

## ***Fusarium* species producers of trichothecenes in wheat – characteristics and control – Review**

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### **Abstract**

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Wheat is a major cereal crop culture, with highest part of global production and consumption. It represents a favorable substrate for the development of numerous fungal pathogens, of which the members of the genus *Fusarium* are of great importance. *Fusarium* spp. causes a variety of diseases affecting cereal crops in wheat manifested in two forms – *Fusarium* root and stem rot and *Fusarium* head blight (FHB) when it occurs at a later stage. Apart from the fact that these infections dramatically reduce the yields and the quality of the grain, there is a risk of secondary *Fusarium* metabolites known as mycotoxins entering the production. Among them, the trichothecenes deoxynivalenol (DON), nivalenol (NIV), T-2, HT-2 toxin are of great concern. These secondary metabolites can accumulate in high concentrations in the rain and enter the food chain, causing various diseases in both humans and animals. The production of mycotoxins is not only species-specific but also strain-specific characteristic. Therefore, the knowledge of the pattern of *Fusarium* species spread on the wheat, will allow accurate prediction and early management of the potential presence of mycotoxins in food and feed.

*Keywords: Fusarium; Fusarium infections; wheat; Fusarium mycotoxins; Fusarium species identification*

### **Introduction**

Wheat (*Triticum aestivum* L.) is one of the most important cereal crops for humanity. According Food and Agriculture Organization of the United Nations, the world production of wheat for 2021, is more than 770 million tons, and over the years it has been continuously growing (FAO, 2022). There are more than 220 million hectares available for its cultivation. Data indicate that about 70% of wheat produced worldwide is used for food, 20% for livestock feed, and only 2% to 3% is used in the industrial sector. The European Union (EU)'s production of 126,658,950 tons ranks 2<sup>nd</sup> among the

5 largest wheat producers. In Bulgaria, a total of 4,847,940 tons were produced in 2020 (FAO, 2020). Unfortunately, the quality of wheat can be negatively affected by microscopic fungi that could infect the grain in the field (Beccari et al., 2018), as well as during storage. Infected grain become shriveled and pink-white in color, perithecia appear on their surface and yield is greatly reduced. (Gagkaeva et al., 2021).

One of the most important causative agents of wheat diseases are the representatives of genus *Fusarium*. It is a complex, widespread genus that has been known for over 200 years. Some of its species are well-known phytopathogens. The American Phytopathological Society report at least one

*Fusarium*-related disease affects 81 out of 101 commercial plants (Babadoost, 2018). Therefore, it is crucial to consider these ascomycete fungi's influence. The members of *Fusarium* spp. cause a variety of diseases affecting cereal crops, including wheat, manifesting in two forms – *Fusarium* head blight (FHB) and *Fusarium* root and stem rot. When it occurs at a later stage, *Fusarium* infection appears as *Fusarium* head blight. Predominant species causing the diseases may vary depending on wheat types, geographic region, treatment methods, and environmental conditions during the growing season (Van der Lee et al., 2015). They colonize the underground and above-ground parts and seeds of cereals, as well as the soil, reducing the yield and quality of the crop and indirectly causing significant economic losses to agriculture. *Fusarium* related diseases are caused by single species or more often by co-occurring species in the field (Ferrigo et al., 2016) that produce one or more mycotoxins, weakening the defense of the crop. Therefore, they are an interesting example of biodiversity. According to Desjardins et al. (1993) in order to better adapt and colonize new ecological niches *Fusarium* spp. have altered their biosynthetic metabolism in the evolutionary process, producing a wide range of secondary metabolites known as mycotoxins that vary in chemical structure and toxicity (Desjardins et al., 1993). Trichothecenes, fumonisins, zearalenone, etc., have the greatest impact on public health. During colonization of cereals and feed, some of them could accumulate to high levels, posing a significant risk to animal and human health (Chandra et al., 2011).

The aim of the current review is to address the most important aspects of the distribution of toxigenic *Fusarium* spp. on wheat worldwide and the dynamics in the populations of the *Fusarium* species complex that cause FHB in different climatic regions. The main emphasis is placed on their mycotoxigenic profile, particularly the appearance of the trichothecenes DON, NIV, T-2, HT-2, as well as on the risks they cause to both animal and human health. The review also takes into account the regulations that control the mycotoxins levels, as well as the methods for identification of mycotoxigenic *Fusarium* spp.

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### ***Fusarium* spp., causing FHB of wheat**

The FHB was first described by W.G. Smith in 1884 in England as “Wheat Scab”. Due to the frequent outbreaks of FHB throughout the world and the devastating properties of this wheat disease, the attention of a large part of the world's scientists is focused on the strategies of combating its causative agents.

The species causing FHB of wheat differ in their geographical distribution and the optimal climatic conditions they require. Despite their extreme adaptability, different dominant species causing FHB have been established in certain regions in the respective climatic zones (Moretti et al., 2019). Different *Fusarium* spp. have been found to infect cereals at different temperatures. The most favorable temperature for grain infection for *F. avenaceum* and *F. graminearum* is 28.0 to 29.0°C, while for *F. culmorum* it is 26.5°C (Ejar et al., 2023). FHB has been found to be promoted by rainfall during and after flowering and is caused by nearly 19 *Fusarium* spp. comprising a complex of toxigenic pathogens (Liddell, 2003), (Ferrigo et al., 2016). Among them, *F. graminearum* is the most prevalent species worldwide and is considered the most aggressive (Kazan et al., 2012). According to the Molecular Plant Pathology journal, *F. graminearum* (teleomorph *Gibberella zaeae*) is currently ranked fourth among plant fungal pathogens, based on its virulence properties and economic importance (Dean et al., 2012). In Europe, the most dominant are *F. graminearum*, *F. avenaceus*, *F. culmorum*, the less common are *F. poae*, *F. cerealis* and *F. equiseti*, lesser extent are *F. oxysporum* and *F. solani* (Bottalico and Perrone, 2002). According to latest studies *F. poae*, which until recently had underestimated virulence properties, is shown to be dominant in certain geographical areas under suitable agrometeorological conditions (Stenglein, 2019).

As Europe is characterized by climate variations, differences in species composition are found between northern, central and warm Mediterranean regions. In all Scandinavian countries at the turn of the century, *F. culmorum* was reported as dominant, found on cereal roots and the base stem (Logrieco and Moretti, 2008). *F. graminearum* is the main producer of DON in Central and Southern Europe (Logrieco and Moretti, 2008). However, variation in the population structure of mycotoxigenic fungi is also increasing in more countries such as the United Kingdom, Germany, Poland, etc. and *F. graminearum* becomes dominant in the FHB complex and displaces the previously abundant *F. culmorum*, as higher temperatures stimulate its development (Miedaner et al., 2008; Stępień and Chelkowski, 2008; Edwards, 2009). Gagkaeva et al. (2021) investigated the causes of FHB in 2019 in the Amur region, which comprises 60–70% of grain pro-

duction in the Russian Far East. Through the use of morphological and molecular methods, 51–98% of wheat samples were found to be infected with *F. graminearum* and it was the most abundant species. Although according to the recent study by Refai (2015) that indicate the main pathogenic species between 50% and 96% of FHB wheat infections is *F. graminearum*, this is not always the case (Refai, 2015).

Tittlemeier et al. reported that *F. avenaceum* is the main causative agent of FHB of durum wheat in Canada (Tittlemeier et al., 2013). In Italy, the molecular identification of *Fusarium* spp. in durum wheat (*Triticum durum* Desf.) showed that *F. avenaceum* was predominant (61%), followed by *F. graminearum* (22%), and *F. poae* (9%) (Beccari et al., 2018). According to Abdallah-Nekache et al. (2019) in Algeria, *F. culmorum* is the most common of the invasive species distributed on wheat seedlings. A similar *Fusarium* profile was also observed in North and Northwestern Iran – *F. acuminatum* was the most prevalent of the *Fusarium* isolates, followed by *F. incarnatum-equiseti* species complex and *F. equiseti*, respectively 27.7% and 16.6% (Imane et al., 2022). Few investigations have so far been conducted in Bulgaria to confirm the validity of the above-mentioned trend related to the prevalence of *Fusarium* spp. in cereal crops (Valcheva, 2003; Beev, 2004; 2011; 2013). *F. tricinctum* was the most common species found among the *Fusarium* isolates. The second most frequently identified was *F. poae*, followed by *F. proliferatum*, *F. graminearum* and *F. equiseti*, which is consistent with previous information from 2011 (Gencheva and Beev, 2020).

In their research from 2023 Ejaz et al. (2023) suggested that crop diseases caused by *Fusarium* spp. will increase worldwide because of future climate changes. Correlative models used for species distribution analysis (SDMs) show how the ecological niche of *Fusarium* spp. will change by 2050 and 2070. The gathered predictive data could benefit farmers and the scientists from all continents, in their efforts to create strategies for stopping *Fusarium* spp. spreading and for selecting the right fungicides to minimize disease damage to cereal crops, including wheat.

### **Mycotoxins and chemotypes of *Fusarium* spp.**

Pathogenic *Fusarium* spp. are believed to possess complex mechanisms for penetration, infection and colonization of plants (Rauwane, 2020). They have modified their biosynthetic metabolism, producing „an arsenal of compounds such as virulence factors“ – mycotoxins (Chandra et al., 2011; Nathanail et al., 2015). These are complex organic compounds with low molecular weight (0,3–0,7 kDa), natural contaminants of cereals and feed. They have the capacity to accumulate to high levels in grain and foods (Majeed et al.,

2018). Due to their relatively resistance to heat treating they can easily be transmitted through food and feed commodities along the food chain and pose a health threat to animals and humans. Analyzes show that by-products of grain grinding, such as bran, feed flour, polishing products, etc. often contain higher levels of mycotoxins, namely they are used as feed for domestic animals (Vanara, 2018).

The most well-known *Fusarium* mycotoxins on cereals, including wheat, are: trichothecenes type B, deoxynivalenol (DON) also known as vomitoxin can be present as mono-acetylated (3-AcDON, 15-AcDON) and di-acetylated (3,15-AcDON) derivatives, and nivalenol (NIV). The A group mainly includes T-2 and HT-2 toxins. Moniliformin (MON), zearalenone (ZEA), fusarin C are also present (Ferrigo et al., 2016; Refai et al., 2015). Each *Fusarium* spp. possesses a specific mycotoxigenic profile that has been well studied and clarified in recent years (Table 1). *F. graminearum* and *F. culmorum* generate trichothecenes type A and ZEA (Refai et al., 2015; Beccari et al., 2018). The type A trichothecenes are produced by several *Fusarium* spp., mainly *F. poae* and *F. sporotrichioides*. NIV and its di-acetylated derivatives, i.e., 4,15-AcNIV are formed by some *F. poae*, *F. culmorum* and *F. cerealis* strains (Ferrigo, 2016). Fusarin C could be a secondary metabolite of *F. culmorum*, *F. avenaceum*, *F. sporotrichioides*, *F. poae*, etc.

The production of a particular type of mycotoxins is a specific characteristic not only of the species but also of individual strains. The chemotype of a individual strain of a given *Fusarium* spp. refers to the variety of secondary metabolites that strains of that species can produce. From a toxicological point of view toxigenic strains of *F. graminearum* were classified into NIV and DON chemotypes. The strains can produce different acetylated forms of trichothecenes: chemotypes 3-acetyl-DON (3-ADON) and 15-acetyl-DON (15-ADON), depending on whether a C-15 or C-3 acetyl group is present in their 15-carbon skeleton. This further necessitated their subclassification into DON-chemotype IA and DON-chemotype IB, respectively (Bottalico and Perrone, 2002). The population associated with the 3-ADON chemotype is thought to be more aggressive than the 15-ADON. The 3-ADON chemotype is more prevalent in the USA and Canada (Kelly et al., 2015; Walkowiak et al., 2015). The results of Polish scientists on wheat samples from 2016–2017 indicated that 15-ADON was the predominant chemotype, although 3-ADON and NIV were also observed (Bilska et al., 2018). In a study conducted in Romania, the chemotypes of fungal isolates from local populations of the pathogen were determined. After performing pathogenicity tests, it was shown that the isolate of *F. culmorum* from Romania has a high prevalence of chemotype 3-ADON and its aggres-

siveness has been established. The species composition of *Fusarium* spp. isolated from winter wheat was examined in Luxembourg between 2009 and 2012, with *F. graminearum sensu stricto* (15AcDON chemotype) strains predominating (Beyer, 2014). The co-occurrence of DON, 15ADON and 3ADON was observed in Chinese wheat. In Bulgaria are established infestations of wheat ears by *F. graminearum*. DON, 3-AcDON and 15-AcDON were found in 67%, 2.1% and 0.7% of the samples, respectively (Vrabcheva and Vrabchev, 1997).

The monitoring of *Fusarium* mycotoxins DON, FUM, ZEN and T-2 for the period 2010–2019 was carried out by European Food Safety Agency (EFSA) and agribusiness BIOMIN (BIOMIN, World Mycotoxin Survey). The report indicated that in European food and feed wheat samples, DON was the most abundant mycotoxin, with DON-producing *F. graminearum* and *F. culmorum* dominating species (Johns et al., 2022). According to the survey, the share of T-2 has increased. This suggests that the population of *Fusarium* pathogens in Europe has changed with T-2 producing *F. langsethiae* or *F. sporotrichioides*. DON and T-2 co-infection of wheat crops is a cause for concern. There is a real

risk of mycotoxin exposure over acceptable levels when pigs consume straw and grain contaminated with DON and ZEN (Hägglblom and Nordkvist, 2015).

The global trends in mycotoxin levels during the past few years in different commodities are presented in Table 2 and Figure 1, respectively (DSM, 2022).

According to Johns (2022), *Fusarium* mycotoxins are ubiquitous across Europe, with DON persistently detected in wheat, raising concerns about the health effects of chronic dietary exposure. Alarming high concentrations of DON in feed wheat and severe mycotoxin outbreak have been reported in lower latitude regions of Europe, possible due to agronomic and climatic changes. Although lower contamination in food suggest that EU legal restrictions are having a positive effect, strict monitoring and outbreak-responsive management of *Fusarium* mycotoxins should continue to protect human and livestock health. Changing mycotoxin profiles, such as increasing DON and T-2 co-contamination, indicate changing dynamics in FHB pathogen populations and may have synergistic negative health consequences. The economic evaluation made by the study authors show the significant cost of DON contamination in European wheat.

**Table 1. Mycotoxigenic species isolated from FHB of wheat (Adapted from Refai, M., Hassan, A., & Hamed, M., 2015)**

Species	Mycotoxin	MON	NIV	DON	ZEN	TRI	T-2	FUS C
	<i>F. avenaceum</i>		+					
<i>F. graminearum</i>			+	+	+			
<i>F. culmorum</i>		+	+	+	+	+		+
<i>F. equiseti</i>			+		+	+	+	
<i>F. cerealis</i>			+		+			+
<i>F. sporotrichioides</i>		+			+		+	+
<i>F. poae</i>						+		+
<i>F. tricinctum</i>						+		+

MON = moniliformin; NIV = nivalenol; DON = deoxynivalenol; ZEN = zearalenone; TRI = trichothecenes; T-2 = T-2 toxin; FUS C = fusarin C.

**Table 2. Mycotoxin levels in corn, wheat grain, barley, rye, oats, rice, sorghum, and millet in Europe, Asia, Africa and South and Central America according to the DSM World Mycotoxin survey data, 2022 (<https://www.biomin.net/solutions/mycotoxin-survey>)**

			ZEN	DON	T-2	FUM	Maximum (ppb)			
			ZEN	DON	T-2	FUM	ZEN	DON	T-2	FUM
% Contaminated samples	Europe	Corn	32%	48%	9%	64%	13 498	15 640	1 892	38 481
		Cereals*	30%	49%	25%	19%	2 145	21 200	1 069	2 028
	South and Central America	Corn	32%	48%	9%	64%	3 513	16 000	197	22 610
		Cereals*	30%	49%	25%	19%	4 539	5 157	230	1 900
	Asia	Corn	66%	78%	23%	95%	8 305	17 743	124	37 850
		Cereals*	61%	45%	16%	72%	702	3 620	73	10 998
Africa	Corn	64%	79%	0%	64%	739	4 576	24	34 995	
	Cereals*	72%	82%	1%	87%	1 147	4 818	24	10 660	

\*Cereals include: wheat grain, barley, rye, oats, rice, sorghum, millet

## Global trends of Fusarium mycotoxins in all commodities (last 10 years)

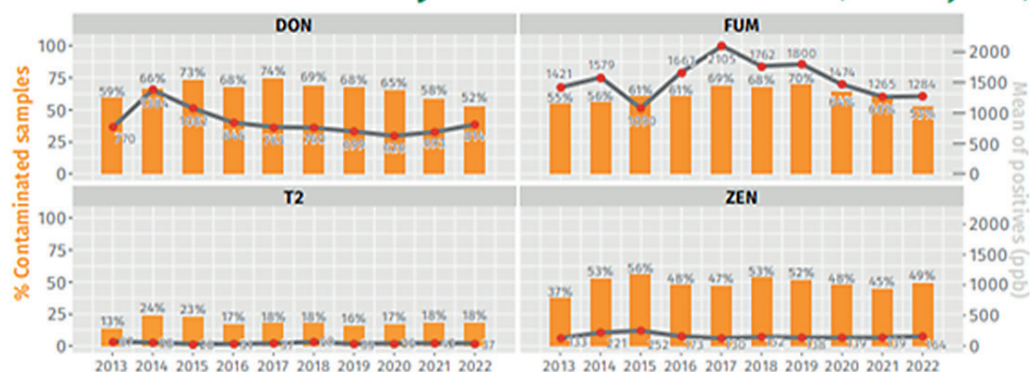


Fig. 1. Level of *Fusarium* mycotoxins in all commodities for the period 2013–2022, according to data of DSM World Mycotoxin Survey, 2022 (<https://www.biomin.net/solutions/mycotoxin-survey>)

The study quantifies the threat and cost of *Fusarium* mycotoxins in order to predict future food security scenarios, support legislation and implement appropriate mycotoxin mitigation strategies.

Toxins type A like T-2 toxin (T2) and its deacetylated form HT-2 toxin inhibit protein synthesis in plants, causes metabolic transformations, as well as acute or chronic intoxication of humans and animals, a growth retardation, hematotoxicity, and myelotoxicity (Nathanail et al., 2015). These types of toxins have been shown to „modify“ DNA matrices to alter the transcription process (Pleadin, 2018). Type A trichothecenes, including T-2 and HT-2 toxins, are considered more toxic than type B trichothecenes (e.g. deoxynivalenol and nivalenol). Toxicological studies have shown that T-2 toxin can cause acute intoxication and chronic disease in both humans and animals (Van der Fels-Klerx, 2010) and has been shown to be a potent cytotoxic and immunosuppressive agent. As a result of some evidence of the carcinogenic properties of T-2 toxin in experimental animals, it has been classified by the International Agency for Research on Cancer (IARC) into Group 3 carcinogens because of the lack of data regarding its ability to cause cancer in humans and only limited evidence supporting its carcinogenicity in experimental animals (IARC, 1993).

Clinical symptoms of trichothecene exposure in animals include food refusal and weight loss, vomiting and necrosis of various tissues, as well as hemorrhage (Polak-Śliwińska and Paszczyk, 2021).

Trichothecenes have a proven ability to inhibit protein synthesis in eukaryotic cells. Clinical symptoms of DON exposure are associated with vomiting, weight loss, and hemorrhage. Additionally, it could suppress nutrient uptake by human epithelial cells and directly affect proper uterine

function (Ferrigo, 2016; Maresca et al., 2002). According to recent studies based on animal models, trichothecenes have been found to cause necrotic lesions in the gastrointestinal tract (Kolf-Clauw et al., 2013; Liew and Mohd-Redzwan, 2018).

The complexity of exposure to *Fusarium* mycotoxins in humans and animals is further complicated by the fact that plants are able to modify mycotoxins into masked mycotoxins. They cannot be extracted by conventional extraction solvents used in assays and are difficult to be proven (Berthiller et al., 2013). The possible hydrolysis of masked mycotoxins, which may be present in high amounts during mammalian digestion, raises the concern that the underlying toxin may be released and absorbed in the gut and thus contribute to exposure.

Sometimes, contamination in cereals can occur when different mycotoxins are produced by the same fungi or by different fungi growing simultaneously in the same product. In addition, the composition of feed and food with different grains may expose consumers to multiple mycotoxins that have originated from various sources.

In addition to their individual toxic effects, mycotoxins can also produce interactive effects when combined, resulting in synergistic, antagonistic or additive toxicity (Corrêa et al., 2018).

According to the Pancosma and associates study from 2015 mycotoxin analysis survey, covering 1740 raw material and animal feed samples in Europe, it became clear that monogastric animals were highly exposed to the mycotoxins trichothecenes type A and B. In the same study, it was shown that wheat in Europe is not as contaminated as maize, but it may also pose a threat due to the co-occurrence of  $\geq 2$  mycotoxins in 46.6 % of the samples (Pancosma, 2015).

## Methods for the identification of *Fusarium* spp.

Morphological identification is a slow and laborious process and very often closely related species in more than 20 species complexes are misidentified (Crous et al., 2021). Variation was observed in the size and shape of conidia and spores, both within species and between strains (Ko et al., 2011). Because of the advances in molecular biology, it has become possible to identify unknown species based on their nucleotide sequences. Various techniques and molecular biological tools have been developed. They help not only in taxonomy, but also provide information on genetic relationship and assist scientists in studying the differences between fungal isolates. Molecular markers used for identification of *Fusarium* spp. are amplified fragment length polymorphism (AFLP), randomly amplified polymorphic DNA (RAPD), restriction fragment length polymorphism (RFLP), single nucleotide polymorphism (SNP), sequence related amplified polymorphism (SRAP), sequence characterized amplified regions (SCAR), SNP-based multilocus genotyping assay (Refai et al., 2015). Currently, real-time PCR, as well as a number of modern markers, such as DNA barcoding, DNA microarrays, etc. are successfully applied (Chandra et al., 2011). The use of molecular methods for species identification of molds began after the ribosomal operon was discovered as a functional unit of the DNA molecule containing a „package“ of genes under the control of a single regulatory signal and the primers described for it. Barcoding of Internal transcribed spacer (ITS), mitochondrial small subunit (mtSSU) *rRNA* gene, translational elongation factor 1 $\alpha$  (*TEF-1 $\alpha$* ), Beta-tubulin ( *$\beta$ -tub*), and RNA polymerase II subunit (RPB2) is increasingly used (Al-Hatmi et al., 2016). Micro-satellite inter simple sequence repeats (ISSR) is one of the most important marker to study the genetic diversity for identifying highly polymorphic multilocus markers (Laraba, 2017). Microsatellite fingerprints differentiate closely related species complexity, genetic diversity, genome mapping, and evolutionary lineages (Navale et al., 2023). Methods for the design of primers from specific sequences of *Fusarium* spp. have also been developed (Arif et al., 2012).

Although all molecular methods are a useful tool for the differentiation of fungal species, they are not able to distinguish between strains based on their mycotoxigenic potential. In recent years, it has been proven that a biosynthetic gene cluster (BGC) in fungi represents, a group of two or more genes physically located as a cluster in a specific location in the genome“ (Medema et al., 2014). They are expressed simultaneously as a BGC. These BGCs usually consist of genes that encode polyketide synthases (PKS), terpene cyclases (TCs), non-ribosomal peptide synthetases (NRPS), and prenyltrans-

ferases (PTs) in addition to adjacent genes that assist in gene expression regulation and metabolites transport. Each cluster contains one or more regulatory genes encoding a protein that binds to the promoter of biosynthetic genes and assists RNA polymerase II to initiate transcription (Lazarus et al., 2014). The progress in the knowledge of the type of genes, their location in the trichothecenes gene cluster, as well as the role of each one in the synthesis of the different mycotoxin analogs, lay the basis for the development of PCR-based approaches as a reliable tool for chemotype prediction (Kulik, 2011; Nielsen, 2014). The enzymes responsible for trichothecene mycotoxins in *Fusarium* spp. are encoded by genes in 3 loci: single gene locus *TRI 101*, two gene locus *TRI 1- TRI 16* and 12 gene *TRI* cluster. It has been established that the different alleles of the *TRI 1* gene play the primary role for the production of the different types of trichothecenes type A and type B (McCormick et al., 2006). A number of scientists have successfully demonstrated mycotoxigenic potential using various genes from the trichothecene gene cluster. In 2013, Cornea C. et al. successfully determined the chemotype of 85 *F. graminearum*, *F. culmorum* and *F. sporotrichoides* isolates from Romanian wheat using the four genes *TRI3*, *TRI 7*, *TRI 2* and *TRI 13*. In their studies Lee et al. have identified that *TRI3* and *TRI7* *Fusarium* trichothecene biosynthetic cluster genes are responsible for conversion of DON to NIV (*TRI13*) and acetylation of NIV to 4-acetyl-nivalenol (4-ANIV), (*TRI7*) (Lee, et al., 2002). A number of scientists have successfully demonstrated the chemotype diversity of strains isolated from wheat. By using sequence of elongation factor-1 $\alpha$  gene (*EF-1 $\alpha$* ) in 28 strains, scientists identify presence of *F. graminearum sensu stricto*, *F. asiaticum*, *F. meridionale* and *F. cortaderiae*. It has been proven that in Paraguayan isolates, the 15-ADON chemotype of *F. graminearum sensu stricto* was predominant and by using *TRI3* and *TRI12* and specially developed primers, the trichothecene genotype was determined (Arrua Alvarenga et al., 2022). In Japan, based on *TRI 3* and *TRI 12*, the NIV, 15ADON and 3ADON chemotypes for *F. graminearum* and *F. asiaticum* were determined by the diagnostic PCR-RFLP method (Suga et al., 2008).

## Regulations of mycotoxin levels control

Taking into account the *Fusarium* potential risk to human and animal health, as well as the global trade in cereals and feed, various international institutions and organizations have developed regulations outlining acceptable levels of mycotoxins in food. For example, In the European Union, the presence of *Fusarium* toxins in foodstuffs is regulated by Regulation (EC) No 1126/2007 (Regulation EC, 2007) which is in addition to the recommendation of Regulation (EU) No

1881/2006 (EC, 2006). Recommendation 2013/165/EU (Ec., 2013) lays down the indicative levels of presence of T-2 and HT-2 toxins for cereals and cereals products and The Commission encourages Member States to perform monitoring of the both toxins. Regulation EC No 1126/2007 defines the maximum permissible level for DON, FUM and ZEN in different types of cereal-based foods (EC, 2007). Considering DON maximum levels are as follows: 1250 µg/kg on unprocessed cereals other than durum wheat, oats and maize, 1750 µg/kg on unprocessed durum wheat and oats, 750 µg/kg on cereals intended for direct human consumption, cereal flour, bran and germ, and 500 µg/kg on bread (including small bakery wares), pastries, biscuits, cereal snacks and breakfast cereals (Regulation (EC, 2006 r). According to the Guidance for Industry and FDA, the advisory levels for DON are as follows: 1000 µg/kg DON on finished wheat products, e.g. flour, bran, and germ, 10 000 µg/kg on grain and grain by-products and 30 000 µg/kg in distillers grain, brewers grain, and gluten feeds and gluten meals derived from grain, and 5 000 µg/kg DON on grain and grain by-products destined for all other animals (US FDA, 2010). As the EU has some of the highest food safety standards in the world, the Rapid Alert System for Food and Feed (RASFF) was created. Information is exchanged between Member States in case of risks to public health arising from the food chain. The RASFF portal provides information on recent food recalls from markets and at international borders ([https://food.ec.europa.eu/safety/rasff\\_en](https://food.ec.europa.eu/safety/rasff_en)).

## Conclusion

The worldwide contamination of cereals and feed with mycotoxins is a global environmental, economic, social and health problem. Pathogenic representatives of the genus *Fusarium* are one of the contamination primary causes. They can cause serious wheat diseases such as Fusarium head blight and Fusarium root and stem rot, causing serious damage to the agricultural and food sectors. The fact that in low concentrations, the potential synergism between them can lead to significant negative changes in the health status of people and animals is also worrying. A number of countries, international health organizations and the European Commission establish either recommendatory or restrictive in character regulations, in order to prevent wheat and wheat-based products from being contaminated with mycotoxins. Having the knowledge of *Fusarium* spp. affecting wheat, their mycotoxigenic profile and distribution in relation to climatic regions, their early and reliable identification is crucial. New molecular methods are widely used for accurate determination of *Fusarium* chemotypes, aiming at early prediction and development of new strate-

gies to improve this important raw material for both Bulgaria and rest of the world.

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