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Article

Influence of *Fusarium graminearum* infection on the accumulation of mycotoxins in wheat grains

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Abstract: *Fusarium graminearum* is a dangerous pathogen of the cereals producing mycotoxins (trichothecene and zearalenone) harmful for human and animal health. There were evaluated sixteen winter wheat varieties for their response in conditions of natural infection with *F. graminearum* in the epidemic year 2019, being well known that accumulation of mycotoxins (DON, ZON and T-2) is induced by different biotic and abiotic factors. Field plot was organized in Latin rectangle randomized with three replicates. For all evaluated wheat varieties were collected field data (incidence, severity and infection degree of the fungus *F. graminearum*) and laboratory data (mycotoxins concentration in grains) that have been processed using the software JASP (Version 0.14) for descriptive statistics, and exploratory factor analysis (EFA). Microsoft Excel 2019 was used to calculate Pearson's correlation coefficients. The results showed negative corelation between plants' density and *F. graminearum* attack frequency. Positive correlations were found between DON and T-2 and between DON and fungus attack intensity. This work highlights that during a *F. graminearum* epidemic year some of the most influential factors in the contamination with harmful mycotoxins (DON, ZON and T-2) are: plants density, frequency of the attack on ear, diseased ears and attack intensity on ears.

Keywords: Fusarium Head Blight **(**FHB), *Fusarium graminearum;* mycotoxins; wheat; natural infection; epidemic year

1. Introduction

F. graminearum is a phytopathogen fungus from the genus *Fusarium* which causes one of the most devastating disease of the wheat and of the other small grain cereals known as Fusarium Head Blight (FHB) or scab, determining great yield losses and affecting grains quality due to its capacity to produce mycotoxins. Contamination with mycotoxins is considered to be a global problem there being considered that about 25% of agricultural products are contaminated each year, this fact determining economic losses [1 - 2]. In cereals the harvest damages are caused mainly due to the sterility of the ears, low TGW (1000 grain mass) and to the presence of the mycotoxins in infected grains. During the period 1991 – 1997 in USA the losses due to FHB in wheat and barley were surpassed 1.3 billion dollars [3].

Among the mycotoxins produced in the cereal grains by toxigenic phytopathogens, aflatoxins (AFs), ochratoxins (OTs), fumonisins (FUMs), T-2 toxin, deoxynivalenol (DON)

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and zearalenone (ZON) are considered harmful due to their toxic potential to humans, animals and plants, being in the attention of the researchers from worldwide as real reason of concern [4 - 5]. Therefore, the development of new physical, chemical and biological methods for the detoxification of the mycotoxins are highly desirable [6 - 7].

In FHB of wheat can be implied several *Fusarium* species, such as *F. graminearum, F. culmorum, F. nivale, F. poae* and *F. sporotrichioides* [8 - 9]. The mycotoxins produced by *F. graminearum* are framed in the chemical group of trichothecene. Often in the cereal grain samples analysed are found deoxynivalenol or vomitoxin (DON), toxin T-2, monoacetoxyscirpenol (MAS), diacetoxyscirpenol (DAS) and nivalenol (NIV). In the favourable epidemic years, the mycotoxins DON and T-2 are accumulating in cereals in high amounts [10, 11]. *F. graminearum* is considered the most important *Fusarium* species that produces DON [12 - 13]. Deoxynivalenol (DON) belongs to the chemical family of sesquiterpene, being derived from trichodiene (the biochemical precursor of all the trichothecene) and it is very thermostable, persisting during the storage of the cereals and later in the foodstuff. In case of ingestion, DON can produce food intoxications that are manifesting through sickness, vomiting, diarrhoea, headache, abdominal pains and fever [14, 15]. Among domestic animals, pigs are the most sensitive to DON, showing haemorrhages and necroses of the intestinal mucosa, skin and bone marrow. In comparison with the pigs, the ruminants are more resistant to DON. The forages contaminated with 10-15 ppm DON were tolerated by cattle and sheep without adverse effects [16 - 17].

T-2 mycotoxin appears in significant amounts in cereals together with DON [18]. The disease produced by it is named alimentary toxic aleukia or ATA. The intoxication with T-2 mycotoxin is manifesting by symptoms as fever, vomiting, convulsions, anaemia and acute inflammations of the digestive system, kidney and neurological diseases and cancer [19 - 20]. From the domestic animals the most sensitive to T-2 mycotoxin are poultry [21 - 22].

Other toxic metabolite produced by the fungus *F. graminearum* is zearalenone (ZON). It appears in the wheat grains and raw material affected by FHB together with DON and T-2. Most often it appears in the maize grains infected with *Fusarium*, but also it was found in oils, being potentially harmful for the health of humans and animals [23 - 24]. The estrogenic syndrome, induced by this toxin, appears after the ingestion of contaminated feed or food and is characterized by the swelling of the mammal glands, uterine hypertrophy, infertility and swelling of the vulva. The most sensitive to this mycotoxin are the pigs [25 - 26].

The technologies applied nowadays by farmers can influence positively the infection with *Fusarium* sp. and the accumulation of mycotoxins. The cultivation systems as "minimum tillage" or "no tillage" (useful for the conservation of soil fertility), high plant densities or the absence of the crop rotation have leaded to the increase of the inoculum source in the death biomass that is remaining on the soil surface [27, 28]. *Fusarium* can survive as mycelia, chlamydospores and perithecia with asci with ascospores on the decaying biomass. At this inoculum source can be added the infected seeds that can spread the disease [13, 29]. The prolonged humid weather during the vegetation period favours the fungus growth and sporulation. The spores of the fungus are carried by the wind and raindrops to the wheat ears. The wheat is susceptible to be infected during the flowering season and when the grains start to develop [30].

Among all *Fusarium* species, *F. graminearum* is most often present in the temperate regions with warmer climate in comparison with *F. culmorum* that prefers the cooler regions [31 - 32]. In Banat Plain from Romania, the most prevalent species that produces infections on wheat ear is *F. graminearum* [33].

Importance of this research consisted in the investigation of the wheat varieties response to *F. graminearum* attack and mycotoxins accumulation in conditions of natural infection, this type of research being possible only in the years that have specific climatic conditions.

The aim of the current research was to investigate some aspects involved in the contamination of the wheat grains with several harmful mycotoxins (DON, ZON and T-2), for this purpose being used 16 winter wheat varieties with different origin infected in

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natural conditions with *F. graminearum*, due to the favourable climatic background for *Fusarium* epidemics from the year 2019.

2. Material and Methods

2.1. Description of the field plot conditions and biological material

The experimental plot was set up in Timișoara (90 m a.s.l.) in the perimeter of the Didactic Station of Banat's University of Agricultural Sciences and Veterinary Medicine "King Michael I of Romania" from Timișoara. The soil in the experimental fields is chernozem type [34]. The multiannual average air temperature in the experimental area is 10.6 ^{o}C and the multiannual rainfall amount is 592 mm [35]. The plots' size was 7 m length and 2 m width and the organization of the field plot was in Latin rectangle, randomized with three replicates. The biological material was represented by 16 winter wheat varieties, with different origins: Anapurna, Apertus, Glosa, Exotic, Arezzo, Illico, Ingenio, Alex, Rubisco, Crișana, Airbus, Ciprian, Altigo, Șofru, Lovrin 6x and Lovrin 5x.

2.2. Field data and grain samples collection

The winter wheat varieties were monitored during the *Fusarium* epidemics from the year 2019, knowing that the year effect is substantial in the case of this disease [36]. The assessment of the incidence (F%) and severity (I%) of *Fusarium* attack on wheat ear was performed for every variety after flowering stage. The first evaluation was performed on 25 May 2019 and the second one at the middle of June (when most of the varieties were ended the flowering). The incidence of the attacked ears was calculated as rate of ears with symptoms of whitening reported to the total number of analysed ears (*diseased ears/analysed ears x 100*) (1). Severity classes of the *Fusarium* attack on ear were assessed using the current method described by Miedaner [37] and Trottet and Rolland [38]. In this way there were given marks from 1-9 to every analysed ear from the inside of the metric frame (0.5 m x 0.5 m). The infection degree (AD%) was calculated as the report between the frequency and attack intensity multiplied with 100.

Having in view the determination of the mycotoxins produced by *Fusarium* in wheat grains there were collected samples at harvesting time directly from the harvester grain tank.

2.3. Mycotoxins concentration determination

The determination of the concentrations of the mycotoxins DON (deoxynivalenol), ZON (zearalenone), and T-2 (toxin T2) was performed at the Platform for Interdisciplinary Research of USAMVB from Timișoara using ELISA mycotoxin test kit according with their analysis protocol.

Mycotoxins' extraction method had consisted in the grinding of the cereal samples, weighting 20 g from each sample followed by mixing it with 100 ml of distilled water. Then the obtained sample was mixed strongly with a mechanic agitator for 5 minutes and after it was filtered. From the obtained filtrate were taken 100 µl that were diluted with 400 µl dilution solution. These stages are followed by the immunoenzymatic determining using ELISA kit. In every sampler capsule were introduced 50 µl solution of concentrated antibody, was agitated slightly and was left to rest for 10 minutes at room temperature. After that time interval the liquid from the sampler was dried and washed three times with 300 µl washing solution. Then 100 µl of chromogen substrate was added and the sample was agitated again slightly and after it was kept 10 minutes in dark at room temperature. At the end were added 100 µl stopping solution and the solutions from the samples turned to yellow. From the moment of stopping solution addition the samples were read. The reading was realized with the ELISA PR 1100 apparatus at the wave length of 450 nm. The minimum detectable concentrations for the used tests were: 0.08 ppm for DON, 1 ppb for zearalenone and 13 ppb for T-2. In Table 1 is presented the maximum allowable concentrations of mycotoxins produced by *F. graminearum* in the unprocessed wheat grains according with the EU regulations [39, 40].

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Table 1. Maximum allowable concentrations of mycotoxins produced by *Fusarium graminearum* in the unprocessed wheat grains [39, 40].

Mycotoxins produced by *Fusarium sp.* in wheat grains	Maximum allowable concentration allowed in unprocessed cereal grains
Deoxynivalenol (DON)	1250 ppb [39]
Zearalenone (ZON)	100 ppb [39]
Toxin T-2 + HT-2	100 ppb [40]

The climate data (rainfall amounts and air temperatures) were registered at the Meteorological Station from Timișoara and were used to assess the favourability of the weather conditions for the development of the fungus *Fusarium graminearum*.

2.4. Statistical analysis of the data

The statistical analysis characterized the relationships among the wheat varieties, mycotoxins (DON, ZON and T-2) concentrations, plants density and infection features (diseased ears, F% on ears, I% on ears). The data were processed with the software JASP (Version 0.14) [41] respectively descriptive statistics, ANOVA (using Tukey test as post-hoc test) and Exploratory Factor Analysis (EFA) by path diagram. The Pearson's correlation coefficient ($r_{calc.}$) was determined using Microsoft Excel [42].

The statistical analyses performed for the processing of the field and laboratory data were structured considering first the interrelation among the field data, secondary the laboratory data and finally the interaction among all the variables considered here.

3. Results

3.1. Weather conditions for the infection of wheat with F. graminearum

The spring of the year 2019 was characterized by a humid and warm climate (Figure 1 and Figure 2), mainly in the second part of the season.

The rainfall amount from May was 109.7 mm, surpassing the multiannual monthly average with 46.7 mm. Analysing the daily data there was noticed that only in 8 days from May wasn't rain (Figure 1 and Figure 3). The maxim temperatures during the day were framed in the demands of the fungus, respectively 20.4 °C – 30.9 °C. The minimum temperatures of May at Timișoara were comprised in the interval from 4.3°C (the beginning of the month) to 17°C (to the end of the month), they being registered during the night or in the morning. The thermal regime of the month was characterized by deviations. The average temperature registered was 15.1 °C, much lower than the multiannual average with 6.9 °C (the normal temperature of the month in the area is 22 °C) – (Figure 2 and Figure 3).

These weather conditions have leaded to the conclusion that continuous moisture and maximal temperatures during the day were in the favourable values interval for great attack frequencies and intensities of the fungus *F. graminearum,* the conditions being proper for the mycotoxins' accumulation in wheat grains in the research area, 2019 being a *Fusarium* epidemic year.

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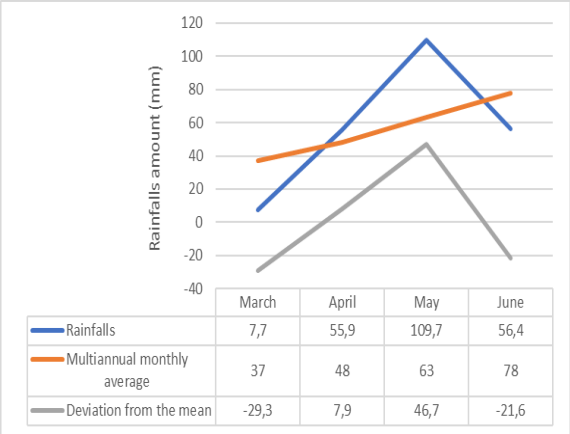
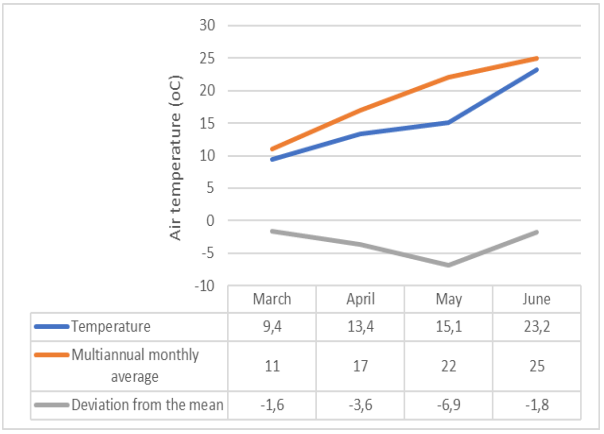


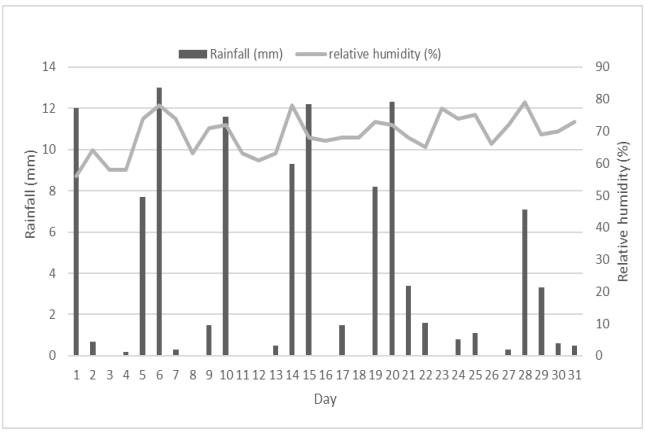
	March	April	May	June
Rainfalls	7,7	55,9	109,7	56,4
Multiannual monthly average	37	48	63	78
Deviation from the mean	-29,3	7,9	46,7	-21,6

Figure 1. Monthly average rainfall amount from Timișoara from March - June 2019 [35].



	March	April	May	June
Temperature	9,4	13,4	15,1	23,2
Multiannual monthly average	11	17	22	25
Deviation from the mean	-1,6	-3,6	-6,9	-1,8

Figure 2. Monthly average air temperature from Timișoara from March - June 2019 [35].



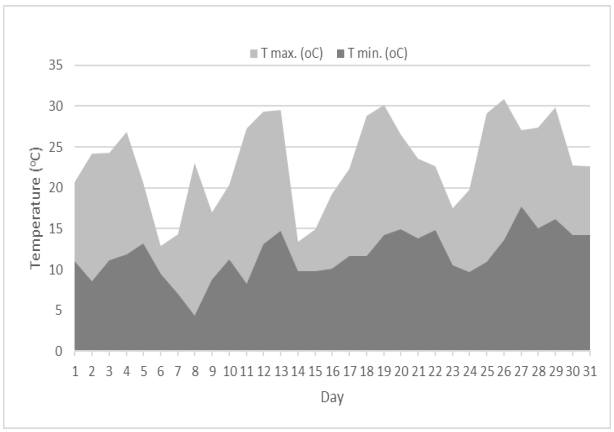


Figure 3. Rainfall amount and air temperature from Timișoara (Romania) (in May 2019) [35].

3.2. F. graminearum natural infection situation

Attack frequency and intensity of *Fusarium* in 2019 was high. All the varieties from the experience were infected. The highest attack frequencies were registered in the varieties Ingenio (95%), Șofru (84%), Anapurna (69%), Glosa (74%) and Altigo (60%), and the lowest in the varieties Lovrin 5x and Lovrin 6x (below 20%). The average severity of the attack on ear had great values in the varieties Anapurna (67%), Glosa (61%), Șofru (60%) and Arezzo (54%). Varieties Lovrin 5x and Lovrin 6x have registered low attack intensities ranging between 19 - 22% (Figure 4).

The infection degree (AD%) calculated on the background of attack frequency and intensity has changed the hierarchy of assessed wheat varieties. On the first position as infection level was the variety Șofru with 50.79% infection rate, this variety registering the highest concentration of DON too (figure 5). This is followed by the varieties Anapurna with 47.02%, Glosa with 45.35% and Altigo with 31.33%. Some varieties from the experience are highlighted by low infection rate. Varieties Lovrin 5x and Lovrin 6x have the infection rate below 5% and Apertus 6.11% (Figure 4). In all these varieties the DON level surpassed the maxim allowed concentration (Figure 5).

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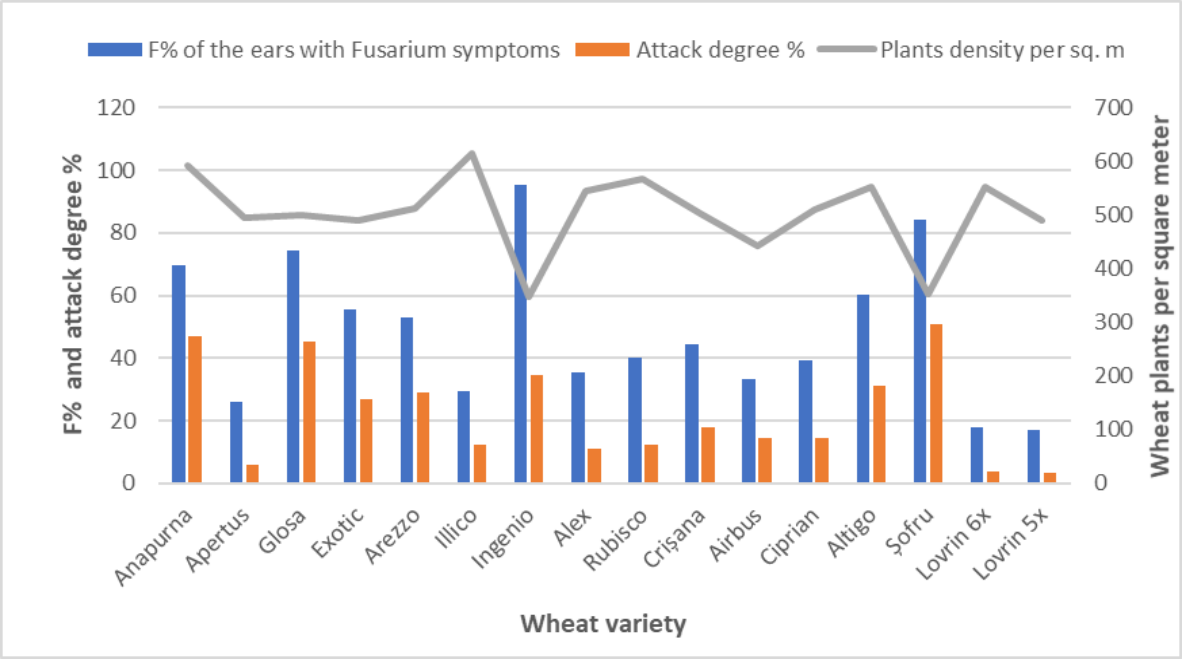

Figure 4. Attack frequency (F%) and attack degree (%) of the fungus *Fusarium graminearum* in ears related with wheat plants density, in the epidemic year 2019.

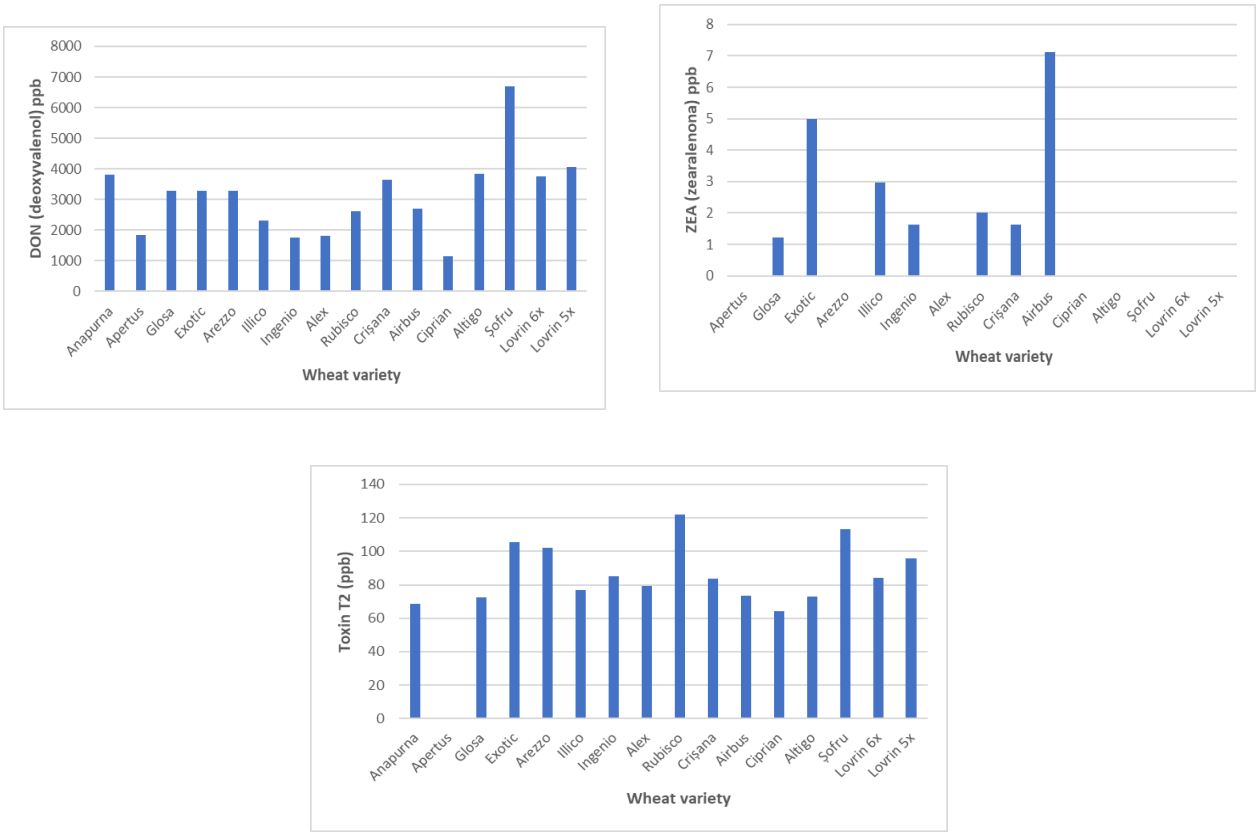

Figure 5. Mycotoxins found in the wheat grains of the varieties analysed in 25 July 2019 in Western Romania.

3.3. Variety influence on F. graminearum atack

In Table 2 are presented the results for ANOVA using for post-hoc analysis the Tukey test having in view the influence of wheat variety on *F. graminearum* attack. According with the obtained results three varieties (Apertus, Lovrin 5x and Lovrin 6x) recorded significant differences compared with the control Șofru ($p < 0.05$), all these varieties

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registering the lowest values of the attack degree. The results suggest that all winter wheat varieties were susceptible to the *Fusarium* infection when the climatic conditions were favouring the development of the pathogen, and variety can influence the attack intensity.

Table 2. ANOVA for wheat variety influence on *Fusarium* attack (%) (post-hoc Tukey method).

Variety	Mean Difference	t	p tukey
Șofru (control)	-	-	-
Airbus	-35.403	-2.781	0.328
Alex	-39.310	-3.088	0.190
Altigo	-17.743	-1.394	0.987
Anapurna	-5.330	-0.419	1.000
Apertus	-47.460	-3.728	0.048*
Arezzo	-19.883	-1.562	0.964
Ciprian	-35.347	-2.777	0.330
Crișana	-31.200	-2.451	0.527
Exotic	-22.600	-1.775	0.908
Glosa	-5.510	-0.433	1.000
Illico	-37.917	-2.978	0.233
Ingenio	-11.040	-0.867	1.000
Lovrin 5x	-52.040	-4.088	0.020*
Lovrin 6x	-50.633	-3.977	0.026*
Rubisco	-37.640	-2.957	0.243

* $p < 0.05$

Note. P-value and confidence intervals adjusted for comparing a family of 16 estimates (confidence intervals corrected using the Tukey method).

In Figure 6 is presented the matrix of the plots between the analysed field variables, with the scatterplots between the analysed field variables, with the histograms and density plots. The aspect of the scatterplots and the trendlines suggest the existence of the correlation among some field data sets as plants density and attack frequency, the number of diseased ears and frequency and attack intensity and attack frequency and attack intensity. The obtained data were very well correlated with the low plant density per square meter, suggesting that low plant density influenced positively the evolution of the pathogen (Figure 6). Low densities of the wheat plants from 2019 were due to the drought from the autumn of 2018, when the wheat hasn't germinated well because of the absence of moisture into the soil. The germination was delayed and the plants haven't sprouted or had few shoots. In the case of the density influence on the infection there were registered positive correlations in the varieties Ingenio and Șofru that have low densities comprised between 348 and 352 plants/m^2 (Figure 4).

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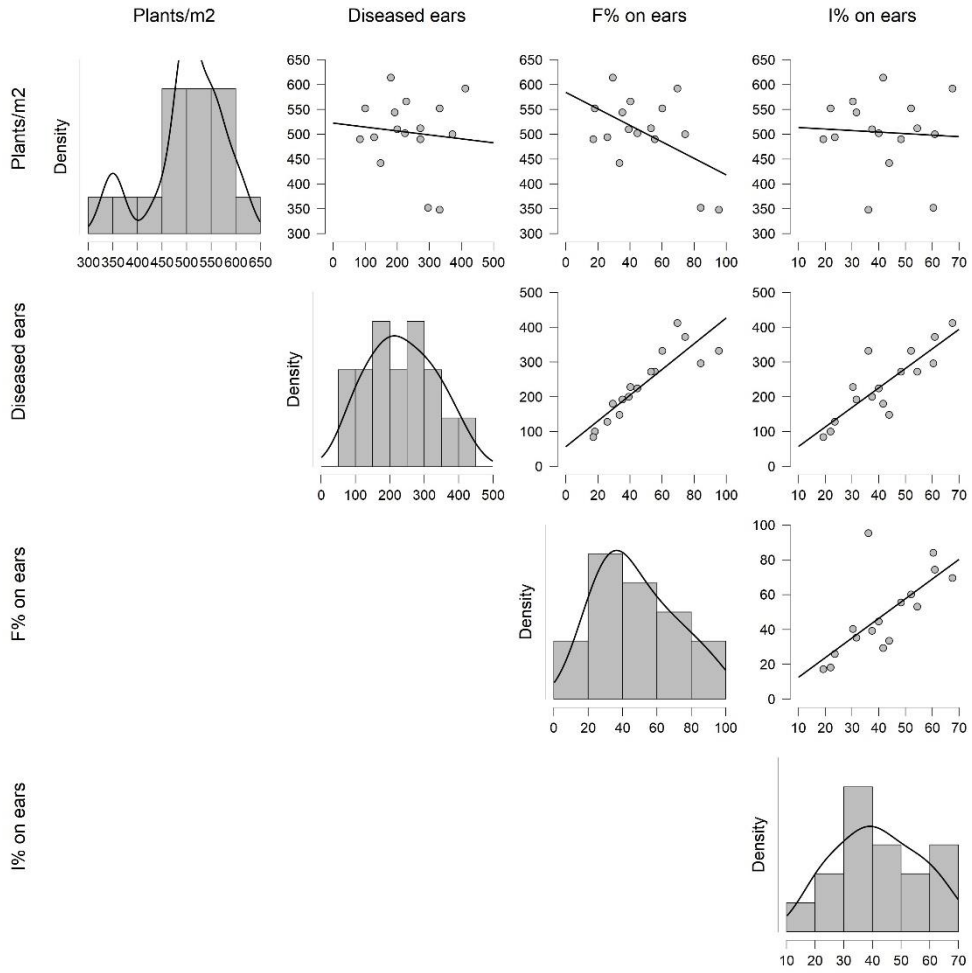

Figure 6. Correlation matrix among winter wheat plants density per square meter and *Fusarium* symptoms assessment with the histograms and density plots.

3.4. Mycotoxins concentrations and their relationships with wheat variety

DON concentration was determined in all the samples included in the experiment (Figure 5). The highest DON level was detected in the variety Șofru, respectively 6.69 ppm (four time surpassed the maxim allowed concentration in unprocessed cereals) and the lowest was in the variety Ciprian with 1.16 ppm (below the allowed limit, but very close of it). The average value of DON concentration of the wheat grain samples was 3.34 ppm. In some varieties with low *Fusarium* infection rate was accumulated DON over the maxim allowed concentrations (Figure 4 and Figure 5).

Zearalenone was detected only in some samples in the varieties Anapurna, Glosa, Exotic, Ingenio, Illico, Rubisco, Crișana and Airbus. The ZON amounts found in the analysed samples weren't surpassed the maxim allowed concentration for EU, respectively 100 ppb. The ZON concentrations from wheat grains were very low, being comprised between 1.21 and 7.11 ppb (Figure 5). Toxin T – 2 was found in 15 samples from all 16 analysed. The highest level of T-2 was registered in the variety Rubisco (121.93 ppb) and the lowest in the variety Ciprian (64.45 ppb). The maxim allowed concentration of 100 ppb was surpassed slightly by the varieties Exotic (105.5 ppb), Arezzo (102.22 ppb), Rubisco (121.93 ppb) and Șofru with 113.19 ppb (Figure 5).

In Table 3 is presented ANOVA analysis regarding the wheat variety influence on the accumulation of DON mycotoxin in wheat grains. The results showed that all the varieties have registered highly significant differences in comparison with the control Șofru, according with the post-hoc Tukey method ($p < 0.001$). In the case of ZON and T-2 concentrations weren't obtained significant results from the point of view of variety influence,

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the distribution of the data being not normal, from this reason these results weren't presented here.

Table 3. Wheat variety influence on DON (deoxynivalenol) - ppm (Tukey method).

Variety	Mean Difference	t	p tukey
Șofru (control)	-	-	-
Airbus	-3.990	-42.645	< 0.001 ***
Alex	-4.880	-52.157	< 0.001 ***
Altigo	-2.850	-30.461	< 0.001 ***
Anapurna	-2.890	-30.888	< 0.001 ***
Apertus	-4.850	-51.836	< 0.001 ***
Arezzo	-3.400	-36.339	< 0.001 ***
Ciprian	-5.530	-59.104	< 0.001 ***
Crișana	-3.040	-32.491	< 0.001 ***
Exotic	-3.400	-36.339	< 0.001 ***
Glosa	-3.400	-36.339	< 0.001 ***
Illico	-4.380	-46.813	< 0.001 ***
Ingenio	-4.920	-52.584	< 0.001 ***
Lovrin 5x	-2.640	-28.216	< 0.001 ***
Lovrin 6x	-2.950	-31.529	< 0.001 ***
Rubisco	--4.060	-43.393	< 0.001 ***

Note. P-value adjusted for comparing a family of 16
* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

The matrix of the mycotoxin variables with the scatterplots among them and histograms and density plots are presented in Figure 7, the trendlines indicating the interrelation between the concentration of DON and T-2 mycotoxins.

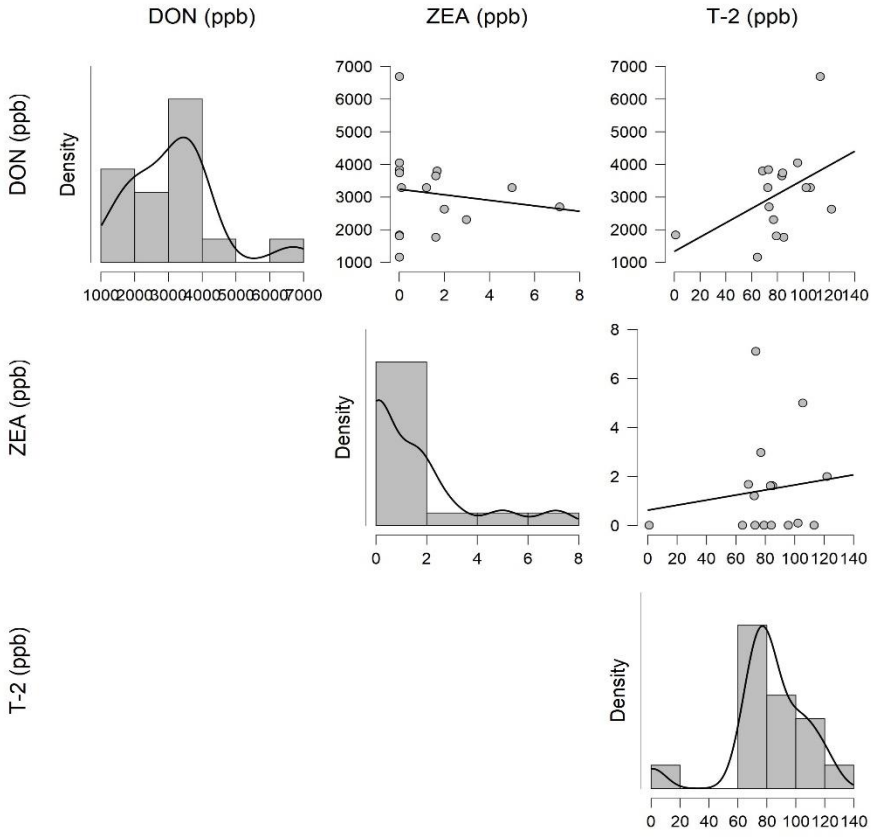

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Figure 7. Correlation matrix among the mycotoxins' concentration (DON – deoxynivalenol, ZEA – zearalenone an T-2) from winter wheat grains with the histograms and density plots.

3.5. Correlations among some FHB infection variables

Analysis of the relationships among the mycotoxin's concentrations, plants densities per square meter, diseased ears, FHB infected ears and attack intensity (I%) and frequency (F%) are presented in Table 4. In the case of DON mycotoxin were determined two correlations, respectively with T-2 and attack intensity on ears (I%). The r_{calc} = 0.457 showed the existence of a positive correlation between the concentration of DON and T-2 mycotoxins.

The r_{calc} = 0.424 showed the positive relationship between attack intensity and DON concentration from wheat grains. Plants density per square meters and *Fusarium* attack frequency have a negative correlation, this fact being highlighted by r_{calc} = -0.525.

The highest significant positive correlation coefficients were determined between the diseased ears and attack frequency (r_{calc} = 0.888) and attack intensity (r_{calc} = 0.845), they being followed by the relationship between the attack frequency and attack intensity (r_{calc} = 0.709) (Table 4).

Table 4. Matrix of the Pearsons' r_{calc} values among the mycotoxin concentrations in wheat grains, wheat plants density and fusarium blight infection features (α = 0.05; n = 16; $df = n - 1$ one-tailed; p 0.1 ≥ 0.412 *; p 0.05 ≥ 0.558 **; p 0.005 ≥ 0.606 ***).

Specification	DON (ppb)	ZON (ppb)	T-2 (ppb)	Density (plants/m²)	Diseased ears	F% on ears	I% on ears
DON (ppb)	-	-0.134	**0.457***	-0.255	0.226	0.292	**0.424***
ZON (ppb)		-	0.136	-0.073	-0.009	-0.012	0.172
T-2 (ppb)			-	-0.133	0.178	0.242	0.186
Density (plants/m²)				-	-0.104	**-0.525***	-0.061
Diseased ears					-	**0.888*****	**0.845*****
F% on ears						-	**0.709*****
I% on ears							-

Using Exploratory Factors Analysis (EFA) (Table 5 and Figure 8) there were assessed the possible joint variations in the response of the analysed variables. Thus, for the achievement of this objective there were performed preliminary several statistical tests as Kaiser-Meyer-Olkin test, Bartlett's test, Chi-squared test and all of them were rejected the null hypothesis (Table 5), this fact demonstrated the existence of potential multiple relationships among the considered variables. In the same table group are presented the results regarding the Factor Loadings considering the Factor 1 and Uniqueness of every of the analysed potential factors involved in the contamination of the wheat grains with mycotoxins. The greatest factor loadings determined were for F% on ear (0.948), diseased ears (0.892) and I% on ears (0.824), the high values obtained showing their relevance among the considered variables multiple correlation. Regarding the results for the uniqueness of the variance of the analysed data sets, the high values were obtained for ZEA (zearalenone) (0.998), T-2 (0.914), plants/m² (0.91) and DON (0.836), their variances being not shared with the other variables. Cumulative loading of the factor was 0.388% of the total variance of the cumulated variables and the multiple correlation coefficient obtained was 0.388. These results were expressed graphically in figure 8.

Table 5. Exploratory Factor Analysis (EFA) among mycotoxins concentration, wheat plant density and fusarium blight infection features.

Kaiser-Meyer-Olkin test	Bartlett's test		
MSA	**X^2**	**df**	**p**

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Overall MSA	0.406
DON (ppm)	0.397
ZEA (ppb)	0.090
T-2 (ppb)	0.342
Plants/m2	0.166
Diseased ears	0.451
F% on ears	0.462

75.511	21.000	< .001

Chi-squared Test

	Value	Df	**p**
Model	41.240	14	< .001

Factor loadings

	Factor 1	**Uniqueness**
DON (ppm)	0.405	0.836
ZEA (ppb)		0.998
T-2 (ppb)		0.914
Plants/m²		0.910
Diseased ears	0.892	0.204
F% on ears	0.948	0.101
I% on ears	0.824	0.321

Note. No rotation method applied.

Factor Characteristics

	Sum Sq.	**Loadings proportion**	**Var. Cumulative**
Factor	2.716	0.388	0.388

Path diagram (Figure 8) represents the direction and strength of the relationship among the analysed variables and factors and shows the order of the causal relationships according with the path coefficients. The results obtained highlighted the factors that are influencing the variables in the following order: F% on ear, diseased ears and I% on ears. The variables with the highest loading from the considered factors were ZEA (zearalenone), T-2, plants density per square meter and DON.

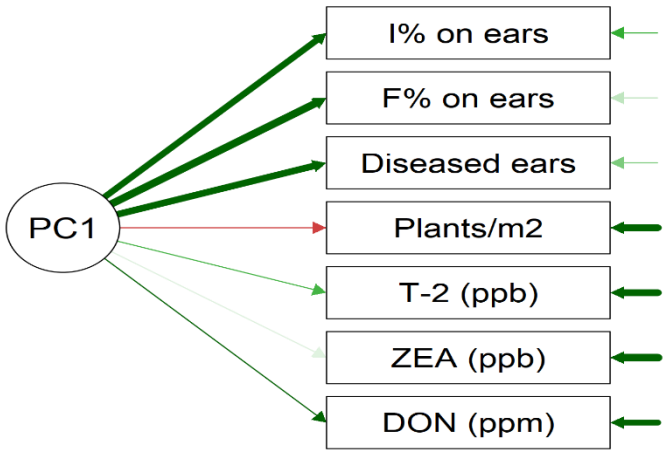

Figure 8. Path diagram among the variables and factors implied in the concentration of mycotoxin from wheat grains.

4. Discussions

The climate parameters with great importance in the favouring of *Fusarium* sp. infection in field are: temperature, rainfall amount and the relative air humidity [30, 43, 44,]. The climate changes of our planet have influenced the climatic factors leading to long drought periods or to long intervals with excessive rainfalls, affecting the interaction between cereals and pathogens by changing host-pathogen relationship [45 - 46]. Thus, climate changes

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from the last years have influenced positively the development of the fungi from the genus *Fusarium* favouring the appearance of the epidemics in wheat crops. The most recent epidemics of *Fusarium* in cereals from Banat Plain (western Romania) was in the year 2019, when wheat grains quality was very low, mainly due to the presence of the mycotoxins in rates that have overpassed the maxim allowed concentrations, according with EU standards [39, 40]. The continuous moisture from rainfalls in May and the temperatures registered in the period before flowering, after flowering and grain development were favoured the appearance of *Fusarium* epidemics in wheat [9, 47]. As the exposure to moisture is longer the attack intensity increases. De Wolf et al. [48] showed that for infection the most important are the duration in hours of the rainfalls prior to flowering with seven days and the temperatures comprised between 15 and 30°C. It is a certitude that in warm weather conditions with temperatures comprised between 15 and 30°C and continuous moisture the *Fusarium* symptoms on ear (whitening) can appear in 2 – 4 days from the infection [9]. Thus, a crop with healthy appearance can develop disease symptoms suddenly. Chandelier et al. [49], in a research developed during seven years, shows also a strong correlation between the average air relative moisture greater than 80% and the DON amount accumulated in grains, the hard rainfall registered during the research determining high moisture conditions.

The factors that are facilitating the occurrence of the mycotoxins produced by FHB in wheat grains are numerous, some of the most important being climatic context, variety, cropping technology, and reliability of the measured mycotoxins [45, 50 - 51]. Many researches showed positive correlations between the attack intensity of *F. graminearum* in field and the amount of DON accumulated [52 - 53]. The attack severity in field is considered a major factor in the accumulation for the mycotoxins in cereal grains [47, 54]. Jansen et al. [55] suggested that DON seems to play a key role in the spread of *F. graminearum* from the wheat flower to the rachis. Hallen - Adams et al. [56] showed that as far as the fungus spreads from the infected spikelet in the upper or lower part of the wheat ear, the biosynthesis of DON is maxim in the proximity of the initially infected zone before the symptoms' appearance. As far as the grains are colonized by fungus the biosynthesis of DON decreases.

Berca [57] mentions that DON is present in Romania in wheat and triticale in amounts that are surpassing the maxim concentrations allowed by European regulations. According with Wegulo [9], as high is the rate of the grains affected by fusarium ear blight the DON concentration will be higher. Thus, most of the researchers are correlating the presence of deoxynivalenol in grains with attack intensity from field and the rate of diseased grains [58]. According with IARC [18] the mycotoxin T-2 appears in significant amounts in cereals together with deoxynivalenol, this fact being confirmed by the results of this research. Hernandez Nopsa et al. [47] found that the accumulation of high amounts of DON in the wheat grains is favoured especially by the rainfalls during the anthesis, even when the most tolerant varieties are severely affected. Researches developed in Italy during 2009-2010 showed the simultaneous occurrence of zearalenone and T-2 toxin in wheat grains in conditions climate favourable to *F. graminearum* infection [59].

After many years of research in the context of climate change and epidemic years, the association between FHB intensity and mycotoxins accumulation in harvested grains, is not fully understood. In U.S.A. the concerns for the wheat inbreeding for *Fusarium* resistance exists from 1929. Schroeder and Christensen [60] showed that after nine years of research all wheat plants can be infected in a lower or a higher rate.

There were described five resistance types: type I – resistance to the initial infection (defensive reactions), type II – resistance to the spread of the pathogen in the infected tissue: type III – resistance to the infection of the seeds: type IV – tolerance to infection: and type V – resistance to mycotoxins [50, 61 - 63].

The evaluation in field of the resistant genotypes is difficult to be achieved because the *Fusarium* epidemics are rare and the inoculation methods aren't very accurate. Kumar et al. [64] showed that the methods used have leaded often to experimental errors. On other side, the link between DON and the infection severity is very well correlated in the years

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with *Fusarium* epidemics, but this isn't happening in the years unfavourable to the infection.

The varietal effect observed in the present research suggests that during epidemic years the choice of a susceptible wheat variety is the second risk factor, after favourable weather conditions, in FHB development. Therefore, breeding for resistance is still the best option to control this disease and to limit mycotoxins accumulation in harvested grains [65].

5. Conclusions

F. graminearum infection rate of the analysed wheat varieties is influenced by the climate conditions from the spring of the year 2019, especially those recorded in May are favourable to *Fusarium* blight epidemics. This epidemic had a negative impact on the quality of wheat yield in all analysed samples. Among all mycotoxins detected in wheat grains (DON, ZON and T-2 toxin), the main factor for low yield quality was the presence of the mycotoxin DON in high concentrations, 15 wheat varieties from 16 overpassing the minimum allowed concentration (wheat variety Șofru recorded the greatest infection rate and the greatest DON concentration - four times higher than the maximum allowed concentration according with EU regulations). At the opposite pole was the Romanian variety Ciprian that had registered the lower infection rate and the lowest DON concentration, being the only variety from the experience with DON concentration below the maximum level allowed. The increase of the *Fusarium graminearum* attack frequency was strongly correlated with the decrease of the plants' density. Other challenging positive interrelations were noticed between DON and T-2 and DON and fungus attack frequency. The most influential factors in the contamination with the mycotoxins were frequency of the attack on ear, diseased ears, attack intensity on ears and plants density per square meter. All of these factors have influenced the contamination of the wheat grains with mycotoxins (DON, ZON and T-2) in the field plots.

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