UNIVERSITY OF LJUBLJANA
BIOTECHNICAL FACULTY

Sasho POPOVSKI

WHEAT (*Triticum aestivum* L.) AND MAIZE (*Zea mays* L.) KERNEL FUSARIOSIS (*Fusarium* spp.): RELATIONSHIPS BETWEEN SPECIES COMPOSITION OF PATHOGENS, INFECTION RATE AND CONTAMINATION BY MYCOTOXINS

DOCTORAL DISSERTATION

Ljubljana, 2016

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FUZARIOZE (*Fusarium* spp.) NA ZRNJU PŠENICE (*Triticum aestivum* L.) IN KORUZE (*Zea mays* L.): POVEZAVE MED VRSTNO SESTAVO PATOGENOV, OKUŽENOSTJO IN ONESNAŽENJEM Z MIKOTOKSINI

DOKTORSKA DISERTACIJA

Ljubljana, 2016

Based on the Statute of the University of Ljubljana and by decision of the Senate of the Biotechnical Faculty and decision of the University Senate of 14 January 2014, it was confirmed that the candidate qualifies for a PhD postgraduate study of biological and biotechnical sciences and the pursuit of a doctorate degree in the field of agronomy. Prof. dr. Franci Aco Celar was appointed as the supervisor, and for co-advisor dr. Alenka Munda.

Na podlagi Statuta Univerze v Ljubljani ter po sklepu Senata Biotehniške fakultete in sklepa Senata Univerze z dne 14. januarja 2014 je bilo potrjeno, da kandidat izpolnjuje pogoje za doktorski Podiplomski študij bioloških in biotehniških znanosti ter opravljanje doktorata znanosti s področja agronomije. Za mentorja je imenovan prof. dr. Franci Aco Celar, za somentorico je imenovana višja znan. sod. dr. Alenka Munda.

The PhD thesis has been accomplished at the Chair of Phytomedicine, Agricultural Engineering, Crop Production, Pasture and Grassland Management, Department of Agronomy, Biotechnical Faculty, University of Ljubljana, Slovenia.

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AB Macro field trials were conducted and analyzed different varieties of wheat and maize, grown on two different test locations (Rakičan and Jablje) in Slovenia. Standard phytopathological methods were used to identify *Fusarium* species and study their representation in connection with the environmental conditions during the trials. The content of mycotoxin deoxynivalenol (DON) in the grain samples was determined by ELISA test. Particularly we were interested in how the location and weather conditions on test locations affect infection with *Fusarium* spp. and contamination of each variety/hybrid of wheat/corn with DON. Based on the statistical analysis of acquired data, the length of flowering and the rainfall during flowering did not affect the infection rate of *Fusarium* spp. on the ears of wheat. Awned wheat from Rakičan was statistically significantly less infected with *F. culmorum*+*F. graminearum* (FC+FG) than awnless wheat. The ecologically produced wheat was less infected with *Fusarium* spp. and less contaminated with DON than wheat from integrated production. Maize samples from Rakičan were minimally infected with FC+FG as opposed to Jablje. The correlation analysis showed a highly significant correlation between kernel infection with FC+FG and DON contamination.

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AI Na terenu smo izvedli poljske poskuse in analizirali različne sorte pšenice in koruze iz dveh različnih testnih lokacij (Rakičan in Jablje) v Sloveniji. Uporabili smo standardne fitopatološke metode za ugotavljane fitopatogenih gliv iz rodu *Fusarium* in njihovo zastopanost v povezavi z okoljskimi dejavniki v času poskusov. Vsebnost mikotoksina deoksinivalenola (DON) v vzorcih zrnja smo določili s pomočjo ELISA testa. Posebej nas je zanimalo kako lokacija oz. vremenski pogoji na testnih lokacijah vplivajo na okuženost z glivami *Fusarium* spp. in kontaminacijo posamezne sorte/hibrida pšenice/koruze z DON. Na podlagi statistične analize pridobljenih podatkov smo ugotovili, da dolžina cvetenja in padavine v času cvetenja niso vplivale na stopnjo okuženosti klasa pšenice s fuzariozami. Pšenica tipa resnica je bila v Rakičanu statistično značilno manj okužena s *F. culmorum*+*F. graminearum* (FC+FG) kot pšenica tipa golica. Ekološko pridelana pšenica je bila manj okužena s fuzariozami in tudi tudi manj kontaminirana z DON. Vzorci koruze iz Rakičana so bili minimalno okuženi s FC+FG v nasprotju s tistimi iz Jabelj. Korelacijska analiza je pokazala zelo signifikantno povezavo med okužbo zrnja s FC+FG in onesnaženostjo z DON.

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ABBREVIATIONS AND SYMBOLS

Ac-DON	Acetyldeoxynivalenol
AEI	Average ear infection
AP	Anthesis period
Aw	Awned wheat
Awl	Awnless wheat
BBCH	Phenological development of cereals
CLA	Carnation leaf Agar
DAS	Diacetoxyscirpenol
DNA	Deoxyribonucleic Acid
DON	Deoxynivalenol
ELISA	Enzyme-linked Immunosorbent Assay
EU	European Union
FA	*Fusarium avenaceum*
FC	*Fusarium culmorum*
FCe	*Fusarium cerealis*
FG	*Fusarium graminearum*
FP	*Fusarium poae*
FPr	*Fusarium proliferatum*
FS	*Fusarium subglutinans*
Fso	*Fusarium solani*
FSp	*Fusarium sporotrichioides*
FT	*Fusarium tricinctum*
FV	*Fusarium verticillioides*
FDK	*Fusarium* damaged kernels
FHB	*Fusarium* Head Blight
FUM	Fumonisins
GC	Gas Chromatography
HPLC	High-performance Liquid Chromatography

HR	Hypersensitive Reaction
LC-MS	Liquid Chromatography with Mass Spectrometry
LC-MS/MS	Liquid Chromatography with tandem Mass Spectrometry
NaOCl	Sodium hypochlorite
NIV	Nivalenol
PCR	Polymerase Chain Reaction
PDA	Potato Dextrose Agar
ppb	Part per Billion
QTL	Quantitative Trait Loci
r	Coefficient of correlation
R^2	Coefficient of determination
RDF	Rainy days during flowering
Std Dev	Standard Deviation
T	Temperature
T-2	T-2 toxin
TLC	Thin-layer Chromatography
TRF	Total rainfall during flowering
ZEA	Zearalenone

GLOSSARY

Aggressiveness	The relative ability of a plant pathogen to colonize and cause damage to plants. The term is often used in epidemiology and describes differences among isolates of the same species.
Anamorph	The imperfect (asexual) stage of a fungus.
Antagonist	An organism that is able to suppress pathogenic fungi and bacteria in artificial systems, often detected as inhibition zones in dual culture plates. The term is often confused with biocontrol agent.
Anthesis	A period during which a flower is fully open and functional.
Antibiosis	A mode of action in biocontrol. The antagonist produces one or more substances that inhibits or kills the pathogen.
Biological control	Exploitation by humans of natural competition, parasitism and/or antagonism of organisms for management of pests and pathogens.
Facultative parasite	An organism that usually lives on decomposing dead material (saprotroph), but under certain conditions can turn pathogenic, e.g. if the host is stressed or weakened.
Fusarios(es)	Common name for all the various diseases caused by fungi in the genus *Fusarium.*
Haustorium	Specialized branch of a parasite formed inside host cells to absorb nutrients.
in vitro	in glass, on artificial media, or in an artificial environment; outside the host.
in vivo	within a living organism, here sometimes used synonymously with *in situ* - in its original place or environment.
Pathogenicity	Term that describes whether or not an organism is able to cause disease.
Seed treatment	Application of a biological agent, chemical substance or physical treatment of seed, in order to protect the seed or plant

	from pathogens or to stimulate germination and plant growth.
Seed borne	Carried on or in a seed.
Virulence	The relative capacity of an organism to cause disease, or its ability to overcome the resistance of the host.

1 INTRODUCTION

The genus *Fusarium* is composed of various species that are of great importance to the field of agriculture and human and veterinary medicine due to their pathogenicity, as well as their commonness in nature. The genus contains both pathogenic and saprophytic species which colonize aerial plant organs, plant debris and other organic substrates, however, they can also be found in soil and air, as well as on seeds, food or in tap water. It was pointed out by Summerell et al. (2003) that the genus is responsible for a wide range of plant diseases, some of its species are known to produce mycotoxins, such as deoxynivalenol (DON), zearelenone (ZEA), and fumonisins (FUM), which are hazardous to both human and animal health. The *Fusarium* toxins can also be found in various feeds (Leslie and Summerell, 2006; Nelson et al., 1983, 1994; Alm et al., 2006).

In the areas of moderate climate, maize can be infected by several species of the genus *Fusarium*. These species can cause root and stalk rot and ear rot of maize. Because of the infections they cause, yield reduction is possible in an average between 10 and 30% (Parry et al., 1995). Some *Fusarium* species are capable of forming mycotoxins, already in the field as well as after the harvest. Most mycotoxicological research has been done with regard to mycotoxins in grains, although some studies found mycotoxins also in rotten stalks, infected leaves even on the whole plant. This may pose a serious risk for maize, which is used as a fresh feed and silage (Parry et al., 1995).

Fusarium graminearum and *F. subglutinans* were the most common fungi infecting maize in Slovenia according to the data from monitoring researches, while to a lesser extend i.e. sporadically *F. avenaceum, F. culmorum, F.verticillioides, F. poae, F. equiseti* and some other *Fusarium* species were also detected (Milevoj, 2002). These dominant *Fusarium* species in Slovenia, according to the literature data form important mycotoxins such as DON, NIV (nivalenol), ZEN and MON (moniliformin) (D'Mello and Macdonald, 1997; D'Mello et al., 1999; Placinta et al., 1999).

Beside maize, wheat is also heavily infected by many *Fusarium* species. So far, 12 species were recorded as the causative agents of fusariosis in wheat. They primarily infect the roots, stems and the grains. Fusariosis can cause yield reduction in an average between 10 to 40% (Sutton, 1982). Mycotoxins can form as well as the result of infection before and after the harvest. Fusariosis, after all, can highly affect the yields of wheat and barley, which represent 80% of cereal production in Europe. The rest of the cereal species (rye, triticale, oat) are less susceptible to ear infection with *Fusarium* species and therefore their grains are less contaminated with mycotoxins (Parry et al., 1995; Miedaner, 1997; Mesterhazy et al., 1999).

The most commonly represented *Fusarium* species isolated from infected wheat ears in Europe are *F. graminearum, F. avenaceum,* and *F. culmorum,* slightly less *F. poae, F. equiseti* and to a lesser extent i.e. sporadically *F. tricinctum, F. cerealis, F. acuminatum, F. sporotrichioides, F. subglutinans. F. oxysporum* and *F. solani* are detected (Sutton, 1982; Parry et al., 1995; Miedaner, 1997; Tekauz et al., 2000; Brennan et al., 2003).

Both maize and wheat are infected by almost the same *Fusarium* species, which allows their conservation in the narrow crop rotation maize-wheat and consequently cause greater economic losses. Slovenian agriculture is in general livestock-oriented, because of that on the arable areas are produced mainly plants that are used for animal feed (maize, wheat, etc.). It may be noted that in Slovenia the agricultural production is dominated by a narrow crop rotation (maize-wheat), or in some cases even monocultures (maize). Predominant fungal species, the causative agent of fusariosis, for both wheat and maize are *F. graminearum* and *F. avenaceum,* which form major mycotoxins (DON, NIV, ZEN and MON). In maize, the third most important species is *F. subglutinans* (mycotoxin MON) while in the case of wheat it is *F. culmorum* (mycotoxins DON and ZEN) (Sutton, 1982; Leslie et al., 1986; Pomeranz et al., 1990; Odiemah and Manninger, 1994; Vigier et al., 1997; Velluti et al., 2000; Torres et al., 2001).

We must be aware that the *Fusarium* fungi do not live only on the growing maize or wheat, but they may develop as saprophytes on stored crops, silage maize and even on products of the food industry. The incidence of *Fusarium* spp. in crops, feed products and in food processing industry depends on a numerous environmental factors as well as the method of production and storage of products (choice of varieties, fertilization, tillage, crop rotation, chemical protection, technology of storage and processing of grain, etc.), (Andersen, 1948; Goswami and Kistler, 2004).

Some researches indicate that the infections with *Fusarium* species on wheat are less frequent, in extensive organic production than in the conventional, but in contrary to that, there were no differences in the content of DON and ZEN in grains. With regard to the small extent of Slovenian agriculture there is a tendency to increase the volume of organic production. Cereals and cereal products in Slovenia today are the basic market items of the ecologically oriented farms. If there is a possibility to determin that the Slovenian organic grain contains less mycotoxin, it would be certainly a market opportunity and advantage for both the domestic and foreign markets (Windels, 2000; Nganje et al., 2004).

1.1 AIMS AND GOALS

The specific aims of this work were to explore:

- Whether the weather condition during and after the flowering period affect the contagion of the different wheat types, and subsequently, the mycotoxin concentration;
- Whether awnless wheat is more prone to *Fusarium* infection;
- Whether the immaturity or maturity of the wheat/corn varieties/hybrids affects the *Fusarium* infection of the grains;
- Whether there are differences in sensitivity, i.e. the resistance to fusariosis between the studied varieties/hybrids of wheat/corn, independently of the studied region (Rakičan - Jablje);
- Whether the *Fusarium* species and the percentage of individual *Fusarium* species in the infected wheat/corn grains depends on the region itself;
- Whether the share of *Fusarium* infected grains in wheat/corn correlates to the mycotoxin content, especially DON;
- Whether there is a possibility to approximately forecast the grain contamination in the wheat, i.e. the grain's mycotoxin contamination, based on a field evaluation of the ear infection;
- Whether the ecologically grown wheat is less infected by fusariosis, i.e. less contaminated with mycotoxins, than the wheat grown in integrated production.

1.2 WORKING HYPOTHESIS

We assume that:

- There is a correlation between the quantity, i.e. the period of precipitation during the wheat flowering period and the level of *Fusarium* infection;
- The wheat types that have a longer flowering period are more infected;
- The awnless wheat types are more receptive to *Fusarium* infection when compared to the awned wheat types;
- The maturity grade of the wheat/corn varieties/hybrids affects the *Fusarium* infection of the grains;

- The test area (Rakičan - Jablje), and by that the related climate conditions (arid - humid) affect the infection in the varieties/hybrids of wheat/corn;
- There are differences in sensitivity, i.e. the resistance to fusariosis between the studied varieties/hybrids of wheat/corn, independently of the studied region;
- The *Fusarium* species and the percentage of individual *Fusarium* species in the infected wheat/corn grains depend on the region itself;
- The share of *Fusarium* infected grains in wheat/corn does not correlate to the mycotoxin content, especially DON;
- We cannot forecast the mycotoxin contamination of grain based on a field estimation of ear infection in the wheat;
- The wheat grown in ecological production is less infected by *Fusarium* spp., i.e is less contaminated with mycotoxins than the one grown in integrated production.

2 LITERATURE REVIEW

2.1 THE GENUS *Fusarium*

The genus *Fusarium* is commonly represented in nature, where its pathogenic and saprophytic species are found (Liddell, 1991), and because the genus consists of a large number of species, it is responsible for diseases in several crops as well as cereals and it can also be pathogenic to humans and animals. Due to their devastating effect on wheat and maize, which can lead to world-wide yield losses in inferior quality grain, the *Fusarium* infections are considered to be very significant to the economy, as they can not only lead to economical difficulties, but their mycotoxins could also affect human and animal welfare through consumption of infected grain in food products (McMullen et al., 1997; Parry et al., 1995; Sutton, 1982).

Leslie and Summerell (2006) who reviewed all *Fusarium* species that have a connection with plant disease and especially cereals, stated that in at least 80% of all cultivated plants, a disease caused by *Fusarium* species could be found, allowing for isolation from various sources, such as different plant organs, plant debris as well as soil.

Wheat and maize in particular can be infected by *Fusarium* during any of the growth stages, from the seed germination stage to full-grown vegetative tissue, depending on the *Fusarium* species that was involved and the host plant, with the possibility of multiple *Fusarium* species infections in the same plant, which lead to complex diseases with difficult etiology capable of mycotoxins production (Logrieco et al., 2007). That is why it is of the highest importance to precisely identify the *Fusarium* spp. as early as possible during any stage of infection, so that the potential toxicological exposure risk can be predicted, as opposed to preventing these metabolites to be formed, since the majority of the species have specific mycotoxin profiles.
However, the identification of mycotoxigenic *Fusarium* species is continuing to be a critical problem due to the ambiguity of the identification process, which results in large and constantly evolving number of species in the genus, because of different taxonomic systems that caused controversies among researchers (Asan, 2011). Three different species concepts, morphological, biological and phylogenetic, have been applied in species recognition during the last century and consequently the number of species was constantly changing.

Fusarium ear blight can be manifested through different symptoms which vary according to the stage of the disease, as well as the age of the plant. The first signs often occur in younger plants and are recognized as the whitening of the florets in the head, while a fully developed infection can lead to the early decay or whitening of the entire head or spike. The symptoms are often displayed at the head of the plant during the growth stage of the dough from soft to

hard, and they appear like the plant is early maturing and lead to dark brown blemishes of the peduncle and shriveled kernels that have a white, chalky ("tomsbstone") appearance (Sutton, 1982; Parry et al., 1995; Miedaner, 1997). Furthermore, in cases where sufficient humidity is present, the molds at the base of the florets might also display orange color.

The *Fusarium* genus consists of a number of pathogens and saprotrophes, like *F. oxysporum*, *F. graminearum*, *F. culmorum*, *F. avenaceum*, *F. verticillioides*, *F. equiseti*, *F. poae*, *F. sporotrichoides* which are adapted to saprophytic growth and survival. There are some difficulties which arise when an isolation is to be done, due to the fact that the literature in this field is quite confusing, but it is clear that individual isolates of most of the above-mentioned species have the ability to cause a number of various diseases (Garrett, 1970; Deacon, 1984; Bruehl, 1987). Therefore isolation is a complex process, not only due to the variety of diseases, but also due to the probability of isolating more than one species from symtomatic tissue, which in fact makes the etiology a complex process (Parry, 1990; Parry et al., 1995; Pettitt et al., 1996; Smiley and Patterson, 1996; Paveley et al., 1997).

Further problems arise due to the varying virulence among the different isolates present in a species and also the ones between species (Miedaner et al., 1996; Gang et al., 1998; Carter et al., 2002), as well as the fact that a large number of isolates could not be essentially treated as pathogens, but as saprotrophes with an ability to become pathogens. The fungi of the *Fusarium* genus are such, and despite their ability to produce mycotoxins (Vesonder et al., 1992; Tóth et al., 1993; Perkowski et al., 1996; Langseth et al., 1997; Gang et al., 1998; Hörberg, 2001; Magan et al., 2002; Proctor et al., 2002, Table 1) they can also create extra-cellular enzymes like the β-glucosidase, cellulase, pectinase, and xylanase (Kang and Buchenauer, 2002).

2.2 *Fusarium* SPECIES AS PATHOGENS OF WHEAT AND MAIZE

The diseases caused by the pathogens of the *Fusarium* genus are commonly named fusariosis. They don't have a specially developed structure for entering of host cells, like the appresorria or haustoria, but their attack is mostly focused on host plants which are either: damaged, immature or aged. Infection is carried out at the anthesis stage of wheat (during flowering when pollen is mature) during which the parts that would construct the grain are fragile and exposed (Parry et al., 1990; Parry et al., 1995; Pettitt et al., 1996).

However, if there are no lacerations, the hyphae of *F.culmorum* can also penetrate through the lateral root tips of wheat and then breach the vascular bundle, via cells of the Casparian strip that lack suberin lamellae, and once inside, the fungus can systematically spread. In the case of

wheat scab however, the flowers are the road through which *F.culmorum* infection occurs, with the help of a penetration peg (Kang and Buchenauer, 2002) which infiltrates the host cells that were firstly weakened by extracellular enzymes, after a heavy structure of hyphae was previously built. Once inside the plant, *Fusarium* fungi can firm their hold on the plant by living endophytically, without provoking visible symptoms. And once the plant is aged and wilted, the fungi have the upper hand by having been previously established, when the saprophytic soil microflora get access to the nutrition source (Clement and Parry, 1998).

The seriousness with which the head blight disease is taken is mainly owed to the production of mycotoxins (Table 1), which are considered to be responsible for the damaging effect of the disease on yield reduction as well as grain quality. One such example are the trichothecenes, which are treated as one of the toxins that are directly related to the process of contamination (Kang and Buchenauer, 2002; Proctor et al., 2002). Furthermore, there is in generally connection between the degree of infection and the concentration of mycotoxins in the kernels of wheat (Perkowski et al., 1996; Gang et al., 1998). However, this is not always the instance, as can be observed in the case when fungicides are applied and the observable symptoms decrease while the concentration of mycotoxins increases (Magan et al., 2002).

Fusarium species in regard to soils can be found everywhere. They are commonly considered to be soil-borne fungi that infest 50% and more of maize grains prior to harvest (Robledo-Robledo, 1991). A number of phytopathogenic species of *Fusarium* are related with maize, including *F. subglutinans* (Wollenweb. & Reinking) P.E. Nelson, T.A. Toussoun, & Marasas, *F. verticillioides* (Saccardo) Nirenberg, and *F. graminearum* Schwabe (Lawrence et al., 1981; Scott, 1993; Munkvold and Desjardins, 1997). Among them, *F. verticillioides* is probably the most commonly isolated species from diseased maize on a global scale (Munkvold and Desjardins, 1997). Each of the three species has the ability to produce mycotoxins in the grain. The most concering toxins are the ones produced by *F. verticillioides* (fumonisin) and *F. graminearum* (deoxynivalenol and zearalenone) (Prelusky et al., 1994).

Fusarium spp. can infect maize ears with spores germinating on the silks and growing down the silks to the kernels and cob (rachis), (Hesseltine and Bothast, 1977; Sutton, 1982) or via wounds through the husk made by insects or birds (Attwater and Busch, 1983; Sutton et al., 1980).

F. graminearum produces a mold that has a pink- to reddish hue on the kernels that commonly spreads from the tip of the ear downwards or outwards from an insect wound. The mold growth of *F. subglutinans* resembles the one of *F. graminearum* but it is colored somewhat more orange than pink. *F. verticillioides* produces a whitish-colored mold which is mostly

dispersed accros the ear. According to reports, not every *F. verticillioides* -infected kernel is symptomatic. It has been proposed that this fungus causes systemic infection in the maize plant and can be found everywhere in nature (Munkvold and Carlton, 1997).

F. verticillioides is an endophyte of maize that is long associatied with the plant (Munkvold and Desjardins, 1997). An infection without symptoms is possible in the grains, roots, stems and leaves, and the presence of the fungus is in the majority of cases ignored because the damage it causes to the plant is not visible (Munkvold and Desjardins, 1997). This suggests that certain strains of this fungus cause disease in maize while others do not (Bacon and Williamson, 1992). *F. verticillioides* infects maize at every stage of plant development, either through infected seeds, the silk channel or wounds, producing grain rot during the periods of before and after the harvest (Munkvold and Desjardins, 1997).

Table 1: Mycotoxins produced by *Fusarium* species pathogenic to cereals (Johansson, 2003)
Preglednica 1: Mikotoksini, ki jih tvorijo *Fusarium* vrste patogene za žita (Johansson, 2003)

Species of *Fusarium*	Toxin	Crop[b]	References
F. avenaceum	Deoxynivalenol	Wheat	Tóth et al., 1993
F. culmorum	Zearalenone	Wheat, maize,	Perkowski et al., 1996
	Deoxynivalenol	Wheat, rye	Tóth et al., 1993; Gang et al., 1998
	Nivalenol	Wheat, rye, Barley	Gang et al., 1998; Perkowski et al., 1996 Hörberg, 2001
	Deoxynivalenoles[a]	Barley, wheat, oats	Perkowski et al., 1996; Hörberg, 2001 Magan et al., 2002
	Fusarenone	Wheat, oats	Hörberg, 2001; Magan et al., 2002
	HT-2 toxin	Wheat, barley	Hörberg, 2001
F. equiseti	Deoxynivalenol	Wheat	Tóth et al., 1993
	Nivalenol	Wheat	Tóth et al., 1993
	Zearalenone	Wheat	Tóth et al., 1993
F. graminearum	Zearalenone	Wheat, maize	Magan et al., 2002
	Deoxynivalenol	Wheat Wheat,	Tóth et al. 1993; Magan et al., 2002
	Trichothecenes[c]	maize	Proctor et al., 2002; Magan et al., 2002
	Fusarenone	Cereals	Magan et al., 2002

Continued

continuation of Table 1:

Species of *Fusarium*	Toxin	Crop[b]	References
F. oxysporum	Moniliformin	Cereals	Magan et al., 2002
	Wortmannin	Cereals	Magan et al., 2002
	Fusaric acid	Cereals	Magan et al., 2002
F. poae	Trichothecenes	Cereals	Magan et al., 2002
	T-2 toxin	Cereals	Magan et al., 2002
	HT-2 toxin	Cereals	Magan et al., 2002
F. sporotrichoides	T-2 toxin	Wheat	Tóth et al., 1993; Magan et al., 2002
	HT-2 toxin	Cereals	Magan et al., 2002
	Neosolaniol	Cereals	Magan et al., 2002
	Diacetoxyscirpinol	Cereals	Magan et al., 2002
	Fusarenone	Cereals	Magan et al., 2002
	Zearalenone	Cereals	Magan et al., 2002
F. verticillioides	Fumonisins	Maize	Proctor et al., 2002; Magan et al., 2002
	Moniliformin	Cereals	Vesonder et al., 1992; Magan et al., 2002
	Fusarin C	Cereals	Magan et al., 2002
	Fusaric acid		Vesonder et al., 1992

a 3-acetyldeoxynivalenol and 15-acetyldeoxynivalenol
b Maize (*Zea mays*), oats (*Avena sativa*), barley (*Hordeum vulgare*), rye (*Secale cereale*)
c Deoxynivalenol and nivalenol are examples of trichothecenes

2.3 FUNGAL SECONDARY METABOLITES - MYCOTOXINS

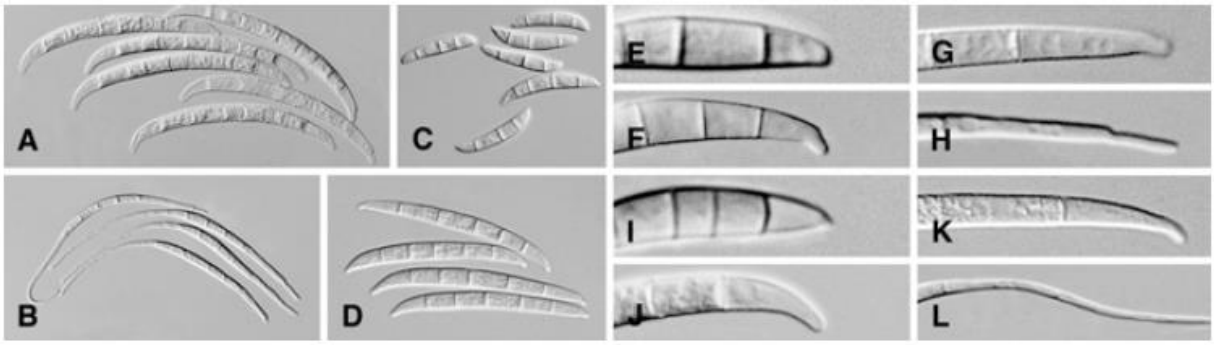
Mycotoxins are organic compounds that are not produced via normal metabolic pathways, after a cycle of growth or reproduction of organisms. They are characterized as fungal secondary metabolites that have a lower molecular weight and are toxic to the vertebrates (Desjardins and Hohn, 1997). A magnificently differing category of cellular products, these metabolites often display taxonomic uniqueness and there is a large speculation over the true organic function of mycotoxins. They are considered not crucial to the organism growth under culture conditions (Bennett, 1995; Bode et al., 2002), while still they are believed to have an important part in fungal defense, substrate colonization, as well as interspecies contests for its producer in its ecological niche.

2.4 MORPHOLOGICAL FEATURES

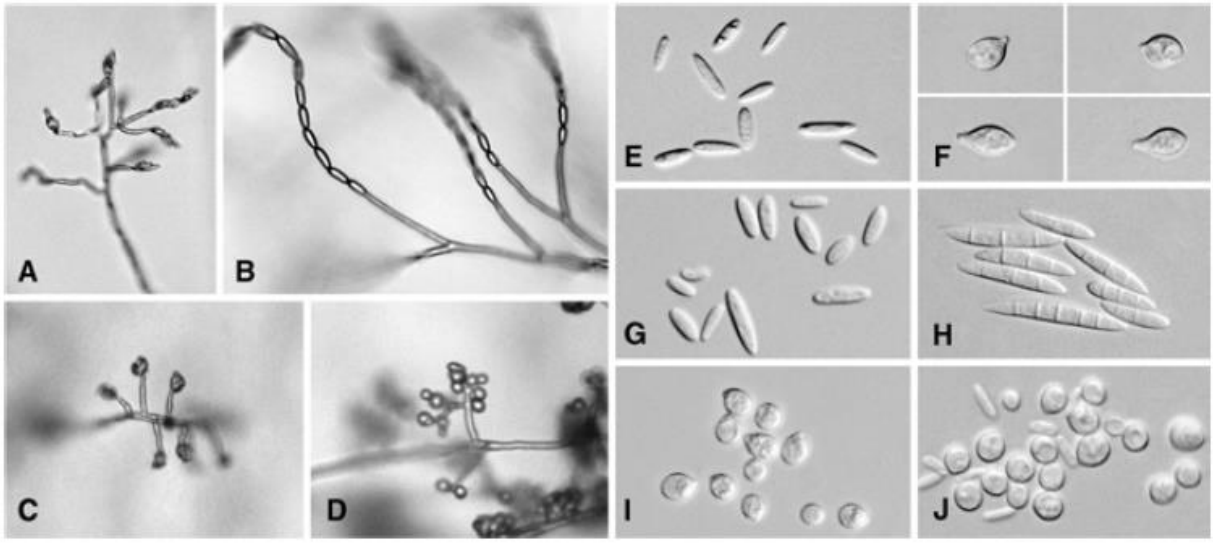
As previously mentioned, the diversity and complexity of the *Fusarium* genus creates certain issues in the identification of the most significant toxic and pathogenic species. However, there are certain morphological characteristics present in this genus which were proven to be quite convenient in the differentiation of the various species, and have their roots in conventional morphological procedure. Such specific characteristics are the chlamydospores, microconidia, macroconidia or the colony features (Dongyou, 2009), which could be used for distinguishing those *Fusarium* species that are considered to be significantly toxigenic or pathogenic (Dongyou, 2009).

By analyzing the various shapes or the existence or lack of the above-mentioned structures as well as the features of the micro- and macro-conidiogenous cells, the various *Fusarium* species can be identified. The primary characteristic needed for placing the species in the *Fusarium* genus is the appearance of asexual spores, as well as the easily distinguished banana shaped macroconidia (Moretti, 2009). And as recommended by various taxonomists, the proper procedure to successfully complete the characterization and identification of the species is to use strain cultures that were retrieved from single spore isolations, grown on appropriate media, under optimal conditions (Dongyou, 2009).

The macroconidia can be identified by their place of origin, such is the case the septated macroconidia whose birthplace is the aerial mycelium, especially on mono or polyphialides, but their most common production site are the specialized structures referred to as sporodochia, which are found on short monophialides. The distinction between these two phialides is that the monophialides are conidiation cells that have a single characteristic pore that releases endoconidia, while polyphialides have multiple pores. However, it is the shape which still remains to be the most significant characteristic in the process of macroconidia recognition. Thus the *Fusarium* macroconidia can be distinguished by their shape that resembles a sickle, a canoe, or a banana, with multisepta (Figure 1). The microconidia are yet another feature that can be used to identify the *Fusarium* species. The microconidia are produced from phialides and they are either one or two celled. However, their shape and size varies so they are more easily identified by their place of origin - the aerial mycelium, where they are produced in clumps or chains, on both monophialides and polyphialides (Figure 1) (Leslie and Summerell, 2006).



a)



b)

Figure 1: Spore morphology of *Fusarium* species: a) Macroconidia of *Fusarium* species. A-D: Variation in macroconidial shape and length. A, *F. decemcellulare*. B, *F. longipes*. C, *F. culmorum*. D, *F. chlamydosporum*. E-H: Variation in basal cells of macroconidia. E, *F. culmorum*. F, *F. crookwellense*. G, *F. avenaceum*. H, *F. longipes*. I-L: Variation in apical cells of macroconidia. I, *F. culmorum*. J, *F. decemcellulare*. K, *F. verticillioides*. L, *F. longipes*. b) Formation and types of microconidia produced by *Fusarium* species. A, Microconidia produced in short chains (*F. brevicatenulatum*). B, Microconidia produced in long chains (*F. decemcellulare)*. C, Microconidia produced in false heads (*F. circinatum*). D, Napiform microconidia in false heads (*F. konzum*). E, Oval microconidia (*F. babinda)*. F, Pyriform microconidia (*F. anthophilum*). G, Clavate microconidia (*F. anthophilum*). H, Fusiform microconidia (*F. semitectum*). I, Napiform microconidia (*F. poae*). J, Globose microconidia (*F. anthophilum*) (Summerell et al., 2003).

Slika 1: Morfologija spor *Fusarium* vrst: a) makrokonidiji *Fusarium* vrst. A-D: Različne oblike in velikosti makrokonidijev. A, *F. decemcellulare*. B, *F. longipes*. C, *F. culmorum*. D, *F. chlamydosporum*. E-H: Različne oblike bazalnih celic makrokonidijev. I, *F. culmorum*. J, *F. decemcellulare*. K, *F. verticillioides*. L, *F. longipes*. b) Tvorba in oblike mikrokonidijev pri vrstah iz rodu *Fusarium*. A, mikrokonidiji nanizani v kratkih verižicah (*F. brevicatenulatum*). B, mikrokonidiji nanizani v dolgih verižicah (*F. decemcellulare)*. C, mikrokonidiji združeni v »kvazi« glavicah (*F. circinatum*). D, repasti mikrokonidiji v »kvazi« glavicah (*F. konzum*). E, ovalni mikrokonidiji (*F. babinda*). F, hruškasti mikrokonidiji (*F. anthophilum*). G, kijasti mikrokonidiji (*F. anthophilum*). H, vretenasti mikrokonidiji (*F. semitectum*). I, repasti mikrokonidiji (*F. poae*). J kroglasti mikrokonidiji (*F. anthophilum*) (Summerell in sod., 2003).

Furthermore, the persistent structures, the chlamydospores, with walls with high lipid content, are yet another distinctive feature that can be used by researchers to identify various *Fusarium* species. Thick walled chlamydospores are present in some species (Leslie and Summerell, 2006) and they are formed either in the middle or the apex of the hyphae (Figure 2) (Sen and Asan, 2009).

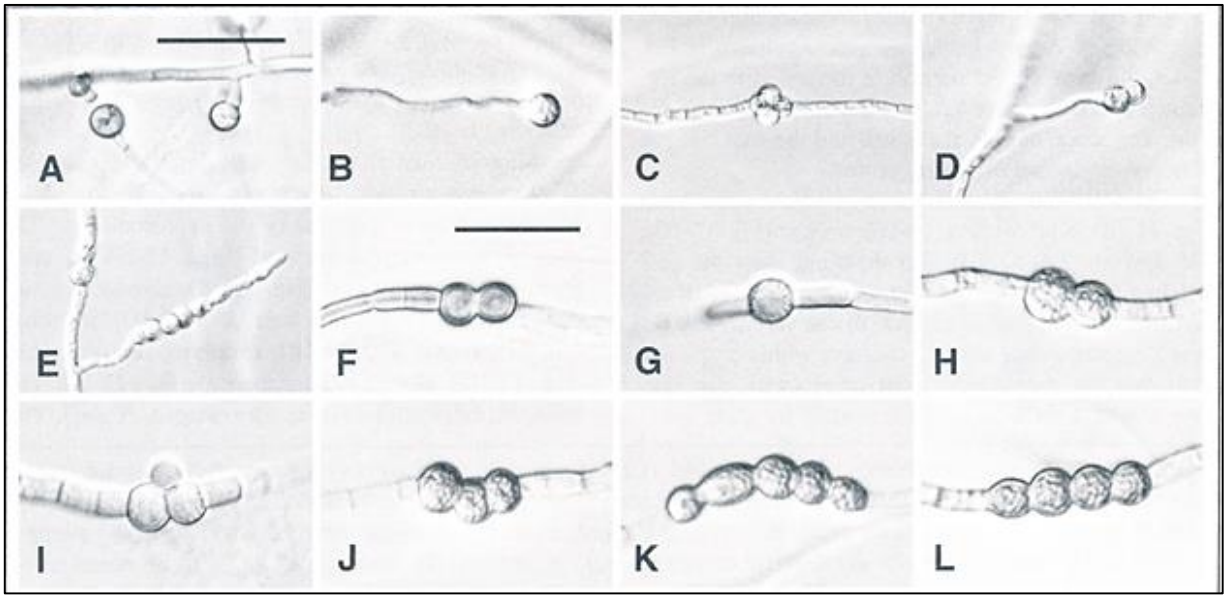


Figure 2: Chlamydospores of *Fusarium* species. A-B: Single, verrucose chlamydospores of *F. solani*. C-D: Clustered chlamydospores of *F. compactum*. E: Chain of verrucose chlamydospores of *F. compactum*. F: Paired, smooth-walled chlamydospores of *F. solani*. G: Single, verrucose chlamydospore of *F. scirpi*. H: Paired, verrucose chlamydospores of *F. compactum*. I: Clustered, smooth-walled chlamydospores of *F. scirpi*. J and L: Chains of verrucose chlamydospores of *F. compactum*. K: Chain of verrucose chlamydospores of *F. scirpi*. A-E: bar = 50 µm; F-L: bar = 25 µm. (Leslie and Summerell, 2006)
Slika 2: Klamidospore *Fusarium* vrst. A-B: posamezne, bradavičaste klamidospore *F. solani*. C-D: klamidospore v grozdih *F. compactum*. E: veriga bradavičastih klamidospor *F. compactum*. F: vparu, gladke klamidospore *F. solani*. G: posamezne, bradavičaste klamidospore *F. scirpi*. H: v paru, bradavičaste klamidospore *F. compactum*. I: v grozdih, gladke klamidospore *F. scirpi*. J in L: veriga bradavičastih klamidospor *F. compactum*. K: veriga bradavičastih klamidospor *F. scirpi*. A-E: merilna skala = 50 µm; F-L: merilna skala = 25 µm. (Leslie in Summerell, 2006)

On the other hand, the phylogenetic studies based on molecular analysis of various genes revealed that the real diversity of the genus *Fusarium* is underestimated. Based on the phylogenetic species recognition system the presence of several cryptic species was demonstrated within existing morpho-species. At least 16 distinct species were thus recognized within the *F. graminearum* species complex (O'Donnell et al., 2004; Sarver et al., 2011). Further DNA sequencings as well as species- specific PCR assays need to be performed in order to further understand this issue.

2.5 *Fusarium* HEAD BLIGHT (FHB)

One of the most prominent diseases of wheat and maize, induced by the widely spread pathogenic fungi of the *Fusarium* genus, are the *Fusarium* head blight (FHB) and the *Fusarium* and *Giberella* ear rots of maize (Goswami and Kistler, 2004). The *Fusarium* head blight can appear rapidly, its contagious incidence depending on whether there are suitable environmental circumstances for the appearance of the disease, such as precipitation during the period of flowering, but only under the condition that vulnerable hosts and aggressive isolates of the pathogen are present (Xu and Nicholson, 2009). Such epidemics were noted in recent history in Europe, Asia, and South America (Parry et al., 1995; McMullen et al., 1997), and the effects of FHB have been devastating. For example, in Europe yield losses caused by FHB ranged from 10 to 30% (Bottalico and Perrone, 2002), while in Canada the damage was more catastrophic, reaching a concerning 70% (Bai and Shaner, 1994). Furthermore, epidemics of FHB in the USA occurring during the period from 1991-1997 were responsible for a total of $2.6 billion loss in damages, as well as the following mycotoxin infections of wheat and maize (Windels, 2000).

2.5.1 Epidemiology

The *Fusarium* head blight, due to the seriousness of the devastation that it is known to cause, is rightfully treated as the most destructive disease induced by a complex of *Fusarium* species, of which *F. graminearum*, *F. culmorum* and *F. avenaceum* are most frequently involved, slightly less *F. poae* and *F. tricinctum*.

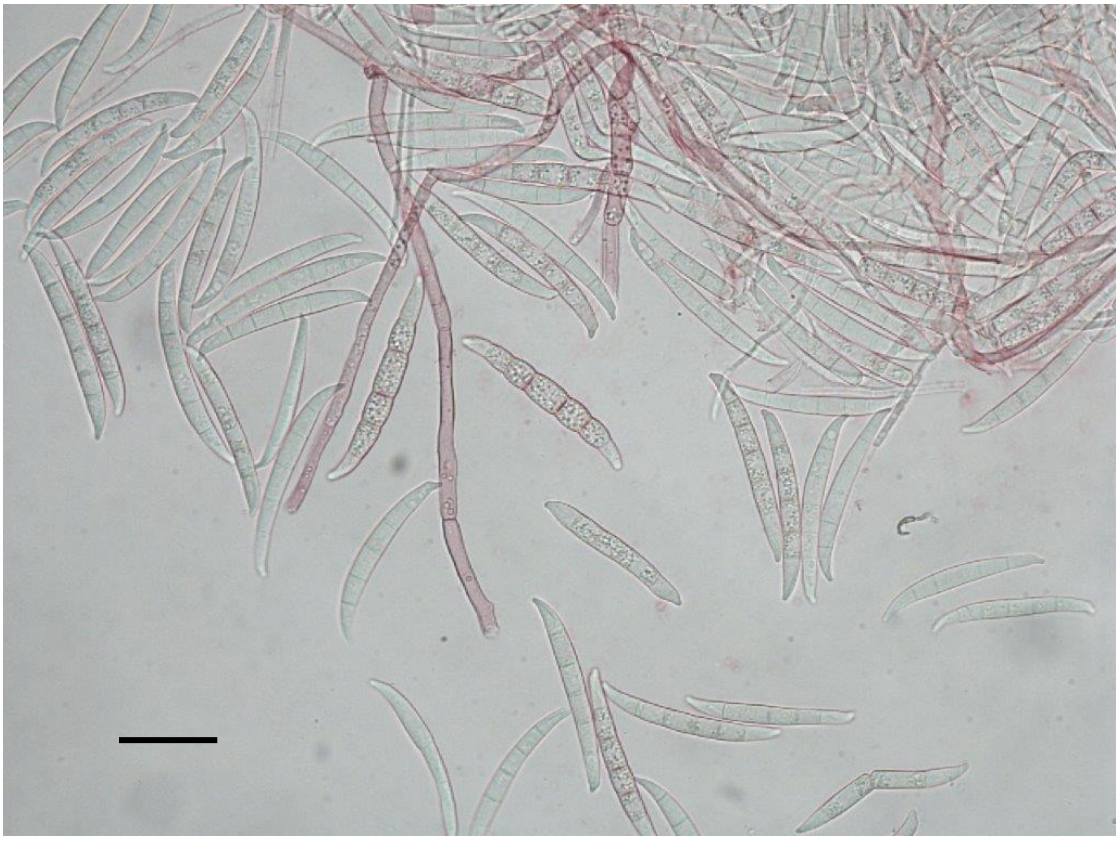


Figure 3: Macroconidia of *Fusarium graminearum*. Bar = 25 µm (photo: Celar F.A.)
Slika 3: Makrokonidiji glive *Fusarium graminearum*. Merilna skala = 25 µm (foto: Celar F.A.)

F. graminearum according to its geographical and host distribution is a cosmopolitan, and it is most commonly found in wheat, maize, and barley, however, it can also be found in other annual and perennial plants as well (Leslie and Summerell, 2006).

Characteristical features for *F. graminearum* on PDA media: fast growing colonies with abundant dense mycelia variable in hue (from white to pale orange to yellow in color). Redbrown to orange sporodochia are produced after long incubation (more than 30 days). Cultures form red pigments in the agar, however, the pigment varies from red to yellow, depending on the pH levels (the lower the value, the yellower the pigment) (Leslie and Summerell, 2006).

Macroconidia features:

- Sporodochia: Pale orange, but often rare or hard to find. Macroconidia in the sporodochia are commonly of the same shape and size.
- General morphology: Slim, thick-walled, and of medium length (48-50 x 3-3.5 µm). They are only slightly curved, with a straight ventral surface and smoothly curved dorsal side (Figure 3).
- Apical cell morphology: Conical and sporadically constricted to a snout-like shape.
- Basal cell morphology: Foot shape - properly developed.
- Number of septa: 5- to 6-septate.
- Abundance: Macroconidia are relatively rare in *F. graminearum* cultures. Macroconidia are mostly concentrated in sporodochia.

Microconidia features:

- Absent.

Chlamydospores features:

- Most commonly slowly formed. Diagnosis is not related to lack of chlamydospore production.
- Mostly located in the macroconidia, even though they may also form in the mycelia.
- Individually produced, in clusters, and chains. Usually are globose with a finely roughened, but not verrucose, appearance (Leslie and Summerell, 2006).

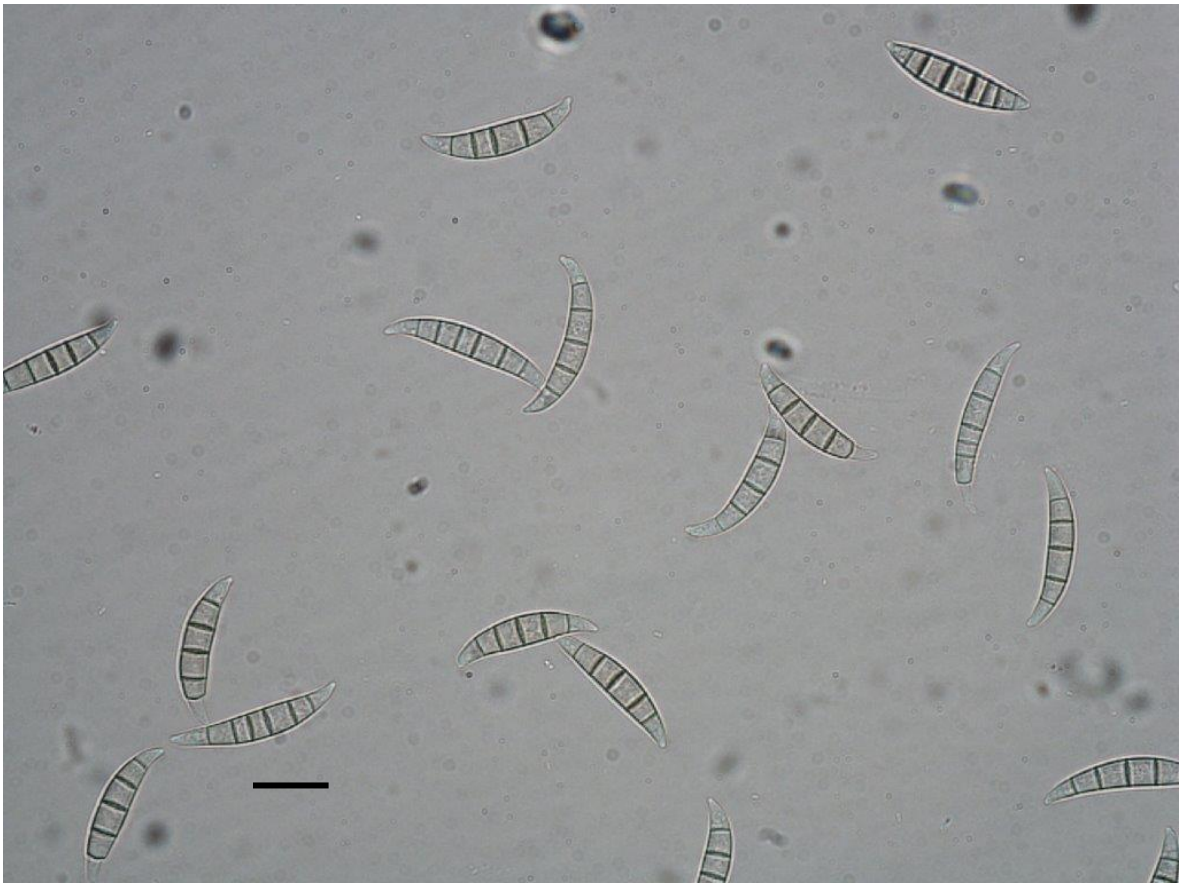


Figure 4: Macroconidia of *Fusarium culmorum*. Bar = 25 µm (photo: Celar F.A.)
Slika 4: Makrokonidiji glive *Fusarium culmorum*. Merilna skala = 25 µm (foto: Celar F.A.)

F. culmorum according to its geographical and host distribution is often present in temperate areas. It is associated with cereal crowns and grain, and plant debris in soil (Leslie and Summerell, 2006).

Characteristical features for *F. culmorum* on PDA media: *F. culmorum* is fast growing and produces abundant sporodochia in a large central spore mass (1 to 2 cm diameter), that is primarily in the color of pale orange, which consequently changes to brown-dark brown as it ages. If grown in different light and temperature conditions, it may form rings of spore masses. In most cases, the strains form red pigments in the agar, however few strains may have olive brown mycelium and olive brown pigment in the agar instead (Leslie and Summerell, 2006).

Macroconidia features:

- Sporodochia: Commonly found, orange to brown in color.
- General morphology: Robust, relatively short, and thick walled. Widest at the midpoint of the macroconidium. The dorsal side is slightly curved; however, its ventral side is nearly straight. Depending on their length, they can be quite wide (34-50 x 5-7 µm) (Figure 4).
- Apical cell morphology: Rounded and not sharp.
- Basal cell morphology: Tooth edged form (no distinct foot shape).
- Number of septa: Most commonly 3- or 4-septate.
- Abundance: Most commonly concetrated in sporodochia, with similar shape and size.

Microconidia features:

- Absent.

Chlamydospores features:

- Fast forming (3-5 weeks on CLA) and often abundant. Lack of chlamydospores is not a dependable source of identification.
- Located in hyphae and macroconidia. In field conditions, chlamydospores found in macroconidia endure longer than those found in the hyphae.
- Individually found, in clumps or chains (Leslie and Summerell, 2006).

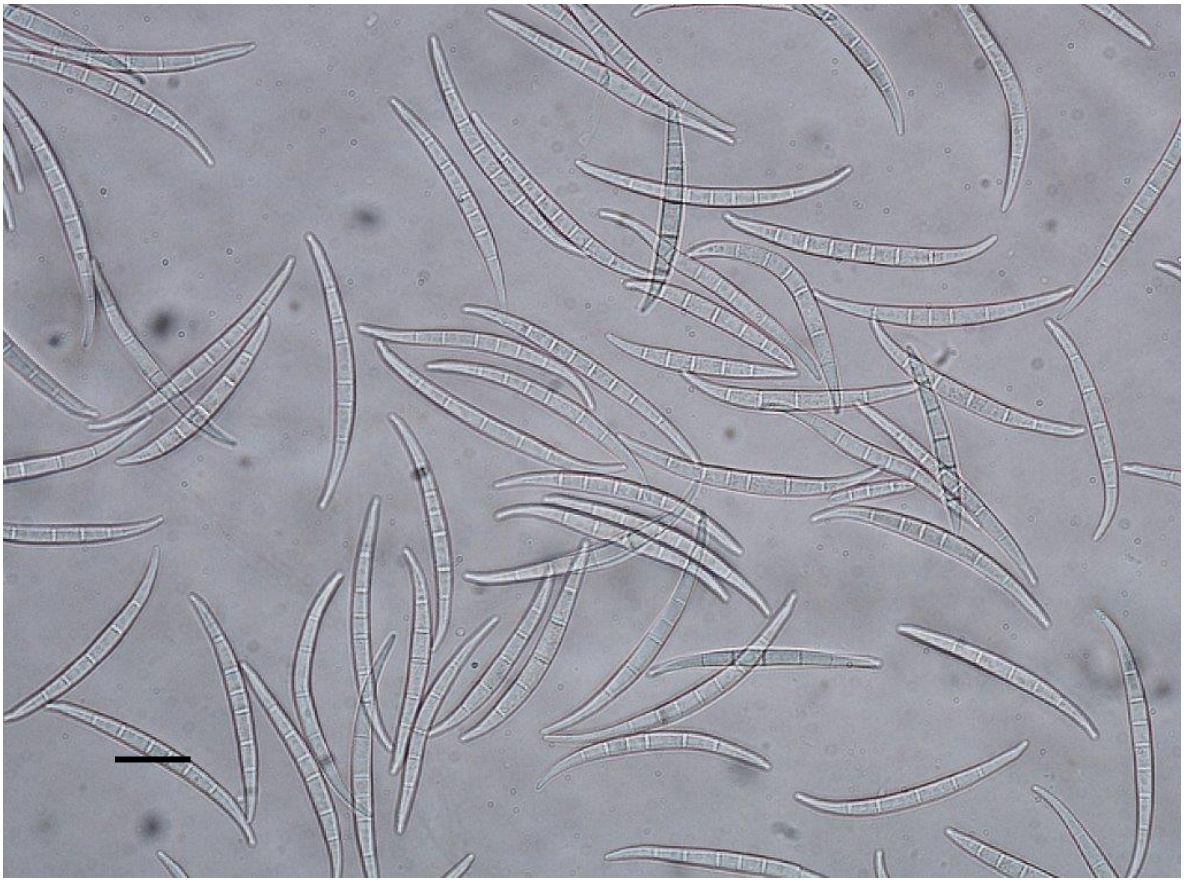


Figure 5: Macroconidia of *Fusarium avenaceum*. Bar = 25 µm (photo: Celar F.A.)
Slika 5: Makrokonidiji glive *Fusarium avenaceum*. Merilna skala = 25 µm (foto: Celar F.A.)

F. avenaceum according to its geographical and host distribution is mostly present in temperate areas as a soil saprophyte and as a pathogen of legumes, carnations, and different perennial plant species, although it may be also common on some cereal grains, e.g., wheat and barley (Leslie and Summerell, 2006).

Characteristical features for *F. avenaceum* on PDA media: They have variable growth speed which ranges from slow to relatively fast. *Fusarium avenaceum* forms abundant mycelium with variable color (from white to light yellow to grayish rose). Abundant pale orange to brown sporodochia are formed in a central spore mass. The pigment formed in the agar is grayish rose to burgundy, although due to the light reflected from the central spore mass it may appear brownish. The colony morphology varies highly. Culture mutation on PDA is commonly to a pionnotal form and ocassionaly in a white mycelial form (Leslie and Summerell, 2006).

Macroconidia features:

- Sporodochia: pale orange,located on carnation leaf pieces and on the agar surface of CLA.
- General morphology: Long (40-80 µm) and slim (3.5-4 µm), with thin walls. Either straight or somewhat curved (Figure 5).
- Apical cell morphology: Long and narrow to a point, might be curved.
- Basal cell morphology: Mostly tooth edged, however, some isolates may have foot-shaped basal cells.
- Number of septa: Mostly 5-septate, although 3- and 4-septate macroconidia may also be found.
- Abundance: Abundant in moderation, in sporodochia.

Microconidia/Mesoconidia features:

- Shape/septation: Fusoid. 1- to 2-septate. Size variations might be possible (8-50 x 3.5-4.5 µm).
- Presentation in aerial mycelium: Mostly individually found.
- Conidiogenous cells: Monophialides and polyphialides.
- Abundance: Restricted to some isolates. When produced they are commonly rare.

Chlamydospores features:

- Absent (Leslie and Summerell, 2006).

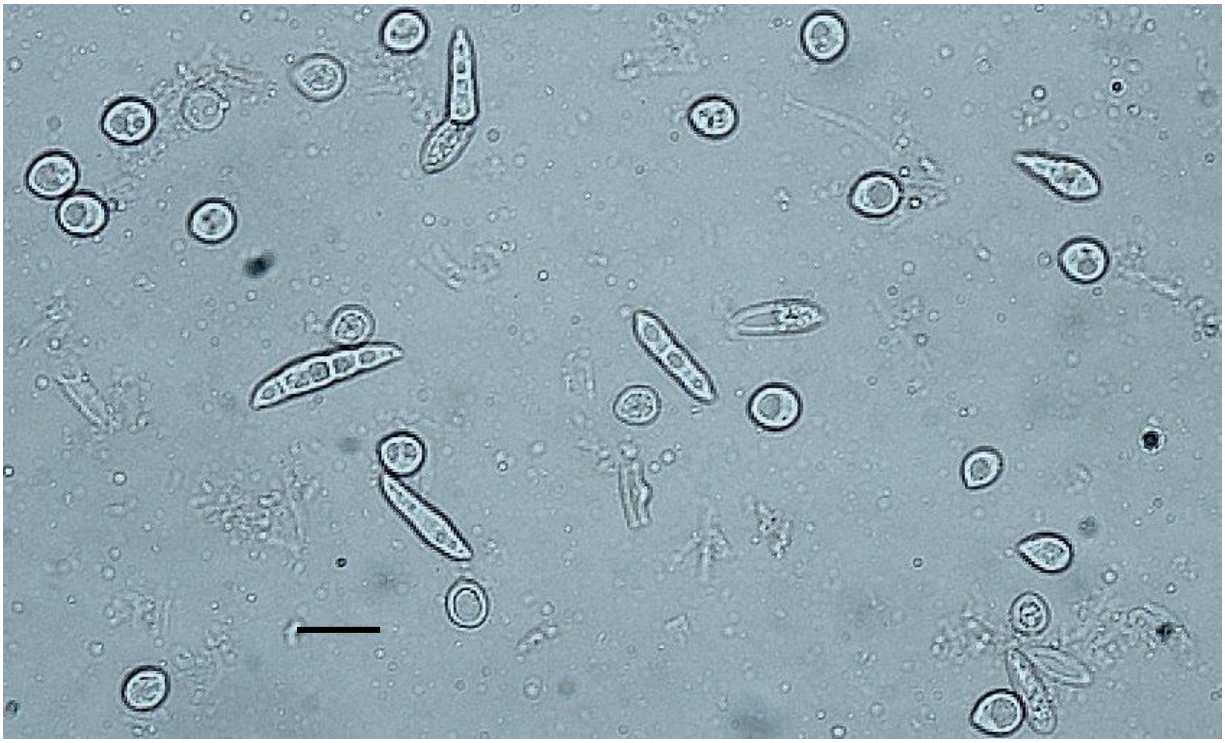


Figure 6: Macroconidia and microconidia of *Fusarium poae*. Bar = 25 µm (photo: Celar F.A.)
Slika 6: Makrokonidiji in mikrokonidiji glive *Fusarium poae*. Merilna skala = 25 µm (foto: Celar F.A.)

F. poae according to its geographical and host distribution is widely spread, most commonly in temperate areas where it is mostly isolated from seed and grain heads, or woody seedlings (Leslie and Summerell, 2006).

Characteristical features for *F. poae* on PDA media: the aerial mycelium is abundant, with a hairy or felted appearance which can change to powdery during microconidia formation. The mycelium is primarily pale-colored, but as it ages it darkens to a reddish brown. The pigments produced in the agar are most commonly red, although they can also be yellow. The cultures may have a characteristically sweet smell (Leslie and Summerell, 2006).

Macroconidia features:

- Sporodochia: Every strain does not form sporodochia, but when it does they may be located on the carnation leaves and the agar surface of both CLA and PDA cultures.
- General morphology: Slim, somewhat short, and falcate to almost lunate (20-40 x 3-4.5 µm).
- Apical cell morphology: Bent and narrow.

- Basal cell morphology: Foot cell.
- Number of septa: 3- to 5-septate, but mostly 3-septate.
- Abundance: Usually rare and not formed by all isolates even after UV light stimulation (Figure 6).

Microconidia features:

- Shape/septation: Round or napiform, often with a distinctive papilla, and usually 0- but occasionally 1-septate (8-12 x 7-10 µm; round with a diameter 7-10 µm).
- Presentation in aerial mycelium: Found in clusters resembling bunches of grapes.
- Conidiogenous cells: Urn-shaped monophialides with a distinctive collarette.
- Abundance: Abundant in the aerial mycelia (Figure 6), (Leslie and Summerell, 2006).

Chlamydospores features:

- Seldomly formed and not a dependable taxonomic character.
- Formed in clumps or chains in mycelia of older cultures.
- As stated by Gerlach and Nirenberg (1982), true chlamydospores are not formed by *F. poae*. They descrbe inflated cells with thick walls and granulated plasma that occur in older cultures.

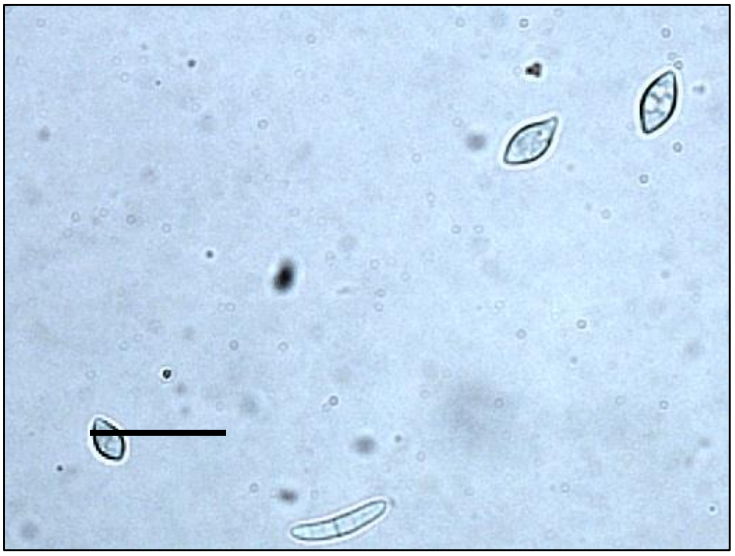


Figure 7: Microconidia of *Fusarium tricinctum* - oval with 1 septa and citriform type. Bar = 25 µm (photo: Celar F.A.)
Slika 7: Mikrokonidiji glive *Fusarium tricinctum* - ovalen z eno pregrado in limonast tip. Merilna skala = 25 µm (foto: Celar F.A.)

F. tricinctum according to its geographical and host distribution has been isolated from various substrates in many parts of the world. It seems to be more commonly found in temperate areas (Leslie and Summerell, 2006).

Characteristical features for *F. tricinctum* on PDA media: fast growing and forming abundant dense mycelia which is at first white, but with age changes to pink, red or purple. The pigments formed in the agar are red (Leslie and Summerell, 2006).

Macroconidia features:

- Sporodochia: pale orange in hue, and located on carnation leaves in CLA.
- General morphology: Mostly slender and falcate to almost lunate.
- Apical cell morphology: Bent and narrow.
- Basal ell morphology: Easily observable foot shape.
- Number of septa: Mostly 3- , sometimes 4- to 5-septate.
- Abundance: Mostly abundant.

Microconidia features:

- Shape/septation: Napiform, oval-shaped, pyriform and sometimes citriform (7-11 x 4,5-7,5 µm) with usually 0 but in certain cases 1 septa. Some microconidia may have papilla at the base of the microconidium (Figure 7).
- Presentation in aerial mycelium: Small false heads with a few microconidia that may be clustered and resemble a bunch of grapes.
- Conidiogenous cells: Monophialides.
- Abundance: Abundant in the aerial mycelia.

Chlamydospore features:

- Only sporadically formed by some isolates after 6+ weeks of incubation on CLA. If formed they don't have a diagnostic value.
- May be intercalary, terminal or on short lateral branches. Mostly in hyphae either individually or in chains.
- Globose with a smooth outer wall which turns brown with age (Leslie and Summerell, 2006).

The process of epidemic spread of the disease begins during the winter period when the fungi overwinter in crop debris, particularly in maize residues, as a saprophytic mycelia. When the weather shifts during subsequent seasons to a warm and moist, suitable circumstances are created that facilitate the growth and maturity of conidia and perithecia, which produce ascospores, while the cereal crops are in the flowering period (Goswami and Kistler, 2004). Ascospores are then forcibly released from the matured perithecia that have formed on the debris' surface (Trail et al., 2005) and are then spread with the help of rain, wind or insects to host plants (Parry et al., 1995; Sutton, 1982).

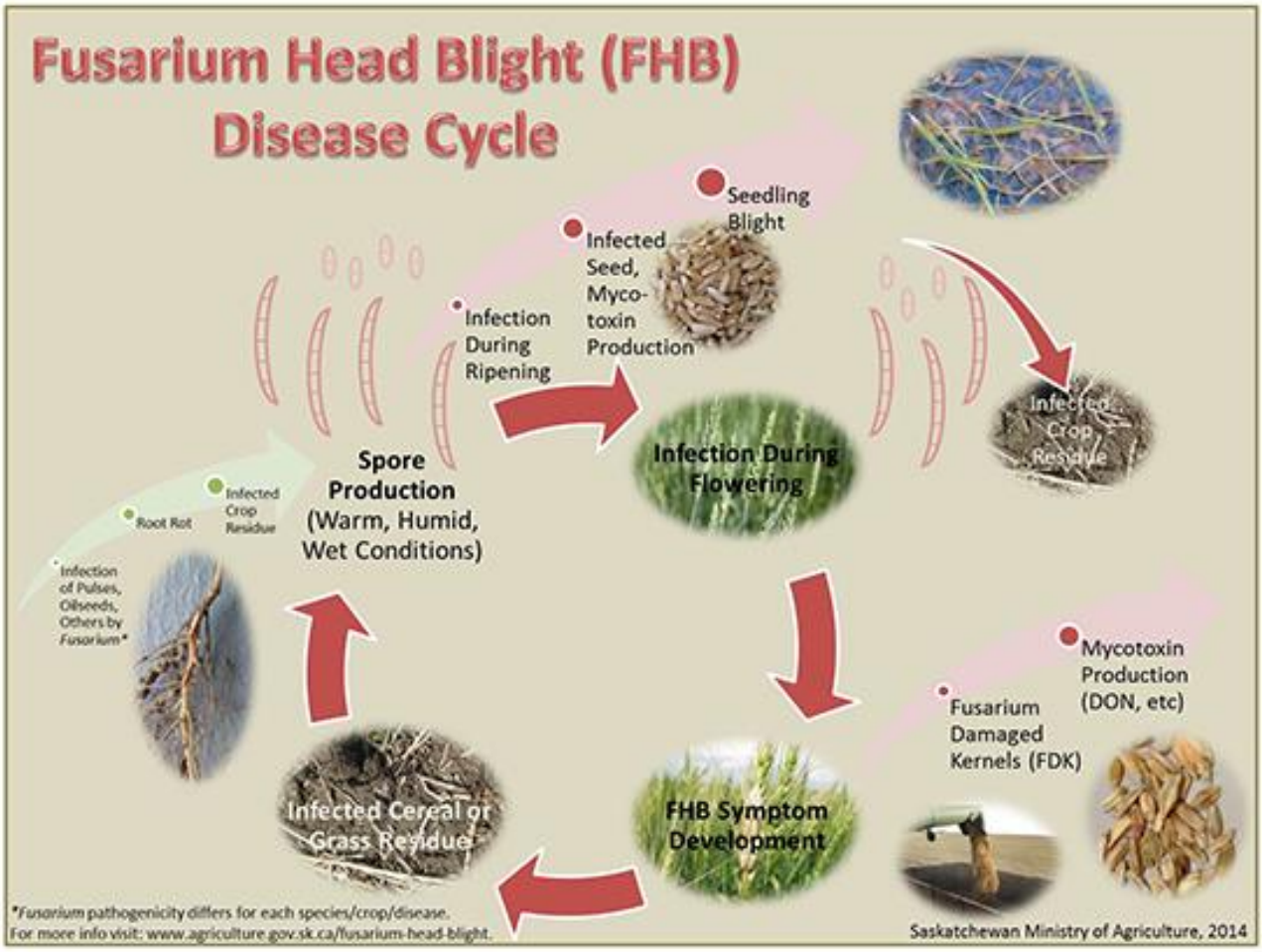


Figure 8: Disease cycle of the causal agents of FHB in wheat (Dokken-Bouchard, 2014)
Slika 8: Razvojni krog povzročiteljev fuzarioz klasa na pšenici (Dokken-Bouchard, 2014)

In order to properly grasp the connection between seedling blight and head blight in small grains, wheat included, we must primarily comprehend the life cycle of *Fusarium* pathogens (Figure 8). According to Boshoff (1996) some species of *Fusarium* are able to endure in the soil through longer periods of time and due to their pathogen's saprophytic survival, they are able to pass on the inoculum from one season to another (Shaner, 2003). Once infected with *Fusarium*, any cereal seed planted into that soil could consequently produce infected plants or allow for the appearance of seedling rot, while any grain that is infected with *Fusarium*, as a result of FHB development, could, if used as seed, be a vital source of inoculum that would lead to seedling blight and finalization of the disease cycle (Dill-Macky, 2003). Also important for the infection are the airborne inocula, significant for infection of plant ears late in the season due to the hyphal fragments that are considered a significant source of inoculum for root infection.

The production of FHB occurs if certain prerequisites are satisfied. The infection occurs via asexual or sexual spores. The former occurring when rain-splashed condiospores are transported from the stem base, the latter by the dispersing of ascospores from the surface of the soil to the leaves, with the assistance of weather conditions, like the wind (Trail et al., 2005). Primarily, for the FHB to occur, the level of humidity needs to be high so the ascospores could be released during the process of anthesis (Trail et al., 2002). However, if the discharge of ascospores does not correlate to the anthesis, the severity of the infection will be diminished (Nelson et al., 1981). There are certain symptoms of the disease, which can be easily recognized. They usually appear in the form of whitened head spikes, or when highly suitable circumstances to FHB are present, the infection could expand over the entire head, with visible pink-red mycelium and conidia on the spikelets (Figure 9). And the final stage of infection could also be observed in the kernels, which start to shrivel and lose their color, appearing pale-white (Von der Ohe, 2010).

a) b)

Figure 9: FHB symptoms: a) orange sporodochia formed at the base of the glumes; b) bleached spike (photo: Celar F.A.)
Slika 9: FHB simptomi: a) oranžni sporodohiji na bazah plev; b) obeljen klas (foto: Celar F.A.)

Special attention must be given to the anthesis period, due to the fact that the head of the wheat is most vulnerable to infections during this period (Figure 10) (Bushnell et al., 2003; Sutton, 1982). The infection primarily begins on the spike tissue where the accumulation of spores occurs. Then, the hyphae grow on the outer side of the florets and glumes, allowing for further growth in the direction of the stomata, within the inflorescence (Bushnell et al., 2003; Goswami and Kistler, 2004), while occasionally, a formation of hyphae among the cuticle and epidermal cell wall on the glume surface occurs (Pritsch et al., 2000). And it is this subcuticular development on the glume, lemma and palea that is believed to be the instrument for fungal spread (Bushnell et al., 2003).

There are however, alternative means of entering, like the gap between lemma and palea on the spikelet during dehiscence (Bushnell et al., 2003), through unprotected anthers, underlying parenchyma and stomata, or via the wheat glumes base where the walls of epidermis and parenchyma are thin. And as soon the floret is breached by the pathogen, it is a very simple task to establish in the anthers and stigmas and spread among the florets in the spikelet, as well as further on the remaining spikelets via the vascular bundles in the rachis and rachilla (Ribichich et al., 2000).

Figure 10: Anthesis stage in wheat (photo: Celar F.A.)
Slika 10: Pšenica v fazi cvetenja (foto: Celar F.A.)

Furthermore, if the climate conditions are favorable for fungal development, i.e. if there is a damp weather, the mycelia can further expand to the outer side of the lemma, palea and glume, an expansion possible in both wheat and maize (Bushnell et al., 2003). There are also two stages between which the pathogen can occasionally shortly shift, a biotrophic stage and a necrotrophic one, the latter connected with a extensive colonization and the consequent dying of the plant. *F. graminearum,* can also, in certain instances, asymptomatically colonize plant tissues, corn stalks for instance (Bushnell et al., 2003).

2.5.2 Germination and growth of *Fusarium* spp.

In order for *Fusarium* spp. to grow and germinate, there are certain environmental conditions which must be satisfied, such as light, pH, aeration, humidity, temperature, as well as enough available nutritiens. The nutritional availability is not restrictiveduring the infection and host tissue colonization, but it can be restrictive or development-impeding during the saprophytic

survival (Moliszewska and Pisarek, 1996). Considering that the availability of nutrition is not in the focus of our research, we will focus more on the impact of climatic or environmental factors on the infections of cereals.

The majority of studies dealing with the connection between FHB and environmental factors have been established during field monitoring, by which it was determined that a similarity existed between the conditions needed for every causative agent of FHB: a damp and wet environment during the flowering period (De Wolf et al., 2003). However, even though the conditions are very similar for all causative agents of. FHB, certain differences exist between the causative agents as the temperature needed for infection and germination varies among them. The place of origin is one factor contributing to this diversity, as was proven by *in vitro* studies which demonstrated that different isolates have different optimal temperatures to grow and germinate (De Wolf et al., 2003).

2.5.2.1 Germination

Apart from the climatic prerequisites needed for germination, such as temperature and moisture, there is also another factor which affects this phase of development - water availability (*a*w). The *a*w minimum for a microconidial germination of Spanish isolates of *F. verticillioides* and *F. proliferatum* were found by Marín et al. (1996) to be 0.88 on maize meal extract medium. The optimal germination temperature for microconidia of *F. verticillioides* was found to be 25-37ºC, when the *a*w was 0.96-0.98, but when the temperature was 30ºC, the *a*w value was 0.90-0.94, with intraisolate variation. On the other hand, in the case of *F. proliferatum* no matter how high the *a*w was, the optimal temperature for germination of microconidia was 30ºC, with significant intra-isolate variation.

When different conditions were created, different conclusions were reached, as it happened in Argentina, where Etcheverry et al. (2002) discovered that the growth of *F. moniliforme* and *F. proliferatum* isolates was very slow or hardly present at all, at *a*w of 0.93 and temperatures of 25ºC. Furthermore, it was discovered by Francis and Burgess (1977) that if the water potential is reduced from -1 to -20 bars, it would also decrease the percentage of germination of the chlamydospores, ascospores and conidia of *F. graminearum* isolates. And if both the temperature and water availability are reduced to a minimal level, the lag time of the germination would correspondingly increase (Marín et al., 1996; Etcheverry et al., 2002).

2.5.2.2 Growth

When it comes to the development of *Fusarium* species, the two major factors -water availability and temperature have a different effect (Table 2, Figure 11). The temperature conditions for optimal growth are different among the various *Fusarium* species. In the case of potato dextrose agar (Cook and Christen, 1976; Pettitt et al., 1996; Brennan et al., 2003), *F. poae* and *F. culmorum* achieve optimal growth at temperature of 20-25°C, *F. avenaceum* at 20°C and *F. graminearum* at 25°C, as proven by *in vitro* experiments, regardless of the origin of the isolates.

And apart from depending on temperature, the growth rate also depends 51-63% on the species itself and 23-52% on the place of origin, each species having a different growth rate according to the temperature, some achieving faster rates at high, while others at low temperatures. When provided with a temperature spanning from 10-30°C, *F. culmorum* has the fastest growth rate of the four species studied. Likewise, the maize pathogens *F. verticillioides* and *F. proliferatum,* grow best on sterile layers of maize at temperature of 30°C (Marín et al., 1998a). Furthermore, as it was discovered by Cook and Christen (1976), *F. graminearum* does not grow on temperatures higher than 35°C, even after a period of 30 days (Table 2).

Water availability is yet another important factor as it affects the optimal temperature for *Fusarium* spp. growth. In order to achieve the optimal growth temperature, water availability should be at a different value with different species. *F. culmorum* reaches the optimal at -8 to -14 bars while *F. graminearum* at -10 to -20 bars. However, in some cases if the water potential is lowered, it might lead to a raise in optimal temperature, as is the case with European isolates of *F.graminearum,* for which Cook and Christen (1976) established that the optimal temperature (24-28°C) rose when *a*w was lowered. On the other hand, increasing the *a*w can also affect the optimal growth temperature of particular species. Such examples would be *F. verticillioides* and *F. proliferatum,* which grew best on sterile layers of maize at 30°C, when *a*w was increased to 0.925 (Marín et al., 1995). However, there is not enough evidence to comprehend the effect of *a*w on the development of *F. culmorum* and *F. poae.* Furthermore, growth rate also relies heavily on the type of substrate used. Such an instance would be *F. subglutinans* which grew optimally on maize culture media at temperature of 20-25°C, but faster when grown on rice culture media, at a temperature of 15°C (Castellá et al., 1999).

Table 2: Optimum temperature and water potential/availability for the *in vitro* growth of *Fusarium* species (Johansson, 2003)
Preglednica 2: Optimalne temperature in vodni potencial/dostopnost za *in vitro* rast *Fusarium* vrst (Johansson, 2003)

Species	Substrate[a]	Optimum growth conditions		References
		Temperature (°C)	Water potential/availability[b]	
F. graminearum	BM, PDA	24–28	−10 to −20 bars	Cook and Christen (1976), Brennan et al. (2003)
F. culmorum	BM, CMA, PDA	20–25	−8 to −14 bars	Cook and Christen (1976), Parry et al. (1994), Brennan et al. (2003)
F. avenaceum	PDA	20–25	ND	Parry et al. (1994), Brennan et al. (2003)
F. poae	PDA	20–25	ND	Brennan et al. (2003)
F. verticillioides	Sterile maize layers	30	a_w > 0.925	Marín et al. (1995)
F. proliferatum	Sterile maize layers	30	a_w > 0.925	Marín et al. (1995)
F. subglutinans	MCM, RCM	15–25	ND	Castellá et al. (1999)

[a] BM = basal medium, PDA = potato dextrose agar, CMA = corn meal agar, MCM = maize culture media, RCM = rice culture media.
[b]ND = no data.

Finally, whether the *Fusarium* head blight specie will survive or largely expand, depends most on the specie itself and the climatic conditions the specie prefers. A dry and warm environment is beneficial for the spreading and growth of *F. poae*, a wet, humid and cooler one is favorable to both *F. culmorum* and *F. avenaceum*, while a warm and humid environment is the most suitable for the expansion of *F. graminearum* (Xu and Nicholson, 2009).

2.5.3 Control

According to several researchers, there are various procedures to control FHB which can be conducted, such as chemical control, biological antagonists, cultural control methods as well as the use of genetic resistance (Parry et al., 1995).

The use of chemical treatments to control FHB is currently problematic. There have been instances where tebuconazol and prothioconazol were used, but not without difficulties, as tebuconazol and prothioconazol not only rely on the environmental conditions and the

genotype, but also the fungicide treatments need to be very frequent in order to be effective. Because the period during which the heads are most sensitive to FHB is short (only during anthesis and for a short period after), the optimal opportunity for application is during the flowering period, a time-frame further limited by precipitation (Von der Ohe, 2010). There are even greater problems with the use of bacterial or fungal antagonists. Even though there are many studies of fungal or bacterial antagonism, the majority of results retrieved from the field, are inconstant or a total failure (Xu and Nicholson, 2009). As the current circumstances are such, the use of biological antagonists are still very distant to our reach (Parry et al., 1995).

There are several agronomical procedures which have been proven to have an effect on FHB. One such procedure is using fertilizers. If nitrogen fertilizers from 0 to 80 kg/ha are used, it would cause an increase of the severity of FHB and DON grain contamination, an effect explainable from the fact that fertilizers are increasing the plant density and alteration of microclimate (Lemmens et al., 2004). Other effective practices would be tillage and stubble management. These two procedures were proven to be effective in affecting FHB when previously cultivated crops were either maize or wheat. The type of tillage can have a significant effect on the FHB infection potency. The infection is lowest when the tillage is deep and rises to alarming levels without it. The effect that tillage has on the development of FHB is best demonstrated if the crop residues are left to prevent soil erosion, and thus create a source of fungal inoculum on the soil surface (Dill-Macky, 2008), while when the residues are destroyed the source of the inoculum is restrained (Paul et al., 2004; Bateman et al., 2007). However, even though the type of the previously cultivated crop and the tillage can have an effect on severity of the disease, they do not cause a meaningful change of the composition of the species, while stubble burning leads to a reduction in *F. graminearum* survival (Dill-Macky, 2008). Another practice would be crop rotation. In the case of FHB caused by *F. graminearum* the most useful practice would be to rotate maize with oats, soybeans or pumpkins, however there are also other crops that could likewise affect the population of FHB pathogens. In cases where the previously grown crop instead of wheat or maize was soybeans, a 25-50% reduction in FHB occurrence was observed (Dill-Macky, 2008).

And finally, genetic resistance as a solution for FHB control was proposed by Lemmens et al., (2004), who recommended the development and cultivation of resistant cultivars, as a cure to the aggregation of mycotoxins in wheat and maize. However, for the purpose of all the above - referred procedures to be achieved, and the symptoms and mycotoxin accumulation to be effectively reduced, we need to consider species composition of the FHB pathogens as well. It is of vital importance to comprehend the community structure of the FHB pathogen, in order to successfully regulate the disease in practice (Xu and Nicholson, 2009).

2.5.4 FHB resistance

When the pathogen is not able to further grow, reproduce or expand in a certain plant, there is some form of resistance to that pathogen present in the plant. Resistance is usually expressed in the form of a hypersensitive reaction (HR) that restrains the pathogen to the infection site in the form of a necrotic lesion (Van Loon, 1997). When discussing wheat resistance to FHB, according to Mesterhazy (2002), there is no single form of resistance that can be analyzed, but a diverse group of various kinds of resistances, such as (I) primary infection resistance (Schroeder and Christensen, 1963); (II) expansion resistance (Schroeder and Christensen, 1963); (III) kernel infection resistance (Mesterhazy, 1995; Mesterhazy et al., 1999); (IV) infection tolerance (Mesterhazy, 1995; Mesterhazy et al., 1999) and (V) DON accumulation resistance (Miller et al., 1985).

Furthermore, the majority of researchers agree that there is no such thing as immunity in wheat cultivars, and that nearly all of the cultivars are receptive to infections, but some of them are, to some extent, resistant (Parry et al, 1995). For example, *Triticum aestivum* L. is more resistant to *Fusarium* infections than *Triticum durum* L., grains of which are exposed to high mycotoxin accumulations (Stack et al., 2002). Common wheat contains 46 separate measurable trait loci (QTLs) that were diagnosed for resistance to FHB, as opposed to durum wheat, which contains only four QTLs, as reported by Buerstmayr et al. (2009). Likewise resistance against *F. graminearum* and *F. culmorum* were discovered by Mesterhazy et al. (1999), which was also accurate for *Fusarium* damaged kernels (FDK), FHB, yield loss and the scale of DON contagion. Moreover, it is a fact that there is a quantitative inheritance of FHB resistance present in all cereal species however there is also a very important genetic variation present between the breeding elements (Snijders, 1990; Miedaner, 1997).

According to Buerstmayr et al. (2000), the most efficient disease control procedure is to grow genetically resistant cultivars. However, since such a genetic difference in resistance to FHB among wheat varieties exists, the true test would be to create wheat cultivars that are not only resistant against most diseases, FHB included, but also ones that are capable of yields that are both large, reliable and of a good quality (Buerstmayer et al., 2009). In order to successfully grow resistant cultivars, we must first know the sources that create resistance. The genes are naturally one source to which attention must be paid, as there are several known genes that are responsible for resistance activation, however, there is also one more factor that must be taken into consideration, and that is the environmental factor (Bai et al., 2000). That is why, even though the QTL method has been largely used in the search for resistance cultivars, with the addition of molecular markers, durum wheat sources that are effective in their resistance to FHB still haven't been discovered. Assessment of FHB resistance in whole plants should be

done during a cultivation that would span over several years and various environments (Browne and Cooke, 2004).

According to Toth et al. (2008), in order to successfully cultivate FHB resistant wheat, a single pathogenic isolate of *Fusarium* sp. would be enough to assist us in the breeding process. However, he also believes that the pathogen used should be an aggressive isolate, due to the fact that isolates with lower aggressiveness might not grant us the possibility to differentiate the various degrees of resistance in the wheat lines and cultivars.

2.6 *Gibberella* AND *Fusarium* EAR ROTS OF MAIZE

Fusarium pathogens are responsible for two types of maize (corn, *Zea mays* L.) ear rot disease. Species from the *F. fujicuroi* species complex, mainly *F. verticillioides* and *F. subglutinans*, are responsible for the *Fusarium* ear rot of maize, while *F. graminearum* is responsible for another type of ear rot, traditionally called the *Gibberella* ear rot of maize (Figure 11). On the other hand, depending on the geographical location and climate, other causal agents of ear rot may also include *F. culmorum* and *F. equiseti* (Leslie et al., 1986; Sutton, 1982). Even though both diseases are globally distributed and occur in every climate that is suitable for corn growth, there are certain climate types where these diseases are especially problematic. These two ear rots pose a serious threat to new sweet and waxy corns, grown and produced in the tropics (Smith and White, 1988).

Their survival ability especially in tropical and sub-tropical areas is significantly worrying due to not only the numerous annual cropping cycles that permit the pathogens to develop great populations, but also to their creation of mycotoxins which are hazardous to human and animal welfare, in the case of consummation (Smith and White, 1988).

Figure 11: *Gibberella* and *Fusarium* ear rot on maize (photo: Celar F.A.)
Slika 11: Fuzarijska plesnivost storžev koruze (foto: Celar F.A.)

2.6.1 Pathogens

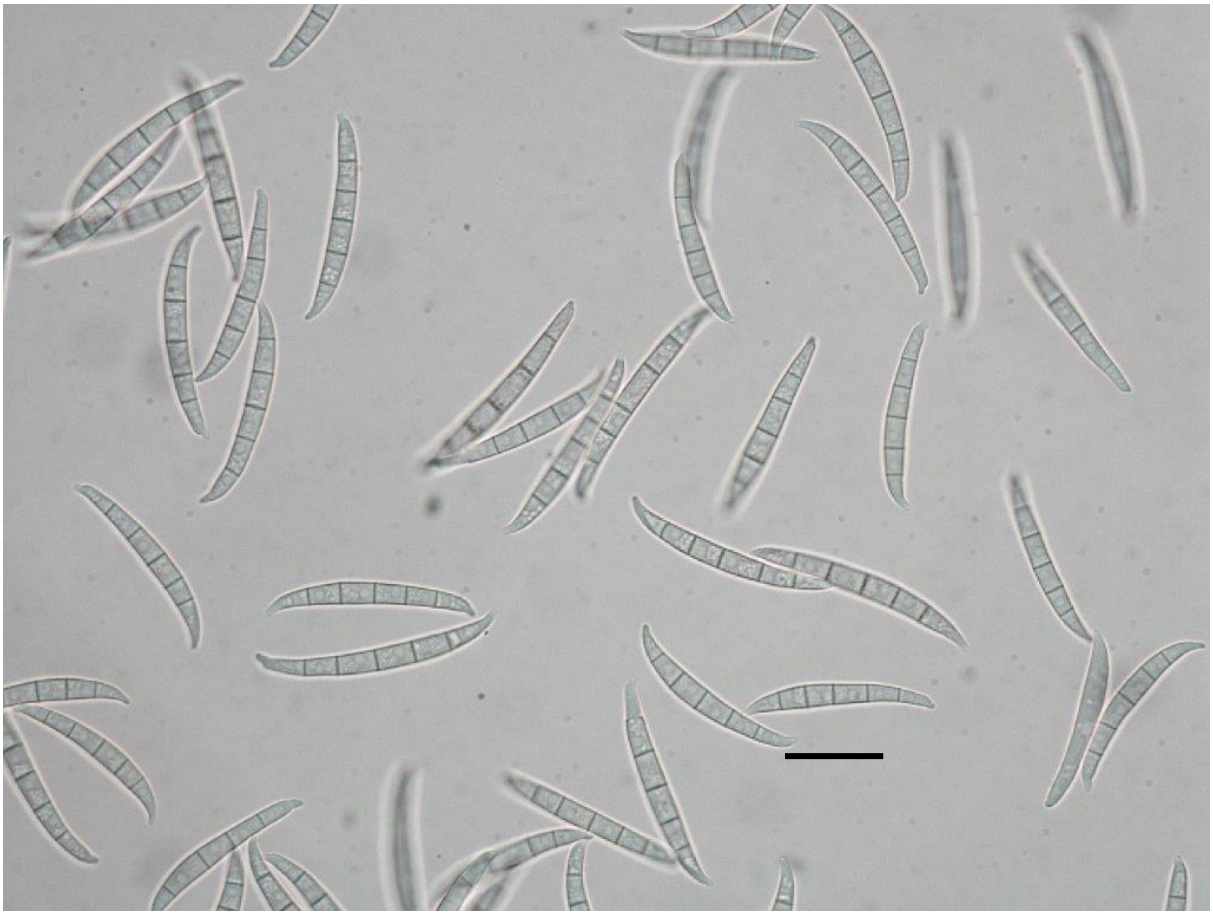


Figure 12: Macroconidia of *Fusarium graminearum*. Bar = 25 µm (photo: Celar F.A.)
Slika 12: Makrokonidiji glive *Fusarium graminearum*. Merilna skala = 25 µm (foto: Celar F.A.)

F. graminearum can be recognized by the production of macroconidia, which are distinctly septate, falcate to almost straight (Figure 12), and the absence of microconidia. However, chlamydospores can also develope after long incubation (Leslie and Summerell, 2006).

Microconidia and macroconidia are also developed by *F. verticillioides,* however there are no chlamydospores (Figure 13). When compared to *F. graminearum*, they are generally similar in shape with a bit longer and narrower macroconidia. The microconidia, on the other hand, are small in size, oval, egg-shaped, and single-celled, but they are more easily identified not by their shape and size, but by their arrangement in long chains above the mycelium (Leslie and Summerell, 2006).

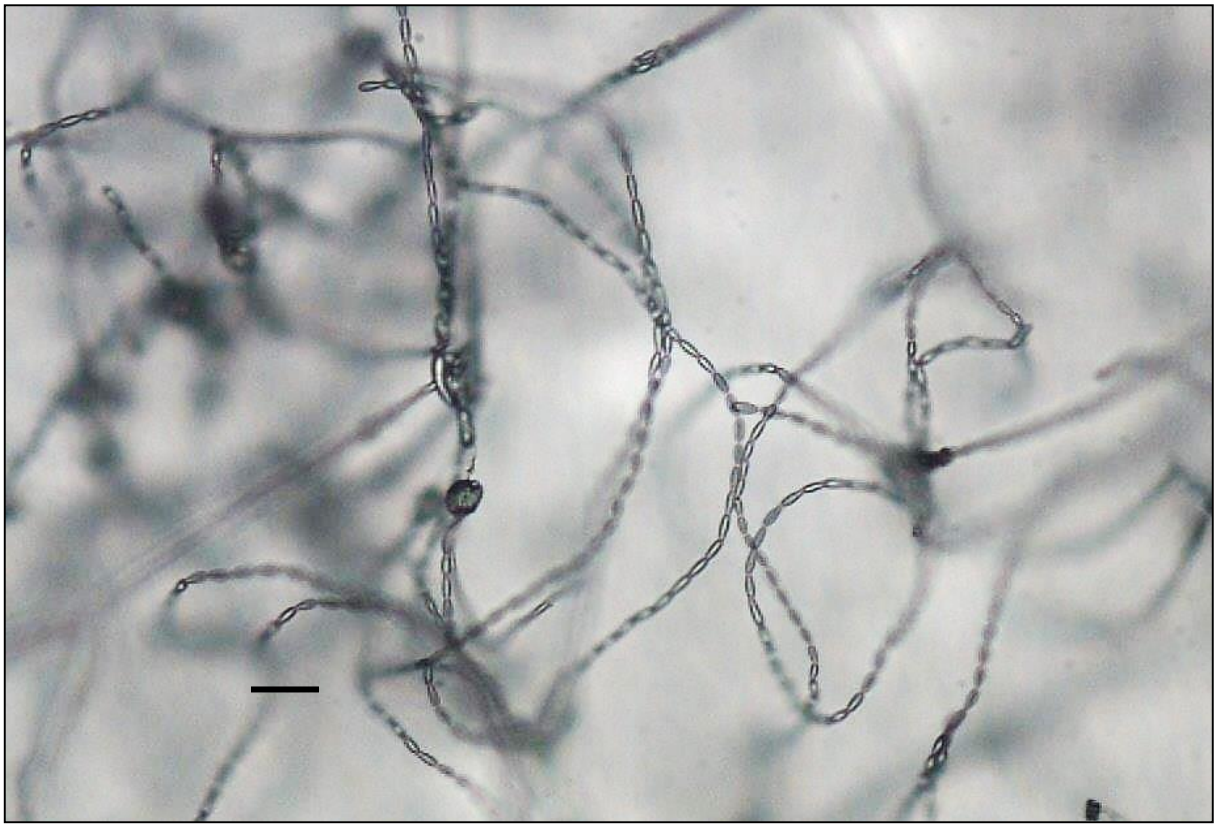


Figure 13: Microconidia of *Fusarium verticillioides* in long chains. Bar = 25 µm (photo: Celar F.A.)
Slika 13: Mikrokonidiji glive *Fusarium verticillioides* v dolgih verižicah. Merilna skala = 25 µm (foto: Celar F.A.)

Microconidia and macroconidia, bur not chlamydospores are developed by *Fusarium subglutinans*, a species most common in colder areas (Figure 14). The microconidia though (7-11 x 4,5-7,5 µm), are not as long as the ones of *F. graminearum* and *F. verticillioides*, they are oval in shape, and form in aerial bunches, or false heads (Leslie and Summerell, 2006).

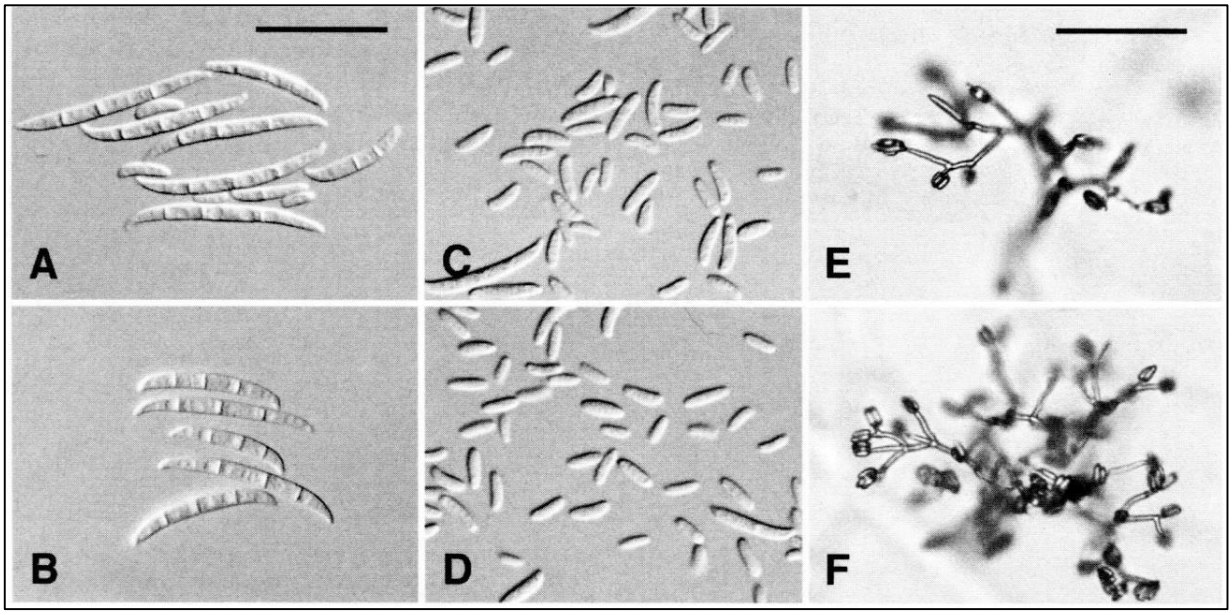


Figure 14: Macroconidia (A-B) and microconidia (C-F) of *Fusarium subglutinans*. A-D, bar = 25 μm; E-F, bar = 50 μm. (Leslie and Summerell, 2006)
Slika 14: Makrokonidiji (A-B) in mikrokonidiji (C-F) glive *Fusarium subglutinans*. A-D, merilo = 25 μm; E-F, merilo = 50 μm. (Leslie in Summerell, 2006)

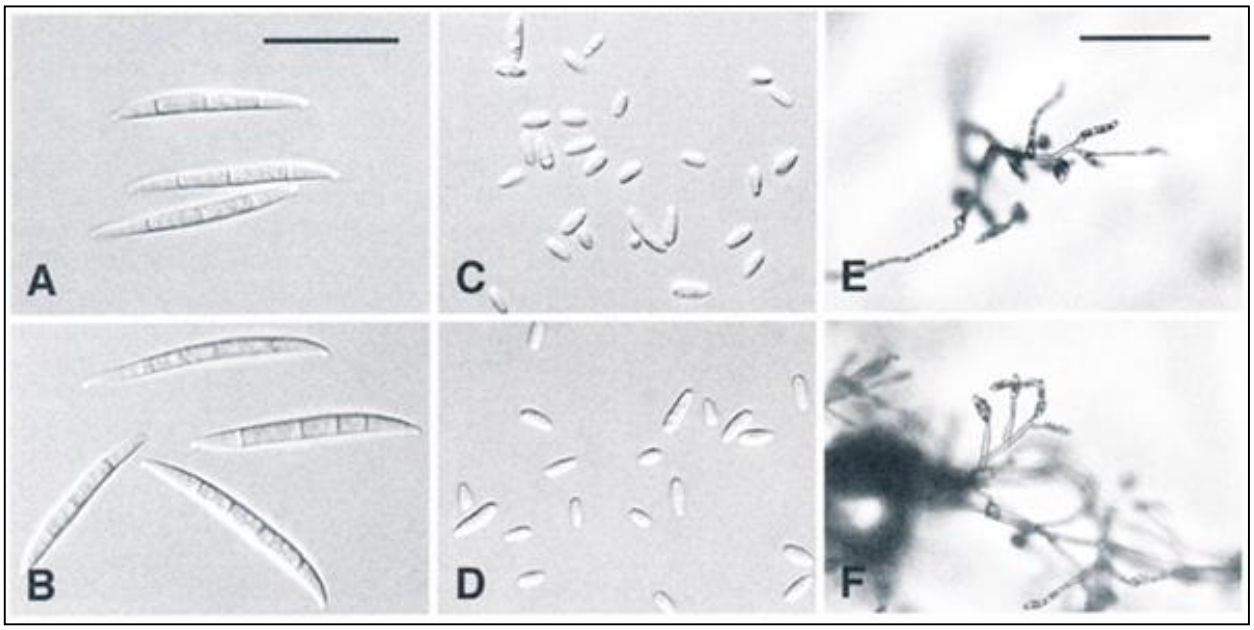


Figure 15: Macroconidia (A-B) and microconidia (C-F) of *Fusarium proliferatum*. A-D, bar = 25 μm; E-F, bar = 50 μm. (Leslie and Summerell, 2006)
Slika 15: Makrokonidiji (A-B) in mikrokonidiji (C-F) glive *Fusarium proliferatum*. A-D, merilo = 25 μm; E-F, merilo = 50 μm. (Leslie in Summerell, 2006)

Another pathogen is *F. proliferatum*, which produces microconidia that are in the shape of a club with a flattened base, as well as macroconidia that are visually much alike to the ones of *F. graminearum* (Figure 15). There are no chlamydospores present in this species (Leslie and Summerell, 2006).

2.6.2 Pathogen dissemination and disease cycle

Various factors are responsible for the dissemination of *Fusarium* species, the most important of all being the weather factor, as the primary agents of *Fusarium* distribution are wind and rain. Other agents are mostly insects, like the European corn borer which can spread the pathogen during corn ears infestation or by contact with corn silks (Parsons, 2008).

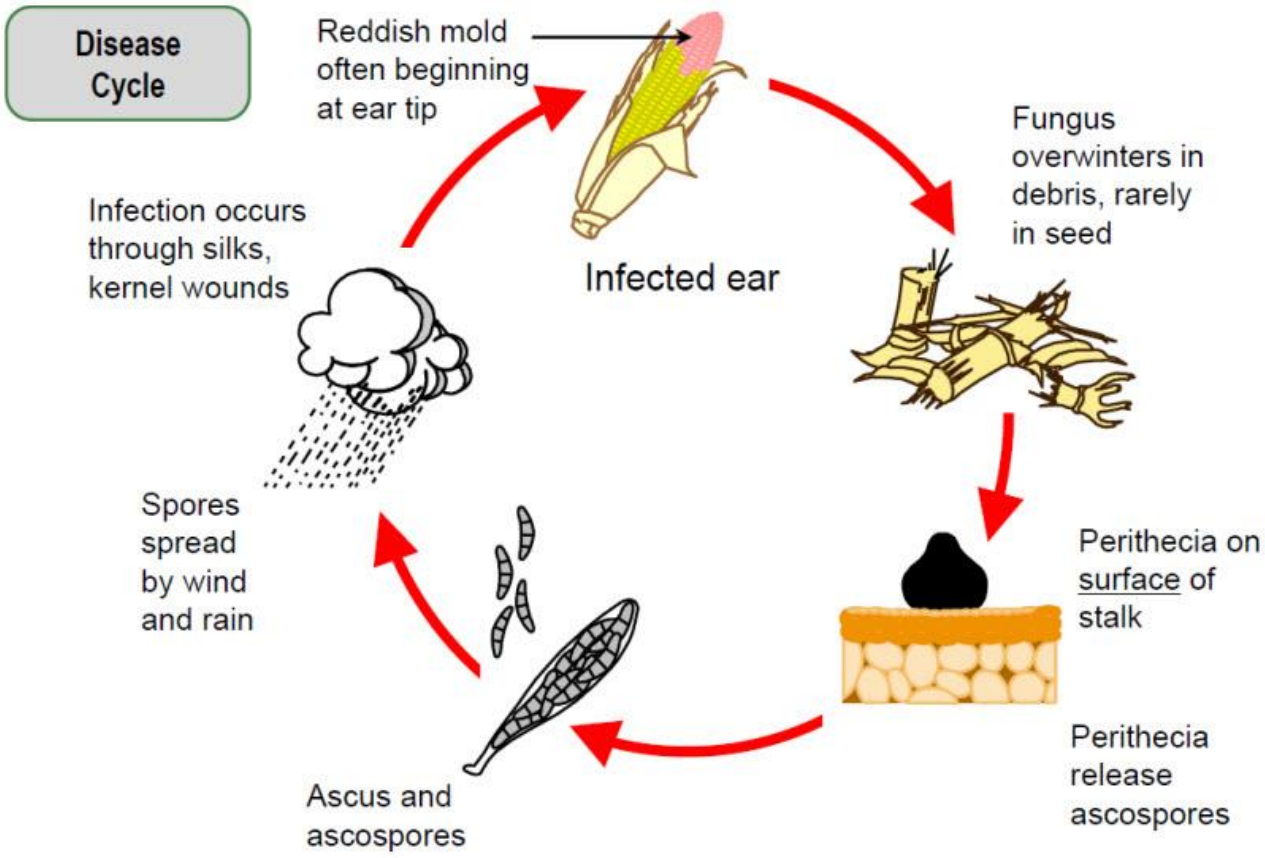


Figure 16: Disease cycle of *Gibberella* and *Fusarium* ear rots on maize (Corn Insect and Disease Guide, 2015)
Slika 16: Razvojni krog gliv *Gibberella* spp. in *Fusarium* spp., povzročiteljic plesnivosti koruznih storžev (Corn Insect and Disease Guide, 2015)

The corn ear silks are the primary site of *F. graminearum* infection, from where the disease spreads basipetally, starting from the tip and moving to the ear base, possibly reaching the ear peduncle, in the case of serious epidemics. The mature perithecia will then produce ascospores which will further start subsequent disease cycles within or among the fields.
The inoculum that would be responsible for contamination in subsequent crops can be usually located in the crop debris, but it could also be traced to infected corn seeds (Figure 16), (Munkvold et al., 1997).

Since the wounds are the place of entering for the majority of *Fusarium* species from *F. fujikuroi* species complex, insects are considered vital agents in the infection and dissemination processes. During their feeding, insects can cause injuries through which the infection can enter (Parsons, 2008), and they can further spread the disease by transporting *Fusarium* spores that can attach to them, to uncontaminated plants. However, the fungus can also survive as a saprophyte in the crop debris after harvest has been done, and then further move through wind or splashing rain on the next, freshly planted corn crop. The climatic factor is also important for survival and spread of the pathogens and develpoment of the disease. *Gibberella* ear rot has been known to thrive in humid environments during the initial 21 days following the silking (Woloshuk et al., 2010), with the most suitable temperature being from 26 to 28°C (Parsons, 2008), but it can also occur at lower temperatures (Willyerd et al., 2010; Woloshuk et al., 2010). On the other hand, dry and insufficiently irrigated environments are most favorable for *Fusarium* ear rot induced by *F. verticillioides*. The ear rot appears when the corn is mature and kernels begin to lose moist (Parsons, 2008).

2.6.3 Pathogen host ranges

There is a great number of hosts of the *Fusarium* and *Gibberella* ear rot causative agents, and that is why identifying possible inoculums sources in the vicinity of the cultivation area is of great importance. Currently, there is a variety of host ranges of disease generating pathogens that have been discovered up to the present, and they can be seen in Table 3.

Table 3: The host ranges of the pathogens causing *Gibberella* ear rot and *Fusarium* ear rot of corn (Leslie and Summerell, 2006; Burlakoti et al., 2008; Bacon et al., 1996; Viljoen et al., 1997; Proctor et al., 2010)
Preglednica 3: Gostitelji patogenov, ki povzročajo plesnivost storžev koruze (Leslie in Summerell, 2006; Burlakoti in sod., 2008; Bacon in sod., 1996; Viljoen in sod., 1997; Proctor in sod., 2010)

	Hosts
Fusarium graminearum	Maize, corn (*Zea mays*), wheat (*Triticum* sp.), barley (*Hordeum vulgare*), oats (*Avena sativa*), rye (*Secale cereal*), and species of Lycopersicon, Pisum, Trifolium, and Solanum, such as potato, as well as sugar
F. verticillioides	Hundreds of plants important to agriculture including maize, rice (*Oryza sativa*), Sorghum, sugarcane (*Saccharum officinale*), wheat, cotton (*Gossypium hirsutum*), banana (*Musa* spp.), pineapple (*Ananas comosus*), and tomato (*Solanum lycopersicum*)
F. subglutinans	Maize, mango (*Mangifera indica*), pine (*Pinus* sp.), sugarcane, pineapple, various grasses/reeds (family: Poaceae)
F. proliferatum	Maize, sorghum, mango, asparagus (*Asparagus officinalis*), fig (Ficus), onion (*Allium cepa*), palm (family: Arecaceae), pine, rice, cucumber (*Cucumis sativus*), garlic (*Allium sativum*), salt cedar (*Tamarix* sp.)

2.6.4 Symptoms, signs, and disease diagnosis

Gibberella ear rot caused by *F. graminearum* produces visual symptoms that can be easily recognized, thus providing for a better diagnosis and earlier control measurements. The symptoms are visible at the corn ear, where a pinkish-red fungal mycelium grows, initially appearing at the ear tip and further moving downward toward the base, seldom infecting the whole ear. It can also cause the husk to stick to the kernels and create difficulties when being removed. However, these symptoms can also vary and cause confusions as sometimes the mycelium can have a pale pink color, causing it to be mistaken as the symptom of another disease, like the *Diplodia,* which has gray-colored mycelium. Another observable symptom are the little, black perithecia that can appear on the stalk, husk or kernels (Sutton, 1982; Parry et al., 1995; Miedaner, 1997).

Fusarium ear rot caused by *F. verticillioides* and *F. subglutinans*, on the other hand, has different symptoms. The mycelium on rotten ear is either white, pale pink, or the color of pale lavender. Other distinctions include the absence of perithecia as well as association of the disease with the vicinity of the feeding injuries caused by thrips or corn earworms invasions (Goswami and Kistler, 2004).

It is due to these easily recognized symptoms that more expensive methods, like the molecular or serological molecular detections, such as the Polymerase Chain Reaction (PCR) or Enzyme-Linked Immunosorbent Assay (ELISA), can be omitted when a simple visual diagnosis is to be made.

2.6.5 Integrated management of corn ear rots

When discussing disease management, primary importance must be given to an early, efficient diagnosis, because disease control is much more problematic during subsequent disease stages. However, there are also some other methods and approaches that are similarly essential to proper disease control. First of all, and probably one of the most efficient management procedures is to prevent spread of the pathogen by quarantine. Such an example would be to establish a state quarantine program in the case of dangerous insects, like the European corn borer, which inflicts injuries on corn ears and by that allows infection by *Fusarium* species. Alternatively, another form of eliminating the potential pathogen presence form a certain area is to address the problem at the planting stage and plant seeds that are disease-free. This could also, along with rotating the crop to non-host plants, efficiently help with the decreasing of the initial inoculum (Parsons, 2008).

Another successful tactic is selection of resistant corn types. By choosing genotypes that are more resistant to *Fusarium* species, spread of the disease could be significantly lowered. Such an example would be corn types that dehydrate fast, or types that have swirled rows, spiky tips, and looser husks, as those types are known to be less prone to diseases. Alternatively, disease management could also be made easier by more closely understanding corn types that are known to be more disease-prone, such as types that have rectangular tips, tight-fitted husks and linear-rowed ears (Burlakoti et al., 2008). All the corn types and their various degrees of resistance can be found in seed catalogs.

Furthermore, changes in the approach we take on the harvest can also improve control of the disease. One such change would be to do the harvest earlier than usual, which could lead to a lower disease manifestation as well as a reduction in the severity of the disease, due to the fact that the disease has a tendency of appearing in plants that have reached physiological maturity. Another change would be to set up the machinery we use in processing to decrease damaging the kernels and take out the lightest-infected kernels, in order to elude post-harvest losses. Also, we can improve control by managing the moisture levels of the stored grain and keep it at, or lower it to, 15% with a swift drying, done by heating the kernels (Das, 2014).

And finally, there are some other approaches not mentioned thus far that are also effective in disease control. One such approach would be to sanitize the field by removing debris and earlier crop residues, to decrease the initial inoculums levels. Or we can also manage the alternate hosts by controlling the weed and remove the alternate hosts, to keep the level of pathogen inoculum at minimum, as well as decrease the number of receptive hosts in a certain area (Das, 2014).

2.7 The *Fusarium* SPECIES INVOLVED IN INDUCING WHEAT AND MAIZE KERNEL FUSARIOSIS AND CONTAMINATION WITH MYCOTOXINS

Mycotoxins are toxic secondary metabolites that contaminate agricultural products, if the environmental conditions are suitable for their production. Mycotoxins are produced by molds, or fungi, and it is due to their presence in soil and plant debris as well as their ability to be disseminated through air by wind, rain and insects that they are often present, together with their related mycotoxins, in/on food (Pittet, 2001). The presence of mycotoxins in food as well as their effect on causing acute and chronic diseases in animals, has raised a serious awareness to their harm, and thus created the urgent need to focus on more efficient analytical methods for mycotoxin analysis, as well as the minimization of their occurrence in food (Pittet, 2001).

Nevertheless, even though such a vital need exists, the design of an approach that would measure and evaluate mycotoxins is a difficult and demanding mission. In order to calculate the necessary grain mycotoxin concentration at the μg/kg or parts-per-billion levels needed for the majority of relevant mycotoxins, we must develop a truly reliable approach, as opposed to the usual method of retrieving a comparatively big initial sample out of a lot, and then largely diminishing it to a practical amount, and ultimately analyzing the small representative segment (Pohland and Trucksess, 2001).

Due to the large number of approaches that were created to efficiently detect mycotoxin levels in the majority of food, in order to select the most optimal method of analyses we must judge each method according to certain criteria, so that we can choose the most suitable. There are some criteria, which apart from the basic efficiency criteria like precision and veracity, need to be satisfied in order for the method to be considered the most practical one. The time needed for the procedure or the speed with which it could be done is the first criteria, the specialized skills needed for the procedure to be conducted is the second one, and the result of the procedure, i.e. whether the assay provides a qualitative or quantitative result is the third one (Pittet, 2001). It is obvious that the best method would be the one that would include all the criteria, that would be fast, easily conducted as well as quantitative, however, the majority of methods known to practice usually do not meet all the criteria, and it is usually left to the

researcher to decide which of the criteria is vital to his/her application. In our instance, since the analysis generally includes the extraction, cleaning and identification of mycotoxins, the criteria most relevant to us would be the cost needed to conduct the analyses, as well as the required skills for the successful completion of the process.

2.7.1 The *Fusarium* species involved in kernel fusariosis and accumulation of mycotoxins

There *Fusarium* species responsible for inducing kernel fusariosis are globally disseminated and responsible for a variety of diseases to their host plants. They causediseases like stalk rot, ear rot, and seedling blight of maize and foot rot, seedling blight, and head blight (scab) of small grain cereals (wheat, barley, triticale, rye, oats), the most worrying of which, from a mycotoxicological standpoint, are definitely the maize ear rot and head blight of small cereals, due to the possible mycotoxin buildups in the grains during the disease phases. Furthermore, in both cases it is quite usual for a number of *Fusarium* species, i.e. a complex, to appear in a fast sequence, so usually from a single piece of contaminated tissue up to nine species could be isolated, while a newly picked wheat sample that was freshly harvested from the test location, could have even up to seventeen *Fusarium* species. Out of all these species, there is only a limited number that could be treated as pathogenic and a very small number that could be predominant in an appropriate host-agroclimatic system (Burgess et al., 1997), however this does not imply that the species which are less pathogenic or opportunistic are not competent of generating serious toxin problems. That is why a full toxicogenic profile of infected crop should include both the predominant pathogenic as well as the opportunistic species, found in a complex (Burgess et al., 1997).

The species most commonly related to scab of wheat and other cereals are *F. avenaceum*, *F. culmorum*, as well as *F. graminearum*, while the less frequent species are *F. cerealis*, *F. equiseti*, *F. poae*, *F. sporotrichioides*, as well as *F. tricinctum*. However, there are also some other species that could infrequently appear, such as the *F. solani*, *F. subglutinans*, *F. acuminatum*, *F. incarnatum*, as well as *F. oxysporum* (Burgess et al., 1997).

In the case of maize ear rot, on the other hand, there are at least two types of the disease of which *Fusarium* species are suspected to be responsible for. The dominantly isolated species of *Fusarium* ear rot or random kernel rot are *F. subglutinans*, *F. proliferatum* and *F. verticillioides*, while the less often isolated species from molded maize ears are *F. sporotrichioides*, *F. acuminatum*, *F. poae*, *F. equiseti*, *F. solani*, *F. incarnatum* as well as *F. oxysporum* (Burgess et al., 1997). In the case of Gibberella ear rot or 'red ear rot', the most frequent species are *F. culmorum*, *F. graminearum* and *F. cerealis*, while the less frequently isolated are *F. avenaceum*, *F. verticillioides* and *F. subglutinans* (Burgess et al., 1997).

There is also a great number of remaining species that are isolated less frequently from cereals, but are still described, in some instances, as a problem that is yet to be prominent. Among those species are *F. chlamydosporum*, *F. flocciferum*, *F. sambucinum*, *F. anthophylum*, *F. heterosporum*, *F. venenatum*, *F. lateritium*, *F. torulosum* and *F. compactum* (Burgess et al., 1997).

The differences between species in relation to the chemotype dissemination could also be a factor of sub-classifications. Thus, the *F. graminearum* toxigenic strains could also be sub-divided into two different chemotypes in relation to the main type B trichothecenes produced: NIV and DON producers. The DON chemotype strains can be then further subdivided into two distinct types of producers: 15-AcDON and 3-AcDON producers (Miller et al., 1991; Logrieco et al., 1992; Szécsi and Bartok, 1995; Yoshizawa, 1997). Similarly, the *F. culmorum* toxigenic strains are also divided into two chemotypes, DON and NIV, in relation to the main B type trichothecenes produced, of which the DON strains were likewise known to produce AcDON (3-AcDON) (Gang et al., 1998; D'Mello et al., 1999).

2.7.2 Mycotoxin production

Mycotoxin contamination of the grains is one of the most significant results of FHB and ear rot of corn (D'Mello and Macdonald, 1997; D'Mello et al., 1999; Placinta et al., 1999), and it primarily appears in the field and proceeds to affect the grains during storage also. The growth of the fungus and development of mycotoxins, as well as their appearance, relies primarily on two major factors: temperature and *a*w. However, the optimum environmental factors for the production of mycotoxins in contaminated grains depend on the *Fusarium* species, substrate and isolate. The effect of the temperature and *a*w on the production of mycotoxin by *Fusarium* species is not so completely straightforward, but it is a kind of function of the influence of these, more vital, parameters on fungal growth (D'Mello and MacDonald, 1997; D'Mello et al., 1999).

The most significant of *Fusarium* mycotoxins, according to their hazard on human and animal health, are the classes known as FUM, MON, ZEA and the trichothecenes (D'Mello et al., 1999). The fumonisin class consist of a a group of metabolites which are similar in their construction, B_1 (FB_1) and B_2 (FB_2) types which, together with moniliformin, most frequently occur in maize grain. On the other hand, trichothecene mycotoxins are tricyclic sesquiterpenes, with two types A and B which, together with the oestrogenic mycotoxin ZEA, most frequently occur in cereals (D'Mello and MacDonald, 1997; D'Mello et al., 1999).

2.7.2.1 Trichothecenes and zearalenone (ZEA)

ZEA and trichothecenes are usually the products of various *Fusarium* species, some of which are *F. sporotrichioides, F. oxysporum, F. graminearum*, *F. culmorum*, and *F. poae* (D'Mello and Macdonald, 1997; D'Mello et al., 1999) (Table 4).

Table 4: The major classes of *Fusarium* mycotoxin, their principal producers and optimal production conditions on cereal grains (Johansson, 2003)
Preglednica 4: Glavne skupine fuzarijskih mikotoksinov, vrste gliv, ki jih tvorijo in optimalni pogoji za njihovo tvorbo na žitnem zrnju (Johansson, 2003)

Toxin	Species	Substrates	Optimum production conditions[a]	References
Type A trichothecenes [T-2 toxin, HT-2 toxin, neosolaniol and diacetoxyscirpenol (DAS)]	*F. sporotrichioides* *F. poae*	Barley, oats, rice, wheat, maize	Moderately warm and humid (20–25 °C, a_w = 0.990)	Mateo et al. (2002), Miller (1994), Rabie et al. (1986)
Type B trichothecenes [deoxynivalenol (DON), 3-acetyl DON, 15-acetyl DON, nivalenol (NIV)]	*F. graminearum* *F.culmorum*	Barley, wheat, rice, Maize	Warm and humid (25–28 °C, a_w = 0.97)	Greenhalgh et al. (1983), Lori et al. (1990), Beattie et al. (1998), Homdork et al. (2000)
ZEA	*F. graminearum* *F. culmorum*	Wheat, rice, maize	Warm (17–28 °C), or temperature cycles (e.g. 25–28 °C for 14–15 days; 12–15 °C for 20–28 days) and humid (a_w = 0.97 or 90% RH)	Jiménez et al. (1996), Lori et al. (1990), Ryu and Bullerman (1999), Homdork et al. (2000), Martins and Martins (2002)
Fumonisins	*F. verticillioides* *F. proliferatum* *F. subglutinans*	Maize	Cool to warm conditions and humid (15–30 °C, a_w = 0.98)	Cahagnier et al. (1995), Marín et al. (1999a,b)
Moniliformin	*F. subglutinans* *F. verticillioides* *F. avenaceum*	Wheat, rye, barley, oats, maize	Warm temperatures (25–30 °C)	Kostecki et al. (1999), Schütt (2001)

[a] Optimum temperature and humidity vary depending on substrate, species and isolate, typical conditions are given in parentheses, time of production varies from 3 to 8 weeks
[a] Optimalna temperatura in vlažnost se razlikujejo glede na substrat, vrsto in izolat, značilni pogoji so podani v oklepaju, čas tvorjenja je od 3 do 8 tednov

There are two types of trichothecens as we know type A and B. *F. sporotrichioides* and perhaps *F. poae* predominately produce type A trichothecenes, HT-2 toxin, T-2 toxin, diacetooxyscirpenol (DAS) and neosolaniol. On the other hand, the type B, including NIV, DON, its 3-acetyl and 15-acetyl derivatives (3-ACDON and 15-ACDON, respectively), are mostly the products of *F. graminearum* and *F. culmorum*. And as previously mentioned, in the majority of studies one of the most important factors for the occurrence of both of these mycotoxin classes is high humidity. However, the optimal temperatures for the development of ZEA and trichothecenes in grains infected with *Fusarium*, seem to be specific to the species, substrate and individual metabolites (Table 4). In the case of type A trichothecenes production by *F. sporotrichioides*, the most suitable environment for the mycotoxin formation was moderate climate (Miller, 1994; Mateo et al., 2002) (Table 4). Thus the optimal production conditions varied depending on the substrate and toxic metabolite. Overall *F. sporotrichioides* infected grains of maize, rice and wheat contained more type A trichothecenes when moistened with 35% water (aw = 0.990) and the incubation was done at 20^{o}C for three weeks, as opposed to incubations with higher aw and temperature. However, an exception was noted by Rabie et al. (1986) who observed that in *F. acuminatum* infected oats, there were rather large quantities of T-2 toxin formed at 25^{o}C, but the varying incubation circumstances were not compared.

On the other hand, type B trichothecenes are mostly produced when the grain infected with *F. graminearum* and *F. culmorum* is stored in a warm and moist environment (Greenhlagh et al., 1983; Lori et al., 1990; Beattie et al., 1998; Homdork et al., 2000; Martins and Martins, 2002) (Table 4). Thus was the case in the research of Martins and Martins (2002) who proved that when *F. graminearum* infected corn (aw = 0.97) was incubated at 28^{o}C for 35 days, i.e. the temperature was kept constant throughout the incubation period, the production of type B trichothecene DON was higher than when the temperature was at first 22 or 28^{o}C for 15 days and then lowered to 12^{o}C for 20 days. The same results were previously reached by Greenhlagh et al. (1983). A similar temperature was favorable for *F. graminearum* to produce maximal content of DON in infected wheat, polished rice and hulled rice, but with a variation in lighting conditions. In polished rice and wheat high DON amounts were produced at 27^{o}C in dark, while incubation at the same temperature and light was more influential in the case of hulled rice (Lori et al., 1990).

Depending on the toxic metabolite, another important factor that contributes to the severity of mycotoxin infections and might even surpass the environmental factor, is the degree of the initial infection. When barley grain with high *Fusarium* infection (85%) was stored for 7 months, no notable variation in DON levels was observed regardless of the conditions (-4, 20 or 24^{o}C, quiescent or forced aeration), though the lowest levels were found to be in the malt of

the grain kept at 24ºC (Beattie et al., 1998). Furthermore, it was discovered by Homdork et al. (2000) that DON levels were notably higher in low to moderate *F. culmorum* infected grain (4-15%), stored for 6 to 8 weeks in a warm and moist environment (25ºC, 90% RH), while it was the opposite case with highly infected grains (> 50%). However, despite the degree of initial infection, the mycotoxin levels might be specific to the toxin, because the mentioned conditions were also favorable for the production of NIV, and its levels, despite not being detectedt or having low initial infection at harvest, increased under the same circumstances.

Whereas in the case of thrichothecenes, the circumstances that favor the ZEA production vary from the ones that favour DON production, and they rely on the species, isolate or substrate. In maize contaminated with in *F. oxysporum* and *F. graminearum,* highest ZEA levels were reached at *a*w 0.97 when incubation temperatures were rotated, primarily incubating for 14 to 15 days at 25-28ºC temperatures, and then lowering the temperatures from 12-15ºC and continuing incubation for 20-28 days (Jiménez et al., 1996; Ryu and Bullerman, 1999; Martins and Martins, 2002) (Table 4). Nevertheless, further researches proved that the most suitable temperature varies from isolate to isolate and substrate to substrate, as was discovered by Jiménez et al. (1996) who revealed that the previously described conditions were suitable for ZEA production in maize grain that was infected by two isolates of *F. graminearum* and *F. oxysporum*, but a different climate was needed for *F. culmorum* and *F. graminearum* isolates, which reached maximum ZEA production at room temperatures (16-25ºC), following a 30 day incubation period, as opposed to the previously stated temperature levels of 28 or 37ºC (*a*w = 0.97).

Another example, when wheat and polished rice that were infected by *F. graminearum* are incubated in the dark at temperatures of 17 and 21ºC respectively, maximum ZEA production is reached, while a temperature of 27ºC as well as light is needed for maximum ZEA levels in hulled rice (Lori et al., 1990). Furthermore, when wheat grain that had a moderate to high initial *F. culmorum* infection (4-15%) was stored under warm and moist conditions (25ºC, 90% RH), ZEA levels were higher than when stored under opposite circumstances. And finally, in most cases, the majority of ZEA was produced close to the end of the storage period (6-8 weeks) (Homdork et al., 2000).

2.7.2.2 Fumonisins and moniliformin

Fumonisins and moniliformin are most commonly found mycotoxins in corn contaminated with *F. proliferatum* and *F. verticillioides*, species which prefer higher temperatures to grow, (Keller et al., 1997; Kostechi et al., 1999; Miller, 2001; Marín et al., 1999a, 1999b).

Moniliformin, who was also found in cereals infected by *F. subglutinans* and *F. avenaceum* (Kostechi et al., 1999; Torres et al., 2001; Kiecana et al., 2002).

Fumonisins are usually formed when the water availability is at *a*w ~ 0.98, while if temperature is lowered, and *a*w is increased, it will lead to lower fumonisin productions (Cahagnier et al., 1995; Marín et al., 1999a, 1999b). This is also the case in *F. proliferatum* and *F. moniliforme* infected ground maize and maize grain, where it was similarly discovered that *a*w had a higher impact on fumonisin production than the temperature levels (Marín et al., 1999a, 1999b). Nevertheless, the production of fumonisin and fungal biomass in most circumstances decreases when the temperatures and *a*w are lowered, the optimal conditions, relying on the isolate, being water availability level at 0.98 *a*w and temperature at 15-30°C. However, if the *a*w is decreased to 0.92 and 0.95 and the temperature is kept at marginal levels, particularly 15°C, it will lead to higher fumonisin levels than the ones given at increased temperature and *a*w levels. Exceptions apply though, because even at 37°C, an *F. moniliforme* isolate was discovered to be producing notable fumonisin quantities (Marín et al., 1999b).

Other significant factors conditioning the fumonosin production are precipitation and oxygen availability. According to Ono et al. (1999), there was a higher fumonisin concentration in the maize of the North of Párana, Brazil than the South-central area, due to the higher amounts of rain in the month before the harvest (202 and 92.8 mm, respectively), while in conditions where oxygen was limited, the development of *F. moniliforme* and *F. proliferatum* was delayed, and no FB1 had been produced (Keller et al., 1997).

On the other hand, for higher production of moniliformins by *F. subglutinans* or *F. avenaceum* infected cereal grains, higher temperatures are needed (Kostecki et al., 1999; Schütt, 2001). Such was the case with production of moniliformin by *F. subglutinans*, isolated from maize, which was greater at higher temperatures at 30°C, as opposed to lower temperatures at 20 to 25°C, and was also higher in rice than rye, barley, wheat, oat or maize grains (Kostecki et al., 1999). Furthermore, it was found that in the case of wheat, *F. avenaceum* produced more moniliformin in Mediterranean, than in temperate environments (Schütt, 2001).

3 MATERIALS AND METHODS

3.1 EXPERIMENTAL LOCATIONS

In order to determine the representation of the various *Fusarium* species, as well as the mycotoxin contamination of grains of diverse wheat varieties and maize hybrids, during the period of two years, in 2012 and 2013 we have conducted field experiments in arable plots of the Rakičan Biotechnical School (Prekmurje) and the Center for rural and agricultural development Jablje (Central Slovenia). The soil at site Rakičan is typical distric brown on holocene gravel debris and moderately gley soil on siliceous limestone base at site Jablje. For the experiments we have sown a total of 30 wheat varieties and 33 maize hybrids. The sown varieties are perspective for cultivation in Slovenia, varieties that are different in origin, type and maturing period. The test locations differ significantly in terms of typical weather conditions, but above all in terms of precipitation, which strongly favors the development of fusariosis. In every plot, an integrated manner of maize and wheat prouction was conducted.

The experiments were performed at two different locations: Rakičan and Jablje, with the same crop rotation (maize-wheat) for at least a 6-year time period, in order to allow greater infection pressure carried by the *Fusarium* spp. In the both years of the field trial and at both locations at the beginning of wheat flowering (BBCH 61), the fungicide Prosaro (prothioconazole + tebuconazole), a product of Bayer CS, was applied at a dosage of 1 l per hectare.

3.1.1 Meteorological stations of reference and meteorological data received

Rakičan (46°38'N, 16°11'E, altitude: 184 m) is located in the central part of the Prekmurje region near Murska Sobota and the meteorological station of reference is the Murska Sobota station, likewise located in Rakičan in the immediate vicinity of the experiment. The average mean air temperature for the period 2011-2013 was found to be 11°C, the coldest month being February with an average temperature of - 4.6°C, while the maximum average monthly temperature was measured in August with 28.9°C. Furthermore, the observed average annual rainfall during this period was 795.5 mm (ARSO, 2016).

On the other hand, Jablje (46°8'N, 14°34'E, altitude: 305 m) is located in the central part of the Ljubljana basin in pre-alpine Slovenia. The meteorological station of reference is the Brnik station where the recorded average mean annual temperature (2011-2013) was 9.9°C, with the coldest month being February with an average monthly temperature of - 5.6°C, while the warmest was August with temperatures up to 28.6°C. The recorded average annual precipitation was 1218.9 mm (ARSO, 2016).

The meteorological stations were performing measurements and observations, three times a day, at 07:00, 14:00 and 21:00 hours after local or solar time, while the atmospheric phenomena were observed and recorded continuously, even during observation terms. Precipitation is measured once a day at 07:00 am. Observations and measurements in the meteorological stations were done by professional observer. All the measurements and specific observations were entered into the climatological logs that were submitted monthly to the bureau of meteorology.

3.1.2 Implementation of the field trials

The field experiments began in October 2011, during which we had begun and finished the sowing of 30 different cultivars of wheat, designated for cultivation in Slovenia, chosen for that occasion. In April of 2012 we had sowed 33 hybrid types of corn, also designated for cultivation in Slovenia. In May 2012, a monitoring was conducted during which we have established the length of the flowering period, for each wheat varieties separately (BBCH: 61-69), all the while monitoring the weather conditions, with a special emphasis on rainfall during the same period. Furthermore, we have also determined the percenti representation of *Fusarium* spp. in ears (average plot severity), for each wheat variety separately (BBCH:71-75), of the samples retrieved from the test fields, using the Stack and McMullen (1995) method. In July 2012, after the harvest we retrieved some suitable wheat samples from each cultivar in order to conduct a laboratory analysis of mycotoxin contamination and grain infection with *Fusarium* spp. In the period between August and October of the same year, we have determined the *Fusarium* spp. infection in wheat grains, while at the same time analyzing the mycotoxin representation, or concentration, in the same grains. Analyses were also conducted for determination of *Fusarium* spp. and presence of mycotoxins on samples obtained from ecologically produced wheat, harvested from areas nearby our test locations. Similar tests were made in the period from October until December of 2012 with 33 hybrid species of corn. During that month, we have also sowed the same wheat types as in 2011. And in 2013, the field and laboratory analyses were conducted in the same order as in 2012.

3.2 IDENTIFICATION OF *Fusarium* spp. AND ELISA FOR DON DETECTION

3.2.1 Materials - chemicals and reagents

The analyses were performed in the Laboratory of phytopathology at the Department of Agronomy, Biotechnical Faculty, University of Ljubljana, Slovenia. The laboratory analyses were carried out on individual grain samples of wheat and maize from different varieties on which an accurate representation of all types of *Fusarium* spp. was determined.
Potato dextrose agar (PDA) and technical agar were supplied by Biolife Italiana S.r.l. (Milan, Italy), while penicillin G, and streptomycin sulfate were purchased from Merck (Kenilworth, New Jersey, United States). We have used 14 g of potato dextrose agar (Biolife), 10 g technical agar (Biolife), 0.121 g penicillin G (Merck), and 0.542 g streptomycin sulfate (Merck) per liter of medium.

3.2.2 Methods used for the determination of *Fusarium* spp. and DON in wheat and maize grain

Firstly, a monitoring was conducted during the months of May and June at both test locations, the two year experimental period included (2012-2013), to determine the length of flowering of all wheat varieties, and each variety was separately catalogued in our database, a procedure of crucial significance to our research, due to the fact that the *Fusarium* spp. are the most infective at that stage of wheat development. We recorded the beginning of flowering, the full flowering, as well as the end of flowering (BBCH 61, 65 and 69).

Several methods were used to determine the *Fusarium* spp. grain infection in the various wheat and maize cultivars, as well as the mycotxin contamination. After the harvest and storage of the wheat and maize grains according to designated standards (Direktiva komisije 2005/38/ES ..., 2005), a test sample was retrieved from each variety/hybrid, from which a fragment was analyzed for mycotoxin representation and another fragment was used in a laboratory phytopathological analysis, in order to determine *Fusarium* infection of grain. In the laboratory, we have identified, in accordance with the prescribed standard phyto-pathological methods, the *Fusarium* species and the percent of infected grains (Leslie and Summerell, 2006).

From the data retrieved from the conducted analysis, we have gathered information on the *Fusarium* species representation in different wheat and maize varieties, the percentage of infection as well as the mycotoxin content in the same varieties. All wheat and maize samples were tested for DON with ELISA. In addition, two-tail t-test of type 3 (Independent samples

with different variance), was used to evaluate the significant statistical differences in the data gathered during the two-year time period.

In order to make a comparison of the presence of mycotoxins in eco-wheat versus integrated, we have obtained a number of representative samples of ecologically grown wheat, gathered from areas near our test locations. This is necessary because of the similarity in the weather conditions, which can, to a greater or lesser extent affect the occurrence of infections and thus contamination with mycotoxins. The samples were analyzed in order to determine presence of mycotoxins and later on were compared with the results obtained from our test locations, where integrated production method of wheat is applied.

3.2.2.1 Identification of *Fusarium* spp.

The number of samples that were included in this research, obtained from each growing trial for 2012 are 30 wheat varieties and 33 maize hybrids in the 2 distinct locations, while for 2013 31 wheat varieties were acquired from Rakičan and 34 from Jablje, and 21 maize hybrids were obtained from Rakičan and 27 from Jablje. After the harvest, all the samples were taken from 30 kg sacks in a total of 2 kg, half of the amount was used for mycotoxin analysis, and the other half was used for *Fusarium* spp. identification. Out of those samples, for the identification of *Fusarium* spp., 100 randomly selected wheat and corn grains were surface sterilized in 1% sodium hypochlorite NaOCl in Erlenmeyer flasks for 10 min, and further rinsed with sterile distilled water and dried on previously sterilized filter paper.

Afterwards, all the grains were deployed by 5, under sterile conditions, on 20 petri dishes, 90 mm in diameter, containing 15 mL of potato dextrose agar (PDA) supplemented with 0.121 g penicillin G (Merck), and 0.542 g streptomycin sulfate (Merck) per liter (Figure 17). They were further incubated under dark conditions at 20°C, RH 60%, for the period of 7 days for wheat and 14 days for maize while being daily observed. Following the incubation period, each colony resembling *Fusarium* spp. was selected, subcultred on water agar or used directly to pruduce single spore isolates. The isolates obtained were then examined microscopically under 100 and 400X magnification (Zeiss Axio Scope, Germany), the focus of the observations was on macroconidia morphology, the presence/absence of microconidia and chlamoidospores as well as perithecium production. Besides PDA the carnation leaf agar (CLA) and the synthetic nutrition agar (SNA) were used in identification. The CLA and SNA media were prepared according to Burgess et al. (1994) and Nirenberg (1976), respectively. Isolates were incubated in 12h day/night regime, at 20°C, RH 60%, using combined fluorescent and near ultraviolet light during the daily period. Identification was performed by utilizing the keys of Gerlach and Nirenberg (1982), Burgess et al. (1994) and Leslie and

Summerell (2006), noting the considerations of Summerell et al. (2003). Identified isolates were subcultured on potato dextrose agar (PDA) and SNA and deposited in the refrigerator for the purpose of further analysis.

Figure 17: *Fusarium* spp. on PDA medium at 20°C - red pigmented colonies (photo: Celar F.A.)
Slika 17: *Fusarium* spp. na PDA gojišču pri 20°C - rdeče obarvane kolonije (foto: Celar F.A.)

3.2.2.2 ELISA for the determination of DON

The final step in an analytical protocol involves determining whether the toxin is present, using at least one detection method. However, a distinction must be made between reference (confirmatory) methods, which allow for the detection, identification and numbering mycotoxins in various matrices, and rapid screening methods (also often classified as alternative methods) which are aimed essentially at detecting the presence of a mycotoxin or group of toxins.

From the rapid screening methods we have used ELISA for DON detection in wheat and maize kernels. The process consisted of the following steps. Firstly, a 20 g sample was taken from the main sample (1 kg) and put into a 250 mL extraction flask and then 100 mL of deionized water was added. After an hour of stirring and filtering the extract, 1 mL was poured into a tube and diluted with 3 mL of deionized water. Using a microtiter plate, we applied 200 µL of the conjugate, then 100 µL of the extract solution in the designated spots. The mixture

was moved to a different microtiter plate that contained antibodies and left for 15 minutes. We further washed the plate with a mild detergent solution and added another 100 µL of the substrate, leaving it for 5 minutes, and then adding another 100 µL of the mild detergent solution to stop the reaction. The DON levels were measured with an ELISA reader.

4 RESULTS AND DISCUSSION

4.1 VISUAL ASSESSMENT ON INFECTED EARS, INFLUENCE OF THE ENVIRONMENTAL FACTORS AND WHEAT TYPE ON *Fusarium* spp. OCCURRENCE

The climatic conditions are one of the most vital factors influencing *Fusarium* spp. infections as the infection primarily occurs during the flowering period which is governed by these conditions. A wide open flower combined with a longer flowering period allows spores to enter the grain, also providing more severe infection with *Fusarium* spp. to occur (Gilsinger et al., 2004). Prolonged periods of openness are possible not only because of the breed characteristics but also the climatic factor. Raised temperatures and dry conditions have been known to reduce the period of openness during flowering as much as the opposite circumstances have been known to increase it (De Vries, 1971), which leads us to conclude that temperature and rainfall are the two most important climatic factors that influence the development and occurrence of *Fusarium* infection in wheat. Such a conclusion was reached by Parry et al. (1995) who have pointed out that temperatures above 15°C favour the development of *F. graminearum* and *F. culmorum* ear infections in a minimal wetting time of at least 24 hours, with the optimal temperature for infection and disease spread being 25°C (Ćosić and Vrandečić, 2002; Hope et al., 2005). And it is due to the importance of these factors for the contamination process that we have conducted some field tests, in order to determine the relationship between the weather conditions and the occurrence and development of *Fusarium* spp. in various wheat varieties.

In addition to the comments on the weather conditions through the years in both locations (Rakičan, Jablje) we have added figures which show the measured temperature and precipitation for each year and their comparison with the average for twenty years (1991-2010). At the locations where the experiment took place, we weren't able to obtain long-term data and compare them to the data from each year of the experiment, and that's why we specify only those from the nearest meteorological station; in the case of Rakičan - Murska Sobota and Jablje - Brnik station. In some cases there are minor discrepancies, but they don't deviate significantly from the actual situation in the pilot locations.

Rakičan 2012: the average monthly air temperature in May was 0.1°C higher than the long-term average (May; 1991-2010; 15.8°C). However, it was in the middle of May, during the flowering of most varieties of wheat, when a cold period with average daily temperature of 13.7°C (10-20 May; 2012) occurred, which is 2.1°C colder than the long-term average. June was warmer by 1.9°C from the long-term average (June; 1991-2010; 19.2°C). Since the end of

June till the harvest there was a notable heatwave, during which the average daily temperatures were above the average and around 25°C. During May there was a 119 mm of precipitation, which is 62.2% more than the long-term average (May; 1991-2010; 74 mm). However, they were in the form of downpours. On May 23rd there was as much as 50.7 mm of rainfall. In June there was a 78% drop in the long-term average precipitation, while the first decade of July was also dry (Figure 18).

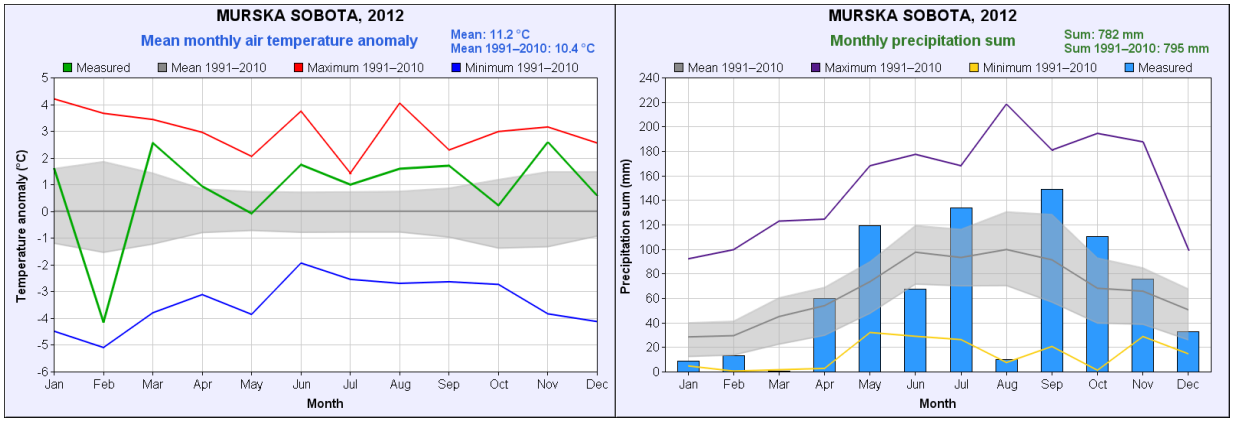


Figure 18: Data for mean monthly air temperature and sum of monthly precipitation for Murska Sobota - 2012 (ARSO, 2016).
Slika 18: Podatki za povprečne mesečne temperature zraka in vsote mesečnih padavin za Mursko Soboto - 2012 (ARSO, 2016).

Rakičan 2013: the development of wheat during this year was "delayed" due to exceptionally snowy winter and a very cold March. During May it was averagely warm, with a distinctly cold period in the last ten days of the month. The last decade of May (22-31 May; 2013; 12.9°C) was 2.9°C colder than the long-term average (May; 1991-2010; 15.8°C). The mean daily air temperature in June (19.3°C) was at the long-term average level (June; 1991-2010; 19.2°C), but there were two distinct cold periods, during the beginning and the end of the month. Temperatures in July until the harvest were also at the long-term average level. In May, there was 22.11% more rain than the long-term average (May; 1991-2010; 74 mm). In June the precipitation amounted to three quarters (66 mm) of the long-term average rainfall (June; 1991-2010; 86 mm). The rainy season was at the beginning of June, but most of the precipitation fell during three rainy days in the last decade of this month. In July, only 15 mm of rain fell until the wheat harvest, with three-quarters of the precipitation occurring in one day (Figure 19).

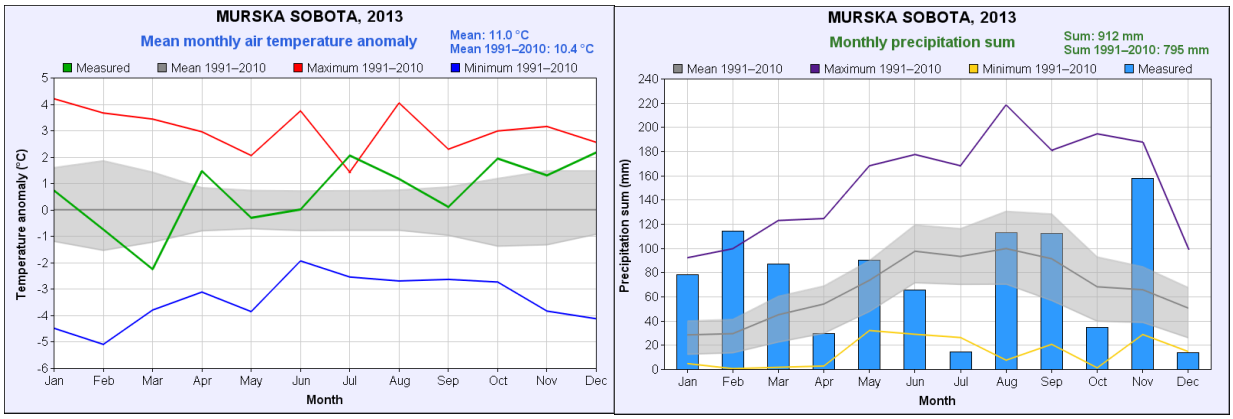


Figure 19: Data for mean monthly air temperature and sum of monthly precipitation for Murska Sobota - 2013 (ARSO, 2016).
Slika 19: Podatki za povprečne mesečne temperature zraka in vsote mesečnih padavin za Mursko Soboto - 2013 (ARSO, 2016).

Jablje 2012: the average monthly air temperature in May (16.1°C) was at the same level with the long-term average (May; 1991-2010; 16.1°C). However, during the second decade of May (11-20 May; 2012) it was 3°C colder than the long-term average. June (21.3°C) was warmer than the long-term average (June; 1991-2010; 19.6°C), especially during the last decade (21-30 June; 2012; 21.7°C), when the average air temperature was 2.1°C higher than the long-term average. May was an averagely wet month (124 mm), the same can be said for the first two decades of June (1-20 June; 2012; 98.5 mm) (Figure 20).

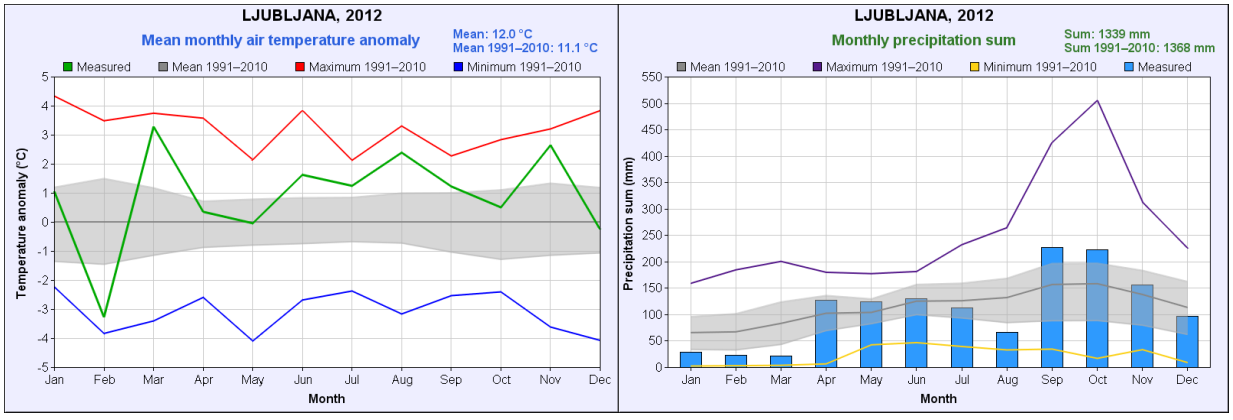


Figure 20: Data for mean monthly air temperature and sum of monthly precipitation for Jablje - 2012 (ARSO, 2016).
Slika 20: Podatki za povprečne mesečne temperature zraka in vsote mesečnih padavin za Jablje - 2012 (ARSO, 2016).

Jablje 2013: just like in Rakičan, the wheat development during this year was "delayed" due to the exceptionally snowy winter and a very cold March. May was averagely warm (14.7°C), with a distinctly cold period in the last ten days of the month (10.9°C). The last decade of May (22-31 May; 2013) was 5.2°C colder than the long-term average (May; 1991-2010; 16.1°C). In May 2013, there was 50% more rain (217 mm) than the long-term average (May; 1991-2010; 109 mm). The mean daily air temperature in June (19.8°C) was almost at the same level as the long-term average (June; 1991-2010; 19.6°C). The average air temperature in the second decade of June was 22.3°C. In July, the average daily temperature (23.5°C) was 2.1°C higher than the long-term average (July; 1991-2010; 21.4°C). Precipitation during the first decade of June was 58 mm, or 49.2% of the long-term average level (June; 1991-2010; 118 mm), while the second two decades were dry. In the first two decades of July there was 38 mm of rainfall during 8 days (Figure 21).

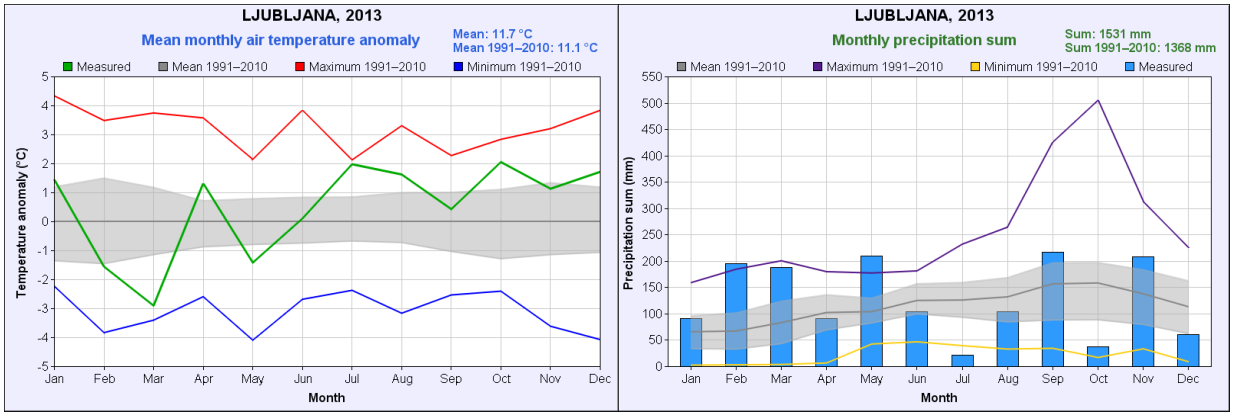


Figure 21: Data for mean monthly air temperature and sum of monthly precipitation for Jablje - 2013 (ARSO, 2016).
Slika 21: Podatki za povprečne mesečne temperature zraka in vsote mesečnih padavin za Jablje - 2013 (ARSO, 2016).

With the help of the 10-point scales (Stack and McMullen, 1995) we have visually evaluated the annual ear infestation and calculate an average infection of each variety with fusariosis (Table 6). Table 5 present's the average infection of all wheat varieties at individual locations in the years 2012 and 2013. The evaluation was conducted in order to determine whether there is correlation between the field assessment of ear infection, the laboratory evaluation of grain infection and DON content.

From the conducted evaluation we can conclude that the average ear infection of all studied varieties of wheat in both years (2012 - 2013) and both locations was relatively small, it was 3.52 or 1.40% in Jablje, and in Rakičan - 1.87 or 1.81%. If we compare these results with the ones where we have determined the actual *Fusarium* spp. presence on the grains of wheat in

the laboratory (Table 9 and 11), we can conclude that the field assessment actually underrates the real picture of the infection. This can be explained by the fact that the ear fusariosis progressively develops further from the developmenal phase BBCH: 71-75 on until the harvest of wheat, and therefore, the subsequent infection is greater.

Table 5: Average ear infection (%) of all wheat varieties examined separately for each year
Preglednica 5: Povprečna okuženost klasov vseh preučevanih sort pšenice po posameznih letih

Location	Average ear infection (%)	
	2012	2013
Rakičan	1.87	1.81
Jablje	3.52	1.40

It was indeed the climatic factor, which was one of the most prominent reasons for which we had to make some exceptions in our analyses, i.e. a part of all of our analyzed wheat and maize samples was not taken into consideration in our further laboratory tests. This decision was based on our inability to isolate, determine and identify *Fusarium* spp. from the collected samples. This inability to conduct the research necessary in all samples was due to the fact that some of them were contaminated with *Mucor* sp. and *Penicillium* sp. so heavily that the isolation of *Fusarium* spp. was impossible. The mycelia of these contaminating fungi, especially *Mucor* spp., overgrew the whole petri dish, preventing the isoaltion of mycelia resembling *Fusarium* spp. Thus, in all our further analyses, only those maize hybrids and wheat varieties, for which we have obtained complete laboratory results during the two-year trial period of 2012 and 2013 in both locations, were taken into consideration.

One of the test locations where 30 different varieties of wheat were sown - Rakičan, is located in the central part of the Prekmurje region, near Murska Sobota. Out of all sown varieties, only 19 were used in our further research, due to their consistency during the trial period. The meteorological station of reference from where our data of the weather conditions during the wheat's flowering period in that area were recorded during 2012 and 2013 is located in Murska Sobota, in the immediate vicinity of the test field. According to the data, during the flowering period for 2012 in Rakičan a minimum of 0.1 mm, maximum of 52.9 mm, and mean value of 15.4 mm of rainfall during flowering were observed. The mean number of rainy days during flowering was 2, while the minimal, maximal and mean temperature for the same period was detected to be 10.5°C, 17.9°C and 13.7°C, respectively (Table 6).

Table 6: Average amount of rainy days, total rainfall during flowering, ear infection and average temperature during anthesis on 19 different varieties of wheat for Rakičan in years 2012 and 2013
Preglednica 6: Povprečno število deževnih dni, skupna količina padavin, okuženost klasov in povprečna temperatura med cvetenjem pri 19 različnih sortah pšenice v Rakičanu v letih 2012 in 2013

Wheat varieties and ear types	Rakičan – 2012					Rakičan – 2013				
	AP days	RDF	TRF mm	AEI %	T °C	AP days	RDF	TRF mm	AEI %	T °C
aw BOLOGNA	**6**	**2**	**15.4**	**0.74**	**10.6**	**6**	**1**	**4.9**	**2.34**	**16.4**
aw INGENIO	**7**	**2**	**15.4**	**8.76**	**12.6**	**8**	**2**	**5.1**	**0.86**	**15.1**
aw LUKULLUS	**5**	**2**	**2.2**	**0.86**	**15.1**	**6**	**4**	**16.5**	**0.49**	**12.9**
aw MIHELCA	**6**	**1**	**14.9**	**5.55**	**15.2**	**6**	**2**	**5.6**	**1.73**	**17.0**
aw NS 40S	**6**	**2**	**15.4**	**0.12**	**10.6**	**6**	**0**	**0.0**	**0.00**	**15.6**
aw SY MOISSON	**4**	**1**	**0.1**	**1.97**	**15.0**	**5**	**1**	**0.2**	**0.00**	**13.4**
awl ALIXAN	7	2	15.4	0.12	11.2	7	3	16.3	3.33	13.0
awl BC RENATA	5	3	52.9	0.00	16.8	5	2	15.9	0.37	12.4
awl GARCIA	6	2	15.4	0.25	10.6	5	0	0.0	0.49	16.2
awl ILLICO	7	2	0.6	1.85	13.2	7	1	0.2	0.49	14.7
awl KATARINA	5	2	15.4	0.37	10.5	7	1	4.9	1.48	15.8
awl KETCHUM	4	1	1.1	14.19	16.6	4	4	6.0	2.10	15.1
awl LORD	5	3	52.9	4.56	17.9	5	2	15.9	2.71	12.4
awl LUCIJA	7	2	19.1	0.00	15.3	6	2	5.6	0.74	17.3
awl NINA	7	2	15.4	4.56	12.6	7	1	4.9	0.12	15.8
awl SIMONIDA	5	1	14.9	0.12	13.7	7	2	5.6	1.23	17.2
awl SRPANJKA	7	2	19.1	0.00	15.3	7	2	5.6	4.56	17.2
awl ZDENKA	4	1	0.1	1.11	15.0	5	0	0.0	0.86	14.8
awl ŽITARKA	7	2	15.4	0.62	11.4	7	1	4.9	2.59	15.8
MEAN (AEI)				2.41		**MEAN**			1.39	
MIN (RDF, TRF and T)		1	0.1		10.5	**MIN**	0	0.0		12.4
MAX (RDF, TRF and T)		3	52.9		17.9	**MAX**	4	16.5		17.3
MED (RDF, TRF and T)		2	15.4		13.7	**MED**	2	5.1		15.6
MEAN Awned wheat				3.00		**MEAN**			0.90	
MEAN Awnless wheat				2.13		**MEAN**			1.62	

awl-awnless wheat; **aw**-awned wheat; AP-anthesis period; RDF-rainy days during flowering; TRF-total rainfall during flowering; AEI-average ear infection; T-average temperature during anthesis
awl-golica; **aw**-resnica; AP-obdobje cvetenja pšenice; RDF-deževnih dni med cvetenjem; TRF-skupna količina padavin v času cvetenja; AEI-povprečna okuženost klasov; T-povprečna temperatura med cvetenjem

On the other hand, during the following year 2013, the weather conditions in Rakičan were quite different from the ones in the same period of the previous year. The minimal amount of rainfall was 0.0 mm, the maximum 16.5 mm and the mean was 5.1 mm. The average for rainy days during flowering in 2013 was 2. The minimal, maximal and mean temperature during the same period was detected to be 12.4°C, 17.3°C and 15.6°C, respectively (Table 6). From the observations and their descriptive statistics, we can conclude that no correlation has been observed on this location for 2012 and 2013 between the weather conditions and the appearance of *Fusarium* head blight. Consequently, we can also confirm that there is no direct

influence of the total amount of precipitation and temperature during flowering on the average level of ear infection (Figure 22 and Figure 23).

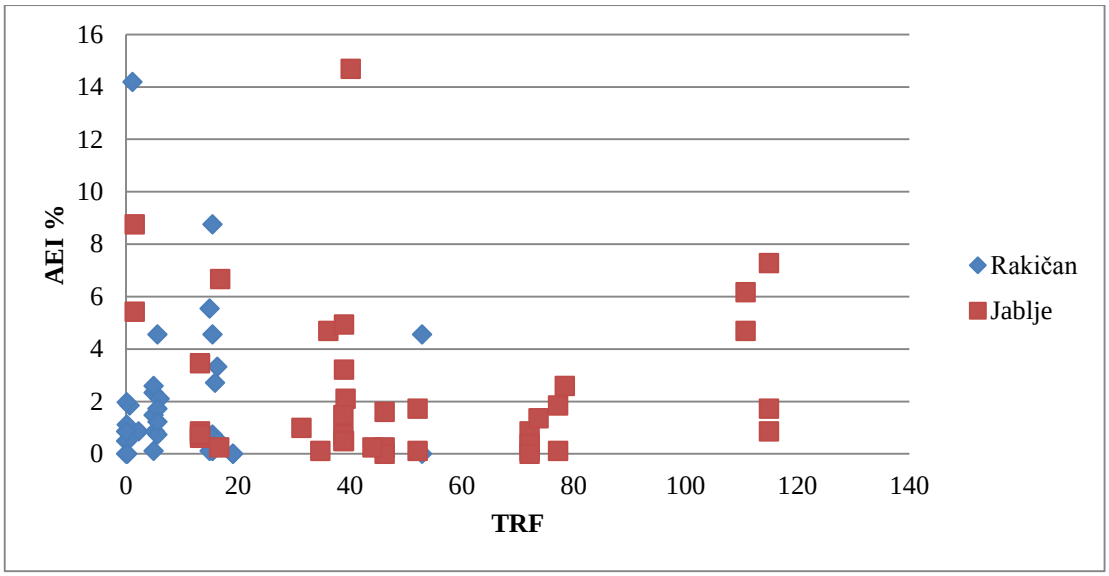


Figure 22: Visual comparison of the records between AEI (average ear infection) and TRF (total rainfall during flowering) on 19 different wheat varieties for Rakičan and Jablje in years 2012 and 2013
Slika 22: Vizualna primerjava podatkov med AEI (povprečna okuženost klasov) in TRF (skupna količina padavin med cvetenjem) na 19 različnih sortah pšenice v Rakičanu in Jabljah v letih 2012 in 2013

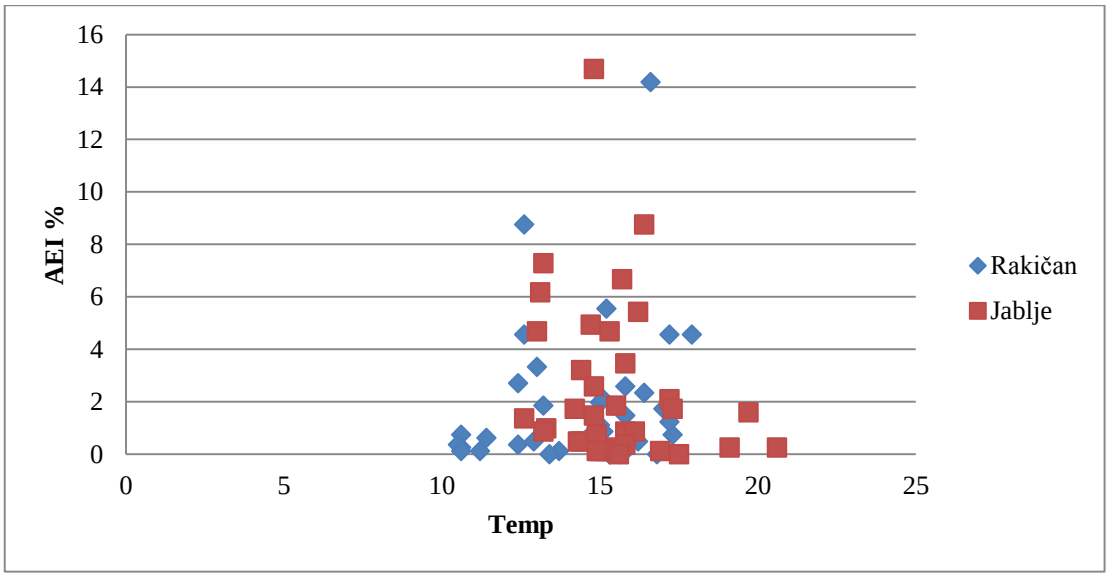


Figure 23: Visual comparison of the records between AEI (average ear infection) and Temp (average temperature during flowering) on 19 different wheat varieties for Rakičan and Jablje in years 2012 and 2013
Slika 23: Vizualna primerjava podatkov med AEI (povprečna okuženost klasov) in Temp (povprečna temperatura med cvetenjem) na 19 različnih sortah pšenice v Rakičanu in Jabljah v letih 2012 in 2013

Another test location where the same wheat varieties were sown was Jablje, which is located in the central part of the Ljubljana basin, where mostly pre-alpine climate prevails. Similarly to our first location, out of all sown varieties only 19 were taken into consideration, the ones that showed consistency during the two-year trial period, in both locations. The meteorological station of reference, from where we recorded our data for the weather conditions during the anthesis in that area for 2012 and 2013, was Brnik. According to the data for the wheat flowering period for 2012 in Jablje, significant difference in precipitation was observed as opposed to Rakičan, with minimal amount of 1.5 mm, maximum of 40.1 mm and mean amount of 34.7 mm of rainfall. The mean for rainy days during flowering was 5, while the minimal, maximal and mean temperature for the same period was 13.3°C, 17.2°C and 15.3°C, respectively (Table 7).

Table 7: Average amount of rainy days, total rainfall during flowering, ear infection and average temperature during anthesis on 19 different varieties of wheat for Jablje in years 2012 and 2013
Preglednica 7: Povprečno število deževnih dni, skupna količina padavin, okuženost klasov in povprečna temperatura med cvetenjem pri 19 različnih sortah pšenice v Jabljah v letih 2012 in 2013

Wheat varieties and ear types	Jablje – 2012					Jablje – 2013				
	AP days	RDF	TRF mm	AEI %	T °C	AP days	RDF	TRF mm	AEI %	T °C
aw BOLOGNA	**6**	**5**	**16.6**	**0.25**	**15.5**	**12**	**8**	**72.1**	**0.12**	**15.2**
aw INGENIO	**7**	**6**	**38.9**	**4.94**	**14.7**	**16**	**9**	**77.2**	**0.12**	**14.9**
aw LUKULLUS	**6**	**3**	**1.5**	**5.43**	**16.2**	**14**	**4**	**44.0**	**0.25**	**20.6**
aw MIHELCA	**6**	**5**	**38.8**	**0.74**	**14.9**	**16**	**11**	**73.7**	**1.36**	**12.6**
aw NS 40S	**7**	**5**	**13.2**	**0.74**	**15.8**	**13**	**8**	**72.1**	**0.00**	**15.6**
aw SY MOISSON	**7**	**5**	**13.2**	**3.46**	**15.8**	**14**	**6**	**52.1**	**1.73**	**17.3**
awl ALIXAN	8	7	36.1	4.69	15.3	17	5	46.2	0.25	19.1
awl BC RENATA	7	5	13.2	0.62	15.8	15	8	72.1	0.86	16.1
awl GARCIA	8	7	40.1	14.69	14.8	14	8	72.1	0.37	15.8
awl ILLICO	7	5	13.2	0.86	15.8	13	6	52.1	0.12	16.9
awl KATARINA	6	6	38.9	0.49	14.4	23	14	114.9	1.73	14.2
awl KETCHUM	6	2	39.2	2.10	17.2	17	5	46.2	1.60	19.7
awl LORD	7	3	1.5	8.76	16.4	12	5	46.2	0.00	17.5
awl LUCIJA	9	5	31.3	0.99	13.3	19	13	110.7	6.17	13.1
awl NINA	6	6	38.9	3.21	14.4	17	10	78.4	2.59	14.8
awl SIMONIDA	8	5	38.8	0.49	14.3	19	14	114.9	7.28	13.2
awl SRPANJKA	7	5	38.8	1.48	14.8	20	14	114.9	0.86	13.2
awl ZDENKA	7	6	16.8	6.67	15.7	17	9	77.2	1.85	15.5
awl ŽITARKA	6	5	34.7	0.12	15.0	18	13	110.7	4.69	13.0
MEAN (AEI)				3.20		**MEAN**			1.68	
MIN (RDF, TRF and T)		2	1.5		13.3	**MIN**	4	44.0		12.6
MAX (RDF, TRF and T)		7	40.1		17.2	**MAX**	14	114.9		20.6
MED (RDF, TRF and T)		5	34.7		15.3	**MED**	8	72.1		15.5
MEAN Awned wheat				2.59		**MEAN**			0.60	
MEAN Awnless wheat				3.47		**MEAN**			2.18	

Continued

Continuation of Table 7:

awl-awnless wheat; **aw**-awned wheat; AP-anthesis period; RDF-rainy days during flowering; TRF-total rainfall during flowering; AEI-average ear infection; T-average temperature during anthesis
awl-golica; **aw**-resnica; AP-obdobje cvetenja pšenice; RDF-deževnih dni med cvetenjem; TRF-skupna količina padavin v času cvetenja; AEI-povprečna okuženost klasov; T-povprečna temperatura med cvetenjem

In 2013, the minimal amount of rainfall was 44.0 mm, the maximum was recorded 114.9 mm and the mean data was 72.1 mm, respectively. The mean for rainy days during flowering in 2013 was 8. The minimal, maximal and mean temperature during the same period was calculated to be 12.6°C, 20.6°C and 15.5°C, respectively (Table 7). For both years (2012 and 2013) in Jablje, the descriptive statistics shows that there is no correlation between weather conditions and AEI (Figure 22 and Figure 23). Despite such results from our statistical analysis, the weather factor still represents a crucial factor for the appearance of fusariosis, according to a number of authors. Large efforts are invested worldwide to determine the main factors responsible for FHB in cereal crops. According to Schaafsma et al. (2001), Moschini et al. (2001), Hooker et al. (2002) and Klem et al. (2007), the effect of weather conditions during anthesis on FHB incidence is strong. Heavy rains during the flowering period, which represent the most sensitive growth stage for infection, spreads the *Fusarium* inoculum from crop residues and provides FHB infection. At the milky growth stage substantial infection is possible and the extended periods of warm and humid conditions enablemould growth and secondary infections (Parry et al., 1995; Hooker et al., 2002; Wagacha and Muthomi, 2007).

Apart from the influence of the weather conditions, great importance should also be assigned to the influence of the wheat type on *Fusarium* spp. ear infection. From the the data retrieved from Rakičan 2012, a 3% mean value of ear infection can be observed for the awned wheat, while the same value for the awnless wheat type was calculated to be 2.13% (Table 6).

On the other hand, during 2013, a reduction was noticed in both of the wheat types i.e. awned wheat showed 0.90% mean value of ear infection while the mean value for awnless wheat was 1.62% (Table 6). We reached the same conclusion by analysing the data from Jablje for both years as well, where the results were also in favor of the awned wheat whose ear infection mean value was 2.59% in 2012 (Table 7), while the same value for the awnless wheat was 3.47% (Table 7).

Furthermore, the smaller proneness to infection of awned wheat was again proven in 2013, where the average mean value was only 0.60% for the awned wheat, while it amounted to 2.18% for the awnless wheat (Table 7).

However, the statistical analysis shows that overall (for both locations and years) there is no statistical difference between the two wheat types, neither for each location, Rakičan and Jablje, separately nor for both locations merged together (Table 8). With our results we can confirm the research of Parry et al. (1995), Ćosić and Vrandečić (2002) and Hope et al. (2005).

Table 8: Two-tailed t-test of Type 3 for both types of wheat based from date of average ear infection (%) (data for both locations and years), with significance level $\alpha = 0.05$
Preglednica 8: Dvostranski t-test tipa III za oba tipa pšenice, ki temelji na povprečni okuženosti klasov (%) (podatki za obe leti in obe lokaciji), s stopnjo signifikantnosti $\alpha = 0.05$

Location	Rakičan and Jablje		Rakičan		Jablje		Rakičan	Jablje
Wheat types	Awnless wheat	Awned wheat	Awnless wheat	Awned wheat	Awnless wheat	Awned wheat	Both awl+aw	Both awl+aw
Mean (%)	2.35	1.77	1.87	1.95	2.82	1.59	1.90	2.44
Std Dev	3.20	2.27	2.91	2.63	3.46	1.94	2.79	3.09
df	74		36		36		74	
p-value	0.37		0.93		0.17		0.43	

Std Dev-Standard Deviation; df-degrees of freedom; *-statistical significant difference; awl-awnless wheat; aw-awned wheat
Std Dev-Standardna deviacija; df-stopnje prostosti; *-statistično značilne razlike; awl-pšenica tip golica; aw-pšenica tip resnica

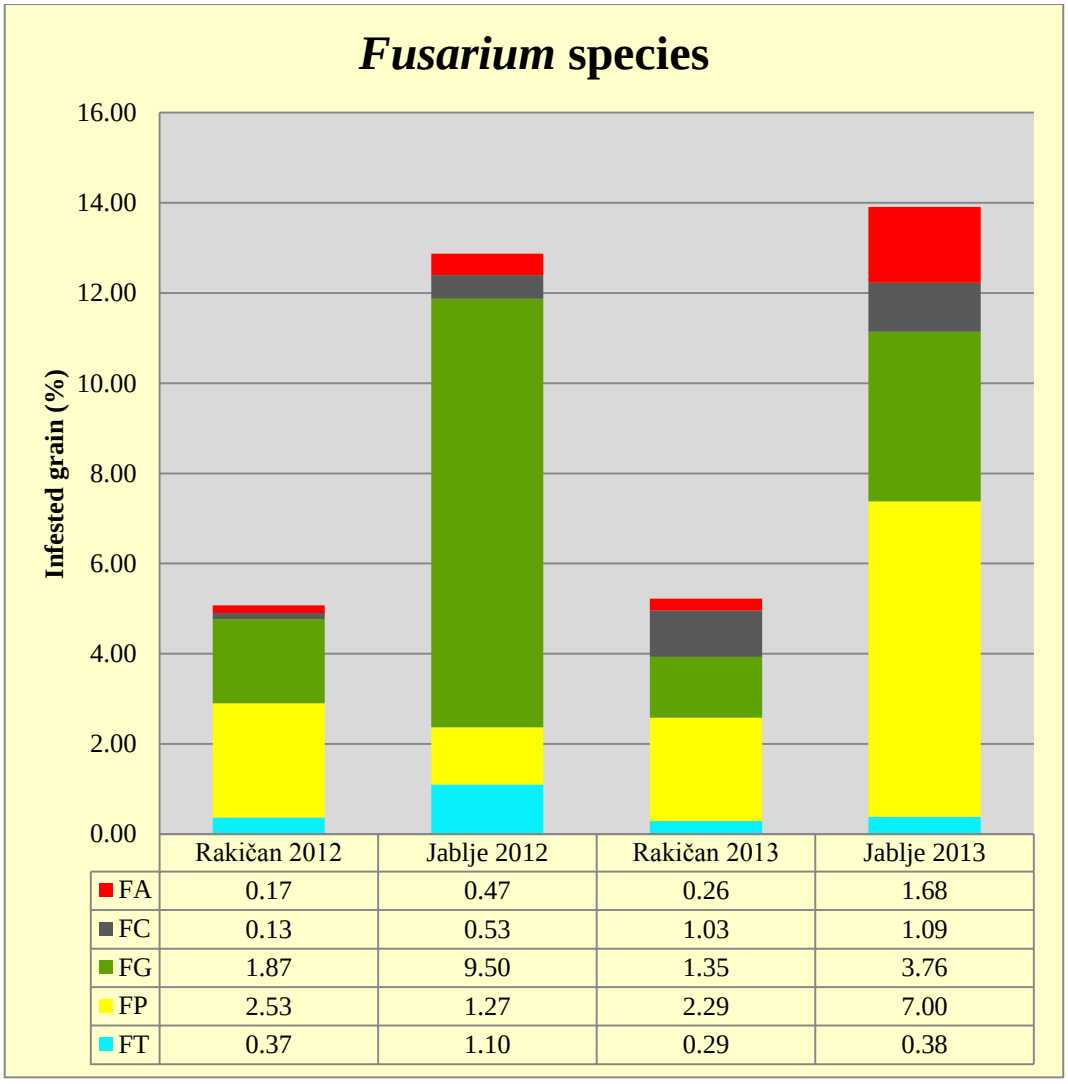
4.2 *Fusarium* spp. INCIDENCE AND DON CONTENT FOR INTEGRALLY PRODUCED WHEAT

The appearance of the primary toxic secondary metabolite of *Fusarium* - DON is also considered to be a sign of potential existence of other, more hazardous trichothecenes. This is why, along with the fact that cereal products derived from garin infectedwith *Fusarium* spp. may lead to contamination with mycotoxicosis, DON is considered to be the most significant trichothecene in Europe, in fact, it is so significant that the majority of European countries have set regulations to control its occurrence in grains and related products for human and animal consumption. The limitations for DON were imposed by the EU commission for unprocessed corn and food products with the EC Regulation 1126/2007 for unprocessed cereals other than durum wheat, and they amount to 1250 µg/kg (Commission Regulation (EC) No 1126/2007 of 28 September 2007). That is why in this study, samples of grain collected from the two regions of Slovenia in the period of 2012-2013, were also used for DON analysis.

DON is the product of type B trichothecene producing species of the *F. graminearum* species complex and *F. culmorum* (D'Mello and Macdonald, 1997; D'Mello et al., 1999). However, symptoms of FHB do not always imply that there is a potential existence of DON in the grain, but a large number of infected kernels present in the grain collected, do imply that strong possibility of DON presence exist. In order to more precisely measure the presence of DON in our grain samples collected from the two regions of Slovenia in the period of 2012-2013, we used an enzyme-linked immunosorbent assay Ridascreen® Fast DON.

In addition to the aforementioned data, we have included a graphical representation of the average grain infections in all analyzed wheat varieties from the integrated production, during the two year experimental period. The goal was to reach a more clear understanding of the difference in degree of infection caused the *Fusairum* spp. in wheat.

The wheat varities grown in Jablje during the summers of 2012 and 2013 were, on average, more infected with *Fusarium* spp. than the varieties grown in Rakičan. The total grain infection rate by *Fusarium* spp. in Jablje was 12.9% during 2012 and 13.9% in 2013, while the grain infection in Rakičan during those same years was 5.1%, and 5.2%, respectively. The *Fusarium* species, their composition, and their percentage in the infected grains changed according to location and years (Figure 24).



	Rakičan 2012	Jablje 2012	Rakičan 2013	Jablje 2013
FA	0.17	0.47	0.26	1.68
FC	0.13	0.53	1.03	1.09
FG	1.87	9.50	1.35	3.76
FP	2.53	1.27	2.29	7.00
FT	0.37	1.10	0.29	0.38

Figure 24: The average infection rate of all the grain samples (varieties) of wheat in Rakičan and Jablje in integrally produced wheat with various species of *Fusarium* (FA-*F. avenaceum*, FC-*F. culmorum*, FG-*F. graminearum*, FP-*F. poae*, FT-*F. tricinctum*) in the years 2012 and 2013
Slika 24: Povprečna okuženost zrnja vseh vzorcev (sort) pšenice v Rakičanu in Jabljah v integralni pridelavi z različnimi vrstami gliv iz rodu *Fusarium* (FA-*F. avenaceum*, FC-*F. culmorum*, FG-*F. graminearum*, FP-*F. poae*, FT-*F. tricinctum*) v letih 2012 in 2013

We have also created a graphical representation where we have presented the average amount of DON content in all analyzed grain samples (varieties) of wheat of the integrated production, during the two year experimental period. The obtained results refer to all wheat samples analyzed for DON content that were initially taken into consideration. In 2012, for each location (Rakičan and Jablje), a total of 30 diferent wheat varieties were analyzed. On the other hand, 31 different wheat varities from Rakičan were analyzed for DON for 2013, while from Jablje 34 varieties of wheat were used (Figure 25).

Considering the climatic circumstances, we have expected the mycotoxin levels in Rakičan to be at their minimum (Figure 25), which was proven by the ELISA test, according to which the 1250 µg/kg DON content limit imposed by the European Union (EU) was not exceeded in the studied samples, which again proved that the DON presence in Rakičan was at a low level during 2012 and 2013 (Table 9).

In Jablje, on the other hand, the weather conditions allowed greater accumulation of DON (Figure 25), which was proven by the ELISA test conducted on the samples from Jablje in 2012 and 2013. The results reflected that the determined limit of 1250 µg/kg imposed by the European Union (EU) for DON content was exceeded in two samples in Jablje in 2012, as well as two samples in 2013 (Table 11).

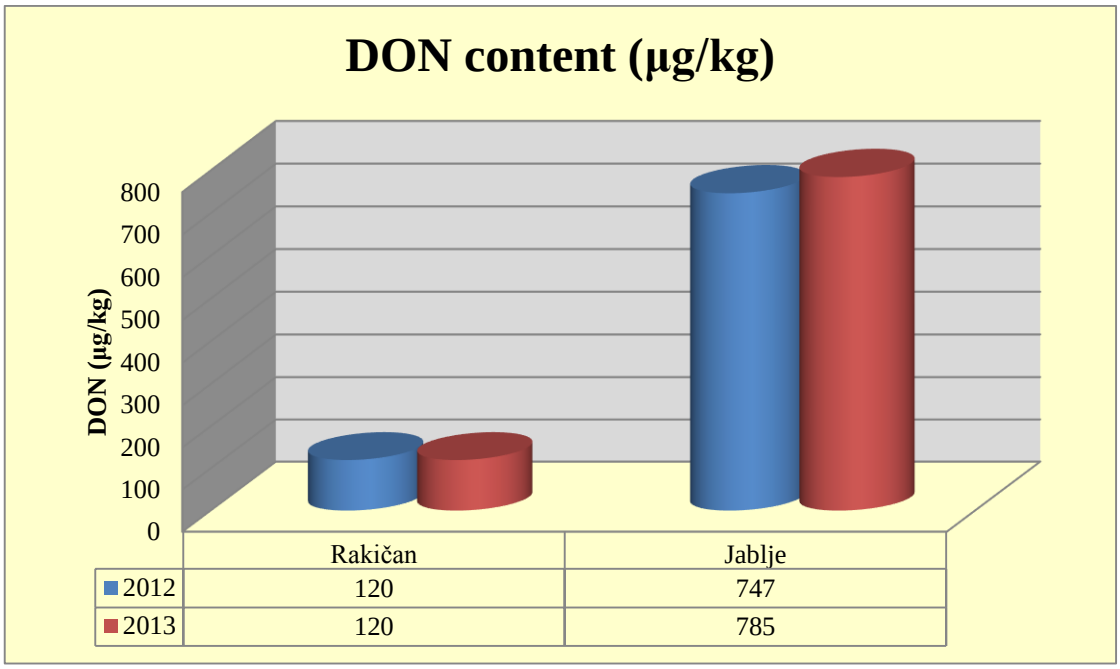


Figure 25: Average amount of DON content (µg/kg) of all the grain samples (varieties) of wheat in Rakičan and Jablje in the years 2012 and 2013
Slika 25: Povprečna vsebnost DON (µg/kg) vseh vzorcev (sort) pšenice v Rakičanu in Jabljah v letih 2012 in 2013

All wheat varieties that were primarily taken into consideration as varieties designated for cultivation in Slovenia and were later sown in Rakičan in 2012 and 2013, are presented (see Annex A). Out of all samples, we managed to retrieve complete laboratory results for a total of 19 wheat varieties, which were consistent in both locations during the two-year trial period.

The results obtained were from analyses of *Fusarium* presence as well as DON concentrations, and they are presented in the following tables.

Table 9: DON content (µg/kg) and percentage of identified *Fusarium* species in 19 different wheat varieties from Rakičan in years 2012 and 2013
Preglednica 9: Vsebnost DON (µg/kg) in odstotek ugotovljenih vrst iz rodu *Fusarium* v 19 različnih sort pšenice iz Rakičana v letih 2012 in 2013

Wheat varieties	Rakičan – 2012							Rakičan – 2013						
	FA	FC	FG	FP	FT	Σ	DON µg/kg	FA	FC	FG	FP	FT	Σ	DON µg/kg
BOLOGNA	0	0	2	4	0	6	32	0	0	0	2	0	2	17
INGENIO	2	0	2	2	0	6	135	0	2	1	2	0	5	117
LUKULLUS	0	0	2	1	0	3	133	0	0	1	1	1	3	93
MIHELCA	0	0	1	1	0	2	173	0	1	1	4	0	6	71
NS 40S	0	1	0	0	2	3	106	0	2	1	1	0	4	114
SY MOISSON	0	0	2	6	0	8	155	0	0	1	3	1	5	40
ALIXAN	1	0	1	6	0	8	104	0	2	0	3	0	5	136
BC RENATA	0	0	1	2	0	3	47	0	3	0	0	0	3	95
GARCIA	0	0	2	4	2	8	159	0	0	1	1	0	2	57
ILLICO	2	2	1	0	0	5	98	0	1	1	0	0	2	77
KATARINA	0	1	5	2	1	9	115	0	0	1	1	0	2	86
KETCHUM	0	0	1	2	0	3	**297**	1	2	9	6	1	19	**1033**
LORD	0	0	3	0	1	4	**240**	0	0	3	0	0	3	140
LUCIJA	0	0	2	5	0	7	149	0	4	2	0	0	6	15
NINA	0	0	4	5	0	9	180	0	1	1	7	1	10	71
SIMONIDA	0	0	1	2	0	3	156	0	0	2	0	0	2	8
SRPANJKA	0	0	4	4	1	9	148	0	2	2	2	0	6	3
ZDENKA	0	0	2	0	1	3	144	0	0	2	4	0	6	61
ŽITARKA	0	0	3	6	0	9	83	0	0	1	4	1	6	53
Σ	5	4	39	52	8	108	2654	1	20	30	41	5	97	2287
AVERAGE	0.3	0.2	2.1	2.7	0.4	5.7	139.7	0.1	1.1	1.6	2.2	0.3	5.1	120.4

FA-*Fusarium avenaceum*; FC-*Fusarium culmorum*; FG-*Fusarium graminearum*; FP-*Fusarium poae*; FT-*Fusarium tricinctum*; Σ-Sum; DON-Deoxynivalenol; **bold numbers**: positive samples i.e. mycotoxin concentration above 200 µg/kg; green marked: awnless wheat; grey marked: awned
FA-*Fusarium avenaceum*; FC-*Fusarium culmorum*; FG-*Fusarium graminearum*; FP-*Fusarium poae*; FT-*Fusarium tricinctum*; Σ-vsota; DON-deoksinivalenol; **poudarjene številke**: pozitivni vzorci oz. vsebnost mikotoksinov nad 200 µg/kg; zelena oznaka: pšenica tipa golica; siva oznaka: pšenica tipa resnica

From a phytopathological stand point, *F. culmorum* and *F. graminearum* which are according to literature, the main producers of DON (Miller, 1994; Mateo et al., 2002), present the greatest importance to us. According to the results obtained from Rakičan in 2012, an average incidence 0.2% was recorded for *F. culmorum*, while *F. graminearum* was represented on average by 2.1% (Table 9 and Figure 26). On the other hand, in 2013, the average incidence of *F. culmorum* was 1.1%, while the incidence of *F. graminearum* decreased to an average of 1.6% (Table 9 and Figure 27). The awned wheat, showed statistically smaller infection levels

when compared to the awnless wheat, according to the analysis of the data from Rakičan based on FC+FG presence.

Table 10: Two-tailed t-test of Type 3 for both types of wheat based on FC+FG data (%) (data for both locations and years), with significance level α = 0.05
Preglednica 10: Dvostranski t-test tipa III za oba tipa pšenice glede na podatke FC+FG (%) (podatki za obe lokaciji in leti) s stopnjo signifikantnosti α = 0.05

Location	Rakičan and Jablje		Rakičan		Jablje		Rakičan	Jablje
Wheat types	Awnless wheat	Awned wheat	Awnless wheat	Awned wheat	Awnless wheat	Awned wheat	Both awl+aw	Both awl+aw
Mean (%)	4.81	4.66	2.81	1.66	6.81	7.66	2.45	7.08
Std Dev	5.17	6.71	2.19	0.89	6.44	8.59	1.94	7.08
df	74		36		36		74	
p-value	0.93		0.029*		0.76		0.00015*	

Std Dev-Standard Deviation; df-degrees of freedom; *-statistical significant difference; awl-awnless wheat; aw-awned wheat
Std Dev-Standardni odklon; df-stopnje prostosti; *-statistično značilna razlika; awl-pšenica tipa golica; aw-pšenica tipa resnica

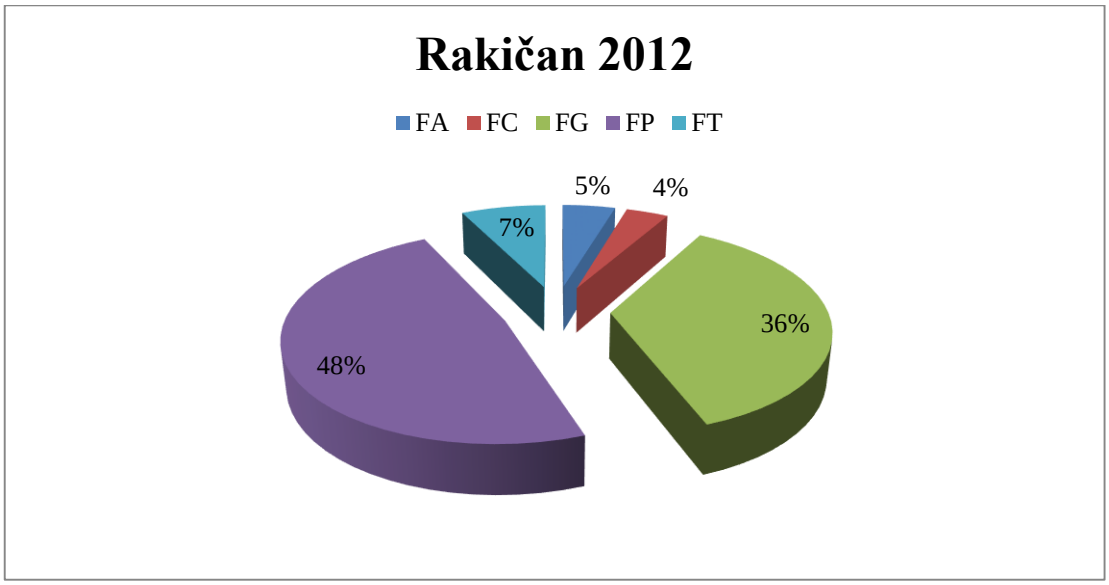


Figure 26: Percentage proportion of *Fusarium* spp. on grains of 19 different varieties of wheat from Rakičan in the year 2012 (FA-*Fusarium avenaceum*; FC-*Fusarium culmorum*; FG-*Fusarium graminearum*; FP-*Fusarium poae*; FT-*Fusarium tricinctum*)
Slika 26: Odstotni delež posameznih *Fusarium* vrst na zrnju 19 različnih sort pšenice iz Rakičana v letu 2012 (FA-*Fusarium avenaceum*; FC-*Fusarium culmorum*; FG-*Fusarium graminearum*; FP-*Fusarium poae*; FT-*Fusarium tricinctum*)

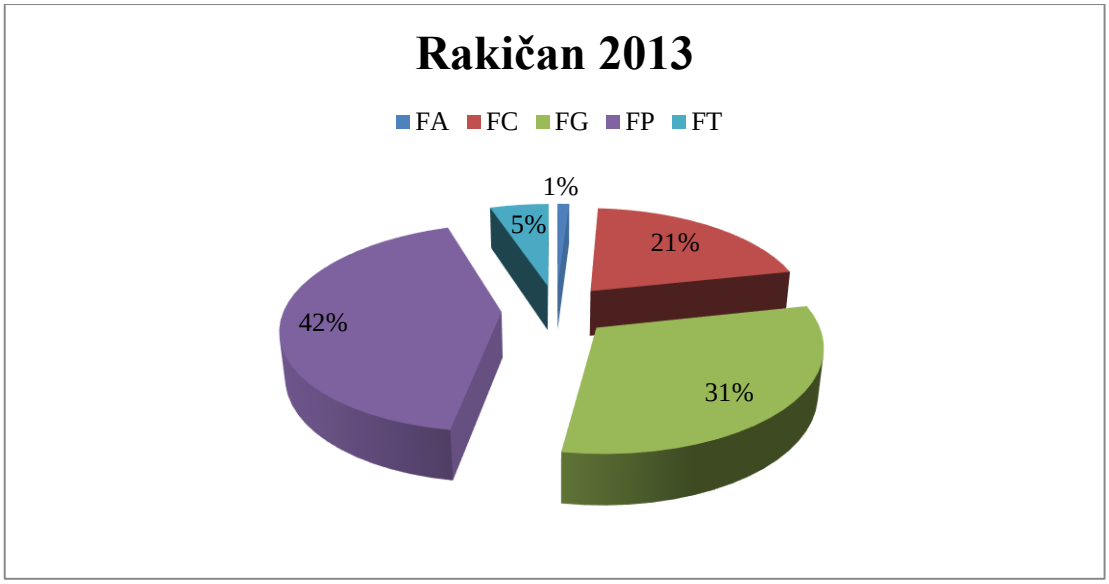


Figure 27: Percentage proportion of *Fusarium* spp. on grains of 19 different varieties of wheat from Rakičan in the year 2013 (FA-*Fusarium avenaceum*; FC-*Fusarium culmorum*; FG-*Fusarium graminearum*; FP-*Fusarium poae*; FT-*Fusarium tricinctum*)
Slika 27: Odstotni delež posameznih *Fusarium* vrst na zrnju 19 različnih sort pšenice iz Rakičana v letu 2013 (FA-*Fusarium avenaceum*; FC-*Fusarium culmorum*; FG-*Fusarium graminearum*; FP-*Fusarium poae*; FT-*Fusarium tricinctum*)

As previously mentioned, the specie composition of the *Fusarium* fungi and their relative share in the infected grains varied between both locations and years. Sometimes, the total grain infection with *Fusarium* spp. slightly blurs the picture of how much is the actual possibility of greater or lesser contamination of grains with DON. According to available literature (Miller, 1994; Mateo et al., 2002), only two *Fusarium* species can be considered as potential producers of this toxin, as mentioned above. The above presented figures, apart from the representation of *F. graminearum* and *F. culmorum* (producers of DON), show the relative shares of individual identified *Fusarium* species on wheat, for Rakičan in 2012 and 2013.

In both years of the experiment in Rakičan we have detected quite a high representation of *F. poae* among other *Fusarium* spp. (from 42 to 48%), (Figure 26 and 27). However, with regards to the high proportion of this specie it is worth mentioning that this specie is not a potential DON producer, but a producer other important mycotoxins, such as nivalenol and beauvericin. Representation of the species *F. tricinctum* and *F. avenaceum* in both years (2012 and 2013) was at relatively low levels (1 to 7%), (Table 26 and 27).

According to the analyses, two positive samples which exceeded the minimal detection limit of 200 µg/kg with DON were detected in Rakičan for 2012, with a maximum DON content of 297 µg/kg, and one positive sample in 2013, with a maximum DON content of 1033 µg/kg.

The total mean value for the DON content for 2012 was 140 µg/kg, and 120 µg/kg for 2013 (Table 9). The limit of 1250 µg/kg imposed by the European Union (EU) for DON content was not exceeded in the studied samples, which indicates that DON presence in Rakičan was at a low level during 2012 and 2013.

All the wheat varieties designated for cultivation in Slovenia, sown at our second test location in Jablje, in 2013 and 2012, are presented in Annex B. Out of all samples, the same 19 wheat varieties as in the previous location, for which we have retrieved complete laboratory results for the determination of *Fusarium* spp. and DON content, were used.

Table 11: DON content (µg/kg) and percentage of identified *Fusarium* species in 19 different wheat varieties from Jablje in the years 2012 and 2013
Preglednica 11: Vsebnost DON (µg/kg) in odstotek ugotovljenih vrst iz rodu *Fusarium* v 19 različnih sort pšenice iz Jabljah v letih 2012 in 2013

Wheat varieties	Jablje – 2012							Jablje – 2013						
	FA	FC	FG	FP	FT	Σ	DON µg/kg	FA	FC	FG	FP	FT	Σ	DON µg/kg
BOLOGNA	0	0	2	0	0	2	162	2	0	2	7	0	11	128
INGENIO	2	0	30	0	0	32	**703**	1	3	4	10	0	18	**1040**
LUKULLUS	0	0	12	2	2	16	**814**	0	3	1	2	4	10	**1135**
MIHELCA	0	0	2	4	4	10	**222**	0	0	2	0	0	2	**339**
NS 40S	0	0	4	1	0	5	**576**	0	0	2	22	0	24	**382**
SY MOISSON	0	0	18	0	0	18	**946**	2	1	6	6	0	15	**1176**
ALIXAN	0	3	3	1	0	7	**467**	1	0	8	6	0	15	***1385**
BC RENATA	0	0	1	1	2	4	**242**	2	0	3	9	0	14	**246**
GARCIA	0	0	18	1	2	21	**800**	3	1	2	5	0	11	**585**
ILLICO	0	3	0	0	1	4	**307**	1	0	1	2	0	4	136
KATARINA	1	0	0	7	1	9	**227**	3	4	4	5	1	17	**486**
KETCHUM	0	0	23	0	1	24	***2498**	0	6	8	3	0	17	***2905**
LORD	2	1	23	1	0	27	***3550**	0	1	4	15	0	20	**793**
LUCIJA	0	0	6	1	2	9	**308**	6	3	4	5	0	18	**595**
NINA	0	0	1	4	0	5	**862**	0	0	3	10	0	13	**533**
SIMONIDA	0	0	6	0	0	6	138	5	2	7	5	3	22	**543**
SRPANJKA	1	0	7	1	1	10	**229**	2	0	5	6	0	13	**231**
ZDENKA	0	0	8	0	1	9	**396**	3	0	1	1	0	5	**224**
ŽITARKA	0	2	5	1	5	13	118	0	0	0	8	0	8	140
Σ	6	9	169	25	22	231	13565	31	24	67	127	8	257	13002
AVERAGE	0.3	0.5	8.9	1.3	1.2	12.2	713.9	1.6	1.3	3.5	6.7	0.4	13.5	684.3

FA-*Fusarium avenaceum*; FC-*Fusarium culmorum*; FG-*Fusarium graminearum*; FP-*Fusarium poae*; FT-*Fusarium tricinctum*; Σ-Sum; DON-Deoxynivalenol; **bold numbers**: positive samples i.e. mycotoxin concentration above 200 µg/kg; *-samples that exceed the limit of 1250 µg/kg imposed by the EU for DON; green marked: awnless wheat; grey marked: awned
FA-*Fusarium avenaceum*; FC-*Fusarium culmorum*; FG-*Fusarium graminearum*; FP-*Fusarium poae*; FT-*Fusarium tricinctum*; Σ-vsota; DON-deoksinivalenol; **poudarjene številke**: pozitivni vzorci oz. vsebnost mikotoksinov nad 200 µg/kg; *- vzorci ki presegajo mejo 1250 µg/kg uvedeno z EU za DON; zelena oznaka: pšenica tipa golica; siva oznaka: pšenica tipa resnica

The results from Jablje in 2012 give us an entirely different picture, in relation to *Fusarium* spp. frequency and DON content, than the results for the same period in Rakičan. In this location, the average presence of *F. culmorum* was recorded to be 0.5%, while the average of *F. graminearum* amounted up to 8.9%, which is also the highest amount recorded at both location for the two-year period (Table 11 and Figure 28). On the other hand, in 2013, the presence of *F. culmorum* was greater than the previous year with an average of 1.3%, while the average for *F. graminearum* decreased to 3.5% (Table 11 and Figure 29). In 2012, the relative proportion of *F. poae* when compared to Rakičan was relatively low, 11%, but in 2013 it reached 50%, which is comparable with the results from Rakičan (Figure 28 and Figure 29). In this case, the statistical analysis of the data from Jablje showed that there is no statistically significant difference between the awned and awnless wheat, in relation to FC+FG presence (Table 10). Nevertheless, the data for both wheat types together, showed that the wheat (grain) in Jablje is singnificantly more infected by FC+FG when compared to Rakičan (Table 10). Our results of the occurrence of *Fusarium* spp. in different clamatic regions are consistent with the findings of Doohan et al. (2003).

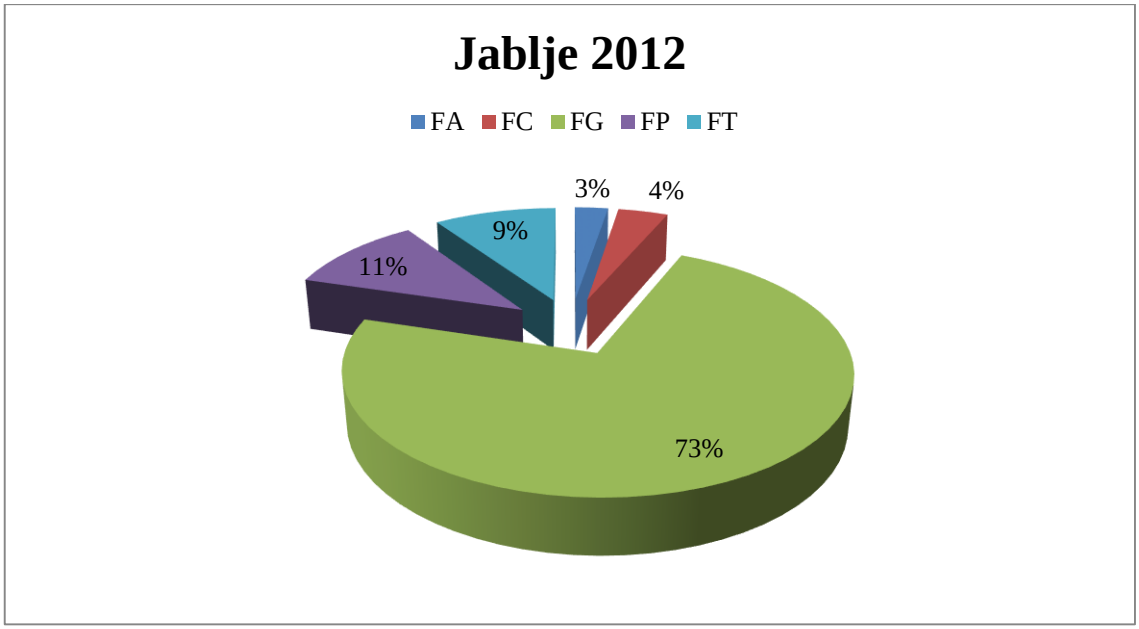


Figure 28: Percentage proportion of *Fusarium* spp. on grains of 19 different varieties of wheat from Jablje in the year 2012 (FA-*Fusarium avenaceum*; FC-*Fusarium culmorum*; FG-*Fusarium graminearum*; FP-*Fusarium poae*; FT-*Fusarium tricinctum*)
Slika 28: Odstotni delež posameznih *Fusarium* vrst na zrnju 19 različnih sort pšenice iz Jabelj v letu 2012 (FA-*Fusarium avenaceum*; FC-*Fusarium culmorum*; FG-*Fusarium graminearum*; FP-*Fusarium poae*; FT-*Fusarium tricinctum*)

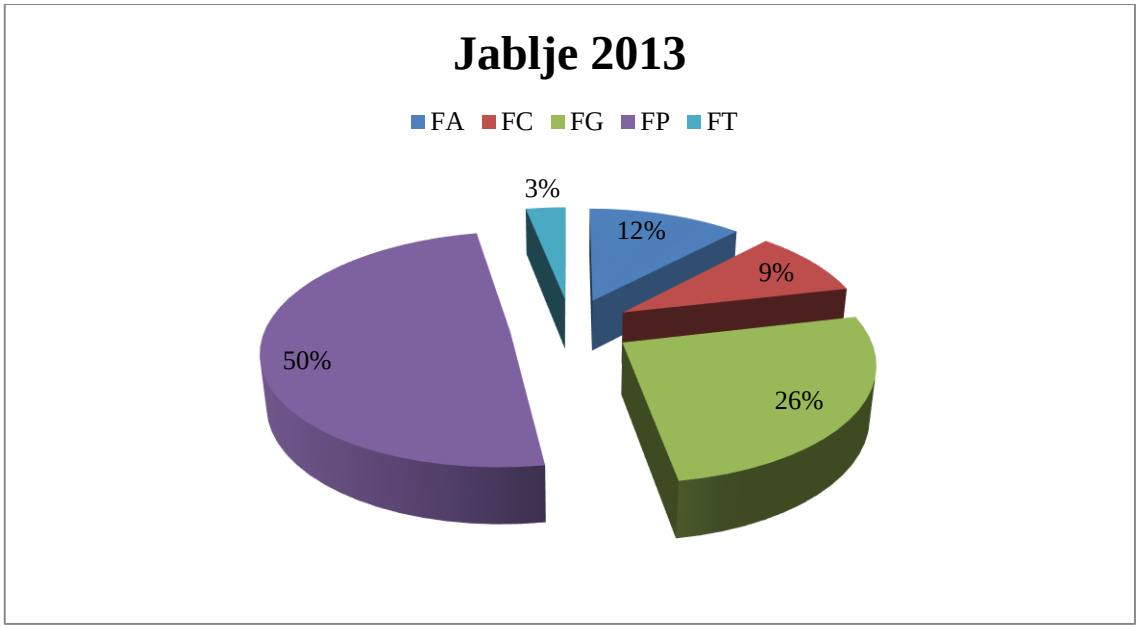


Figure 29: Percentage proportion of *Fusarium* spp. on grains of 19 different varieties of wheat from Jablje in the year 2013 (FA-*Fusarium avenaceum*; FC-*Fusarium culmorum*; FG-*Fusarium graminearum*; FP-*Fusarium poae*; FT-*Fusarium tricinctum*)
Slika 29: Odstotni delež posameznih *Fusarium* vrst na zrnju 19 različnih sort pšenice iz Jabelj v letu 2013 (FA-*Fusarium avenaceum*; FC-*Fusarium culmorum*; FG-*Fusarium graminearum*; FP-*Fusarium poae*; FT-*Fusarium tricinctum*)

As the tests revealed, the occurrence of samples with DON in Jablje during 2012 was determined in 16 out of 19 samples which showed mycotoxin concentrations that exceeded the established limit of 200 µg/kg, with a maximum DON content of 3550 µg/kg, while in 2013, 16 out of 19 samples were also detected, with a maximum DON content of 2905 µg/kg. The total mean value for DON in 2012 was 714 µg/kg and 684 µg/kg in 2013, respectively (Table 11). The limit of 1250 µg/kg imposed by the European Union (EU) for DON content was exceeded in two samples in Jablje for 2012, as well as two samples of 2013 (Table 11).

4.2.1 Correlation between grain infection with FC+FG and DON content

With the help of statistical analysis, and in order to determine whether there is a connection between the infected grains of wheat with FC+FG and the content of DON, we have conducted a test correlation. This test was conducted for every location separately, as well as for both locations together.
As it can be seen from Figure 30, a correlation r=0.68 can be observed between FC+FG and DON over data from location Rakičan ($y=56.742x - 8.8426$, $R^2=0.46$).

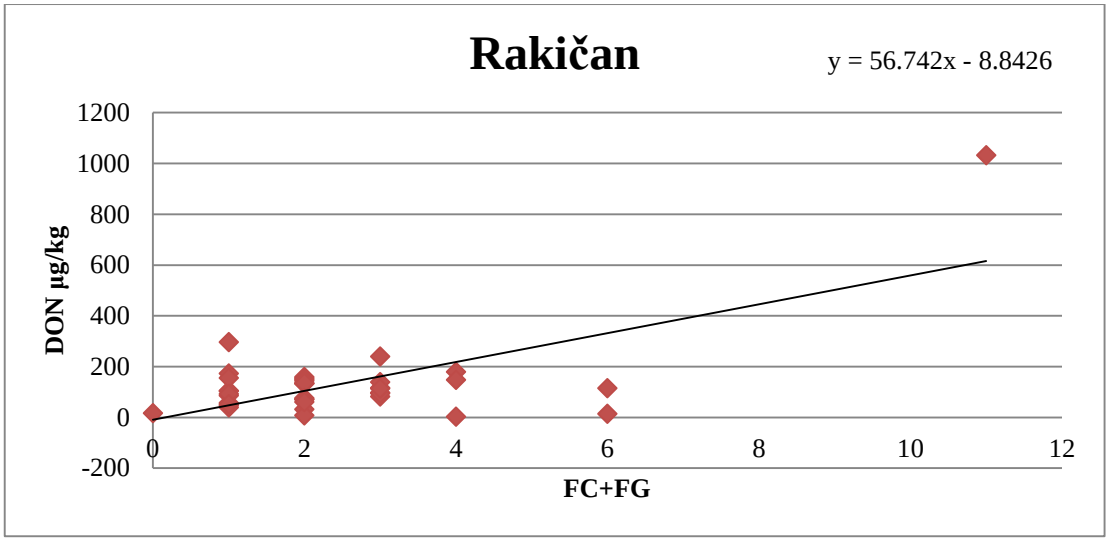


Figure 30: Correlation between grains infection with FC+FG (*F. culmorum* + *F. graminearum*) and DON content on 19 different wheat varieties for Rakičan in years 2012 and 2013 (y-DON µg/kg, x-grain infested with FC+FG in %)
Slika 30: Korelacija med okuženostjo zrnja z FC+FG (*F. culmorum* + *F. graminearum*) in vsebnostjo DON pri 19 različnih sortah pšenice v Rakičanu za leti 2012 in 2013 (y-DON µg/kg, x-zrna okužena s FC+FG v %)

As shown in Figure 31, in Jablje a correlation of r=0.64 was observed between FC+FG and DON (y=69.507x + 207.1, R^2=0.41).

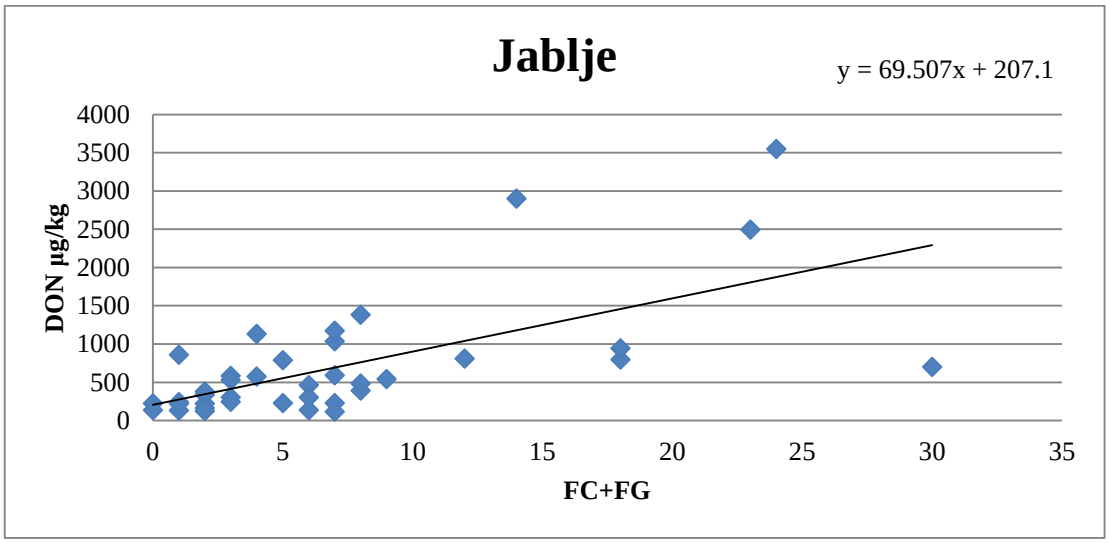


Figure 31: Correlation between grains infection with FC+FG (*F. culmorum* + *F. graminearum*) and DON content on 19 different wheat varieties for Jablje in years 2012 and 2013 (y-DON µg/kg, x-grain infested with FC+FG in %)
Slika 31: Korelacija med okuženostjo zrnja z FC+FG (*F. culmorum* + *F. graminearum*) in vsebnostjo DON pri 19 različnih sortah pšenice v Jabljah za leti 2012 in 2013 (y-DON µg/kg, x-zrna okužena s FC+FG v %)

Finally, by combining the data from both locations (Rakičan and Jablje) and both years (2012 and 2013), as presented in Figure 32, the descriptive statistics shows highest correlation (r=0.71) with correlation function y=77.816x + 43.931, where coefficient of determination (R^2) is 0.51.

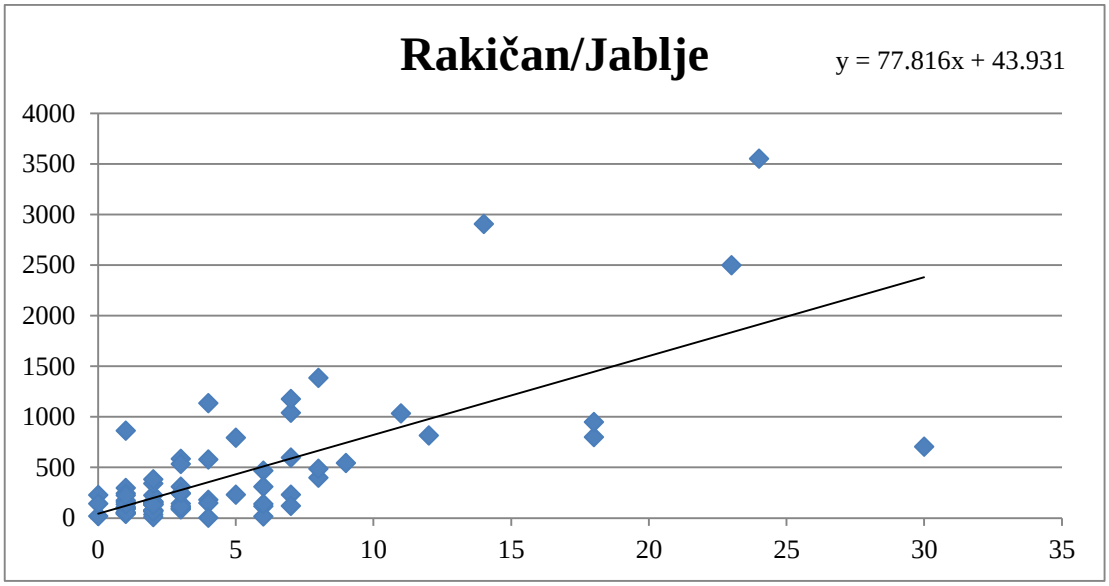


Figure 32: Correlation between grains infection with FC+FG (*F. culmorum* + *F. graminearum*) and DON content on 19 different wheat varieties for both locations Rakičan/Jablje in years 2012 and 2013 (y-DON µg/kg, x-grain infested with FC+FG in %)
Slika 32: Korelacija med okuženostjo zrnja z FC+FG (*F. culmorum* + *F. graminearum*) in vsebnostjo DON pri 19 različnih sortah pšenice za obe lokaciji Rakičan/Jablje za leti 2012 in 2013 (y-DON µg/kg, x-zrna okužena s FC+FG v %)

In both locations, the main differences in the infection levels of wheat samples with FC+FG were found in those locations situated in a humid area, and showed higher levels of DON content than those in dry area. Such statement is supported by Figure 30, 31 and 32 where correlation is noticed between FC+FG and DON for every location separately, as well as for both locations together. Furthermore, following our previous finding that FC+FG infection is higher in Jablje compared to Rakičan due to the weather conditions (Table 6 and 11), a conclusion can be drawn that weather conditions have influenced DON accumulation.
This claim is substantiated by a statistical analysis which indicates that the level of infection is significantly lower in Rakičan as opposed to Jablje where p=3.5859E-05 for FC+FG and p=2.38246E-05 for DON at α=0.05 level of significance was calculated. Results of our studies were in accordance with the studies conducted by Landschoot et al. (2012).

4.3 *Fusarium* spp. INCIDENCE AND DON CONTENT FOR ECOLOGICALLY PRODUCED WHEAT

In order to draw a comparison between mycotoxin presence in ecologically produced wheat and mycotoxin presence in integrally produced wheat, we have also obtained a representative number of samples from ecologically grown wheat originating from the areas found in the vicinity of our test (experimental) locations. These samples were obtained simultaneously with the samples from the integrated production in the same time intervals during the course of two years, 2012-2013. The need for this comparison has arisen due to the similar weather conditions which can, to some extent, either less or more, have an effect on the occurrence of wheat infection and subsequently mycotoxin contamination. The samples were analyzed in order to determine the presence of mycotoxins and the results were compared to the ones from the test locations where the production was integrated.

The total number of eco-samples retrieved from various regions of Slovenia was 18 (see Annex C and D). A part of those samples (7) was obtained from the Gorenjska region and another part from the Prekmurje region (4), while the rest were obtained from other production regions in Slovenia. In order to conduct the necessary comparison between the eco-samples and the integrally produced ones, only those samples which were gathered from the immediate vicinity of our test locations were used in our research.

Signiticantly lower infection levels were detected in the wheat from the ecological (ECO) production, which can be attributed to the fact that in this production system a much wider crop rotation was performed comparing to the rotation in intergrated production (maize-wheat). In addition, it is worth mentioning that the majority of the organic producers do not include maize in their crop rotation regularly, but only eventually, every three to four years, on their arable plots. The infected maize residues present a considerably high infection potential for infection of wheat or some other stubble cereals. Figure 33 indicates *Fusarium* species composition on wheat grains from the ECO production. The specie composition found in the ECO samples is more diverse than the one found in the wheat from integrated production. Also, some species that have been identified in the ECO grain samples weren’t found on the kernels from the integrated production.

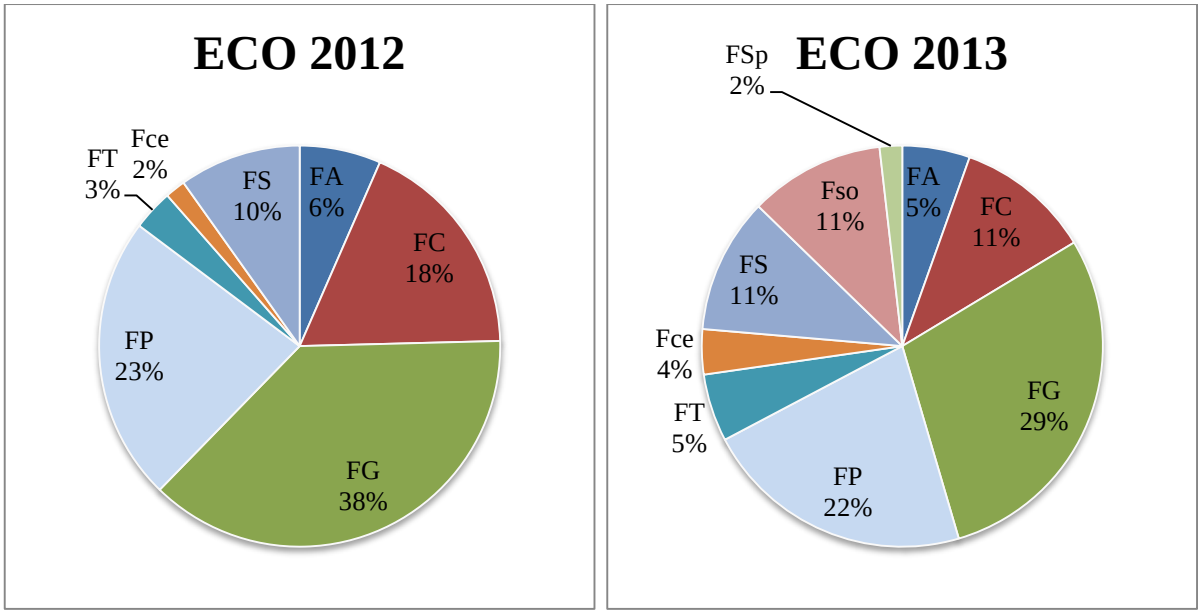


Figure 33: Percentage proportion of *Fusarium* species detectedin grain samples from ecological wheat production in individual years (2012-2013) (FA-*Fusarium avenaceum*; FC-*Fusarium culmorum*; FG-*Fusarium graminearum*; FP-*Fusarium poae*; FT-*Fusarium tricinctum*; Fce-*Fusarium cerealis*; FS-*Fusarium subglutinans*; Fso-*Fusarium solani*; FSp-*Fusarium sporotrichoides*)
Slika 33: Odstotni delež posameznih *Fusarium* vrst v posameznih letih (2012-2013) na vzorcih ekološko pridelane pšenice (FA-*Fusarium avenaceum*; FC-*Fusarium culmorum*; FG-*Fusarium graminearum*; FP-*Fusarium poae*; FT-*Fusarium tricinctum*; Fce-*Fusarium cerealis*; FS-*Fusarium subglutinans*; Fso-*Fusarium solani*; FSp-*Fusarium sporotrichoides*)

The percentage proportion of *F. graminearum* in grains from ecological production was comparable with the proportion in Rakičan, however, it was considerably smaller than the one in Jablje, especially in the year 2012. The percentage proportion of *F. culmorum* was 18 and 11%, *F. poae* 23 and 22%, *F. avenaceum* 6 and 5%, and *F. tricinctum* 3 and 5% in the years 2012 and 2013, respecitively. In contrast to Rakičan and Jablje, a broader spectrum of *Fusarium* species was isolated from the ecologically grown wheat grains, including *F. subglutinas* which was represented with approximately equal proportions of about ten percent (10 and 11%), while the percentage proportion of *F. cerealis* was 2 and 4%, in the years2012 and 2013, respectively. In 2013, we determined two new species, namely *F. solani* (11%) and *F. sporotrichoides* (2%), (Figure 33). Once again it must be emphasized that the overall infection of wheat grains with fusariosis in the years 2012 and 2013 was significantly lower when compared to the wheat from the integrated production. Our results are consistent with the research of Birzele et. al (2002) in Germany.

In figure 34 we present the average amount of DON content in all analyzed wheat varieties from ecological production, collected during the two year experimental period. The obtained

results for DON content refer to all ECO samples that were initially taken into consideration (Figure 34).

Considering the climatic conditions, we have expected the mycotoxin levels in Prekmurje region to be at their minimum (Figure 34), which was confirmed by ELISA test, according to which the 1250 µg/kg DON content limit imposed by the European Union (EU) was not exceeded in the studied samples during 2012 and 2013.

Gorenjska region, on the other hand, has different weather conditions which would presumably enable higher DON contamination, but still only one sample exceeded the limit of 1250 µg/kg in 2013 (Table 13). The results obtained again emphasised the need for a broader crop rotation in wheat production.

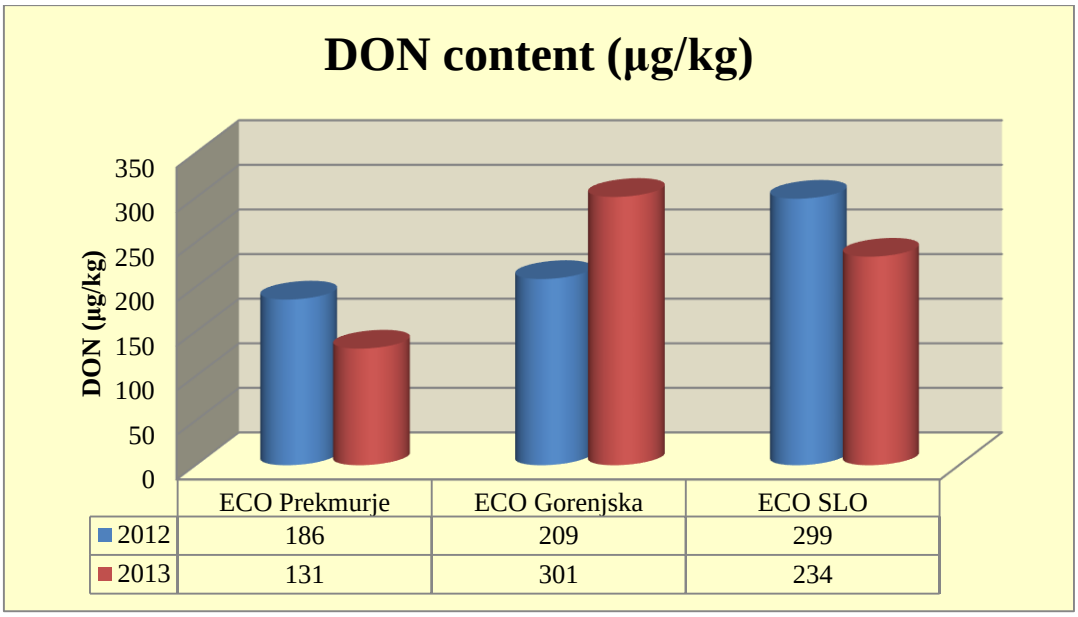


Figure 34: Average amount of DON content of all the grain samples (varieties) of ECO wheat in the years 2012 and 2013
Slika 34: Povprečna količina vsebine DON vseh vzorcev (sort) EKO pšenice v letih 2012 in 2013

The Gorenjska region was one of the locations from where a part of our ecologically produced wheat samples were obtained, or to be more precise, 7 different samples of wheat were obtained which were immediately subjected to phytopathological analysis, performed in the same manner as the analysis on the wheat samples retrieved from the test locations. This analysis revealed the incidence of *Fusarium* species infecting eco-wheat (Table 12), our primary focus being on *F. culmorum* and *F. graminearum*, the two main producers of DON.

Table 12: *Fusarium* spp. incidence (%) and DON content (µg/kg) in ecologically produced wheat samples from Gorenjska and Prekmurje region for 2012
Preglednica 12: Zastopanost *Fusarium* vrst (%) in vsebnost DON (µg/kg) v ekološko pridelani pšenici iz Gorenjske in Prekmurja za leto 2012

Eco wheat	Gorenjska region – 2012										
	FA	FC	FG	FP	FT	Fce	FS	Fso	FSp	Σ	DON µg/kg
ECO - 6	0	0	2	1	0	0	0	0	0	3	**279**
ECO - 7	0	0	0	1	0	0	0	0	0	1	94
ECO - 8	0	1	0	1	0	0	0	0	0	2	111
ECO - 9	0	0	0	2	1	0	0	0	0	3	161
ECO - 10	0	1	2	0	0	0	0	0	0	3	**477**
ECO - 11	0	0	1	1	0	0	0	0	0	2	176
ECO - 12	0	0	1	0	0	0	0	0	0	1	163
Σ	0	2	6	6	1	0	0	0	0	15	1461
AVERAGE	0.0	0.3	0.9	0.9	0.1	0.0	0.0	0.0	0.0	2.1	208.7
Eco wheat	Prekmurje region – 2012										
ECO - 15	2	1	1	1	0	1	3	0	0	9	**247**
ECO - 16	2	1	0	1	0	0	2	0	0	6	176
ECO - 17	0	1	1	0	0	0	0	0	0	2	187
ECO - 18	0	1	0	0	0	0	0	0	0	1	133
Σ	4	4	2	2	0	1	5	0	0	18	743
AVERAGE	1.0	1.0	0.5	0.5	0.0	0.3	1.3	0.0	0.0	4.5	185.8

FA-*Fusarium avenaceum*; FC-*Fusarium culmorum*; FG-*Fusarium graminearum*; FP-*Fusarium poae*; FT-*Fusarium tricinctum*; Fce-*Fusarium cerealis*; FS-*Fusarium subglutinans*; Fso-*Fusarium solani*; FSp-*Fusarium sporotrichoides*; ECO-Ecologically produced wheat; Σ-Sum; DON-Deoxynivalenol; **bold numbers**: positive samples i.e. mycotoxin concentration above 200 µg/kg
FA-*Fusarium avenaceum*; FC-*Fusarium culmorum*; FG-*Fusarium graminearum*; FP-*Fusarium poae*; FT-*Fusarium tricinctum*; Fce-*Fusarium cerealis*; FS-*Fusarium subglutinans*; Fso-*Fusarium solani*; FSp-*Fusarium sporotrichoides*; ECO-ekološko pridelana pšenica; Σ-vsota; DON-deoksinivalenol; **poudarjene številke**: pozitivni vzorci oz. koncentracija mikotoksinov nad 200 µg/kg

According to the results from our analyses, the average incidence of *F. culmorum* and *F. graminearum* in the eco-wheat from the Gorenjska region in 2012 were 0.3% and 0.9%, respectively (Table 12 and Figure 35). On the other hand, the data for 2013 from the same region showed 0.0% incidence of *F. culmorum* which is significantly lower than the previous year and at the same time the lowest percentage in all test locations during the two-year period, while the average incidence of *F. graminearum* has increased to 1.1% (Table 13 and Figure 36).

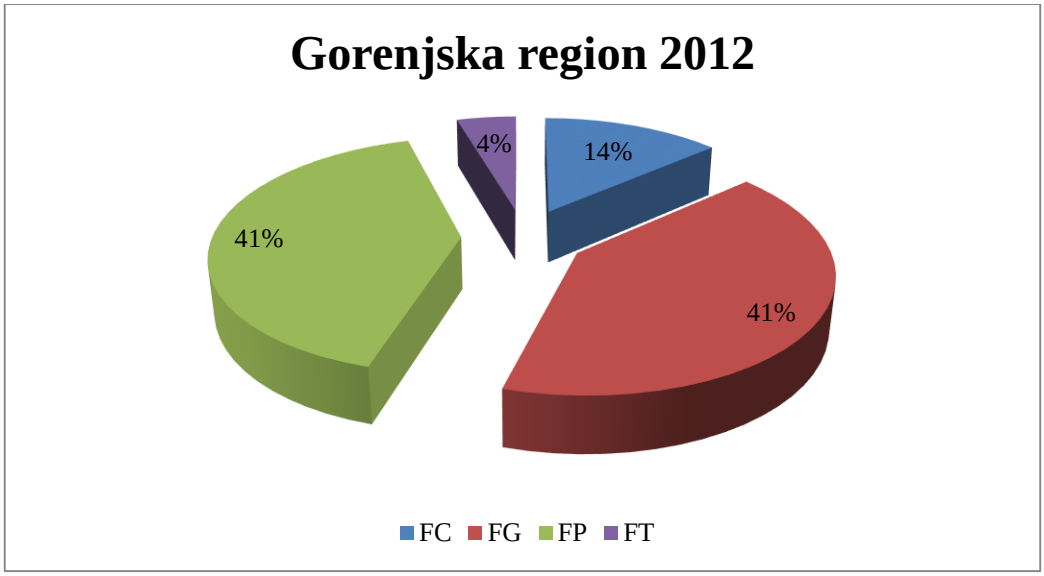


Figure 35: Percentage proportion of *Fusarium* spp. on grains of 7 different samples of ecologically produced wheat from Gorenjska region 2012 (FC-*Fusarium culmorum*; FG-*Fusarium graminearum*; FP-*Fusarium poae*; FT-*Fusarium tricinctum*)
Slika 35: Odstotni delež posameznih *Fusarium* vrst na zrnju 7 različnih vzorcev ekološko pridelane pšenice iz Gorenjske 2012 (FC-*Fusarium culmorum*; FG-*Fusarium graminearum*; FP-*Fusarium poae*; FT-*Fusarium tricinctum*)

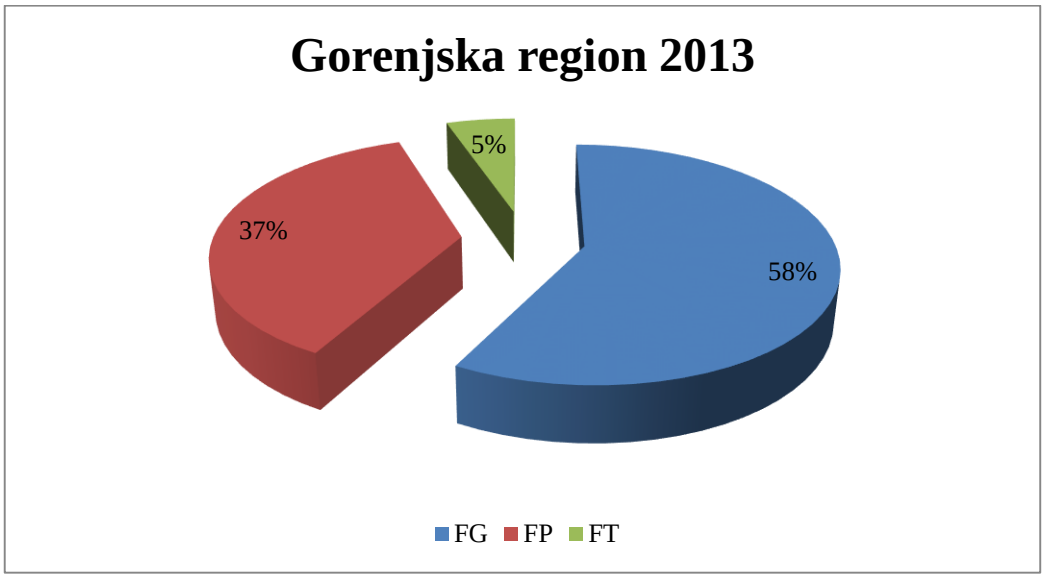


Figure 36: Percentage proportion of *Fusarium* spp. on grains of 7 different samples of ecologically produced wheat from Gorenjska region 2013 (FG-*Fusarium graminearum*; FP-*Fusarium poae*; FT-*Fusarium tricinctum*)
Slika 36: Odstotni delež posameznih *Fusarium* vrst na zrnju 7 različnih vzorcev ekološko pridelane pšenice iz Gorenjske 2013 (FG-*Fusarium graminearum*; FP-*Fusarium poae*; FT-*Fusarium tricinctum*)

As the results imply, the presence of both *Fusarium* species in the eco wheat samples indicates contamination with DON, which was confirmed by the ELISA test Two samples which exceeded the detection limit of 200 µg/kg were detected in the Gorenjska region in 2012, maximum DON contentwas 477 µg/kg (Table 12), and one positive sample in 2013, maximum DON content was 1456 µg/kg (Table 13). The total average value for DON was 209 µg/kg in 2012 (Table 12), and 301 µg/kg in 2013 (Table 13). The limit of 1250 µg/kg, imposed by the European Union (EU) for the presence of DON, was not exceeded in the tested samples of 2012 (Table 12), while in 2013 one sample exceed the imposed limit (Table 13).

Another region from where we have obtained 4 samples of ecologically produced wheat was Prekmurje. The results from this region for 2012 differ significantly regarding *Fusarium* spp. composition and DON content form the data obtained for Gorenjska region. According to our results, the average incidence of *F. culmorum* was 1.0%, while the average incidence of *F. graminearum* was 0.5% (Table 12 and Figure 37). In 2013, the detected average incidence of *F. culmorum* decreased to 0.8%, similar reduction was also observed in the case of *F. graminearum*, where the average incidence was 0.3% (Table 13 and Figure 38).

Table 13: *Fusarium* spp. incidence (%) and DON content (µg/kg) in ecologically produced wheat samples from Gorenjska and Prekmurje region for 2013
Preglednica 13: Zastopanost *Fusarium* vrst (%) in vsebnost DON (µg/kg) v ekološko pridelani pšenici iz Gorenjske in Prekmurja za leto 2013

Eco wheat	Gorenjska region – 2013										
	FA	FC	FG	FP	FT	Fce	FS	Fso	FSp	Σ	DON µg/kg
ECO - 6	0	0	1	0	0	0	0	0	0	1	134
ECO - 7	0	0	0	3	0	0	0	0	0	3	117
ECO - 8	0	0	0	1	0	0	0	0	0	1	75
ECO - 9	0	0	7	1	1	0	0	0	0	9	***1456**
ECO - 10	0	0	0	0	0	0	0	0	0	0	92
ECO - 11	0	0	0	0	0	0	0	0	0	0	111
ECO - 12	0	0	0	0	0	0	0	0	0	0	122
Σ	0	0	8	5	1	0	0	0	0	14	2107
AVERAGE	0.0	0.0	1.1	0.7	0.1	0.0	0.0	0.0	0.0	2.0	301.0
Eco wheat	Prekmurje region – 2013										
ECO - 15	2	1	1	1	0	2	4	3	0	14	182
ECO - 16	1	0	0	1	0	0	1	2	0	5	102
ECO - 17	0	1	0	0	0	0	0	0	0	1	108
ECO - 18	0	1	0	0	0	0	0	0	0	1	132
Σ	3	3	1	2	0	2	5	5	0	21	524
AVERAGE	0.8	0.8	0.3	0.5	0.0	0.5	1.3	1.3	0.0	5.3	131.0

Continued

Continuation of Table 13:

FA-*Fusarium avenaceum*; FC-*Fusarium culmorum*; FG-*Fusarium graminearum*; FP-*Fusarium poae*; FT-*Fusarium tricinctum*; Fce-*Fusarium cerealis*; FS-*Fusarium subglutinans*; Fso-*Fusarium solani*; FSp-*Fusarium sporotrichoides*; ECO-Ecologically produced wheat; Σ-Sum; DON-Deoxynivalenol; **bold numbers**: positive samples i.e. mycotoxin concentration above 200 µg/kg; *-samples that exceed the limit of 1250 µg/kg imposed by the EU for DON

FA-*Fusarium avenaceum*; FC-*Fusarium culmorum*; FG-*Fusarium graminearum*; FP-*Fusarium poae*; FT-*Fusarium tricinctum*; Fce-*Fusarium cerealis*; FS-*Fusarium subglutinans*; Fso-*Fusarium solani*; FSp-*Fusarium sporotrichoides*; ECO-ekološko pridelana pšenica; Σ-vsota; DON-deoksinivalenol; **poudarjene številke**: pozitivni vzorci oz. koncentracija mikotoksinov nad 200 µg/kg; *- vzorci ki presegajo mejo 1250 µg/kg uvedeno z EU za DON

The occurrence of samples contaminated with DON in the Prekmurje region during 2012 was determined in 1 out of 4 samples which showed mycotoxin concentration that exceeded the established limit of 200 µg/kg, with a maximum DON content of 247 µg/kg (Table 12). In the following year 2013, we have not detected any contaminated samples with DON which exceeded the established limit of 200 µg/kg, a maximum DON content was 182 µg/kg (Table 13). The total average value for DON in 2012 was 186 µg/kg (Table 12), and in 2013 it amounted to 131 µg/kg (Table 13), while the 1250 µg/kg limit for DON content imposed by the European Union was not exceeded not even once in the two-year test period.

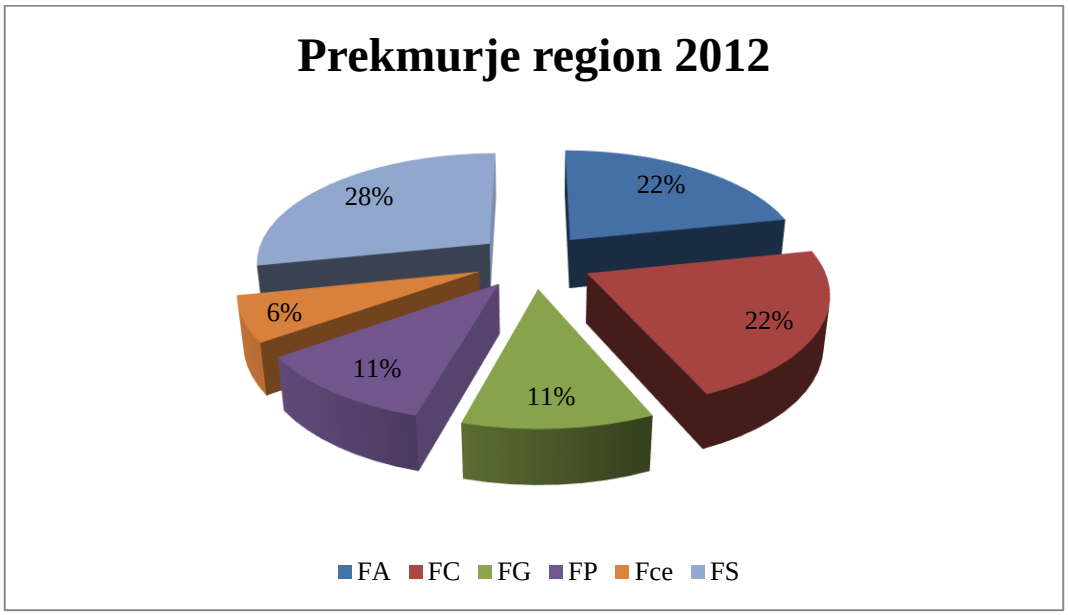


Figure 37: Percentage proportion of *Fusarium* spp. on grains of 4 different samples of ecologically produced wheat from Prekmurje region 2012 (FA-*Fusarium avenaceum*; FC-*Fusarium culmorum*; FG-*Fusarium graminearum*; FP-*Fusarium poae*; Fce-*Fusarium cerealis*; FS-*Fusarium subglutinans*)
Slika 37: Odstotni delež posameznih *Fusarium* vrst na zrnju 4 različnih vzorcev ekološko pridelane pšenice iz Prekmurja 2012 (FA-*Fusarium avenaceum*; FC-*Fusarium culmorum*; FG-*Fusarium graminearum*; FP-*Fusarium poae*; Fce-*Fusarium cerealis*; FS-*Fusarium subglutinans*)

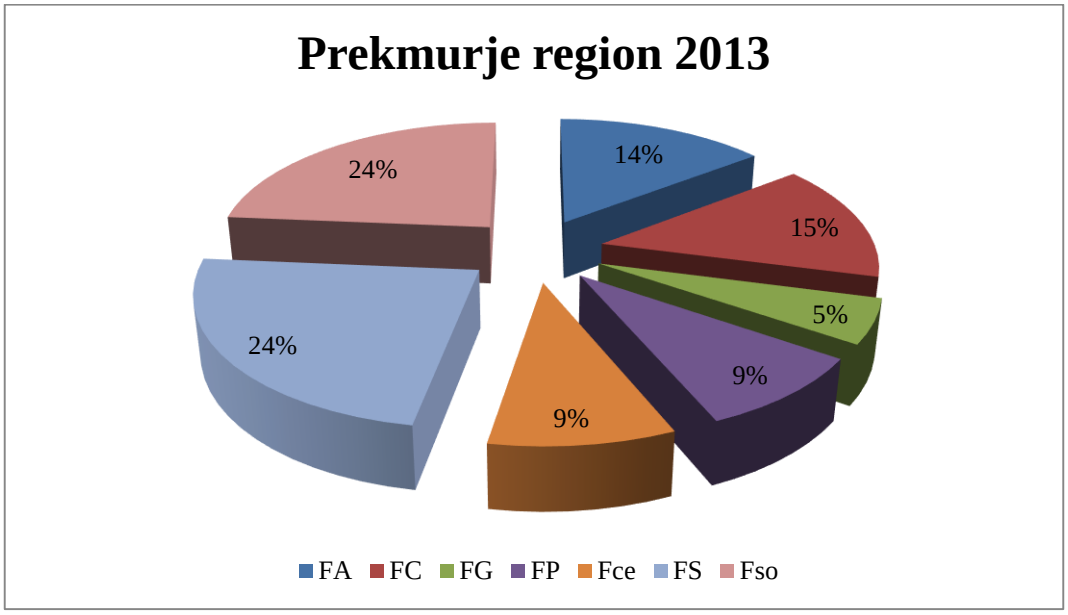


Figure 38: Percentage proportion of *Fusarium* spp. on grains of 4 different samples of ecologically produced wheat from Prekmurje region 2013 (FA-*Fusarium avenaceum*; FC-*Fusarium culmorum*; FG-*Fusarium graminearum*; FP-*Fusarium poae*; Fce-*Fusarium cerealis*; FS-*Fusarium subglutinans*; Fso-*Fusarium solani*)
Slika 38: Odstotni delež posameznih *Fusarium* vrst na zrnju 4 različnih vzorcev ekološko pridelane pšenice iz Prekmurja 2013 (FA-*Fusarium avenaceum*; FC-*Fusarium culmorum*; FG-*Fusarium graminearum*; FP-*Fusarium poae*; Fce-*Fusarium cerealis*; FS-*Fusarium subglutinans*; Fso-*Fusarium solani*)

The descriptive statistics presented below reveals a high correlation between FC+FG infection and DON content.

As presented in Figure 39, a correlation between FC+FG infection and DON content over data from the Gorenjska region is calculated to be r=0.97 with R^2=0.93 (y=182.07x+46.778). The coeficient (182.07) of the FC+FG in the regression equation showes that FC+FG infecton is of high importance, when compared to the second term (46.778).

In comparison with Gorenjska region smaler correlation was observed in this case and the regression equation indicates less importance of FC+FG infection in overall DON content (Figure 40).

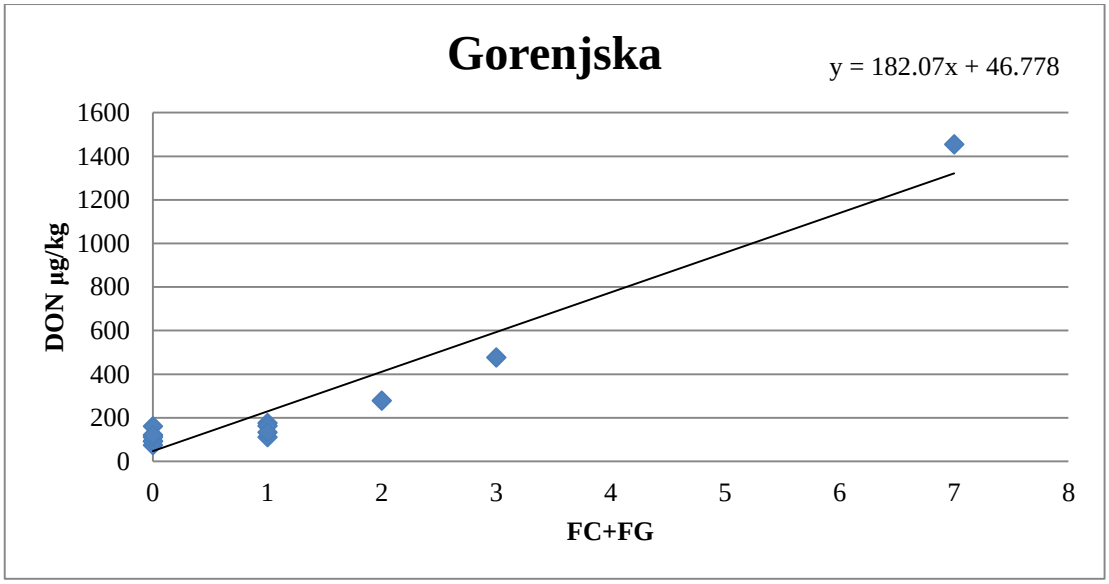


Figure 39: Correlation between grains infection with FC+FG (*F. culmorum* + *F. graminearum*) and DON content on 7 different wheat varieties for Gorenjska region in years 2012 and 2013 (y-DON µg/kg, x-grain infested with FC+FG in %)
Slika 39: Korelacija med okuženostjo zrnja z FC+FG (*F. culmorum* + *F. graminearum*) in vsebnostjo DON pri 7 različnih sortah pšenice v Gorenjsko za leti 2012 in 2013 (y-DON µg/kg, x-zrna okužena s FC+FG v %)

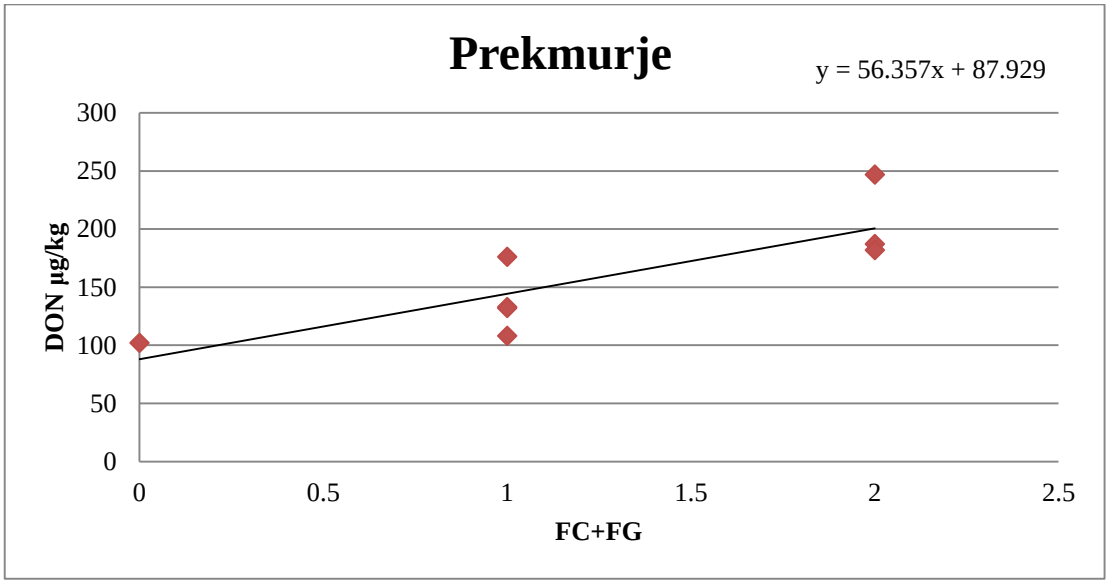


Figure 40: Correlation between grains infection with FC+FG (*F. culmorum* + *F. graminearum*) and DON content on 4 different wheat samples for Prekmurje region in years 2012 and 2013 (y-DON µg/kg, x-grain infested with FC+FG in %)
Slika 40: Korelacija med okuženostjo zrnja z FC+FG (*F. culmorum* + *F. graminearum*) in vsebnostjo DON pri 4 različnih sortah pšenice v Prekmurje za leti 2012 in 2013 (y-DON µg/kg, x-zrna okužena s FC+FG v %)

By combining the data from both locations (Gorenjska and Prekmurje) and both years (2012 and 2013) as shown in Figure 41, the descriptive statistics shows that correlation coeficient is very high (r=0.93) with coeficient of determination R^2=0.87 (y=172.25x+16.2). In ECO production very high correlation (0.93) between FC+FG infection and DON content was revealed, unlike in the integrated production, where such statistics shows smaller correlation (0.71). Furthermore, the coeficient of determination emphasizes that the correlation function (y=172.25x+16.2) confirms FC+FG infecton as the dominant factor in contamination with DON in ECO production, unlike integrated production where the influence of FC+FG inection is not very significant due to small coefficient of determination (0.51).

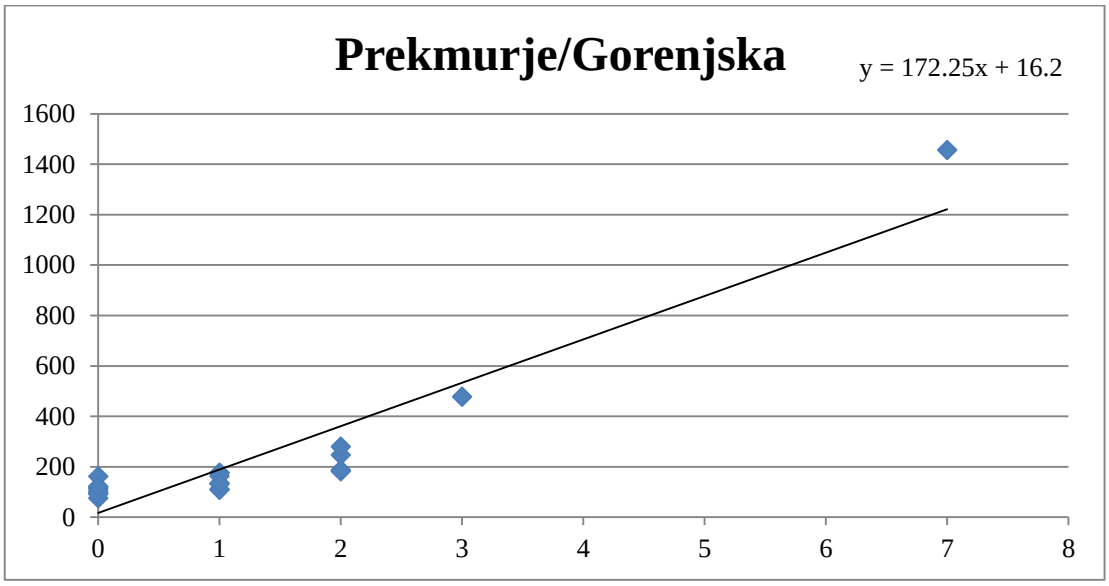


Figure 41: Correlation between grains infection with FC+FG (*F. culmorum* + *F. graminearum*) and DON content on wheat samples for both locations Prekmurje/Gorenjska, in years 2012 and 2013 (y-DON µg/kg, x-grain infested with FC+FG in %)
Slika 41: Korelacija med okuženostjo zrnja z FC+FG (*F. culmorum* + *F. graminearum*) in vsebnostjo DON pri vzorcih pšenice iz obeh lokacij, Prekmurje/Gorenjska za leti 2012 in 2013 (y-DON µg/kg, x-zrna okužena s FC+FG v %)

We have also conducted a statistical analysis in order to draw a comparison of the presence of this mycotoxin among ecologically and integrally produced wheat, by using two-tailed t-test of Type 3 for both types of wheat production, separately done for FC+FG and DON data.

Table 14: Two-tailed t-test of Type 3 for both systems of wheat production (integrated and ecological) based on FC+FG data (%) (data for both locations and years), with significance level α = 0.05
Preglednica 14: Dvostranski t-test tipa III za oba načina pridelave (integirano in ekološko) ki temelji na FC+FG podatkih (%) (za obe lokaciji in leta) s stopnjo signifikantnosti α = 0.05

Location	Rakičan	Prekmurje	Jablje	Gorenjska	Rakičan/Jablje	Prekmurje/Gorenjska
Mean (%)	2.45	1.25	7.08	1.14	4.76	1.18
Std Dev	1.94	0.71	7.08	1.92	5.66	1.56
df	44		50		96	
p-value	0.006*		0.00002*		0.000004*	

Std Dev-Standard Deviation; df-degrees of freedom; *-statistical significant difference
Std Dev-Standardna deviacija; df-stopnje prostosti; *-statistično značilne razlike

Table 15: Two-tailed t-test of Type 3 for both systems of wheat production (integrated and ecological) based on DON (µg/kg) data (data for both locations and years), with significance level α = 0.05
Preglednica 15: Dvostranski t-test tipa III za oba načina pridelave (integirano in ekološko), ki temelji na DON (µg/kg) podatkih (za obe lokaciji in leta) s stopnjo signifikantnosti α = 0.05

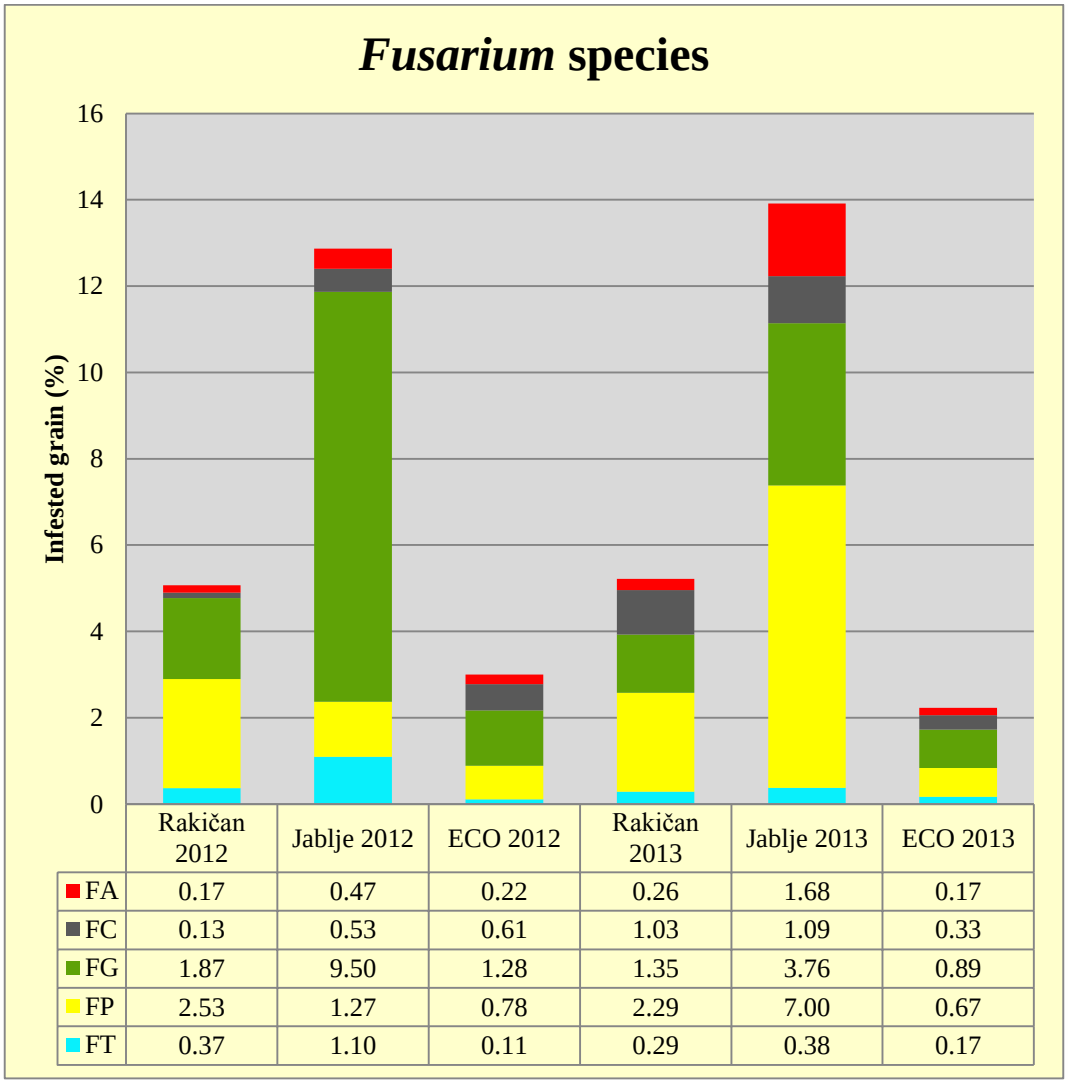
Location	Rakičan	Prekmurje	Jablje	Gorenjska	Rakičan/Jablje	Prekmurje/Gorenjska
Mean (µg/kg)	130.03	158.38	699.13	254.86	414.58	219.77
Std Dev	162.75	48.69	764.06	360.93	618.96	289.30
df	44		50		96	
p-value	0.374		0.007*		0.042*	

Std Dev-Standard Deviation; df-degrees of freedom; *-statistical significant difference
Std Dev-Standardna deviacija; df-stopnje prostosti; *-statistično značilne razlike

The statistical analysis over FC+FG data (Table 14) from integrated and ECO production reveals statistically significant difference among both locations, as well as in combined data accross both locations. In addition to Table 14, statistical analysis were conducted comparing ECO vs integrally produced wheat for both years, location by location with data for DON content (Table 15). The Gorenjska region when compared to Jablje overall (2012-2013) showed statistically significant diference between the means of DON contamination, at p=0.007 (estimated by Two-tail t-test of type 3) which is far lower then the significant level of α=0.05. Overall, the combined data from both locations and years shows that there is a statistically significant difference confirming that the wheat from integrated production is more infected with fusariosis than the wheat from ECO production and consequently has higher DON content.

A graphical representation of the average grain infections in all analyzed wheat varieties from both the integrated and ecological production during the two year experimental period presents a more clear view of the difference in the degree of infection caused by *Fusairum* spp. in wheat.

The wheat varities grown in Jablje during the summers of 2012 and 2013 were averagely more infected with *Fusarium* spp. than the varieties grown in Rakičan. The total grain infection by *Fusarium* spp.in Jablje was 12.9% during 2012, and 13.9% in 2013, while the grain infection in Rakičan during the same years was 5.1%, and 5.2%, respectively. The *Fusarium* species, their composition, and their percentage in the infected grains varied according to location, and years (Figure 42).



	Rakičan 2012	Jablje 2012	ECO 2012	Rakičan 2013	Jablje 2013	ECO 2013
FA	0.17	0.47	0.22	0.26	1.68	0.17
FC	0.13	0.53	0.61	1.03	1.09	0.33
FG	1.87	9.50	1.28	1.35	3.76	0.89
FP	2.53	1.27	0.78	2.29	7.00	0.67
FT	0.37	1.10	0.11	0.29	0.38	0.17

Figure 42: The average infection of all the grain samples (varieties) of wheat in Rakičan, Jablje and in ecologically produced wheat (ECO) with various species of *Fusarium* (FA-*F. avenaceum*, FC-*F. culmorum*, FG-*F. graminearum*, FP-*F. poae*, FT-*F. tricinctum*) in the years 2012 and 2013
Slika 42: Povprečna okuženost zrnja vseh vzorcev (sort) pšenice v Rakičanu, Jabljah in v ekološki pridelavi (ECO) z različnimi vrstami gliv iz rodu *Fusarium* (FA-*F. avenaceum*, FC-*F. culmorum*, FG-*F. graminearum*, FP-*F. poae*, FT-*F. tricinctum*) v letih 2012 in 2013

The average grain infection from the ecologically produced wheat (18 samples) during 2012 and 2013 was notably smaller than the infection in integrated wheat production. The total percentage of infection during 2012 was 3% in average, and 2.2% in 2013. If we look at the percentage of *F. graminearum* and *F. culmorum* infections, which are the potential producers of the mycotoxin DON, during the growing season of 2012 and 2013 their mean percentage of grain infection was 10%, and 4.9%, respectively in Jablje but notably less in Rakičan, where 2% and 2.4% were observed. The percentual representation of the aforementioned species in the ecologically produced wheat was 1.9% (2012) and 1.2% (2013). The notably small infection percentage in the ecologically produced wheat was due to the application of broader crop rotation, unlike the case in out test plots where only maize-wheat rotation was used. We also have to mention that the majority of ecological wheat producers didn't have maize in their crop rotation, but only sow it every 3-4 years. The residues of infected maize represent larger infection potential for the subsequently sowed wheat, than other wheats. Our results are consistent with the research of Birzele et al. (2002) from Germany.

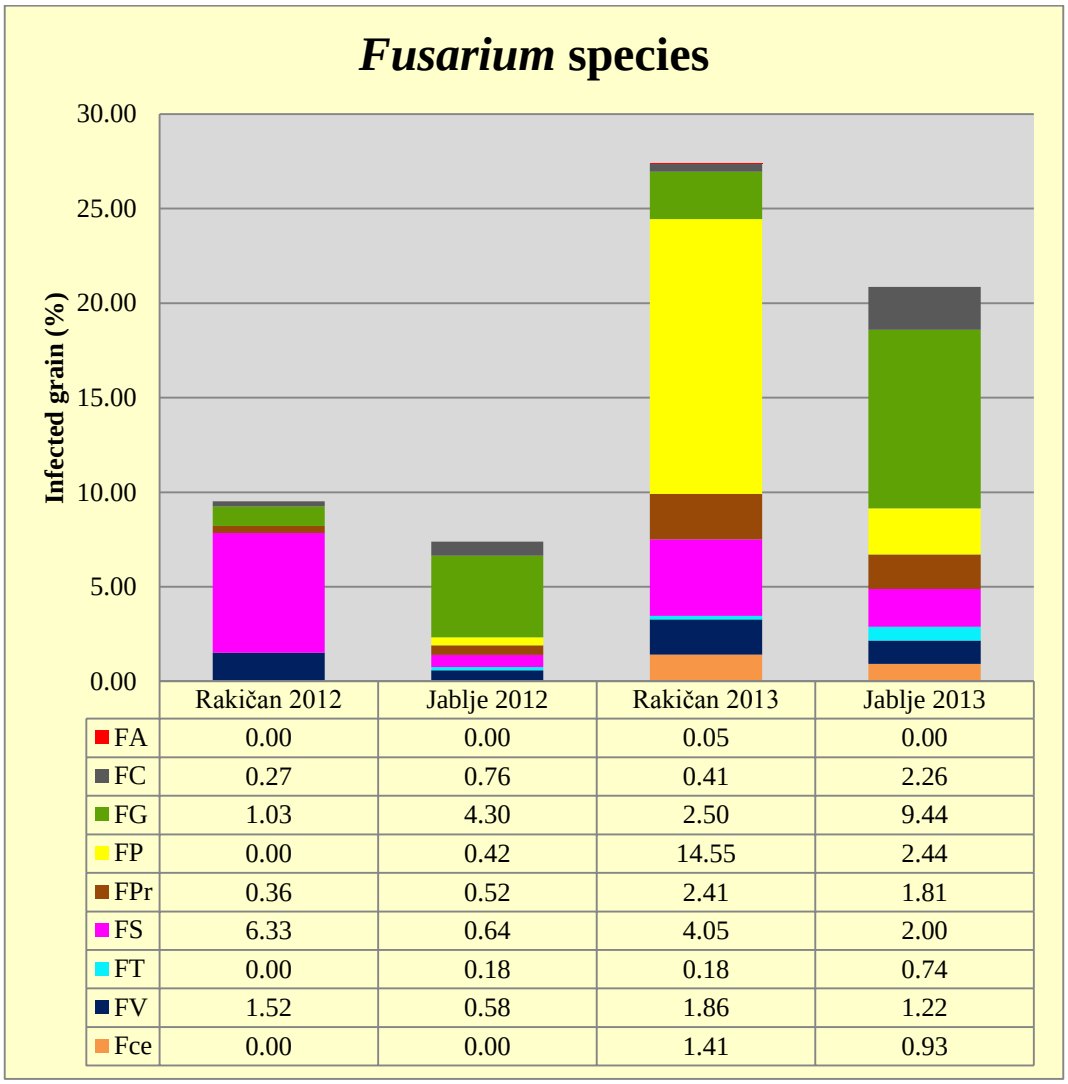
4.4 *Fusarium* spp. INCIDENCE AND DON CONTENT FOR MAIZE

Apart from the analyses on wheat from integrated and ecological productions, in our experiment maize was also included, on which we have likewise conducted the appropriate analyses. Maize, as opposed to small grain cereals which are primarily infected by *F. graminearum*, *F. culmorum*, *F. avenaceum*, *F. poae* as well as *F. cerealis* (Bottalico and Perrone, 2002), is most frequently infected by significantly larger number of *Fusarium* species (Logrieco et al., 2002). Such a variety of species present in maize plants indicates existence of inter-species interaction (Reid et al., 1999).

The most frequent contaminators of maize and its silage are the *Fusarium* mycotoxins, like the trichothecenes, of which deoxynivalenol (DON) is the most common, but also zearalenone (ZEA) and fumonisins (FUM) are frequently detected. The main producers of deoxynivalenol are *F. culmorum*, *F. graminearum* and *F. cerealis* (Logrieco et al., 2002).

Thus, to determine the infection levels caused by the *Fusarium* species, and at the same time determine DON contamination in maize, we have conducted the appropriate phytopathological analysis on a number of different hybrids. The tests were done in the same manner as in the case of wheat, during the same time interval of two-years, i.e. in 2012, on both locations (Rakičan and Jablje) a total of 33 different maize hybrids were sown, while in 2013 a total of 21 different maize hybrids were sown in Rakičan (Annex E), and 27 in Jablje (Annex F).
The average infection rate of maize grain by all of the detected *Fusarium* spp. was approximately ⅔ lower in 2012 than in 2013 in both locations. In Jablje it reached 7.4%, while

in Rakičan 9.5%. In 2013 in Rakičan *F. poae* was more prominent, infecting 14.6% of the grains. During both years, the infection rate of *F. subglutinans* was higher (6.3 and 4.1%) in Rakičan than in Jablje (0.6 and 2%) (Figure 43). Both of these fungi are not the potential producers of DON.



	Rakičan 2012	Jablje 2012	Rakičan 2013	Jablje 2013
FA	0.00	0.00	0.05	0.00
FC	0.27	0.76	0.41	2.26
FG	1.03	4.30	2.50	9.44
FP	0.00	0.42	14.55	2.44
FPr	0.36	0.52	2.41	1.81
FS	6.33	0.64	4.05	2.00
FT	0.00	0.18	0.18	0.74
FV	1.52	0.58	1.86	1.22
Fce	0.00	0.00	1.41	0.93

Figure 43: The average infection rate of all the grain samples (hybrids) of maize in Rakičan and Jablje with various species of *Fusarium* (FA-*Fusarium avenaceum*; FC-*Fusarium culmorum*; FG-*Fusarium graminearum*; FP-*Fusarium poae*; FPr-*Fusarium proliferatum*; FS-*Fusarium subglutinans*; FT-*Fusarium tricinctum*; FV-*Fusarium verticillioides*; Fce-*Fusarium cerealis*) in the years 2012 and 2013
Slika 43: Povprečna okuženost zrnja vseh vzorcev (hibridov) koruze v Rakičanu in Jabljah z različnimi vrstami gliv iz rodu *Fusarium* (FA-*Fusarium avenaceum*; FC-*Fusarium culmorum*; FG-*Fusarium graminearum*; FP-*Fusarium poae*; FPr-*Fusarium proliferatum*; FS-*Fusarium subglutinans*; FT-*Fusarium tricinctum*; FV-*Fusarium verticillioides*; Fce-*Fusarium cerealis*) v letih 2012 in 2013

Although the overall average infection rate with *Fusarium* spp. for the two years period was higher in Rakičan, this was not reflected in the levels of DON content. The reason for this is that maize in Rakičan was much less infected with *F. culmorum* and *F. graminearum*, the potential producers of DON, than maize in Jablje. The average infection rate with these fungi was 5.1% (2012) and 11.7% (2013) in Jablje, while in Rakičan it was 1.3% (2012) and 2.9% (2013). This has indirectly affected the average contamination of DON, which was 788 µg/kg (2012) and 987 µg/kg (2013) in Jablje, while it was 130 µg/kg (2012) and 160 µg/kg (2013) in Rakičan (Figure 44).

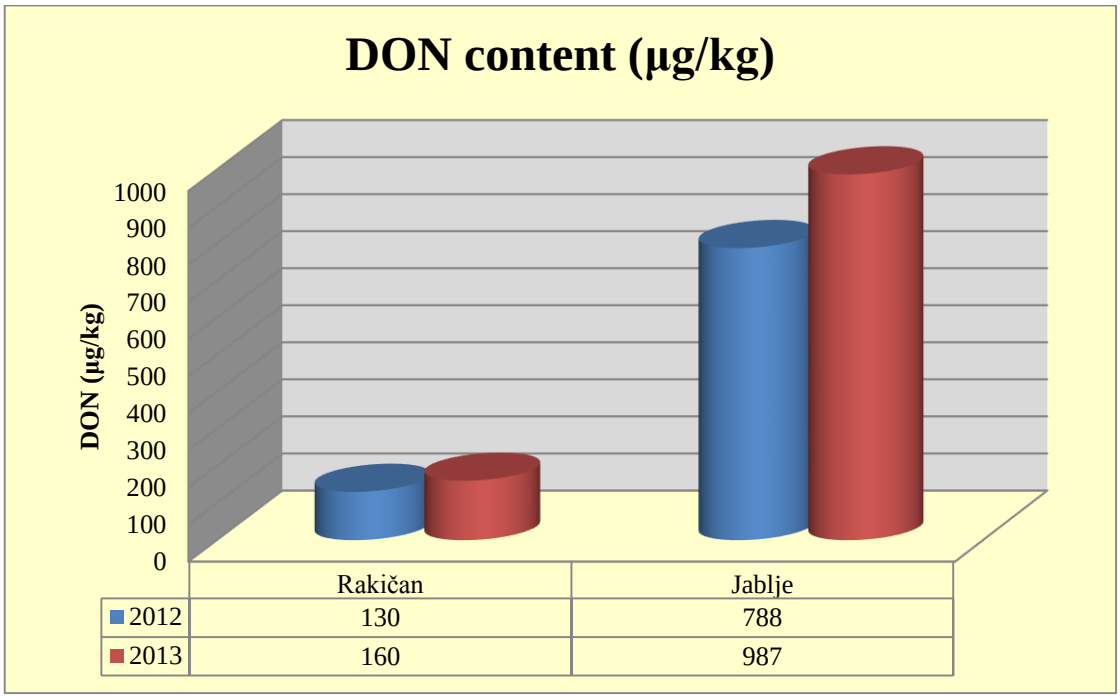


Figure 44: Average amount of DON content of all the grain samples (hybrids) of maize in the years 2012 and 2013
Slika 44: Povprečna vsebnost DON vseh vzorcev (hibridov) koruze v letih 2012 in 2013

Out of all the sown maize hybrids, only 15 displayed results which were consistent, i.e. repetitive, during the two-year trial period, in both locations. Apart from the detailed analyses for *Fusarium* spp. infection on our samples we also conducted a ELISA test for DON content (Table 16 and Table 17). The results obtained gave us a more detailed insight of the DON contamination in grains, which resulted from infection with *Fusarium* spp. that is closely linked to the influence of the climatic factor. The results of the analyses were also compared with analysed parameters in the integrally produced wheat.

From data obtained from the analysed maize samples collected in Rakičan in 2012, we determined an average incidence 0.2% for *F. culmorum*, while the average incidence for *F. graminearum* was 0.9% (Table 16 and Figure 45). In 2013, on the other hand, the data displayed an average incidence 0.4% for *F. culmorum*, while the incidence for *F. graminearum* 2.5% (Table 17 and Figure 46).

Table 16: *Fusarium* spp. incidence (%) and DON content (µg/kg) in 15 different hybrids of maize from Rakičan in the year 2012
Preglednica 16: Zastopanost *Fusarium* spp. (%) in vsebnost DON (µg/kg) v 15 različnih hibridih koruze iz Rakičana v letu 2012

Maize hybrid	Rakičan – 2012								
	FC	FG	FP	FPr	FS	FT	FV	Σ	DON µg/kg
BC 244	0	0	0	0	0	0	0	0	53
BC 416	0	1	0	0	0	0	1	2	65
DANUBIO	0	1	0	0	7	0	11	19	121
DKC 4590	0	2	0	3	16	0	0	21	148
FUTURIXX	0	1	0	5	18	0	0	24	115
NK LUCIUS	0	0	0	0	0	0	1	1	99
NK TIMIC	0	1	0	0	4	0	3	8	128
NS 288	0	1	0	0	0	0	0	1	131
P9175	0	1	0	0	8	0	0	9	94
P9400	1	1	0	1	11	0	0	14	116
P9494	0	1	0	0	5	0	0	6	107
PR38A79	1	1	0	0	5	0	3	10	129
PR38N86	0	1	0	0	3	0	0	4	134
PR38Y34	1	2	0	0	5	0	0	8	**272**
SY FLOVITA	0	0	0	0	1	0	0	1	60
Σ	3	14	0	9	83	0	19	128	1772
AVERAGE	0.2	0.9	0.0	0.6	5.5	0.0	1.3	8.5	118.1

FC-*Fusarium culmorum*; FG-*Fusarium graminearum*; FP-*Fusarium poae*; FPr-*Fusarium proliferatum*; FS-*Fusarium subglutinans*; FT-*Fusarium tricinctum*; FV-*Fusarium verticillioides*; Σ-Sum; DON-Deoxynivalenol; **bold numbers**: positive samples i.e. mycotoxin concentration above 200 µg/kg
FC-*Fusarium culmorum*; FG-*Fusarium graminearum*; FP-*Fusarium poae*; FPr-*Fusarium proliferatum*; FS-*Fusarium subglutinans*; FT-*Fusarium tricinctum*; FV-*Fusarium verticillioides*; Σ-vsota; DON-deoksinivalenol; **poudarjene številke**: pozitivni vzorci oz. koncentracija mikotoksinov nad 200 µg/kg

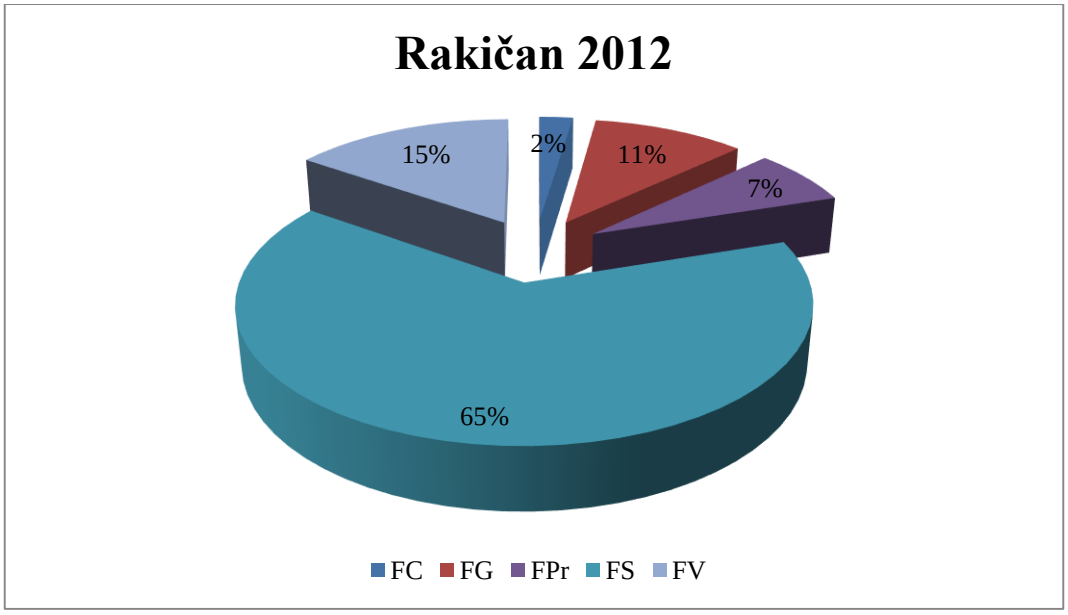


Figure 45: Percentage proportion of *Fusarium* spp. on 15 different hybrids of maize for Rakičan in the year 2012 (FC-*Fusarium culmorum*; FG-*Fusarium graminearum*; FPr-*Fusarium proliferatum*; FS-*Fusarium subglutinans*; FV-*Fusarium verticillioides*)
Slika 45: Odstotni delež posameznih *Fusarium* vrst na 15 različnih hibridih koruze iz Rakičana v letu 2012 (FC-*Fusarium culmorum*; FG-*Fusarium graminearum*; FPr-*Fusarium proliferatum*; FS-*Fusarium subglutinans*; FV-*Fusarium verticillioides*)

Based on our records of the tested maize samples from the Rakičan during the two-year trial period, we have registered a minimal level of infection from both *Fusarium* causers of DON producers, *F. graminearum* and *F. culmorum*. The results from the ELISA test have confirmed that the contamination levels for this mycotoxin in our maize samples were very low. The results have shown that in Rakičan, during 2012, only 1 out of 15 analyzed samples from this region exceeded the prescribed limit of 200 µg/kg, with a maximum DON content of 272 µg/kg, while the total mean value for DON in 2012 was 118 µg/kg (Table 16). On the other hand, in 2013, 3 out of 15 samples exceeded the 200 µg/kg minimal threshold, with a maximally registered DON content of 354 µg/kg, while the total mean value for DON in 2013 was 163 µg/kg (Table 17). During the two-year trial period the 1250 µg/kg limit established by the European Union (EU) was never exceeded.

Table 17: *Fusarium* spp. incidence (%) and DON content (µg/kg) in 15 different hybrids of maize from Rakičan in the year 2013
Preglednica 17: Zastopanost *Fusarium* spp. (%) in vsebnost DON (µg/kg) v 15 različnih hibridih koruze iz Rakičana v letu 2013

Maize hybrid	Rakičan – 2013										
	FC	FG	FP	FPr	FS	FT	FV	FCe	FA	Σ	DON µg/kg
BC 244	0	1	11	4	0	0	0	0	0	16	66
BC 416	1	1	1	0	2	2	13	8	0	28	84
DANUBIO	0	2	10	0	0	0	3	1	0	16	127
DKC 4590	1	2	22	2	1	1	1	0	0	30	144
FUTURIXX	0	3	31	0	2	0	3	0	0	39	163
NK LUCIUS	0	2	14	0	0	0	0	0	0	16	116
NK TIMIC	0	4	21	11	25	0	0	0	0	61	**238**
NS 288	0	2	1	3	2	0	0	4	0	12	77
P9175	0	3	5	0	1	0	1	0	0	10	139
P9400	0	2	46	2	2	0	0	0	0	52	144
P9494	1	2	14	4	15	0	3	4	0	43	169
PR38A79	2	5	29	1	10	1	0	0	0	48	**335**
PR38N86	0	2	0	0	6	0	0	0	0	8	160
PR38Y34	0	2	2	0	0	0	0	0	1	5	122
SY FLOVITA	1	5	45	17	4	0	1	1	0	74	**354**
Σ	6	38	252	44	70	4	25	18	1	458	2438
AVERAGE	0.4	2.5	16.8	2.9	4.7	0.3	1.7	1.2	0.1	30.5	162.5

FC-*Fusarium culmorum*; FG-*Fusarium graminearum*; FP-*Fusarium poae*; FPr-*Fusarium proliferatum*; FS-*Fusarium subglutinans*; FT-*Fusarium tricinctum*; FV-*Fusarium verticillioides*; FCe-*Fusarium cerealis*; FA-*Fusarium avenaceum*; Σ-Sum; DON-Deoxynivalenol; **bold numbers**: positive samples i.e. mycotoxin concentration above 200 µg/kg
FC-*Fusarium culmorum*; FG-*Fusarium graminearum*; FP-*Fusarium poae*; FPr-*Fusarium proliferatum*; FS-*Fusarium subglutinans*; FT-*Fusarium tricinctum*; FV-*Fusarium verticillioides*; FCe-*Fusarium cerealis*; FA-*Fusarium avenaceum*; Σ-vsota; DON-deoksinivalenol; **poudarjene številke**: pozitivni vzorci oz. koncentracija mikotoksinov nad 200 µg/kg

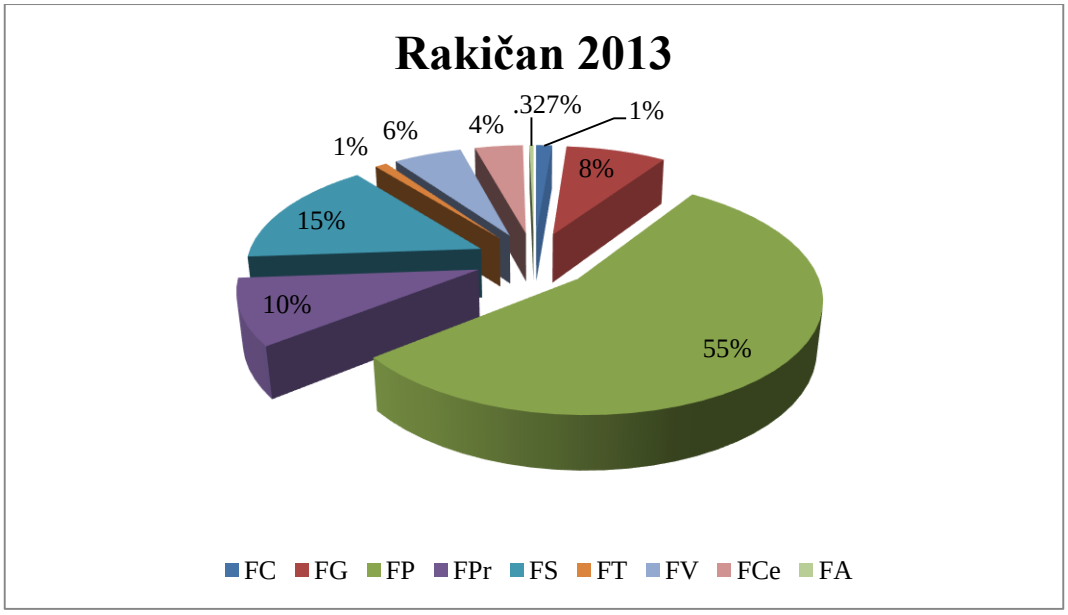


Figure 46: Percentage proportion of *Fusarium* spp. on 15 different hybrids of maize for Rakičan in the year 2013 (FC-*Fusarium culmorum*; FG-*Fusarium graminearum*; FP-*Fusarium poae*; FPr-*Fusarium proliferatum*; FS-*Fusarium subglutinans*; FT-*Fusarium tricinctum*; FV-*Fusarium verticillioides*; FCe-*Fusarium cerealis*; FA-*Fusarium avenaceum*)
Slika 46: Odstotni delež posameznih *Fusarium* vrst na 15 različnih hibridih koruze iz Rakičana v letu 2013 (FC-*Fusarium culmorum*; FG-*Fusarium graminearum*; FP-*Fusarium poae*; FPr-*Fusarium proliferatum*; FS-*Fusarium subglutinans*; FT-*Fusarium tricinctum*; FV-*Fusarium verticillioides*; FCe-*Fusarium cerealis*; FA-*Fusarium avenaceum*)

In the same manner, we have also simultaneously calculated both the percentile presence of all detected *Fusarium* species as well as the amount of DON content in the maize samples from the other test location - Jablje (see Annex F).

Like in the first location, we obtained samples from the same 15 maize hybrids on which *Fusarium* infection was determined via the standard phytopathological methods, and conducted an ELISA - DON test for those samples. The tests were performed during the same two-year time interval, 2012-2013 (Table 18 and Table 19). We have observed that in these samples the incidence of *Fusarium* infection was also noticeably higher than in the samples from the other test location.

The data obtained from the analyzed maize samples from Jablje in 2012 showed an average incidence 0.7% for *F. culmorum* and 4.5% for *F. graminearum* (Table 18 and Figure 47). On the other hand, data from the following year 2013, revealed that the incidence of *F. culmorum* was higher than in the previous year, with an average of 2.4%. The same situation was noted

for *F. graminearum*, where the average incidence increased to 10.1% (Table 19 and Figure 48).

Table 18: *Fusarium* spp. incidence (%) and DON content (µg/kg) in 15 different hybrids of maize from Jablje in the year 2012
Preglednica 18: Zastopanost *Fusarium* spp. (%) in vsebnost DON (µg/kg) v 15 različnih hibridih koruze iz Jabelj v letu 2012

Maize hybrid	Jablje – 2012								
	FC	FG	FP	FPr	FS	FT	FV	Σ	DON µg/kg
BC 244	0	3	0	3	0	1	3	10	**413**
BC 416	0	8	0	0	1	0	4	13	***1440**
DANUBIO	0	2	0	0	0	1	0	3	**209**
DKC 4590	0	2	0	3	0	0	0	5	**336**
FUTURIXX	0	5	0	0	0	0	4	9	**931**
NK LUCIUS	0	4	0	0	0	1	0	5	**423**
NK TIMIC	6	15	2	0	1	0	0	24	***3561**
NS 288	0	3	0	0	0	0	0	3	**240**
P9175	0	5	0	0	1	0	0	6	**952**
P9400	0	2	3	0	0	0	0	5	**269**
P9494	2	8	2	1	1	0	0	14	***1625**
PR38A79	0	1	0	0	0	0	1	2	**203**
PR38N86	0	5	0	2	0	0	0	7	**601**
PR38Y34	0	2	1	0	0	0	0	3	125
SY FLOVITA	3	3	0	0	4	0	0	10	**987**
Σ	11	68	8	9	8	3	12	119	12315
AVERAGE	0.7	4.5	0.5	0.6	0.5	0.2	0.8	7.9	821.0

FC-*Fusarium culmorum*; FG-*Fusarium graminearum*; FP-*Fusarium poae*; FPr-*Fusarium proliferatum*; FS-*Fusarium subglutinans*; FT-*Fusarium tricinctum*; FV-*Fusarium verticillioides*; Σ-Sum; DON-Deoxynivalenol; **bold numbers**: positive samples i.e. mycotoxin concentration above 200 µg/kg; *-samples that exceed the limit of 1250 µg/kg imposed by the EU for DON
FC-*Fusarium culmorum*; FG-*Fusarium graminearum*; FP-*Fusarium poae*; FPr-*Fusarium proliferatum*; FS-*Fusarium subglutinans*; FT-*Fusarium tricinctum*; FV-*Fusarium verticillioides*; Σ-vsota; DON-deoksinivalenol; **poudarjene številke**: pozitivni vzorci oz. koncentracija mikotoksinov nad 200 µg/kg; *- vzorci ki presegajo mejo 1250 µg/kg uvedeno z EU za DON

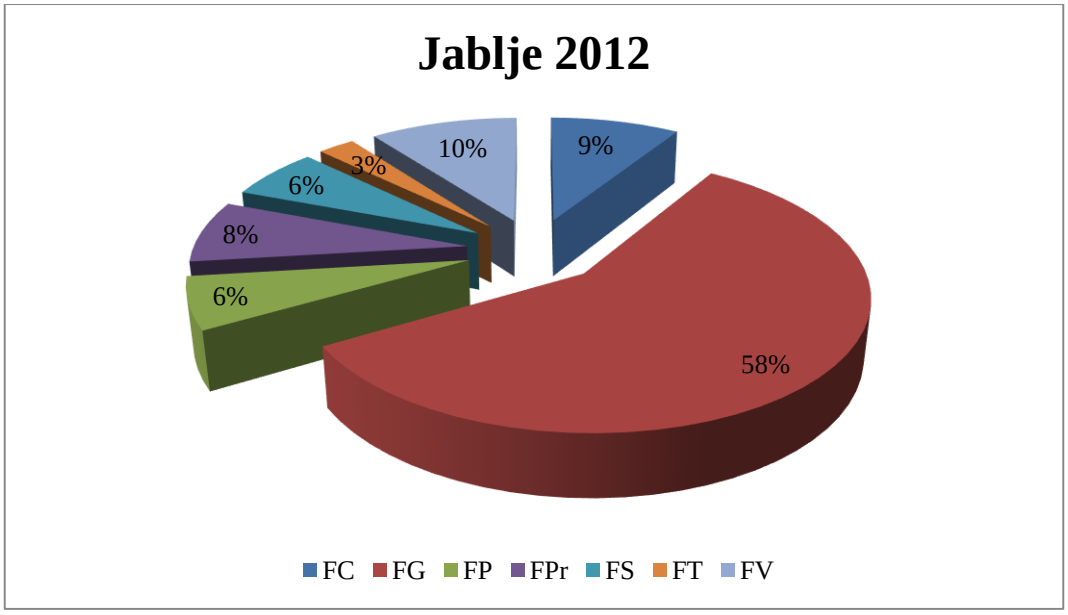


Figure 47: Percentage proportion of *Fusarium* spp. on 15 different hybrids of maize for Jablje in the year 2012 (FC-*Fusarium culmorum*; FG-*Fusarium graminearum*; FP-*Fusarium poae*; FPr-*Fusarium proliferatum*; FS-*Fusarium subglutinans*; FT-*Fusarium tricinctum*; FV-*Fusarium verticillioides*)
Slika 47: Odstotni delež posameznih *Fusarium* vrst na 15 različnih hibridih koruze iz Jabelj v letu 2012 (FC-*Fusarium culmorum*; FG-*Fusarium graminearum*; FP-*Fusarium poae*; FPr-*Fusarium proliferatum*; FS-*Fusarium subglutinans*; FT-*Fusarium tricinctum*; FV-*Fusarium verticillioides*)

ELISA test for DON presence in the maize samples from Jablje, collected during the two-year trial period, showed a much higher contamination level when compared to the levels detected in the samples obtained from Rakičan, during the same period. According to the resultsfrom 2012, high contamination with DON was detected in Jablje, since 14 out of 15 samples were discovered to contain mycotoxin in higher amounts than the established limit of 200 µg/kg, the maximal DON content being 3561 µg/kg, while the total mean value for DON content was 821 µg/kg (Table 18). In 2013, all 15 hybrids were detected to contain DON in amounts which exceeded the 200 µg/kg minimal threshold, with a maximal DON content of 7645 µg/kg, while the total mean value for DON in 2013 was 1028 µg/kg (Table 19). The 1250 µg/kg limit imposed by the European Union (EU) was exceeded three times in 2012 in Jablje, while in 2013, there were two hybrids exceeding the limit for DON contnet.

Table 19: *Fusarium* spp. incidence (%) and DON content (µg/kg) in 15 different hybrids of maize from Jablje in the year 2013
Preglednica 19: Zastopanost *Fusarium* spp. (%) in vsebnost DON (µg/kg) v 15 različnih hibridih koruze iz Jabelj v letu 2013

Maize hybrid	Jablje – 2013									
	FC	FG	FP	FPr	FS	FT	FV	FCe	Σ	DON µg/kg
BC 244	1	3	0	8	1	2	7	0	22	**299**
BC 416	15	69	0	0	2	2	4	6	98	***7645**
DANUBIO	1	10	1	0	0	1	0	2	15	**1084**
DKC 4590	0	4	7	9	0	1	0	0	21	**282**
FUTURIXX	1	3	5	0	1	0	6	0	16	**305**
NK LUCIUS	2	3	3	0	1	2	0	0	11	**233**
NK TIMIC	3	10	5	2	5	0	0	0	25	**1007**
NS 288	2	5	1	0	0	0	0	2	10	**453**
P9175	0	4	0	0	2	1	0	0	7	**397**
P9400	0	6	8	0	0	0	0	0	14	**440**
P9494	6	12	2	3	4	0	0	3	30	***1356**
PR38A79	2	3	6	0	0	1	3	0	15	**243**
PR38N86	2	12	0	5	2	0	0	0	21	**1199**
PR38Y34	1	4	0	0	0	0	0	1	6	**242**
SY FLOVITA	0	3	5	2	7	0	0	0	17	**237**
Σ	36	151	43	29	25	10	20	14	328	15422
AVERAGE	2.4	10.1	2.9	1.9	1.7	0.7	1.3	0.9	21.9	1028.1

FC-*Fusarium culmorum*; FG-*Fusarium graminearum*; FP-*Fusarium poae*; FPr-*Fusarium proliferatum*; FS-*Fusarium subglutinans*; FT-*Fusarium tricinctum*; FV-*Fusarium verticillioides*; FCe-*Fusarium cerealis*; Σ-Sum; DON-Deoxynivalenol; **bold numbers**: positive samples i.e. mycotoxin concentration above 200 µg/kg; *-samples that exceed the limit of 1250 µg/kg imposed by the EU for DON
FC-*Fusarium culmorum*; FG-*Fusarium graminearum*; FP-*Fusarium poae*; FPr-*Fusarium proliferatum*; FS-*Fusarium subglutinans*; FT-*Fusarium tricinctum*; FV-*Fusarium verticillioides*; FCe-*Fusarium cerealis*; Σ-vsota; DON-deoksinivalenol; **poudarjene številke**: pozitivni vzorci oz. koncentracija mikotoksinov nad 200 µg/kg; *- vzorci ki presegajo mejo 1250 µg/kg uvedeno z EU za DON

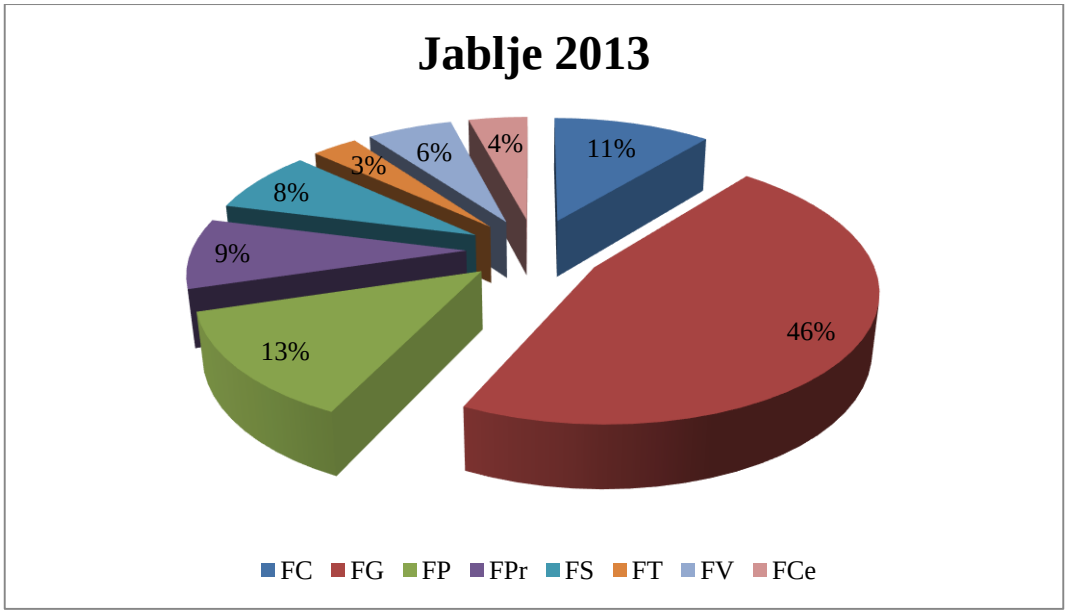


Figure 48: Percentage proportion of *Fusarium* spp. on 15 different hybrids of maize for Jablje in the year 2013 (FC-*Fusarium culmorum*; FG-*Fusarium graminearum*; FP-*Fusarium poae*; FPr-*Fusarium proliferatum*; FS-*Fusarium subglutinans*; FT-*Fusarium tricinctum*; FV-*Fusarium verticillioides*; FCe-*Fusarium cerealis*)
Slika 48: Odstotni delež posameznih *Fusarium* vrst na 15 različnih hibridih koruze iz Jabelj v letu 2013 (FC-*Fusarium culmorum*; FG-*Fusarium graminearum*; FP-*Fusarium poae*; FPr-*Fusarium proliferatum*; FS-*Fusarium subglutinans*; FT-*Fusarium tricinctum*; FV-*Fusarium verticillioides*; FCe-*Fusarium cerealis*)

The increased content of DON in both maize and wheat is owed mainly to the increased incidence of infection caused by the *Fusarium* DON producers, which were directly correlated to higher contamination levels in both maize and wheat, as presented in the correlation test below.

From Figure 49, a correlation r=0.89 and R^2=0.79 can be observed between FC+FG infection and DON content considering data from Rakičan. The correlation function (y=41.268x + 56.421) shows that FC+FG infection has high impact on DON contamination, but not the highest, since other factors also influence DON contamination, as can be observed from high value of the second parameter in the correlation function (56.421).

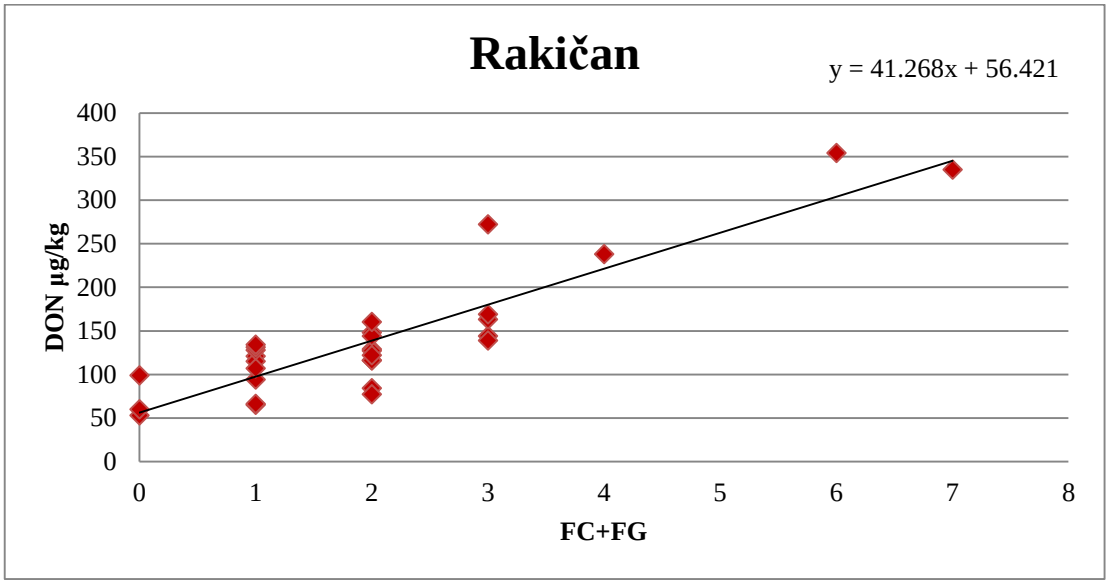


Figure 49: Correlation between grains infection with FC+FG (*F. culmorum* + *F. graminearum*) and DON content on 15 different hybrids of maize for Rakičan in the years 2012 and 2013 (y-DON µg/kg, x-grain infested with FC+FG in %)
Slika 49: Korelacija med okuženostjo zrnja z FC+FG (*F. culmorum* + *F. graminearum*) in vsebnostjo DON pri 15 različnih hibridih koruze v Rakičanu za leti 2012 in 2013 (y-DON µg/kg, x-zrna okužena s FC+FG v %)

From Figure 50 it can be seen that high correlation, with r=0.96 and R^2=0.93, exists between FC+FG infection and DON content also if considering data from Jablje. In the correlation function (y=92.866x + 101.15), as previously noted in Rakičan, the second parameter has higher value than parameter FC+FG, which leads to the conclusion that beside FC+FG other factors influence the DON contamination as well.

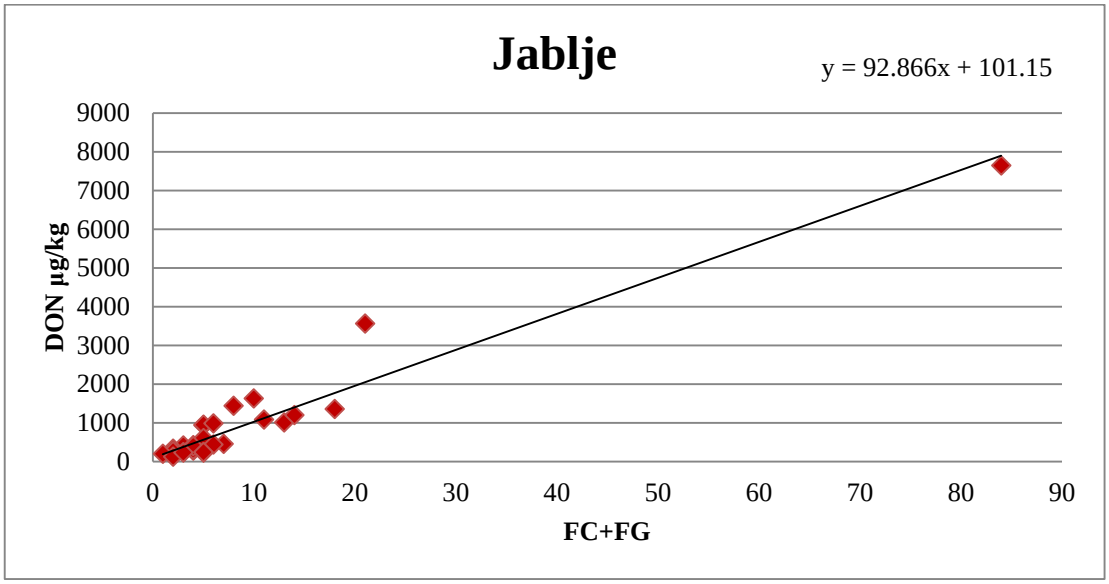


Figure 50: Correlation between grains infection with FC+FG (*F. culmorum* + *F. graminearum*) and DON content on 15 different hybrids of maize for Jablje in the years 2012 and 2013 (y-DON µg/kg, x-grain infested with FC+FG in %)
Slika 50: Korelacija med okuženostjo zrnja z FC+FG (*F. culmorum* + *F. graminearum*) in vsebnostjo DON pri 15 različnih hibridih koruze v Jablje za leti 2012 in 2013 (y-DON µg/kg, x-zrna okužena s FC+FG v %)

Finally, by combining the data from both locations (Rakičan and Jablje) and both years (2012 and 2013), as shown in Figure 51, the descriptive statistics show very high correlation (r=0.96) with correlation function y=94.47x+17.586, where the coefficient of determination (R^2) is 0.93. The correlation function shows that overall FC+FG infection is the most important factor influencing DON contamination, due to the hight value of the parameter of this factor.

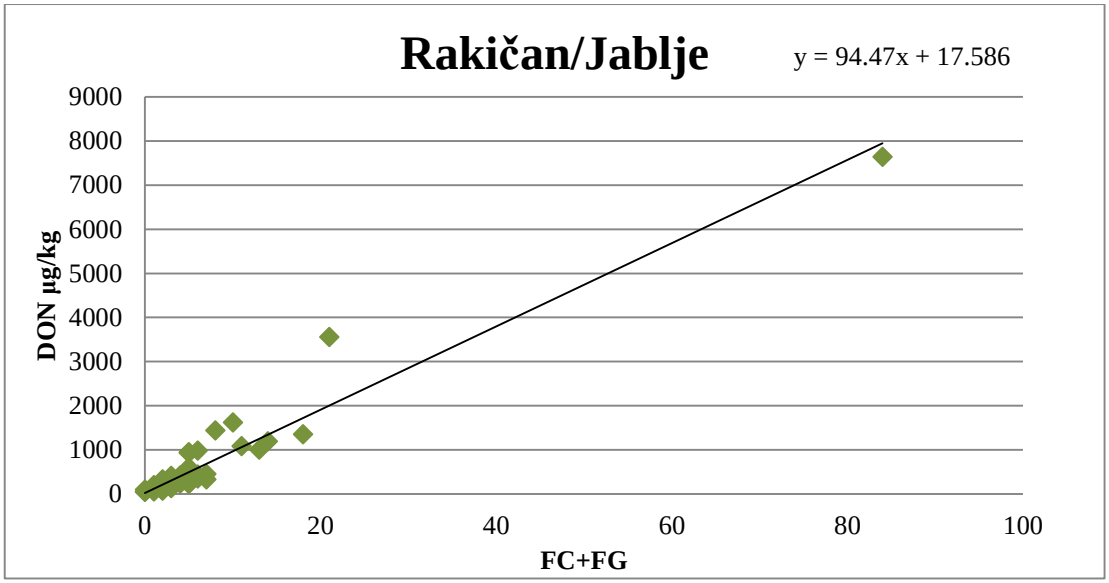


Figure 51: Correlation between grains infection with FC+FG (*F. culmorum* + *F. graminearum*) and DON content on 15 different hybrids of maize for both locations Rakičan/Jablje in the years 2012 and 2013 (y-DON µg/kg, x-grain infested with FC+FG in %)
Slika 51: Korelacija med okuženostjo zrnja z FC+FG (*F. culmorum* + *F. graminearum*) in vsebnostjo DON pri 15 različnih hibridih koruze za obe lokaciji Rakičan/Jablje za leti 2012 in 2013 (y-DON µg/kg, x-zrna okužena s FC+FG v %)

Furthermore, by comparison of the data from the ELISA - DON test for maize samples from Rakičan and Jablje in 2012 and 2013 and the data obtained for wheat samples originating from integrated production from the same regions in the two-year period, we have also determined the difference in percentile contamination between both crops.

Table 20: Two-tailed t-test of Type 3 for both wheat and maize, based on DON (µg/kg) contamination data (data for both locations and years), with significance level $\alpha = 0.05$
Preglednica 20: Dvostranski t-test tipa III za žito in koruzo, ki temelji na podatkih o kontaminiranosti z DON (µg/kg) (podatki za obe lokaciji in leti) s stopnjo signifikantnosti $\alpha = 0.05$

Location	Rakičan and Jablje		Rakičan		Jablje	
Wheat/Maize	Wheat	Maize	Wheat	Maize	Wheat	Maize
Mean (µg/kg)	414.58	532.45	130.03	140.33	699.13	924.57
Std Dev	618.96	1088.36	162.75	72.58	764.06	1444.48
df	134		66		66	
p-value	0.46		0.73		0.44	

Continued

Continuation of Table 20:

Std Dev-Standard Deviation; df-degrees of freedom; *-statistically significant difference
Std Dev-Standardna deviacija; df-stopnje prostosti; *-statistično značilne razlike

The statistical analysis did not show any statistical difference between contamination of wheat and maize, neither overall for both locations and years together nor considering location by location separately (Table 20).

These results proved again that the amount of rainfall has greater impact on the infection level than temperature. However, it is difficult to infer trends from recent developments regarding high DON contamination in grains because contamination is influenced by many other factors besides environmental factors (Wegulo, 2012). High contamination levels might also be associated with factors other than climate conditions, such as mycotoxin formation (Škrbič et al., 2012), crop rotation (maize as a pre-crop for wheat) as well as with growing of highly susceptible wheat cultivars with no fungicide application (Koch et al., 2006).

5 CONCLUSIONS

We begun our tests by analyzing wheat samples from integrated production, obtained from our test locations. In this initial stage of research we have determined the percent of infection with *Fusarium* spp. in the ear of each wheat type individually, via an analysis based on a field estimate, in accordance with Stack and McMullen's method (1995). By doing so, we have obseved at the very beginning, that it was impossible to even nearly predict the mycotoxin contamination by field estimates.

By comparing the data for the average ear infection and our statistics on the weather conditions, we can confirm, based on our observations and the statistical analysis, that no correlation exists between the weather conditions and the appearance of *Fusarium* head blight. Furthermore, we have also conducted statistical analyses of the potential influence of both wheat types (awned and awnless) on the contamination level, from which it was concluded that overall, there is no statistically significant difference between both wheat types.

In addition, we have also included statistical analysis of the influence of FC+FG infection on DON content, conducted on samples from both locations and years together, as well as on samples from both locations in relation to year, and on merged samples from the two years in relation to location.

A high correlation between FC+FG infection and DON content was observed in the samples collected from both locations Rakičan and Jablje in the years 2012 and 2013. The same samples were used to determine whether there is a statistically significant difference regarding the influence of FC+FG infection on DON contamination. It was concluded that the samples collected from Rakičan in 2012 and 2013 showed a statistically significantly lower contamination when compared to the samples from Jablje (based on the data for FC+FG).

By merging samples from each location, we confirmed lower contamination in Rakičan in comparison with Jablje (based on our FC+FG data). Furthermore, the statistical analysis shows that the level of contamination between both wheat types differs only in Rakičan, where the awned wheat type showed a significantly smaller contamination when compared to the awnless wheat. In all other instances there were no significant differences observed.

When we merged data according to each year, statistical analysis revealed no significant difference between the years of 2012 and 2013 (based on FC+FG data).

According to the climatic conditions we have also expected that mycotoxin levels in Rakičan will be at their minimum. This assumption was proven by ELISA tests that determined the total mean value for DON content for 2012 to be 140 μg /kg, and 120 μg/kg for 2013 (Table 9). In addition, the 1250 μg/kg DON content limit imposed by the European Union (EU) was not exceeded in the studied samples, which again proved that DON contamination in Rakičan was at a low level during both years of the experiment.

Climatic conditions on the other test location indicated on the possibility of higher DON accumulation, which was further confirmed by ELISA test conducted on samples collected in Jablje in 2012 and 2013. The total mean value for DON in 2012 was 714 μg/kg and 684 μg/kg in 2013 (Table 11). The limit of 1250 μg/kg imposed by the European Union (EU) for DON content was exceeded in Jablje in two samples from 2012, as well as two samples from 2013 (Table 11).

We have also acquired a representative number of samples from ecologically produced wheat originating from the areas close to our test locations.

On the same samples comparisons were conducted with the purpose to determine statistically significant differences based on the two parameters, infection of wheat with FC+FG and contamination with DON. The former (FC+FG comparison), showed strong statistically sifnificant difference in all types of comparison that were conducted: Rakičan compared to Prekmurje region, Jablje compared to Gorenjska region and overall in locations with integral wheat production compared to ECO production. The latter (DON comparison), however, did not show statistically significant difference only in comparison of data from Rakičan and Prekmurje region, while on the other location and in overall comparison statistically significant difference for the mean DON content were confirmed.

In addition, we analysed correlation between the incidence of FC+FG infection and DON content in ECO production, similar as we did for integrated production. The results showed strong correlation and emphasized high importance of FC+FG infection for DON contamination.

With this analysis, we have also confirmed our hypothesis that the ecologically produced wheat is less infected by *Fusarium* spp. and consequently less contaminated with mycotoxins than wheat grown in the integrated production. The notably small infection percentage in the ecologically produced wheat was due to the fact that a larger spectre of crop rotation was used there, as opposed to the integrated production, where only maize-wheat rotation was used.

Apart from the analyses on wheat samples from integrated and ecological production, we have also analyzed maize samples. This analysis was conducted in order to determine to what extent the test areas (Rakičan - Jablje) or the climate conditions in those areas (arid-humid) affect the contamination of each corn variety.

In Rakičan minimal infection rates of both *F. culmorum* and *F. graminearum* were detected in maize samples collected in 2012, with a slight decrease in infection rate observed in 2013. The data that we collected from this region during the two-year period, apart from assisting us in concluding that there was a minimal infection rate by both *Fusarium* DON producers, also helped us to determine that mycotoxin contamination in these samples was at a very low level as confirmed by ELISA test. According to these test, the total mean value for DON content recorded in Rakičan was 118 μg/kg in 2012 (Table 16), and 163 μg/kg in 2013 (Table 17). Furthermore, the 1250 μg/kg limit for DON content imposed by the European Union (EU) was never exceeded during the two-year trial period.

We calculated also average incidence of infection and the percent proportion of all detected *Fusarium* species as well as the amount of DON content in the samples from the other test location in Jablje. The tests were conducted during the same two-year time interval from 2012 to 2013. The statisticaly significant difference in the incidence of *Fusarium* species in this location comparing to Rakičan proved that the infection level in Jablje was evidently higher. Both *Fusarium* producers of DON during the trial period exhibited a significantly higher percent infection levels in this location than maize samples from the other test location in Rakičan. This was due to the weather condition which, regardless of the crop or location, were again confirmed to be the predominant factor for *Fusarium* infection. According to ELISA results the total mean value for DON content in Jablje in 2012 was 821 μg/kg (Table 18), and 1028 μg/kg in2013 (Table 19). The 1250 μg/kg DON presence limit imposed by the European Union (EU) was exceeded three times in 2012 and two times in 2013.

In order to complete the analysis of influence of FC+FG infection for DON contamination we conducted the correlation analysis for the data obtained for maize crop. The analysis showed very high correlation between FC+FG infection and DON content, which confirms the importance of FC+FG infection for DON contamination.

Finally, by comparing overall DON contamination between wheat and maize, no statistical difference has been observed. Such results confirm the above mentioned theoretical claim that in both hosts the same *Fusarium* pathogens are responsible for DON contamination.

6 SUMMARY (POVZETEK)

6.1 SUMMARY

One of the main diseases of wheat and maize, induced by the widely spread pathogenic fungi of the *Fusarium* genus, are the *Fusarium* head blight (FHB) and the ear rots of maize (Goswami and Kistler, 2004).

FHB is a fungal disease that is considered a serious threat to worldwide grain production in a large variety of weather conditions, but also an important factor in the reduction of cereal quality as well as the decrease of yield (Ireta and Gilchrist, 1994). Its significance is mostly due to the effect of the mycotoxin-producing *Fusarium* species, which render the infected grains useless for feeding and malting purposes. There are up to 17 *Fusarium* species that can be isolated from grain cereals (Parry et al., 1995) and their representation varies according to location and climate. The weather conditions during the period of our research in 2012 and 2013 varied significantly which allowed us to obtain an exhaustive picture of the variability of *Fusarium* spp. that naturally contaminated wheat in both locations studied. Because of this variability we were able to understand better the correlation between the weather conditions and the appearance of *Fusarium* species and contamination with mycotoxin DON.

FHB is induced by a complex of *Fusarium* species, of which *F. graminearum*, *F. culmorum* and *F. avenaceum* are the most prominent ones.

The development of FHB depends on specific prerequisites. The infection occurs in two ways – by asexual or sexual spores. The former happens when rain-splashed condiospores are transported from the stem base, the latter when the ascospores are dispersed from the surface of the soil to the leaves, with the help of weather conditions, such as wind (Trail et al., 2005). For the emergence of FHB, the levels of humidity need to be high so that the ascospores can be released during anthesis (Trail et al., 2002). However, if the discharge of ascospores does not correlate to the anthesis, the severity of the infection will be diminished (Nelson et al., 1981). There are certain visual symptoms of the disease that can be easily recognized. They usually appear in the form of whitened head spikes. When circumstances that are highly suitable for the development of FHB exist, the infection might expand over the entire head and visible pink-red mycelium and conidia develop on the spikelets. Furthermore, the final stage of infection can also be observed in the kernels, which start to shrivel and lose their color until their appearance is pale-white (von der Ohe, 2010).

Fusarium pathogens induce two types of maize ear rot disease, the *Fusarium* ear rot and the *Gibberella* ear rot of maize. Species from the *F. fujicuroi* species complex, mainly *F. verticillioides* and *F. subglutinans* are responsible for the *Fusarium* ear rot of maize, while *F.graminearum* caueses ear rot, traditionally called the *Gibberella* ear rot of maize.

F. graminearum infections starts from the corn ear silks and spreads basipetally, starting from the tip and moving down to the ear base, while in the case of serious epidemics it reaches the ear peduncle as well. Perithecia will then develop and when mature produce ascospores which will further start subsequent disease cycles. The inoculum that is responsible for the contamination in subsequent crops can usually be found in the crop debris, but it could also be traced to infected corn seeds (Munkvold et al., 1997).

Visual symptoms of *Gibberella* ear rot caused by *F. graminearum* can be easily recognized, thus enabling faster diagnosis and earlier control measurements. The symptoms are visible at the corn ear where a pinkish-red fungal mycelium develops, initially appearing at the ear tip and later moving downward toward the base, seldom infecting the whole ear. Other observable symptoms are the little, black perithecia that can appear on the stalk, husk or kernels (Sutton, 1982; Parry et al., 1995; Miedaner, 1997).

Fusarium ear rot caused by *F. verticillioides* and *F. subglutinans* on the other hand, shows different symptoms. The mycelium that develops on infected maize ear is either white, pale pink, or pale lavender (Goswami and Kistler, 2004).

Deoxynivalenol (DON), also known as vomitoxin, is a trichothecene mycotoxin produced by the fungal plant pathogens *Fusarium graminearum* and *F. culmorum*. In addition to DON, the trichothecenes nivalenol (NIV) and T-2 toxin and the sterol zearalenone (ZEA) are also produced by the FHB-causing pathogens, and all of these mycotoxins are harmful to humans and animals. Although DON is the least toxic of them, it is the most commonly detected *Fusarium* mycotoxin, but also the predominant and most economically important mycotoxin in small grain production (D'Mello and MacDonald, 1997; D'Mello et al., 1999). Therefore, we have also examined the factors that influence the accumulation of DON in wheat grain under field conditions. Understanding of these factors can be useful in devising strategies aimed at reducing the amounts of DON in grain before the harvest, and ultimately mitigating the human and animal health risks associated with DON contamination of food and feed.

Our research includes field trials but also studies the natural incidence of maize and wheat disease, which is intensely variable and very closely connected to the environmental factors, particularly the weather factor. Our task was very versatile.

During our research we set several aims, the main ones being to determine whether there are any differences in infection levels between various wheat/maize varieties/hybrids in relation to the weather conditions during the flowering period, the wheat type and the location; to find whether the share of *Fusarium* infected grains in wheat/maize correlates to the mycotoxin content, especially DON; to discover if there is a possibility to approximately forecast the grain's mycotoxin contamination in wheat - via a field evaluation of the ear infection; and to simultaneously determine whether the ecologically grown wheat is less infected by fusariosis, i.e. less contaminated with mycotoxins, than the wheat grown in integrated production.

With our research we have contributed to a more clear understanding of the occurence of *Fusarium* spp. and mycotoxins in the grains of various types of wheat and maize, as well as their influence on crop quality.

Rakičan and Jablje are the locations were we have performed the experiments, repeatedly useing maize-wheat crop rotation for at least a 6-year time period, in order to allow greater infection pressure carried by the *Fusarium* spp. In the both years of the field trial and at both locations at the beginning of wheat flowering (BBCH 61), the fungicide Prosaro (prothioconazole + tebuconazole), a product of Bayer CS, was applied at a dosage of 1 l per hectare.

During May and June a monitoring was conducted at both test locations, the two year experimental period included (2012-2013), to determine the length of flowering of all wheat varieties, and each variety was separately catalogued in our database, a procedure of crucial significance to our research, due to the fact that the *Fusarium* spp. are the most infective at that stage of wheat development. We recorded the beginning of flowering, the full flowering, as well as the end of flowering (BBCH 61, 65 and 69).

Several methods were used to determine the *Fusarium* spp. grain infection in the various wheat and maize cultivars, as well as the mycotxin contamination. After the harvest and storage of the wheat and maize grains according to designated standards (Direktiva komisije 2005/38/ES ..., 2005), a test sample was retrieved from each variety/hybrid, from which a fragment was analyzed for mycotoxin representation and another fragment was used in a laboratory phytopathological analysis, in order to determine *Fusarium* infection of grain. In the laboratory, we have identified, in accordance with the prescribed standard phyto-pathological methods, the *Fusarium* species and the percent of infected grains.

From the data retrieved from the conducted analysis, we have gathered information on the *Fusarium* species representation in different wheat and maize varieties, the percentage of infection as well as the DON mycotoxin content in the same varieties. All wheat and maize samples were tested for DON with ELISA. In addition, two-tail T-TEST of type 3 (Independent samples with different variance), was used to evaluate the significant statistical differences in the data gathered during the two-year time period.

We gathered data related to the impact of the various environmental factors on the incidence of fusariosis and mycotoxin content at harvest during our two-year trial period. The weather conditions are one of the most important factors during the wheat's flowering period since they have a direct effect on the infections and subsequently on mycotoxin contamination. However, if we compare the data for the average ear infection of wheat with statistics on the climatic conditions, we can confirm that no correlation was found between the weather conditions and the occurence of *Fusarium* spp in our test locations. This analysis managed to deny our hypothesis that there is a correlation between the quantity, i.e. the period of precipitation during the wheat flowering period and the level of *Fusarium* infection. Other important factors that affect the rate of the *Fusarium* infection in grains and consequently the mycotoxin contamination are the sensitivity or the resistance of the cultivar, the wheat type used (awned, awnless), as well as the region itself. With that in mind, we have also conducted statistical analyses of the potential influence of both wheat types (awned and awnless) on the infection and contamination levels and concluded that overall, there is no statistically significant difference between both wheat types. Our hypothesis that the awnless wheat types are more receptive to *Fusarium* infection when compared to the awned wheat types was denied.

In addition, we have also included a statistical analysis of the influence of FC+FG infection on DON content, conducted on samples from both locations and years together, as well as on samples from each location and year separately.

In the first examination, a high correlation between FC+FG infection and DON content was observed in the samples collected from both locations, Rakičan and Jablje, for 2012 and 2013. The same samples were subjected to statistical analysis to evaluate whether there is a statistically significant difference regarding the influence of FC+FG infection of grains on DON contamination, from which it was concluded that the samples collected from Rakičan in 2012 and 2013 showed a statistically smaller infection level when compared to the other location (based on the data for FC+FG). The descriptive statistics confirms our hypothesis that the *Fusarium* species and the percentage of individual *Fusarium* species in the infected grains depend on the region itself.

In our second examination, we evaluated the samples from each location, and we concluded that in overall Rakičan had a smaller infection level as opposed to Jablje (based on our FC+FG data). Furthermore, the statistical analysis showed that the level of infection between both wheat types statistically significantly differs only in Rakičan, where the awned wheat type showed a significantly lower infection rate when compared to the awnless wheat. In all other instances there were no significant differences observed.
Moreover, when we grouped the data from each year, no significant difference was observed between the years of 2012 and 2013 according to our statistical analysis (based on FC+FG data).

According to the climatic conditions, we expected that mycotoxin levels in Rakičan will be at their minimum. This assumption was proven by ELISA tests that determined the total mean value for DON content for 2012 to be 140 μg /kg, and 120 μg/kg for 2013 (Table 9). In addition, the 1250 μg/kg DON content limit imposed by the European Union was not exceeded in the studied samples during 2012 and 2013.

Furthermore, the weather conditions also indicated on the possibility of higher DON contamination, which was confirmed by ELISA test conducted for the samples collected from Jablje in 2012 and 2013. The total mean value for DON in 2012 was 714 μg/kg and 684 μg/kg in 2013 (Table 11). The limit of 1250 μg/kg imposed by the European Union (EU) for DON content was exceeded in in Jablje in two samples from 2012, as well as two samples from 2013 (Table 11).

We have also acquired a representative number of samples from ecologically produced wheat originating from the areas close to our test locations (ECO-Prekmurje region, ECO-Gorenjska region).

The comparisons we have conducted were based on the contamination of wheat with FC+FG, as well as the appearance of DON. In both cases, a statistically significant difference has been observed between the mean presence of FC+FG. The former (FC+FG comparison), showed a statistical difference that was larger than every other type of comparison that was conducted: the comparison between Rakičan and the ECO-Prekmurje region, between Jablje and the ECO-Gorenjska region, and an overall comparison of each location between the integrally and the ecologically produced wheat. The latter (DON comparison), however, showed an insignificant difference only in regards to the comparison between Rakičan and the ECO-Prekmurje region, while the other locations and overall comparisons showed statistically significant differences in the mean presence of DON.

We analysed also the correlation between FC+FG infection and DON content in the ecological production and the same in the integrated production. The results showed a strong correlation and again proved that FC+FG is a factor of high importance in DON contamination.

The analysis that we have conducted on wheat samples obtained from ecological production have also helped us to confirm our hypothesis that the ecologically produced wheat is less contaminated with the mycotoxin DON compared to wheat obtained from the integrated production. Finally, based on a field estimate of the *Fusarium* spp. infection of wheat ears, which was carried out by the Stack and McMullen (1995) method, we have confirmed our hypothesis that it was impossible to predict even nearly the extent of mycotoxin DON contamination.

Our experiment included also analysis of maize kernels harvested in the same test locations, Rakičan and Jablje. Maize samples from Rakičan were minimally infected with FC+FG as compared to Jablje. In order to complete the analysis of the influence of FC+FG infection on DON content we conducted correlation analysis over data collected for maize crop, similar as we did for wheat. The correlation analysis showed a highly significant correlation between maize kernel infection with FC+FG and DON contamination. By comparing overall DON contamination between wheat and maize, no statistically significant difference has been found.

All our results will be further used in practice, i.e. we will be able to produce a list of wheat varieties and maize hybrids that are suitable for cultivation in specific conditions.

6.2 POVZETEK

Dve izmed najpomebnejših bolezni pšenice in koruze, ki jih povzročajo široko razširjene patogene glive iz rodu *Fusarium*, sta fuzarioza klasov (FHB) in plesnivost koruznih storžev (Goswami in Kistler, 2004).

Fuzarioza klasov je glivična bolezen, ki je resna grožnja v pridelavi žit v najrazličnejših pridelovalnih območjih in zelo pomemben dejavnik zmanjševanja pridelka ter kakovosti žit (Ireta in Gilchrist, 1994). Njen največji pomen je, da glive iz rodu *Fusarium* tvorijo mikotoksine, ki povzročijo, da so zrna neuporabna za prehranske in pivovarske namene. Na podlagi raziskav obstaja do 17 *Fusarium* vrst, ki jih lahko izoliramo iz zrn žit (Parry in sod., 1995). Njihova zastopanost se spreminja glede na lokacijo in podnebje. Vremenske razmere v času naših poskusov v letih 2012 in 2013 so se spreminjale dovolj močno, da so omogočile

izčrpen vpogled v raznolikost *Fusarium* vrst, ki so naravno okuževale pšenico in koruzo na obeh lokacijah v Sloveniji. Zaradi te raznolikosti smo lahko lažje ugotovili povezavo med vremenskimi razmerami in pojavom gliv iz rodu *Fusarium* ter onesnaženostjo z mikotoksinom deoksinivalenol (DON).

Fuzariozo klasov povzroča kompleks vrst iz rodu *Fusarium*, med katerimi so najpomembnejše *F. graminearum, F. culmorum* in *F. avenaceum*.

Za pojav fuzarioze klasov morajo biti izpolnjeni nekateri določeni pogoji. Okužba se lahko izvrši na dva načina, in sicer z nespolnimi in spolnimi sporami. Prva se zgodi, ko se z dežnimi kapljicami konidiji prenesejo od baze bili, slednja pa, ko se askospore s pomočjo vetra prenesejo iz površja tal na liste (Trail in sod., 2005). Za okužbo klasov je potrebna visoka stopnja vlažnosti, ki omogoča sproščanje askospor med cvetenjem (Trail in sod., 2002). Vendar če sproščanje askospor ne sovpada s cvetenjem, bo obseg okužb manjši (Nelson in sod., 1981). Bolezen povzroča lahko prepoznavna bolezenska znamenja. Najbolj značilna so obeljeni klaski v klasu. V primeru ugodnih razmer, ko se okužba razširi po celotnem klasu, lahko na klaskih opazimo s prostim očesom rožnato rdeč micelij oziroma z mikroskopom konidije. Poleg tega lahko v končni fazi okužbe opazimo spremembe na zrnju, ki se začne grbančiti in izgubi svojo značilno barvo in postane bledo belo (Von der Ohe, 2010).

Patogeni iz rodu *Fusarium* povzročajo tudi plesnivost storžev koruze, fuzarijski in *Giberella* tip. Vrste iz kompleksa *F. fujikuroi*, zlasti *F. verticillioides* in *F. subglutinans* povzročajo plesnivost koruznih storžev fuzarijskega tipa, medtem ko *F. graminearum* povzroča plesnivost storžev *Giberella* tipa.

Gliva *F. graminearum* okuži koruzne storže prek svile in se razširja od vrha do baze storža. V primeru resnih epidemij lahko okužba doseže pecelj storža. Gliva bo tvorila peritecije in razširjala askospore, ki bodo povzročile nadaljnje bolezenske cikle. Kužilo, ki je odgovorno za okužbo nadaljnjih posevkov, običajno najdemo v rastlinskih ostankih, vendar ga je mogoče najti tudi na okuženih koruznih semenih (Munkvold in sod., 1997).

Plesnivost storžev *Gibberella* tipa povzroča vidne simptome, ki jih zlahka prepoznamo, kar omogoča boljšo diagnostiko in s tem zgodnejše varstvene ukrepe. Na storžih opazimo rožnato rdeč micelij glive, sprva na vrhu storža, ki se kasneje širi navzdol proti njegovi bazi. Redko okuži cel storž. Drug viden simptom so majhni črni periteciji, ki se lahko pojavijo na steblu, ličju ali zrnju (Sutton, 1982; Parry in sod., 1995; Miedaner, 1997).

Fuzarijski tip plesnivosti storžev povzroča bolezenska znamenja, ki se razlikujejo od znamenj *Gibberella* tipa. Za razliko od slednjega, kjer se pojavlja rožnato rdeč micelij, je micelij fuzarijskega tipa lahko bel, bledo rožnat ali vijoličasto siv (Goswami in Kistler, 2004).

DON, znan tudi kot vomitoksin, je mikotoksin iz skupine trihotecenov, ki ga tvorita fitopatogeni glivi *Fusarium graminearum* in *F. culmorum*. Poleg DON tvorijo patogeni povzročitelji plesnivosti klasov tudi mikotoksine NIV, T-2 toksin in ZEA. Vsi omenjeni mikotoksini so škodljivi za ljudi in živali. Čeprav je DON najmanj toksičen od vseh, je najpogosteje zaznan fuzarijski mikotoksin in je gospodarsko najpomembnejši mikotoksin v pridelavi strnih žit (D'Mello in MacDonald, 1997; D'Mello in sod., 1999). Zato smo v naši raziskavi v poljskih razmerah preučili tudi dejavnike, ki vplivajo na kopičenje DON v zrnju pšenice. Razumevanje teh dejavnikov je koristno pri oblikovanju strategij, katerih cilj je zmanjšanje količine DON v zrnju pred žetvijo ter posledično ublažitev tveganj za zdravje ljudi in domačih živali, povezanih s kontaminacijo hrane in krme z DON.

Naša raziskava je vključevala poljske poskuse in med drugim tudi naravno pojavnost bolezni na pšenici in koruzi, ki je zelo variabilna in močno podvržena okoljskim dejavnikom, predvsem vremenu. Pomen naloge je zelo vsestranski.

V naši raziskavi smo si postavili več ciljev, osnovni so bili: ugotoviti, ali na stopnjo okužb različnih sort/hibridov pšenice/koruze vplivajo vremenske razmere v času cvetenja, tip pšenice in lokacija pridelave; ugotoviti, ali je delež s fuzariozami okuženih zrn pšenice/koruze v korelaciji z vsebnostjo mikotoksinov, zlasti DON; preučiti, ali lahko na podlagi poljske ocene okuženosti klasov okvirno napovemo okuženost zrnja pšinice oziroma njegovo kontaminiranost z mikotoksini; in hkrati ugotoviti, ali je pšenica pridelana v ekološki pridelavi manj okužena s fuzariozami oziroma kontaminirana z mikotoksini kot pšenica iz integrirane pridelave.

Z raziskavami smo prispevali k natančnejšemu poznavanju vrstne zastopanosti *Fusarium* vrst in mikotoksina DON v zrnju različnih sort pšenice in koruze ter njihov vpliv na kakovost pridelka.

Poskuse smo izvedli na dveh lokacijah, Rakičanu in Jabljah, kjer že več kot 6 let uporabljajo dvoletni kolobar (koruza-pšenica). Razlog za izbiro tako ozkega kolobarja je predvsem v tem, da čim bolj povečamo infekcijski pritisk *Fusarium* vrst. Obe leti poljskega poskusa smo na začetku cvetenja (BBCH 61) na obeh lokacijah pšenico, poškropili s fungicidnim pripravkom Prosaro (Bayer CS) v odmerku 1 liter na hektar.

Obe leti, 2012 in 2013, smo v maju in juniju na obeh lokacijah pri vsaki sorti pšenice časovno zabeležili posamezne faze cvetenja: začetek cvetenja, polno cvetenje in konec cvetenja (BBCH 61, 65 in 69) ter dolžino cvetenja. Razlog za tako natačno spremljanje pšenice v času cvetenja je bil predvsem v tem, ker ravno v teh razvojnih fazah pšenice pride do okužb klasov z glivami *Fusarium* spp.

Za ugotavljanje okuženosti pšeničnih in koruznih zrn z glivami *Fusarium* spp. in onesnaženosti z mikotoksini smo uporabili več metod. Po žetvi in uskladiščenju zrnja pšenice in koruze smo vzorce posameznih sort/hibridov, ki smo jih uporabili za nadaljnje analize, odvzeli po standardizirani metodi (Direktiva komisije 2005/38/ES ..., 2005). Vsak odvzeti vzorec je bil razdeljen na dva enaka dela. Eno polovico smo uporabili za analize, kjer smo ugotavljali kontaminiranost zrnja z mikotoksini. Drugo polovico zrnja pa smo uporabili za standardne laboratorijske fitopatološke analize, s katerimi smo ugotavljali okuženost zrnja z glivami *Fusarium* spp. (vrstna sestava in delež).

S temi analizami smo pridobili podatke o okuženosti zrnja posameznih sort pšenice in koruze z glivami *Fusarium* spp., določili njihovo vrstno sestavo in izračunali delež posameznih vrst ter onesnaženost zrnja z mikotoksinom DON. Za ugotavljanje statistično značilnih razlik med zbranimi podatki, pridobljenimi v dveh letih, smo uporabili dvostranski t-test-tip 3 (neodvisni vzorci z različno varianco).

Med dvoletnim poskusnim obdobjem smo pridobili podatke, ki se nanašajo na vpliv različnih okoljskih dejavnikov na pojavnost fuzarioz in vsebnost mikotoksinov. Vremenske razmere v času cvetenja pšenice naj bi bil eden izmed najpomembnejših dejavnikov, ki vpliva na okuženost različnih sort pšenice ter posledično na vsebnost mikotoksinov. Če primerjamo podatke o povprečni okuženosti klasov pšenice z našimi statističnimi podatki o klimatskih razmerah, ugotavljamo, da ni bilo korelacije med vremenskimi razmerami in pojavom *Fusarium* spp. S to analizo nismo uspeli potrditi hipoteze, da obstaja povezava med količino padavin oziroma številom padavinskih dni v času cvetenja pšenice in stopnjo okuženosti klasov s fuzariozami. Drugi pomembni dejavniki, ki vplivajo na obseg okužb zrnja s fuzariozami in s tem na vsebnost mikotoksinov, so tudi občutljivost oziroma odpornost sorte, tip pšenice (resnica, golica), zgodnost oz. zrelostni razred ter pridelovalno območje. Iz tega vidika smo opravili tudi statistične analize potencialnega vpliva obeh tipov pšenice (resnica in golica) na raven okužbe in kontaminacije in ugotovili, da ni statistično značilne razlike med obema tipoma pšenice. Naša hipoteza, da so sorte pšenice tipa golica bolj občutljive na okužbe s fuzariozami v primerjavi z resnicami, tudi ni bila potrjena. Dodatno smo preučili, če okuženost s FC+FG vpliva na vsebnost DON. Analiza je bila opravljena skupaj za vse vzorce

iz obeh lokacij in obeh let kot tudi posebej za vzorce iz obeh lokacij in obeh let (2012 in 2013).

Kot prvo smo ugotovili visoko korelacijo med okuženostjo s FC+FG in vsebnostjo DON v vzorcih, zbranih na obeh lokacijah, Rakičanu in Jabljah, za leti 2012 in 2013. Za iste vzorce smo opravili statistično analizo in preučili, če obstajajo statistično značilne razlike glede vpliva okuženosti zrnja s FC+FG na kontaminacije z DON. Ugotovili smo, da so vzorci zbrani iz Rakičana tako v letu 2012 kot leta 2013 statistično značilno manj okuženi v primerjavi z vzorci iz Jabelj (na podlagi FC + FG podatkov). Deskriptivna statistika je potrdila našo hipotezo, da sta vrstna sestava in delež gliv *Fusarium* spp. na okuženem zrnju pšenice/koruze odvisni od pridelovalnega območja.

Kot drugo smo po analizi vzorcev iz posamezne lokacije za obe leti skupaj ugotovili, da je bila v Rakičanu manjša raven okužb v primerjavi z Jabljami (na osnovi FC+FG podatkov). Poleg tega, je statistična analiza pokazala, da se raven okužb med obema tipoma pšenice v Rakičanu statistično značilno razlikuje. Resnice so bile pomembno manj okužene v primerjavi z golicami. V vseh drugih primerjavah nismo ugotovili statistično značilnih razlik.

Poleg tega smo ugotovili, da ni statistično značilnih razlik v okuženosti zrn s FC+FG med leti 2012 in 2013.

Glede na vremenske razmere smo pričakovali, da bodo vsebnosti mikotoksina DON v Rakičanu minimalne. To smo tudi potrdili z ELISA testom. Skupna povprečna vsebnost DON za leto 2012 je bila 140 µg/kg in 120 µg/kg za leto 2013 (preglednica 9). Poleg tega mejna vsebnost za DON 1250 µg/kg, določena s strani Evropske unije, v analiziranih vzorcih v letih 2012 in 2013 ni bila nikoli presežena.

Za razliko od Rakičana so vremenske razmere v Jabljah omogočile večje vsebnosti DON, kar je bilo potrjeno z ELISA testom, izvedenim na vzorcih iz Jabelj v letih 2012 in 2013. Kot je prikazano v preglednici 11, je bila v letu 2012 skupna povprečna vsebnost DON 714 µg/kg in 684 µg/kg v letu 2013. Mejna vrednost za DON, 1250 µg/kg, je bila presežena pri dveh vzorcih iz Jabelj v letu 2012, kot tudi v dveh vzorcih v letu 2013 (preglednica 11).

Pridobili smo tudi reprezentativno število vzorcev ekološko pridelane pšenice, ki so izvirali iz območij v bližini naših testnih lokacij (ECO-prekmurska regija, ECO-gorenjska regija).

Primerjave, ki smo jih opravili, temeljijo na okuženosti pšenice s FC+FG kot tudi na vsebnosti DON. V obeh primerih je bila ugotovljena statistično značilna razlika med povprečji

zastopanosti FC+FG. Predhodna primerjava (FC+FG) je pokazala večjo statistično značilno razliko od vseh drugih vrst primerjav, ki so bile izvedene: primerjava med Rakičanom in ECO-prekmurska regija, med Jabljami in ECO-gorenjska regija, splošne primerjave obeh lokacij med integrirano in ekološko pridelano pšenico. Pri primerjavi vzorcev glede vsebnosti DON nismo ugotovili statistično značilne razlike le med vzorci iz Rakičana in ekološko pridelanimi vzorci iz prekmurske regije, medtem ko so bile razlike v vsebnosti DON med vsemi ostalimi vzorci različnega porekla statistično značilne.

Prav tako smo analizirali povezavo med okuženostjo z FC+FG in vsebnostjo DON v ekološki pridelavi in v integrirani pridelavi. Ugotovili smo močno povezavo in s tem znova dokazali, da okuženost zrnja s FC+FG pomembno vpliva na vsebnost DON.

Analize, ki smo jih izvedli v vzorcih pšenice, pridelane v ekološki pridelavi, so tudi potrdile našo hipotezo, da je ekološko pridelana pšenica manj onesnažena z mikotoksini kot pšenica, pridelana v integrirani pridelavi. Ugotovili smo, da na podlagi poljske ocene okuženosti klasov s fuzariozami, izvedene po metodi Stack in McMullen (1995), ni mogoče napovedati onesnaženosti zrnja pšenice z mikotoksinom DON.

Naš poskus je vključeval tudi analizo vzorcev zrnja koruze pridelanega na istih poskusnih lokacijah, v Rakičanu in Jabljah. Vzorci koruze iz Rakičana so bili minimalno okuženi s FC+FG v primerjavi s tistimi iz Jabelj. Podobno kot pri pšenici smo pri koruzi preučili vpliv okuženosti zrnja s FC+FG na vsebnost DON s pomočjo korelacijske analize. Ta je pokazala zelo signifikantno povezavo med okuženostjo zrnja koruze s FC+FG in onesnaženostjo z DON. Ko smo primerjali povprečno kontaminacijo pšenice in koruze z DON, med njima nismo ugotovili statistično značilne razlike.

Vsi dobljeni rezultati se bodo lahko uporabili v praksi pri izbiri sort pšenice in hibridov koruze, ki so primerni za pridelavo na posameznih območjih.

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ANNEX A

Annex A: DON content (µg/kg) and percentage of identified *Fusarium* species in 41 different wheat varieties from Rakičan in years 2012 and 2013
Priloga A: Vsebnost DON (µg/kg) v zrnju 41 različnih sort pšenice iz Rakičana v letih 2012 in 2013

Wheat varieties	Rakičan – 2012							Rakičan – 2013						
	FA	FC	FG	FP	FT	Σ	DON µg/kg	FA	FC	FG	FP	FT	Σ	DON µg/kg
ALIXAN	1	0	1	6	0	8	104	0	2	0	3	0	5	136
AMICUS								2	1	0	1	0	4	70
ANĐELKA								0	1	0	3	1	5	73
APACHE	0	0	1	0	0	1	14							
AS DE COEUR	0	0	1	5	0	6	46							
BC RENATA	0	0	1	2	0	3	47	0	3	0	0	0	3	95
BC TENA								2	1	1	1	0	5	184
BOLOGNA	0	0	2	4	0	6	32	0	0	0	2	0	2	17
CHEVALIER	0	0	2	3	0	5	93							
ELEMENT	0	0	1	2	0	3	36							
ENERGO	0	0	1	0	0	1	59							
EUCLIDE	0	0	2	2	0	4	125							
FARMEUR	0	0	2	0	0	2	37							
FELIX								0	0	0	5	1	6	27
GARCIA	0	0	2	4	2	8	159	0	0	1	1	0	2	103
ILLICO	2	2	1	0	0	5	98	0	1	1	0	0	2	57
INGENIO	2	0	2	2	0	6	135	0	2	1	2	0	5	77
JANA								0	1	1	2	0	4	177
KATARINA	0	1	5	2	1	9	115	0	0	1	1	0	2	86
KETCHUM	0	0	1	2	0	3	**297**	1	2	9	6	1	19	**1033**
LORD	0	0	3	0	1	4	**240**	0	0	3	0	0	3	140
LUCIJA	0	0	2	5	0	7	149	0	4	2	0	0	6	15
LUKULLUS	0	0	2	1	0	3	133	0	0	1	1	1	3	93
MIHELCA	0	0	1	1	0	2	173	0	1	1	4	0	6	71
NINA	0	0	4	5	0	9	180	0	1	1	7	1	10	71
NS 40S	0	1	0	0	2	3	106	0	2	1	1	0	4	114
PANNONICUS								0	3	2	1	1	7	249
PANONIJA	0	0	4	2	1	7	124							
POBEDA	0	0	1	3	0	4	102							
SAILOR								0	1	1	2	0	4	147
SIMONIDA	0	0	1	2	0	3	156	0	0	2	0	0	2	8
SRPANJKA	0	0	4	4	1	9	148	0	2	2	2	0	6	3
SY ALTEO	0	0	1	2	2	5	185							
SY MOISSON	0	0	2	6	0	8	155	0	0	1	3	1	5	40
TACITUS	0	0	1	5	0	6	131	0	1	2	7	0	10	128
VULCANUS								2	1	3	4	0	10	147
VULKAN								0	0	1	0	0	1	103
ZDENKA	0	0	2	0	1	3	144	0	0	2	4	0	6	61
ZLATKA								0	1	1	0	0	2	70
ZVEZDANA								1	1	0	4	1	7	64
ŽITARKA	0	0	3	6	0	9	83	0	0	1	4	1	6	53

FA-*Fusarium avenaceum*; FC-*Fusarium culmorum*; FG-*Fusarium graminearum*; FP-*Fusarium poae*; FT-*Fusarium tricinctum*; Σ-Sum; DON-Deoxynivalenol; **bold numbers**: positive samples i.e. mycotoxin concentration above 200 μg/kg; green marked: awnless wheat; grey marked: awned
FA-*Fusarium avenaceum*; FC-*Fusarium culmorum*; FG-*Fusarium graminearum*; FP-*Fusarium poae*; FT-*Fusarium tricinctum*; Σ-vsota; DON-deoksinivalenol; **poudarjene številke**: pozitivni vzorci oz. vsebnost mikotoksinov nad 200 μg/kg; zelena oznaka: pšenica tipa golica; siva oznaka: pšenica tipa resnica

ANNEX B

Annex B: DON content (µg/kg) and percentage of identified *Fusarium* species in 41 different wheat varieties from Jablje in years 2012 and 2013
Priloga B: Vsebnost DON (µg/kg) v zrnju 41 različnih sort pšenice iz Jabelj v letih 2012 in 2013

Wheat varieties	Jablje – 2012							Jablje – 2013						
	FA	FC	FG	FP	FT	Σ	DON µg/kg	FA	FC	FG	FP	FT	Σ	DON µg/kg
ALIXAN	0	3	3	1	0	7	**467**	1	0	8	6	0	15	***1385**
AMICUS								3	1	4	7	0	15	**691**
ANDELKA								3	3	1	5	2	14	**271**
APACHE	2	0	9	0	0	11	**273**							
AS DE COEUR	1	0	7	6	2	16	**338**							
BC RENATA	0	0	1	1	2	4	**242**	2	0	3	9	0	14	**246**
BC TENA								2	0	6	8	0	16	**926**
BOLOGNA	0	0	2	0	0	2	162	2	0	2	7	0	11	128
CHEVALIER	2	0	11	1	0	14	**394**	0	3	2	3	1	9	**330**
ELEMENT	0	2	7	2	1	12	**526**	3	2	7	4	0	16	***1809**
ENERGO	0	0	14	0	1	15	**694**	3	1	3	6	2	15	***2284**
EUCLIDE	1	0	8	0	1	10	**870**							
FARMEUR	0	1	26	0	1	28	***3506**							
FELIX								1	0	0	7	0	8	**396**
GARCIA	0	0	18	1	2	21	**800**	3	1	2	5	0	11	**585**
ILLICO	0	3	0	0	1	4	**307**	1	0	1	2	0	4	136
INGENIO	2	0	30	0	0	32	**703**	1	3	4	10	0	18	**1040**
JANA								1	0	2	11	0	14	191
KATARINA	1	0	0	7	1	9	**227**	3	4	4	5	1	17	**486**
KETCHUM	0	0	23	0	1	24	***2498**	0	6	8	3	0	17	***2905**
LORD	2	1	23	1	0	27	***3550**	0	1	4	15	0	20	**793**
LUCIJA	0	0	6	1	2	9	**308**	6	3	4	5	0	18	**595**
LUKULLUS	0	0	12	2	2	16	**814**	0	3	1	2	4	10	**1135**
MIHELCA	0	0	2	4	4	10	**222**	0	0	2	0	0	2	**339**
NINA	0	0	1	4	0	5	**862**	0	0	3	10	0	13	**533**
NS 40S	0	0	4	1	0	5	**576**	0	0	2	22	0	24	**382**
PANNONICUS								0	0	11	13	0	24	***1520**
PANONIJA	1	0	6	0	3	10	**437**							
POBEDA	1	0	5	0	1	7	**429**	2	0	1	10	0	13	**483**
SAILOR								0	0	4	5	0	9	**806**
SIMONIDA	0	0	6	0	0	6	138	5	2	7	5	3	22	**543**
SRPANJKA	1	0	7	1	1	10	**229**	2	0	5	6	0	13	**231**
SY ALTEO	0	1	3	0	0	4	**333**							
SY MOISSON	0	0	18	0	0	18	**946**	2	1	6	6	0	15	**1176**
TACITUS	0	3	20	4	1	28	**1044**							
VULCANUS								0	0	18	7	0	25	***2667**
VULKAN								1	0	0	5	0	6	**241**
ZDENKA	0	0	8	0	1	9	**396**	3	0	1	1	0	5	**224**
ZLATKA								7	3	2	7	0	19	**839**
ZVEZDANA								0	0	0	13	0	13	**241**
ŽITARKA	0	2	5	1	5	13	118	0	0	0	8	0	8	140

FA-*Fusarium avenaceum*; FC-*Fusarium culmorum*; FG-*Fusarium graminearum*; FP-*Fusarium poae*; FT-*Fusarium tricinctum*; Σ-Sum; DON-Deoxynivalenol; **bold numbers**: positive samples i.e. mycotoxin concentration above 200 μg/kg; *-samples that exceed the limit of 1250 μg/kg imposed by the EU for DON; green marked: awnless wheat; grey marked: awned

FA-*Fusarium avenaceum*; FC-*Fusarium culmorum*; FG-*Fusarium graminearum*; FP-*Fusarium poae*; FT-*Fusarium tricinctum*; Σ-vsota; DON-deoksinivalenol; **poudarjene številke**: pozitivni vzorci oz. vsebnost mikotoksinov nad 200 μg/kg; *- vzorci ki presegajo mejo 1250 μg/kg uvedeno z EU za DON; zelena oznaka: pšenica tipa golica; siva oznaka: pšenica tipa resnica

ANNEX C

Annex C: *Fusarium* spp. incidence (%) and DON content (µg/kg) in 18 ecologically produced wheat samples from Gorenjska and Prekmurje region for 2012
Priloga C: Zastopanost *Fusarium* vrst (%) in vsebnost DON (µg/kg) v 18 vzorcih ekološko pridelane pšenice iz Gorenjske in Prekmurje za leto 2012

Eco wheat	2012										
	FA	FC	FG	FP	FT	Fce	FS	Fso	FSp	Σ	DON µg/kg
ECO - 1	0	0	1	1	0	0	0	0	0	2	131
ECO - 2	0	1	1	2	0	0	1	0	0	5	**272**
ECO - 3	0	0	2	2	1	0	0	0	0	5	**292**
ECO - 4	0	2	2	0	0	0	0	0	0	4	**496**
ECO - 5	0	1	8	1	0	0	0	0	0	10	***1520**
ECO - 6	0	0	2	1	0	0	0	0	0	3	**279**
ECO - 7	0	0	0	1	0	0	0	0	0	1	94
ECO - 8	0	1	0	1	0	0	0	0	0	2	111
ECO - 9	0	0	0	2	1	0	0	0	0	3	161
ECO - 10	0	1	2	0	0	0	0	0	0	3	**477**
ECO - 11	0	0	1	1	0	0	0	0	0	2	176
ECO - 12	0	0	1	0	0	0	0	0	0	1	163
ECO - 13	0	0	0	0	0	0	0	0	0	0	139
ECO - 14	0	1	1	0	0	0	0	0	0	2	**325**
ECO - 15	2	1	1	1	0	1	3	0	0	9	**247**
ECO - 16	2	1	0	1	0	0	2	0	0	6	176
ECO - 17	0	1	1	0	0	0	0	0	0	2	187
ECO - 18	0	1	0	0	0	0	0	0	0	1	133

FA-*Fusarium avenaceum*; FC-*Fusarium culmorum*; FG-*Fusarium graminearum*; FP-*Fusarium poae*; FT-*Fusarium tricinctum*; Fce-*Fusarium cerealis*; FS-*Fusarium subglutinans*; Fso-*Fusarium solani*; FSp-*Fusarium sporotrichoides*; ECO-Ecologically produced wheat; Σ-Sum; DON-Deoxynivalenol; **bold numbers**: positive samples i.e. mycotoxin concentration above 200 µg/kg; *-samples that exceed the limit of 1250 µg/kg imposed by the EU for DON

FA-*Fusarium avenaceum*; FC-*Fusarium culmorum*; FG-*Fusarium graminearum*; FP-*Fusarium poae*; FT-*Fusarium tricinctum*; Fce-*Fusarium cerealis*; FS-*Fusarium subglutinans*; Fso-*Fusarium solani*; FSp-*Fusarium sporotrichoides*; ECO-ekološko pridelana pšenica; Σ-vsota; DON-deoksinivalenol; **poudarjene številke**: pozitivni vzorci oz. koncentracija mikotoksinov nad 200 µg/kg; *- vzorci ki presegajo mejo 1250 µg/kg uvedeno z EU za DON

ANNEX D

Annex D: *Fusarium* spp. incidence (%) and DON content (µg/kg) in 18 ecologically produced wheat samples from Gorenjska and Prekmurje region for 2013
Priloga D: Zastopanost *Fusarium* vrst (%) in vsebnost DON (µg/kg) v 18 vzorcih ekološko pridelane pšenice iz Gorenjske in Prekmurje za leto 2013

Eco wheat	2013										
	FA	FC	FG	FP	FT	Fce	FS	Fso	FSp	Σ	DON µg/kg
ECO - 1	0	1	0	0	0	0	0	0	0	1	122
ECO - 2	0	0	2	3	0	0	1	0	1	7	**235**
ECO - 3	0	0	1	1	2	0	0	0	0	4	149
ECO - 4	0	1	1	0	0	0	0	0	0	2	**319**
ECO - 5	0	0	1	0	0	0	0	0	0	1	**214**
ECO - 6	0	0	1	0	0	0	0	0	0	1	134
ECO - 7	0	0	0	3	0	0	0	0	0	3	117
ECO - 8	0	0	0	1	0	0	0	0	0	1	75
ECO - 9	0	0	7	1	1	0	0	0	0	9	***1456**
ECO - 10	0	0	0	0	0	0	0	0	0	0	92
ECO - 11	0	0	0	0	0	0	0	0	0	0	111
ECO - 12	0	0	0	0	0	0	0	0	0	0	122
ECO - 13	0	1	0	0	0	0	0	0	0	1	188
ECO - 14	0	0	2	1	0	0	0	1	0	4	**346**
ECO - 15	2	1	1	1	0	2	4	3	0	14	182
ECO - 16	1	0	0	1	0	0	1	2	0	5	102
ECO - 17	0	1	0	0	0	0	0	0	0	1	108
ECO - 18	0	1	0	0	0	0	0	0	0	1	132

FA-*Fusarium avenaceum*; FC-*Fusarium culmorum*; FG-*Fusarium graminearum*; FP-*Fusarium poae*; FT-*Fusarium tricinctum*; Fce-*Fusarium cerealis*; FS-*Fusarium subglutinans*; Fso-*Fusarium solani*; FSp-*Fusarium sporotrichoides*; ECO-Ecologically produced wheat; Σ-Sum; DON-Deoxynivalenol; **bold numbers**: positive samples i.e. mycotoxin concentration above 200 µg/kg; *-samples that exceed the limit of 1250 µg/kg imposed by the EU for DON

FA-*Fusarium avenaceum*; FC-*Fusarium culmorum*; FG-*Fusarium graminearum*; FP-*Fusarium poae*; FT-*Fusarium tricinctum*; Fce-*Fusarium cerealis*; FS-*Fusarium subglutinans*; Fso-*Fusarium solani*; FSp-*Fusarium sporotrichoides*; ECO-ekološko pridelana pšenica; Σ-vsota; DON-deoksinivalenol; **poudarjene številke**: pozitivni vzorci oz. koncentracija mikotoksinov nad 200 µg/kg; *- vzorci ki presegajo mejo 1250 µg/kg uvedeno z EU za DON

ANNEX E

Annex E: *Fusarium* spp. incidence (%) and DON content (µg/kg) in 38 different hybrids of maize from Rakičan in years 2012 and 2013
Priloga E: Zastopanost *Fusarium* spp. (%) in vsebnost DON (µg/kg) v 38 različnih hibridih koruze iz Rakičana v letih 2012 in 2013

Maize hybrid	Rakičan – 2012									Rakičan – 2013										
	C	G	P	Pr	S	T	V	Σ	DON µg/kg	C	G	P	Pr	S	T	V	Ce	A	Σ	DON µg/kg
ALFREDO										1	3	24	0	1	0	1	0	0	30	**223**
ALTIUS	1	0	0	0	7	0	0	8	62											
AMANDO										1	2	4	0	0	0	0	0	0	7	167
ANDORO										0	4	23	2	10	0	1	0	0	40	198
APOLLO										1	2	2	7	3	0	14	13	0	42	149
BC 244	0	0	0	0	0	0	0	0	53	0	1	11	4	0	0	0	0	0	16	66
BC 416	0	1	0	0	0	0	1	2	65	1	1	1	0	2	2	13	8	0	28	84
DANUBIO	0	1	0	0	7	0	11	19	121	0	2	10	0	0	0	3	1	0	16	127
DKC 3705	0	1	0	0	9	0	0	10	129											
DKC 3871	0	2	0	0	0	0	4	6	163											
DKC 3912	0	2	0	0	14	0	0	16	149											
DKC 4014	1	0	0	0	8	0	0	9	102											
DKC 4117	1	1	0	0	45	0	0	47	111											
DKC 4371	1	0	0	0	11	0	7	19	73											
DKC 4490	0	1	0	0	5	0	0	6	147											
DKC 4590	0	2	0	3	16	0	0	21	148	1	2	22	2	1	1	1	0	0	30	144
DODIXX										0	2	14	0	4	0	0	0	0	20	164
FUTURIXX	0	1	0	5	18	0	0	24	115	0	3	31	0	2	0	3	0	0	39	163
KAMELIAS	0	2	0	0	0	0	4	6	174											
KEKEC	0	1	0	0	0	0	4	5	110	0	4	1	0	1	0	0	0	0	6	186
LEON	0	1	0	3	14	0	0	18	119											
LG 30290	0	0	0	0	6	0	0	6	77											
NK LUCIUS	0	0	0	0	0	0	1	1	99	0	2	14	0	0	0	0	0	0	16	116
NK OCTET	0	1	0	0	1	0	0	2	117											
NK TIMIC	0	1	0	0	4	0	3	8	128	0	4	21	11	25	0	0	0	0	61	**238**
NS 288	0	1	0	0	0	0	0	1	131	0	2	1	3	2	0	0	4	0	12	77
NS 375	1	2	0	0	2	0	2	7	**202**											
P9175	0	1	0	0	8	0	0	9	94	0	3	5	0	1	0	1	0	0	10	139
P9400	1	1	0	1	11	0	0	14	116	0	2	46	2	2	0	0	0	0	52	144
P9494	0	1	0	0	5	0	0	6	107	1	2	14	4	15	0	3	4	0	43	169
P9578	1	4	0	0	1	0	0	6	**472**											
PR37F73	0	2	0	0	3	0	0	5	145											
PR38A79	1	1	0	0	5	0	3	10	129	2	5	29	1	10	1	0	0	0	48	**335**
PR38N86	0	1	0	0	3	0	0	4	134	0	2	0	0	6	0	0	0	0	8	160
PR38Y34	1	2	0	0	5	0	0	8	**272**	0	2	2	0	0	0	0	0	1	5	122
SY FLOVITA	0	0	0	0	1	0	0	1	60	1	5	45	17	4	0	1	1	0	74	**354**
TOPOLA	0	0	0	0	0	0	3	3	83											
ZP 341	0	0	0	0	0	0	7	7	97											

C-*Fusarium culmorum*; G-*Fusarium graminearum*; P-*Fusarium poae*; Pr-*Fusarium proliferatum*; S-*Fusarium subglutinans*; T-*Fusarium tricinctum*; V-*Fusarium verticillioides*; Ce-*Fusarium cerealis*; A-*Fusarium avenaceum*; Σ-Sum; DON-Deoxynivalenol; **bold numbers**: positive samples i.e. mycotoxin concentration above 200 µg/kg

C-*Fusarium culmorum*; G-*Fusarium graminearum*; P-*Fusarium poae*; Pr-*Fusarium proliferatum*; S-*Fusarium subglutinans*; T-*Fusarium tricinctum*; V-*Fusarium verticillioides*; Ce-*Fusarium cerealis*; A-*Fusarium avenaceum*; Σ-vsota; DON-deoksinivalenol; **poudarjene številke**: pozitivni vzorci oz. koncentracija mikotoksinov nad 200 µg/kg

ANNEX F

Annex F: *Fusarium* spp. incidence (%) and DON content (µg/kg) in 38 different hybrids of maize from Jablje in years 2012 and 2013
Priloga F: Zastopanost *Fusarium* spp. (%) in vsebnost DON (µg/kg) v 15 različnih hibridih koruze iz Jabljah v letih 2012 in 2013

Maize hybrid	Jablje – 2012									Jablje – 2013									
	C	G	P	Pr	S	T	V	Σ	DON µg/kg	C	G	P	Pr	S	T	V	Ce	Σ	DON µg/kg
ALFREDO										2	9	7	0	8	3	9	0	38	**1039**
ALTIUS	0	2	0	1	0	0	0	3	**249**										
AMANDO										2	14	2	0	1	1	0	0	20	***1424**
ANDORO										6	40	6	1	2	0	0	0	55	***4273**
APOLLO										3	8	0	6	6	2	1	8	34	**861**
BC 244	0	3	0	3	0	1	3	10	**413**	1	3	0	8	1	2	7	0	22	**299**
BC 416	0	8	0	0	1	0	4	13	***1440**	15	69	0	0	2	2	4	6	98	***7645**
DANUBIO	0	2	0	0	0	1	0	3	**209**	1	10	1	0	0	1	0	2	15	**1084**
DKC 3705	0	4	0	0	0	0	0	4	**552**	0	3	0	0	2	0	0	0	5	**204**
DKC 3871	0	1	0	0	0	0	0	1	186										
DKC 3912	0	2	0	0	0	0	0	2	**353**										
DKC 4014	1	2	0	0	1	0	0	4	**255**	2	2	0	0	3	2	0	1	10	**294**
DKC 4117	0	3	0	2	0	1	0	6	**440**										
DKC 4371	0	6	0	0	3	1	5	15	***1095**										
DKC 4490	0	10	0	3	0	0	1	14	***1699**	2	2	0	8	0	0	3	1	16	**252**
DKC 4590	0	2	0	3	0	0	0	5	**336**	0	4	7	9	0	1	0	0	21	**282**
DODIXX										1	2	0	0	2	0	0	0	5	**227**
FUTURIXX	0	5	0	0	0	0	4	9	**931**	1	3	5	0	1	0	6	0	16	**305**
KAMELIAS	0	3	0	0	2	0	0	5	**423**										
KEKEC	0	4	0	0	0	0	0	4	**615**										
LEON	0	4	0	0	2	0	0	6	**710**										
LG 30290	0	4	0	0	0	0	0	4	**567**										
NK LUCIUS	0	4	0	0	0	1	0	5	**423**	2	3	3	0	1	2	0	0	11	**233**
NK OCTET	0	7	0	0	0	0	0	7	**883**	4	19	0	0	0	0	0	0	23	***2040**
NK TIMIC	6	15	2	0	1	0	0	24	***3561**	3	10	5	2	5	0	0	0	25	**1007**
NS 288	0	3	0	0	0	0	0	3	**240**	2	5	1	0	0	0	0	2	10	**453**
NS 375	6	1	0	0	0	0	0	7	**755**	2	2	2	0	0	0	0	1	7	**299**
P9175	0	5	0	0	1	0	0	6	**952**	0	4	0	0	2	1	0	0	7	**397**
P9400	0	2	3	0	0	0	0	5	**269**	0	6	8	0	0	0	0	0	14	**440**
P9494	2	8	2	1	1	0	0	14	***1625**	6	12	2	3	4	0	0	3	30	***1356**
P9578	5	8	0	0	2	0	1	16	***2585**										
PR37F73	0	5	4	0	0	1	0	10	**742**										
PR38A79	0	1	0	0	0	0	1	2	**203**	2	3	6	0	0	1	3	0	15	**243**
PR38N86	0	5	0	2	0	0	0	7	**601**	2	12	0	5	2	0	0	0	21	**1199**
PR38Y34	0	2	1	0	0	0	0	3	125	1	4	0	0	0	0	0	1	6	**242**
SY FLOVITA	3	3	0	0	4	0	0	10	**987**	0	3	5	2	7	0	0	0	17	**237**
TOPOLA	0	1	2	1	2	0	0	6	**368**	0	1	6	2	3	1	0	0	13	131
ZP 341	2	7	0	1	1	0	0	11	**1217**	1	2	0	3	2	1	0	0	9	188

C-*Fusarium culmorum*; G-*Fusarium graminearum*; P-*Fusarium poae*; Pr-*Fusarium proliferatum*; S-*Fusarium subglutinans*; T-*Fusarium tricinctum*; V-*Fusarium verticillioides*; Ce-*Fusarium cerealis*; Σ-Sum; DON-Deoxynivalenol; **bold numbers**: positive samples i.e. mycotoxin concentration above 200 μg/kg; *-samples that exceed the limit of 1250 μg/kg imposed by the EU for DON

C-*Fusarium culmorum*; G-*Fusarium graminearum*; P-*Fusarium poae*; Pr-*Fusarium proliferatum*; S-*Fusarium subglutinans*; T-*Fusarium tricinctum*; V-*Fusarium verticillioides*; Ce-*Fusarium cerealis*; Σ-vsota; DON-deoksinivalenol; **poudarjene številke**: pozitivni vzorci oz. koncentracija mikotoksinov nad 200 μg/kg; *- vzorci ki presegajo mejo 1250 μg/kg uvedeno z EU za DON