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Factors influencing the occurrence of *Fusarium* mycotoxins in the grain of winter wheat

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Abstract. Fusarium mycotoxins are secondary metabolites, biosynthesized by filamentous fungi of the genus *Fusarium,* which, due to their diverse toxicity and difficulty in their removal from food and animal feeds, are the subject of research by scientists around the world. The most important mycotoxins found in wheat are: deoxynivalenol (DON), zearalenone (ZEN), and the T-2 and HT-2 toxins. Bearing consumer safety in mind, the levels of these substances in grain, food and animal feeds are regulated by law. In order to meet the health safety requirements imposed on grain, it is important to minimise the risk of fungal infection at individual production stages. Weather conditions are the most important factor influencing the development of fungi and the accumulation of mycotoxins in grain. High temperature, combined with rainfall, are factors favouring the spread of fungal spores. Apart from weather conditions, individual elements of the production technology, such as appropriate variety selection, soil cultivation method, preceding crop, use of fungicides, and fertilization may reduce the quantity of mycotoxins in grain. In the conditions of the emerging climatic changes, favouring the development of fungal diseases, creating wheat varieties resistant to fungal diseases is a considerable challenge. Acquiring more in-depth knowledge on the relationship between the phenotypic features of a wheat variety and mycotoxin content would be helpful. This work is of review character and aims to indicate the basic factors influencing the production of fusarium mycotoxins in winter wheat grain. The focus was placed on factors independent of human activity (weather conditions) and on individual elements of cultivation technology.

Keywords: fungal diseases, synthesis of mycotoxins, cereal protection

INTRODUCTION

Humanity has been struggling for centuries with the harmful effects of secondary fungal metabolites (mycotoxins) present in cereals. Already in the Middle Ages, there were reports of regular poisoning after eating rye bread made of flour containing ergot. It is currently assumed that about 20–25% of cereal production worldwide is contaminated with these xenobiotics, which results in the deterioration of the health quality of grain and its elimination from human consumption and animal feed use (FAO, 2013). In order to ensure safety of consumers, the European Union has introduced unified norms and legal regulations with regard to the maximum permissible content of some of the most important mycotoxins in cereal grains and cereal products. The safe permissible limit for DON (deoxynivalenol) has been established at the level of 1250 μg kg^{-1} for unprocessed cereals other than durum wheat and oats, for which the value has been set at the level of 1750 μg kg^{-1}. However, in the case of the mycotoxin ZEN (zearalenone) for the above-mentioned products, this level amounts to 100 μg kg^{-1}. Permissible mycotoxin levels for cereal-based products are lower. The level of DON in flour, pasta and bran cannot exceed 750 μg kg^{-1}, in bread 500 μg kg^{-1}, and in products for children – 200 μg kg^{-1}. Similarly, the level of ZEN in flour and bran must not exceed 75 μg kg^{-1}, and in bread 50 μg kg^{-1} (Commission Regulation (EC) No. 1881/2006). The capacity of mycotoxin biosynthesis is a natural and quite frequent feature of fungi. Pathogenic filamentous fungi often infect cereal crops at each stage of their development causing them to contract diseases and the mycotoxins they produce may not only be toxic to humans and animals but may also have phytotoxic effects. Fungal crop diseases also lead to potential economic losses due to decreasing grain production. The occurrence of fungal diseases in crop cultivation and, consequently, the formation of mycotoxins has a significant impact not only on plant development during vegetation, but also on the quality and quantity of the obtained harvest (Mielniczuk et al., 2015). From the agricultural point of view (including food and feed safety), especially in the cultivation and storage of cereals, the fungi of the genus *Fusarium*, *Penicillium* and *Aspergillus* are the most important with

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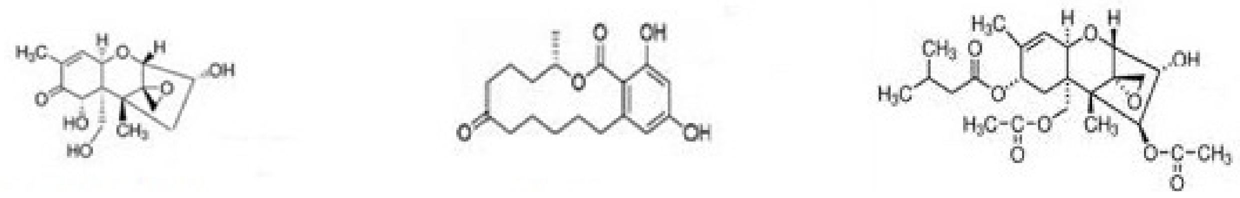

deoxynivalenol (DON) zearalenone (ZEN) T-2 toxin

Figure 1. Structural formula of Fusarium mycotoxins (based on Barabasz and Pikulicka, 2017)

regard to toxin production. Metabolites can be synthesized during plant vegetation and produced during grain storage. *Fusarium* fungi are responsible for the formation of mycotoxins during plant growth, while *Aspergillus* and *Penicillium* during storage. In Europe, the most commonly occurring fusarium mycotoxins produced still on the field during plant vegetation are: deoxynivalenol (DON), zearalenone (ZEN), nivalenol (NIV), fumonisins and T-2 and HT-2 toxins. Ochratoxin A (OTA) is produced during grain storage (Góral, 2006). However, due to the relatively high frequency and content of toxins found in cereal grains, the most important *Fusarium* toxins are DON and ZEN (Bottalico, Perrone, 2002).

Cereal crops constitute the basis of human nutrition all over the world. Wheat plays the leading role, both in Poland and worldwide. Due to the high yield potential and the chemical composition of the grain, wheat is used both for human consumption and animal feed purposes. It is used primarily for the production of flour, bakery and confectionery products, pasta and breakfast cereals. The key task of the producers of cereals, including wheat, is to obtain the raw material (grain) of high health-related and technological quality. The high adaptability of fungi to changing environmental conditions facilitates natural inoculation and the development of plant infections with these fungi. Therefore, protection of crops against fungal diseases can be extremely difficult, and often even impossible, especially during periods of unfavourable weather conditions (e.g., long periods of rainfall and relatively high temperature during vegetation) (Korbas et al., 2013). Therefore, it is important to explore the causes of the contamination of wheat grain with mycotoxins during plant growth and to propose appropriate rules, the observance of which will contribute to reducing the quantity of *Fusarium* toxins in the grain.

FUSARIUM AS A PATHOGEN CAUSING DISEASES OF WINTER WHEAT AND FORMING MYCOTOXINS

Fusarium fungi are commonly found in soil where they live as saprophytes feeding on organic matter. They have the ability to change metabolism as well as to adapt to the substrate they live on, which helps them to quickly infect many plant species grown around the world. They are characterised by high tolerance to environmental factors and they develop in a wide temperature range (0–30 °C). Currently, the fungi of the genus *Fusarium* are considered to be one of the most pathogenic microorganisms in the world (Kwaśna et al., 1991; Pląskowska, 1997; Leslie et al., 2006; Wolny-Koładka, 2014) (Table 1). They infect many plants including wheat and other cereal crops. Wheat plants are infected at various stages of development, most often as a result of mechanical damage to the tissue which becomes the gateway for infection. The spread of *Fusarium* spp. is facilitated by rainfall and air currents carrying propagation spores over long distances (Leslie et al., 2006). The pathogen can cause numerous diseases such as Fusarium seedling blight, cereal snow mould, cereal leaf fusariosis, fusariosis of the stem base and roots, and Fusarium head blight (FHB) (Tratwal et al., 2017). In the case of winter wheat, the paramount importance is held by the fusariosis of the stem base and roots as well as Fusarium head blight, and fusariosis of cereal leaves is of lesser importance (Tratwal et al., 2017). The first symptoms of the fusarium blight of the stem base and roots are already visible in autumn. The fungus infects the roots and the base of the stem, which is

Table 1. The main Fusarium species occurring on wheat ears in Poland and the most important mycotoxins produced by them (Góral et al., 2017).

Species	Importance for Poland	Mycotoxins
F. graminearum (chemotyp DON)	+++	DON, ZEN
F. graminearum (chemotyp NIV)	+	NIV, ZEN
F. avenaceum	+++	moniliformin
F. culmorum (chemotyp DON)	+++	DON, ZEN
F. culmorum (chemotyp NIV)	+	NIV, ZEN
F. poae	+++	NIV, beauvercin, T-2, H-T2
F. langsethiae	+	T-2,H-T2,
F. sporotrichioides	+	T-2, H-T2, beauvercin

manifested by the colour change of the leaf sheaths or the entire base of the stem from green to brown. As a result of this disease, the affected shoots die prematurely, which reduces the plant's ability to obtain water and nutrients supply. Infected plants have less branching, dry out prematurely, the grain fills poorly during ripening and entire ears turn white. The first symptoms of cereal leaf fusariosis may already appear in autumn. Greenish-grey and then yellow spots on leaf blades are the symptoms. FHB is caused by various species of fungi. The most common ones are: *F. culmorum, F. avenaceum, F. graminearum, F. poae* and *F. langsethiae* (Korbas, 2017; Góral et al., 2015; Bottalico, 1998; Bottalico, Perrone, 2002). A single ear may be affected by several species *Fusarium* species (Góral et al., 2015). Fusarium head blight usually occurs when the flowering temperature exceeds 18 °C and the relative humidity is 80%. The disease causes changes in the ears and grains. Yellow, partial or complete discolouration of spikelets, single ones at first, then more numerous, indicates the emergence of this disease. The spikes are covered with a white or pink bloom, on which orange or salmon-coloured sporodochia can be observed. The caryopses from the ears infected at a later stage of development may not differ in size from healthy ones. The shape of diseased wheat ears is the same as the healthy ones, while the affected part is narrowed in the place of visible symptoms. The infection of the ears by pathogens contributes to deteriorating the qualitative and quantitative characteristics of the crop, embryo damage and weakening of the grain germination power. As a result of the infection by some fungi of the genus *Fusarium*, grain may contain mycotoxins which are highly poisonous to humans and animals. (Korbas, 2014; Góral, 2018, 2017).

FACTORS INFLUENCING THE FORMATION OF MYCOTOXINS

Conditions for the synthesis of mycotoxins

The process of creating mycotoxins as secondary metabolites and their role for fungi are not fully understood. On the basis of the available literature, a view may be put forward according to which the process of toxin biosynthesis by mould fungi is the result of mutual dependencies between their development and environmental factors. Fanelli et al. (2004) showed that toxin production aims to reduce the excess of reactive oxygen forms in the plant, while Reverberi (2005) argues that the fungal cell uses toxins to control the oxidative burst. In turn, Voigt et al. (2007) speculated that mycotoxins, including DON and ZEN, could be synthesized to weaken other competing microorganisms during the saprophytic growth phase of the fungus. Many authors (Ponts, 2015; Reverberi et al., 2010; Perincherry et al., 2019) believe that mycotoxins are produced in a defensive response to both biotic and abiotic factors. Reverberi et al. (2010) and Perincherry et al. (2019) believe that the most important stimuli for mycotoxin biosynthesis include oxidative, nutritional and light stress and environmental factors such as pH, temperature, water activity (aw), fungicides and secondary metabolites of plant origin. However, as Popovski and Celar (2013) prove, temperature and water activity play the most important role in the growth of fungi and their production of mycotoxins. The authors note that *Fusarium* fungi require different temperatures for growth and for the production of mycotoxins (Table 2). Llorens et al. (2004) indicate that the most favourable temperature for the growth of *F. garminearum* and *F. culmorum* is a temperature in the range of 20–32 ˚C, with the growth of the fungi being inhibited at temperatures below 15 ˚C. Schmid et al. (2010) indicate that the optimal temperature for DON production is 28 ˚C.

The ability to biosynthesize toxins is not a species trait of a fungus. Wróbel (2014) reports that strains which do not biosynthesize toxic substances may become toxigenic due to stress (e.g., changes in temperature, humidity or the presence of a substance toxic to a given fungus) and vice versa. The basic conditions influencing the development of the fungus and its phenotype denote access to: nutrients (source of carbon and minerals, i.e. zinc, cobalt, magnesium), access to oxygen, temperature usually in the range of 15–30 °C, environmental water activity> 0.9, the pH of the substrate is usually in the range of 4.5–6.5 (Barabasz, Pikulicka, 2017). It has been proved that the synthesis of mycotoxins is genetically determined and related to the ba-

Table 2. Temperature and water activity (aw) for growth and mycotoxin production of *Fusarium* (Sweeney, Dobson, 1998; Popovski, Celar, 2013).

Genotype of *Fusarium*	Growth temperature / aw	Mycotoxins	Mycotoxin production temperature / aw
F. graminearum	optimum 21 °C max. 31 °C	DON, ZEN	25–28 °C / 0.97
F. culmorum	24–26 °C / min. 0.9	DON, ZEN	17–28 °C / 0.97 >37 °C does not produce
F. sporotrichioides	22.5–27.5 °C	T-2, HT-2	20–25 °C / 0.99
F. moniliforme	22.5–27.5 / 0.87	moniliformin	25–30 °C / 0.92

sic metabolic pathways, i.e. the metabolism of amino acids and fatty acids (Piotrowska, 2012).

Species and variety

The resistance of wheat to FHB is genetically determined. Many years of research on the mechanism and development of FHB allowed for selecting five types (mechanisms) of resistance: resistance to infection (type I), resistance to the spread of *Fusarium* in the ear (type II), resistance of caryopses to damage caused by *Fusarium* (type III), tolerance to accumulated toxins (type IV) and resistance to the accumulation of Fusarium toxins in grain (type V) (Mesterhazy, 2002; Boutigny et al., 2008; Góral et al., 2015). Much attention has been given to research on Sumai 3, a highly resistant Chinese spring wheat variety, and its derivatives. The main QTL (quantitative trait locus) of this wheat, which determines its resistance to FHB, is found on the short arm of chromosome 3B (Anderson et al., 2001; Buerstmayr et al., 2002, 2003). QTL was mapped with precision as a Mendelian trait and defined as Fhb1 (Liu et al., 2006). However, over-reliance on the resistance traits of the source may exert selective pressure on the pathogen, leading to the emergence of strains which break the resistance derived from Sumai 3 (Akinsanmi et al., 2006). Breeding for resistance to Fusarium diseases in Polish resistance breeding programs for wheat has gained importance in recent years. The varieties included in the Polish variety register differ in their resistance to the Fusarium disease complex. On a 9-point scale, the variety variation ranges from 6.8 (Tonnage variety) to 8.5 (RGT Specialist variety) (www. COBORU). The varieties and species of wheat differ not only in their resistance to fusarium diseases, but also in their ability to accumulate mycotoxins in the grain (Table 3, Fig. 2) (Konvalina et al., 2011; Podolska, Boguszewska, 2017). Spelt and other harder to thresh wheat species or varieties accumulate lower quantities of toxins in relation to threshable ones. The reason for this is most likely the hard spikelets enveloping the caryopsis,

Table 3. DON content in wheat grain depending on the variety and species (Konvalina et al., 2011).

Species	Cultivar	DON [mg kg^{-1}]
Triticum monococcum	Schwedisches Einkorn	0.132
Triticum monococcum	No. 8910	0.167
Triticum dicoccum	Rudico	0.035
Triticum dicoccum	Weisser Sommer	0.032
Triticum spelta L.	Spalda bila jarni	0.181
Triticum spelta L.	VIR St. Petersburg	0.105
Triticum aestivum	Cervena perla	0.235
Triticum aestivum	Kasticka presivka	0.130
Triticum aestivum L.	Jara	0.320
Triticum aestivum L.	SW Kadrilj	0.332

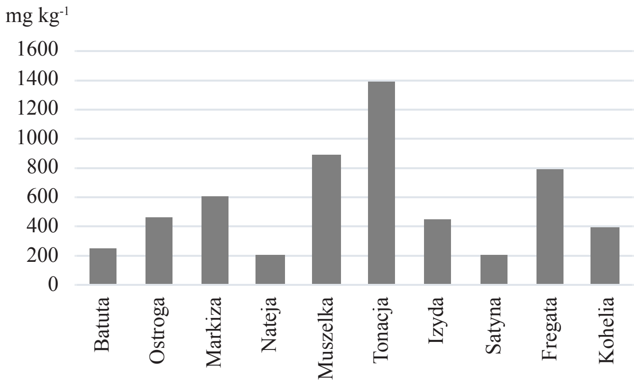

Figure 2. DON content depending on the variety (Podolska, Boguszewska, 2017)

which constitute a barrier to fungal infection. *Triticum durum* wheat compared to *Triticum aestivum* ssp. *vulgare* accumulates more toxins (Rachoń et al., 2016). The variability of common wheat varieties grown under the same environmental conditions in terms of the quantity of *Fusarium* mycotoxins in grain is considerable (Hajšlová et al., 2007; Czaban et al., 2015; Góral et al., 2015; Podolska, Boguszewska, 2017; Weber, Pląskowska, 2017). In the studies by Podolska and Boguszewska (2017), the content of DON in wheat grain ranged from 200 µg kg^{-1} (Nateja variety) to 1400 µg kg^{-1} (Tonacja variety). Bai et al. (2001), employing artificial inoculation of winter wheat *F. graminearum*, noticed significant differences in the level of DON content between wheat varieties. They selected 16 varieties with a low DON content out of 116, indicating these varieties as a source of resistance. Similar studies were performed by Góral et al. (2015, 2019). Infecting 61 wheat genotypes with *F. culmorum* isolates, they established differences in the level of DON from 4.055 mg kg^{-1} to 39.956 mg kg^{-1}, with an average content of 20.515 mg kg^{-1}, and in the case of ZEN from 197 to 6190 mg kg^{-1} (on average 932 mg kg^{-1}). Infecting 27 wheat lines with the chemotype of the fungus *Fusarium culmorum* biosynthesizing DON and NIV, Góral et al. (2019) demonstrated line variation in the content of DON from 0.371 to 8.303 mg kg^{-1}, and NIV in the range of 0.348–4.998 mg kg^{-1}. Podolska et al. (2017) distinguished 3 groups of varieties depending on the content of mycotoxins (DON, ZEN, T-2 / HT-2 toxin) in the grain: varieties with a low degree of contamination, varieties with a low level of some mycotoxins and higher in others, and varieties with a high degree of contamination with all tested fusarium mycotoxins.

Wheat varieties differ in the length of the vegetation period and their morphological features (e.g. plant height, ear structure, stem cross-section). These features affect the quantity of accumulated mycotoxins. Korbas and Mrówczyński (2014) pointed out that the cultivation of varieties with a short vegetation period or those that bloom for a short period of time allows for avoiding or reducing – to a large extent – infestation with fungi of the genus *Fusarium* and the accumulation of toxins. Choo et al. (2004), Góral et al. (2015), Podolska and Boguszewska (2017) showed lower fusariosis infection and lower accumulation of mycotoxins in the grain of tall genotypes. As indicated by Yan et al. (2011) increased infection of short genotypes may result in the presence of the gene responsible for dwarfism (Rht-D1), which mainly influences type I immunity. The genes responsible for resistance to fungal infection are found on all wheat chromosomes except 7D (Buerstmayr, 2009). Weber and Pląskowska (2017) report that the resistance of wheat to *F. culmorum* and *F. graminearum* is inherited polygenically, whereas most of the genes determining this trait exhibit an additive genetic effect. The structure of the stem and ear is of significant importance. Varieties with stems having more filling, accumulate lower quantities of mycotoxins (Podolska, Boguszewska 2017). The presence of specific genes in the plant responsible for colonization conducted by the fungi of the genus *Fusarium* also affects the accumulation of their secondary metabolites. The research on the resistance of wheat varieties to fungal diseases shows that none of the tested genotypes has full resistance to fusariosis (Góral, 2005).

Weather conditions

The development of fungi and, therefore, the content of mycotoxins in cereal grain is closely dependent on the weather conditions, and above all, the temperature and precipitation occurring during the periods of earing, flowering and grain ripening. The research by Sadowski et al. (2010) indicates a significant influence of rainfall and temperature on the colonization of wheat by the fungi of the genus *Fusarium*. The authors state that in 2007, when the average temperature during the flowering of cereals was 21 °C, and the rainfall was 36.8 mm, there occurred a significant increase in the incidence of *Fusarium* diseases on wheat ears compared to 2005 and 2006, when the average temperature and rainfall in the same period were lower. Weather conditions are not the same in individual wheat cultivation locations, therefore both the quantity and the type of mycotoxins in wheat grain are variable (Hajšlová et al., 2007; Bryła et al., 2016; Podolska, Boguszewska, 2017; Podolska et al., 2017; Góral et al., 2019). In the research on the impact of weather conditions on the accumulation of mycotoxins in the grain of 10 winter wheat varieties conducted in various regions of Poland in 2009–2011, the highest average DON content of 256 µg kg^{-1} was demonstrated for wheat grain harvested in 2011, with lower content in 2010 harvest (106 µg kg^{-1}) and the lowest one in 2009 harvest (10 µg kg^{-1}). In the analysed years, the mean DON content in grain depended on rainfall in May ($r> 0.89$), June ($r> 0.80$) and July ($r> 0.84$). Temperature, on the other hand, was of lesser importance (Podolska, Boguszewska, 2017).

Bryła et al. (2016) showed that there is a difference in the level of not only DON, but also ZEN, Enn B (enniatin), and Enn B1 in the grain of the same wheat varieties grown in different parts of Poland. Wheat from the fields in the southern part of Poland was more contaminated with mycotoxins than the samples from the northern or western parts of the country. In their research, the authors showed that high air humidity combined with temperature values in the range of 15–25 ºC create favourable development conditions for the fungi of the genus *Fusarium*. Also, Góral et al. (2015, 2019), conducting artificial inoculation with the strain *F. culmorum*, found significant differences in the content of toxins in wheat grain between localities. The average DON content in the samples of wheat grown near Poznań [a city in western Poland] was relatively low and amounted to 595 µg kg^{-1}, while in the samples from crops near Radzików [a town in south-west Poland] the DON content was four times higher and amounted to 2395 µg kg^{-1}. The NIV content differed in these samples. In the samples of wheat from two localities, the content of this toxin was respectively 5488 µg kg^{-1} and 67 µg kg^{-1}.

Method of soil cultivation

Soil cultivation shapes soil properties. Currently, among the methods of soil cultivation, one can enumerate tillage, ploughless tillage and no-till farming with direct sowing. With the simplification of the classic cultivation and the abandonment of tillage, an increasing quantity of crop residues stays on the field surface. Only tillage cultivation guarantees their complete covering and placement at different depths in the soil. The quantity of crop residues depends on the method of cultivation, with the most, 90%, of the residues being left in the field in the case of no-till farming (Jaskulski, 2016). The plant material acts as soil-protecting mulch, but it is also an excellent substrate for the development of *Fusarium* fungi. Crop residues in the case of cereals and corn can be a reservoir for *Fusarium* fungi (Dill-Macky, Jones, 2000; Steinkellner, Langer, 2004; Sadowski et al., 2010; Weber, Kita, 2010; Korbas, 2014). The subject of research is usually the comparison of conventional tillage with simplified tillage or direct sowing. The available literature shows less infection with *Fusarium* fungi and a lower quantity of mycotoxins in wheat grain with the use of conventional tillage methods (Sadowski et al., 2010; Koch et al., 2006; Weber, Pląskowska, 2017). The research conducted by Weber and Pląskowska showed that the ZEN content in the wheat grain of Muszelka and Kohelia varieties from simplified cultivation exceeded several times (respectively 224 and 1012 µg kg^{-1}) the lowest permissible level of 100 µg kg^{-1} acceptable for this toxin by the European Commission. The tillage cultivation system in relation to the no-till system may reduce *F. graminearum* infestation of wheat by 90% (Miedaner, 2012). Sadowski et al. (2010) proved that by using shallow primary ploughing and subsoiling the occurrence of fungal spore stages is reduced. Koch et al. (2006) compared four methods of soil cultivation: conventional (tillage to a depth of 30 cm), and 3 methods of conservation tillage: soil breaking without turning to a depth of 25 cm in combination with mixing the soil to a depth of 15 cm, using a cultivator to a depth of 15 cm, and direct sowing. The concentration of DON in wheat grain, depending on the compared systems, was respectively: 180, 220, 230 and 490 µg kg^{-1}. The authors proved that the wheat from tillage cultivation contains the lowest quantities of toxins, while the highest quantities are found in the wheat from direct sowing. In addition, they proved the interaction of tillage methods with weather conditions and the forecrop. Weber and Pląskowska (2017) compared the impact of traditional (tillage) cultivation, no-till farming with a cultivator, and no-till farming with a disc harrow on the content of DON and ZEN. They found a lower quantity of DON and ZEN in the grain of wheat grown where the conventional (tillage) farming method was used. The content of DON in wheat grain from tillage cultivation was 6 µg kg^{-1} from simplified tillage performed with a cultivator 561 µg kg^{-1} and from simplified tillage performed with a disc harrow it was 475 µg kg^{-1}. The content of ZEN, depending on the cultivation methods compared, was respectively: 8, 217, 297 µg kg^{-1}.

Crop rotation and forecrop

The fungi of the genus *Fusarium* may remain in the field on crop residues and agricultural plant residues. Therefore, the forecrop also plays an important role in the spread of *Fusarium* diseases, and thus affects the quantity of mycotoxins in the grain (Kaniuczak, Lisowicz, 2000; Weber, Kita, 2014). The currently used short crop rotations, in which cereals dominate, contribute to the increase of the *Fusarium* in the soil (Korbas, Horoszkiewicz-Janka, 2013). Berhoft et al. (2012) noticed in their research that the lack of proper crop rotation resulted in greater pressure of *F. graminearum* and an increase in the DON level in the grain. Unfortunately, a one-year break in cultivation does not entirely eliminate the disease, it may only reduce the infection of the succeeding crop (Korbas, Horoszkiewicz-Janka, 2013). Cereal farming in monoculture is the most dangerous one, especially after maize and cereals, except for oats, which, due to the production of avenacin and scopolatin in its roots, exhibits phytosanitary properties (Morrissey, Osbourn, 1999). The effectiveness of crop rotation in reducing the quantity of mycotoxins in grain is confirmed by the research of Mikos-Szymańska and Podolska (2013). The authors demonstrated that the cultivation of triticale in monoculture led to the accumulation of DON, higher by 2320 µg kg^{-1}, and 7 µg kg^{-1} of T-2 / H-T2 toxin in reference to cultivation with 75% share of cereals in the cropping patterns. These findings are also consistent with the research by Koch et al. (2006) and by Góral et al. (2005), who noted a significant increase in the DON content in the grain of wheat grown after wheat in comparison with the cultivation after sugar beet. Góral et al. (2012) proved that the grain of the Griwa variety spring wheat collected from a site previously occupied by maize contained about 2.5 times more DON than from the site with previous rapeseed. One of the studies of application character on the impact of crop rotation in cereal cultivation on the degree of FHB infection show that the crop rotation: winter wheat – sugar beet – spring barley – winter rapeseed allows for the reduction of FHB pressure (Korbas, Horoszkiewicz-Janka, 2013). Therefore, a properly planned and implemented crop rotation is one of the means of avoiding fusion diseases and reducing the quantity of mycotoxins in wheat grain.

Nitrogen fertilization

Plants with nutrient shortages are more likely to develop diseases, including fungal infections. Appropriate fertilization strengthens the crop and prevents it from diseases. However, the excess of nitrogen in the soil influences the intensive growth and compaction of the crop, thus reducing the possibility of its ventilation under high water activity conditions. Intensive growth of crop cells weakens the cell walls and facilitates access of the pathogen into the cell (Yi et al., 2001). Lemmens et al. (2004) investigated the effect of nitrogen dosage on the occurrence of Fusarium head blight. Nitrogen in the quantity of 160 kg N ha^{-1} increased the proportion of infected ears from 2.2% (sample without nitrogen) to 6.6%. Similar findings were obtained by Czaban et al. (2011) who demonstrate that the strongest infection of winter wheat ears by *Fusarium* spp. and colonization by these fungi occurs in the intensive technology of wheat cultivation. Podolska et al. (2017) observed a significant impact of the quantity of nitrogen on the mycotoxins content in wheat grain. An increase in the dosage of applied nitrogen by 80 kg N ha^{-1} resulted in a signifi-

Table 4. Effect of nitrogen dose on the content of mycotoxins [µg kg^{-1}] in winter wheat grain (Podolska et al., 2017).

Dose of nitrogen [kg N ha^{-1}]	Mycotoxin						
	ZEN	β-ZOL	HT-2	T-2	DON	3-ADON	15-ADON
120	18.3	8.22	6.81	1.78	589	29.5	42.7
200	14.1	7.33	8.56	3.89	652	33.6	48.5

cant increase in the quantity of DON (63.0 μg kg^{-1}). In the case of ZEN, an inverse dependence was found. The use of 200 kg N ha^{-1} compared to 120 kg N ha^{-1} contributed to the reduction of the toxin content by 4.2 μg kg^{-1}. The authors demonstrated a significant impact of nitrogen dosage on the content of T-2 and HT-2 toxin (Table 4). Similar dependencies are confirmed in the literature on the subject (Lori et al., 2009; Subedi et al., 2007a,b; Yang et al., 2010).

Plant protection

A very common practice in reducing the spread of FHB is the use of plant protection products. Using fungicides is recommended during the period of the highest risk of plant infection (flowering period) (Stępień, Chełkowski, 2010). Perkowski et al. (2007), while investigating the mycotoxins content in the grain of 32 wheat varieties from conventional cultivation, observed a lower DON, 3-Ac-DON, HT-2 toxin, NIV + DON content in wheat grain from fields with full fungicide protection compared to the mycotoxins level in wheat grain harvested from fields without chemical protection. A large number of FHB control preparations are registered in the plant protection program. However, their application does not always lead to reducing the fungal infection of grain and thus decreasing the content of mycotoxins (Horoszkiewicz-Janka et al., 2016). Mesterhazy et al. (2003) found an increased DON content in the grain of winter wheat as a result of the use of fungicides containing azoxystrobin and carbendazin compared to other fungicides applied. The authors conclude that some active substances of the fungicide may constitute a stress factor for the fungus, which causes the formation of secondary metabolites. An important aspect in the protection of crops against fungal diseases is also herbicide and insecticide protection. The presence of weeds and insects may increase the infestation of the field. Insects are actively involved in *Fusarium* infection by transferring fungal spores to other plants (Munkvold, 2003). The presence of weeds in the field causes its compaction and at the same time facilitates the development of fungal diseases. However, the use of pesticides in the protection of cereal plants does not fully guarantee that FHB will not occur, and thus mycotoxins will be absent. Moreover, the use of pesticides in plant protection is also questionable from a food safety point of view. In consideration of this fact, new solutions in the field of plant protection based on biocontrol preparations using microorganisms, including bacteria and yeast, are being sought. Their antagonism towards phytopathogen consists of several mechanisms of action: competition for living space and nutrients, secretion of compounds with antifungal properties and mycoparasitism, i.e., the breakdown of the susceptible organism cell walls by the antagonist, which leads to distortion of the mycelium, disturbance of turgor pressure and, consequently, pathogen decomposition (Grzegorczyk et al., 2015).

CONCLUSIONS

Fusarium mycotoxins – secondary metabolites of filamentous fungi are toxic to humans and animals, reduce the yield level and deteriorate the health quality of wheat grain. The high levels of toxins specified in the EU regulation eliminate the use of grain as a material for human consumption and animal feeds. Therefore, reducing their quantity in grain is a priority in modern agriculture. As has been shown, there are many factors which cause them to undergo reduction, but not elimination. The difficulties in reducing them arise from the fact that the most important factor in their production is weather. The stress factors that trigger their production by fungi are not fully understood either. The presented literature review indicates that the greatest importance should be attached to selecting a variety, then a forecrop and soil cultivation. A high correlation between the quantity of mycotoxins and resistance to fusarium diseases does not always occur, hence the connection between the phenotypic features of plants and the quantity of toxins is being searched for. These studies are fragmentary, however, they indicate that the growing season, plant height, stem and ear structure exert their influence on the accumulation of toxins. It seems that this subject should be continued and expanded with new research directions. This study has demonstrated that in order to obtain safe and mycotoxin-free grain (raw material), attention should be paid to the entire wheat production process, starting from the selection of the variety, cultivation method, fertilization, and application of plant protection products. The creation of varieties being not only resistant to fungal diseases but also accumulating small quantities of toxins poses a considerable challenge for breeders. Reducing the quantity of mycotoxins in wheat grain will become more important, especially under the conditions of climate change and global warming.

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