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The Impact of Harvesting Time on *Fusarium* Mycotoxins in Spring Wheat Grain and Their Interaction with Grain Quality

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Abstract: In this work, we studied the impact of harvesting time on *Fusarium* mycotoxin occurrence in spring wheat and the effect of mycotoxin contamination on the quality of these grains. The spring wheat grains (*Triticum aestivum* L.) were collected in 2016–2018 when the crop had reached full maturity, 10 ± 2 days and 17 ± 3 days after full maturity. The grain samples were analyzed for *Fusarium* infection and co-contamination with mycotoxins deoxynivalenol (DON), zearalenone (ZEA), and T-2 toxin (T-2), as well as the quality of the wheat grains (mass per hectolitre, contents of protein, starch, ash and fat, particle size index (PSI), falling number, sedimentation, wet gluten content, and gluten index). The occurrence of *Fusarium* spp. fungi and the mycotoxins produced by them in the grains was mostly influenced by the harvesting time and meteorological conditions. The correlations between *Fusarium* species and the mycotoxins produced by them in the grains of spring wheat showed *F. graminearum* to be a dominant species, and as a result, higher concentrations of DON and ZEA were determined. The co-occurrence of all the three mycotoxins analyzed (deoxynivalenol, zearalenone, and T-2 toxin) was identified in wheat. In rainy years, a delay in harvesting resulted in diminished grain quality of spring wheat, as indicated by grain mass per hectolitre and falling number. Negative correlations were found in highly contaminated grains between mycotoxins (DON, ZEA, and T-2) and falling number and grain mass per hectolitre values.

Keywords: wheat grain; delayed harvesting; grain co-contamination with DON; ZEA; T-2 toxin; spring wheat grain quality

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1. Introduction

Fusarium head blight (FHB) is a common disease of cereals, caused by *F. graminearum* Schwabe (*Gibberella zeae* teleomorph) and *F. culmorum* (Wm.G.Sm.) Sacc., which leads to a diminished wheat grain yield, and lower grade and finished product quality [1–4]. Some of the FHB pathogens produce the trichothecene mycotoxins, and their levels within grain rely on the wet and cool weather conditions [5,6]. Major *Fusarium* mycotoxins that can occur in cereal grains and cereal-based products are deoxynivalenol (DON), T-2 and HT-2 toxins, and zearalenone (ZEA). Their biosynthesis can be affected by a host of factors, including not only temperature, but also humidity, oxygen level, mechanical cereal damage, and the presence of mold spores. The extent of their impact usually depends on climate conditions and widely varies across different world climate zones [7,8]. The weather and plant growth stage interaction affects mycotoxin content in grain at harvest [6]. Delayed harvest could be a further factor that enhances mycotoxin content [9]. It is known that harvest date

influences the incidence of seed-borne *Fusarium* spp. in wheat in Ontario. Xue et al. [10] confirmed that the occurrence of total *Fusarium* spp. increased about twofold, from 9.5% in seed harvested early to 19.8% following delayed harvest.

Consumer satisfaction and health are of utmost importance [11]. International food trade contributes to economic development, but also creates risks. Mycotoxin contamination of crop yield and products is a global problem [12]. The type and amount of mycotoxin produced by a single fungal species may vary from year to year, depending on the environmental conditions, plant varieties, and storage conditions [12]. In addition to the toxicological risk associated with mycotoxin contamination of cereals, grain quality may be impaired because *Fusarium* head blight (FHB) infection can affect grain components such as starch and proteins [13], and therefore the quality characteristics of the end use, as well as pasting properties [14,15] and baking performance [16]. It has been found that the composition of gluten proteins that make a significant contribution to baking wheat properties is altered by FHB [17]. A clear decrease in the content of total glutenin and high molecular weight glutenin subunits was found in wheat after a severe artificial infection [13]. As a result, the baking quality deteriorated, which resulted in poor bread quality. Variations in the biochemical composition of wheat grain and the consequent changes in its quality caused by FHB may be the result of fungal enzymes and impaired synthesis of grain components. It has been reported that *Fusarium* spp. secrete enzymes, such as carbohydrases and proteases, during the invasion of the kernel, thus degrading starch, cell wall components, and gluten proteins [13,18]. *Fusarium* infection could as well lead to an incomplete accumulation of kernel constituents through the mechanical blocking of vascular bundles by fungal mycelium or through impaired synthesis of grain components due to mycotoxin presence [13]. The effect of FHB on grain quality depends on the time of *Fusarium* infection [19]. Early infection is related to a decrease in the physical and chemical quality of grain. The most vulnerable phenological stages of wheat in terms of the disease infection and consequent DON accumulation are between the beginning of flowering until the early milk stage [20].

Fusarium infection can affect the kernel development, resulting in small, shriveled, and lightweight kernels, leading to an increase in ash content. Moreover, *Fusarium*, can be considered as an attacker, modifying, or even destroying, starch granules, storage proteins, and cell walls. According to Magallanes López et al. [21], ash content was greater in the samples that contained DON than in the control sample. Also, Matthäus et al. [22] noticed that ash content increased significantly in grains contaminated by *Fusarium*. It has been documented that *Fusarium* destroys starch granules, resulting in poor quality. The falling number test is used to determine grain sprouting or preharvest germination. In response to the germination, more than a one-thousand-fold increase of α-amylase activity can be found in the spouted kernels. An increase in α-amylase activity results in a decrease of falling number values (below 400 s.). Fungal α-amylase enzyme was identified in grains infected with by *Fusarium culmorum* [14,23]. Compared with winter wheat, there is only limited research evidence worldwide on spring wheat grain mycotoxin contamination by DON, ZEA, and T-2 toxin and its relation to quality.

The aim of this study was to determine the impact of harvesting time on *Fusarium* mycotoxin contamination of spring wheat and the impact on grain quality.

2. Materials and Methods

2.1. Field Plantings

A field experiment was set up in an eight-course crop rotation in Akademija (55°39′ N, 23°88′ E), Kėdainiai district, Lithuania during 2016–2018. The soil of the experimental site is Endocalcari-Epihypogleyic Cambisol (CMg-pw-can), light loam. It contained 1.5–2.0% humus, available phosphorus (P2O5) ranging from 190 to 240 mg kg^{-1}, available potassium (K2O) from 180 to 260 mg kg–1, and pH 6.5–7.0. The rates of PK fertilizers were calculated according to the concentration of PK elements for each individual field. The total N fertilizer application was 115–125 kg ha^{-1} (15 kg ha^{-1} before sowing and 100–110 kg ha^{-1} at the tillering stage).

Weeds were controlled by recommended herbicides. Other pesticides (insecticides and fungicides) were not applied.

Spring wheat cultivar, Triso, which is recommended for cultivation in Lithuania, was selected for trial. The spring wheat cultivar was sown in 5.0 × 1.5 m^2 plots using a randomized design with four replications at a seed rate of 500 seeds m^2. The seeds were not treated with pesticide. The contamination of wheat grains by *Fusarium* species and mycotoxins was studied under natural infection.

The grain samples were collected in three stages: when the crop had reached full maturity (BBCH 89, Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie), 10 ± 2 days, and 17 ± 3 days after first harvesting time (Table 1). The moisture content was determined by an Infratec 1241 analyzer (FOSS, Hilleroed, Denmark) in all grain samples.

Table 1. Flowering and grain harvesting time of spring wheat (2016–2018).

Years	Flowering Date	1st Harvesting Time	2nd Harvesting Time	3rd Harvesting Time
2016	22 06	04 08 full maturity *	16 08 full maturity + 12 days	24 08 full maturity + 20 days
2017	30 06	31 08 full maturity *	08 09 full maturity + 8 days	14 09 full maturity + 14 days
2018	22 06	06 08 full maturity *	16 08 full maturity + 10 days	23 08 full maturity + 17 days

* full maturity (harvesting time at BBCH 89).

Weather Conditions

According to local—Dotnuva—meteorological station (55°23′49.0″ N 23°51′55.0″ E, Kedainiai distr.) data in 2016, the summer period was windy and warm (Figure 1). During the spring wheat flowering stage in June, warm and dry weather prevailed. The rainy August interfered with harvesting.

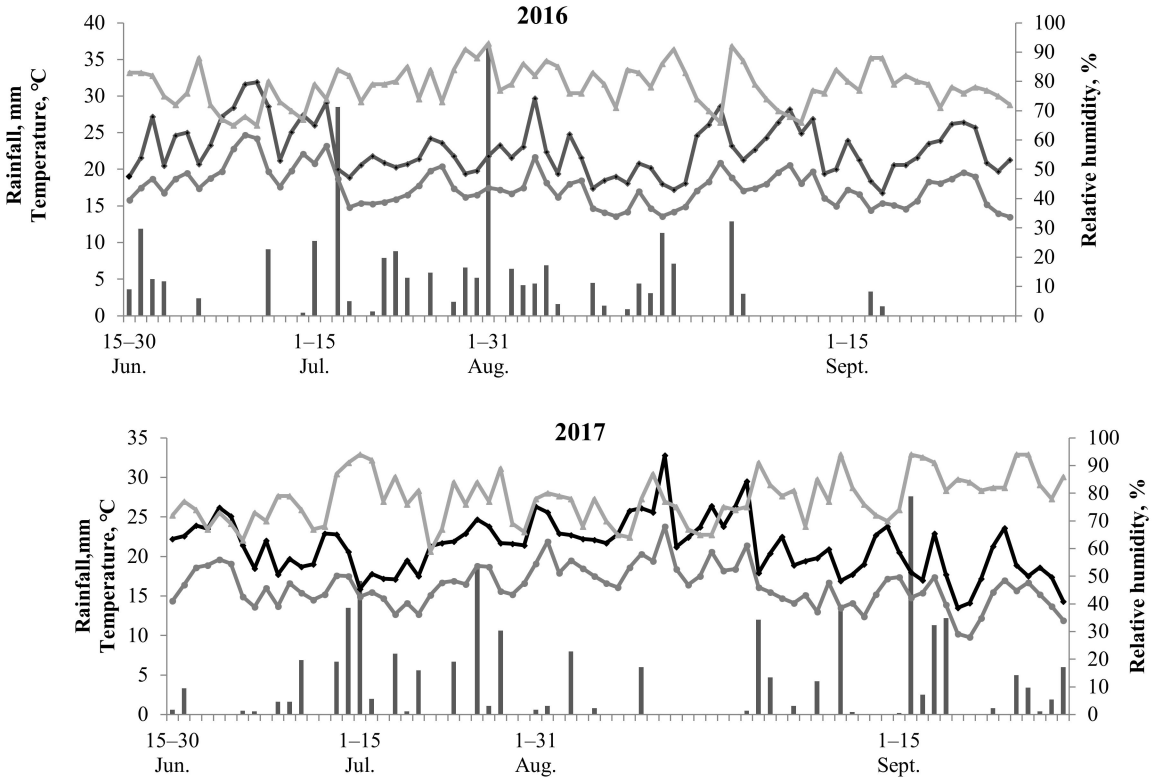

Figure 1. *Cont.*

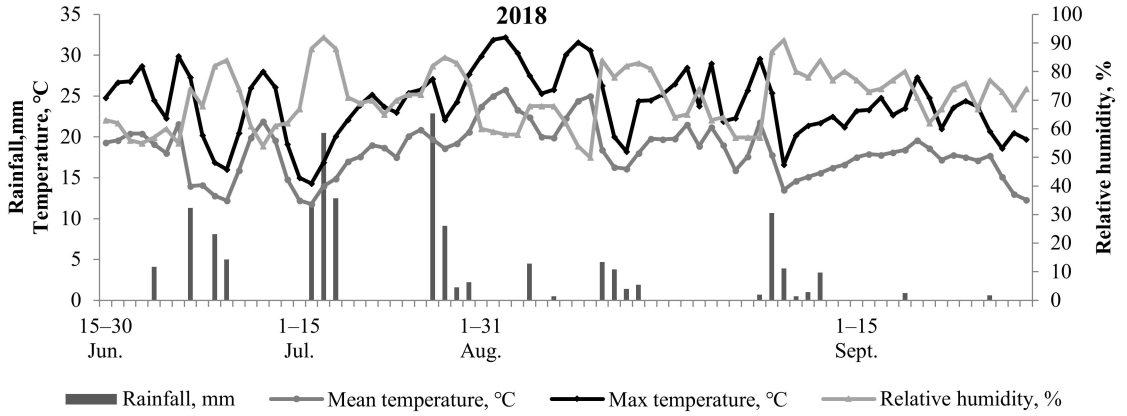

Figure 1. Weather conditions in 2016–2018.

In 2017, spring wheat flowering time in June was cool, with very wet weather conditions. Weather conditions, such as heavy rain in first half of September, were very unfavorable for harvesting.

In 2018, all three summer months were warmer than the long-term mean. The flowering period of spring wheat in June was dominated by extremely warm and dry weather. The weather conditions in August were favorable for harvesting.

2.2. Quantification of Mycotoxins

Quantitative analyses of DON, ZEA, and T-2 were carried out using an enzyme-linked immunoassay (ELISA) commercial kit (RIDASCREEN®FAST for DON Art. No.: R5901; for ZEA Art. No.: R5502; for T2 Art. No.: R5302 R5302, R-Biopharm AG, Darmstadt, Germany). This method is based on antibody antigen interaction and was approved by the AOAC Research Institute (Quality Management System certified ISO 9001). Mycotoxin analyses were done in duplicate.

2.2.1. Reagents

Most of the reagents used were contained in the RIDASCREEN test kit. DON, ZEA, and T-2 standard solution used for the construction of the calibration curve were at levels of DON—0, 222, 666, 2000, 6000 (μg kg^{-1}); ZEA—0, 50, 100, 200, 400 (μg kg^{-1}); and T-2—0, 50, 100, 200, 400 (μg kg^{-1}), all included in the ELISA test kit.

2.2.2. Preparation of Samples and Test Procedure

Five hundred grams of wheat grain was milled in the laboratory mill 3100® with a 0.8 mm sieve (Perten Instruments, Hägersten, Sweden). The milled samples were mixed for homogenization and weighed before separation for mycotoxin and some grain quality analysis. Mycotoxin extraction and tests were performed according to manufacturer's instructions. Extraction of samples was carried out in distilled water for DON, and in methanol:water (70:30 *v/v*) for ZEA and T-2. The basis of the test is the antigen–antibody reaction. The wells in the microtiter plates were coated with antibodies to each mycotoxin. By adding standards of each mycotoxin or the sample solution, the antibody binding sites were occupied in proportion to the concentration of each mycotoxin. Next, (DON, ZEA, T-2) enzyme conjugate and anti-(DON, ZEA, T-2) antibodies were added. Any unbound enzyme conjugate was removed in a washing step. Substrate/chromogen was added to the wells and bound enzyme conjugate converted the chromogen into a blue product. The addition of the stop solution led to a color change from blue to yellow. The optical densities of samples were estimated by a multichannel photometer Multiskan Ascent (Thermo Electron Corp., Vantaa, Finland), supplied with internal software, using a 450 nm filter. A calibration curve of the standards for each toxin dilution (reagents) was plotted using a standard concentration against the percentage inhibition of the respective standard.

For double determinations, cubic spline was used. While assessing our data with regard to food and forage safety, we referred to the European Union (EU) document No.1881/2006 for DON and ZEA [24] and global research recommendations for T-2 [25].

2.3. Grain Infection with Fusarium Spp. Fungi

An agar plate method was used for the estimation of internal grain infection (n = 36). Surface-sterilized (for 3 min in 1% NaOCl solution) grains (200 per sample) were plated in Petri dishes with a potato dextrose agar (PDA) and incubated for 7−14 days at 26 ± 2 °C in the dark [26]. The overgrown *Fusarium* colonies were isolated, purified, and identified according to the manuals [27,28]. The *Fusarium* spp. fungi was carried out using an optical microscope (Nicon Eclipse E 200, Tokyo, Japan). Fungal colonies were identified and the contamination percentage was estimated according to the number of contaminated grains. The infection level of grain was calculated as a percentage (0—all healthy grains, 100%—all infected grains).

2.4. Determination of Wheat Quality Parameters

The hectolitre weight (HLW) of grain was analyzed by measuring using a weighing method (ISO 7971) [29]; grain moisture for analysis was determined by weight method (ISO 712) [30]; the amount of total nitrogen in the grain was determined by Kjeltec digestion distillation systems (FOSS, Hillerød, Denmark), multiplying with 5.7 conversion factor in order to determine total protein content (ISO 20483) [31]; crude fat measured by Soxhlet extraction procedure [32]; and ash was measured by incineration at 550°C. Wet gluten content and quality using gluten index (GI) were determined by the approved Perten method (ICC 155) [33] using a Glutomatic System (Perten Instruments, Hägersten, Sweden), and wet gluten water hydration also was calculated from wet and dry gluten content; starch content analyzed by polarimetry according ICC 123/1 [34], with slight modification using ADP 410 Polarimeter (Bellingham and Stanley Ltd., Tunbridge Wells, UK); flour sedimentation was done by Zeleny method (ISO 5529) [35]; falling number was analyzed by Hagberg method (ISO 3093:2009) [36] using Falling Number 1500 (Perten Instruments, Hägersten, Sweden); particle size index (PSI) for wheat hardness (AACC 55–30) [37] was analyzed using Laboratory Mill 3303® (Perten Instruments, Hägersten, Sweden) and a 0.075 mm sieve sifting with Promylograph Max Egger (Blasen, Austria). The data of chemical composition of grain—protein, fat, ash, starch content—were recalculated on a dry matter (DM) basis, and wet gluten content was calculated on a 86% DM basis.

2.5. Statistical Analyses

Statistical analysis was conducted using the software SAS, version 9.4 (SAS Institute Inc., USA). Significant differences between the samples (Duncan's test) were calculated according to one-way analysis of variance (ANOVA). The results with $p \leq 0.05$ were considered significant. The correlation analysis was performed to examine the quantitative relationship between the investigated compounds. The strength of the relationship varies in degree based on the value of the correlation coefficient (r) at a significance level of $p \leq 0.05$. The data of mycotoxins were expressed as mean ± standard deviation (SD) using the software MS Office Excel (2010).

3. Results

Our experimental evidence determined that *Fusarium* infection in wheat grain was numerically higher in 2017 than in 2016 and 2018 (Figure 2).

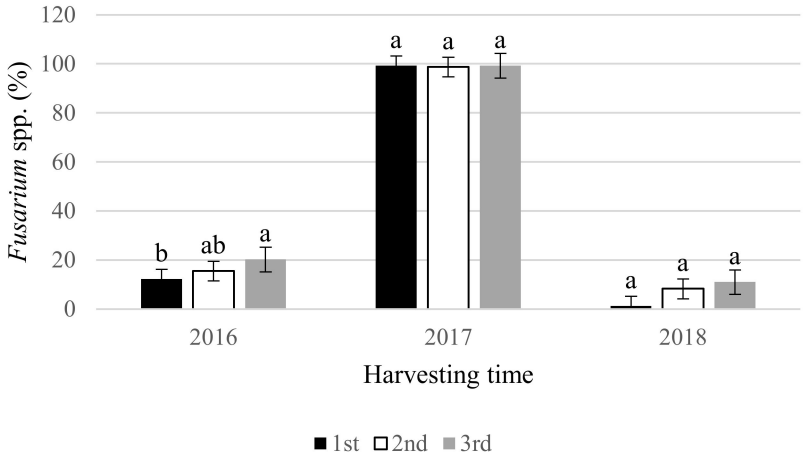

Figure 2. *Fusarium* spp. (%) in spring wheat grain at different harvesting times. Note. Values followed by the same letter for each year are not significantly different (Duncan's multiple-range test, $p < 0.05$); 1st—harvesting time when wheat reached full maturity, BBCH 89; 2nd—full maturity + 10 ± 2 days; 3rd—full maturity + 17 ± 3 days.

The average *Fusarium* infection in 2017 was 99.2%; in 2016, it was 16.0%; and in 2018, it was 6.8%. The wheat grain yields harvested in 2016 at full maturity were significantly less ($p < 0.05$) affected by *Fusarium* than those collected at the second and third harvesting stages. In 2017 and 2018, the *Fusarium* fungi infection in the grains harvested at full maturity stage was similar to that of the grains harvested later.

In our study, higher diversity of *Fusarium* species (*F. poae* (Peck) Wollenw., *F. tricinctum*, (Corda) Sacc., *F. sporotrichioides* Sherb., *F. graminearum* Schwabe, *F. culmorum* (W.G.Sm.) Sacc., and *F. avenaceum* (Fr.) Sacc. in wheat was found in 2017, when the conditions were more conducive to *Fusarium* infection (Figure 3). The prevalent species were *F. graminearum*, *F. sporotrichioides*, and *F. avenaceum.*

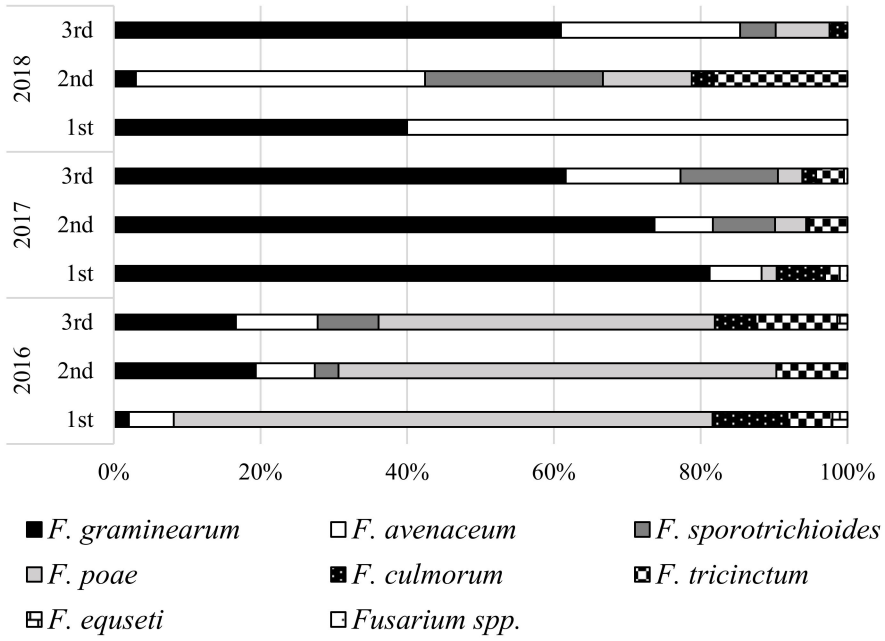

Figure 3. *Fusarium* species distribution (%) and composition in the grains of spring wheat at different harvesting times. Note. 1st—harvesting time when wheat reached full maturity (BBCH 89), 2nd—full maturity + 10 ± 2 days, 3rd—full maturity + 17 ± 3 days.

Significant correlations were observed between the amount of precipitation over a 20-day period after flowering and amount of DON, ZEA, and T-2 toxin (r = 1.00, $p < 0.05$) in grains harvested at all the three harvesting times in 2016–2018. The amount of precipitation over the 20-day period before flowering showed significant correlation with the amount of DON (r = 0.91, $p < 0.05$), ZEA, and T-2 (r = 0.90, $p < 0.05$) at all the three harvesting times. A bit weaker, but still statistically significant, correlation was noticed between the amount of precipitation over a 30-day period before flowering and the amount of DON produced (r = 0.72, $p < 0.05$) at the first harvesting time and (r = 0.71, $p < 0.05$) at the second and the third harvesting times, and for the ZEA and T-2 toxins (r = 0.71, $p < 0.05$) at all the three harvesting times.

The study of wheat grain samples in 2016 showed that DON levels were lower than the permitted level in the grains harvested at full maturity and similar at 12 and 20 days after full maturity (Table 2).

Table 2. Mycotoxin contamination of spring wheat grain at different harvest times during the 2016–2018 growing seasons.

Years	DON (μg kg^{-1}) Harvesting Time			T-2 (μg kg^{-1}) Harvesting Time			ZEA (μg kg^{-1}) Harvesting Time		
	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd
2016	446.0 ^{c}	573.0 ^{b}	783.0 ^{a}	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
2017	2103.0 ^{b}	10250.0 ^{a}	13809.0 ^{a}	30.6 ^{b}	67.3 ^{a}	74.0 ^{a}	50.5 ^{b}	895.0 ^{a}	1055.4 ^{a}
2018	381.0 ^{a}	326.0 ^{a}	352.0 ^{a}	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
EC, 2006		1250.0			100.0			100.0	

Note. Values followed by the same letter in a row are not significantly different (Duncan's multiple-range test, $p < 0.05$). 1st—harvesting time when wheat reached full maturity (BBCH 89), 2nd—full maturity + 10 ± 2 days, 3rd—full maturity + 17 ± 3 days. LOD—limit of detection; DON—deoxynivalenol; T-2—T-2 toxin; ZEA—zearalenone; EC—European Commission.

The concentrations of T-2 and ZEA were below the limit of detection. The concentrations of all mycotoxins in the wheat grains harvested that year were within the allowable limits.

In 2017, wheat grains were more contaminated with mycotoxins than in 2016. The DON levels in the wheat grains harvested at full maturity were significantly different ($p < 0.05$) from those in the grains harvested 8 and 14 days after full maturity. The ZEA contamination significantly increased by 17.7 and 20.9 times 8 and 14 days after full maturity, respectively. The levels of both DON and ZEA mycotoxins exceeded the permitted limit [24]. The contents of T-2 in the wheat grains harvested at full maturity were significantly different ($p < 0.05$) from those in the grains harvested later.

In 2018, the concentration of DON was lower than the allowable limit. The levels of T-2 and ZEA were below the limit of detection.

In 2016, the correlation analyses showed a strong and positive linear relationship between *F. graminearum* and DON in the wheat grains harvested at full maturity and at the 3rd harvesting stage (Table 3).

Table 3. The correlation coefficients (r) between *Fusarium* species and mycotoxins in spring wheat grain.

	2016			2017			2018		
	Harvesting Time			Harvesting Time			Harvesting Time		
	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd
F. graminearum vs. DON	0.65	0.21	0.82	0.28	0.00	0.27	0.00	0.00	0.00
F. graminearum vs. ZEA	0.17	0.00	0.00	0.90	0.21	0.27	0.00	0.00	0.00
F. sporotrichioides vs. T-2	0.00	0.14	0.41	0.00	0.01	0.05	0.00	0.17	0.60
F. tricinctum vs. T-2	0.33	0.00	0.07	0.55	0.10	0.64	0.00	0.01	0.21
F. poae vs. T-2	0.81	0.98	0.76	0.02	0.23	0.00	0.00	0.01	0.00

Note. 1st—harvesting time when wheat reached full maturity, BBCH 89; 2nd—full maturity + 10 ± 2 days; 3rd—full maturity + 17 ± 3 days.

Also, a strong correlation was found between *F. poae* and T-2 in the wheat grains harvested at all harvesting times. In 2017, a strong and positive correlation was identified

between *F. graminearum* and ZEA in the grains harvested at full maturity and between *F. tricinctum* and T-2 in the wheat grains harvested at full maturity and at the 3rd harvesting stage. In 2018, strong correlation was found only between *F. sporotrichioides* and T-2 at the 3rd harvesting stage.

In 2016, wheat grains harvested at full maturity with one mycotoxin were found in the samples harvested at full maturity, 12, and 20 days after full maturity.

In 2017, contamination with two mycotoxins was found in 25% and with three mycotoxins in 75% of the grain samples harvested at full maturity, 100% of the samples harvested later were contaminated with three mycotoxins. In 2018, all grains harvested at full maturity and later were detected with one mycotoxin.

In 2016, it was found that spring wheat grain mass per hectoliter and values of falling number significantly ($p < 0.05$) decreased in the grains harvested later compared to those harvested at full maturity (Table 4).

Table 4. The quality of spring grain in 2016–2018.

Harvesting Time	Mass Per Hectolitre, kg hl^{-1}	PSI, %	Sedimentation Zeleny, mL	Falling Number, s	Wet Gluten Content, %	Gluten Index,%	Gluten Water Hydration, %
				2016			
1—full maturity	80.5 ^{a}	5.46 ^{b}	67.0 ^{a}	399 ^{a}	32.0 ^{b}	91.0 ^{a}	209.1 ^{b}
2—full maturity + 12 days	78.7 ^{b}	6.67 ^{a}	67.0 ^{a}	256 ^{b}	31.2 ^{b}	94.9 ^{a}	207.6 ^{b}
3—full maturity + 20 days	75.9 ^{c}	5.38 ^{b}	67.0 ^{a}	64 ^{c}	34.1 ^{a}	82.0 ^{b}	223.2 ^{a}
				2017			
1—full maturity	78.4 ^{a}	6.85 ^{b}	49.3 ^{b}	351 ^{a}	31.0 ^{a}	91.7 ^{a}	212.7 ^{a}
2—full maturity + 8 days	74.0 ^{b}	7.36 ^{a}	50.4 ^{ab}	191 ^{b}	31.0 ^{a}	90.4 ^{a}	213.1 ^{a}
3—full maturity + 14 days	68.8 ^{c}	7.67 ^{a}	54.4 ^{a}	85 ^{c}	30.3 ^{a}	91.4 ^{a}	214.7 ^{a}
				2018			
1—full maturity	81.9 ^{a}	5.71 ^{b}	53.0 ^{a}	313 ^{c}	30.3 ^{a}	96.9 ^{b}	195.6 ^{a}
2—full maturity + 10 days	72.2 ^{c}	6.64 ^{a}	44.5 ^{b}	378 ^{a}	27.5 ^{c}	98.8 ^{a}	184.3 ^{b}
3—full maturity + 17 days	78.8 ^{b}	6.61 ^{a}	43.8 ^{c}	370 ^{b}	28.3 ^{b}	98.5 ^{a}	192.4 ^{a}

Note. Values followed by the same letter in a column at the same year are not significantly different (Duncan's multiple-range test, $p < 0.05$); PSI—particle size index.

The falling number values at the 3rd harvesting time were below 200. The lower PSI (relatively harder grains) was detected when grains were harvested at full maturity and at the 3rd harvesting stage. The flour Zeleny sedimentation test showed that its volume level was similar in grains harvested at all harvesting times. The gluten index values in the grains harvested at the 3rd harvesting stage were lower (gluten was relatively weaker), while the wet gluten content and the gluten water hydration in those grains were higher.

In 2017, the grain mass per hectoliter and values of falling number significantly ($p < 0.05$) decreased in the grains harvested later compared to those harvested at full maturity. The lower PSI values mean relatively harder grains were determined in the grains harvested at full maturity, while the sedimentation values were lower. The wet gluten content, gluten index, and gluten water hydration were similar in all samples of grains harvested at all harvesting stages.

In 2018, the grain mass per hectoliter and determined falling number values were significantly ($p < 0.05$) lower in the grains harvested later compared to those harvested at full maturity. Lower PSI values were determined in the grains harvested at full maturity, while the flour sedimentation values were higher. The gluten index values were similar in samples from the grains harvested at all harvesting stages. The higher values of gluten content and gluten water hydration were determined from the grain samples harvested at full maturity.

In 2016, it was detected that the spring wheat protein and ash contents were higher from the grains harvested at the 3rd harvesting stage, while higher starch content was determined at full maturity and higher fat level at the 2nd harvesting time (Table 5).

Table 5. Protein, starch, ash, and fat contents in spring wheat grain.

Harvesting Time	Protein, %	Starch, %	Ash, %	Fat, %
		2016		
1—full maturity	11.6 ^{b}	68.3 ^{a}	2.27 ^{ab}	1.07 ^{b}
2—full maturity + 12 days	11.3 ^{c}	67.9 ^{b}	2.24 ^{b}	1.28 ^{a}
3—full maturity + 20 days	12.2 ^{a}	68.1 ^{ab}	2.48 ^{a}	1.06 ^{b}
		2017		
1—full maturity	14.1 ^{a}	65.8 ^{a}	2.51 ^{a}	2.40 ^{b}
2—full maturity + 8 days	14.4 ^{a}	65.6 ^{a}	2.38 ^{a}	2.55 ^{ab}
3—full maturity + 14 days	14.3 ^{a}	65.4 ^{a}	2.16 ^{a}	2.71 ^{a}
		2018		
1—full maturity	15.5 ^{a}	64.1 ^{b}	1.87 ^{a}	2.53 ^{b}
2—full maturity + 10 days	15.5 ^{a}	64.2 ^{b}	1.84 ^{a}	2.38 ^{c}
3—full maturity + 17 days	15.5 ^{a}	64.5 ^{a}	1.91 ^{a}	2.81 ^{a}

Note. Values followed by the same letter in a column at the same year are not significantly different (Duncan's multiple-range test, $p < 0.05$).

It is necessary to mention that grain protein content from the grains harvested in 2016 at the 1st—3rd harvesting time (in contrary to other years) were below 13.0%, therefore they did not achieve a certain market grain quality specification.

In 2017, the protein, starch, and ash levels were not significantly ($p < 0.05$) different from those from the grains harvested at full maturity and later.

In 2018, the protein and ash contents were similar the grains harvested at full maturity and 10 and 17 days after full maturity. Higher levels of starch and fat were detected in the grains harvested at 3rd harvesting time.

In 2016, the DON level in spring wheat was within the allowable limit, while ZEA and T-2 concentrations were below the limit of detection; however, strong negative correlations were found between DON and mass per hectolitre ($r = -0.97$, $p < 0.05$), and DON and falling number ($r = -0.95$, $p < 0.05$).

In 2017, the mycotoxin (DON, T-2, and ZEA) concentrations were the highest and strong negative correlations were determined between DON and mass per hectoliter ($r = -0.71$, $p < 0.05$) and falling number values ($r = -0.89$, $p < 0.05$). Negative correlations were found between T-2 and mass per hectoliter ($r = -0.83$, $p < 0.05$) and falling number ($r = -0.76$, $p < 0.05$), and between ZEA and mass per hectoliter ($r = -0.88$, $p < 0.05$) and falling number ($r = -0.90$, $p < 0.05$).

In 2018, the DON level was within the allowable limit, ZEA and T-2 concentrations were below the limit of detection, and negative weak correlation was found between DON and gluten index ($r = -0.43$, $p < 0.05$) and DON and falling number ($r = -0.46$, $p < 0.05$) values.

4. Discussion

In our study, higher diversity of *Fusarium* species (*F. poae, F. tricinctum, F. sporotrichioides, F. graminearum, F. culmorum, and F. avenaceum*) in spring wheat was found in 2017, when the weather conditions at flowering stage were cooler and wet (Figures 1 and 3). This agrees with the findings of other research [38,39], which showed that the species that make up the *Fusarium* community are related to each other, and this coexistence is particularly affected by climatic factors such as temperature and humidity. It has been noticed that the co-occurrence of different fungal species on the same plant may have a significant negative impact on fungal development and mycotoxin production. The data obtained from our study agree with previous research where *F. graminearum, F. sporotrichioidies*, and *F. avenaceum* were found to be the most predominant species in wheat grains [40,41]. Our investigation is in line with Nordkvist and Häggblom [42], who reported that wheat grains were more contaminated with DON and ZEA (Table 2). Harvesting time in the wet weather conditions significantly influenced DON and ZEA contamination of wheat grain. This agrees with the findings of researchers [9] who showed that a delayed harvest could be a further factor that enhances mycotoxin content in grains. Our results are in line with Xue et al. [10], who observed that a delayed harvest by two weeks was correlated with the

increase in the incidence of the total *Fusarium* and *F. sporotrichioides* in Canada between 2004 and 2005.

Birr et al. [43] documented that correlation results between *F. graminearum* and DON and ZEA concentrations indicate that *F. graminearum* is the main producer of DON and ZEA in wheat grain samples. In our study, we also detected the strong correlations between *F. graminearum* and DON, *F. poae* and T-2, *F. graminearum* and ZEA, and *F. tricinctum* and T-2 (Table 3). Bianchini and Bullerman, [44] reported that T-2 toxin is produced by *Fusarium poae, Fusarium sporotrichioides,* and *Fusarium tricinctum*. The highly toxic HT-2 and T-2 toxins can be synthesized by a few isolates of *F. poae* [43]. The relationship between mycotoxins and *Fusarium spp.* is mainly associated with the weather conditions, this effect being the highest in the wet and cool season. The amount of precipitation during the 20–30-day period before wheat flowering and 20 days after it were the most influential factors. Also, Del Ponte et al., in 2007 [20], detected that the most vulnerable phenological stages of wheat in terms of the disease infection and consequent DON accumulation are between the beginning of flowering until the early milk stage.

The range of mycotoxin co-contaminations in 2017 was much higher than that in 2016 and 2018. In 2016 and 2018, the traces of one mycotoxin in wheat grains were found in 100% of the samples. In 2017, co-contamination with three mycotoxins was detected in 75% of wheat grain samples at full maturity, while with a delay in harvesting, 100% of the samples were found to be co-contaminated with the three mycotoxins.

In 2016, protein content in the grains harvested at the 2nd harvesting time was below 11.5%. While in 2017–2018 protein levels in the grains harvested at all harvesting stages were more than 13.0%. In the two last years (2017–2018), it was detected that protein and ash contents were not significantly ($p < 0.05$) different in the grains harvested at full maturity and later, while fat contents were higher in the grains harvested at the 3rd harvesting stage. The particle size index in the grains harvested at full maturity was the lowest in 2016–2018, and with a delay in harvesting, the grains became softer. The sedimentation values were not significantly different for all harvesting stages only in 2016 but were slightly greater in 2017 or lesser in 2018 (Table 4). Our investigation is in line with Cesevičienė and Mašauskienė, [45], who reported that trends were clear that delayed harvest impacted on protein content and sedimentation rate in wheat grains. The falling number values significantly ($p < 0.05$) decreased in the grains harvested at the 2nd and 3rd harvesting times in 2016–2017. Moreover, in these years, the falling number values established in the grains harvested at the 3rd harvesting stage were lower than 220 s (Table 4). In the EU, this is the minimum falling number limit for intervention of common wheat grain [46]; grain with lower values can be used for animal feed or other non-baking purposes, such as biofuel. The study of Cesevičienė and Mašauskienė [47] also detected that delay in harvesting significantly influenced the falling number value in wheat grains, especially in wet years. The mass per hectoliter (or specific weight) significantly ($p < 0.05$) decreased in the grains harvested later in all three years. Gluten content showed a tendency to increase with a delay in harvesting in 2016–2018. Gluten index values were higher in the grains harvested at the 2nd harvesting time, while gluten water hydration values in these grains were lower in 2016 and 2018.

Negative correlations were determined between high mycotoxin DON, T-2, and ZEA contamination and quality parameters of spring wheat grains, especially in 2017. Strong negative correlations were determined between mycotoxins and falling number values and grain mass per hectoliter. Like in our study, Kreuzberger et al. [13] noticed that sedimentation value was not affected by high mycotoxin contamination. These results have also been confirmed by a few other studies [15,48,49]. Our data have shown that there is no relationship between starch content of spring wheat grains and mycotoxin contamination. This is in accordance with a few authors who noticed that starch content did not differ between the grains free from mycotoxins and contaminated ones [13,17,48,50–52]. Protein content decreased in spring wheat grains that were highly contaminated with mycotoxins DON, ZEA, and T-2. Also, other authors observed a decrease of protein content after severe

Fusarium infection in grain [49,51,53]. In our investigation, the gluten content of highly contaminated wheat grain was not affected by mycotoxin contamination. In another study, it was noticed that gluten content did not differ between the grains free from mycotoxins and contaminated ones [13]. The results of this study show how delay in harvesting time can lead to mycotoxin contamination and losses in grain quality, which presents a potential consumer health hazard. Thus, it demonstrates the importance of control of harvesting time, especially in wet weather conditions, to get a high quality of wheat grains.

5. Conclusions

The occurrence of *Fusarium* spp. fungi and the mycotoxins produced by them in the grains of spring wheat was most influenced by the harvesting time and meteorological conditions of the growing season. The amount of precipitation during a 20–30-day period before wheat flowering and 20 days after it were the most influential factors for mycotoxin accumulation in grains. The correlations between *Fusarium* species and the mycotoxins produced by them in the grains of spring wheat showed *F. graminearum* to be a dominant species; as a result, higher concentrations of deoxynivalenol and zearalenone were determined. Due to the rainy spring wheat flowering and harvesting period (2017), the concentrations of DON and ZEA in the grains were several times higher than the permissible limits set forth in the EU regulation. The co-occurrence of all the three mycotoxins analyzed (DON, ZEA, and T-2 toxin) was identified in wheat. In rainy years, a delay in harvesting resulted in diminished grain quality of spring wheat, as indicated by grain mass per hectoliter and falling number values. A negative correlation was found in highly contaminated grains between mycotoxins (DON, ZEA, and T-2) and several grain quality parameters—specifically, falling number and grain mass per hectoliter.

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