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RESEARCH ARTICLE

Impact of fusarium head blight epidemics on the mycotoxins' accumulation in winter wheat grains

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ABSTRACT

Fusarium graminearum (Fusarium Head Blight - FHB) is a dangerous pathogen of the cereals producing mycotoxins harmful for human and animal health. The quality of the wheat grains is severely affected in the years with FHB epidemics due to the high mycotoxins level that can overpass maximum allowable concentrations, with implications for food safety. The purpose of the work was to investigate some variables involved in the contamination of the wheat grains with several harmful mycotoxins (DON, ZON and T-2) in conditions of FHB natural infection in an epidemic year. The main finding of the results highlighted that the low ears densities of wheat crop canopy determinate high FHB attack frequency, too. Climate conditions from May 2019 were very favourable for the evolution of FHB epidemics in western Romania. This fact was confirmed by the experimental results of FHB attack, and later by the concentrations of mycotoxins (DON) over the maximum allowable concentrations from the wheat grains at the harvesting moment. The overpass of DON concentration was found in 15 from the total 16 wheat varieties from the experiment. Positive significant correlations were found between DON and T-2 and between DON and fungus attack intensity. According with the results, in FHB epidemic year some of the most influential factors in the contamination of the wheat grains with DON, ZON and T-2 in the order of their importance are: ears density, frequency of the attack on ear, number of diseased ears and attack intensity on ears.

Keywords: Epidemic year; Fusarium head blight (FHB); Mycotoxins; Natural infection; Wheat

INTRODUCTION

Fusarium graminearum known as Fusarium Head Blight (FHB) or scab is a phytopathogen fungus from the genus *Fusarium*. It is producing one of the most devastating disease of the wheat and of other small grain cereals. FHB determinates great yield losses and affects grains quality due to its' capacity of producing mycotoxins. Contamination with mycotoxins is considered a global issue there being considered that about 25% of agricultural products are contaminated each year, this fact determining economic losses (Huong et al., 2016; Bai et al., 2018).

After many years of research in the context of climate change and epidemic years, the association between FHB attack intensity and mycotoxins accumulation in harvested grains, is not fully understood. In U.S.A. the concerns for the wheat inbreeding for FHB resistance exists from 1929 (Schroeder and Christensen, 1963).

The prolonged humid weather during the vegetative season favours the fungus growth and sporulation. The spores are carried by the wind and raindrops to the wheat ears. The wheat is susceptible to be infected during the flowering season and at the beginning of the grain development (Popescu 2005).

Abramson (1999) shows that in the condition of FHB epidemics with an incidence of even less than of a quarter of infected wheat ears, in the harvested grains can be found mycotoxins over the maximum allowable concentrations.

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The factors that are leading to the appearance of FHB epidemics are climate conditions, inoculum amount and wheat development phenophase. The primary inoculum source is more important for the setting of the epidemics compared with the secondary one (Gilchrist et al., 1997; Pitt 2014). Besides the mentioned factors, the agricultural technologies applied nowadays by farmers have influence on the FHB infection and on the accumulation of mycotoxins. The minimal cultivation systems as "minimum tillage" or "no tillage", characterised by high plant densities (crops and weeds) or the absence of the crop rotation have leaded to the increase of the volume of the inoculum source in the death biomass that is remaining on the soil surface (Teich and Nelson, 1984 Watkins and Boosali, 1994; Unger, 1994). FHB can survive in the decaying biomass as mycelia, chlamydospores and perithecia with asci and ascospores. At this inoculum source can be added other source as the infected seeds that can spread the disease, too (Paraschivu, 2008; Pereyra and Dill-Macky, 2008).

Schroeder and Christensen (1963) showed that after nine years of research all wheat ears 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 accumulation (Mesterhazy, 1995; Ma et al., 2009; Zhang et al. 2020).

Harvest damages in cereals are caused mainly due to the sterility of the ears, low thousand kernel weights (TKW) and the presence of the mycotoxins in infected grains. During 1991 – 1997 period in USA the losses due to FHB in wheat and barley harvests have surpassed 1.3 billion dollars (Johnson et al., 2003).

The mycotoxins produced in the cereal grains by toxigenic phytopathogens, as aflatoxins (AFs), ochratoxins (OTs), fumonisins (FUMs), T-2 toxin, deoxynivalenol (DON) and zearalenone (ZON), are considered harmful due to their toxic potential to humans and animals. This is the real reason of concern on this issue of the researchers from worldwide (Andretta et al., 2012, Khodaei et al., 2021). Therefore, the development of new physical, chemical and biological methods for the detoxification of the mycotoxins are highly desirable (Pfliegler et al., 2015; Jo et al., 2021).

In FHB of wheat can be implied several *Fusarium* species, such as *F. graminearum, F. culmorum, F. nivale, F. poae* and *F. sporotrichioides* (Miller, 1994; Wegulo, 2012). The mycotoxins produced by *F. graminearum* are framed in the chemical group of trichothecene. Often in the cereal grain samples analysed was 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 (Singh, 1995; Desjardins, 2006). *F. graminearum* is considered the most important *Fusarium* species that produces DON (McMullen et al., 1997; Paraschivu, 2008). DON belongs to the chemical family of sesquiterpene, derived from trichodiene (the biochemical precursor of all the trichothecenes). 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 as sickness, vomiting, diarrhoea, headache, abdominal pains and fever (Liddell, 2003; Sobrova et al., 2010). Amongst the domestic animals, pigs are the most sensitive to DON, the intoxication determining haemorrhages and necroses of the intestinal mucosa, skin and bone marrow. In comparison with the pigs, the ruminants are more resistant to the intoxication with DON. The forages contaminated with 10-15 ppm DON were tolerated by cattle and sheep without side effects (Rotter, 1996; Čonková et al., 2003).

T-2 mycotoxin appears in significant amounts in cereals together with DON (IARC,1994). The disease produced by it is named alimentary toxic aleukia or ATA. The intoxication with T-2 mycotoxin is manifesting with symptoms as fever, vomiting, convulsions, anaemia and acute inflammations of the digestive system, kidney and neurological diseases and cancer (Szczech and Hood, 1978; Pitt and Miller, 2017). Poultry are the most sensitive domestic animals to T-2 mycotoxin (Mézes et al., 1999; Mu et al., 2013).

Other FHB important toxic metabolite is zearalenone (ZON). Usually, it appears in wheat grains and raw material contaminated with FHB together with DON and T-2. Most often it appears in the maize grains infected by FHB, but also it was found in oils. ZON is potentially harmful for the humans' and animals' health (Minervini and Dell'Aquila, 2008; Zheng et al., 2019). 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 domestic animal species to ZON are the pigs (Binder et al., 2017; Skiepko et al., 2020).

From all *Fusarium* species, *F. graminearum* is the most often present in temperate regions with warmer climate in comparison with *F. culmorum* that prefers cooler regions (Wang and Miller, 1988; Miller, 2002). In Banat Plain area from Romania, the most prevalent species that produces infections on wheat ear is *F. graminearum* (Popescu, 2005; Cotuna et al., 2013).

Importance of this research consisted in the investigation of the response to FHB attack and mycotoxins accumulation in grains in the conditions of natural infection of the winter wheat crop. This type of research can be performed successfully in field conditions with natural infection only in the years with favourable climate for the development of FHB, as are FHB epidemic years. The aim of the current research was to investigate some aspects of FHB infection involved in the contamination of the wheat grains with several harmful mycotoxins (DON, ZON and T-2). The objectives of the research were: i) identification of weather conditions favourability for FHB infection in winter wheat crop; ii) characterization of FHB natural infection in field; iii) Assessment of wheat variety impact on FHB infection on plants and on mycotoxins concentrations from grains.

MATERIAL AND METHODS

Description of the field plot conditions and biological material

The experimental plot was set up in Timişoara (90 m a.s.l.) in the farm of the Didactic Station of University of Life Sciences "King Michael I" from Timişoara. The soil from the experimental field is chernozem (Ianoş et al.,1997). The average multiannual air temperature in the area is 10.6°C and the average multiannual rainfall amount is 592 mm. The climate data (rainfall amounts and air temperatures) were registered at the Meteorological Station from Timişoara (2019) and were used to highlight the favourability of the weather conditions for the development of the fungus *F. graminearum*.

The evaluation in field of the resistant genotypes is difficult to be achieved because FHB epidemics are rare and the inoculation methods aren't very accurate. Kumar et al. (2015) showed that the methods that use artificial inoculation have leaded often to experimental errors, that is why there are desirable the experiments with natural infection, even they are rare.

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 most cultivated in Banat region, with different origins: Anapurna, Apertus, Glosa, Exotic, Arezzo, Illico, Ingenio, Alex, Rubisco, Crişana, Airbus, Ciprian, Altigo, Şofru, Lovrin 6x and Lovrin 5x (Fig. 1).

Agronomical practice applied to the experimental field was the classical one. After ploughing was applied a fertilization with a dose of 150 kg/ha^{-1} NPK (14-16-0% active ingredient) on 23 October 2018. The experimental plots were seeded on 30 October 2018 at a distance between rows of 12.5 cm and a density of 400 seeds per square meter. Another fertilisation dose was applied on 27 February 2019, respectively 200 kg/ha^{-1} ammonium nitrate (36% active ingredient). First weed control treatment for dicots control was applied on 28 March 2019 with 20 g/ha^{-1} tribenuron - methyl (Rival Star 75 GD) and the second on 22 April 2019 with 0.5 l/ha^{-1} florasulam + 2.4 D (Vector). The crop was harvested on 22 July 2019.

Diagnosis of *F. graminearum* in field and laboratory

FHB diagnosis was performed at three levels: a) field observations; b) grains observations; c) and microscopic observations (mycelia and conidia grown on ears infected tissue and on grains).

Diagnosis in field was based on visual observations at the flowering development stage (BBCH 60 – 62) (Miedaner, 1986; Trottet and Rolland, 2014). Wheat ears were having FHB symptoms as ears whitening with different intensities and frequencies. Some ears were completely white and other were whitened only partially (Fig. 2a and b). On the surface of the whitened spikelets were visible white – orange mycelia (Fig. 3a and b).

Thus, in 15 June were collected samples of infected ears (10 ears/variety) for detection of FHB mycelia and conidia

Fig 1. Winter wheat experimental field (photo: Cotuna O., 2019).

Fig 2. FHB symptoms on wheat ears: (a and b) ears whitening partial or total (photo: Cotuna O., 2019).

on tissue and grains. There were extracted grains from the sampled wheat ears that had specific symptoms as whitening and shrivelling and pinkish colour (Fig. 4a and b).

The microscopic diagnosis was performed for mycelia from ear tissue (a) and from mycelia grown on grains (b).

a) For the microscopic analysis of the mycelia samples collected from diseased spikelets were sampled 10 ears for every variety. The mycelia samples from slides were prepared in two ways: with distilled water and with lactophenol blue. They were analysed to the microscope for the characterization of the conidia with 40x objective lens.
b) For the sampling of FHB mycelia from the inside of the wheat grains there were prepared samples for every variety, each sample consisting in 28 grains. There were prepared Petri plates by pouring agar growth medium. Wheat grains were washed with tap water and rinsed with distilled water. Sterilization was done by immersing the grains in alcohol 96% for one minute. After sterilization the grains were rinsed twice with distilled water. The grains were dried on sterile filter paper and after were placed with tweezers on the growth medium plates. Petri plates were incubated at 24^{o}C for seven days. From the incubated wheat grains were grown white – pinkish filamentous mycelia (Fig. 5a- d).

Fig 3. FHB symptoms on wheat ears: (a and b) white – orange mycelia on the spikelets surface (photo: Cotuna O., 2019).

Fig 4. FHB symptoms on wheat grains: a) grains shrivelled, decoloured and whitened; b) grain with pinkish mycelium on surface (photo: Cotuna O., 2019).

From the mycelia were collected samples that were placed on microscope slides for the visualization of conidia and macroconidia. The microscopic analysis of the mycelia was performed with 40x objective lens. The observed macroconidia were spindle like, slightly curved, hyaline, with 3 to 7 septa, specific for *F. graminearum* (Fig. 6a and b).

Field data and grain samples collection

The analysed winter wheat varieties were monitored during the FHB epidemics in the vegetative season 2018/2019, knowing that the "*year effect* is substantial in the case of this disease (Van der Burgt and Timmermans, 2009). The assessment of the ears' density, incidence (F%) and severity (I%) of FHB attack on wheat ears was performed for every variety from the experiment. The incidence of the attacked ears was calculated as the rate of the ears with symptoms of whitening reported to the total number of the analysed ears (diseased ears/analysed ears x 100). Severity classes of FHB attack on ear were assessed using the current method described by Miedaner (1986) and Trottet and Rolland (2014). There were attributed marks on a 1-9 scale to every analysed ear from the inside of the

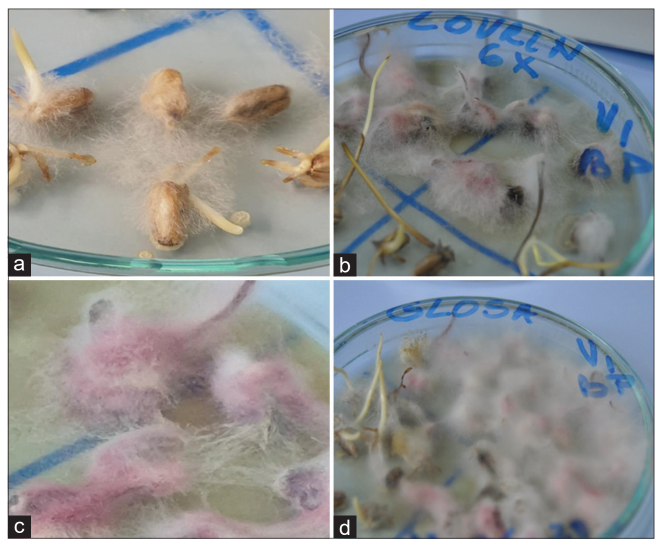

Fig 5. White – pinkish mycelia grown on wheat grains (a-d) (photo: Cotuna O., 2019).

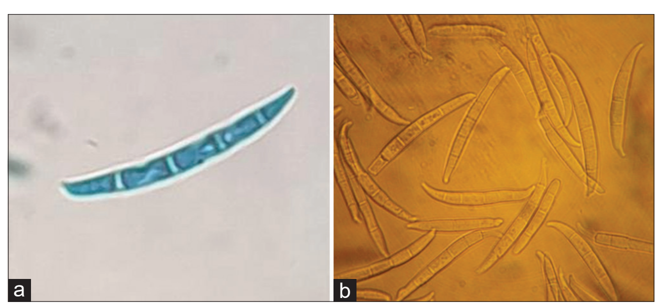

Fig 6. *F. graminearum* macroconidia on microscope (40x objective lens): a) stained with lactophenol blue; b) in distilled water (photo: Cotuna O., 2019).

metric frame (0.5 m x 0.5 m). The infection degree (AD%) was calculated as the report between the attack frequency and intensity multiplied with 100.

There were two data collection phases, respectively on 25 May 2019 and the second one in 15 June 2019. Having in view the analysis of the concentrations of the mycotoxins produced by FHB in wheat grains there were collected samples at harvesting (22 July 2019) directly from the harvester grain tank for every wheat variety from the experimental field.

The field data collected in May and June were used for the FHB diagnosis. For statistical analysis were used the field data collected in June, respectively the ears' density, FHB incidence (F%) and severity (I%), and the mycotoxins data from the laboratory analysis (from 25 July).

Evaluation of mycotoxins' concentration

The mycotoxin data from this paper refers to the situation existent at the moment of wheat harvesting. The grain samples weren't stored, they being sent to the laboratory and analysed the next day after harvesting (23 July 2019), the analysis bulletin being received on 25 July 2019.

The analyses of the concentrations of the mycotoxins DON (deoxynivalenol), ZON (zearalenone), and T-2 (toxin T2) were performed at the Platform for Interdisciplinary Research of USAMVB from Timişoara using ELISA mycotoxin test kit according with the analysis protocol of the laboratory (Turner et al., 2009).

The assessment was done with ELISA PR 1100 apparatus at the wave length of 450 nm. The minimum detectable concentrations for the used tests were: 800 ppb 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 FHB in the unprocessed wheat grains according with the EU regulations (from 2006 and 2013).

Statistical analysis of the data

The statistical analysis characterizes the relationships among the following variables: wheat varieties, mycotoxins (DON, ZON and T-2) concentrations, ears density and infection features (diseased ears, F% on ears, I% on ears). The data were processed with the software JASP (Version 0.14) (2020) respectively descriptive statistics, ANOVA (using Tukey test as post-hoc test) and Exploratory Factor Analysis (EFA) and path diagram. The Pearson's correlation coefficient (r_{calc}) amongst the analysed variables was calculated with Microsoft Excel (version 2019).

Table 1: Maximum allowable concentrations of mycotoxins produced by FHB in the unprocessed wheat grains (according with the Commission Regulation (EC) no 1881/2006 of 19 December 2006[1] and Commission Recommendation 2013/165/EU of 27 March 2013[2])

Mycotoxins produced by *Fusarium sp.* in wheat grains	Maximum allowable concentration allowed in unprocessed cereal grains
Deoxynivalenol (DON)	[1]1250 ppb
Zearalenone (ZEA)	[1]100 ppb
Toxin T-2 + HT-2	[2]100 ppb

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 in this research, starting from the hypothesis that is a complex relationship among them.

RESULTS AND DISCUSSIONS

Weather conditions favourability for FHB infection of wheat

The climate parameters with great importance in the favouring of FHB infection in wheat field are: temperature, rainfall amount and relative air humidity (Cook, 1981; Magan and Lacey, 1984; Popescu, 2005). The climate changes influence the local climatic factors by leading to long time droughts or to excessive rainfalls, affecting the interaction between cereal crops and pathogens by determining changes in the host-pathogen relationships (Doohan et al., 2003; Bajwa et al. 2020). Thus, climate changes from the last years have influenced strongly the development of the fungi from the genus *Fusarium* favouring the appearance of the epidemics in wheat crops. The most recent epidemics of FHB 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 maximum allowed concentrations, according with EU standards.

The spring of the year 2019 was characterized by a humid and warm climate (Figs. 7 and 8), 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 (Fig. 9).

The maximum temperatures during the day were framed in the demands of the fungus, respectively 20.4^{o}C – 30.9^{o}C. The minimum temperatures from May at Timişoara were comprised between 4.3^{o}C (at the beginning of the month) and 17^{o}C (at the end of the month), these values being registered during the night or in the morning. The thermal regime from May was characterized by deviations from the multiannual averages. The average temperature registered was 15.1ºC, much lower than the multiannual average with 6.9ºC (the normal temperature of May in the research area is 22ºC) (Figs. 7 and 8).

The continuous moisture determined by hard rainfalls in May and the temperatures registered before flowering stage, after flowering and during the grain development are favouring the FHB epidemics in wheat (Hernandez Nopsa et al., 2012; Wegulo, 2012). As the exposure to moisture is longer the attack intensity increases. De Wolf et al. (2003)] showed that the most important issues for infection are: the duration in hours of the rainfalls with seven days prior to flowering and the air temperatures comprised between 15 and 30ºC. Chandelier et al. (2011), in research developed during seven years, found a strong correlation between the average air relative moisture greater than 80% and DON amount accumulated in grains, the hard rainfall registered during the research determining high moisture conditions. Thus, it is well known that in warm weather conditions with temperatures comprised between 15 and 30ºC and continuous moisture, the FHB symptoms on ear (whitening) can appear in 2 – 4 days from the initiation of the infection (Wegulo, 2012), these aspects being accomplished in our experimental field. All the wheat varieties analysed were infected with FHB. Hernandez Nopsa et al., (2012) observed that the accumulation of high amounts of DON in the wheat grains is favoured especially by the rainfalls during the anthesis, when even the most tolerant varieties can be severely affected. Researches developed in Italy during 2009-2010 showed the simultaneous occurrence of ZON and T-2 toxin in wheat grains in climate conditions favourable for FHB infection (Bertuzzi et al., 2014).

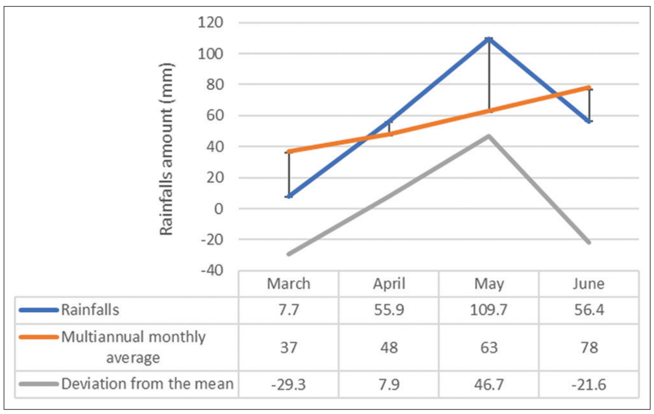

Fig 7. Average monthly rainfall amount in March - June 2019 from Timișoara, Romania (Meteorological Station Timișoara, 2019).

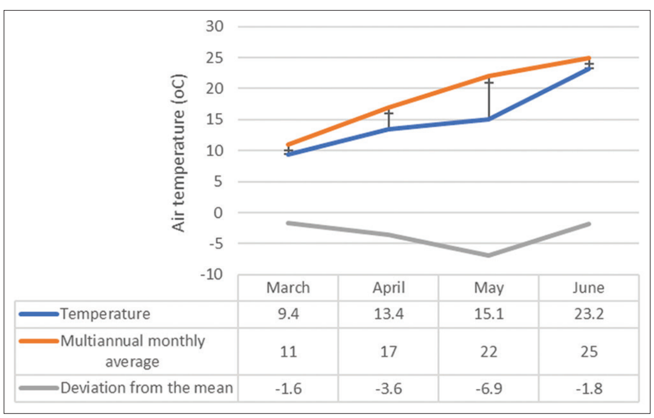

Fig 8. Average monthly air temperature in March - June 2019 from Timișoara, Romania (Meteorological Station Timișoara, 2019).

Thus, the specific weather conditions have leaded to the confirmation of the hypothesis that continuous moisture and maximum temperatures during the day were in the favourability interval for great FHB attack frequencies and intensities, and in the same time have leaded to the accumulation of the mycotoxins in wheat grains in the research area. Thus, the climatic characteristics in the key moments of wheat development were favourable for FHB epidemics, 2019 being an epidemic year.

Situation of FHB natural infection

Attack frequency and intensity of FHB in 2019 was high. All the varieties from the experiment 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%).

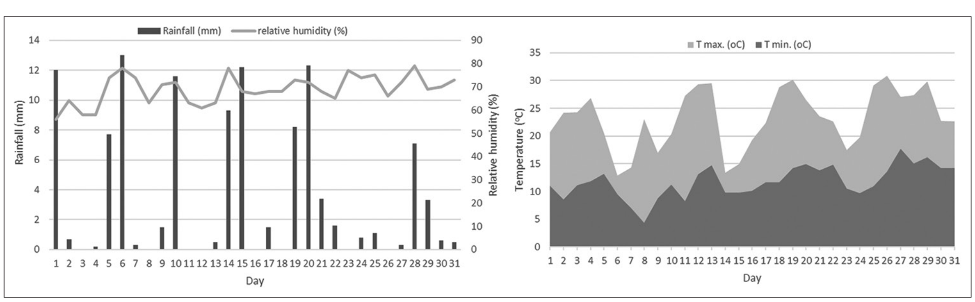

Fig 9. Daily rainfall amount and air temperature from Timișoara (Romania) (in May 2019) (Meteorological Station Timișoara, 2019).

Varieties Lovrin 5x and Lovrin 6x have registered low attack intensities ranging between 19 - 22% (Fig. 10).

The attack degree (AD%) calculated on the background of attack frequency (F%) and attack intensity has changed the hierarchy regarding the response of the assessed wheat varieties to the FHB infection. On the first position as infection level was the variety Şofru with 50.79% infection rate, this variety registering the highest concentration of DON too (see Fig. 10), this being the reason of considering it as control in ANOVA analysis regarding the attack intensity and DON concentration. It was followed by the varieties Anapurna with 47.02%, Glosa with 45.35% and Altigo with 31.33%. Some varieties from the experiment were characterised by low FHB infection rates, as Lovrin 5x and Lovrin 6x with the infection rate below 5% and Apertus with 6.11% (Fig. 4). In all these varieties the level of DON surpassed the maximum allowed concentration (see Fig. 11).

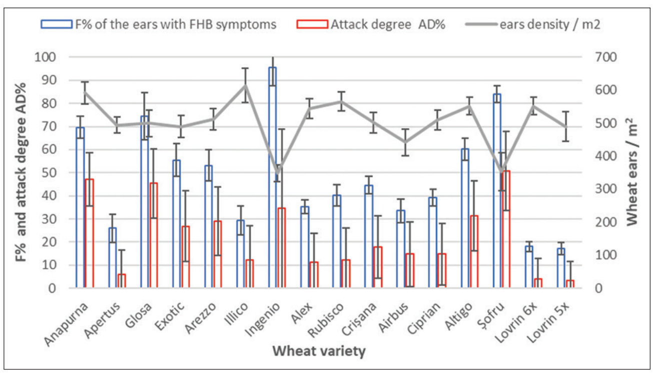

Fig 10. FHB attack frequency (F%) and attack degree (AD%) on ears related with wheat ears density, in the epidemic year 2019.

Variety influence on FHB attack

In Table 2 are presented the results regarding the influence of wheat variety on FHB attack (ANOVA, *post-hoc* Tukey test). Variety Şofru was used as control because it had registered the highest FHB attack degree. According with the obtained results three varieties (Apertus, Lovrin 5x and Lovrin 6x) have recorded significant differences in comparison with the control Şofru ($p < 0.05$), all these varieties registering the lowest values of the attack degree. The results suggest that all winter wheat varieties were susceptible to the FHB infection when the climatic conditions were favouring the development of the pathogen, but the variety can influence the attack intensity.

According with Wegulo (2012) a crop with healthy appearance can develop disease symptoms suddenly. Our results confirmed the fact that variety can influence the FHB attack degree and frequency, respectively some genotypes (Apertus, Lovrin 5x and Lovrin 6x) had registered a significant low attack intensity. The varietal effect noticed in the present research suggests that during epidemic years the choice of a susceptible wheat variety is the second risk factor in FHB development in crops, after favourable weather conditions. Therefore, breeding for resistance is still the one of the best options to control this disease and to limit mycotoxins accumulation in harvested grains (Bai and Shaner, 2004).

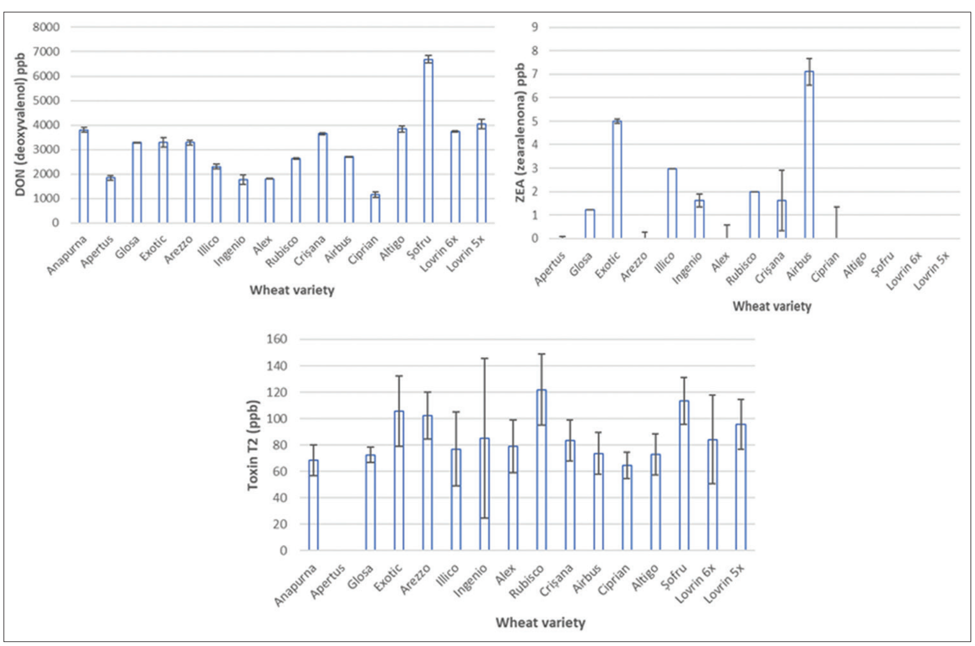

Fig 11. Mycotoxins concentrations (ppb) in the winter wheat grains of the varieties analysed immediately after harvesting in Timişoara, Western Romania.

In Fig. 12 is presented the correlation matrix of the scatterplots with the histograms and density plots characterizing the analysed field variables implied in FHB infection. The aspect of the scatterplots and the trendlines suggest the existence of the correlation among some field variables. The following correlations were statistically significant: ears density and FHB attack frequency (r_{calc} = -0.525*), number of diseased ears and FHB attack frequency (r_{calc} = 0.888***), number of diseased ears and FHB attack intensity (r_{calc} = 0.845***) and FHB attack intensity and attack frequency (r_{calc} = 0.709***) (see Table 3).

Table 2: Wheat variety influence on FHB attack (%) (ANOVA, *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. P-value and confidence intervals adjusted for comparing a family of 16 estimates (confidence intervals corrected using the Tukey method)

The most interesting finding is that low ears' density per square meter determinates the increase of FHB attack intensity (Fig. 12). Low densities of the wheat ears from 2019 were due to the drought from the autumn of 2018, when the wheat hasn't germinated well because of the absence of soil moisture. The germination was delayed and the plants haven't tillered or had few tillers. Regarding the ears density influence on FHB attack frequency, the varieties Ingenio and Şofru had registered the lowest attack densities and the highest attack frequencies (see Fig. 10).

Our results showed that a low ears density favoured high attack frequencies of FHB, contrary with the general

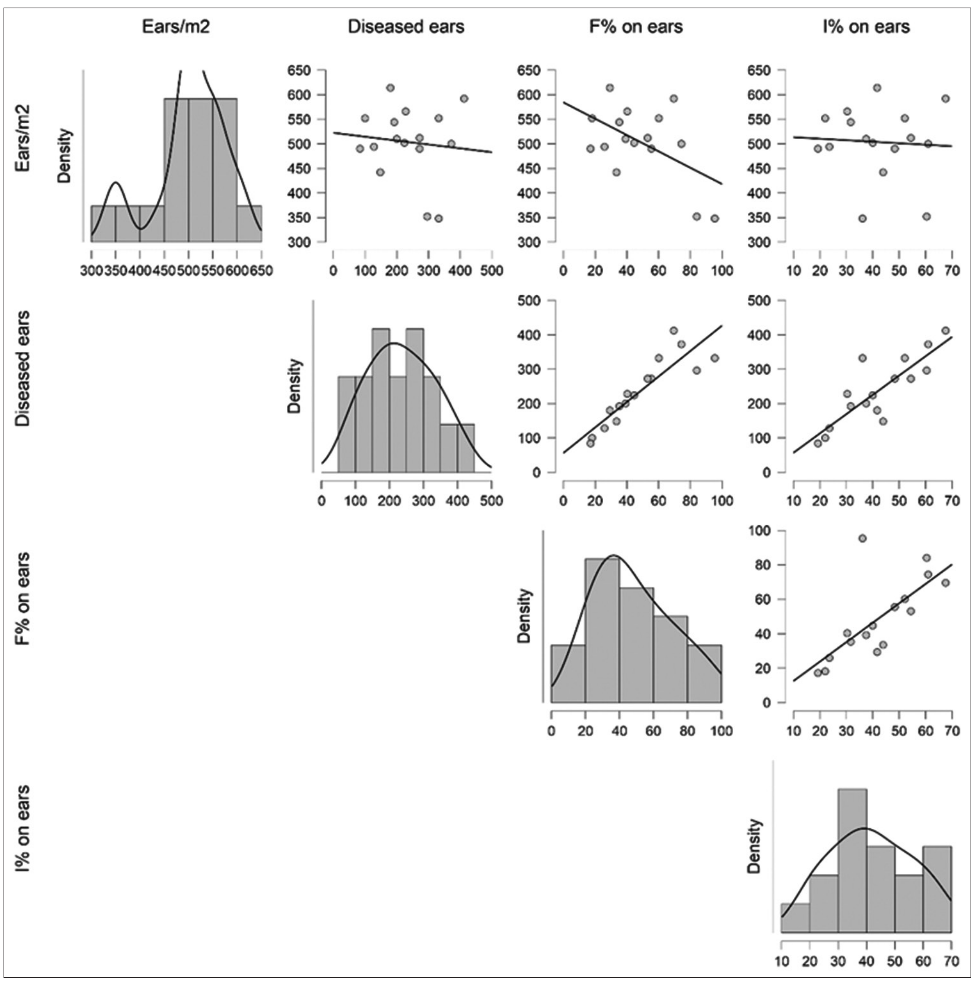

Fig 12. Correlation matrix among winter wheat ears density per square meter and analysed FHB symptoms with the histograms and density plots.

Table 3: Matrix of the Pearsons' rcalc values among the mycotoxin concentrations in wheat grains, wheat ears density and FHB infection features (α=0.05; n=16; df=n – 1 one-tailed test; p0.1 ≥ 0.412 *; p0.05 ≥ 0.558 **; p0.005 ≥ 0.606 *)**

Specification	DON (ppb)	ZON (ppb)	T-2 (ppb)	Density (ears/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 (ears/m²)				-	-0.104	-0.525*	-0.061
Diseased ears					-	0.888***	0.845***
F% on ears						-	0.709***
I% on ears							-

opinion on this topic. But in literature are data that confirm our finding, respectively the explanation is that low ears density favours the dispersal of the spores vertically to the ear level of the tillers because in those conditions are less obstacles in the primary way of FHB spores' dispersal that is splashing (Wegulo, 2012).

Concentrations of mycotoxins in relationships with wheat variety

DON concentration was determined in all the samples included in the experiment (Fig. 11). The highest DON level was detected in the variety Șofru (control), respectively 6690 ppb (surpassing four time the maximum allowed concentration in unprocessed cereals) and the lowest concentration was determined in the variety Ciprian with 1160 ppb (below the allowed concentration, but very close to it). The average value of DON concentration of the grains from the experimental field was 3340 ppb. In some varieties with low FHB infection rates was accumulated DON over the maximum allowed concentration according with EU regulations (see Fig. 11).

Zearalenone was detected only in some samples, respectively in the varieties Anapurna, Glosa, Exotic, Ingenio, Illico, Rubisco, Crișana and Airbus. ZON amounts determined in the analysed samples weren't surpassed the maximum allowed concentration for EU, respectively 100 ppb. Measured ZON concentrations were very low, being comprised between 1.21 and 7.11 ppb (Fig. 10). 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 maximum 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 (113.19 ppb) (Fig. 11).

In Table 4 is presented the influence of wheat variety on the accumulation of DON mycotoxin in wheat grains (ANOVA). The results showed that all the considered varieties have registered highly significant differences in comparison with the control Șofru (p < 0.001). In the case of ZON and T-2 concentrations weren't obtained significant results from the point of view of variety

Table 4: Wheat variety influence on DON (ppb) (ANOVA, *post-hoc* Tukey method)

Variety	Mean difference	t	p_{Tukey}
Șofru (control)	-	-	-
Airbus	-3990	-42.645	<0.001 ***
Alex	-4880	-52.157	< 0.001 ***
Altigo	-2850	-30.461	< 0.001 ***
Anapurna	-2890	-30.888	< 0.001 ***
Apertus	-4850	-51.836	< 0.001 ***
Arezzo	-3400	-36.339	< 0.001 ***
Ciprian	-5530	-59.104	< 0.001 ***
Crișana	-3040	-32.491	< 0.001 ***
Exotic	-3400	-36.339	< 0.001 ***
Glosa	-3400	-36.339	< 0.001 ***
Illico	-4380	-46.813	< 0.001 ***
Ingenio	-4920	-52.584	< 0.001 ***
Lovrin 5x	-2640	-28.216	< 0.001 ***
Lovrin 6x	-2950	-31.529	< 0.001 ***
Rubisco	-4060	-43.393	< 0.001 ***

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

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

Our results regarding the role of the variety in the occurrence of mycotoxins was demonstrated for DON. For ZON and T-2 could be a sum of factors that are influencing their occurrence in grains. According with the literature, the factors that are facilitating the occurrence of the mycotoxins produced by FHB in wheat grains are numerous, some of the most important being climate, variety, cropping technology and the reliability of the measured mycotoxins (Mesterhazy, 1995; Doohan et al., 2003; Hietaniemi et al., 2016).

The matrix of the mycotoxins variables as scatterplots, histograms and density plots are presented in Fig. 13, the trendlines indicating the existence of a significant interrelation between DON and T-2 concentrations (r_{calc} = 0.457*) (see Table 4).

Correlations among some FHB infection variables

Analysis of the relationships amongst the mycotoxin's concentrations, ears densities per square meter, diseased ears, FHB infected ears and attack intensity (I%) and

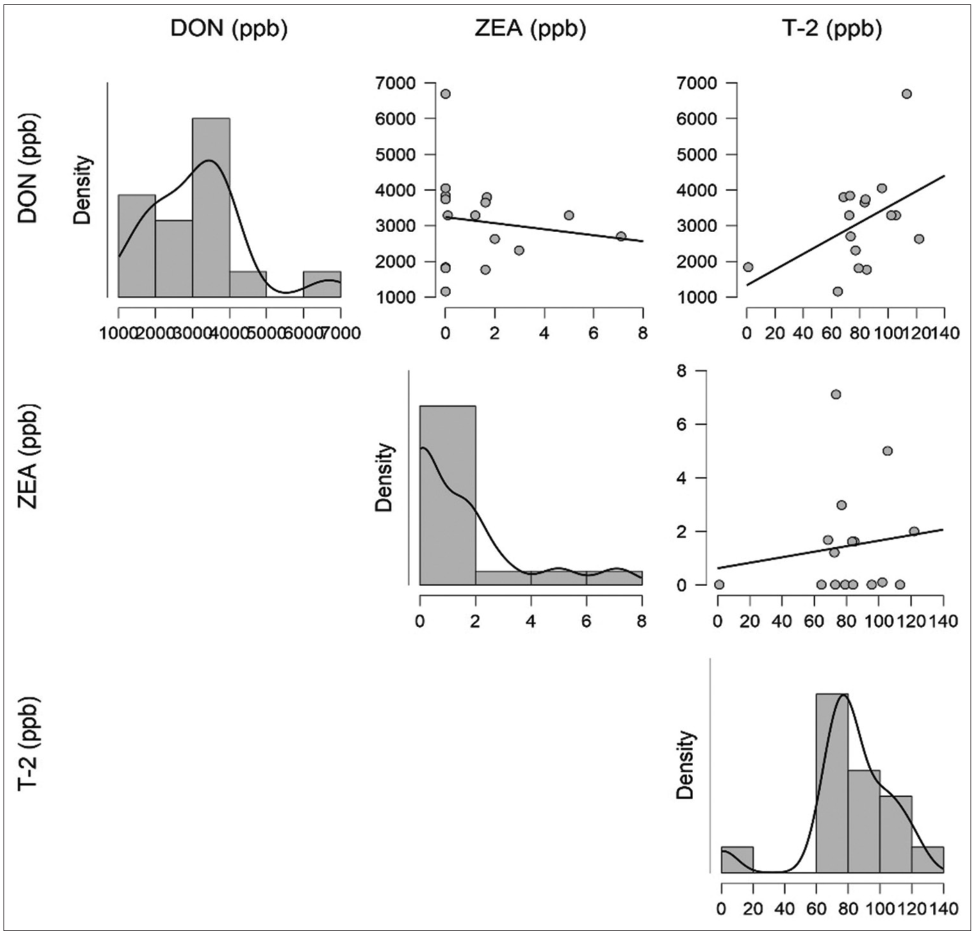

Fig 13. Correlation matrix among the concentration of the mycotoxins (DON, ZEA and T-2) from winter wheat grains with the histograms and density plots.

frequency (F%) are presented in Table 4. In the case of DON mycotoxin there were found 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 FHB I% and DON concentration from wheat grains. Ears density per square meters and FHB 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 diseased ears 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).

Numerous researches found positive correlations between the FHB attack intensity in field and the amount of DON accumulated (Haidukowski et al., 2005; Alisaac et al., 2020). The attack severity in field is considered a major factor in the accumulation for the mycotoxins in cereal grains (Hernandez Nopsa et al., 2012; Paul et al., 2005). Jansen et al., (2005) 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. (2011) 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 maximum in the proximity of the initially infected zone, before the appearance of the symptoms. As far as the grains are colonized by FHB fungus the biosynthesis of DON decreases.

Berca (2003) mentions that DON is present in Romania in wheat and triticale in amounts that surpass the maximum concentrations allowed by the European regulations. According with Wegulo (2012), as high is the rate of the grains affected by FHB, DON concentration will be higher. Thus, most of the researchers are correlating the presence of DON in grains with attack intensity from field and the rate of diseased grains (Cowger and Arellano, 2013). According with IARC (1994) the mycotoxin T-2 appears in significant amounts in cereals together with DON, this fact being confirmed by the results of our research too.

Using Exploratory Factors Analysis (EFA) (Table 5 and Fig. 14) there were assessed the possible joint variations in the response of the analysed variables. Thus, for the achievement of this objective there were performed several preliminary 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.

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

Kaiser-Meyer-Olkin test			
	MSA		
Overall MSA	0.406		
DON (ppm)	0.397		
ZON (ppb)	0.090		
T-2 (ppb)	0.342		
Plants/m^2	0.166		
Diseased ears	0.451		
F% on ears	0.462		
Bartlett's test			
X^2	df	p	
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	
ZON (ppb)		0.998	
T-2 (ppb)		0.914	
Plants/m^2		0.910	
Diseased ears	0.892	0.204	
F% on ears	0.948	0.101	
I% on ears	0.824	0.321	
Factor Characteristics			
	Sum Sq.	Loadings proportion	Var. Cumulative
Factor	2.716	0.388	0.388

No rotation method applied

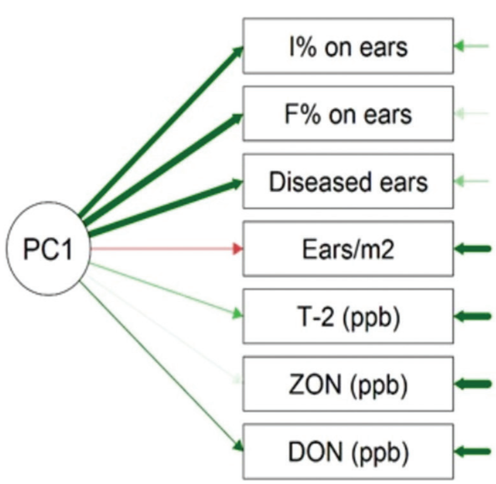

Fig 14. Path diagram among the variables and factors implied in the FHB infection and concentration of mycotoxin from wheat grains.

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 for the considered variables in the multiple correlation analysed here. Regarding the results for the uniqueness of the variance of the analysed data sets, the high values were obtained for ZON (0.998), T-2 (0.914), ears/m^2 (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 as path diagram (Fig. 14).

Path diagram (Fig. 14) represents the direction and strength of the relationship among the analysed variables and factors and show 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 ZON, T-2, ears density per square meter and DON.

T the link between DON and the infection severity is very well correlated in the years with FHB epidemics, but this isn't happening in the years unfavourable to the infection (Champeil, 2004). Our data showed a low but significant correlation, because there were various responses from a wheat variety to other regarding the synchronization of the FHB infection with DON concentration. According with the results obtained, there were various responses (see Figs. 9 and 10), respectively varieties with low FHB infection and high DON concentrations (e.g. Lovrin 5x and Lovrin 5x) and varieties with high infection frequencies and intensities and low DON concentrations (e.g. Ingenio and Glosa).

In general, high ears densities in wheat crop are considered to be determinant for high FHB attack frequencies (Mesterhazy, 1995; Wegulo, 2012). Other approach, according with Champeil et al. (2004) is that canopy density plays a role in FHB infection, but the magnitude of the phenomenon is not enough known.

Future research in the environmentally friendly control of FHB in wheat can approach the hypothesis that the canopy density plays an important role on the magnitude of the FHB infection in wheat crop, together with genetic resistance of varieties.

CONCLUSIONS

FHB infection rate of the analysed wheat varieties was influenced by the climate conditions from the spring of the year 2019, mainly the values recorded in May were favourable to FHB epidemics. This epidemic had a negative impact on the quality of wheat yield in all analysed samples. The main factor for low yield quality was the high concentration of the mycotoxin DON, 15 wheat varieties from 16 overpassing the minimum allowed concentration according with EU regulations (wheat variety Şofru recorded the greatest infection rate and the greatest DON concentration. At the opposite pole was the Romanian variety Ciprian that had registered the lowest infection rate and the lowest DON concentration, being the only variety from the experiment with DON concentration below the maximum level allowed. The response of some Romanian varieties with low mycotoxins concentrations could be assumed to the genetic resistance to the local FHB strains, but this aspect needs to be investigated in future research. The most interesting result obtained refers to the increase of the FHB attack frequency with the decrease of the wheat ears' density, similar results being isolated obtained in some researches too, contrary with the general opinion that FHB is favoured only by high ears densities. Thus, positive correlations were determined between DON and T-2 concentrations and DON and FHB attack frequency. The most influential factors in the FHB infection and the contamination with mycotoxins of the wheat grains in hierarchical order were: frequency of the attack on ear, diseased ears, attack intensity on ears and ears 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 wheat field plots in the conditions of FHB epidemic year.

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Authors' contributions

Otilia Cotuna has coordinated the field experiment, collected the data and samples and documented and wrote a part of the manuscript. Mirela Paraschivu has documented and wrote a part of the manuscript. Veronica Sărățeanu performed the statistical analysis, has documented and wrote a part of the manuscript and translated it in English. Elena Partal has given advices on the paper writing and revised the manuscript. Carmen Claudia Durău has participated to data and sample collection in field and revised the manuscript. All authors have read and agreed to the published version of the manuscript.

REFERENCES

Abramson, D. 1999. Mycotoxins toxicology. In: Robinson, R. K. (Ed), Encyclopedia of Food Microbiology, Elsevier, Berlin, pp. 1539-1547.

Alisaac, E., A. Rathgeb, P. Karlovsky and A. K. Mahlein. 2020. *Fusarium* head blight: Effect of infection timing on spread of *Fusarium graminearum* and spatial distribution of deoxynivalenol within wheat spikes. Microorganisms. 9: 79.

Andretta, I., M. Kipper, C. R. Lehnen and P. A. Lovatto. 2012. Meta-analysis of the relationship of mycotoxins with biochemical and hematological parameters in broilers. Poult. Sci. 91: 376-382.

Bai, G. and G. Shaner. 2004. Management and resistance in wheat and barley to *Fusarium* head blight. Ann. Rev. Phytopathol. 42: 135-161.

Bai, X., C. Sun, J. Xu, D. Liu, Y. Han, S. Wu and X. Luo. 2018. Detoxification of zearalenone from corn oil by adsorption of functionalized GO systems. Appl. Surf Sci. 430: 198-207.

Bajwa, A. A., M. Farooq, A. M. Al-Sadi, A. Nawaz, K. Jabran and K. H. M. Siddique. 2020. Impact of climate change on biology and management of wheat pests. Crop Prot. 137: 105304.

Berca, M. 2003. Mycotoxins, an old problem, but new for alimentary security. Rev. Prot. Plantelor. 51: 5-24.

Bertuzzi, T., M. C. Leggieri, P. Battilani and A. Pietri. 2014. Cooccurrence of Type A and B trichothecenes and zearalenone in wheat grown in northern Italy over the years 2009-2011. Food Addit. Contam. Part B Surveill. 7: 273-281.

Binder, S., H. Schwartz-Zimmermann, E. Varga, G. Bichl, H. Michlmayr, G. Adam and F. Berthiller. 2017. Metabolism of zearalenone and its major modified forms in pigs. Toxins. 9: 56.

Champeil, A., T. Dore and J. Fourbet. 2004. *Fusarium* head blight: Epidemiological origin of the effects of cultural practices on head blight attacks and the production of mycotoxins by *Fusarium* in wheat grains. Plant Sci. 166: 1389-1415.

Chandelier, A., C. Nimal, F. André, V. Planchon and R. Oger. 2011. *Fusarium* species and DON contamination associated with head blight in winter wheat over a 7-year period (2003-2009) in Belgium. Eur. J. Plant Pathol. 130: 403-414.

Čonková, E., A. Laciaková, G. Kováč and H. Seidel. 2003. Fusarial toxins and their role in animal diseases. Vet. J. 165: 214-220.

Cook, R. J. 1981. *Fusarium* diseases of wheat and other small grains in North America. In: Nelson, P. E., T. A. Toussoun and R. J. Cook (Ed), *Fusarium* Diseases, Biology and Taxonomy. United States Pennsylvania State University Press, United States, pp. 39-52.

Cotuna, O., V. Sărățeanu, C. C. Durău, M. Paraschivu and G. Rusalin. 2013. Resistance reaction of some winter wheat genotypes to the attack of *Fusarium graminearum* L. Schw. in the climatic conditions of Banat plain. Res. J. Agric. Sci. 45: 117-122.

Cowger, C. and C. Arellano. 2013. *Fusarium graminearum* infection and deoxynivalenol concentrations during development of wheat spikes. Phytopathology. 103: 460-471.

De Wolf, E. D., L. V. Madden and P. E. Lipps. 2003. Risk assessment models for wheat *Fusarium* head blight epidemics based on within-season weather data. Phytopathology. 93: 428-435.

Desjardins, A. E. 2006. *Fusarium* mycotoxins: In: Chemistry, Genetics, and Biology. APS Press, American Phytopathological Society, St. Paul, MN.

Doohan, F. M., J. Brennan and B. M. Cooke. 2003. Influence of climatic factors on *Fusarium* species pathogenic to cereals. Eur. J. Plant Pathol. 109: 755-768.

Gilchrist, L., S. Rajaram, A. Mujeeb-Kazi, M. van Ginkel, H. Vivar and W. Pfeiffer. 1997. In: Dubin, H. J., L. Gilchrist, J. Reeves and A. McNab (Eds.) *Fusarium* Head Scab: Global Status and Future Prospects. Mexico, DF, CIMMYT.

Haidukowski, M., M. Pascale, G. Perrone, D. Pancaldi, C. Campagna and A. Visconti. 2005. Effect of fungicides on the development of *Fusarium* head blight, yield and deoxynivalenol accumulation in wheat inoculated under field conditions with *Fusarium graminearum* and *Fusarium culmorum*. J. Sci. Food Agric. 85: 191-198.

Hallen-Adams, H. E., N. Wenner, G. A. Kuldau and F. Trail. 2011. Deoxynivalenol Biosynthesis-related gene expression during wheat kernel colonization by *Fusarium graminearum*. Phytopathology. 101: 1091-1096.

Hietaniemi, V., S. Rämö, T. Yli-Mattila, M. Jestoi, S. Peltonen, M. Kartio, E. Sieviläinen, T. Koivisto and P. Parikka. 2016. Updated survey of *Fusarium* species and toxins in Finnish cereal grains. Food Addit. Contam. Part A Chem. Anal. Control Expo Risk Assess. 33: 831-848.

Huong, B. T. M., L. D. Tuyen, T. T. Do, H. Madsen, L. Brimer and A. Dalsgaard. 2016. Aflatoxins and fumonisins in rice and maize staple cereals in Northern Vietnam and dietary exposure in different ethnic groups. Food Control. 70: 191-200.

Ianoș, G., I. Pușcă and M. Goian. 1997. Solurile Banatului. Condițiile Naturale și Fertilitate. Mirton, Timișoara, Romania.

Jansen, C., D. von Wettstein, W. Schafer, K. H. Kogel, A. Felk and F. J. Maier. 2005. Infection Patterns in Barley and Wheat Spikes Inoculated with Wild-type and Trichodiene Synthase Gene Disrupted *Fusarium graminearum*. In: Proceedings of the National Academy of Sciences. Vol. 102, pp. 16892-16897.

Jo, H. W., M. K. Park, H. M. Heo, H. J. Jeon, S. D. Choi, S. E. Lee and J. K. Moon. 2021. Simultaneous determination of 13 mycotoxins in feedstuffs using QuEChERS extraction. Appl. Biol. Chem. 64: 34.

Johnson, D. D., G. K. Flaskerud, R. D. Taylor and V. Satyanarayana. 2003. Quantifying economic impacts of *Fusarium* head blight in wheat. In: Leonard, K. J. and W. R. Bushnell, (Eds), *Fusarium* Head Blight of Wheat and Barley. American Phytopathological Society, St. Paul, MN, USA, pp. 461-483.

Khodaei, D., F. Javanmardi and A. M. Khaneghah. 2021. The global overview of the occurrence of mycotoxins in cereals: A three-year survey. Curr. Opin. Food Sci. 39: 36-42.

Kumar, A., S. Karre, D. Dhokane, U. Kage, S. Hukkeri and A. C. Kushalappa. 2015. Real-time quantitative PCR based method for the quantification of fungal biomass to discriminate quantitative resistance in barley and wheat genotypes to *Fusarium* head blight. J. Cereal Sci. 64: 16-22.

Liddell, C. M. 2003. Systematics of *Fusarium* species and allies associated with *Fusarium* head blight. In: Leonard, K. J. and W. R. Bushnell, (Eds), *Fusarium* Head Blight of Wheat and Barley, American Phytopathological Society, St. Paul, MN, USA.

Ma, H., H. Ge, X. Zhang, W. Lu, D. D. Yu, H. Chen and J. Chen. 2009. Resistance to *Fusarium* head blight and deoxynivalenol accumulation in Chinese barley. J. Phytopathol. 157: 166-171.

Magan, N. and J. Lacey. 1984. Water relations of some *Fusarium* species from infected wheat ears and grain. Transac. Br. Mycological Soc. 83: 281-285.

McMullen, M., R. Jones and D. Gallenberg. 1997. Scab of wheat and barley: A re-emerging disease of devastating impact. Plant Dis. 81: 1340-1348.

Mesterhazy, A. 1995. Types and components of resistance to *Fusarium* head blight of wheat. Plant Breeding. 114. 377-386.

Mézes, M., M. Barta and G. Nagy. 1999. Comparative investigation on the effect of T-2 mycotoxin on lipid peroxidation and antioxidant status in different poultry species. Res. Vet. Sci. 66. 19-23.

Miedaner, T. 1986. Entwicklung von Methoden zur Bestimmung der *Fusarium*-Resistenz in Frühen Wachstumsstadien des Weizens. University of Hohenheim, Germany.

Miller, J. D. 1994. Epidemiology of *Fusarium* ear diseases of cereals. In: Miller, J. D. and H. L. Trenholm, (Eds), Mycotoxins in Grain. Compounds other than Aflatoxin. Eagan Press, St. Paul, MN, USA, pp. 19-36.

Miller, J. D. 2002. Aspects of the ecology of *Fusarium* toxins in cereals. In: DeVries, J.W., M. W. Trucksess and L. S. Jackson, (Eds), Mycotoxins and Food Safety. Kluwer Academic/Plenum Publishers, New York, USA, pp. 19-28.

Minervini, F. and M. E. Dell'Aquila. 2008. Zearalenone and reproductive function in farm animals. Int. J. Mol. Sci. 9: 2570-2584.

Mu, P., M. Xu, L. Zhang, K. Wu, J. Wu, J. Jiang, Q. Chen, L. Wang, X. Tang and Y. Deng. 2013. Proteomic changes in chicken primary hepatocytes exposed to T-2 toxin are associated with oxidative stress and mitochondrial enhancement. Proteomics. 13: 3175-3188.

Nopsa, J. F. H., P. S. Baenziger, K. M. Eskridge, K. H. S. Peiris, F. E. Dowell, S. D. Harris and S. N. Wegulo. 2012. Differential accumulation of deoxynivalenol in two winter wheat cultivars varying in FHB phenotype response under field conditions. Can. J. Plant Pathol. 3: 380-389.

Official Journal of the European Union. 2006. Commission Regulation (EC) No 1881/2006 of 19 December 2006 Setting Maximum Levels for Certain Contaminants in Foodstuffs. Official Journal of the European Union, European Union.

Official Journal of the European Union. 2013. Commission Recommendation 2013/165/EU of 27 March 2013 on the Presence of T-2 and HT-2 Toxin in Cereals and Cereal Products. Official Journal of the European Union, European Union.

Paraschivu, M. 2008. The impact of tillage, crop rotation and residue management on *Fusarium* head blight in wheat. In: Govaerts, `B., and A. Castellanos-Navarrete, (Eds), Compendium of Deliverables of the Conservation Agriculture Course, Mexico, Mexico, pp. 6-14.

Paul, P. A., P. E. Lipps and L. V. Madden. 2005. Relationship between visual estimates of *Fusarium* head blight intensity and deoxynivalenol accumulation in harvested wheat grain: A meta-analysis. Phytopathology. 95: 1225-1236.

Pereyra, S. A. and R. Dill-Macky. 2008. Colonization of the residues of diverse plant species by *Gibberella zeae* and their contribution to *Fusarium* head blight inoculum. Plant Dis. 92: 800-807.

Pfliegler, W. P., T. Pusztahelyi and I. Pócsi. 2015. Mycotoxins-prevention and decontamination by yeasts: Mycotoxins-prevention and decontamination by yeasts. J. Basic Microbiol. 55: 805-818.

Pitt, J. I. 2014. Mycotoxins: Deoxynivalenol and other trichothecenes. In: Motarjemi, Y. (Ed.), Encyclopedia of Food Safety, Academic Press, Cambridge, pp. 295-298.

Pitt, J. I. and J. D. Miller. 2017. A concise history of mycotoxin research. J. Agric. Food Chem. 65: 7021-7033.

Popescu, G. 2005. Tratat de Patologia Plantelor. Eurobit, Timișoara.

Rotter, B. A. 1996. Invited review: Toxicology of deoxynivalenol (Vomitoxin). J. Toxicol. Environ. Health. 48: 1-34.

Schroeder, H. W. and J. J. Christensen. 1963. Factors affecting resistance of wheat to scab caused by *Gibberella zeae*. Phytopathology. 53: 831-838.

Singh, R. P. 1995. Genetic analysis of resistance to scab in spring wheat cultivar Frontana. Plant Dis. 79: 238.

Skiepko, N., B. Przybylska-Gornowicz, M. Gajęcka, M. Gajęcki and B. Lewczuk. 2020. Effects of deoxynivalenol and zearalenone on the histology and ultrastructure of pig liver. Toxins (Basel). 12: 463.

Sobrova, P., V. Adam, A. Vasatkova, M. Beklova, L. Zeman and R. Kizek. 2010. Deoxynivalenol and its toxicity. Interdiscip. Toxicol. 3: 94-99.

Szczech, G. M. and R. D. Hood. 1978. Animal model of human disease: Alimentary toxic aleukia, fetal brain necrosis, and renal tubular necrosis. Am. J. Pathol. 9: 689-692.

Teich, A. H. and K. Nelson. 1984. Survey of *Fusarium* head blight and possible effects of cultural practices in wheats fields in Lambton County in 1983. Can. Plant Dis. Surv. 64: 11-13.

Trottet, M., and B. Rolland. 2014. QuantiPest-Assessment of *Fusarium* Head Blight on Wheat-INRA, IPM Network, France.

Turner, N. W., S. Subrahmanyam and S. A. Piletsky. 2009. Analytical methods for determination of mycotoxins: A review. Anal. Chim. Acta. 632: 168-180.

Unger, P. W. 1994. Managing Agricultural Residues. Lewis Publishers, Boca Raton.

Van der Burgt, G. J. H. M. and B. G. H. Timmermans. 2009. *Fusarium* in Wheat. The Effect of Soil Fertility Strategies and Nitrogen Levels on Mycotoxins and Seedling Blight. Louis Bolk Instituut, Netherlands.

Wang, Y. Z. and J. D. Miller. 1988. Screening techniques and sources of resistance to fusarium head blight. In: Klatt, A. R. (Ed), Wheat Production: Constraints in Tropical Environments. CIMMYT, Mexico, pp. 239-250.

Watkins, J. E. and M. G. Boosali. 1994. Plant disease incidence as influenced by conservation tillage systems. In: Unger, P. W. (Eds), Managing Agricultural Residues. Lewis Publishers, Boca Raton, pp. 261-283.

Wegulo, S. 2012. Factors influencing deoxynivalenol accumulation in small grain cereals. Toxins (Basel). 4: 1157-1180.

World Health Organization. 1994. IARC Monographs on the Evaluation of Carcinogenic Risk to Humans. Vol. 56. Some Naturally Occurring Substances: Food Items and Constituents, Heterocyclic Aromatic Amines and Mycotoxins. World Health Organization, Geneva, p. 341.

Zhang, W., K. Boyle, A. L. Brûlé-Babel, G. Fedak, P. Gao, Z. R. Djama, B. Polley, R. D. Cuthbert, H. S. Randhawa, F. Jiang, F. Eudes and P. R. Fobert. 2020. Genetic characterization of multiple components contributing to *Fusarium* head blight resistance of FL62R1, a Canadian bread wheat developed using systemic breeding. Front. Plant Sci. 11: 580833.

Zheng, W., N. Feng, Y. Wang, L. Noll, S. Xu, X. Liu, N. Lu, H. Zou, J. Gu, Y. Yuan, X. Liu, G. Zhu, J. Bian, J. Bai and Z. Liu. 2019. Effects of zearalenone and its derivatives on the synthesis and secretion of mammalian sex steroid hormones: A review. Food Chem. Toxicol. 126: 262-276.