Hungarian University of Agriculture and Life Sciences

Doctoral PhD Dissertation

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Hungarian University of Agriculture and Life Sciences

INFLUENCE OF AGROTECHNOLOGY ON *FUSARIUM* INFECTION, MYCOTOXIN PRODUCTION AND TECHNOLOGICAL QUALITY OF WINTER WHEAT (*Triticum aestivum L.*)

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Influence of Agrotechnology on *Fusarium* Infection, Mycotoxin Production and Technological Quality of Winter Wheat (*Triticum aestivum L.*)

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LIST OF ABBREVIATIONS

%	:	percent
°C	:	degree Celsius
cm	:	centimeter
df	:	degree of freedom
DON	:	deoxynivalenol
F	:	F statistic
FAO	:	Food and Agriculture Organization of the United Nations
FHB	:	*Fusarium* Head Blight
FUM	:	fumonisins
g/l	:	gram per liter
kg N ha^{-1}	:	kilogram nitrogen per hectare
l	:	liter
m	:	meter
ml	:	milliliter
mm	:	millimeter
Mv	:	Martonvasar
N	:	nitrogen
NIV	:	nivalenol
P	:	significance value
ppb	:	part per billion
ppm	:	Part per million
Sig.	:	Significance
Spp.	:	species
SPSS	:	Statistical Program for Social Sciences
Std	:	standard
t/ha	:	tonne per hectare
ZEA	:	zearalenone

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1. INTRODUCTION

Cereals and cereal by-products constitute a major part of the daily human and animal diet. According to the Food and Agriculture Organization of the United Nations (FAO), rice, maize, and wheat are staple foods for 4 billion people and make up about 60% of the world's food energy intake. The FAO estimated that the global consumption for wheat is about 66 kg/per capita. Latest estimates for world cereal production in 2021/2022 are 2813.2 million tonnes. In 2021/2022, approximately 478.5 million tonnes of cereal were exported worldwide. Among the most important risks associated with cereal consumption are mycotoxins, heavy metals, pesticide residues, and alkaloids. Cereal and cereal products can be contaminated with mycotoxins produced by a variety of fungi that colonize crops in the field (Coulombe, 1993; Scudamore and Livesey, 1998; Storm et al., 2008; Cheli et al., 2013).

Wheat (*Triticum aestivum L.*) is the most widely grown cereal crop in the world. The major exporters of wheat are Argentina, Australia, Canada, the European Union, Kazakhstan, Russian Federation, Ukraine, and the United States. Latest estimates for world wheat production in 2021/2022 are 778.1 million tonnes. In 2021/2022, approximately 194.9 million tonnes of wheat were exported worldwide. Wheat is grown across a wide range of environments around the world with the broadest adaptation of all the cereal crop species. It is a cool season crop requiring a minimum temperature for growth of 3 °C to 4 °C, with optimal growth occurring around 25 °C and tolerance of temperatures to a maximum of about 32 °C. Wheat flourishes in many different agro-climatic zones with production concentrated between latitudes 30 °and 60 °N and 27 ° and 40 °S, but there are examples of wheat production beyond these limits (Briggle and Curtis, 1987; Kimber and Sears, 1987). Wheat grows best on well drained soils anywhere from sea level up to heights of about 4500 m above sea level. It will grow in areas receiving 250 to 1750 mm annual precipitation, but most wheat production occurs in areas receiving 375 to 875 mm annually (Briggle and Curtis, 1987; Kimber and Sears, 1987). The primary use of bread wheat is for bread manufacture. Wheat flour is also used to produce biscuits, confectionery products, pasta and vital wheat gluten or seitan (a powdered form of purified wheat gluten, used as an alternative to soy-based products in vegetarian cooking). Other than its primary use as a human food source, wheat has several alternatives uses around the world. These include, but are not limited to, use in animal feed, conversion of wheat starch to ethanol, brewing of wheat beer, the production of wheat-based cat and pet litter, wheat-based raw materials for cosmetics, wheat protein in meat substitutes and to make wheat straw composites.

The wheat kernel is composed of endosperm (81-84 %), bran (14-16 %), and germ (2-3 %). The endosperm is rich in carbohydrate, lipid, and protein (Hung, 2016). The bran is the outer layer protecting the kernel. The germ is the kernel’s embryo that will grow into a new plant (Shewry, 2004). Wheat flour is mainly composed of starch (about 75-85 %) and protein (about 10 %) and it is derived from the endosperm of the wheat kernel. The provision of essential mineral nutrients by fertilizers is critical for the proper growth and development of wheat plants, thereby improving yield and quality of wheat grains and flour (Xue et al., 2016; Ma et al., 2019; Guerrini et al., 2020). Among the nutrients supplied through fertilization, nitrogen is an element required in large amounts by crops (Bazzo et al., 2016; Souza et al., 2021; Marinho et al., 2022). Nitrogen is one of the most important nutrients for wheat because it is an essential constituent of the cell wall, chlorophyll, nucleic acids, and participates in key metabolic pathways and reactions essential for plant survival. Nitrogen application favors the tillering of the wheat plant and, consequently,

increases the density of fertile spikes per area. On the other hand, nitrogen deficiency in wheat can negatively affect the formation of leaves and tillers (Neumann et al., 2009), which negatively influences the number of fertile spikes and often reduces grain yield (Ferreira et al., 2021; Souza et al., 2021; Xue et al., 2019; Lollato et al., 2021). Thus, plants fertilized and nourished with adequate amounts of nutrients mainly nitrogen produce grains with high nutritional value and morphological quality that are suitable for the consumer, market, and food industry (Pataco et al., 2015; Souza et al., 2021; Lollato et al., 2021).

The contamination of food and feed with mycotoxigenic fungi is a persistent problem contributing to food safety and security worldwide. The infection of crops by these fungal pathogens affects crop yield and quality but of greater concern are the secondary metabolites they produce, collectively known as mycotoxins. Mycotoxins are toxic secondary fungal metabolites that can cause a variety of adverse health effects in humans and animals, depending on the type of mycotoxin and the contamination levels. The major food and feed crops affected by mycotoxigenic fungi and mycotoxins include rice, maize, wheat, soybean, sorghum, and groundnut. Three major groups of mycotoxigenic fungi are associated with mycotoxin contamination namely *Aspergillus, Fusarium and Penicillium*. The most important mycotoxins in wheat are mainly *Fusarium* toxins, such as deoxynivalenol, zearalenone and fumonisins (Placinta et al., 1999; Binder et al., 2007; Neuhof et al. 2008; Rodrigues and Naehrer 2012). Multi-mycotoxin contamination is the most common type of contamination (Streit et al., 2012; Schatzmayr and Streit, 2013; Grenier and Oswald, 2014; Streit et al., 2013a; Streit et al., 2013b). This is a topic of great concern, as co-contaminated samples might still exert adverse health effects due to additive/synergistic interactions of the mycotoxins.

More than 100 countries have established mycotoxin regulations, including 15 African countries (Barug et al., 2003; Fellinger, 2006). The globalization of the trade in agricultural commodities and the lack of legislative harmonization have contributed significantly to the discussion about the awareness of mycotoxins entering the food supply chain. The European Union harmonized regulations for the maximum levels of mycotoxins in food and feed (Cheli et al., 2013; Cheli et al., 2014). The European Union and United States Food and Drug Administration established maximum allowable levels for certain food contaminants, including mycotoxins, with the aim to reduce their presence in foodstuffs to the lowest levels reasonably achievable by means of good manufacturing or agricultural practices (Cheli et al., 2016). Most countries have mycotoxin regulations to aid in minimizing food safety concerns. Although fewer countries regulate *Fusarium* mycotoxins, a marked increase in the regulation of this mycotoxin has been observed recently. These regulations have globally significant implications for the importation and exportation of products. Regulatory infrastructure, however, does not enable inspection and enforcement (Warburton and Williams, 2014), making the regulatory control of mycotoxins in Africa largely ineffective (Strosnider et al., 2006).

Fungal growth and mycotoxin contamination can occur during several steps of the food supply chain. Despite efforts in controlling fungal growth, mycotoxin co-contamination represents an unavoidable risk, occurring pre- and postharvest and resulting in reduced nutritional value and possible risks for human and animal health. Because mycotoxins are “natural” contaminants of foods, their formation is often unavoidable. Many factors with preharvest origins must be considered to manage the challenge of mycotoxins in wheat. Preharvest events are predominantly dictated by environmental factors and good agronomic and cultural practices. Methods for

controlling mycotoxins are largely preventive, they include good agricultural practice (Lisker and Lillehoj, 1991). Integrated management practices that reduce the incidence of mycotoxigenic fungi as well as the management of abiotic factors that contribute to mycotoxin contamination are required before and following harvest. However, preharvest management is considered the most important in limiting the overall contamination of crops. Therefore, the use of tolerant varieties is deemed the most proficient and environmentally sound approach to manage fungi and their toxins. In addition, several other management approaches such as crop rotation, well managed irrigation, soil tillage and fertilization, weed control, insect control, biological control and chemical control could further reduce fungal incidence and subsequent mycotoxin contamination.

1.1 Objectives

The aim of this research lies in studying the influence of agrotechnology on *Fusarium* infection, mycotoxin production and technological quality of wheat.

While taking the following questions into account:

Part one:

Does nitrogen fertilization affect *Fusarium* infection and mycotoxin contamination?

Does wheat variety affect *Fusarium* infection and mycotoxin contamination?

Do environmental conditions affect *Fusarium* infection and mycotoxin contamination?

Part two:

Does nitrogen fertilization affect protein content, gluten content, falling number, Zeleny sedimentation Index, thousand kernel weight and test weight?

What is the highest nitrogen fertilizer rate after which no effect is found on wheat quality parameters?

Are excessively high nitrogen fertilization rates needed for good quality wheat?

2. LITTERATURE REVIEW

2.1 Wheat

The cultivation of wheat (*Triticum spp.*) reaches far back into history. Wheat was one of the first domesticated food crops and for 8 000 years has been the basic staple food of the major civilizations of Europe, West Asia, and North Africa. Today, wheat is grown on more land area than any other commercial crop and continues to be the most important food grain source for humans. Its production leads all crops, including rice, maize, and potatoes (FAO, 2011).

Wheat is grown across a wide range of environments around the world with the broadest adaptation of all the cereal crop species. Although the crop is most successful between the latitudes of 30° and 60°N and 27° and 40°S (Nuttonson, 1955), wheat can be grown beyond these limits, from within the Arctic Circle to higher elevations near the equator. In altitude, the crop is grown from sea level to more than 3 000 m above sea level, and it has been reported at 4 570 m above sea level in Tibet. The optimum growing temperature is about 25°C, with minimum and maximum growth temperatures of 3° to 4°C and 30° to 32°C, respectively (Briggle, 1980). Although about three-fourths of the land area where wheat is grown receives an average of between 375 and 875 mm of annual precipitation, it can be grown in most locations where precipitation ranges from 250 to 1 750 mm (Leonard and Martin, 1963).

The primary use of wheat is for bread manufacture. Wheat flour is also used to produce biscuits, confectionery products, pasta, noodles and vital wheat gluten or seitan (a powdered form of purified wheat gluten, used as an alternative to soy-based products in vegetarian cooking). Wheat is the best of the cereal foods and provides more nourishment for humans than any other food source. Wheat starch is easily digested, as is most wheat protein. Wheat contains minerals, vitamins, and fats (lipids), and with a small amount of animal or legume protein added is highly nutritious. A predominately wheat-based diet is higher in fiber than a meat-based diet (Johnson *et al*., 1978). Other than its primary use as a human food source, wheat has several alternatives uses around the world. These include, but are not limited to, use in animal feed, conversion of wheat starch to ethanol, brewing of wheat beer, the production of wheat-based cat and pet litter, wheat-based raw materials for cosmetics, wheat protein in meat substitutes and to make wheat straw composites.

Cultivated wheat is classified into two major types: the hexaploid bread wheat (2n = 6x = 42, BBAADD) and the tetraploid durum wheat (2n = 4x = 28, BBAA). Based on growth habit, wheat is classified into spring wheat and winter wheat, covering about 65 and 35% of the total global wheat production area, respectively (Braun et al., 2010; Braun and Sãulescu, 2002). Classification into spring or winter wheat is common and traditionally refers to the season during which the crop is grown. For winter wheat, the heading is delayed until the plant experiences a period of cold winter temperatures (0° to 5°C). It is planted in the autumn to germinate and develop into young plants that remain in the vegetative phase during the winter and resume growth in early spring. This provides the advantage of using autumn moisture for germination and making effective use of early spring sunshine, warmth, and rainfall. Spring wheat, as the name implies, is usually planted in the spring, and matures in late summer but can be sown in autumn in countries that experience mild winters, such as in South Asia, North Africa, the Middle East and the lower latitudes.

2.2 Wheat taxonomy

Wheat belongs to the Kingdom: *Plantae,* Order: *Poales,* Family: *Poaceae,* Subfamily: *Pooideae,* Supertribe: *Triticodae,* Tribe: *Triticeae,* Genus: *Triticum L.*

Most common species:

Common or bread wheat (*T. aestivum*), Spelt (*T. spelta*), Durum (*T. durum*), Emmer (*T. dicoccon*), Khorasan (*T. turanicum*), Einkorn (*T. monococcum*) (Clayton et al., 2015).

2.3 Wheat morphology

The mature wheat plant consists of a central stem from which leaves emerge at opposite sides. It is made up of repeating segments, called phytomers, which contain a node, a hollow internode, a leaf, and a tiller bud found in the axil of the leaf. The leaf sheath wraps around the stem providing support to the shoot. The stem terminates in the ear of the wheat plant. The leaf structure consists of the sheath and the leaf blade which form from separate meristems. At the base of the leaf blade, where it joins the sheath, are a membranous ligule and a pair of small hairy projections known as auricles, which are characteristic of cereal species. Leaves are produced on alternate sides of the stem. The final leaf before the ear is called the flag leaf. Tillers are lateral branches which are produced off the main stem of the wheat plant. They produce leaves on opposite sides of their central stem in the same manner as the leaves of the main stem are produced and are also able to produce an ear at their terminal. Not all tillers will survive and produce an ear, and this is thought to be due to competition for light and nutrients.

A mature wheat plant has two distinct root types. The seminal roots develop from the root primordia contained within the grain and are the first root type to emerge. The nodal roots which emerge while tiller development starts. The root system can grow 1-2 m deep, but most roots are concentrated in the top 30cm of soil. The inflorescence of wheat is a composite ear or spike; the main axis (rachis) bears several spikelets. Each spikelet has a short spikelet axis (rachilla) along which the glumes are arranged alternately on opposite sides. The ear of a wheat plant is made up of two rows of spikelets. The spikelets contain the florets and are arranged on opposite sides of a central rachis. The spikelet is surrounded by two sterile glumes which enclose up to 10 individual flowers (florets). The florets are enclosed by a lemma and a palea. The tip of the lemma may be extended to form an awn in some varieties. The florets are composed of the carpel (the ovary and the stigmas) and three stamen and anthers. Each anther consists of four loculi enclosing the pollen grains. The caryopsis or grain of the wheat plant is made up of the bran coat (14.5 %) and the endosperm (83 %) surrounding the embryo (2.5 %). The endosperm stores the starch that provides the developing plant with an energy source until its roots are established and newly expanded leaves allow it to harvest energy from the sun. The embryo makes up only a small percentage of the grain but contains the root radicle which becomes the first root and the shoot apex surrounded by the coleoptile which protects the first leaf as it pushes its way through the soil to the surface during germination (Setter and Carlton, 2000; Kirby, 2002).

2.4 Wheat growth stages

Germination starts with the uptake of water by a wheat kernel that has lost its post-harvest dormancy. With the resumption of growth, the radicle and coleoptile emerge from the seed. The first three seminal roots are produced and then the coleoptile elongates pushing the growing point toward the soil surface. The seedling stage begins with the appearance of the first leaf and ends with the emergence of the first tiller. Up to six seminal roots and three leaves support the plant at this stage. The crown of the plant usually becomes noticeably distinct after the third leaf has emerged. Crown formation is soon followed by the appearance of tillers and development of a secondary or crown root system. The crown root system provides the plant with most of its nutrients and water during the growing season. While the first tiller is not produced until the third leaf has fully emerged, the appearance of later tillers is usually synchronized with the emergence of each subsequent new leaf that develops on the main shoot. For example, emergence of the fifth leaf is normally accompanied by the appearance of the second crown tiller which originates from an auxiliary bud located in the node at the base of the second leaf (leaf axil). Similarly, a tiller can start producing its own sub tillers once it has three fully developed leaves. Each tiller that is produced represents the potential for a wheat plant to develop an additional stem complete with its own leaves, roots, and head. Consequently, tillers that do not produce at least three leaves are not competitive and usually die off once the stem elongation stage starts.

A major change in the development of the wheat plant occurs at the end of the tillering stage because the growing points of the main shoot and tillers stop initiating new leaves and start producing reproductive structures. Conversion of the growing point signals the end of the vegetative and the start of the reproductive period. The nodes from which leaves develop are telescoped at the crown during the tillering stage. Once jointing starts, the internode region elongates, moving the nodes and the growing point upward from the crown to produce a long stiff stem that will carry the head. Each successive tiller on a wheat plant normally has one less leaf than its predecessor. This synchronizes the start of the stem elongation stages of the main stem and tillers. Synchronization of growth and development at this stage ensures there will be no more than a few days difference in the maturity of all heads on the plant. The stem elongation or jointing stage comes to an end with the appearance of the last (flag) leaf. The developing head within the sheath of the flag leaf becomes visibly enlarged during the booting stage. The booting stage ends when the first awns emerge from the flag leaf sheath and the head starts to force the sheath open. The heading stage extends from the time of emergence of the tip of the head from the flag leaf sheath to when the head has completely emerged but has not yet started to flower. The flowering or anthesis stage lasts from the beginning to the end of the flowering period. Pollination and fertilization occur during this period. All heads of a properly synchronized wheat plant flower within a few days and the embryo and endosperm begin to form immediately after fertilization. Early kernel formation occurs during the milk stage. The developing endosperm starts as a milky fluid that increases in solids as the milk stage progresses. Kernel size increases rapidly during this stage. Kernel formation is completed during the dough development stage. The kernel accumulates most of its dry weight during dough development. The transport of nutrients from the leaves, stems, and spike to the developing seed is completed by the end of the hard dough stage. The developing kernel is physiologically mature at the hard dough stage even though it still contains approximately. The seed loses moisture, and any dormancy it may have had, during the ripening stage (Figure 1) (Zadoks et al., 1974; Nelson et al., 1988; Fowler, 2018).

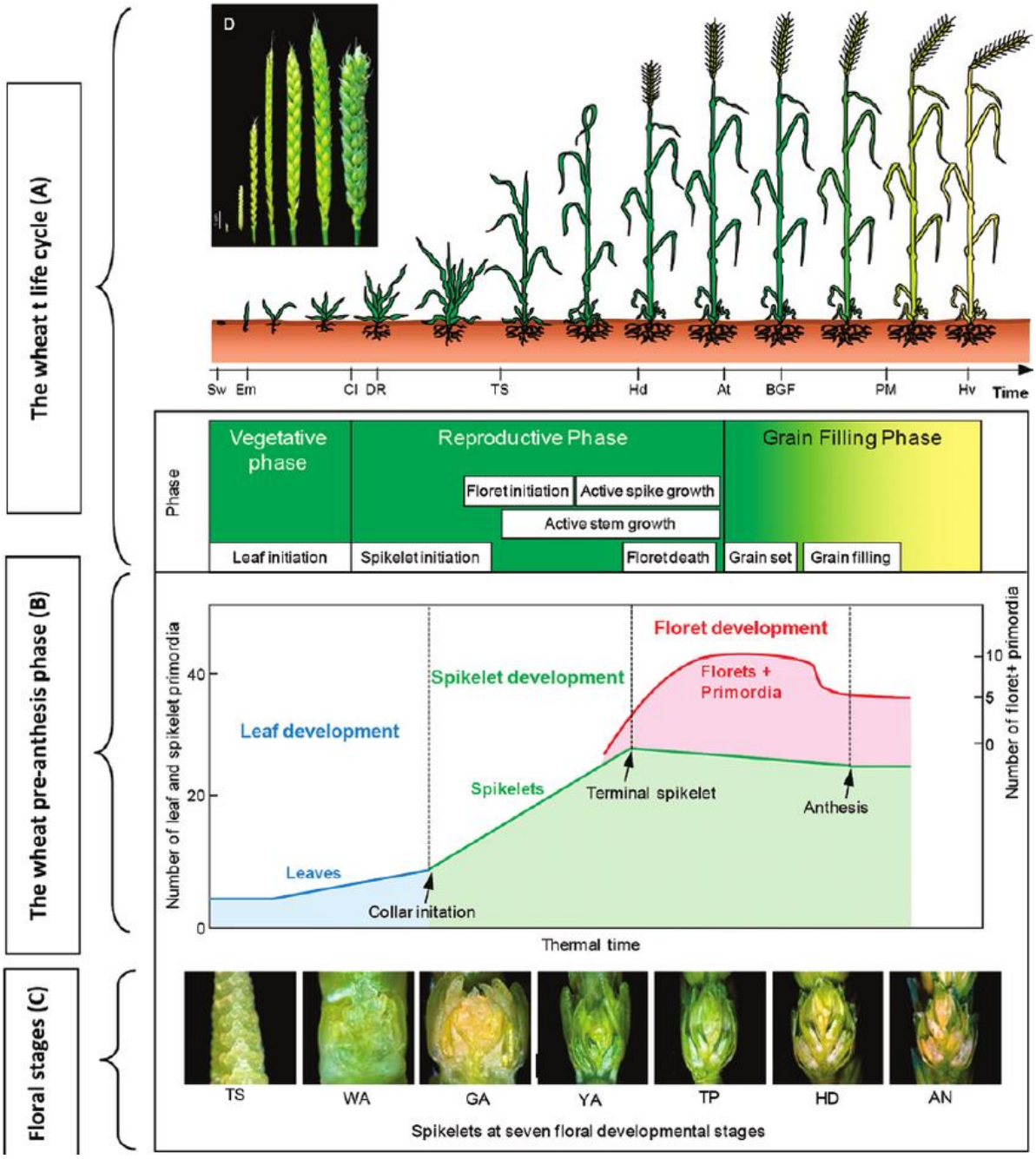

Figure 1. Different developmental stages in the wheat life cycle (Kirby and Appleyard, 1987)

(A) The wheat life cycle; Sw (sowing); Em (emergence); DR (double ridge appearance); TS (terminal spikelet initiation); Hd (heading); At (anthesis); BGF (beginning of grainfilling period); Pm (physiological maturity); Hv (harvesting).

(B) The wheat pre-anthesis phase, including leaf, spikelet, and floret initiation.

(C) The spikelets at the seven floral developmental stages (after Kirby and Appleyard, 1987): TS (terminal spikelet stage), WA (white anther stage), GA (green anther stage), YA (yellow anther stage), TP (tipping stage), HD (heading stage), and AN (anthesis stage) (from left to right).

(D) The spikes at the seven floral developmental stages: TS, WA, GA, YA, TP, HD, and AN (from left to right) during floral development (floret initiation).

2.5 Wheat production

Planting time is determined by several factors, including soil moisture and temperature, avoidance of sub-optimal conditions, particularly early and late in the growing season ensuring optimal flowering time. Winter wheat requires a period of cold stimulus (vernalization) to initiate floral development. Seeds of winter wheat need 40 to 70 days of vernalization with a temperature between -1°C and +8°C. Hömmö and Pulli (1993) reported a maximum cold tolerance for winter wheat of about -25°C. Winter wheat is sown in mid-October to mid-November and harvested in summer. Although wheat is being harvested somewhere in the world in any given month, harvest in the temperate zones occurs between April and September in the Northern Hemisphere and between October and January in the Southern Hemisphere (GRDC, 2014; 2015).

The optimum sowing time for wheat is 15-30 November. Since the winter is prolonged to some extent in the northern region, seeds could be sown up to the first week of December for optimum yield. If seeds are sown beyond this time, yield may be decreased by 1.3% for each day delay. Winter wheat grows well in cold climate and ripens in a warm, dry climate. The cold winters and the hot summers are conducive to a good crop. Winter rainfall is ideal. A cloudless sky having bright sunshine during ripening and harvesting periods will make better quality wheat (Rajan et al., 2018).

Wheat grows best in well-drained loamy soil. Wheats are easily cultivated due to their ability to grow in regions of sparse rainfall, for their roots are able to take up nutrients from dry upper soil as long as they have access to moist lower soil (Ma et al. 2007). Another function of their roots, which makes wheat particularly adaptable to drought and nutrient deficiency, is the ability to extend their roots into deep soil to gain access to nutrient-rich patches which would be normally unreachable (Ma et al., 2007).

Sowing depth depends on soil moisture, timing and seasonal outlook, the variety sown, seedbed temperature and moisture, as well as seed and soil applications such as fungicides and herbicides. Deeper sowing can delay emergence and can result in weaker seedlings which are poorly tillered (Jarvis et al. 2000). The use of treated seeds is excellent for the control of seedborne diseases such as common bunt and loose smut. Treated seeds can reduce seed rot and seedling blight, they may increase stands when planting in poor conditions. It was common to cultivate the field prior to seeding, but more farmers are opting for no-till or low till practices which can help to conserve moisture and improve soil structure, reduce erosion, increase yields and in some cases decrease disease (Jarvis et al., 2000). The three main nutrients required for successful production of a wheat crop are nitrogen (N), phosphorus (P) and potassium (K). Protein production in the wheat grain is reliant on nitrogen levels in the soil. Wheat requires nitrogen-rich soils, infused with inorganic materials, such as potassium, lime, and phosphoric acid. Nitrogen fertilizer is commonly added to a field before sowing the crop but can be added to the field again prior to flowering to boost grain yield and the level of protein in the grains. Legumes can also be used to fix nitrogen in the soil for subsequent crop use. Phosphorus is applied to the field at sowing. It is required for different stages of wheat growth and reproduction, such as germination, root development or grain ripening (Laffan, 1999).

The introduction of crop rotation in the wheat production cycle is extremely important as it reduces the carryover of diseases, insects, and weeds between crops. One year of rotation or fallow is

enough to break the cycle for most diseases. A three-year crop rotation may include the following cycles:1st year canola, 2nd year wheat, 3rd year rye or 1st year fallow, 2nd wheat, 3rd year canola or rye. Crop rotation can affect pest populations and can reduce the need for pesticides. Different crops often break pest cycles and prevent pest and disease organisms from building to damaging levels. Crop rotation may reduce severity of scab, root rot, seedling blight, leaf blotch, glume blotch, and sharp eyespot (GRDC, 2014; 2015).

2.6 Economic importance of wheat

The economic importance of wheat and its contribution to the diets of humans and livestock cannot be disputed. Wheat has played a fundamental role in human civilization and improved food security at the global and regional levels. It provides about 19% of the calories and 21% of protein needs of daily human requirements at the global level (Braun et al., 2010). It is a staple food for 40% of the world's population, mainly in Europe, North America, and the western and northern parts of Asia. The demand for wheat is growing fast in new wheat growing regions of the world such as eastern and southern Africa (5.8%), West and Central Africa (4.7%), and South Asia and the Pacific (4.3%). Demand is also growing in the traditional wheat growing regions of Central Asia (5.6%), Australia (2.2%), and North Africa (2.2%) (Shiferaw et al., 2013).

Wheat is the most traded agricultural commodity at the global level with a trade volume of 144 million tons, with a total value of 36 billion US dollars (Shiferaw et al., 2013). Currently available figures show an average annual global production of about 715.3, 735.6, 737.3, 763.4, 761.6, 731.4, 759.7, 776.0, 778.1 million tonnes over the period from 2013 to 2021 (FAO). According to FAO (2015), about 732 million tons of wheat was produced on an average of 218.5 million ha with a productivity level of 3.3 t/ha in 2013, a highly significant increase from 1961, which stood at 222 million tons with a productivity level of only 1.2 t/ha.

Wheat is the most widely grown food crop in the world. It is grown on more than 240 million ha, larger than for any other crop, and world trade is greater than for all other crops combined. The major exporters of wheat are Argentina, Australia, Canada, the European Union, Kazakhstan, Russian Federation, Ukraine, and the United States. The global production of wheat in 2021 is estimated at 778.1 million tonnes. In 2021/2022, approximately 194.9 million tonnes of wheat were exported worldwide. The accelerated increase in wheat production is attributed to improved high-yielding varieties with better response to inputs (e.g., fertilizers), improved irrigation systems, and improved disease resistance and pesticides as well as better management practices, coupled with conducive policies and stronger institutions (Baum et al., 2013).

2.7 Wheat quality

Wheat (*Triticum aestivum L.*) is one of the most cultivated crops in the world. It is a worldwide cereal grown across a wide range of environments. The primary use of wheat is in the production of bread, bakery, and confectionery products such as noodles, pasta, cakes, and biscuits. In addition, it is used in the production of animal feed, and ethanol. The wheat kernel is composed of endosperm (81–84%), bran (14–16%), and germ (2–3%). The endosperm is rich in carbohydrate, lipid, and protein (Hung 2016). The bran is the outer layer protecting the kernel. The nutrients present in the seeds are essential for the initial seedling establishment, as they undergo hydrolysis during the germination process to meet the nutritional demand of the embryo. The germ is the

kernel's embryo that will grow into a new plant (Shewry 2004). Wheat flour is mainly composed of starch (about 75–85%) and protein (about 10%) and it is derived from the endosperm of the wheat kernel. The starch is made up of two types, namely amylose (about 22%) and amylopectin (about 78%). The protein fraction of the grain consists of gliadin and glutenin, which when hydrated form the gluten that is responsible for the dough elasticity and fermentation. Therefore, protein and starch are the two major components that determine the quality of flour. Protein and starch content is determined by genetics and influenced by environmental conditions and availability of nutrients and water (Guarienti et al. 2004; Rozbicki et al. 2015; Savill et al. 2018). In the milling and baking industries, the technological quality of wheat kernels essential to produce bread and wheat-based products may be determined by protein and gluten content, falling number, Zeleny sedimentation index, test weight, and thousand kernel weight (Min et al. 2017; Guarienti et al. 2004; Hellemans et al. 2018; Xue et al. 2019).

In the wheat kernel, the content of starch, protein, and gluten is determined by genetics and influenced by environmental conditions, such as temperature and availability of nutrients and water (Guarienti et al., 2004; Rozbicki et al., 2015; Savill et al., 2018). Depending on the water supply during the grain-filling stage, the nutrients accumulated in the plant biomass may be mobilized to increase the production of starch or protein in the kernel (Silva et al., 2019; Lollato et al., 2021). Hence, when the water supply is abundant the protein/starch ratio changes in the direction of starch, resulting in big wheat kernels with high starch content. Oppositely, in the condition of drought stress, the protein/starch ratio changes in the direction of protein, resulting in small wheat kernels with high protein content (Blumenthal et al., 1993).

Technological wheat quality parameters may be affected by wheat genotype, environment, and crop management practices such as fertilization, irrigation, plant growth regulators, and control of pests and diseases (Guarienti et al. 2004; Yong et al. 2004; Franceschi et al. 2009; Denčić et al. 2011; Kaya and Akcura 2014; Rozbicki et al. 2015; Ferreira et al. 2021; Rekowski et al. 2021; Faria et al. 2022). Among the available resources to improve the quality of grains produced, fertilizer management is efficient (Sousa and Lobato, 2004). The supply of essential mineral nutrients by fertilizers is fundamental for the suitable growth and development of wheat plants, favoring the yield and quality of wheat grains and flour (Xue et al. 2016; Ma et al. 2019; Guerrini et al. 2020). Optimal nutrient provision is an important factor to get high yield with high grain quality. Among the nutrients supplied through fertilization, nitrogen is an element required in large amounts by crops (Bazzo et al. 2016; Souza et al. 2021; Marinho et al. 2022). The importance of nitrogen for the plants belonging to the *Poaceae* family, especially wheat, is consensual. Nitrogen is one of the most mobile plant nutrients in the soil. Nitrogen fertilizer is a main factor in increasing yield and regulating grain protein content and quality in wheat. Among the available nitrogen sources, urea is the principal fertilizer used in agriculture; however, it has great loss by volatilization and leaching (Civardi et al., 2011; Silva et al., 2012). On the other hand, ammonium nitrate is an alternative to reduce losses and increase the efficiency of these nitrogen fertilizers on wheat culture (Yano et al., 2005). Nitrogen is one of the most important nutrients for wheat because it is an essential constituent of the cell wall, chlorophyll, nucleic acids, and important biomolecules such as ATP, NADH and NADPH, and participates in key metabolic pathways and reactions essential for plant survival (Taiz et al. 2017). Nitrogen determines to a great extent the wheat yield level and the wheat quality. It is also one of the most mobile plant nutrients in the soil. Nitrogen application favors the tillering of the wheat plant and, consequently, increases the density of fertile

spikes per area. On the other hand, nitrogen deficiency in wheat can negatively affect the formation of leaves and tillers (Neumann et al. 2009), which negatively influences the number of fertile spikes and often reduces grain yield (Ferreira et al. 2021; Souza et al. 2021; Xue et al. 2019; Lollato et al. 2021). Thus, plants fertilized and nourished with adequate amounts of nutrients mainly nitrogen produce grains with high nutritional value and morphological quality that are suitable for the consumer, market, and food industry (Pataco et al. 2015; Souza et al. 2021; Lollato et al. 2021). Therefore, it is important to evaluate the use of high nitrogen fertilizer rates because unsuitable nitrogen doses lead to increased nitrate leaching (Huang et al., 2018) which contributes to eutrophication of surface waters.

2.8 *Fusarium spp.*

The *Fusarium species* belong to the Kingdom: *Fungi*, Order: *Hypocreales*, Phylum: *Ascomycota*, Family: *Hypocreaceae*, Class: *Euascomycetes*, Genus: *Fusarium*.

The genus *Fusarium* comprises a high number of fungal species that can be plant-pathogenic, causing diseases such as wilts, blights, rots, and cankers in several agriculturally important crops, including cereals, and can also be harmful for humans and animals (Booth, 1971). From a toxicological and economic point of view, *Fusarium* head blight (FHB) accompanied by mycotoxin grain contamination is one of the most dangerous *Fusarium* diseases causing the greatest worldwide damage to cereals, especially wheat (Salgado et al., 2014; McMullen et al., 2012). *Fusarium* head diseases lead to the reduction of the grain yield and its contamination with mycotoxins. The optimal conditions for the growth of *Fusarium spp.* take place at a relative humidity of 90% and above and a temperature range between 25 to 30 ℃ (Parry et al., 1995). Several species can cause head blight, although *F. graminearum, F. culmorum* and *F. avenaceum* are the predominant pathogens in most regions of the world (Goliński et al., 2010).

Members of the genus *Fusarium* are cosmopolitan and prevalent components of different ecosystems in a wide range of environmental and climatic zones, because they can colonize a wide variety of substrates. The widespread distribution of *Fusarium species* may be attributed to the ability of these fungi to grow on a wide range of substrates and their efficient mechanisms for dispersal (Burgess, 1981). *Fusarium species* are widely distributed in soil and on subterranean and aerial plant parts, plant debris, and other organic substrates (Gordon, 1959). They are common in tropical and temperate regions and are also found in desert, alpine, and arctic areas, where harsh climatic conditions prevail (Burgess et al., 1988). Many *Fusarium species* are abundant in fertile cultivated and rangeland soils but are relatively uncommon in forest soils (Burgess et al., 1975). *Fusarium species* are often regarded as soilborne fungi because of their abundance in soil and their frequent association with plant roots, as either parasites or saprophytes. However, many have active or passive means of dispersal in the atmosphere and are common colonizers of aerial plant parts, where they may result in diseases of considerable economic importance (Burgess et al., 1988).

In the last 20 years, *Fusarium species* have been studied extensively because the mycotoxins they produce can be a threat to animal and human health (Marasas et al., 1984). Mycotoxins are secondary metabolites produced by fungi that are associated with a variety of animal diseases and some human health problems (Marasas et al., 1987). *Fusarium spp.* are generally associated with high temperature excessively wet growing season (Munkvold, 2014; Reyneri, 2006). Plant

infections by *Fusarium* can occur at all developmental stages, from germinating seeds to mature vegetative tissues, but the most susceptible to infection by these fungi are cereals in the flowering stage and immediately after flowering, especially in warm and humid weather conditions, and abundant dew and prolonged rainfall during this period (Osborne and Stein, 2007; Hjelkrem et al., 2017). Disease symptoms on infected head are visible during the milk maturity stage of the grain. Spikes infected by *Fusarium spp.* become all white. Pink or salmon-colored sporodochia with conidial spores, as well as mycelium layer, appear on infected chaff in spikes during persistent high humidity, after a few days of infection. Dying of infected spikelets inhibits the development of kernels, which causes grain number reduction in the spike. The remaining kernels developing in infected heads are usually smaller, gray, shriveled, with a loose consistency and often covered with sporodochia and *Fusarium spp.* mycelium (Kiecana et al., 2002). Damage to starch granules and changes in storage protein composition were observed in kernels infected by *Fusarium spp.* (Packa et al., 2012). The ability of these fungi to produce mycotoxin is a very important factor determining the harmfulness of *Fusarium spp.* and reducing grain quality (Desjardins, 2006).

2.9 *Fusarium* head blight

Fusarium head blight (FHB), also known as scab, is an economically devastating disease of small grain cereal crops. It affects all small grains including wheat (*Triticum spp.*), barley (*Hordeum vulgare*), rye (*Secale cereale*), oats (*Avena sativa*), and triticale (*x Triticosecale*). The majority of economic losses occur in wheat and barley production. FHB was first described in England by W.G. Smith in 1884 and several years later it was reported in the United States by F.D. Chester in 1890 and J.C. Arthur in 1891 (Parry et al., 1995; Stack, 2003). *Fusarium* head blight (FHB) is caused primarily by the fungus *Fusarium graminearum* (sexual stage: *Gibberella zeae*). In addition to *Fusarium graminearum* other species of *Fusarium* may cause FHB, of which, *Fusarium culmorum*, and *Fusarium avenaceum* predominate (Dill-Macky, 2010; Parry et al., 1995). The fungus is a facultative parasite, that is, it normally exists as a saprophyte but can live as a parasite on plants, causing disease. In addition to causing damage to heads, it also may infect roots and crowns and often is, together with other soilborne fungi, the cause of seedling blights and root and crown rots. *F. graminearum* also causes stalk and ear rots in corn. *F. graminearum* and *F. culmorum* are the most common and most virulent, and their geographical distribution appears to be related to temperature and moisture. *F. graminearum* occurs mostly in warmer and wetter climatic regions of the world including North America, Eastern Europe, Australia, and Southern China whereas *F. culmorum* occurs mostly in cooler climatic regions such as Western Europe (Miller, 1994; Parry et al., 1995).

2.9.1 Symptoms

FHB in wheat is recognized in the field by premature bleaching of one or more infected spikelets in the wheat spike. Initial symptoms generally are visible near the middle of the spike but can occur anywhere. Premature bleaching progresses with time to most or all the spikelets, causing the entire head to be bleached. The bleached heads are readily visible in a green field. During prolonged wet weather, there may be whitish or pinkish, fluffy fungal growth on infected heads in the field. *Fusarium* infection occurring early during anthesis causes the spikelets or even the entire head to become sterile leading to yield reduction due to a reduced number of kernels being developed. FHB infection occurring slightly later causes shriveled, shrunken, chalky white or

discolored kernels. These kernels are often referred to as *Fusarium* damaged kernels, scabby kernels, or "tomb stones. FHB infection occurring late in kernel development produces apparently healthy kernels that have elevated infection levels and mycotoxin contamination (Wegulo et al., 2008).

2.9.2 Disease Cycle

Fusarium graminearum overwinters as spores or mycelia in the soil, host crop residues or seeds which serve as a source of primary (initial) inoculum in the spring and are especially suitable for survival and reproduction of FHB causing fungi (Pereyra and Dill-Macky, 2008; Trail, 2009). In the spring as the temperature warms up, spores are released from crop residues and are spread by wind or splashing water. They land on wheat heads and during wet, warm weather they germinate and infect glumes, flower parts, or other parts of the head. Infections can occur any time from full spike emergence until maturity; however, most infections occur during anthesis partly because anthers contain stimulants for spore germination and pathogen growth mainly pollen which serves as a food base for the germinating spores (Figure 2) (Dill-Macky, 2010). Wheat heads are susceptible from anthesis until the soft dough stage. Infections that occur during anthesis are the most damaging. During warm temperatures and wet conditions, blight symptoms develop within two to four days after infection. Therefore, an apparently healthy crop can show symptoms suddenly. FHB is considered a monocyclic or one-cycle disease, that is, after the initial or primary infection, little or no secondary infection occurs by conidia formed on infected heads (Fernando et al., 1997). FHB is favored by prolonged wet, warm weather prior to and during anthesis. Excessive rainfall during the growing season and especially during a one-to-three-week period prior to anthesis can lead to epidemics of FHB. The disease usually is more severe in irrigated than rain-fed fields. Yield loss results mainly from sterility of infected spikelets and reduction in kernel size. Based on estimates from natural FHB epidemics, fungicide trials, and more precise measurements in inoculation studies, yield reductions of up to 74% have been reported in small grain cereals (McMullen et al., 2012; Parry et al., 1995).

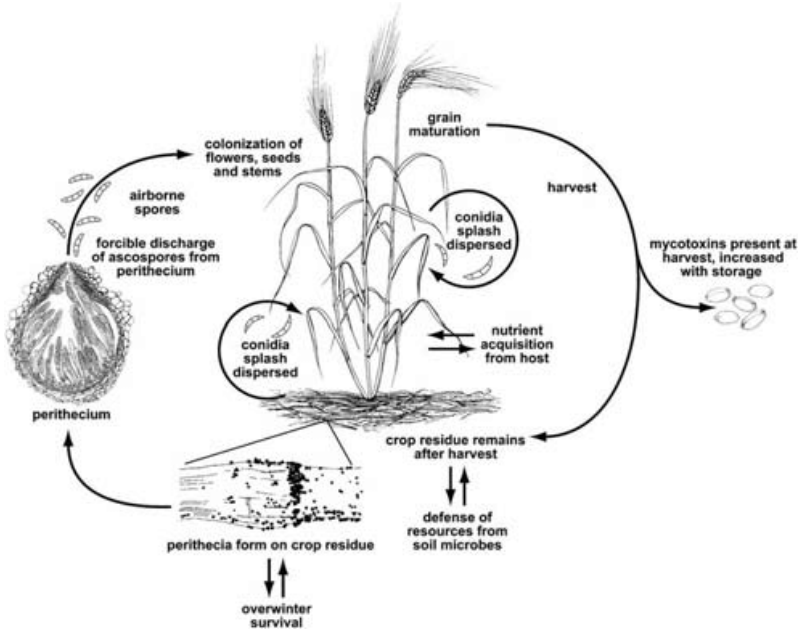

Figure 2. *Fusarium spp.* life cycle (Trail 2009)

2.10 Mycotoxin

The term mycotoxin was coined in 1962 in the aftermath of an unusual veterinary crisis near London, England, during which approximately 100 000 turkey poults died (Blout, 1961; Forgacs, 1962). When this mysterious turkey disease was linked to a peanut meal contaminated with secondary metabolites from *Aspergillus flavus* (aflatoxins), it sensitized scientists to the possibility that fungal secondary metabolites might be deadly.

Mycotoxins are secondary metabolites of fungi, which commonly contaminate cereal grains (wheat, barley, oat, rye, maize, and rice) and cereal products, as well as other food products (Bottallico and Perrone, 2002; Logrieco et al., 2003). These metabolites constitute a toxigenically and chemically heterogeneous assemblage that are grouped together only because the members can cause disease and death in human beings and other vertebrates. Not surprisingly, many mycotoxins display overlapping toxicities to invertebrates, plants, and microorganisms (Bennett, 1987). Mycotoxins exert various effects on the human and animal bodies, among others they are mutagenic, teratogenic, and estrogenic (Bottallico and Perrone, 2002; Logrieco et al., 2003). They also have a significant impact on the economy because, in accordance with the provisions of the legal acts, the presence of mycotoxins at certain levels results in the exclusion of agricultural crops, feed and food products from commercial trade (European Commission).

While all mycotoxins are of fungal origin, not all toxic compounds produced by fungi are called mycotoxins. The target and the concentration of the metabolite are both important. Fungal products that are mainly toxic to bacteria (such as penicillin) are usually called antibiotics. Fungal products that are toxic to plants are called phytotoxins. Mycotoxins are made by fungi and are toxic to vertebrates and other animal groups in low concentrations.

Depending on the definition used and recognizing that most fungal toxins occur in families of chemically related metabolites, some 300 to 400 compounds are now recognized as mycotoxins, of which approximately a dozen groups regularly receive attention as threats to human and animal health (Cole and Cox, 1981).

Contamination of cereals with toxic metabolites of fungi, both pathogenic and saprotrophic, is one of the particularly important problems in global agriculture. This is evidenced by numerous literature references and reports of the European Commission (Bryła et al., 2016; Stanciu et al., 2017). *Fusarium species* are among the dangerous cereal pathogens with a high toxicity potential. Secondary metabolites of these fungi, such as deoxynivalenol, zearalenone and fumonisins are among the most important mycotoxins on a European and world scale (Bottallico and Perrone, 2002; Logrieco et al., 2003). The presence of these metabolites in the grain is the result of the development of *Fusarium* head blight. The epidemic occurrence of this disease is the cause of significant economic losses because infestation of heads and panicles of cereals and maize by *Fusarium spp.* leads to a significant reduction in the size and quality of grain yield (Goliński et al., 2010). The level of contamination of cereal grain with *Fusarium* mycotoxins depends on many factors, among others weather conditions, cultivation system, and date of grain harvest, as well as the degree of resistance of cultivated varieties to *Fusarium spp.* infection (Goliński et al., 2010).

The major mycotoxins occurring in wheat, at levels of potential concern for human and animal health, are *Fusarium* mycotoxins (Placinta et al., 1999; Binder et al., 2007; Zinedine et al., 2007;

Neuhof et al., 2008; Rodrigues and Naehrer, 2012). Studies indicate that deoxynivalenol, zearalenone and fumonisins are the most common mycotoxin contaminant of wheat and wheat-based products. Moreover, studies highlighted the presence of considerable differences regarding the type and prevalence of mycotoxin contamination in different regions of the world, confirming that contamination is strongly dependent on regional climatic conditions (Streit et al., 2012; Schatzmayr and Streit, 2013; Grenier and Oswald, 2014). In general, environmental conditions, such as excessive moisture, temperature extremes, humidity, drought conditions, insect damage, crop systems, and some agronomic practices, can cause stress and predispose wheat in the field to mold and determine the severity of mycotoxin contamination (Hussein and Brasel, 2001; Munkvold, 2014; Cotty and Jaime-Garcia, 2007).

Another important point highlighted from mycotoxin research is that mycotoxin co-contamination is more the rule than the exception. Several studies reported a high incidence of multi-mycotoxin contamination in cereals and agricultural commodities (Streit et al.,2012; Schatzmayr and Streit, 2013; Grenier and Oswald, 2014). A recent survey showed that in 2015, 46% of wheat samples were co-contaminated by two to six mycotoxins (Pancosma, 2015). A study carried out in Italy showed that at least 80% of wheat samples were contaminated with one mycotoxin, while two mycotoxins were found in 27% of contaminated samples; 38% of the analyzed samples were contaminated with three or more mycotoxins (Alkadri et al., 2014).

2.11 Mycotoxicosis

Dietary, respiratory, dermal, and other exposures to toxic fungal metabolites produce the diseases collectively called mycotoxicosis. Mycotoxicosis are examples of “poisoning by natural means” and thus are analogous to the pathologies caused by exposure to pesticides or heavy metal residues. The symptoms of a mycotoxicosis depend on the type of mycotoxin; the amount and duration of the exposure; the age, health, and sex of the exposed individual; and many synergistic effects involving genetics, dietary status, and interactions with other toxic insults. Thus, the severity of mycotoxin poisoning can be compounded by factors such as vitamin deficiency, caloric deprivation, alcohol abuse, and infectious disease status. In turn, mycotoxicosis can heighten vulnerability to microbial diseases, worsen the effects of malnutrition, and interact synergistically with other toxins.

Mycotoxicosis, like all toxicological syndromes, can be categorized as acute or chronic. Acute toxicity generally has a rapid onset and an obvious toxic response, while chronic toxicity is characterized by low-dose exposure over a long time period, resulting in cancers and other generally irreversible effects (James, 1985). Almost certainly, the main human and veterinary health burden of mycotoxin exposure is related to chronic exposure (e.g., cancer induction, kidney toxicity, immune suppression). However, the best-known mycotoxin episodes are manifestations of acute effects.

In general, mycotoxin exposure is more likely to occur in parts of the world where poor methods of food handling and storage are common, where malnutrition is a problem, and where few regulations exist to protect exposed populations. However, even in developed countries, specific subgroups may be vulnerable to mycotoxin exposure. In the United States, for example, Hispanic populations consume more corn products than the rest of the population, and inner-city populations are more likely to live in buildings that harbor high levels of molds (Barrett, 2000). A further

scenario is represented by climate changes. Estimates suggest that climate change will reduce wheat production globally by 29–34% by 2050 in developing countries (Hellin et al., 2012). This will have a great impact on food security. In terms of food safety and mycotoxin contamination, problems also concerning *Fusarium* toxins may represent a challenge if the temperature increases in cool or temperate climate countries (Marroquín-Cardona, 2014; Wu and Mitchell, 2016).

2.12 Major *Fusarium* Mycotoxins

2.12.1 Fumonisins

Fumonisins are carcinogenic mycotoxins mainly produced by *Fusarium verticillioides* and *Fusarium proliferatum* in wheat and maize. Fumonisins B1 and B2 are of toxicological significance, while the others (B3, B4, A1 and A2) occur in very low concentrations and are less toxic. Fumonisins affect animals in different ways by interfering with sphingolipid metabolism (Dutton, 1996; Marasas, 1995; Merrill et al., 2001; Wang et al., 1991). They cause leukoencephalomalacia (hole in the head syndrome) in equines (Marasas et al., 1988) and rabbits (Bucci et al., 1996); pulmonary edema and hydrothorax in swine (Harrison et al., 1990) and hepatotoxic and carcinogenic effects (Gelderblom et al., 1996) and apoptosis in the liver of rats (Pozzi et al., 2000). In humans, there is a probable link with esophageal cancer (Sydenham et al., 1991). The occurrence of fumonisins is correlated with the occurrence of a higher incidence of esophageal cancer in regions of Transkei (South Africa), China, and northeast Italy (Peraica et al., 1999). The Joint FAO/WHO Expert Committee on Food Additives (JECFA) set a maximum allowable daily intake of 2 μg kg^{-1} body weight/day for FB1, FB2 and FB3 (separately or in combination) (WHO, 2001/2002).

2.12.2 Deoxynivalenol

Deoxynivalenol is a mycotoxin belonging to the trichothecenes family, it is primarily produced in cooler climates by *F. graminearum* and *F. culmorum*. Deoxynivalenol is commonly found in barley, corn, rye, safflower seeds, wheat, and mixed feeds (Miller et al., 2001). When ingested in high doses by agricultural animals, it causes nausea, vomiting, and diarrhea; at lower doses, pigs and other farm animals exhibit immunosuppressive activity (Otakawa, 1983), weight loss and food refusal (Rotter et al., 1996). For this reason, deoxynivalenol is sometimes called vomitoxin or food refusal factor.

2.12.3 Zearalenone

Zearalenone is produced mainly by *Fusarium graminearum, Fusarium culmorum, Fusarium equiseti, and Fusarium crookwellense*. All these species are regular contaminants of cereal crops worldwide. Zearalenone and its derivatives produce estrogenic effects: infertility, vulval oedema, vaginal prolapse and mammary hypertrophy in females and feminization of males, atrophy of testes and enlargement of mammary glands (Hagler et al., 2001). An association between zearalenone and hyperestrogenism in swine has been observed since the 1920s; modern work shows that dietary concentrations of zearalenone as low as 1.0 ppm may lead to hyperestrogenic syndromes; higher concentrations can lead to disrupted conception, abortion, and other reproductive problems (Kurtz et al., 1978; El-Nezami et al., 2002; Kuiper-Goodman et al., 1987).

2.13 Preharvest strategies

Fungi can invade, colonize, and produce mycotoxins during either preharvest or postharvest stages (Coulombe, 1993; Scudamore and Livesey, 1998; Storm et al. 2008; Cheli et al., 2013). Therefore, to properly manage mycotoxin contamination in wheat, the primary strategy is the prevention, by reducing fungi proliferation in field and during storage (Kabak and Dobson, 2006; Magan and Aldred, 2007; Choudhary et al., 2010; Magan et al., 2010). Commonly and usually, mycotoxinogenic fungi are divided into two groups: preharvest (mainly *Fusarium species*) and postharvest (mainly *Aspergillius* and *Penicillium species*) fungi.

Mycotoxigenic fungi that contaminate grain crops can lead to reduced grain quality, crop yield reduction and mycotoxicosis among humans and livestock. Preharvest management of fungi and mycotoxin contamination is considered among the most important mitigating strategies. There are several preharvest practices and management approaches to reduce the risk of mycotoxin contamination in wheat, whose combination in an integrated strategy represents the best mitigation measure. Preharvest events are predominantly dictated by environmental factors and good agronomic/cultural practices. All preharvest practices can be controlled, while climatic and environmental conditions cannot. Conditions, such as excessive moisture, temperature extremes, humidity, drought conditions, insect damage, crop systems, and some agronomic practices, can cause stress and predispose plants in the field to fungal infection and determine the severity of mycotoxin contamination (Wegulo et al., 2015; Munkvold, 2014). Computer models, integrating field parameters and weather variables (temperature, rainfall, and moisture level) have been developed to predict the occurrence and risk of *Fusarium* and mycotoxin contamination in wheat (Schaafsma and Hooker, 2007; van der Fels-Klerx et al., 2008; Prandini et al., 2009; Rossi et al., 2015; Giroux et al., 2016). Moreover, forecasting systems have been developed to optimize the use and application of chemical treatments (Wegulo et al., 2015).

The control of infection by *Fusarium* fungi in field is the first critical step in mitigating mycotoxin accumulation in the harvested products. To reduce the risk of *Fusarium* fungi and mycotoxin contamination, the most important preharvesting strategy is the application of appropriate good agriculture practices, such as crop selection, crop rotation, tillage, irrigation, and the proper use of chemicals (Wegulo et al., 2015).

2.13.1 Effect of Stress Factors and Climate Events

Climate is among the most important factors influencing the occurrence and distribution of *Fusarium*. Different climatic conditions (e.g., temperature and rainfall) in different geographical locations affect the incidence of *Fusarium* infection of small grain cereals. Warm and moist conditions, especially during the period of anthesis, are considered critical factors for *Fusarium* infection. The fungal species vary on a regional and continental scale and during any given season (Oerke et al., 2010). As for temperature requirements, most fungal species grow within a temperature range of 5 to 35 ℃ with optimum growth taking place at a range between 25 to 30 ℃ (Dix and Webster, 1995). Changes in climatic extremes would have direct impacts on *Fusarium* diseases and mycotoxin production because weather factors can strongly affect epidemics and the proportions of the species responsible for FHB (Paterson and Lima, 2010). In fact, temperatures that may be optimal for growth are different from those optimal for mycotoxin synthesis (Schmidt-

Heydt et al., 2011; Medina et al., 2013). However, generally, a temperature range between 25 and 30 ℃ and relative humidity between 88% and 95% are considered as favorable for fungal growth and subsequent mycotoxin production (Thanushree et al., 2019). Field crops are continuously challenged by several environmental stresses that occur naturally in a certain area. Stress conditions imposed on developing crops, especially during the reproductive stage, can facilitate fungal infection, mycotoxin production and grain contamination (Bruns, 2003). Biotic factors such as insects, pathogens, and weeds (Afifi et al., 2011) and abiotic factors such as drought and hailstorms (Roberston et al., 2011) can affect crop physiology and productivity (Lobell and Asner, 2003) and may result in conditions that are favorable for mycotoxin accumulation. There is evidence that the abiotic and biotic factors that predispose plants to diseases can activate several plant responses to stress, which can indirectly influence mycotoxin production (Cao et al., 2013). One of the primary biotic stress factors that influence fungal colonization and mycotoxin contamination are the insects. The harmful action of insects occurs in two ways: by producing wounds that are favorable entry sites for conidia already present on the ear tissues and by causing stress conditions in plant tissues (Avantaggiato et al., 2003). Although the effect of insect activity on small cereal is low, insects can still be considered a potential risk for the occurrence of FHB. In fact, pre-exposal of wheat ears to aphids, can co-occur with FHB appearance and lead to a significant increase in *F. graminearum* colonization and DON accumulation (Liu et al., 2005). Weeds represent a threat to the crop and an indirect stress affecting the crop performance. *Fusarium* species have been isolated from a wide range of grasses (Holmes, 1983; Lager and Wallenhammer, 2003) and broad-leaved weed species (Jenkinson and Parry, 1994), and a high weed density has been shown to result in increased *Fusarium* infection (Teich and Nelson, 1984). Preliminary analysis of data from a 4-year field study has shown that the use of glyphosate in the spring was correlated to an increase of *Fusarium* infection in wheat in Eastern Saskatchewan, Canada (Fernandez, 2003). An earlier study does indicate that such an effect may exist, as Levesque et al. (1987) showed that glyphosate increased the colonisation of roots of six weed species by *Fusarium* species and increased their propagule density within soils. Abiotic stress, such drought conditions, strongly alters the efficiency of photosystems and the stability of membranes and is associated with oxidative stress in plants (Sharma et al., 2016). Studies suggest that water and drought stress play an important role in *Fusarium* infection and mycotoxin accumulation in kernels. During kernel filling, drought is conducive to mycotoxin contamination (Abbas et al., 2002). Environmentally damaging conditions such as hailstorms have also been reported to decrease quality and increase mycotoxin contamination (Roberston et al., 2011), favoring the entry of a fungal pathogen and causing plant stress. These authors reported that fumonisins were more frequently detected in grain from hail-damaged fields compared with undamaged fields. Globally, there is evidence that climate change, by modifying the environment to favor disease development, is associated with increased frequency and severity of FHB epidemics (Jeger and Pautasso, 2008; Garrett et al., 2014; Hernandez et al., 2014; Kriss et al., 2012; Parikka et al., 2012).

2.13.2 Resistance

There are inherent differences in the susceptibility of cereal species to *Fusarium spp.*, which are reflected in differences in mycotoxin contamination of each species. The differences between crop species appear to differ between countries. This is probably due to differences in the genetic pool within each country's breeding program and the different environmental and agronomic conditions

in which crops are cultivated. Tekauz (2002) reported that DON levels in wheat, barley and oats were similar when grown under the same field conditions in Western Canada in 2001. However, Prickett et al. (2000) showed that wheat had higher levels of DON than barley or oats from the UK 1999 harvest, and Langseth and Rundberget (1999) showed that oats had higher levels of DON than barley and wheat in Norway from 1996 to 1999. Such trends can also differ for the different trichothecenes produced by the different *Fusarium* species. Samples of cereals taken in Hungary in the 1990s showed higher levels of DON in wheat compared to barley, which had higher levels of DON compared to oats (Rafai et al., 2000). It is also important to remember that the relative rate of mycotoxin contamination of different cereal species can vary between regions and seasons within a single country as determined for Norway by Langseth and Elen (1997). This may well be due to temporal and spatial differences in weather conditions when each cereal species was in flower.

2.13.3 Crop rotation

It is generally accepted that wheat that follows an alternative host for *Fusarium* pathogens is at greater risk of *Fusarium* infection and mycotoxin contamination of grain. However, there is conflicting evidence that wheat following wheat is more at risk than wheat following a non-cereal crop. In Germany, Obst et al. (1997) reported that Mycotoxin contamination of wheat following small-grain cereals was lower than in wheat following potatoes and sugar beet in 3 years out of a five. The fact that several *Fusarium* species, which are pathogenic to cereals, also have some pathogenicity towards non-cereal crops may explain this (Smith et al., 1988). In Saskatchewan, Canada, *F. avenaceum* was reported to be the most isolated pathogen from *Fusarium* infected ears of wheat. In this region, the incidence of *F. avenaceum* was higher in wheat following pulses than in continuous wheat cultivation (Fernandez et al., 2001). In Germany, wheat following maize was shown to have the highest contamination of DON, and that grain maize was higher than forage maize. It was suggested that the reason for this was that more crop debris results from the harvesting of grain compared to forage maize (Obst et al., 1997). Dill-Macky and Jones (2000) showed that *Fusarium* infection severity and mycotoxin contamination of grain was significantly different when the previous crop was maize, wheat, or soya bean, with the highest levels following maize and the lowest levels following soya bean. In a 4-year study, it was shown that the crop planted 2 years prior to planting wheat had a significant effect in 1 year out of 4 and the crop planted 3 years previously had no significant effect on mycotoxin contamination of wheat grain (Schaafsma et al., 2001). *Fusarium* infection and mycotoxin contamination is high in plots where the same crops are grown over consecutive years (FAO, 2017). Most toxicogenic fungi can survive in crop residues; therefore, properly designed crop rotation can significantly reduce the occurrence of *Fusarium spp.* and grain contamination with mycotoxins (Munkvold, 2003; Janssen et al., 2019). Crop rotation prevents fungal species build-up (Mannaa and Kim, 2017; Mahuku et al., 2019) and reduces inoculum build-up on plant residues (Marocco et al., 2008). For wheat cultivation rotating between legumes, brassicas and potato could significantly reduce *F.graminearum* contamination levels (Gilbert and Tekauz, 2011). It is recommended to avoid maize in the rotation, as maize is very susceptible to *Fusarium* sp. and the presence of maize residues appears to be an important factor contributing to mycotoxin contamination of wheat (Dill-Macky and Jones, 2000). The incidence and severity of *Fusarium graminearum* and mycotoxin contamination levels are higher in wheat grown after maize or wheat compared with wheat grown after soybeans (Schmidt and Nitzsche, 2004).

2.13.4 Tillage

Soil cultivation can be divided into ploughing, where the top 10–30 cm of soil is inverted; minimum tillage, where the crop debris is mixed with the top 10–20 cm of soil; and no-till, where seed is directly drilled into the previous crop stubble with minimum disturbance to the soil structure. Any crop husbandry that results in the removal, destruction or burial of infected crop residues is likely to reduce the *Fusarium* inoculum for the following crop. The benefits of such crop practice are likely to be limited to when wheat follows an alternative host crop for *Fusarium* species. Obst et al. (1997) reported that the use of minimum tillage instead of ploughing after a maize crop could result in a 10-fold increase in mycotoxin contamination of the following wheat crop. Dill-Macky and Jones (2000) showed that no-till (direct drilling) after wheat or maize significantly increased mycotoxin contamination of the following wheat crop compared to ploughing, but no-till had no effect when the previous crop was soya bean. Field preparation and cultivation practices play a central role in the management of *Fusarium* diseases and associated mycotoxins (Magan and Olsen, 2004). High levels of *Fusarium* and mycotoxin contamination in wheat have been reported with minimum tillage or no-till compared to conventional tillage (Dill-Macky and Jones, 2000). This effect can be attributed to inoculum survival and the concentration of *Fusarium* sp. in the soil (Steinkellner, 2004). The burial of plant residues from a previous planting season by deep ploughing can reduce the primary inoculum that causes infections (Blandino et al., 2010). Tillage depth has an impact on *Fusarium spp.* The deeper the tillage, the smaller the number of isolated fungi (Steinkellner and Langer, 2004). In addition, not only plowing crop residues, but also their removal can reduce the likelihood of infection of successive plants by *Fusarium spp.* (Gajęcki et al., 2010). Crop rotation in conjunction with tillage techniques may further mitigate *Fusarium* and mycotoxin contamination (Edwards, 2004).

2.13.5 Fertilization

There is some evidence that *Fusarium* infection can be affected by fertilizer regimes. Teich (1989) and Martin et al. (1991) observed that increasing the amount of nitrogen applied to cereals resulted in increased incidence of *Fusarium* infected grain. The form in which nitrogen is applied may also have an effect. Wheat fertilized by urea had fewer symptoms of *Fusarium* infection than wheat fertilized by ammonium nitrate (Martin et al., 1991; Teich, 1987). *Fusarium* infection was reduced by 31–59% when nitro lime was applied compared to calcium ammonium nitrate; however, subsequent reductions in mycotoxin contamination were inconsistent (Yi et al., 2001). Fertilizer regimes may affect *Fusarium* infection incidence and severity either by altering the rate of residue decomposition, by creating a physiological stress on the host plant or by altering the crop canopy structure. Mineral fertilizers used in the cultivation of agricultural plants may cause higher infection degree by fungi of the genus *Fusarium*, which contaminate the yields mainly through the rate of crop residue decomposition, rate of plant growth and change in soil structure and its biological activity (Gajęcki et al., 2010). Excess nitrogen in the soil increases the frequency of grain infection with *Fusarium* fungi. The type of fertilizer (urea, ammonium nitrate or calcium nitrate) can affect the degree of grain contamination with mold fungi, but not DON content (Yi et al., 2001). In other studies, it was found that more various mycotoxins were accumulated by winter wheat grains fertilized with a higher nitrogen dose, 200 kg N ha−1, than a dose of 120 kg N ha−1. Significant statistical relationships between the concentration of mycotoxins and the amount of nitrogen fertilizer and wheat cultivar were also demonstrated (Podolska et al., 2017). Fertilizers

improve plant health and maintain its resistance towards disease and fungi. Nutrient availability is very important for plant vigor and lack of proper plant nutrition leads to breaking in the stem of the plant making it more exposed to fungal invasion. So, in case nutrients were deficient in the soil, fertilizers can be used to increase soil fertility. However, fertilizer application must be accurate in timing and quantity since over-application may expose the plant to further stress making it more prone to pest and mold attacks (Nganchamung and Robson, 2017).

2.13.6 Planting recommendations

Plants should be planted at recommended row widths and densities to specifically reduce water stress (Mukanga et al., 2011) and ensure optimal nutrient availability. Adhering to planting dates and optimal densities reduces mycotoxin accumulation (Munkvold, 2003; Blandino et al., 2008; Abbas et al., 2012). The risk of plant infection by *Fusarium* fungi, and thus contamination with mycotoxins is always greatest when the flowering period of a given plant is close to the date of fungus spore release (Champeil et al., 2004; Jouany, 2007). Crops should be harvested from the field as soon as possible because favorable conditions for fungal diseases and mycotoxin accumulation may occur if harvest is delayed, thus leading to elevated mycotoxin levels (Chulze et al., 1996; Bush et al., 2004). Although the presence of fungi is not a definite indicator of mycotoxin contamination, their presence may imply an increased risk of contamination in case suitable conditions for mycotoxin production were found. Therefore, early detection of filamentous fungi in crops that allows for corrective measures is crucial.

2.13.7 Crop selection

High quality seed material is an important element preventing the occurrence of pathogenic fungi, such as *Fusarium spp.* and their metabolites in plant cultivation. Seeds should be healthy, without signs of damage that could facilitate pathogen penetration, and they should have adequate viability. Various conditioning techniques can be used to improve seed viability (Siqueira et al., 2014). Only high-quality seeds can compete with adverse factors during growth, such as pathogens and pests (Jard et al., 2011). Seed physical parameters are also important, including the appropriate moisture (Edwards, 2009). The use of genetic varieties more resistant to *Fusarium spp.* represents an effective management strategy to mitigate the mycotoxin challenge in wheat. Planting of resistant cultivars is an effective, affordable, and environmentally sound strategy to control fungal diseases and mycotoxin accumulation (Munkvold and Desjardins, 1997). At present, there are no totally resistant varieties, but partially resistant ones exist that can be used, but those do not provide protection against all genera of fungi. There are differences in the susceptibility of wheat variety to *Fusarium* and differences in the degree of mycotoxin contamination. Moreover, differences between crops appear to differ between countries which can be related to differences in the genetic pool within each country and the different environmental and agronomic conditions in which crops are cultivated (Kabak and Dobson, 2006). Partially resistant seeds are also mostly effective in cooler temperature climates, while the resistance is needed to a bigger extent in tropical and sub-tropical regions where fungal infections are more frequent (FAO, 2017). Commercial hybrids differ in their ability to accumulate mycotoxins, while hybrids grown outside of their adapted range are more susceptible to mycotoxins than those grown within their adapted range (Shelby et al., 1994). Wheat lines have been produced and provide good resistance to *Fusarium spp.* (Snijders, 2004; Góral et al., 2015).

2.13.8 Irrigation management

All plants in the field need adequate water supply. Drought stress and excess irrigation are favorable conditions for *Fusarium* infection. Drought stress should be avoided during the period of wheat seed development and maturation; therefore, crop planting should be timed accordingly. Excessive moisture in irrigated wheat fields during flowering and early grain fill period is a favorable condition for *Fusarium* infection (Lemmens et al., 2004). Nevertheless, the effect of moisture in increasing the levels of mycotoxin contamination is not consistent among published studies (Hernandez et al., 2014). Limiting plant stress to increase plant vigor by adhering to optimum plant dates, preventing drought stress and the optimal use of fertilizers have reduced *Fusarium* infection in several grain crops (Blandino et al., 2008; Parsons and Munkvold, 2010). Extended periods of heat and drought stress lead to increased mycotoxin contamination levels, this could be managed with proper irrigation schedules (Miller, 2001). Managing plant stress conditions is also important as this is considered key in the symptomless endophytic relationship converting to a disease- and/or mycotoxin-producing interaction (Abbas et al., 2006).

2.13.9 Weed and insect control

Weed and insect control is crucial to prevent disease in crops and further fungal invasion. Weeds contribute to contamination by acting as reservoirs of fungal inoculum and by competing for water and nutrients with the crops hence rendering them weak (Reboud et al., 2016). Therefore, weed removal should be continuously practiced. Hence, it is important to keep the area clean from plant debris, since removing any residual plants or vegetable matters makes food unavailable and reduces pest attack possibilities. Insects, on the other hand, can cause fungal dissemination and make the grains more vulnerable to infection by causing physical damage. The use of insecticides can prevent insect wounds that contribute to fungal infection and mycotoxin accumulation in the crops (Parsons and Munkvold, 2010). The application of insecticides at appropriate doses, as well, can help control the frequency of attack.

2.13.10 Chemical control

A wide range of chemicals has been tested against *Fusarium spp.* both in vitro, in glasshouse trials and under field conditions. Of concern is the fact that several fungicides at sub-lethal concentrations stimulate mycotoxin production in vitro (D'Mello et al., 1998; Matthies et al., 1999). Fungicides in the quinone inhibitor class have been shown to increase DON levels in grain (Blandino and Reyneri, 2009; Blandino et al., 2006) and therefore are not recommended for FHB and DON control. In several field trails, the application of azoxystrobin has resulted in a significant increase in mycotoxin contamination of grain (Jennings et al., 2000; Simpson et al., 2001). Although azoxystrobin can induce mycotoxin production in vitro (D'Mello et al., 2001), glasshouse studies have indicated that azoxystrobin does not appear to have a direct effect on mycotoxin production in the field (Pirgozliev et al., 2002), and that the mycotoxin increase is a result of an increase in *Fusarium* infection due to the activity of azoxystrobin on other microorganisms within the wheat ear environment (Pirgozliev et al., 2003). Fungal infection can be controlled by the appropriate use of fungicides. Fungicide treatment reduces *Fusarium* infection and mycotoxin contamination (Haidukowski, 2005; Yoshida et al., 2012). The most widely used fungicides are in the demethylation inhibitor class (McMullen et al., 2012). They include metconazole, propiconazole, prothioconazole, tebuconazole, and prothioconazole + tebuconazole.

Meta-analyses of fungicide trials in the US showed that prothioconazole + tebuconazole, metconazole, and prothioconazole were superior to propiconazole and tebuconazole in suppressing FHB and DON, and all five fungicides resulted in significant yield and test weight increase and reduction in FHB and DON compared to the check (Paul et al. 2008, 2010). Scarpino et al. (2015) also reported that azole fungicides are the most effective active substances in the reduction of mycotoxin and consistently reduce the main emerging and modified mycotoxins of winter wheat in temperate areas. Although fungicides in the DMI class suppress FHB and DON, they do not achieve complete control. To be effective, fungicide application usually is timed at the anthesis growth stage, or up to 6 days after anthesis (D'Angelo et al, 2014) because susceptibility to FHB infections is highest at this stage. In a study, Yoshida et al. (2012) showed that timing of thiophanate-methyl application differentially affected FHB and mycotoxin (DON and NIV) concentration in winter wheat. The results demonstrated that fungicide application 20 days after anthesis reduced mycotoxin concentration in matured grain but had no effect on FHB whereas application at anthesis was critical in reducing FHB. It is clear that a reduction in *Fusarium* infection after the application of a fungicide cannot be assumed to result in a corresponding reduction in mycotoxin contamination of grain. Overall results indicate that the variability of fungicide effects is related to several factors, such as cultivar resistance, the type of fungicide used, fungicide timing, pathogen aggressiveness, and different environmental and agronomic conditions.

2.13.11 Biological control

Biological control agents are primarily antagonistic microorganisms. Several biological control agents have been shown to give promising control of *Fusarim spp.* and reduction of mycotoxin contamination of wheat grain under glasshouse conditions when targeted against spore infection of flowering heads (Schisler et al., 2002). Under field conditions biological control achieved has been shown to be variable (McMullen et al., 2002). However, evidence does indicate that biological control could contribute to the reduction of *Fusarium* infection and mycotoxin contamination of grain in commercial agriculture. Biological control agents can be used alone or as part of an integrated management program. Bacteria and fungi are among the biological control agents that have been identified and tested in vitro, in the greenhouse, and in the field. Bacterial biological control agents that have been shown to have antagonistic activity against *Fusarium spp.* causing FHB include *Bacillus spp.* (Schisler et al., 2002; Zhao et al., 2014), *Pseudomonas spp.* (Schisler et al., 2006), *Lysobacter enzymogenes* (Jochum et al., 2006), and *Streptomyces spp.* (Palazzini et al., 2007). Fungal antagonists include *Cryptococcus spp.* (Schisler et al., 2011), *Trichoderma spp.* (Matarese et al., 2012), *Clonostachys rosea* (Xue et al., 2014), and *Aureobasidium pullulans* (Wachowska and Glowacka, 2014). These biological control agents can be applied directly to spikes to slow down disease progression (Xue et al., 2014) or to residues to suppress production of perithecia (Xue et al., 2014).

3. MATERIALS AND METHODS

3.1 Experimental design and statistical analysis

The experiment was conducted during the growing seasons (2020, 2021 and 2022) at the experimental field and laboratories of the Hungarian University of Agriculture and Life Sciences (MATE), Agronomy Institute, Gödöllő, Hungary (Figure 3). The experimental site is in a hilly area with a close to average climatic zone of the country (47°35'40.8"N 19°22'08.4"E, 210 m above sea level). A three-year crop rotation of soybean, wheat, and maize was implemented in the field. Prior to sowing, the field was cleared, ploughed and rotor-tilled, and the seedbed was prepared. The plots were sown in October and harvested in the middle of July with plot machines. The sowing depth was 5 cm. The rate of sowing was 450 to 500 seeds per square meter. Weeds were controlled by herbicides (Mustang Forte™) and wheat pests and diseases beside *Fusarium* were controlled by pesticides. Rainfall (mm) measurements were collected from the World Weather Online® meteorological service during the flowering period (May) when wheat is most susceptible to *Fusarium* infection. Rainfall during the flowering period (May) was 24.6 mm in 2022, 88.39 mm in 2021 and 42.8 mm in 2020. The soil type of the experimental field was sand-based brown forest soil (Chromic Luvisol). The textural classification of the soil was sandy loam with parameters shown in Table 1. The agronomic characteristic of the soil was neutral sandy soil with variable clay content. The soil structure was susceptible to compaction issues.

The trial design was that of a split plot with main plots consisting of different wheat varieties (Table 2) and subplots consisting of different nitrogen doses. Main plots and subplots were 50 cm apart horizontally and 30 cm apart vertically, and the area of each subplot was 5 m^2. Each treatment had three replications.

During the 2020 and 2021 growing seasons the wheat varieties used were Alföld, Mv Kolompos, and Mv Karéj. Nitrogen fertilizer was applied in the form of granular ammonium (NH_4NO_3) with 34% content of the active ingredient either in single dose application or in split dose application. Single dose nitrogen fertilization was done once during the growing season at the heading stage (April) with the following doses: 40, 80, 120, and 160 kg N ha^{-1}. Split dose nitrogen fertilization was done twice during the growing season, the first application was at tillering stage (March) and the second application at heading stage (April). The doses of nitrogen in the first application were: 40, 80, and 120 kg N ha^{-1}. In the second application 40 kg N ha^{-1} was added only. Plots without nitrogen topdressing were used as control. Wheat was harvested in the middle of July and representative samples were randomly taken from each plot. *Fusarium* percentage was calculated by counting the number of colonies that formed on wheat kernels (100 kernels from each treatment) disinfected for 2 minutes with a solution of pentachloronitrobenzene (PCNB) and chloramphenicol (distilled water 1 L, PCNB 1 g, chloramphenicol 100 ppm) and then incubated for 7 days under laboratory conditions (23 °C ± 0.6 °C and 45% RH ± 5% RH) on Nash and Snider *Fusarium* selective medium (distilled water 1 L, peptone 15 g, KH_2PO_4 1 g, $MgSO_47H_2O$ 0.5 g, agar 20 g, PCNB 1 g, chloramphenicol 100 ppm). Mycotoxin concentrations of deoxynivalenol (DON), zearalenone (ZEA), and fumonisins (FUM) were analyzed using ROSA FAST 5 Quantitative Test by Charm Sciences (DONQ-FAST5 Test, FUMQ-FAST5 Test, ZEARQ-FAST5 Test).

During the 2022 growing season the wheat varieties used were Alföld and Mv Ménrót. Nitrogen fertilizer was applied once at the heading stage (April) in the following doses: 200, 400, 600, 800, and 1000 kg N ha^{-1}. Plots without nitrogen topdressing were used as control. Apart from nitrogen

topdressing, all other agronomic treatments as well as sowing and harvesting were identically applied to all plots to study the impact of nitrogen treatments independently. Wheat was harvested in the middle of July and representative samples were randomly taken from each plot. Protein content, gluten content, and Zeleny sedimentation index were measured with Near-infrared (NIR) spectroscopic equipment Mininfra Scan-T Plus 2.02 version. The falling number was measured with Perten 1400 system (ICC method No. 107/1 1995). Test weight was measured with the Chondrometer Hectoliter grain tester (ISO 7971-3:2019). Thousand kernel weight and test weight were measured with the KERN EMS and the Sartorius MA-30 precision scales.
For the statistical evaluation of the results, the analysis of variance (ANOVA) module of the IBM SPSS V.21 software at a 5% significance level with subsequent Tukey's test was performed.

Figure 3. The experimental field at Gödöllö, Hungary

Table 1. Soil type of the experimental field at Hungarian University of Agriculture and Life Sciences, Agronomy Institute, Gödöllö, Hungary

	Humus %	pH (H_2O)	K_A	Sand %	Silt %	Clay %	$CaCO_3$
Medium	1.32	7.08	40	49	25	26	0

Table 2. Characteristics of the different wheat varieties used (Martonvásár 2020)

Quality values specific to the variety	Alföld	Mv Kolompos	Mv Karéj	Mv Ménrót
Thousand Kernel Weight (g)	39-44	50-55	45-50	45-50
Hectolitre Weight (kg/hl)	78-82	75-78	82-85	80-84
Falling Number (seconds)	350-400	350-400	350-400	350-400
Protein Content (%)	14-17	12-14	11-13	12-13
Gluten Content (%)	34-40	30-35	29-32	30-32
Zeleny Sedimentation Index (ml)	31-55	36-46	31-55	29-42
FHB Disease Resistance (1-9)	6	6	6	6

3.2 Meteorological properties of the experimental field

Figure 4 shows the Rainfall (mm), and Figure 5 shows the temperature (°C) in Godollo during 2020, 2021 and 2022.

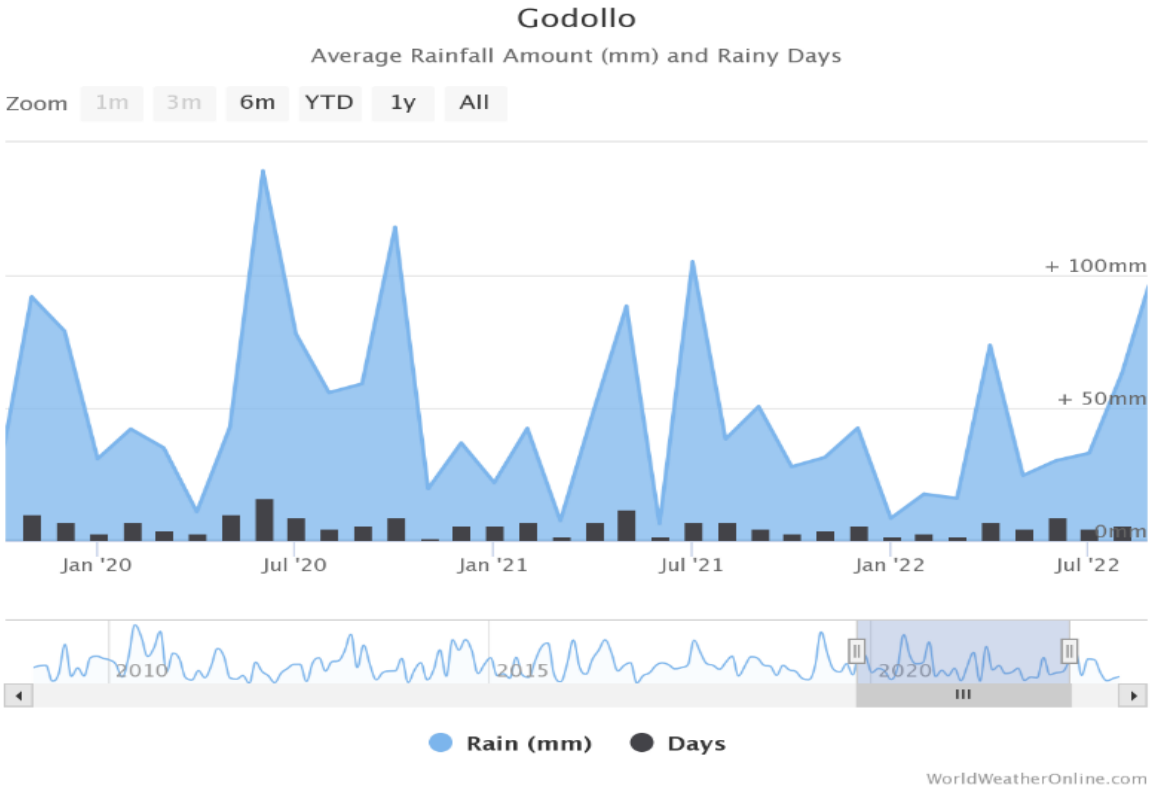

Figure 4. Rainfall in 2020, 2021 and 2022 (worldweatheronline.com)

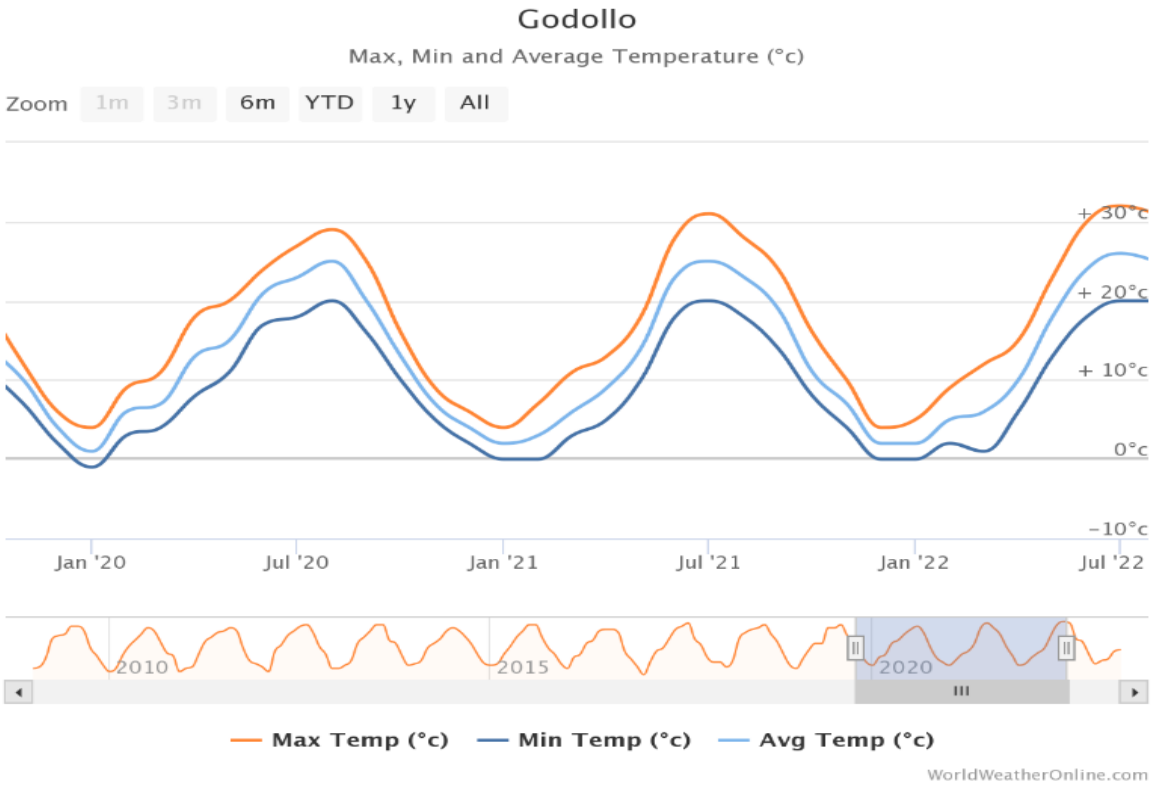

Figure 5. Temperature in 2020, 2021 and 2022 (worldweatheronline.com)

3.3 Determination of wheat quality parameters

Protein content, gluten content and Zeleny sedimentation index

Protein content, gluten content and Zeleny sedimentation index were measured by Near infrared (NIR) spectroscopic equipment Mininfra Scan-T Plus 2.02 version (Figure 6). NIR spectroscopy is a method that makes use of the near-infrared region of the electromagnetic spectrum (from about 700 to 2500 nanometers). By measuring light scattered off and through a sample, NIR reflectance spectra can be used to quickly determine a material's properties without altering the sample. The instrument requires no sample preparation other than cleaning or removal of large impurities.

Figure 6. NIR apparatus

Falling number test

The Hagberg Falling number test is used to measure the enzyme activity in flour. The Falling number of a flour is related to the amount and activity of cereal enzyme α-amylase, which is present in the wheat after harvesting. Wheat kernels with high moisture levels usually exhibit high levels of α-amylase. The Falling number is the time in seconds required for a device to fall a measured distance through a hot flour/water mixture while heat is applied. If the enzymatic activity is high, the starch is broken down (liquefied) rapidly during gelatinization. So then, the device falls through the liquid paste in a relatively short time. On the other hand, if the activity of the enzyme is low, it takes longer for the device to cover the distance of its fall. This means the Falling number is high. Falling numbers over 250 seconds are most suitable for the bread-baking process. In contrast, FNs above 350 seconds may indicate that the flour should be supplemented with a form of amylolytic enzyme or with malted grain flour. Most large-scale bakeries work with an ideal FN range of 250–280 seconds.

The Hagberg Falling number (HFN) Perten Type:1400 system, which meets the requirements of the AACC (American Association of Cereal Chemist) No.56-81.04, ICC (International Cereal Chemist) No. 107/1, and PN EN ISO 3093:2010 standards, was used to determine the falling number (Figure 7).

Procedure:

- Prepare/weigh a representative amount of sample (usually 250–350 g of dry product).
- Specify the moisture content of the flour (use correction table).

- Place the flour into the viscometer tube.
- Using a dispenser/pipette, pour distilled or tap water (approximately 25 mL) into the viscometer tube.
- Thoroughly shake the flour/water mixture in the viscometer tube until a homogeneous suspension is obtained (this step can be performed by a shaking device).
- Place/insert the viscometric tube with the stirring rod in a warm bath at boiling temperature.
- Start the FN instrument. After 5 seconds, the agitation will start automatically.
- After a few seconds the stirring rod is automatically released in its upper position and starts to fall.
- The total time, in seconds, from the moment the device is activated until the rod descends a certain distance is registered by the equipment. This is the Falling Number.

Figure 7. Falling Number apparatus

Thousand kernel weight

Thousand kernel weight is the weight of 1000 wheat kernels. It is a quality test applied to wheat to determine its potential milling yield. Thousand kernel weight of wheat depends on kernel size and density. Large dense wheat kernels normally have a higher ratio of endosperm to non-endosperm; hence the 1000 kernel weight will be higher. Smaller less dense kernels have less weight and hence less yield. The OS 1 type equipment by the ISO 7971-3:2019 standard was used to measure test weight.

Equipment: Digital balance, Wheat samples

Procedure

- Count one thousand wheat kernels
- Using a digital balance measure the weight of the one thousand wheat kernels
- Carry out the test in triplicate for each wheat sample
- Record the weight of the one thousand kernels in grams
- Assess the quality of wheat based on the results. The values range from 30 to 50g. The higher the value, the better the wheat is for milling in terms of flour yield.

Test weight

Test weight is the weight of a known volume of grain expressed in kilograms per hectoliter (kg/hL). The objective of the hectoliter test in wheat is to estimate the flour productivity. When the hectoliter of the wheat increases, the flour productivity increases as well. The hectoliter value varies according to the form, density, size, and uniformity of the grain.

Equipment: Plastic container, Chondrometer, digital balance, plunger weight, cut-off slide

Procedure:

- Insert the cut-off slide into the designated opening of the Chondrometer
- Drop the plunger weight into the Chondrometer, ensuring that it is resting flat on the cut-off slide
- Place the Chondrometer with the cut-off slide and the plunger weight on the weighing platform. Press on the tare button
- Remove the Chondrometer from the balance
- Pour the grain sample into the plastic container
- Slowly fill the Chondrometer to the top with the grain sample
- Remove the cut-off slide carefully, allowing that the plunger weight together with the grain to descent into the lower part of the Chondrometer
- Re-insert the cut-off slide through the slot of the Chondrometer and push it through the grain in one go; intervening grains are cut through
- Tip out the surplus grain
- Place the Chondrometer on the balance
- Read off the mass and take the value for the hectoliter weight [kg/hl] from the calibration chart

3.4 Determination of *Fusarium* infection level and mycotoxin concentration

Grain yields of the winter wheat varieties were sampled and measured from each harvested plot. *Fusarium* infection and Mycotoxin concentration were measured from harvested wheat grain. Analyses were done at the research laboratory of the Hungarian University of Agriculture and Life Sciences, Agronomy Institute. *Fusarium* infection level (%) was calculated by counting the number of colonies that formed on wheat kernels (100 kernels from each treatment) disinfected for 2 minutes with a solution of pentachloronitrobenzene (PCNB) and chloramphenicol (distilled water 1 L, PCNB 1 g, chloramphenicol 100 ppm) and then incubated for 7 days under laboratory conditions (23 °C ± 0.6 °C and 45% RH ± 5% RH) on Nash and Snider *Fusarium* selective medium (Distilled water 1 l, Peptone 15 g, KH_2PO_4 1 g, $MgSO_47H_2O$ 0.5 g, Agar 20 g, PCNB 1 g, Chloramphenicol 100 ppm). Colonies that were developed on kernels were not identified to species level but were classified as either *Fusarium spp.* or other fungal species. Mycotoxin concentrations of deoxynivalenol (DON), zearalenone (ZEA) and fumonisins (FUM) were analyzed using ROSA FAST 5 Quantitative Test by Charm Sciences (DONQ-FAST5 Test, FUMQ-FAST5 Test, ZEARQ-FAST5 Test).

Deoxynivalenol concentration analysis method

- Pipet 1000 µl DON dilution buffer into a clean micro centrifuge tube for each sample. Label tubes as needed
- Obtain a representative ground sample. Mix the sample
- Weigh 5 g ground sample and add to clean extraction container
- Add distilled water equal to five times the sample weight to the sample.
- Shake vigorously or blend for 1 minute
- Allow sample to settle at least 2 minute to obtain sample extract
- Pipet 100 µl extract to predispensed tube with 1000 µl DON dilution buffer
- Mix well and label, this tube contains the diluted extract
- Use the diluted extract in the procedure to quantitate 0 to 1500 ppb (0 to 1.5 ppm)

Zearalenone and Fumonisins concentration analysis method

- Pipet 1000 µl dilution buffer into a clean micro centrifuge tube for each sample. Label tubes as needed
- Obtain a representative ground sample. Mix the sample
- Weigh 5 g ground sample and add to clean extraction container
- Add 70 % methanol equal to two times the sample weight to the sample
- Shake vigorously or blend for 1 minute
- Allow sample to settle at least 2 minute to obtain sample extract
- Pipet 100 µl extract to predispensed tube with 1000 µl dilution buffer
- Mix well and label, this tube contains the diluted extract
- Use the diluted extract in the procedure to quantitate 0 to 1500 ppb (0 to 1.5 ppm)

For quantification of 1000 to 5400 ppb (1 to 5.4 ppm) prepare the second diluted extract:

- Pipet 1000 µl dilution buffer into a clean micro centrifuge tube for each sample
- Pipet 300 µl diluted extract to predispensed tube with 1000 µl dilution buffer
- Mix well and label this tube contains the second diluted extract
- Use the second diluted extract in the procedure to quantitate 1000 to 5400 ppb

Incubation and test strip reading

- Label test strips with sample identification. Avoid crushing the sample compartment.
- Place test strip in ROSA incubator.
- Holding test strip flat in ROSA incubator use tab to expose sample compartment by peeling tape back to peel here line.
- Pipet 300 µl diluted extract into sample compartment at ROSA incubator indicator line
- Reseal tape over sample compartment
- Close lid on ROSA incubator and incubate for 5 minutes.
- After 5 minutes remove test strip from ROSA incubator and insert it into ROSA M reader.
- Read results on appropriate channel and appropriate matrix.

Nash and Snyder *Fusarium* selective medium preparation:

Part 1

- Peptone: 15 g
- KH_2PO_4: 1 g
- $MgSO_47H_2O$: 0.5 g
- Agar: 20 g
- PCNB (thermostable): 1 g
- Distilled water: 1 liter

Part 2

- Chloramphenicol (not thermostable, soluble in ethanol): 100 ppm (0.1 g l^{-1})
- 0.1 g l^{-1} chloramphenicol to be dissolved in 1 ml of ethanol

Procedure

- Combine ingredients from part 1 in a flask and add a stir bar and stir ingredient well before autoclaving.
- Autoclave and cool ingredients from part 1 to 50-55 °C.
- After the medium has cooled, add 0.1 g l^{-1} chloramphenicol dissolved in 1 ml of ethanol

4. RESULTS AND DISCUSSION

4.1 Effect of nitrogen fertilization, wheat variety and growing season on *Fusarium* infection and mycotoxin production

4.1.1 Effect of nitrogen fertilization

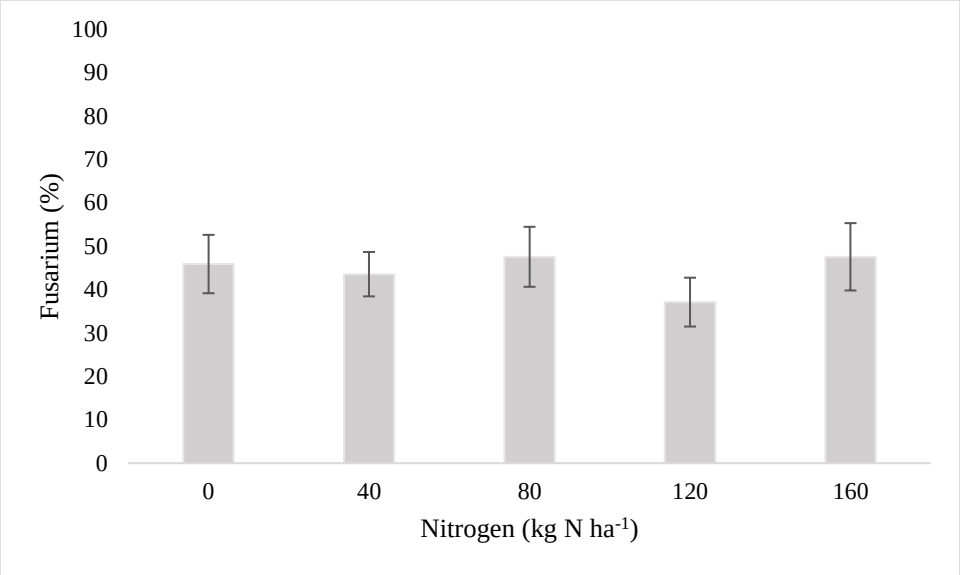

Figure 8. Effect of single dose nitrogen fertilization (kg N ha^{-1}) on *Fusarium* infection (%) in 2020

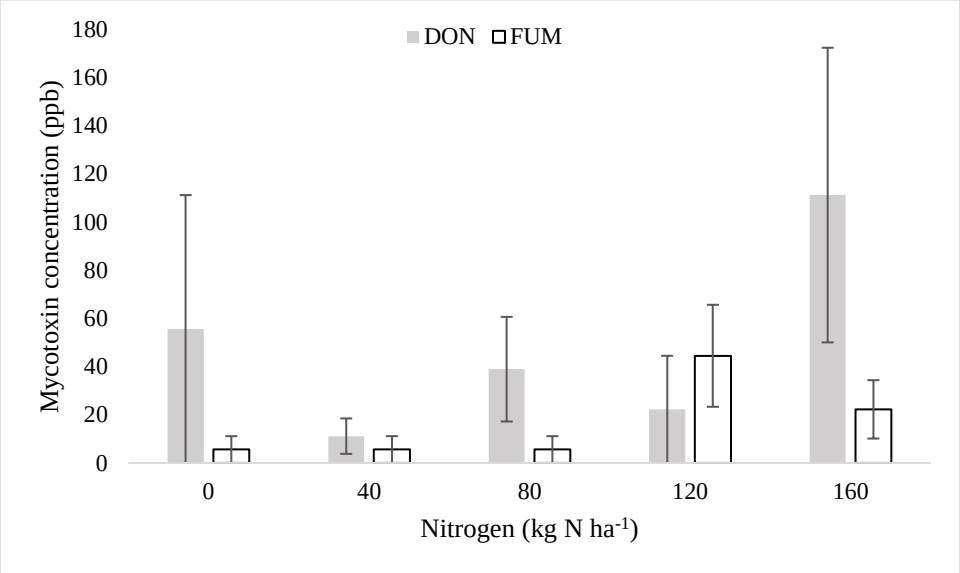

Figure 9. Effect of single dose nitrogen fertilization (kg N ha^{-1}) on mycotoxin concentration (ppb) in 2020

In 2020, the increasing single doses of nitrogen fertilization did not show a statistically significant effect on *Fusarium* infection (F = 0.450, P = 0.772) and mycotoxin production (DON, F = 0. 980, P = 0. 429; FUM, F = 2.135, P = 0.094). There was no statistically significant difference in *Fusarium* infection and mycotoxin contamination between the increasing nitrogen doses. *Fusarium* infection and mycotoxin contamination did not change with the increasing nitrogen doses (Figure 8, 9) (Table 3, 4).

Table 3. Descriptive statistics of *Fusarium* infection (%), DON and FUM concentration (ppb) affected by single dose nitrogen fertilization (kg N ha^{-1}) in 2020

		N	Mean	Std. Deviation	Std. Error	Minimum	Maximum
DON	0	9	55.56	166.67	55.56	0	500
	40	9	11.11	22.05	7.35	0	50
	80	9	38.89	65.09	21.70	0	150
	120	9	22.22	66.67	22.22	0	200
	160	9	111.11	183.33	61.11	0	550
	Total	45	47.78	118.68	17.69	0	550
FUM	0	9	5.56	16.67	5.56	0	50
	40	9	5.56	16.67	5.56	0	50
	80	9	5.56	16.67	5.56	0	50
	120	9	44.44	63.46	21.15	0	200
	160	9	22.22	36.32	12.11	0	100
	Total	45	16.67	36.93	5.50	0	200
Fusarium	0	9	45.89	20.18	6.73	12	72
	40	9	43.56	15.36	5.12	24	66
	80	9	47.56	20.73	6.91	22	82
	120	9	37.11	16.89	5.63	12	66
	160	9	47.56	23.28	7.76	20	88
	Total	45	44.33	19.00	2.83	12	88

0: no nitrogen
40: the nitrogen dose was 40 kg N ha^{-1}
80: the nitrogen dose was 80 kg N ha^{-1}
120: the nitrogen dose was 120 kg N ha^{-1}
160: the nitrogen dose was 160 kg N ha^{-1}

Table 4. Analysis of variance for *Fusarium* infection and DON, FUM concentration affected by single dose nitrogen fertilization in 2020

		Sum of Squares	df	Mean Square	F	Sig.
DON	Between Groups	55333.333	4	13833.333	.980	.429
	Within Groups	564444.444	40	14111.111		
	Total	619777.778	44			
FUM	Between Groups	10555.556	4	2638.889	2.135	.094
	Within Groups	49444.444	40	1236.111		
	Total	60000.000	44			
Fusarium	Between Groups	683.556	4	170.889	.450	.772
	Within Groups	15196.444	40	379.911		
	Total	15880.000	44			

df: degree of freedom; Sig.: significance; Significance level: $P < 0.05$

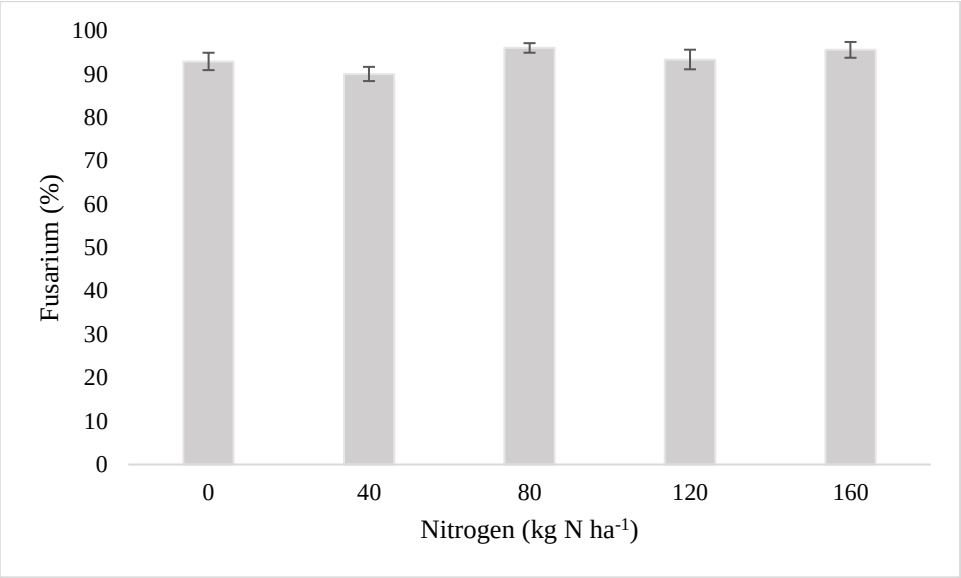

Figure 10. Effect of single dose nitrogen fertilization (kg N ha^{-1}) on *Fusarium* infection (%) in 2021

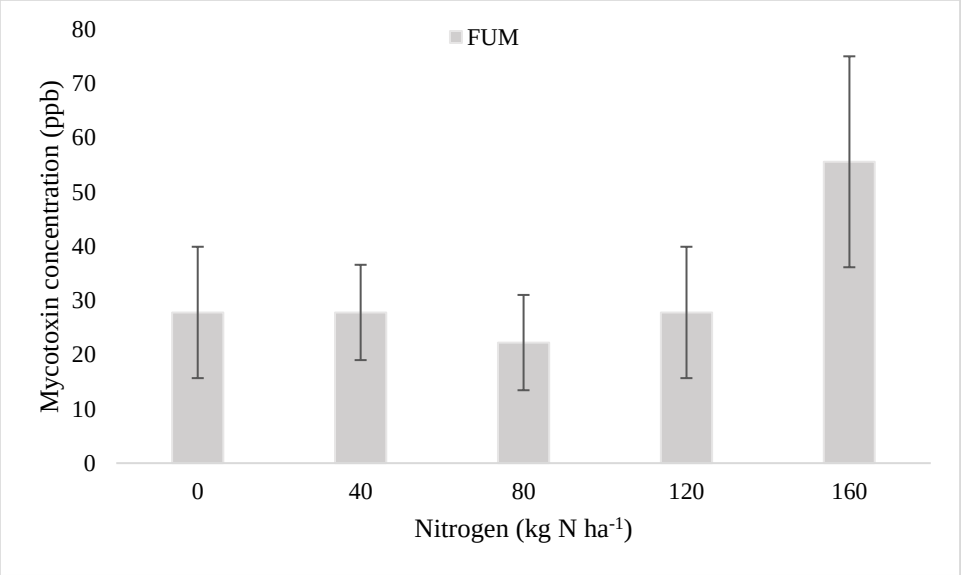

Figure 11. Effect of single dose nitrogen fertilization (kg N ha^{-1}) on mycotoxin concentration (ppb) in 2021

In 2021, the increasing single doses of nitrogen fertilization did not show a statistically significant effect on *Fusarium* infection (F = 1.770, P = 0.154) and mycotoxin production (FUM, F = 1.065, P = 0.386). There was no statistically significant difference in *Fusarium* infection and mycotoxin contamination between the increasing nitrogen doses. *Fusarium* infection and mycotoxin contamination did not change with the increasing nitrogen doses (Figure 10, 11) (Table 5, 6).

Table 5. Descriptive statistics of *Fusarium* infection (%), DON and FUM concentration (ppb) affected by single dose nitrogen fertilization (kg N ha^{-1}) in 2021

		N	Mean	Std. Deviation	Std. Error	Minimum	Maximum
FUM	0	9	27.78	36.32	12.11	0	100
	40	9	27.78	26.35	8.78	0	50
	80	9	22.22	26.35	8.78	0	50
	120	9	27.78	36.32	12.11	0	100
	160	9	55.56	58.33	19.44	0	200
	Total	45	32.22	38.66	5.76	0	200
Fusarium	0	9	92.89	6.01	2.00	84	100
	40	9	90.00	4.90	1.63	80	96
	80	9	96.00	3.32	1.11	90	100
	120	9	93.33	6.78	2.26	82	100
	160	9	95.56	5.46	1.82	86	100
	Total	45	93.56	5.61	0.84	80	100

0: no nitrogen
40: the nitrogen dose was 40 kg N ha^{-1}
80: the nitrogen dose was 80 kg N ha^{-1}
120: the nitrogen dose was 120 kg N ha^{-1}
160: the nitrogen dose was 160 kg N ha^{-1}

Table 6. Analysis of variance for *Fusarium* infection and DON, FUM concentration affected by single dose nitrogen fertilization in 2021

		Sum of Squares	df	Mean Square	F	Sig.
FUM	Between Groups	6333.333	4	1583.333	1.065	.386
	Within Groups	59444.444	40	1486.111		
	Total	65777.778	44			
Fusarium	Between Groups	208.000	4	52.000	1.770	.154
	Within Groups	1175.111	40	29.378		
	Total	1383.111	44			

df: degree of freedom; Sig.: significance; Significance level: $P < 0.05$

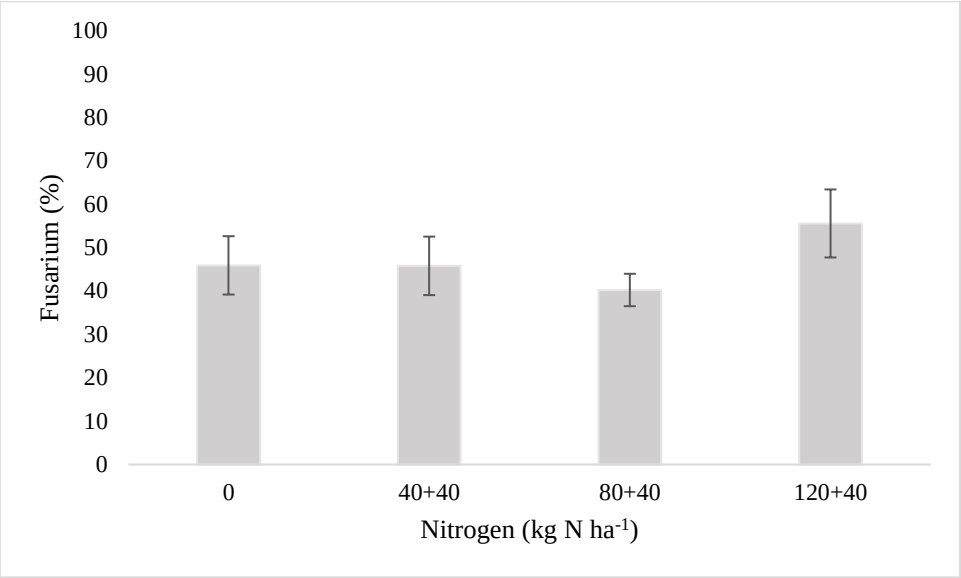

Figure 12. Effect of split dose nitrogen fertilization (kg N ha^{-1}) on *Fusarium* infection (%) in 2020

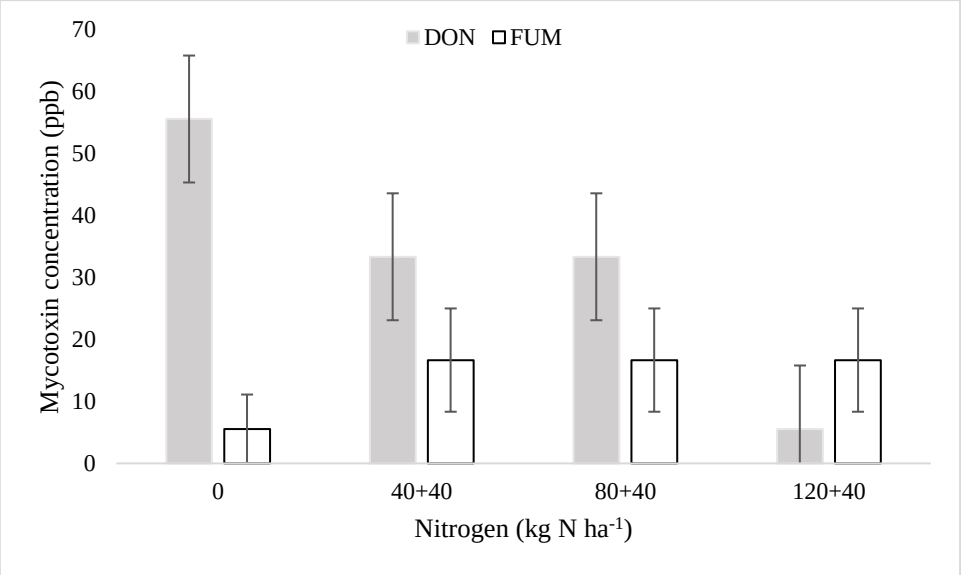

Figure 13. Effect of split dose nitrogen fertilization (kg N ha^{-1}) on mycotoxin concentration (ppb) in 2020

In 2020, the increasing split doses of nitrogen fertilization did not show a statistically significant effect on *Fusarium* infection (F = 0.978, P = 0.415) and mycotoxin production (DON, F = 0.351, P = 0.789; FUM, F = 0.516, P = 0.674). There was no statistically significant difference in *Fusarium* infection and mycotoxin contamination between the increasing nitrogen doses. *Fusarium* infection and mycotoxin contamination did not change with the increasing nitrogen doses (Figure 12, 13) (Table 7, 8).

Table 7. Descriptive statistics of *Fusarium* infection (%), DON and FUM concentration (ppb) affected by split dose nitrogen fertilization (kg N ha^{-1}) in 2020

		N	Mean	Std. Deviation	Std. Error	Minimum	Maximum
DON	0	9	55.56	166.67	55.56	0	500
	40+40	9	33.33	100.00	33.33	0	300
	80+40	9	33.33	70.71	23.57	0	200
	120+40	9	5.56	16.67	5.56	0	50
	Total	36	31.94	100.82	16.80	0	500
FUM	0	9	5.56	16.67	5.56	0	50
	40+40	9	16.67	25.00	8.33	0	50
	80+40	9	16.67	25.00	8.33	0	50
	120+40	9	16.67	25.00	8.33	0	50
	Total	36	13.89	22.71	3.79	0	50
Fusarium	0	9	45.89	20.18	6.73	12	72
	40+40	9	45.78	20.21	6.74	22	80
	80+40	9	40.22	11.20	3.73	22	54
	120+40	9	55.56	23.51	7.84	24	90
	Total	36	46.86	19.31	3.22	12	90

0: no nitrogen
40 + 40: the first nitrogen dose was 40 kg N ha^{-1} and the second was 40 kg N ha^{-1}
80 + 40: the first nitrogen dose was 80 kg N ha^{-1} and the second was 40 kg N ha^{-1}
120 + 40: the first nitrogen dose was 120 kg N ha^{-1} and the second was 40 kg N ha^{-1}

Table 8. Analysis of variance for *Fusarium* infection and DON, FUM concentration affected by split dose nitrogen fertilization in 2020

		Sum of Squares	df	Mean Square	F	Sig.
DON	Between Groups	11319.444	3	3773.148	.351	.789
	Within Groups	344444.444	32	10763.889		
	Total	355763.889	35			
FUM	Between Groups	833.333	3	277.778	.516	.674
	Within Groups	17222.222	32	538.194		
	Total	18055.556	35			
Fusarium	Between Groups	1096.083	3	365.361	.978	.415
	Within Groups	11950.222	32	373.444		
	Total	13046.306	35			

df: degree of freedom; Sig.: significance; Significance level: $P < 0.05$

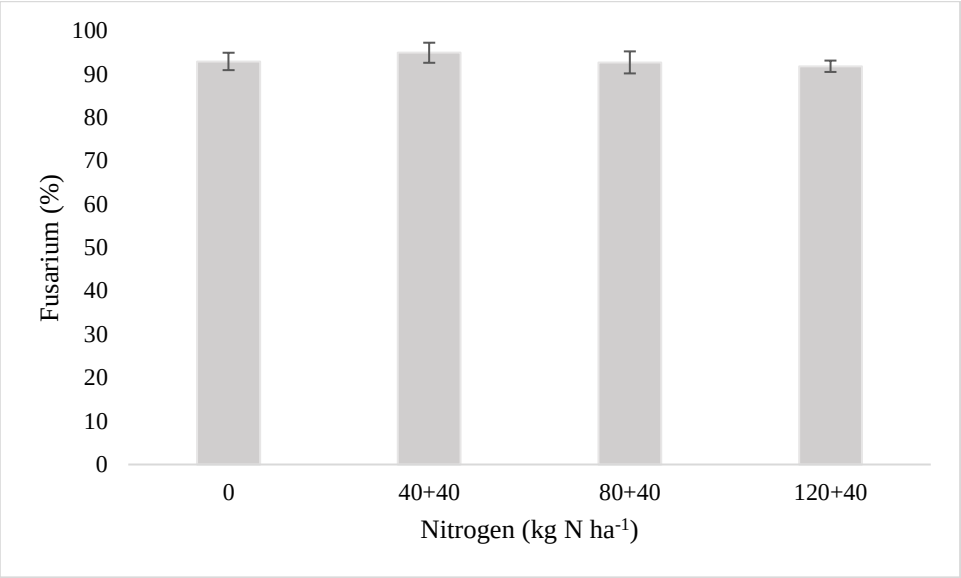

Figure 14. Effect of split dose nitrogen fertilization (kg N ha^{-1}) on *Fusarium* infection (%) in 2021

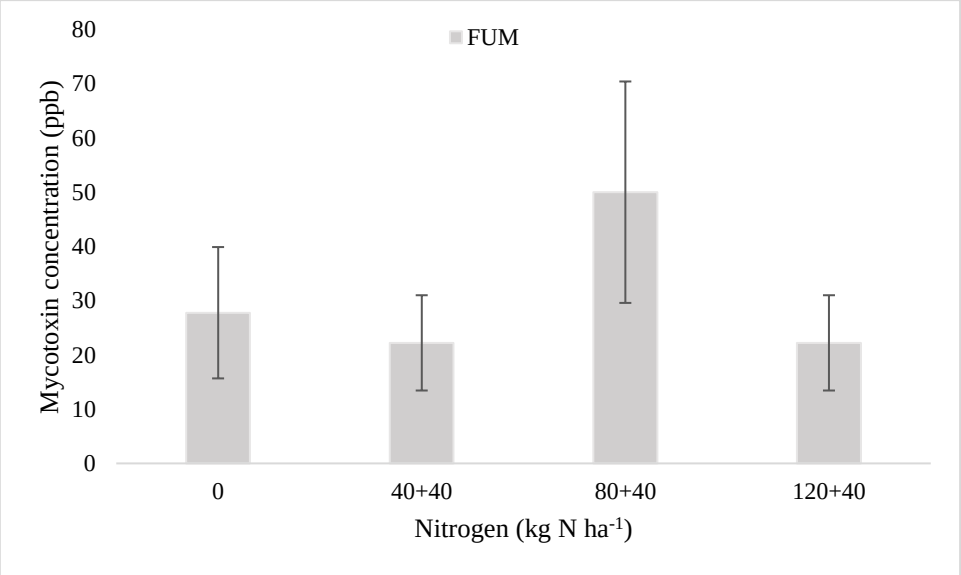

Figure 15. Effect of split dose nitrogen fertilization (kg N ha^{-1}) on mycotoxin concentration (ppb) in 2021

In 2021, the increasing split doses of nitrogen fertilization did not show a statistically significant effect on *Fusarium* infection (F = 0.394, P = 0.758) and mycotoxin production (FUM, F = 0.975, P = 0.417). There was no statistically significant difference in *Fusarium* infection and mycotoxin contamination between the increasing nitrogen doses. *Fusarium* infection and mycotoxin contamination did not change with the increasing nitrogen doses (Figure 14, 15) (Table 9, 10).

Table 9. Descriptive statistics of *Fusarium* infection (%), DON and FUM concentration (ppb) affected by split dose nitrogen fertilization (kg N ha^{-1}) in 2021

		N	Mean	Std. Deviation	Std. Error	Minimum	Maximum
FUM	0	9	27.78	36.32	12.11	0	100
	40+40	9	22.22	26.35	8.78	0	50
	80+40	9	50.00	61.24	20.41	0	200
	120+40	9	22.22	26.35	8.78	0	50
	Total	36	30.56	40.14	6.69	0	200
Fusarium	0	9	92.89	6.01	2.00	84	100
	40+40	9	94.89	6.94	2.31	80	100
	80+40	9	92.67	7.62	2.54	76	100
	120+40	9	91.78	3.93	1.31	86	96
	Total	36	93.06	6.11	1.02	76	100

0: no nitrogen
40 + 40: the first nitrogen dose was 40 kg N ha^{-1} and the second was 40 kg N ha^{-1}
80 + 40: the first nitrogen dose was 80 kg N ha^{-1} and the second was 40 kg N ha^{-1}
120 + 40: the first nitrogen dose was 120 kg N ha^{-1} and the second was 40 kg N ha^{-1}

Table 10. Analysis of variance for *Fusarium* infection and DON, FUM concentration affected by split dose nitrogen fertilization in 2021

		Sum of Squares	df	Mean Square	F	Sig.
FUM	Between Groups	4722.222	3	1574.074	.975	.417
	Within Groups	51666.667	32	1614.583		
	Total	56388.889	35			
Fusarium	Between Groups	46.556	3	15.519	.394	.758
	Within Groups	1261.333	32	39.417		
	Total	1307.889	35			

df: degree of freedom; Sig.: significance; Significance level: $P < 0.05$

4.1.2 Effect of wheat variety

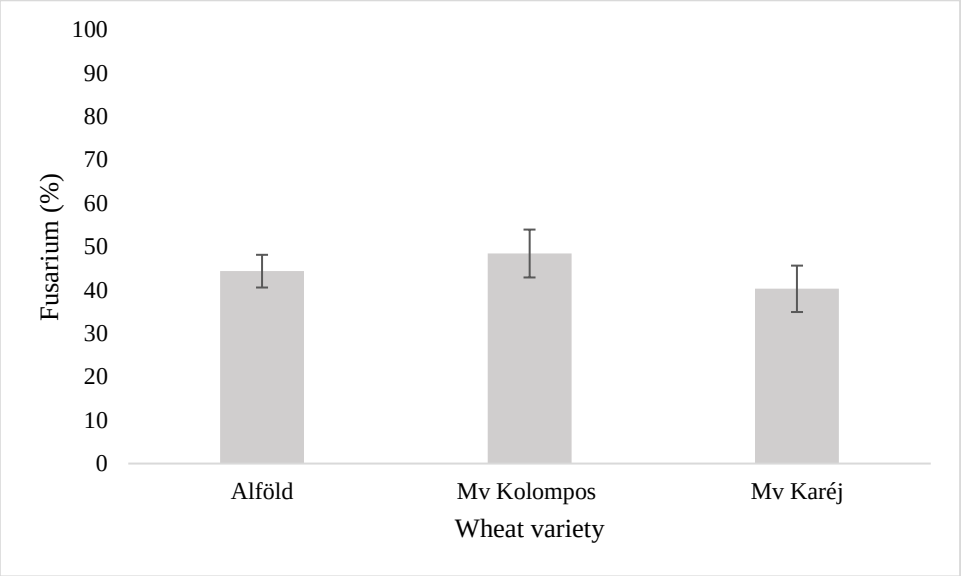

Figure 16. Effect of wheat variety on *Fusarium* infection (%) in single dose nitrogen fertilization in 2020

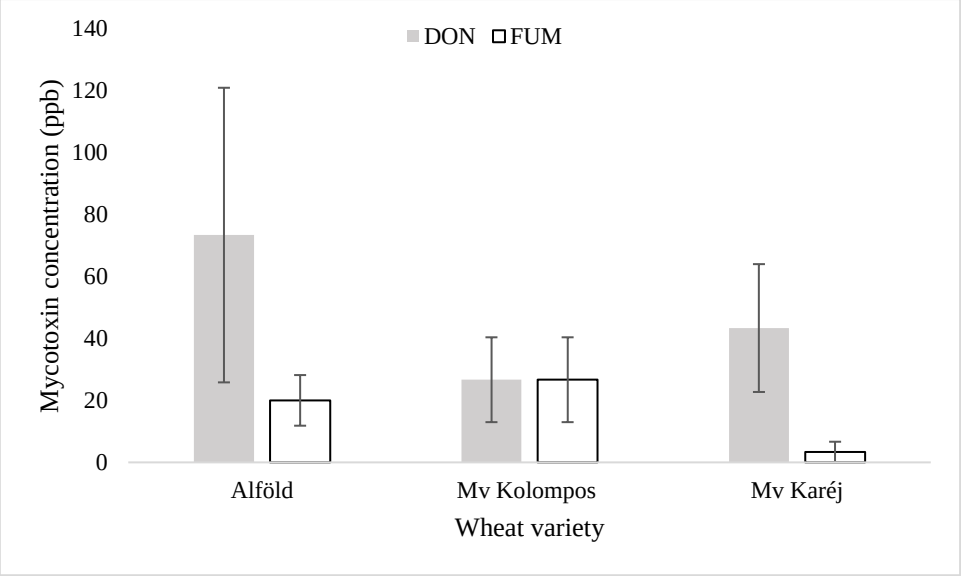

Figure 17. Effect of wheat variety on mycotoxin concentration (ppb) in single dose nitrogen fertilization in 2020

In 2020, wheat variety did not show a statistically significant effect *Fusarium* infection (F = 0.677, P = 0.513) and mycotoxin production (DON, F = 0.584, P = 0.562; FUM, F = 1.635, P = 0.207). There was no statistically significant difference in *Fusarium* infection and mycotoxin contamination between Mv Kolompos, Mv Karéj and Alföld wheat varieties. *Fusarium* infection and mycotoxin contamination did not change between the wheat varieties (Figure 16, 17) (Table 11, 12).

Table 11. Descriptive statistics of *Fusarium* infection (%), DON and FUM concentration (ppb) affected by wheat variety in 2020

		N	Mean	Std. Deviation	Std. Error	Minimum	Maximum
DON	Alföld	15	73.33	184.07	47.53	0	550
	Mv Kolompos	15	26.67	53.00	13.69	0	150
	Mv Karéj	15	43.33	79.88	20.63	0	250
	Total	45	47.78	118.68	17.69	0	550
FUM	Alföld	15	20.00	31.62	8.16	0	100
	Mv Kolompos	15	26.67	53.00	13.69	0	200
	Mv Karéj	15	3.33	12.91	3.33	0	50
	Total	45	16.67	36.93	5.50	0	200
Fusarium	Alföld	15	44.33	14.60	3.77	20	66
	Mv Kolompos	15	48.40	21.37	5.52	12	82
	Mv Karéj	15	40.27	20.71	5.35	12	88
	Total	45	44.33	19.00	2.83	12	88

Table 12. Analysis of variance for *Fusarium* infection and DON, FUM concentration affected by wheat variety in 2020

		Sum of Squares	df	Mean Square	F	Sig.
DON	Between Groups	16777.778	2	8388.889	.584	.562
	Within Groups	603000.000	42	14357.143		
	Total	619777.778	44			
FUM	Between Groups	4333.333	2	2166.667	1.635	.207
	Within Groups	55666.667	42	1325.397		
	Total	60000.000	44			
Fusarium	Between Groups	496.133	2	248.067	.677	.513
	Within Groups	15383.867	42	366.283		
	Total	15880.000	44			

df: degree of freedom; Sig.: significance; Significance level: $P < 0.05$

Figure 18. Effect of wheat variety on *Fusarium* infection (%) in single dose nitrogen fertilization in 2021

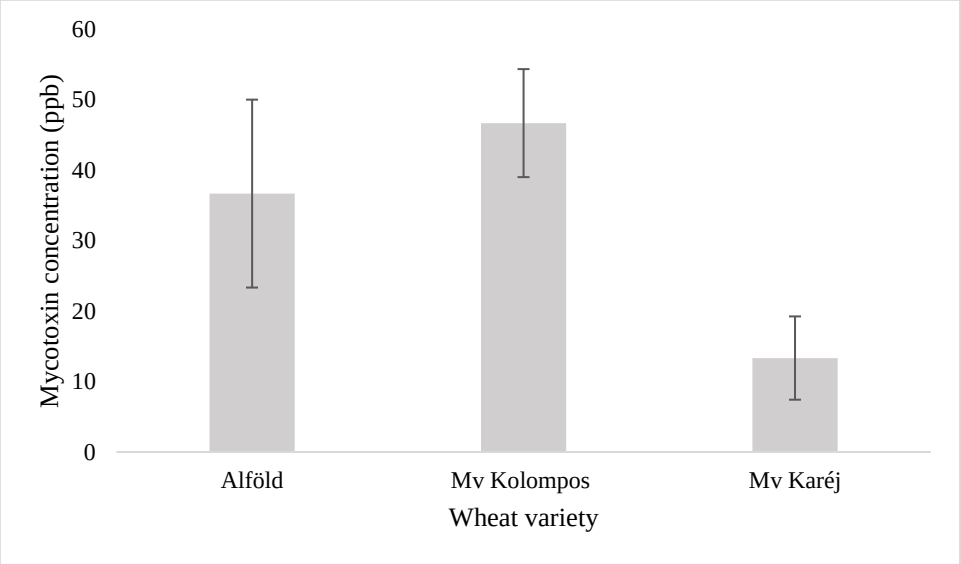

Figure 19. Effect of wheat variety on mycotoxin concentration (ppb) in single dose nitrogen fertilization in 2021

In 2021, wheat variety did not show a statistically significant effect on *Fusarium* infection (F = 1.796, P = 0.178) but it showed a statistically significant effect on fumonisin production (FUM, F = 3.234, P = 0.049). Mv Kolompos had the highest fumonisin concentration (46.67 ppb) followed by Alföld (36.67 ppb) and then Mv Karéj (13.33 ppb) (Figure 18, 19) (Table 13, 14).

Table 13. Descriptive statistics of *Fusarium* infection (%), DON and FUM concentration (ppb) affected by wheat variety in 2021

		N	Mean	Std. Deviation	Std. Error	Minimum	Maximum
FUM	Alföld	15	36.67	51.64	13.33	0	200
	Mv Kolompos	15	46.67	29.68	7.66	0	100
	Mv Karéj	15	13.33	22.89	5.91	0	50
	Total	45	32.22	38.66	5.76	0	200
Fusarium	Alföld	15	91.47	5.68	1.47	80	100
	Mv Kolompos	15	94.00	6.28	1.62	84	100
	Mv Karéj	15	95.20	4.39	1.13	88	100
	Total	45	93.56	5.61	0.84	80	100

Table 14. Analysis of variance for *Fusarium* infection and DON, FUM concentration affected by wheat variety in 2021

		Sum of Squares	df	Mean Square	F	Sig.
FUM	Between Groups	8777.778	2	4388.889	3.234	.049
	Within Groups	57000.000	42	1357.143		
	Total	65777.778	44			
Fusarium	Between Groups	108.978	2	54.489	1.796	.178
	Within Groups	1274.133	42	30.337		
	Total	1383.111	44			

df: degree of freedom; Sig.: significance; Significance level: $P < 0.05$

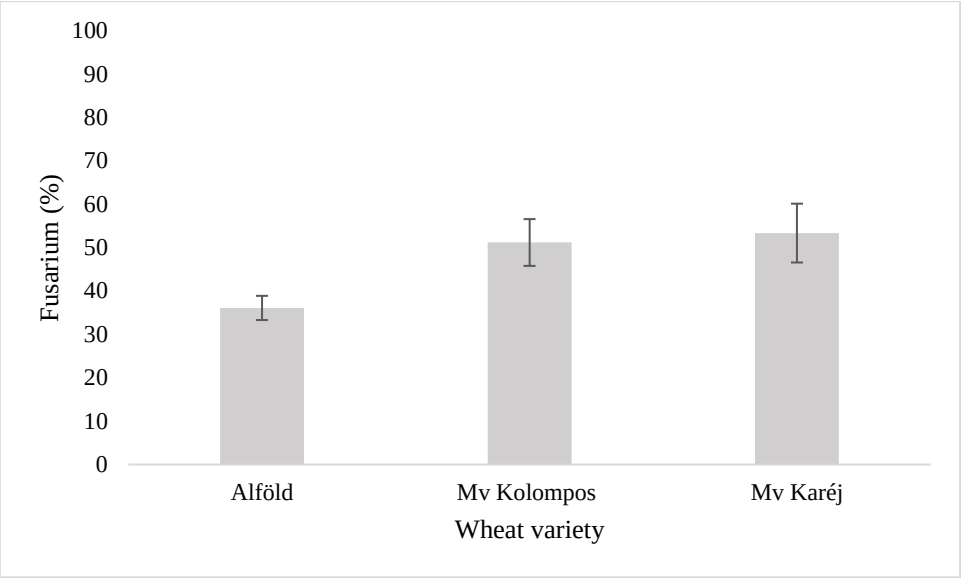

Figure 20. Effect of wheat variety on *Fusarium* infection (%) in split dose nitrogen fertilization in 2020

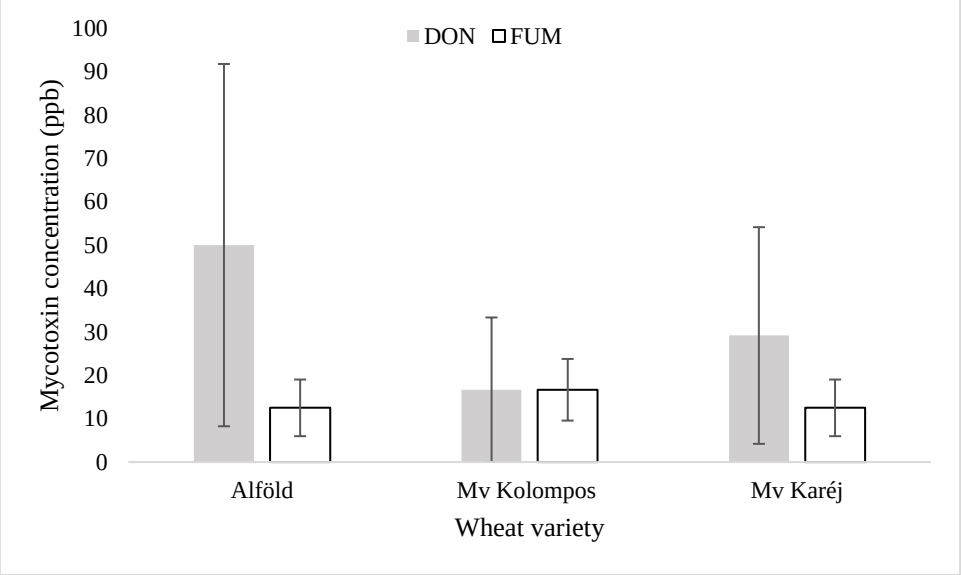

Figure 21. Effect of wheat variety on mycotoxin concentration (ppb) in split dose nitrogen fertilization in 2020

In 2020, wheat variety did not show a statistically significant effect on *Fusarium* infection (F = 3.2, P = 0.054) and mycotoxin production (DON, F = 0.322, P = 0.727; FUM, F = 0.128, P = 0.88). There was no statistically significant difference in *Fusarium* infection and mycotoxin contamination between Mv Kolompos, Mv Karéj and Alföld wheat varieties. *Fusarium* infection and mycotoxin contamination did not change between the wheat varieties (Figure 20, 21) (Table 15, 16).

Table 15. Descriptive statistics of *Fusarium* infection (%), DON and FUM concentration (ppb) affected by wheat variety in 2020

		N	Mean	Std. Deviation	Std. Error	Minimum	Maximum
DON	Alföld	12	50.00	144.60	41.74	0	500
	Mv Kolompos	12	16.67	57.74	16.67	0	200
	Mv Karéj	12	29.17	86.49	24.97	0	300
	Total	36	31.94	100.82	16.80	0	500
FUM	Alföld	12	12.50	22.61	6.53	0	50
	Mv Kolompos	12	16.67	24.62	7.11	0	50
	Mv Karéj	12	12.50	22.61	6.53	0	50
	Total	36	13.89	22.71	3.79	0	50
Fusarium	Alföld	12	36.08	9.65	2.79	22	52
	Mv Kolompos	12	51.17	18.67	5.39	22	78
	Mv Karéj	12	53.33	23.48	6.78	12	90
	Total	36	46.86	19.31	3.22	12	90

Table 16. Analysis of variance for *Fusarium* infection and DON, FUM concentration affected by wheat variety in 2020

		Sum of Squares	df	Mean Square	F	Sig.
DON	Between Groups	6805.556	2	3402.778	.322	.727
	Within Groups	348958.333	33	10574.495		
	Total	355763.889	35			
FUM	Between Groups	138.889	2	69.444	.128	.880
	Within Groups	17916.667	33	542.929		
	Total	18055.556	35			
Fusarium	Between Groups	2119.056	2	1059.528	3.200	.054
	Within Groups	10927.250	33	331.129		
	Total	13046.306	35			

df: degree of freedom; Sig.: significance; Significance level: $P < 0.05$

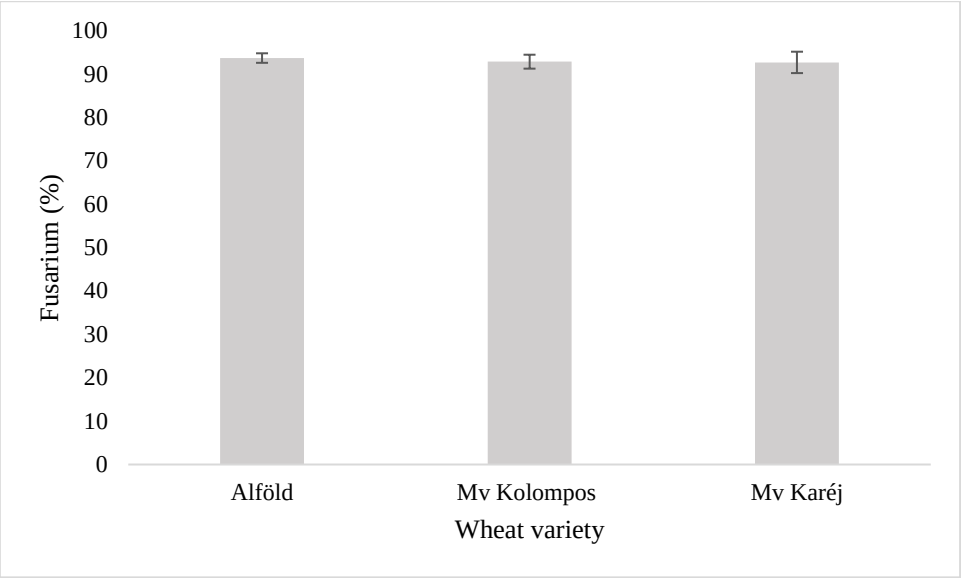

Figure 22. Effect of wheat variety on *Fusarium* infection (%) in split dose nitrogen fertilization in 2021

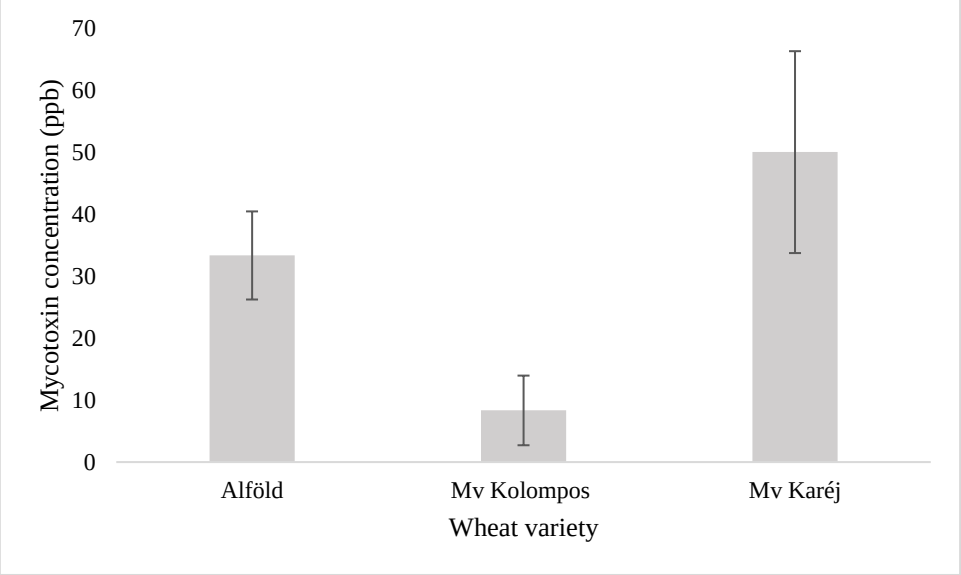

Figure 23. Effect of wheat variety on mycotoxin concentration (ppb) in split dose nitrogen fertilization in 2021

In 2021, wheat variety did not show a statistically significant effect on *Fusarium* infection (F = 0.087, P = 0.917) but it showed a statistically significant effect on fumonisin production (FUM, F = 3.8, P = 0.033). Mv Karéj had the highest fumonisin concentration (50 ppb) followed by Alföld (33.33 ppb) and then Mv Kolompos (8.33 ppb) (Figure 22, 23) (Table 17, 18).

Table 17. Descriptive statistics of *Fusarium* infection (%), DON and FUM concentration (ppb) affected by wheat variety in 2021

		N	Mean	Std. Deviation	Std. Error	Minimum	Maximum
FUM	Alföld	12	33.33	24.62	7.11	0	50
	Mv Kolompos	12	8.33	19.46	5.62	0	50
	Mv Karéj	12	50.00	56.41	16.28	0	200
	Total	36	30.56	40.14	6.69	0	200
Fusarium	Alföld	12	93.67	3.80	1.10	88	100
	Mv Kolompos	12	92.83	5.56	1.60	86	100
	Mv Karéj	12	92.67	8.54	2.47	76	100
	Total	36	93.06	6.11	1.02	76	100

Table 18. Analysis of variance for *Fusarium* infection and DON, FUM concentration affected by wheat variety in 2021

		Sum of Squares	df	Mean Square	F	Sig.
FUM	Between Groups	10555.556	2	5277.778	3.800	.033
	Within Groups	45833.333	33	1388.889		
	Total	56388.889	35			
Fusarium	Between Groups	6.889	2	3.444	.087	.917
	Within Groups	1301.000	33	39.424		
	Total	1307.889	35			

df: degree of freedom; Sig.: significance; Significance level: $P < 0.05$

4.1.3 Effect of growing season

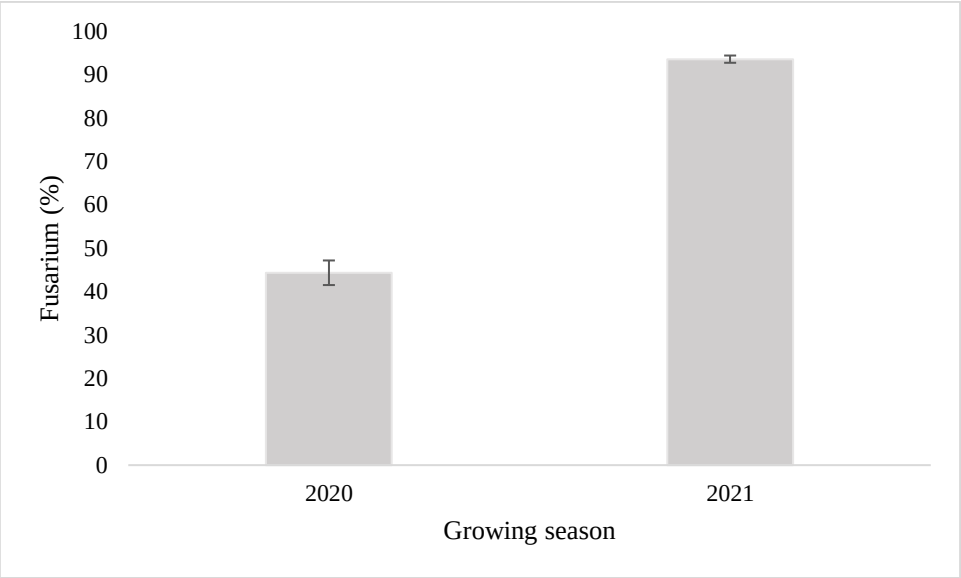

Figure 24. Effect of growing season on *Fusarium* infection (%) in wheat with single dose nitrogen fertilization

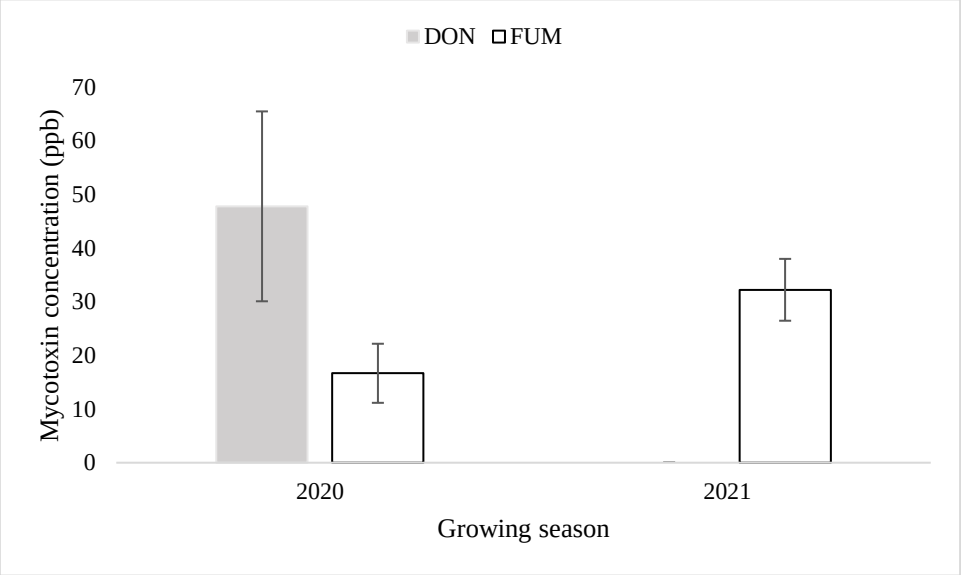

Figure 25. Effect of growing season on mycotoxin concentration (ppb) in wheat with single dose nitrogen fertilization

The growing season in the case of wheat with single dose nitrogen fertilization significantly affected *Fusarium* infection (F = 277.89, P = .000) and mycotoxin production (DON, F = 7.29, P = .008; FUM, F = 3.81, P = 0.05) (Table 20). *Fusarium* infection was higher in 2021 (93.56 %) than in 2020 (44.33 %) (Table 19). Zearalenone was not detected throughout the two growing seasons. Fumonisins concentration (total mean = 24.44 ppb) was higher than that of deoxynivalenol (total mean = 23.89 ppb). Deoxynivalenol was not detected in 2021, its concentration was 47.78 ppb in 2020. Fumonisins concentration was higher in 2021 (32.22 ppb) than in 2020 (16.67 ppb) (Table 19) (Figure 24, 45).

Table 19. Descriptive statistics of *Fusarium* infection (%), DON and FUM concentration (ppb) affected by growing season in wheat with single dose nitrogen fertilization

		N	Mean	Std. Deviation	Std. Error	Minimum	Maximum
DON	2020	45	47.78	118.68	17.69	0	550
	2021	45	0	0	0	0	0
	Total	90	23.89	86.84	9.15	0	550
FUM	2020	45	16.67	36.93	5.50	0	200
	2021	45	32.22	38.66	5.76	0	200
	Total	90	24.44	38.40	4.05	0	200
Fusarium	2020	45	44.33	19.00	2.83	12	88
	2021	45	93.56	5.61	0.84	80	100
	Total	90	68.94	28.40	2.99	12	100

Table 20. Analysis of variance for *Fusarium* infection and DON, FUM concentration affected by growing season in wheat with single dose nitrogen fertilization

		Sum of Squares	df	Mean Square	F	Sig.
DON	Between Groups	51361.11	1	51361.11	7.29	.008
	Within Groups	619777.78	88	7042.93		
	Total	671138.89	89			
FUM	Between Groups	5444.44	1	5444.44	3.81	.05
	Within Groups	125777.78	88	1429.29		
	Total	131222.22	89			
Fusarium	Between Groups	54513.61	1	54513.61	277.89	.000
	Within Groups	17263.11	88	196.17		
	Total	71776.72	89			

df: degree of freedom; Sig.: significance; Significance level: $P < 0.05$

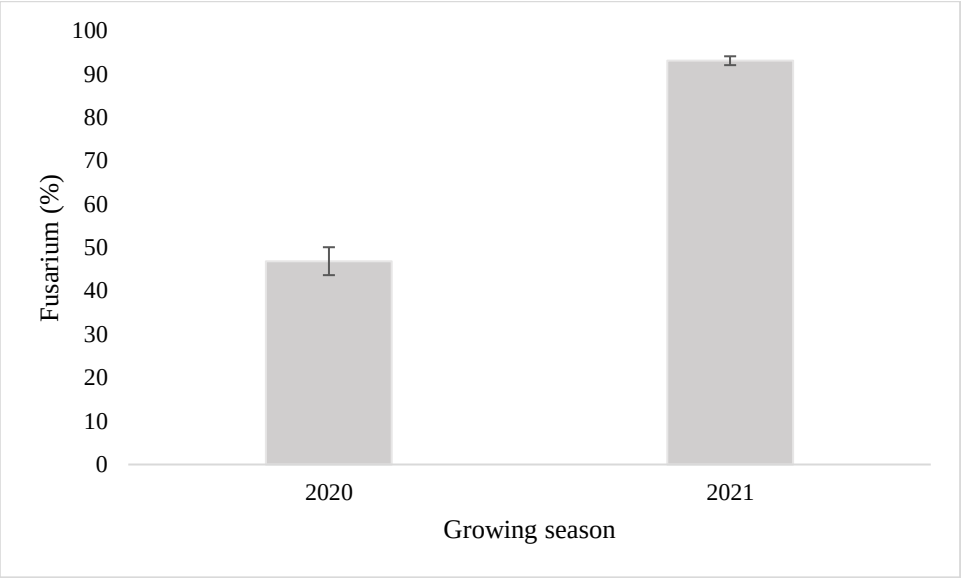

Figure 26. Effect of growing season on *Fusarium* infection (%) in wheat with split dose nitrogen fertilization

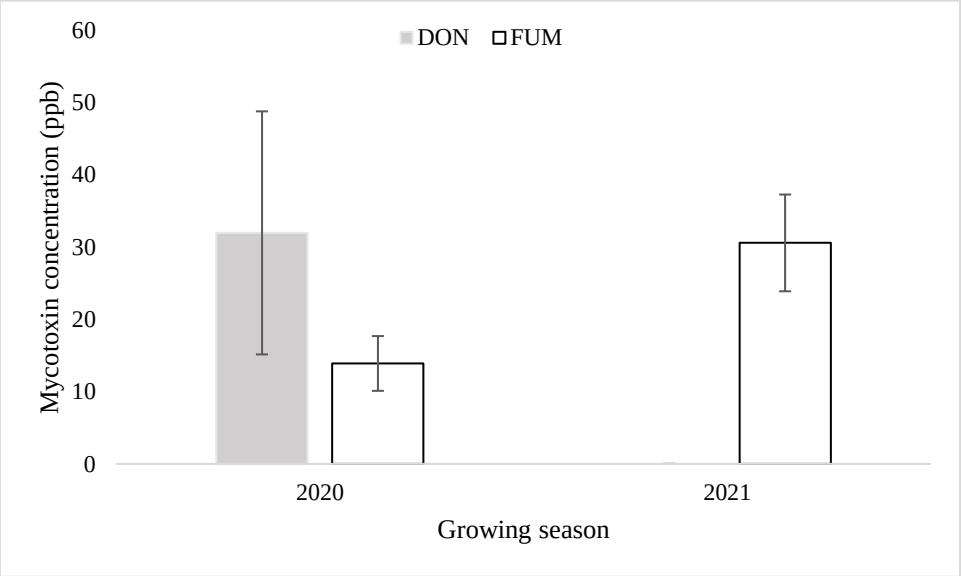

Figure 27. Effect of growing season on mycotoxin concentration (ppb) in wheat with split dose nitrogen fertilization

The growing season in the case of wheat with split dose nitrogen fertilization significantly affected *Fusarium* infection (F = 187.31, P = 0.000) and fumonisins concentration (F = 4.7, P = 0.03) but did not significantly affect deoxynivalenol concentration (F = 3.61, P = 0.06) (Table 22). *Fusarium* infection was higher in 2021 (93.1 %) than in 2020 (46.9 %). Zearalenone was not detected throughout the two growing seasons. Fumonisins concentration (total mean = 22.2 ppb) was higher than that of deoxynivalenol (total mean = 15.97 ppb). Deoxynivalenol was not detected in 2021, its concentration was 31.9 ppb in 2020. Fumonisins concentration was higher in 2021 (30.6 ppb) than in 2020 (13.9 ppb) (Table 21) (Figure 26, 27).

Table 21. Descriptive statistics of *Fusarium* infection (%), DON and FUM concentration (ppb) affected by growing season in wheat with split dose nitrogen fertilization

	Growing season	Mean	Std. Deviation	Std. Error	Minimum	Maximum
DON	2020	31.94	100.82	16.8	0	500
	2021	0	0	0	0	0
	Total	15.97	72.59	8.55	0	500
FUM	2020	13.89	22.71	3.79	0	50
	2021	30.56	40.14	6.69	0	200
	Total	22.22	33.45	3.94	0	200
Fusarium	2020	46.86	19.31	3.22	12	90
	2021	93.06	6.11	1.02	76	100
	Total	69.96	27.26	3.21	12	100

Table 22. Analysis of variance for *Fusarium* infection and DON, FUM concentration affected by growing season in wheat with split dose nitrogen fertilization

	Source of variation	Sum of Squares	df	Mean Square	F	Sig.
DON	Between Groups	18368.06	1	18368.06	3.61	0.06
	Within Groups	355763.89	70	5082.34		
	Total	374131.94	71			
FUM	Between Groups	5000.00	1	5000.00	4.70	0.03
	Within Groups	74444.44	70	1063.49		
	Total	79444.44	71			
Fusarium	Between Groups	38410.68	1	38410.68	187.31	0.00
	Within Groups	14354.19	70	205.06		
	Total	52764.88	71			

df: degree of freedom; Sig.: significance; Significance level: $P < 0.05$

The study of the influence of growing season, wheat variety, and nitrogen fertilization on *Fusarium* infection and subsequent mycotoxin production in wheat kernel was carried out in 2020, 2021.

In our study, the different climatic conditions that prevailed during 2020/2021 could be the reason for the increase in *Fusarium* infection and mycotoxin concentration. The results of this study confirm the results of Schaafsma et al. (2001), who ascertained from a four year-survey that the climatical/weather conditions and the variety are the factors associated with variation in *Fusarium* and mycotoxin levels in wheat grain. Rainfall (mm) measurements were collected from World Weather Online® meteorological service during the flowering period (May) when wheat is most susceptible to *Fusarium* infection. Rainfall during the flowering period (May) in 2021 was 88.39 mm, higher than in 2020 (42.8 mm), this increase in rainfall could explain the increased *Fusarium* infection and mycotoxin concentration. *Fusarium* Head Blight is a disease that poses a serious threat to cereal crops like wheat (Kelly et al., 2015). *Fusarium* infection and mycotoxin contamination cause economic losses because they reduce production and grain quality (Shephard, 2008; Berthiller et al., 2009; Zain, 2011). Wheat heads are most susceptible to *Fusarium* Head blight infection at anthesis, but infection can occur up to the soft dough stage (Lacey et al., 1999; Windels, 1999). Environmental conditions such as precipitation, temperature and humidity in the atmosphere are major factors modulating *Fusarium* infection. There is a consensus that warm and wet conditions at anthesis favor *Fusarium* infection (Berthiller et al., 2009; Kriss et al., 2010; Doohan et al., 2003). Wetness periods of at least 24 h and temperatures above 15 °C are required for successful infections by most of the FHB causing agents (Berthiller et al., 2009; Doohan et al., 2003). Environmental conditions at anthesis promote *Fusarium* infection and mycotoxin contamination (Dufault et al., 2006; Gilbert et al., 2008). In general, precipitation during anthesis is favorable for wheat contamination by *Fusarium spp.* (De Wolf et al., 2003; Fedak et al., 2007; Visconti and Pascale, 2010). Humidity, precipitation, and temperature play an important role in the development of FHB, the production and dispersal of the inoculum and the infection of wheat heads (Brennan et al., 2003). Bryła et al. (2016) also stated that the development, growth, and spread of *Fusarium* fungi and the degree of infection strongly depend on rainfall. According to Czaban et al. (2015) wheat kernel infection by *Fusarium spp.* depends primarily on weather conditions and then on wheat genotype. Covarelli et al., 2015 and Kelly et al., 2015 reported that climatic conditions, especially during wheat anthesis, affect *Fusarium* infection level. Bernhoft et al. (2012) also reported that climatic factors explained the variation in the levels of *Fusarium* infection and mycotoxin contamination in wheat. Mesterházy et al. (1999) pointed out that climatic conditions may play an important role in *Fusarium* infection of wheat. González et al. (2008) suggested that the relatively high level of natural *Fusarium* contamination could be due to a high rainfall period that occurred during the flowering stage. Various studies suggest weather conditions, plant development, and genetic or morphological cultivar characteristics as factors influencing the epidemiology of *Fusarium* species (Osborne and Stein, 2007). The risk of FHB in wheat plants depends also on genetically determined resistance of the given wheat cultivar to *Fusarium spp.* (Zhang et al., 2008). According to these authors, the main factors affecting *Fusarium* contamination of wheat were weather conditions and susceptibility of wheat cultivars to *Fusarium spp.*

Agronomic practices such as tillage, crop rotation, cultivar choice, and chemical or biological control are discussed as keys to disease prevention (Wegulo et al., 2015). Nitrogen (N) fertilization is also considered to affect FHB. In our study, nitrogen dosage did not influence *Fusarium*

contamination and mycotoxin production. However, recent research has produced conflicting results. Some studies found an increasing effect of FHB with increased nitrogen supply (Lemmens et al., 2004, Ma et al., 2004, Muhammed et al., 2010), whereas others reported decreasing nitrogen effects on FHB (Obst et al., 2002, Yang et al., 2010). Other studies could not detect any nitrogen influence (Aufhammer et al., 2000, Fauzi and Paulitz, 1994, Teich and Hamilton, 1985) or generated inconsistent data (Heier et al., 2005, Subedi et al., 2007). Krnjaja et al. (2015) found that nitrogen fertilization did not increase FHB intensity. Kuzdralinski et al. (2014) reported that the rate of autumn N fertilization did not affect the number of *Fusarium* detections. Bernhoft et al. (2012) concluded that farming system (organic versus conventional) impacted *Fusarium* infestation, and that organic management tended to reduce *Fusarium* contamination and mycotoxins. However, *Fusarium* infestation and mycotoxin concentrations may be influenced by a range of factors such as local topography and local climate. Oldenburg et al. (2007) concluded that nitrogen rates of up to 240 kg N ha^{-1} did not influence *Fusarium* growth and their production of mycotoxins in wheat grains. According to Parry et al. (1995), the impact of nitrogen fertilization on *Fusarium* infestation remains unclear. Moreover, Aufhammer et al. (2000) concluded that nitrogen fertilization did not stimulate *Fusarium* infection and mycotoxin production. In addition, Martin et al. (1991) observed that nitrogen rates increasing from 70 to 170 kg N $ha^{-}1$ significantly increased the occurrence of *Fusarium* infected grains in wheat. According to Lemmens et al. (2004), increasing nitrogen fertilization rates up to 80 kg N ha^{-1} significantly affected *Fusarium* infection and subsequent mycotoxin contamination in wheat. However, Lemmens et al. (2004) concluded that the occurrence of *Fusarium spp.* could not be solely based on the nitrogen input in crop production. All these results suggest that the effect of nitrogen fertilization can only partially influence the creation of favorable conditions for the occurrence of *Fusarium spp.*

4.2 Effect of nitrogen fertilization on wheat quality parameters

4.2.1 Effect of nitrogen on thousand kernel weight and test weight

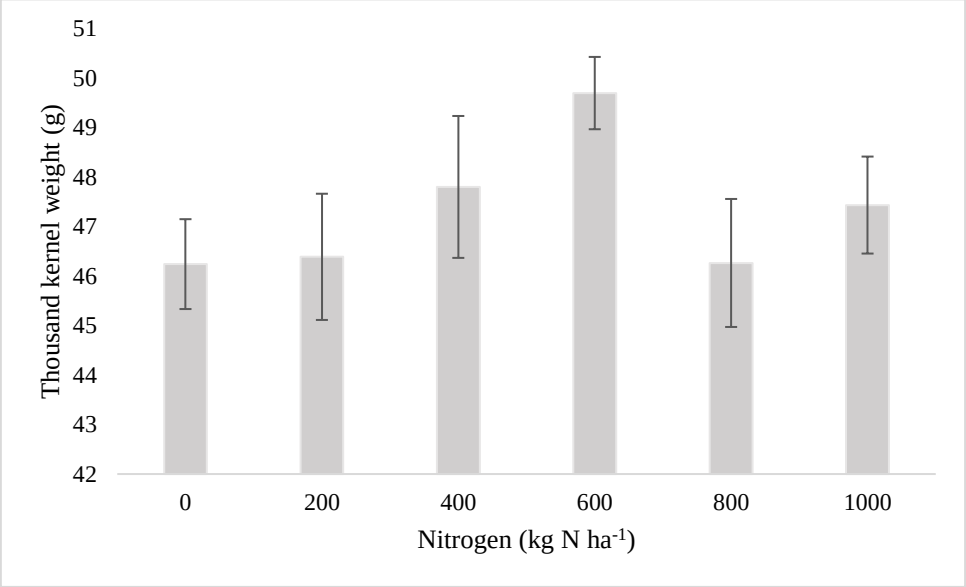

Figure 28. Effect of nitrogen (kg N ha^{-1}) on thousand kernel weight (g) in Mv Ménrót

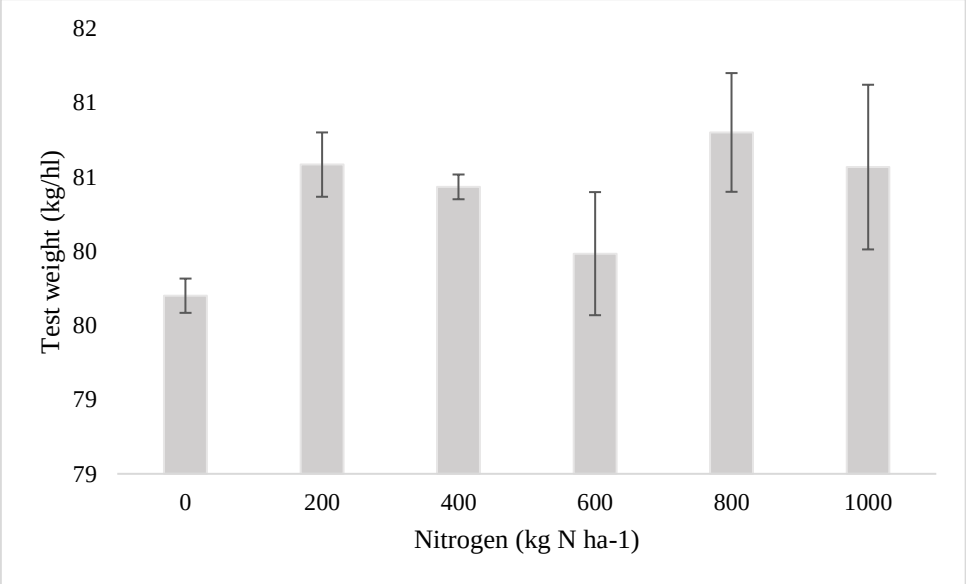

Figure 29. Effect of nitrogen (kg N ha^{-1}) on test weight (kg/hl) in Mv Ménrót

In Mv Ménrót, nitrogen fertilization did not show a significant effect on thousand kernel weight (F = 1.414, P = 0.288) and test weight (F = 1.473, P = 0.269) (Figure 28,29) (Table 24).

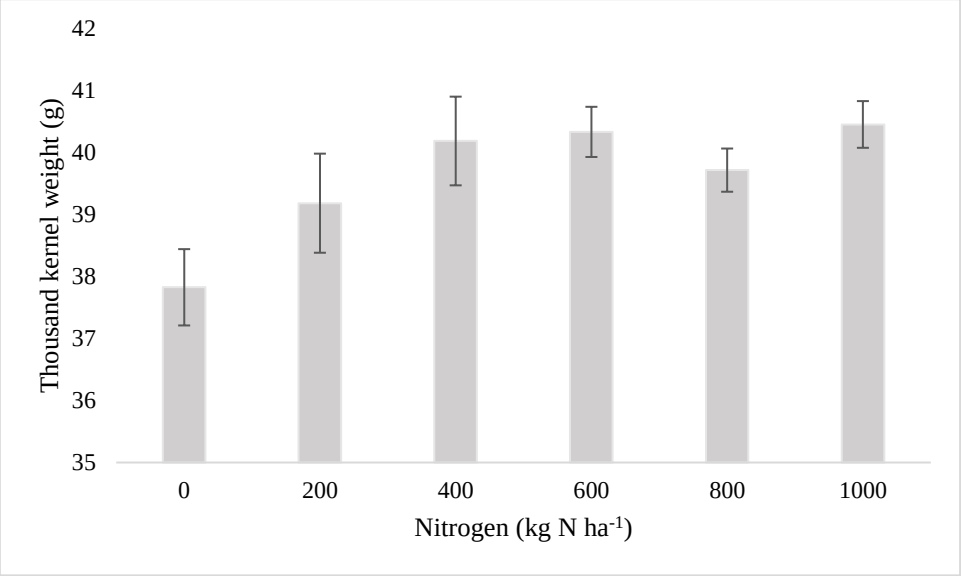

Figure 30. Effect of nitrogen (kg N ha^{-1}) on thousand kernel weight (g) in Alföld

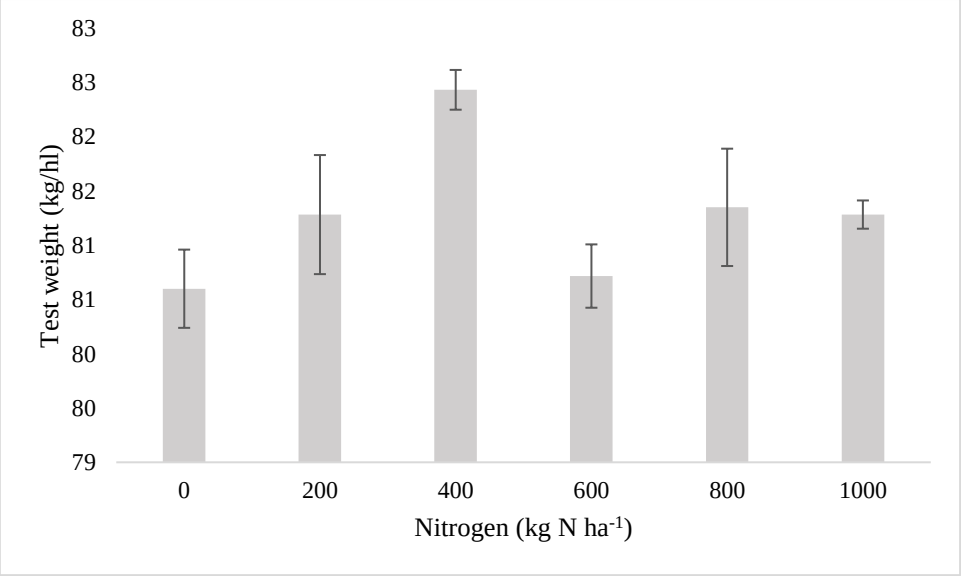

Figure 31. Effect of nitrogen (kg N ha^{-1}) on test weight (kg/hl) in Alföld

In Alföld, nitrogen fertilization did not show a significant effect on thousand kernel weight (F = 3.030, P = 0.054) and test weight (F = 2.953, P = 0.058) (Figure 30,31) (Table 26).

4.2.2 Effect of nitrogen on gluten content

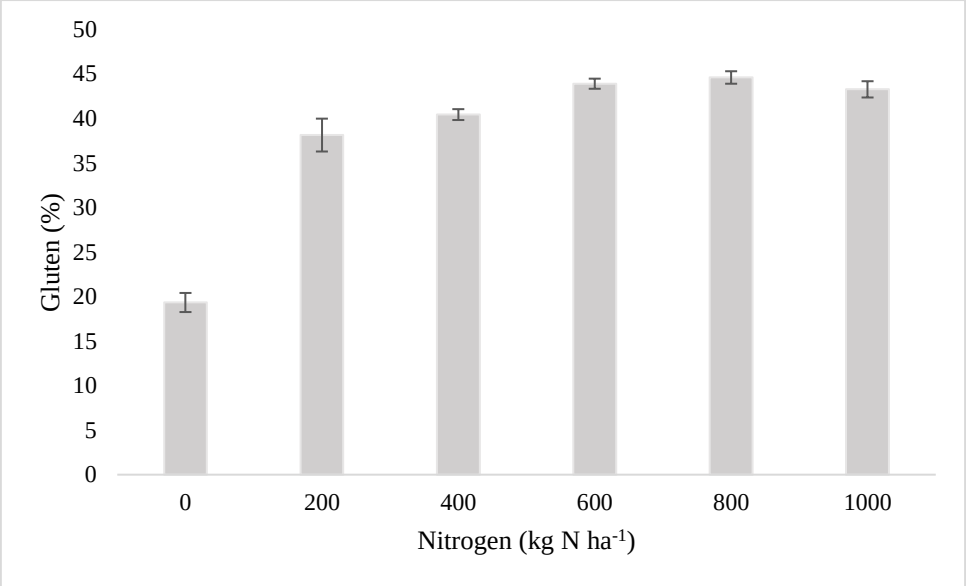

Figure 32. Effect of nitrogen (kg N ha^{-1}) on gluten (%) in Mv Ménrót

In Mv Ménrót, nitrogen fertilization significantly affected gluten content (F = 83.882, P = 0.000) (Table 24). Gluten content was 19.33 % at 0 kg N ha^{-1}, 38.13 % at 200 kg N ha^{-1}, 40.43 % at 400 kg N ha^{-1}, 43.9 % at 600 kg N ha^{-1}, 44.6 % at 800 kg N ha^{-1}, and 43.27 % at 1000 kg N ha^{-1} (Table 23). Gluten content increased with increasing nitrogen dose. Gluten content was the lowest at 0 kg N ha^{-1} followed by 200 kg N ha^{-1} then 400 kg N ha^{-1} and the highest at 600 kg N ha^{-1} (Figure 32).

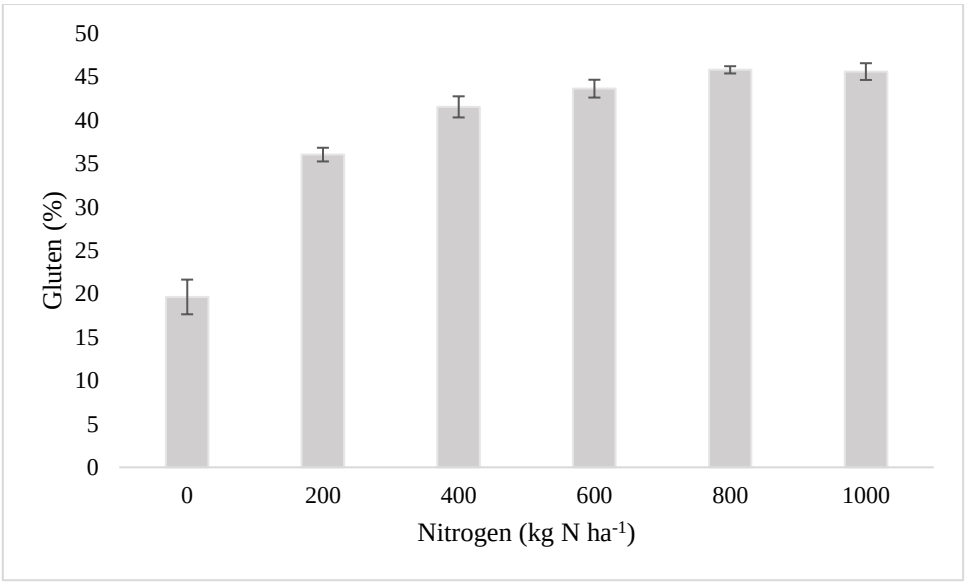

Figure 33. Effect of nitrogen (kg N ha^{-1}) on gluten (%) in Alföld

In Alföld, nitrogen fertilization significantly affected gluten content (F = 72.897, P = 0.000) (Table 26). Gluten content was 19.63 % at 0 kg N ha^{-1}, 36.03 % at 200 kg N ha^{-1}, 41.53 % at 400 kg N ha^{-1}, 43.63 % at 600 kg N ha^{-1}, 45.8 % at 800 kg N ha^{-1}, and 45.6 % at 1000 kg N ha^{-1} (Table 25). Gluten content increased with increasing nitrogen dose. Gluten content was the lowest at 0 kg N ha^{-1} followed by 200 kg N ha^{-1} then 400 kg N ha^{-1} and the highest at 600 kg N ha^{-1} (Figure 33).

4.2.3 Effect of nitrogen on protein content

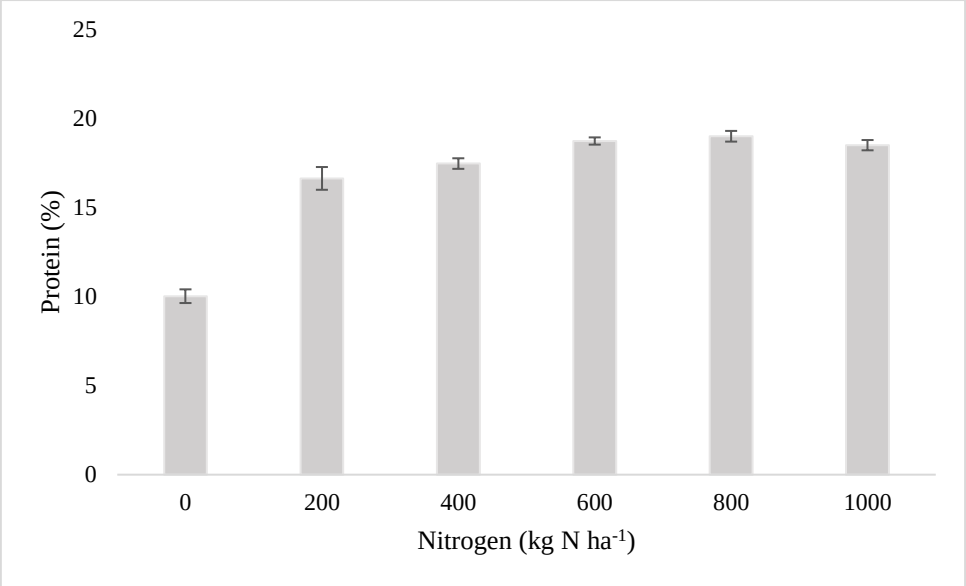

Figure 34. Effect of nitrogen (kg N ha^{-1}) on protein (%) in Mv Ménrót

In Mv Ménrót, nitrogen fertilization significantly affected protein content (F = 80.969, P = 0.000) (Table 24). Protein content was 10.02 % at 0 kg N ha^{-1}, 16.63 % at 200 kg N ha^{-1}, 17.47 % at 400 kg N ha^{-1}, 18.73 % at 600 kg N ha^{-1}, 19 % at 800 kg N ha^{-1}, and 18.5 % at 1000 kg N ha^{-1} (Table 23). Protein content increased with increasing nitrogen dose. Protein content was the lowest at 0 kg N ha^{-1} followed by 200 kg N ha^{-1} then 400 kg N ha^{-1} and the highest at 600 kg N ha^{-1} (Figure 34).

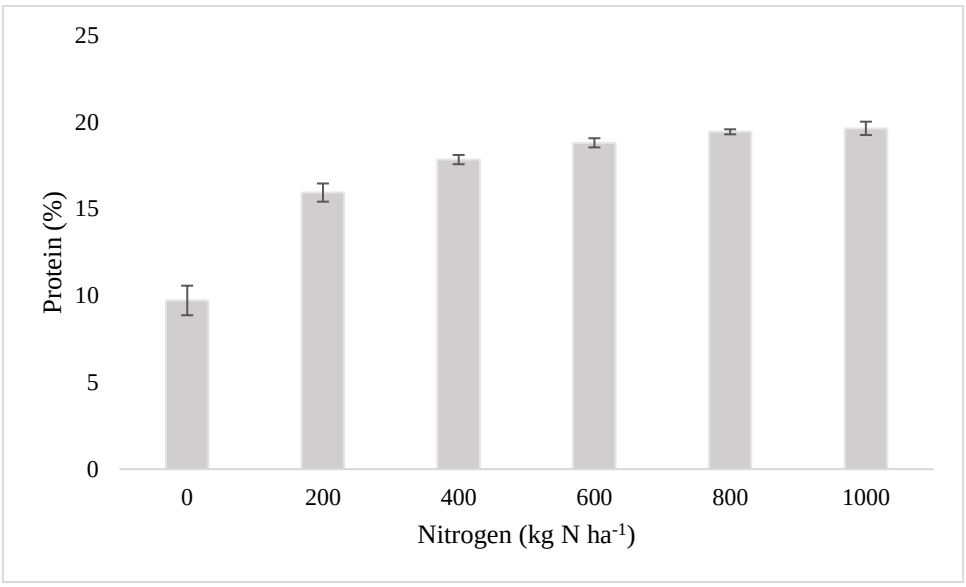

Figure 35. Effect of nitrogen (kg N ha^{-1}) on protein (%) in Alföld

In Alföld, nitrogen fertilization significantly affected protein content (F = 64.941, P = 0.000) (Table 26). Protein content was 9.72 % at 0 kg N ha^{-1}, 15.93 % at 200 kg N ha^{-1}, 17.83 % at 400 kg N ha^{-1}, 18.8 % at 600 kg N ha^{-1}, 19.43 % at 800 kg N ha^{-1}, and 19.63 % at 1000 kg N ha^{-1} (Table 25). Protein content increased with increasing nitrogen dose. Protein content was the lowest at 0 kg N ha^{-1} followed by 200 kg N ha^{-1} then 400 kg N ha^{-1} and the highest at 600 kg N ha^{-1} (Figure 35).

4.2.4 Effect of nitrogen on Zeleny sedimentation index

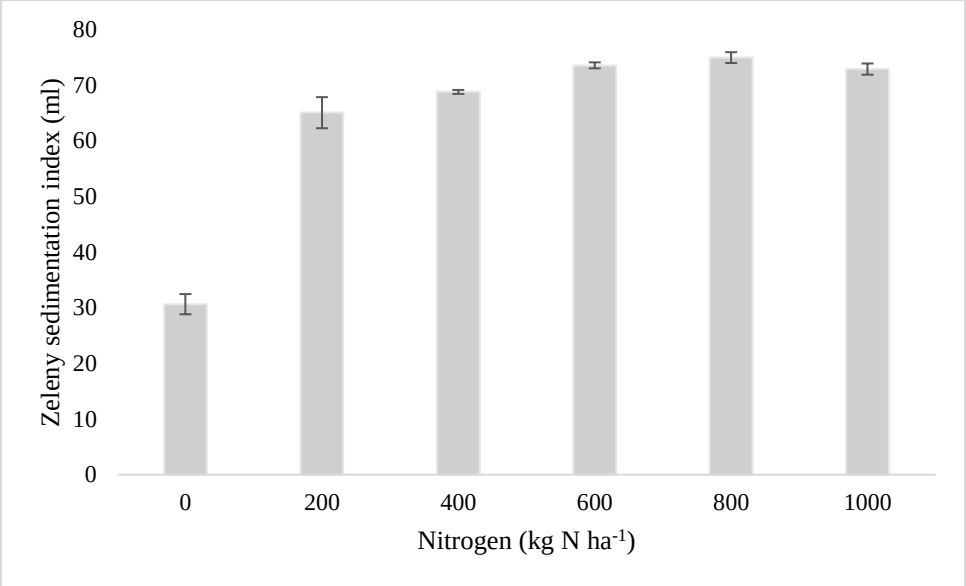

Figure 36. Effect of nitrogen (kg N ha^{-1}) on Zeleny sedimentation index (ml) in Mv Ménrót

In Mv Ménrót, nitrogen fertilization significantly affected Zeleny sedimentation index (F = 127.132, P = 0.000) (Table 24). It was 30.63 ml at 0 kg N ha^{-1}, 65.03 ml at 200 kg N ha^{-1}, 68.76 ml at 400 kg N ha^{-1}, 73.53 ml at 600 kg N ha^{-1}, 74.93 ml at 800 kg N ha^{-1}, and 72.87 ml at 1000 kg N ha^{-1} (Table 23). Zeleny sedimentation index increased with increasing nitrogen dose. It was the lowest at 0 kg N ha^{-1} followed by 200 kg N ha^{-1} then 400 kg N ha^{-1} and the highest at 600 kg N ha^{-1} (Figure 36).

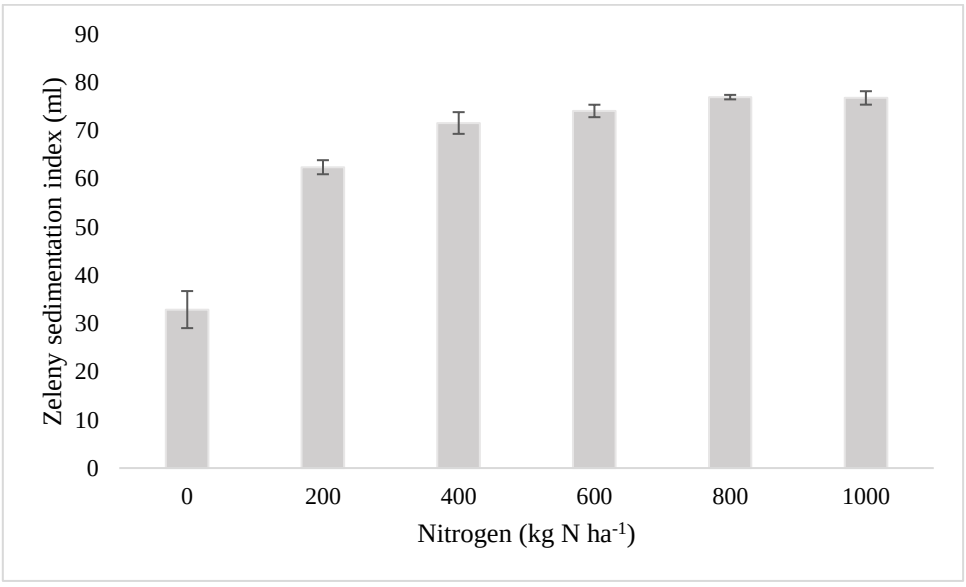

Figure 37. Effect of nitrogen (kg N ha^{-1}) on Zeleny sedimentation index (ml) in Alföld

In Alföld, nitrogen fertilization significantly affected Zeleny sedimentation index (F = 67.123, P = 0.000) (Table 26). It was 32.87 ml at 0 kg N ha^{-1}, 62.40 ml at 200 kg N ha^{-1}, 71.57 ml at 400 kg N ha^{-1}, 74.07 ml at 600 kg N ha^{-1}, 76.93 ml at 800 kg N ha^{-1}, and 76.77 ml at 1000 kg N ha^{-1} (Table 25). Zeleny sedimentation index increased with increasing nitrogen dose. It was the lowest at 0 kg N ha^{-1} followed by 200 kg N ha^{-1} then 400 kg N ha^{-1} and the highest at 600 kg N ha^{-1} (Figure 37).

4.2.5 Effect of nitrogen on falling number

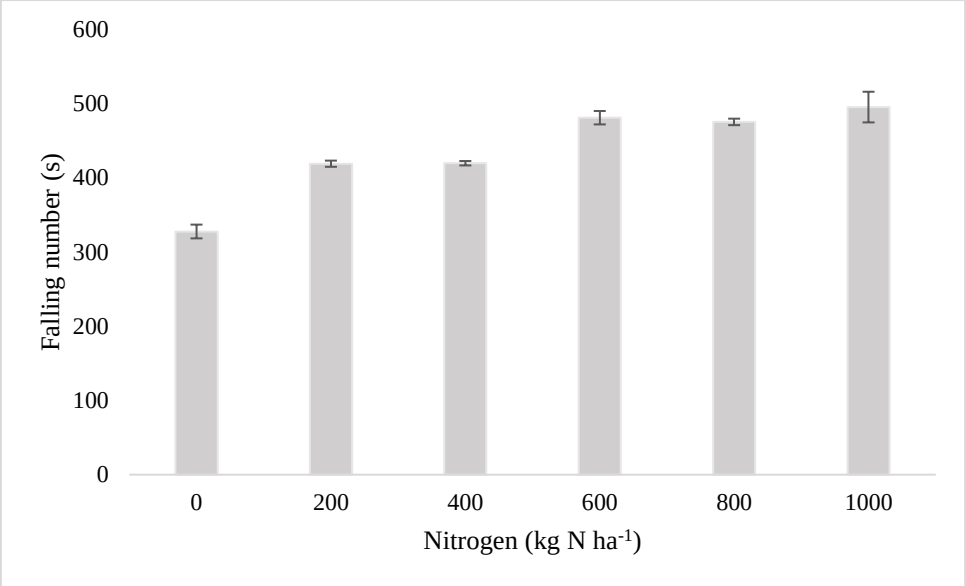

Figure 38. Effect of nitrogen (kg N ha^{-1}) on falling number (s) in Mv Ménrót

In Mv Ménrót, nitrogen fertilization significantly affected the falling number (F = 36.357, P = 0.000) (Table 24). The falling number was 327.67 s at 0 kg N ha^{-1}, 419 s at 200 kg N ha^{-1}, 419.67 s at 400 kg N ha^{-1}, 481 s at 600 kg N ha^{-1}, 475.33 s at 800 kg N ha^{-1}, and 495.33 s at 1000 kg N ha^{-1} (Table 23). The falling number increased with increasing nitrogen dose. It was the lowest at 0 kg N ha^{-1} followed by 200 kg N ha^{-1} then 400 kg N ha^{-1} and the highest at 600 kg N ha^{-1} (Figure 38).

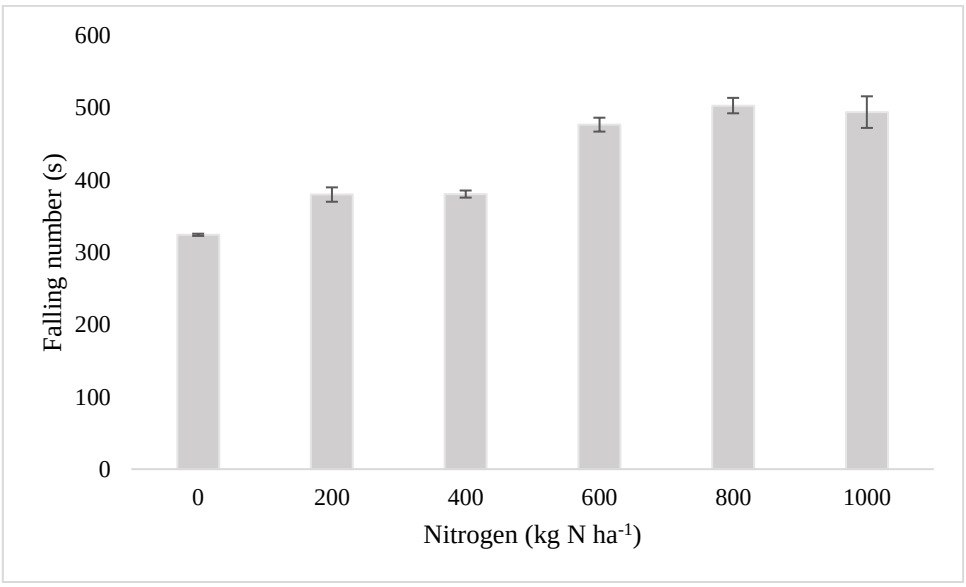

Figure 39. Effect of nitrogen (kg N ha^{-1}) on falling number (s) in Alföld

In Alföld, nitrogen fertilization significantly affected the falling number (F = 40.984, P = 0.000) (Table 26). The falling number was 324 s at 0 kg N ha^{-1}, 379.67 s at 200 kg N ha^{-1}, 380.33 s at 400 kg N ha^{-1}, 476.33 s at 600 kg N ha^{-1}, 502.67 s at 800 kg N ha^{-1}, 493.67 s at 1000 kg N ha^{-1} (Table 25). The falling number increased with increasing nitrogen dose. It was the lowest at 0 kg N ha^{-1} followed by 200 kg N ha^{-1} then 400 kg N ha^{-1} and the highest at 600 kg N ha^{-1} (Figure 39).

Table 23. Descriptive statistics of thousand kernel weight(g), test weight(kg/hl), gluten (%), protein (%), Zeleny sedimentation index (ml) and falling number (s) affected by nitrogen fertilization (kg N ha^{-1}) in Mv Ménrót

		N	Mean	Std. Deviation	Std. Error	Minimum	Maximum
Thousand kernel weight	0	3	46.24	1.57	0.91	45.03	48.02
	200	3	46.39	2.21	1.28	44.05	48.44
	400	3	47.80	2.48	1.43	45.59	50.49
	600	3	49.70	1.27	0.73	48.28	50.71
	800	3	46.27	2.24	1.29	43.68	47.61
	1000	3	47.44	1.70	0.98	45.80	49.19
	Total	18	47.31	2.07	0.49	43.68	50.71
Test weight	0	3	79.70	0.20	0.12	79.50	79.90
	200	3	80.58	0.38	0.22	80.15	80.80
	400	3	80.43	0.14	0.08	80.35	80.60
	600	3	79.98	0.72	0.41	79.45	80.80
	800	3	80.80	0.69	0.40	80.40	81.60
	1000	3	80.57	0.96	0.55	79.70	81.60
	Total	18	80.34	0.63	0.15	79.45	81.60
Gluten	0	3	19.33	1.86	1.07	17.20	20.60
	200	3	38.13	3.20	1.85	35.30	41.60
	400	3	40.43	1.06	0.61	39.30	41.40
	600	3	43.90	0.98	0.57	42.80	44.70
	800	3	44.60	1.21	0.70	43.20	45.30
	1000	3	43.27	1.58	0.91	41.90	45.00
	Total	18	38.28	9.14	2.15	17.20	45.30
Protein	0	3	10.02	0.67	0.38	9.26	10.50
	200	3	16.63	1.11	0.64	15.60	17.80
	400	3	17.47	0.51	0.30	16.90	17.90
	600	3	18.73	0.35	0.20	18.40	19.10
	800	3	19.00	0.52	0.30	18.40	19.30
	1000	3	18.50	0.50	0.29	18.00	19.00
	Total	18	16.73	3.24	0.76	9.26	19.30
Zeleny sedimentation index	0	3	30.63	3.14	1.81	27.10	33.10
	200	3	65.03	4.84	2.79	61.30	70.50
	400	3	68.77	0.61	0.35	68.10	69.30
	600	3	73.53	0.93	0.54	72.90	74.60
	800	3	74.93	1.67	0.97	73.00	75.90
	1000	3	72.87	1.75	1.01	71.10	74.60
	Total	18	64.29	16.01	3.77	27.10	75.90
Falling number	0	3	327.67	16.04	9.26	311.00	343.00
	200	3	419.00	7.21	4.16	411.00	425.00
	400	3	419.67	5.13	2.96	414.00	424.00
	600	3	481.00	15.62	9.02	471.00	499.00
	800	3	475.33	7.57	4.37	470.00	484.00
	1000	3	495.33	35.81	20.67	454.00	517.00
	Total	18	436.33	60.39	14.23	311.00	517.00

0: no nitrogen - 200: the nitrogen dose was 200 kg N ha^{-1} - 400: the nitrogen dose was 400 kg N ha^{-1} - 600: the nitrogen dose was 600 kg N ha^{-1} - 800: the nitrogen dose was 800 kg N ha^{-1} - 1000: the nitrogen dose was 1000 kg N ha^{-1}

Table 24. Analysis of variance for thousand kernel weight, test weight, gluten, protein, Zeleny sedimentation index and falling number affected by nitrogen fertilization in Mv Ménrót

		Sum of Squares	df	Mean Square	F	Sig.
Thousand kernel weight	Between Groups	27.133	5	5.427	1.414	.288
	Within Groups	46.062	12	3.838		
	Total	73.194	17			
Test weight	Between Groups	2.603	5	.521	1.473	.269
	Within Groups	4.242	12	.353		
	Total	6.844	17			
Gluten	Between Groups	1380.084	5	276.017	83.882	.000
	Within Groups	39.487	12	3.291		
	Total	1419.571	17			
Protein	Between Groups	173.625	5	34.725	80.969	.000
	Within Groups	5.146	12	.429		
	Total	178.772	17			
Zeleny sedimentation index	Between Groups	4276.929	5	855.386	127.132	.000
	Within Groups	80.740	12	6.728		
	Total	4357.669	17			
Falling number	Between Groups	58151.333	5	11630.267	36.357	.000
	Within Groups	3838.667	12	319.889		
	Total	61990.000	17			

df: degree of freedom; Sig.: significance; Significance level: $P < 0.05$

Table 25. Descriptive statistics of thousand kernel weight(g), test weight (kg/hl), gluten (%), protein (%), Zeleny sedimentation index (ml) and falling number (s) affected by nitrogen fertilization (kg N ha^{-1}) in Alföld

		N	Mean	Std. Deviation	Std. Error	Minimum	Maximum
Thousand kernel weight	0	3	37.83	1.07	0.62	36.66	38.75
	200	3	39.18	1.38	0.80	38.06	40.73
	400	3	40.19	1.24	0.72	39.01	41.48
	600	3	40.33	0.70	0.40	39.55	40.90
	800	3	39.72	0.60	0.35	39.02	40.08
	1000	3	40.45	0.65	0.38	39.70	40.85
	Total	18	39.62	1.25	0.29	36.66	41.48
Test weight	0	3	80.60	0.62	0.36	79.90	81.10
	200	3	81.28	0.95	0.55	80.35	82.25
	400	3	82.43	0.32	0.18	82.25	82.80
	600	3	80.72	0.51	0.29	80.40	81.30
	800	3	81.35	0.94	0.54	80.60	82.40
	1000	3	81.28	0.23	0.13	81.05	81.50
	Total	18	81.28	0.82	0.19	79.90	82.80
Gluten	0	3	19.63	3.46	2.00	16.60	23.40
	200	3	36.03	1.37	0.79	35.10	37.60
	400	3	41.53	2.11	1.22	39.20	43.30
	600	3	43.63	1.78	1.03	41.60	44.90
	800	3	45.80	0.72	0.42	45.20	46.60
	1000	3	45.60	1.67	0.96	44.10	47.40
	Total	18	38.71	9.56	2.25	16.60	47.40
Protein	0	3	9.72	1.48	0.85	8.38	11.30
	200	3	15.93	0.91	0.52	15.10	16.90
	400	3	17.83	0.46	0.27	17.30	18.10
	600	3	18.80	0.46	0.26	18.30	19.20
	800	3	19.43	0.25	0.15	19.20	19.70
	1000	3	19.63	0.67	0.38	19.20	20.40
	Total	18	16.89	3.60	0.85	8.38	20.40
Zeleny sedimentation index	0	3	32.87	6.65	3.84	26.10	39.40
	200	3	62.40	2.52	1.46	60.30	65.20
	400	3	71.57	3.90	2.25	67.70	75.50
	600	3	74.07	2.24	1.29	71.50	75.60
	800	3	76.93	0.80	0.46	76.10	77.70
	1000	3	76.77	2.42	1.40	74.20	79.00
	Total	18	65.77	16.23	3.83	26.10	79.00
Falling number	0	3	324.00	2.65	1.53	321.00	326.00
	200	3	379.67	17.21	9.94	360.00	392.00
	400	3	380.33	8.50	4.91	372.00	389.00
	600	3	476.33	16.65	9.61	463.00	495.00
	800	3	502.67	18.48	10.67	492.00	524.00
	1000	3	493.67	37.85	21.85	450.00	517.00
	Total	18	426.11	71.84	16.93	321.00	524.00

0: no nitrogen - 200: the nitrogen dose was 200 kg N ha^{-1} - 400: the nitrogen dose was 400 kg N ha^{-1} - 600: the nitrogen dose was 600 kg N ha^{-1} - 800: the nitrogen dose was 800 kg N ha^{-1} - 1000: the nitrogen dose was 1000 kg N ha^{-1}

Table 26. Analysis of variance for thousand kernel weight, test weight, gluten, protein, Zeleny sedimentation index and falling number affected by nitrogen fertilization in Alföld

		Sum of Squares	df	Mean Square	F	Sig.
Thousand kernel weight	Between Groups	14.821	5	2.964	3.030	.054
	Within Groups	11.739	12	.978		
	Total	26.560	17			
Test weight	Between Groups	6.344	5	1.269	2.953	.058
	Within Groups	5.157	12	.430		
	Total	11.501	17			
Gluten	Between Groups	1503.103	5	300.621	72.897	.000
	Within Groups	49.487	12	4.124		
	Total	1552.589	17			
Protein	Between Groups	212.713	5	42.543	64.941	.000
	Within Groups	7.861	12	.655		
	Total	220.574	17			
Zeleny sedimenation index	Between Groups	4325.907	5	865.181	67.123	.000
	Within Groups	154.673	12	12.889		
	Total	4480.580	17			
Falling number	Between Groups	82878.444	5	16575.689	40.984	.000
	Within Groups	4853.333	12	404.444		
	Total	87731.778	17			

df: degree of freedom; Sig.: significance; Significance level: $P < 0.05$

The study was carried out during the 2022 growing season to test the influence of nitrogen fertilization on the following wheat quality parameters: protein content, gluten content, test weight, thousand kernel weight, falling number, and Zeleny sedimentation index and to detect of the appropriate rate of nitrogen fertilization to reduce pollution and environmental impact of fertilization. Rainfall (mm) and temperature (°C) measurements were collected from World Weather Online® meteorological service during the flowering period (May), maturation (June) and harvesting (July). The rainfall was 24.6 mm in May, 30.2 mm in June and 33 mm in July. The temperature was 18 °C in May, 24 °C in June and 26 °C in July. The environmental conditions were not optimal for the wheat quality parameters.

In our study, nitrogen fertilization had no statistically significant influence on thousand kernel weight and test weight. However, it had a statistically significant influence on protein content, gluten content, and Zeleny sedimentation index. The higher the nitrogen dosage the higher those quality parameters were. Protein content, gluten content and Zeleny sedimentation index were the lowest at 0 kg N ha^{-1} followed by 200 kg N ha^{-1} then 400 kg N ha^{-1} and the highest at 600 kg N ha^{-1} after which further increase of nitrogen rate did not give a significant increase. Protein content is a key specification for wheat and flour purchasers since it is related to water absorption and gluten strength. Protein content can also be related to finished product attributes, such as texture and appearance. Low protein content is desired for crisp or tender products, such as snacks or cakes. High protein content is desired for products with chewy texture, such as pan bread and hearth bread. Bakers use protein content results to anticipate water absorption and dough development time for processes and products, because higher protein content usually requires more water and a longer mixing time to achieve optimum dough consistency. Because bread quality is positively correlated with wheat grain protein content (Gooding et al., 1991), high grain protein content is essential for bread wheat cultivars. Increasing nitrogen application rate at tillering stage and/or at flowering stage generally increases grain protein content. Nakano et al. (2008) indicated that nitrogen application at flowering stage is more effective than nitrogen application at tillering stage for increasing grain protein content. Nitrogen fertilization has been shown to improve protein content, gluten content, and the Zeleny sedimentation index (Pechanek et al., 1997; Ralcewicz and Knapowski, 2004; Győri, 2006; Szaframska et al., 2008; Cesevicien and Masauskien, 2009; Kismányoky and Tóth, 2010; Rakszegi et al., 2016). Protein content, gluten content, and Zeleny sedimentation index of the wheat grain samples in our experiment are consistent with previous research findings (Pollhamer, 1981; Vida et al., 1996; Pepó, 2010) which also indicate that nitrogen fertilization had a high positive effect on the examined wheat kernel quality parameters. Horváth et al. (2014) also demonstrated that increasing nitrogen fertilization levels had a positive effect on the protein and gluten content of wheat grain. Szentpétery et al. (2005) found that increasing fertilizer dose applications had a preferable effect on the protein content and gluten content. Pepó et al. (2005) found a medium to high significant correlation between fertilization and gluten content. According to Ozturk and Aydin (2004) and Horvat et al. (2006), nitrogen application is important for protein content and gluten content. In addition, they observed a high positive correlation between protein content and Zeleny sedimentation index. Guerrini et al. (2020), studying the effects of nitrogen fertilization in Italian wheat genotypes, verified that nitrogen fertilizer rates increased the grain protein concentration. Pinnow et al. (2013) also verified a positive effect of nitrogen fertilization on gluten concentration from harvested grains of a Brazilian wheat genotype. Souza et al. (2021), Stankowski et al. (2004), Zecevic et al.

(2004), Varga et al. (2007), and Szaframska et al. (2008) found that nitrogen fertilization had a significant positive influence on protein content, gluten content, and Zeleny sedimentation index.

The falling number is used to estimate alpha-amylase activity in wheat grain (Perten 1964), and it is used as a grain quality measurement for breadmaking by flour millers and European Economic Community Intervention Agencies. During breadmaking, alpha amylase breaks the complex starch in the flour down into sugars. The yeast in the bread recipe cannot digest the starch and needs sugar as a food source. The alpha-amylase therefore provides the yeast with sugar to ferment, so that carbon dioxide gas can be produced, which makes the dough rise (Cauvain and Young, 2001). A high falling number (for example, above 300 seconds) indicates minimal enzyme activity and sound quality wheat or flour. A low falling number (for example, below 250 seconds) indicates substantial enzyme activity and sprout-damaged wheat or flour. Too much enzyme activity means that too much sugar and too little starch are present. Since starch provides the supporting structure of bread, too much activity results in sticky dough during processing and deformed difficult to slice bread loaf with a dark crust. If the falling number is too high, enzymes can be added to the flour in various ways to compensate. If the falling number is too low, enzymes cannot be removed from the flour or wheat, which results in a serious problem that makes the flour unusable. Nitrogen fertilization had a statistically significant influence on the falling number in our study. The nitrogen fertilizer rate significantly increased the falling number. The higher the nitrogen dosage the higher the falling number was. The falling number was the lowest at 0 kg N ha^{-1} followed by 200 kg N ha^{-1} then 400 kg N ha^{-1} and the highest at 600 kg N ha^{-1} after which further increase of nitrogen rate did not give a significant increase. This finding is consistent with the conclusions made by Teesalu and Leedu (2001), Ralcewics and Knapowski, (2004), and Stankowski et al. (2004), who reported that nitrogen fertilizer rates affect the falling number. Cesevičiené and Mašauskiené (2007) reported that when higher nitrogen rates were used, the falling number of wheat was greater than when lower rates were used. Varga et al. (2007), on the other hand, found that under more intensive nitrogen fertilization, some wheat cultivars in Croatia failed to significantly improve the falling number. Eguchi et al. (1969) reported that nitrogen topdressing sometimes delayed maturity and was associated with a decrease in grain quality. Brun (1982) observed that high nitrogen fertilizer application can decrease the falling number. This could be due to damp conditions around the ear promoting germination and thus increasing alpha-amylase activity. The influence of nitrogen application, which can potentially delay development, has been hypothesized to maintain a high dropping number (Stewart 1984). In contrast to Brun's findings, Pushman and Bingham (1976) showed that higher nitrogen application lowered alpha-amylase activity, supporting the earlier hypothesis.

5. CONCLUSION AND RECOMMENDATIONS

Nitrogen fertilization plays a crucial role in crop growth and development. The used nitrogen fertilization doses and the time of nitrogen application did not influence *Fusarium* infection and mycotoxin production.

Breeding programs have made significant progress in developing *Fusarium* tolerant wheat varieties. Wheat varieties exhibit different levels of resistance or tolerance to *Fusarium.* Tolerance reduces the severity of infection and subsequent mycotoxin contamination. The used wheat varieties showed the same level of *Fusarium* infection.

Environmental conditions play a critical role in *Fusarium* infection and mycotoxin production. Factors such as rainfall, temperature, and relative humidity contribute to the development and severity of *Fusarium* infection and mycotoxin contamination especially during crucial growth stages. Optimal temperatures within the range of 25-30°C, aw above 0.78, and RH of 88-95% provide favorable environment for *Fusarium* growth and mycotoxin production, while extreme temperatures can inhibit their development.

It is important to note that the interaction between nitrogen fertilization, wheat variety, and environmental conditions is complex. Understanding the specific environmental requirements and interactions involved in *Fusarium* infection and mycotoxin production is crucial for implementing effective management strategies. By considering these factors, farmers and agricultural practitioners can make informed decisions to minimize *Fusarium* infection and reduce mycotoxin contamination, safeguarding both crop quality and human and animal health. Optimizing nitrogen fertilization practices and selecting *Fusarium*-resistant wheat varieties are essential strategies for reducing *Fusarium* infection and mycotoxin production in wheat, protecting crop quality, and ensuring food and feed safety.

Nitrogen is a crucial nutrient that plays a vital role in wheat growth, development, and grain yield. Nitrogen fertilization significantly affected the quality parameters of wheat crops. Proper nitrogen fertilization can improve wheat quality by enhancing key parameters such as protein content, gluten content, falling number and Zeleny sedimentation index. Adequate nitrogen supply promotes photosynthesis and protein synthesis, leading to higher protein accumulation in wheat grains. This is particularly important for wheat varieties used for bread-making, as higher protein content contributes to better dough strength and bread quality.

Furthermore, nitrogen fertilization influences gluten content, which is crucial for determining the baking quality of wheat. Gluten proteins, specifically glutenin and gliadin, contribute to dough elasticity and the ability to retain gas during fermentation. Optimal nitrogen levels promote the formation of strong gluten networks, resulting in improved dough elasticity and bread volume.

Therefore, achieving the right balance in nitrogen fertilization is crucial for optimizing wheat quality. By carefully managing nitrogen fertilization, farmers can enhance wheat quality parameters, ensuring desirable protein content, gluten content, thousand kernel weight, test weight, falling number and Zeleny sedimentation index, ultimately improving the market value and end-use suitability of their wheat crops.

6. NEW SCIENTIFIC RESULTS

Measurements of this experiment proved that:

- The following nitrogen fertilization doses: 0, 40, 80, 120, 160 kg N ha^{-1} did not show a signification effect on *Fusarium* infection and mycotoxin production.

- The following nitrogen fertilization doses: 0, 40+40, 80+40, 120+40 kg N ha^{-1} did not show a signification effect on *Fusarium* infection and mycotoxin production.

- Increasing nitrogen doses and the time of nitrogen application did not affect *Fusarium* infection and mycotoxin production.

- The following wheat varieties: Mv Karéj, Mv Kolompos and Alföld did not show a significant difference in *Fusarium* infection.

- The growing season's environmental conditions during anthesis showed a significant effect on *Fusarium* infection and mycotoxin production.

7. SUMMARY

Wheat (*Triticum aestivum L.*) is one of the most cultivated crops around the world. It is grown across a wide range of environments. The primary use of wheat is for bread making. In addition, it is used in the production of bakery and confectionery products, animal feed, and ethanol. The annual global wheat output for 2021 and 2022 was estimated at 775.1 million and 778 million metric tons respectively. The genus *Fusarium* is a plant pathogen of wheat. It causes *Fusarium* head blight (FHB), a major fungal disease in wheat production. Initial symptoms of FHB appear on the spike and grain. FHB reduces the quality of the grain and decreases the yield. Wheat is particularly susceptible to FHB infection during the period of anthesis and the early stages of grain development. Diseased grains are shriveled, discolored, and light weight. Under favorable conditions, *Fusarium spp.* can produce mycotoxins, mainly deoxynivalenol (DON), zearalenone (ZEA), and fumonisins (FUM). The presence of mycotoxins in food and feed can cause chronic or acute mycotoxicosis in animals and humans. Deoxynivalenol, also known as vomitoxin, has been identified as the causative agent of feed refusal in animals. Zearalenone is an estrogenic mycotoxin that affects the endocrine and reproductive system of human and animal. Fumonisin is a carcinogenic mycotoxin linked with the incidence of esophageal and liver cancer in human and animal. To minimize the risk of *Fusarium* head blight and mycotoxins, some preventive measures should be applied such as crop rotation, weed control, biological control, and the use of tolerant or resistant varieties. The results indicate that nitrogen fertilization and wheat variety did not have statistically significant influence on *Fusarium* infection and mycotoxin production. The growing season had statistically significant influence on *Fusarium* infection and fumonisins production due to higher rainfall in 2021 compared to 2020 during the flowering period when the wheat spike is the most vulnerable to *Fusarium* infection.

The technological quality of wheat, essential for the production of bread and wheat-derived products, can be evaluated based on several parameters such as protein and gluten content, falling number, Zeleny sedimentation index, test weight, and thousand kernel weight. Technological wheat quality parameters may be affected by wheat genotype, environment, and crop management practices such as fertilization, irrigation, plant growth regulators, and control of pests and diseases. Nitrogen is among the vital nutrients supplied by fertilization, which crops require in significant quantities. Nitrogen is a crucial nutrient for wheat due to its indispensable role as a constituent of the cell wall, chlorophyll, nucleic acids. Wheat crops that receive optimal fertilization and nutrition, particularly in the form of nitrogen, are known to generate grains that exhibit superior morphological quality and substantial nutritional value suitable for consumption, trade, and utilization by the food industry. The results indicate that nitrogen fertilization did not show a significant effect on thousand kernel weight and test weight. However, nitrogen fertilization significantly affected protein content, gluten content, Zeleny sedimentation index, and the falling number. The higher the nitrogen fertilization the better the technological quality parameters of the wheat.

8. SCIENTIFIC PUBLICATIONS

El Chami, J., El Chami, E., Tarnawa, Á., Kassai, K.M., Kende, Z., Jolánkai, M., (2023). Influence of *Fusarium* head blight on technological quality of wheat. *Acta Phytopathologica et Entomologica Hungarica.* https://doi.org/10.1556/038.2023.00179

El Chami, E., El Chami, J., Tarnawa, Á., Kassai, K.M., Kende, Z., Jolánkai, M., (2023). *Fusarium* head blight in wheat: impact of growing season, wheat variety and nitrogen fertilization under natural infection. *Acta Phytopathologica et Entomologica Hungarica.*

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