth of *Fusarium* fungi [24,25]. Zinc sulfate, mainly ZnO-NP, suppressed the growth of *F. graminearum* and the accumulation of DON in wheat grain [26].

*Fusarium graminearum* and *F. culmorum* are important producers of DON and NIV [19,27,28]. Fusarium mycotoxins pose a significant threat to human and animal health, and regulatory limits for mycotoxins in cereals have been introduced by many countries [29,30].

The aim of this study was to determine the effect of foliar fertilization with micronutrients on infections caused by fungal pathogens in winter spelt and mycotoxin concentrations in grain.

2. Materials and Methods

2.1. Field Experiment

Winter spelt (*Triticum aestivum* spp. *spelta* L.) cv. Schwabenkorn was grown in 2012 and 2013 in the Agricultural Experiment Station in Tomaszkowo near Olsztyn (53°72 N; 20°42 E) on podzolic soil of complex 4 and quality class IIIb (with the granulometric composition of light loam according to the FAO) [31]. The soil had the following properties: pH in a 1 molar solution of KCl—4.62, C<sub>org</sub> content—7.93 g·kg<sup>−1</sup>, N<sub>total</sub> content—0.95 g·kg<sup>−1</sup>, plant-available minerals (mg kg<sup>−1</sup>): P—58.9, K—203.4, Mg—8.1, Cu—2.5, Zn—7.9, Mn—189.0 and Fe—1800.0 (mean values for 2012–2013). The analyses were performed in the Chemical and Agricultural Station in Olsztyn.

The plot sown area and harvested area were 8.00 m<sup>2</sup> and 5.20 m<sup>2</sup>, respectively. The experiment had a randomized block design with three replications, and it consisted of seven treatments:

1—control: without fertilizers or the NanoGro biostimulant;

2—NPK: 90 kg N ha<sup>−1</sup> (54 kg N ha<sup>−1</sup> applied to soil/46% urea in the tillering stage BBCH 22-23/36 kg N ha<sup>−1</sup> applied to leaves/10% urea in the stem elongation stage BBCH 30–31); 70 kg ha<sup>−1</sup> P<sub>2</sub>O<sub>5</sub> applied to soil before sowing (46% triple superphosphate) at a rate equivalent to 30.2 kg P ha<sup>−1</sup>; 100 kg ha<sup>−1</sup> K<sub>2</sub>O applied to soil before sowing (56% potassium salt) at a rate of 83.1 kg K ha<sup>−1</sup>;

3—NPK as above + 0.2 kg Cu ha<sup>−1</sup> foliar fertilization with micronutrients at the stem elongation stage BBCH 30–31 (1% solution of CuSO<sub>4</sub>);

4—NPK as above + 0.2 kg Zn ha<sup>−1</sup> foliar fertilization with micronutrients at the stem elongation stage BBCH 30–31 (1% solution of ZnSO<sub>4</sub>);

5—NPK as above + 0.2 kg Mn ha<sup>−1</sup> foliar fertilization with micronutrients at the stem elongation stage BBCH 30–31 (0.5% solution of MnSO<sub>4</sub>);

6—NPK as above + 0.2 kg Cu ha<sup>−1</sup> + 0.2 kg Mn ha<sup>−1</sup> foliar fertilization with micronutrients at the stem elongation stage BBCH 30–31;
VII—NPK as above + the NanoGro biostimulant (organic plant growth stimulant in the form of oligosaccharide pellets containing Fe, Co, Al, Mg, Mn, Ni and Ag sulfates in a concentration of $10^{-9}$ mol) applied to leaves at the stem elongation stage—BBCH scale 30–31 (BBCH scale—Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie, Berlin and Braunschweig, Germany) [32].

The experiment was conducted under natural infection conditions. Seeds were sown on 14 September 2011 and 17 September 2012 (plant density was 550 plants m$^{-2}$ in both years). All operations (sowing, plant protection, fertilization, harvest), identical in all plots, were carried out in accordance with the agronomic requirements of winter spelt. Winter triticale was the preceding crop in both years of the study. Weeds were controlled with herbicides (a.i. florasulam 5 g, aminopyralid 10 g, 2,4-D 180 g, a.i. fenoxaprop-P-ethyl 69.0 g in 2012; a.i. iodosulfuron methyl sodium 2 g, mesosulfuron methyl 10 g, a.i. iodosulfuron methyl sodium 25 g, amidosulfuron 100 g in 2013). Grain was harvested on 31 July in both years.

2.2. Severity of Leaf, Spike and Stem Base Diseases in Winter Spelt

The health status of winter spelt was evaluated in the growing seasons of 2012 and 2013. The severity of leaf, spike and stem base diseases was estimated visually (identification of external infection symptoms) and microscopically. The severity of powdery mildew (*Blumeria graminis*), Septoria leaf blotch (*Mycosphaerella graminicola*, anamorph: *Zymoseptoria tritici*) and brown rust (*Puccinia recondita* f. sp. *tritici*) was evaluated in the medium milk stage BBCH 75 (grain content milky, grains reached final size, still green) on two leaves—the flag leaf and the first leaf below the flag leaf, on 20 plants per plot. The severity of *Fusarium* head blight (*Fusarium* spp.), *Septoria* glume blotch (*Phaeosphaeria nodorum*, anamorph: *Stagonospora nodorum*), powdery mildew (*Blumeria graminis*) and black head mold (*Alternaria alternata*, *Cladosporium* spp. and *Epicoccum nigrum* fungal complex) was evaluated in the late milk stage BBCH 79 on 25 spikes per plot. The severity of leaf and spike diseases was estimated on a five-point scale ($1^\circ$—up to 5% of leaf/spike area has been infected, $2^\circ$—6% to 10% of leaf/spike area has been infected, $3^\circ$—11% to 30% of leaf/spike area has been infected, $4^\circ$—31% to 50% of leaf/spike area has been infected, $5^\circ$—more than 50% of leaf/spike area has been infected). The severity of the stem base diseases take-all (*Gaeumannomyces graminis*), eyespot (*Oculimacula acuformis*, *O. yallundae*, anamorph: *Tapesia acuformis*, *T. yallundae*), Fusarium foot and root rot (*Fusarium* spp.) and sharp eyespot (*Ceratobasidium cereale* anamorph: *Rhizoctonia cerealis*; *Thanatephorus cucumeris* anamorph: *Rhizoctonia solani*) was evaluated during ripening (soft dough stage—grain content soft but dry, hard dough stage—grain content solid, BBCH 85–87) on 30 plants per plot (the plants with the roots were transported to the laboratory for accurate identification of external infection symptoms). The severity of stem base diseases was estimated on a two-point scale ($0^\circ$—absence of disease symptoms, $1^\circ$—weak disease symptoms, $2^\circ$—strong disease symptoms).

The results were presented as the infection index $I_i$, which was expressed as a percentage [33]:

$$I_i = \frac{\Sigma (a \ast b) \ast 100\%}{N \ast I}$$

where $\Sigma (a \ast b)$ is the sum of the products resulting from the multiplication of the number of plants (a) by points on the five-point/two-point scale (b), $N$ is the total number of plants, $I$ is the highest number of points on the scale.

2.3. Fungi Isolated from Spelt Grain

After harvest, 100 kernels were collected randomly (a pooled sample per treatment), they were surface sterilized with 50% ethanol and 0.1% sodium hypochlorite for 30 s and then rinsed with sterile water. Surface sterilized kernels were placed in Petri dishes on potato dextrose agar (PDA) medium, and were incubated for seven days in a thermostat.
at a temperature of 22 °C. Fungal colonies were transferred to agar slants for microscopic identification.

2.4. Mycotoxin Analysis

2.4.1. Standards and Chemical Reagents

Mycotoxins FB$_1$ + FB$_2$, ZEA, DON, NIV and ergosterol (ERG) certified reference materials to be used as analytical standards were purchased from Sigma-Aldrich (Steinheim, Germany). Sodium dihydrophosphate, potassium hydroxide, sodium hydroxide, potassium chloride, acetic acid, hydrochloric acid and orthophosphoric acid were purchased from POCh (Gliwice, Poland). Organic solvents (HPLC grade), disodium tetraborate, n-pentane, 2-mercaptoethanol and sodium acetate as well as the remaining chemical reagents were purchased from Sigma-Aldrich (Steinheim, Germany). Water for preparing a mobile phase for HPLC was purified with the use of a Milli-Q system (Millipore, Bedford, MA, USA).

2.4.2. Extraction and Purification Procedure

Ten-gram samples of winter spelt kernels were ground and homogenized. All mycotoxins (FB$_1$ + FB$_2$, ZEA, DON, NIV) and ERG were extracted and purified in accordance with a procedure described elsewhere [19,34]. The elute was evaporated to dryness at 40 °C under a stream of nitrogen. The dry residue was stored at −20 °C until analysis.

2.4.3. HPLC Analysis

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

Waters 2475 Multi λ Fluorescence Detector (Waters, Milford, MA, USA) ($\lambda_{ex} = 335$ nm, $\lambda_{em} = 440$ nm) with an XBridge column (3.0 × 100 mm) for FB$_1$ and FB$_2$ analysis,

Waters 2996 Photodiode Array Detector (Waters, Milford, MA, USA) with a Nova Pak C-18 column (150 × 3.9 mm) for ERG ($\lambda_{max} = 282$ nm) analysis and a Nova Pak C-18 column (300 × 3.9 mm) for DON and NIV analyses ($\lambda_{max} = 224$ nm),

Waters 2475 Multi λ Fluorescence Detector ($\lambda_{ex} = 274$ nm, $\lambda_{em} = 440$ nm) and the Waters 2996 Photodiode Array Detector (Waters, Milford, MA, USA) with a Nova Pak C-18 column (150 × 3.9 mm) for ZEA analysis.

Mycotoxins were quantified by measuring the peak areas and retention times based on the relevant calibration curves. The limits of detection were as follows: 0.001 µg g$^{-1}$ for ZEA, 0.02 µg g$^{-1}$ for FB$_1$ and FB$_2$ and 0.01 µg g$^{-1}$ for DON, NIV and ERG.

2.5. Statistical Analysis 2

The results were processed statistically by analysis of variance (ANOVA). Mean values were compared by NIR, Tukey’s and Scheffy’s tests at a significance level of 0.05. The differences in the incidence of diseases and the occurrence of mycotoxins under different fertilization variants were assessed to more fully determine the effect of NPK mineral fertilization as well as foliar fertilization with micronutrients (Cu, Zn, and Mn) and the NanoGro biostimulant on the intensity of leaf, spike, stem and root diseases in winter spelt. The hypothesis was formulated that fertilization variants differentiate the intensity of leaf, spike, stem and root diseases as well as mycotoxins in the winter spelt. To this end, the following hypotheses were tested:

**Hypothesis 1.** The fertilization variants applied do not differentiate the intensity of leaf, spike, stem or root diseases or mycotoxins in the winter spelt;

**Hypothesis 2.** The applied fertilization variants differentiate the intensity of leaf, spike, stem and root diseases as well as mycotoxins in the winter spelt.

The PROFIT procedure, which combines two analytical techniques, i.e., multidimensional scaling and multiple regressions, was carried out.
The PROFIT method is a two-step procedure that is a combination of multidimensional scaling (MDS) and multiple regression analysis. Multidimensional scaling (MDS) is a way to visualize the level of similarity of individual dataset cases [35,36]. The advantage of multidimensional scaling is that it expresses the relationships between individual objects not only through correlation matrices, but also as matrices of any distance measures (Euclidean, urban Manhattan, Chebyshev). It is important for the analyses carried out because it allows comparing objects due to quantitative and qualitative characteristics. The result of multidimensional scaling is a space with deployed objects [37]. The PROFIT analysis algorithm, based on information about coordinates and object values relative to each of the analyzed characteristics, performs multiple regression analysis as many regressive analyses as features are included in multidimensional scaling [38]. When interpreting PROFIT results, you must take into account regression factors. They contain information on the extent to which the position of objects relative to a given feature is explained by the location of those objects on the perception map [39,40].

All calculations were performed using STATISTICA® 13.3 (StatSoft, Tulsa, OK, USA).

2.6. Weather Conditions

Mean monthly temperatures and total precipitation from August to the end of October (comparable with the long-term average) promoted spelt emergence in both growing seasons. Winter precipitation in 2012 and 2013, except in January 2012, was below the norm (Tables 1 and 2). Temperatures were also low in this period, particularly during the first ten days of May (−16.7 °C). The spring growing season started early in 2012—above-zero temperatures were recorded from 10 March, and the last ten days of April were particularly warm (14.1 °C). Total precipitation from the last ten days of March to 20 June 2012 was around 192 mm, i.e., 73 mm higher than in 2013. Below-zero temperatures, which were noted until 10 April, slowed down plant development in the second growing season. A warmer May with precipitation comparable with the long-term average promoted plant growth from BBCH stage 39 (flag leaf fully unrolled) to BBCH stage 91 (harvest) in both years. Weather conditions were not conducive to flowering and grain ripening—precipitation was high in June 2012 (103 mm), and it exceeded the long-term average by over 60% in July 2012 and 2013.

Table 1. Ten-day and monthly air temperatures in 2011–2013 meteorological data against the long-term average of 1981–2010 (Meteorological Station in Tomaszkowo).

| Month/Year/10 days | 2011/2012 Mean Temperature (°C) | 2012/2013 Mean Temperature (°C) | 1981–2010 Mean Temperature (°C) |
|--------------------|---------------------------------|---------------------------------|---------------------------------|
|                    | I *                            | II                             | III                             | Av. **  | I                  | II                             | III                             | Av.                             |
| August             | 18.2                            | 16.9                            | 17.7                            | 17.6    | 18.6               | 17.0                            | 17.4                            | 17.7                            | 17.9                             |
| September          | 14.4                            | 14.6                            | 13.4                            | 14.1    | 14.7               | 14.1                            | 11.5                            | 13.5                            | 13.5                             |
| October            | 12.6                            | 6.3                             | 6.2                             | 8.3     | 10.0               | 8.2                             | 4.4                             | 7.4                             | 8.0                              |
| November           | 4.8                             | 0.8                             | 3.7                             | 3.1     | 6.1                | 3.8                             | 4.7                             | 4.9                             | 4.9                              |
| December           | 2.9                             | 2.4                             | 1.7                             | 2.3     | −3.4               | −4.7                            | −2.4                            | −3.5                            | −0.9                             |
| January            | 2.2                             | −6.3                            | −0.6                            | −1.7    | 0.8                | −7.1                            | −7.2                            | −4.6                            | −2.4                             |
| February           | −16.7                           | −5.2                            | 1.1                             | −7.5    | −0.3               | −2.1                            | −0.9                            | −1.1                            | −1.7                             |
| March              | −0.4                            | 4.0                             | 5.3                             | 3.0     | 0.2                | −6.7                            | −4.0                            | −3.5                            | 1.8                              |
| April              | 2.2                             | 7.2                             | 14.1                            | 7.8     | −0.4               | 8.7                             | 9.6                             | 5.9                             | 7.7                              |
| May                | 13.1                            | 12.0                            | 14.8                            | 13.4    | 14.7               | 15.8                            | 13.8                            | 14.8                            | 13.5                             |
| June               | 12.2                            | 16.5                            | 16.4                            | 15.0    | 16.3               | 18.2                            | 18.1                            | 17.5                            | 16.1                             |
| July               | 21.6                            | 15.5                            | 19.9                            | 19.0    | 18.2               | 16.6                            | 19.1                            | 18.0                            | 18.7                             |

* I, II, III—10-day periods in a month. ** Av—averages.
Table 2. Ten-day and monthly rainfall in 2011–2013 meteorological data against the long-term average of 1981–2010 (Meteorological Station in Tomaszkowo).

| Month/Year/10 days | 2011/2012 | Rainfall (mm) | 2012/2013 | 1981–2010 |
|-------------------|-----------|---------------|-----------|-----------|
|                   | I         | II  | III | Sum | I     | II  | III | Sum | Sum |
| August            | 20.4      | 44.9| 16.8| 82.1| 29.2  | 9.5 | 6.4 | 45.1| 59.4|
| September         | 24.5      | 43.0| 0   | 67.5| 2.2   | 34.1| 9.4 | 45.7| 56.9|
| October           | 7.6       | 21.1| 0.8 | 29.5| 46.4  | 18.6| 3.5 | 68.5| 42.6|
| November          | 0.0       | 8.1 | 6.0 | 14.1| 18.7  | 1.2 | 25.3| 45.2| 44.8|
| December          | 5.9       | 10.3| 9.6 | 25.8| 0.0   | 10.2| 1.6 | 11.8| 38.2|
| January           | 32.4      | 18.7| 10.7| 61.8| 18.5  | 6.3 | 19.3| 44.1| 36.4|
| February          | 0.4       | 6.3 | 21.0| 27.7| 14.2  | 8.4 | 0   | 22.6| 24.2|
| March             | 4.6       | 5.9 | 14.2| 24.7| 1.9   | 7.4 | 8.8 | 18.1| 32.9|
| April             | 20.5      | 32.2| 20.4| 73.1| 14.1  | 9.6 | 4.8 | 28.5| 33.3|
| May               | 0.8       | 48.6| 2.3 | 51.7| 6.5   | 20.5| 27.5| 54.5| 58.5|
| June              | 33.8      | 18.5| 50.9| 103.2|26.4  | 0.0 | 34.8| 61.2| 80.4|
| July              | 76.7      | 32.1| 12.2| 121  |16.9  |100.9| 4.1 |121.9|74.2|

*I, II, III—10-day periods in a month.

3. Results

3.1. Severity of Leaf, Spike and Stem Base Diseases in Winter Spelt

Symptoms of Septoria leaf blotch were observed on spelt plants in the growing season of 2012 (approx. 67% of plants were infected in the NPK + Cu treatment), and the disease was significantly less severe in the following year (the highest infection index of 35% was noted in the NPK + Zn treatment) (Figure 1a). The above could be attributed to abundant precipitation in June and early July of 2012 (180 mm) which was more than twice as high than in 2013 (80 mm), as well as high temperature (around 22 °C) in the first ten days of July 2012. NPK + Mn and NPK + NanoGro fertilization treatments decreased the severity of *Zymoseptoria tritici* infections in 2012 and 2013, respectively. Symptoms of brown rust were observed on spelt leaves only in 2012. The severity of infections increased in treatments fertilized with NPK and with NPK and micronutrients relative to unfertilized plants, and the infection index was highest (34%) in the NPK + Mn treatment (Figure 1b). The values of the infection index did not differ significantly between treatments. The symptoms of infection caused by *Blumeria graminis* were observed sporadically (in up to 2.5% plants) in all treatments only in 2013 (Figure 1c). In the early stages of disease (May and June), precipitation was moderate and temperatures exceeded the long-term average, and the spread of infection was probably suppressed by high rainfall and below-average temperatures in July.
Figure 1. Infection of winter spelt leaves by pathogens depending on foliar fertilization with micronutrients: (a) *Zymoseptoria tritici* (2012 and 2013 Y); (b) *Puccinia recondita* (2012 Y); (c) *Blumeria graminis* (2013 Y). a,b—Values followed by the same letters do not differ significantly in Tukey’s (HSD) test (*p* < 0.05). Data without a letter designation do not differ significantly.

In the growing season of 2012, weather conditions also contributed to the spread of Septoria glume blotch, which was most prevalent (maximum infection index of 42%) in the NPK + Cu treatment (Figure 2a). The progression of the disease was significantly inhibited in the remaining mineral fertilization treatments and the control treatment. In 2013, the incidence of Septoria glume blotch was much lower, and it did not exceed 5%. However, more than 25% of the spikes in the NPK treatment displayed symptoms of black head mold (Figure 2c), probably due to high precipitation during grain ripening (approx. 120 mm in the first 20 days of July). The fertilization treatment combining micronutrients and the NanoGro biostimulant significantly reduced the severity of disease symptoms. *Fusarium* head blight affected 0.6% of spelt plants only in the NPK + Cu + Zn + Mn treatment (Figure 2b).
Figure 2. Infection of winter spelt spikes by pathogens depending on foliar fertilization with micronutrients; (a) *Parastagonospora nodorum* (2012 and 2013), (b) *Fusarium* spp. (2013 Y), (c) Fungi of the genera *Alternaria*, *Cladosporium*, *Epicoccum* (2013 Y). a,b—Values followed by the same letters do not differ significantly in Tukey’s (HSD) test ($p < 0.05$). Data without a letter designation do not differ significantly.

Symptoms of eyespot and Fusarium foot and root rot were noted on the stem bases of spelt plants in both years of the study, whereas sharp eyespot was observed only in 2013 (Figure 3a–c). In 2012, unfertilized plants were most severely infected (infection index of 46%) with *Tapesia* spp., whereas the plants fertilized with NPK and the biostimulant were least affected. In 2013, the severity of *Tapesia* spp. infection did not differ significantly between treatments and ranged from around 29% (NPK + Zn) to 41% (NPK + Mn). In 2012, fertilization had a non-significant effect on the severity of Fusarium foot and root rot, and the infection index ranged from 10% to 21%. In the second year of the experiment, the severity of Fusarium foot and root rot increased in all treatments, and it was, significantly, highest (approx. 44%) in the control treatment and lowest (approx. 25%) in plants
fertilized with NPK + Zn and NPK + Cu + Zn + Mn. Sharp eyespot was the least prevalent take-all disease in 2013.

**Figure 3. Cont.**
3.2. Toxigenic Fungi of the Genera Fusarium and Gibberella, and Fusarium Mycotoxins

Fungi of the genus *Fusarium* were isolated from spelt grain in both years of the study, but symptoms of FHB were observed only in 2013. The isolated *Fusarium* species were *Fusarium culmorum*, *F. poae*, *F. sporotrichioides*, *Gibberella avenacea* (teleomorph of *F. avenaceum*), *G. tricincta* (teleomorph of *F. tricinctum*) and *G. zeae* (teleomorph of *F. graminearum*) (Table 3). These toxigenic fungi were more prevalent in 2013 (from 7% in the NPK + Zn treatment to approx. 30% in NPK + Cu + Zn + Mn and NPK + NanoGro treatments) than in 2012 (from 2% in the NPK + Cu treatment to approx. 9% in control, NPK and NPK + Mn treatments). In both years of the study, *G. zeae* was isolated from all treatments, excluding control and NPK treatments in 2012 and the NPK treatment in 2013. *Gibberella avenacea* was also identified in all treatments (excluding NPK + Zn), and it was most prevalent (17%) in the NPK + Cu + Zn + Mn treatment.

Table 3. The prevalence of *Fusarium* spp. and *Gibberella* spp. in winter spelt kernels (%) depending on foliar fertilization with micronutrients.
ERG is an indicator of fungal invasion of grain, and it is used to determine the total biomass content of toxic and non-toxic fungi in cereals. In both years of the study, ERG concentrations were highest in spelt grain from the control treatment (6011.38 ng g\(^{-1}\) in 2012 and 6789.45 ng g\(^{-1}\) in 2013) (Figure 4a). In the remaining treatments, the ERG content of grain was significantly higher in the first year of the experiment. The grain of plants treated with the biostimulant was the least contaminated with ERG in 2013 (1803.33 ng g\(^{-1}\)).

The content of *Fusarium* mycotoxins in spelt grain was significantly higher in 2012 than in 2013. DON concentration exceeded the EU regulatory limits and was highest at around 1920 ng g\(^{-1}\) in the kernels from spelt plants treated with all three micronutrients and the NanoGro biostimulant (Figure 4b). In 2012, mycotoxin concentrations were significantly lower in the remaining treatments, approximating the maximum safe limits, except for the NPK treatment (763 ng g\(^{-1}\)). In 2013, DON accumulation in spelt grain did not exceed 500 ng g\(^{-1}\) and did not differ significantly between treatments, thus posing no threat of contamination. In the second year of the experiment, the NIV content of grain was low (maximum of 210 ng g\(^{-1}\)), and only minor differences were noted between treatments (Figure 4c). In 2012, NIV concentrations were 4- and 13-fold higher than in 2013 (937.18 ng g\(^{-1}\) in the control treatment and 763 ng g\(^{-1}\)). In 2013, DON accumulation in spelt grain did not exceed 500 ng g\(^{-1}\) and did not differ significantly between treatments, thus posing no threat of contamination. In the second year of the study, the NIV content of grain was significantly lower in the unfertilized treatment and in plants fertilized with NPK and NPK + Cu than in the remaining treatments. ZEA concentrations remained considerably lower than the EU regulatory limits in both years of the study. The ZEA content of grain was highest in treatments fertilized with NPK + Mn, NPK + Cu + Zn + Mn and NPK + NanoGro, where it ranged from 30 to 40 ng g\(^{-1}\) in 2012, and from 7.4 to 8.5 ng g\(^{-1}\) in 2013 (Figure 4d). In the first year of the experiment, ZEA contamination of grain was lowest in plants with foliar Cu and Zn fertilization. This mycotoxin was not detected in NPK and NPK + Zn treatments in 2013. The concentrations of FB\(_1\) + FB\(_2\) in spelt grain were twice as high in 2012 than in 2013. Significant differences in FB\(_1\) and FB\(_2\) levels were noted only in the first year of the study between NPK and NPK + Mn treatments (10 to 12 ng g\(^{-1}\)) and the remaining treatments (18 to approx. 24 ng g\(^{-1}\)) (Figure 4e).

![Figure 4. Cont.](image-url)
Figure 4. Cont.
3.3. Map Perception of Leaf, Spike and Stem Base Diseases and Fusarium Mycotoxins

The conducted analysis of variance shows that the fertilization variants differ significantly \((p = 0.05)\) in at least one of the characteristics. NIR, Tukey’s and Scheffe’s tests were applied on three fungi of the genera *Alternaria*, *Cladosporium* and *Epicoccum*, and \(\text{FB}_1 + \text{FB}_2\), ZEA, DON, NIV to six homogeneous groups and ERG. At a further stage, an analysis of contrasts was conducted to compare the obtained mean fungal values depending on the fertilization variant.

Contrast ratios for intergroup comparisons were calculated according to the formula proposed by Rabiej [38], and their values provided a basis for performing a multivariate test of significance for planned comparisons (Table 4). The contrast test results for dependent variables indicate that the applied fertilization variants affected disease severity and myco-
toxin concentrations in winter spelt grain. The determined confidence intervals did not include zero, and the significance level for all dependent variables was 0.0000 < p < 0.00002.

Table 4. Contrast ratios for intergroup comparisons of disease severity and mycotoxin concentrations in winter spelt grain depending on foliar fertilization with micronutrients.

| Treatments            | Contrast.1 | Contrast.2 | Contrast.3 | Contrast.4 | Contrast.5 | Contrast.6 |
|-----------------------|------------|------------|------------|------------|------------|------------|
| NPK                   | 5          | −1         | −1         | −1         | −1         | −1         |
| NPK + Cu              | −1         | 5          | −1         | −1         | −1         | −1         |
| NPK + Zn              | −1         | −1         | 5          | −1         | −1         | −1         |
| NPK + Mn              | −1         | −1         | −1         | 5          | −1         | −1         |
| NPK +Cu + Zn + Mn     | −1         | −1         | −1         | −1         | −1         | 5          |
| NPK + NanoGro         | −1         | −1         | −1         | −1         | −1         | −1         |

The confidence intervals determined for the contrast (Wilks’s test), as well as the t-statistics value and the p-value for this statistic, show that the contrast proved to be highly significant (p = 0.00007). It can be concluded that the fertilizer type differentiates the presence of leaf, spike and stem base diseases and *Fusarium* mycotoxins.

The PROFIT analysis results (Figures 5 and 6) enable the interpretation of similarities and dissimilarities between the analyzed fertilizer treatments applied in the disease incidence experiment. The PROFIT analysis conducted for 2012 did not take into account *Fusarium* spp. due to the absence of this pathogen. The distribution of points and vectors in 2012 indicates that the incidence of fungal diseases is determined by two groups of fertilizer variants. The most strongly correlated diseases include the mycotoxins NIV, DON and ZEA. The occurrence of the mycotoxins NIV, DON and ZEA was determined to the greatest extent by the fertilization treatment NPK + Cu + Zn + Mn. A strong correlation was also observed for the pair FB1 and FB2 and ERG. It follows from the analysis of the data included in the preference map for the year 2013 that, from the perspective of the occurrence of ERG and FB1 and FB2, the application of the fertilizer treatments of NPK, NPK + Cu and NPK + Mn is not preferred. Based on the direction vectors, a negative correlation between *Fusarium* and DON was observed. The conducted analysis shows the small influence of the fertilizer treatments of NPK, NPK + Cu, and NPK + Mn on the occurrence of ZEA.

![Figure 5](image_url)  
**Figure 5.** Map of perception—PROFIT analysis of mycotoxin and ergosterol (ERG) concentrations in the grain of winter spelt depending on foliar fertilization with micronutrients (2012 Y).
3.4. Grain Yield

The impact of infections caused by fungal pathogens and mycotoxin contamination on grain yield was analyzed by Wojtkowiak and Stepień [7]. Significant differences in grain yield could be attributed to the influence of weather conditions. Higher temperatures at the beginning of the growing season in 2013 (2 °C higher in April and May 2013 relative to 2012) and optimal precipitation levels contributed to rapid plant growth and high yields. Mineral fertilization and biostimulant treatments did not induce significant differences in grain yields between years. In comparison with the control treatment, grain yield was 7% higher in the NPK + Cu + Zn + Mn treatment in 2012 and around 10% higher in the NPK + Cu treatment in 2013.

4. Discussion

Symptoms of Septoria leaf blotch were observed on spelt plants in both years of the study, but they were more severe in 2012. Fertilization with NPK, NPK and micronutrients and NPK and the NanoGro biostimulant exerted varied effects on the severity of Septoria leaf blotch, excluding the NPK + Cu treatment where the disease was most prevalent in 2012. Symptoms of brown rust were also observed in the first year of the study (the infection index was highest at approx. 30% in the NPK + Mn treatment), and mineral fertilization induced a minor increase in disease severity in all treatments. Spelt plants were sporadically infected by the dangerous pathogen B. graminis only in 2013. In a study by Cwalina-Ambroziak et al. [41], the incidence of Septoria tritici blotch and brown rust in winter wheat was also higher in 2012 than in 2013. The authors also found that the effects of fertilization with NPK as well as NPK, micronutrients and the NanoGro biostimulant on the severity of the above diseases in 2012 were ambiguous. In the present study, spelt spikes were colonized only by Parastagonospora nodorum in 2012. The severity of Septoria glume blotch and Septoria leaf blotch was significantly higher in the NPK + Cu treatment than in the remaining fertilization treatments. In 2013, Septoria glume blotch was less prevalent, and symptoms of FHB were observed only sporadically in selected treatments. High precipitation in the second year of the experiment contributed to the spread of
black head mold. Fertilization with NPK, micronutrients and the biostimulant reduced the severity of the infection. Symptoms of FHB are observed between heading and grain ripening [16], but they are most pronounced during anthesis [42]. Buerstmayr et al. [43] observed a correlation between FHB severity and the time of anthesis in winter wheat artificially inoculated with F. culmorum. Wickiel and Filoda [44] reported a low incidence of Fusarium spp. infections in spelt that was naturally infected and artificially inoculated with F. culmorum, and concluded that spelt was resistant to the above pathogens. In contrast, Sadowski et al. [45] isolated Fusarium spp. more frequently from spelt than from winter wheat kernels. According to Backhouse [46], FHB in wheat is caused mainly by Fusarium graminearum in warm and wet seasons, and Fusarium poae in warm and dry seasons.

In the current study, symptoms of eyespot and Fusarium foot and root rot were observed in both years of the experiment, but they were more pronounced in 2013. Fertilization decreased the incidence of infections caused by Oculimacula spp. in both years of the study (excluding the NPK + Mn treatment in 2013) and Fusarium spp. in 2013 (where the lowest infection index of 25% was noted in NPK + Zn and NPK + Cu + Zn + Mn treatments). In the second year of the study, the incidence of sharp eyespot did not differ significantly between fertilization treatments. Eyespot is a widespread disease in regions with a cold and humid climate, including Poland [47]. Fusarium foot rot caused by Fusarium equiseti and F. sporotrichioides is also a serious disease of cereals [48]. Grzebisz et al. [49] found that foliar Zn fertilization reduced the severity of root infections caused by Fusarium spp. According to Thompson and Huber [50], ammonium is more effective than nitrate in increasing plant resistance to soil pathogens and enhancing the activity of micronutrients, including Mn which is toxic for some pathogens. In contrast, Bhaduri et al. [51] postulated that only nitrate fertilizers can effectively decrease the incidence of diseases caused by soil-borne pathogens, including Fusarium spp. and Rhizoctonia solani.

In the present study, symptoms of FHB were observed sporadically only in 2013, but Fusarium spp. were isolated from spelt grain in both years of the experiment. Spelt kernels were colonized by Gibberella zeae in both years and by G. avenacea in 2013 in all treatments, with very few exceptions. According to Wiwart et al. [52], the lower incidence of natural infections caused by Fusarium spp. in winter spelt and low susceptibility to inoculation with F. culmorum spores suggest that winter spelt is resistant to these pathogens. In a study by Sadowski et al. [45], spelt grain was colonized mainly by F. poae, and less frequently by F. avenaceum and F. tricinctum, whereas F. sporotrichioides, F. culmorum and F. graminearum were isolated only in single cases. Wickiel and Filoda [44] argued that spelt is less susceptible to pathogens than other cereals because spelt glumes remain more tightly closed over the kernels. According to Golitški et al. [19] and Gräfenhan et al. [53], mycotoxins are produced mainly by F. avenaceum, F. graminearum and F. culmorum. Fusarium culmorum and F. graminearum generally produce type B trichothecenes, mostly DON and NIV [54]. Cereals are less contaminated with NIV, which does not pose health risks, than with DON [55]. Zearealenone is produced mainly by F. graminearum, F. culmorum, F. cerealis, F. equiseti and F. semitectum, and it often contaminates grain together with DON. This Fusarium mycotoxin is widely encountered in regions with a temperate climate, including in Europe [56]. Fumonisins produced by F. verticillioides are isolated from cereal grain around the world, but they are most prevalent in regions with a warm temperate climate [57]. In Poland, DON, NIV, ZEA and FB₁ and FB₂ are the most widespread Fusarium mycotoxins in cereal grain [58]. In the current study, the above trichothecenes, ZEA and FB₁ + FB₂ were isolated from all treatments, and their concentrations were significantly higher in 2012. Deoxynivalenol levels approximated or exceeded the maximum regulatory limit (1250 ng g⁻¹) in 2012, with the exception of the NPK treatment. Kernels were not contaminated with DON in 2013 and with the remaining mycotoxins in either year of the study. According to Schollenberger et al. [59], DON is the only mycotoxin to contaminate spelt grain. In the group of mycotoxins isolated by Blajet-Kosicka et al. [60] from the grain of organically grown spelt, DON was the most prevalent mycotoxin whose
content was determined at only 140 ppb (which is nearly 10 times lower than the maximum regulatory limit for DON in unprocessed cereals), whereas the average NIV content of grain was below the limit of detection. According to Cowger and Arrellano [61] and Yoshida and Nakajima [62], DON concentrations in grain can exceed the safe limits when FHB infections occur late (after anthesis) and produce mild symptoms or no symptoms. Wickiel and Filoda [44] found no correlation between the severity of FHB and DON concentrations in spelt. Cwalina-Ambroziak et al. [28] observed a negative correlation between the severity of FHB and the accumulation of DON and NIV in winter wheat grain, but found no significant correlation between FHB severity and the content of ZEA and FB$_1$ and FB$_2$ in grain. In the present study, ERG concentrations in grain were significantly lower in treatments fertilized with NPK and in treatments supplied with NPK, micronutrients and the biostimulant than in unfertilized treatments in both years of the experiment (approx. 6011 ng g$^{-1}$ in 2012 and 6790 ng g$^{-1}$ in 2013). According to Gutarowska and Zakowska [63], ERG levels in grain should not exceed 7.0 µg g$^{-1}$. High ERG levels are not always correlated with mycotoxin concentrations in plant samples [64].

Weather conditions did not induce significant differences in the grain yield of winter spelt across the analyzed mineral fertilization treatments. Blandino et al. [65] demonstrated that the foliar application of nitrogen and fungicide (strobilurin) did not improve grain yield or quality. In a study by Chattha et al. [66], Zn fertilizer applied to both soil and leaves increased wheat grain yields.

5. Conclusions

The study was conducted in north-eastern Poland. Weather conditions during the experiment considerably affected the development of spelt diseases and grain yield. Septoria leaf blotch, brown rust and Septoria glume blotch were most prevalent in the growing season of 2012. The severity of black head mold and stem base diseases caused by Tapesia spp. and Fusarium spp. was highest in 2013. Mineral fertilization, foliar fertilization with micronutrients and the NanoGro biostimulant exerted varied effects on the prevalence of Septoria leaf blotch, brown rust and Septoria glume blotch, had a non-significant stimulatory effect on the development of brown rust and inhibited the spread of black head mold (excluding the NPK treatment) and eyespot in both years of the study (excluding the NPK + Mn treatment in 2013), and Fusarium foot and root rot in 2012. Throughout the experiment, ERG concentrations were highest in the grain of unfertilized spelt plants. The concentrations of DON in grain exceeded the maximum permissible levels in 2012 (excluding the NPK treatment), but not in 2013. The concentrations of NIV, ZEA and FB$_1$ and FB$_2$ remained within the EU regulatory limits throughout the study. The influence of fertilization on the content of Fusarium mycotoxins in spelt grain was ambiguous.

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