FUSARIUM SPECIES SPECTRUM IN ASYMPTOMATIC KERNELS OF SPRING BARLEY

Bíliková J., Hudec K.

Department of Plant Protection, Faculty of Agrobiology and Food Resources, Slovak University of Agriculture in Nitra, Tr. A. Hlinku 2, 949 76 Nitra, Slovak Republic

E-mail: bilikova.jana86@gmail.com

ABSTRACT

Fusarium head blight as a dangerous disease of cereals, including barley, is a current problem worldwide. Intense *Fusarium* infections of barley can render it technologically non useable for malt production, therefore the aim of the study was to identify *Fusarium* species spectrum in barley kernels in Slovakian conditions and to evaluate their frequency of occurrence. The samples were collected during vegetation seasons 2011 and 2012 from growth stage milk ripe (BBCH 73) to harvest (BBCH 99) at two localities (Hronovce and Sklabiná). The average infestation by *Fusarium* spp. in barley grain was low, ranging from 2.14 – 3.57 %. Totally, seven *Fusarium* species were identified in grains: *F. poae*, *F. equiseti*, *F. graminearum*, *F. langsethiae*, *F. sporotrichoides*, *F. avenaceum*, *F. tricinctum*. In both localities, the most predominant species each year was *F. poae* with frequency of occurrence ranging from 1 to 4 % with high relative density 40 - 100 %. Generally, *Fusarium graminearum* had frequency of occurrence 1-3 % with low relative density – 16.67 %. *Fusarium equiseti* and *Fusarium avenaceum* achieved frequency of occurrence – 3 %. The less frequent species - *F. tricinctum*, *F. sporotrichioides*, *F. langsethiae* achieved low relative density in population structure. These results confirmed the change in *Fusarium* species spectrum and their relative density in Slovakia, when comparing our recent results and literature.

Key words: barley, grains, *Fusarium*, spectrum, relative density, frequency of occurrence

Acknowledgments: The authors acknowledge the support of the scientific grant agency VEGA (Grant No 1/0797/11)

INTRODUCTION

Fusarium head blight (FHB) disease of small-grain cereals such as barley, wheat and oats is also referred to as Fusarium ear blight, scab or head fusariosis and is caused by several species of the genus Fusarium (Parry et al., 1995). This destructive disease is widespread and is predominantly caused by *F. avenaceum, F. culmorum, F. graminearum, F. poae* and *M. nivale* (Parry et al., 1995; Xu et al., 2005). *Fusarium* head blight (FHB) symptoms on barley (*Hordeum vulgare* L.) include premature necrosis and a brown/grey discoloration of the spike (Boddu et al., 2006; Geddes et al., 2008). More intense *Fusarium* infections of barley can render it technologically non useable for malt production (Schwarz et al., 1997). The major negative effect of contamination of malt barley with these molds is reduced test weight of infected grains, which implies less endosperm, decreased protein ratio, resulting in reduced malting yields (Duijnhouwer et al., 1993). *Fusarium* molds also synthesize unidentified compounds called hydrophobins which cause beer gushing (Kleemola et al., 2001; Denschlag et al., 2012). Moreover, detrimental effects of *Fusarium*, infection on brewing performance and beer flavor have also been noted (Haikara, 1983; Oliveira, 2012). *Fusarium* epidemics can result in significant economic losses to producers due to yield losses, lower prices and mycotoxin contaminations (Schaafsma, 2002; Mesterházy et al., 2003).

Climate change can increase the range and severity of plant disease epidemics (Garett et al., 2006). Except this, climate can provide more or less favorable conditions for the pathogen (West et al., 2012). *Fusarium* head blight is dangerous disease of wheat, barley and other cereals, and is caused by different *Fusarium* species (Osborne, Stein, 2007). Some species tend to occur predominantly in tropical and subtropical regions, some appear to be restricted to cold climatic and alpine zones, whereas others have a cosmopolitan distribution (Burgess et al., 1988). The prevailing species in colder areas are *F. culmorum, F. avenaceum, F. poae*, in warmer areas dominate *F. graminearum, F. solani, F. equiseti and F. oxysporum*. The frequency of occurrence and the spectrum of species are not stable; they vary depending on the year, varietal composition and development of weather in the time of infection (Šudyová, Šliková, 2011).

In view of this interaction, the aim of our research was to examine the influence of localities and weather conditions on FHB occurrence on barley grains, under natural infections**.**

MATERIAL AND METHODS

Grain samples of commercial cultivars of spring barley, a total of 1400, were collected from 2 localities of Slovakia situated in different climatic region (Table 1) and in different agronomical conditions (Table 2). Samples were collected every week from growth stage milk ripe (BBCH 73) to harvest (BBCH 99).

In laboratory, grains were surface-sterilized in 1 % NaOCl solution for 2 minutes. They were subsequently rinsed three times in re-distilled water and cultured in Petri dishes on potato-dextrose agar (PDA) in a climatic box at 21º C for 7-10 days. From the developed colonies mycelium was re-isolated and re-cultured in Petri dishes on synthetic nutrient medium (SNA) under UV-light, photoperiod 12 hours by day/12 hours by night, temperature of 24º C. To determine the species the classical identification based on microscopic characteristics according to the laboratory manuals Nelson et al. (1983) and Leslie, Summerell (2006) were used. Frequency of occurrence and relative density of *Fusarium* spp. in kernel samples were calculated using of formula of Gonzáles et al. (1996).

Table 1: Climatic region and soil characteristics (Linkeš et al., 1996) of evaluated localities in Slovakia

Code of climatic areas	Localities	Coordinates	Sum of average daily temperature ≥ 10°C	Rainfall VI – VIII [mm]	Soil type
01 - Warm, very dry, lowland	Hronovce	48º02´40´´N 18º39´23´´E	3000 - 2800	200 - 150	regosol brown soil
04 - Hot, very dry, hollow basin	Sklabiná	48º07´27´´N 19º21´22´´E	3030 - 2800	200 - 100	brown soil

Table 2: Average temperature in Hronovce, Sklabiná, 2011, 2012

		Average rainfall				Average temperature			
Year	Locality	April	May	June	July	April	May	June	July
2011	Hronovce	37.7	26	84.6	118	13.7	16.8	20.1	20.4
2012		45.5	8.1	47.3	109.4	12.5	17.7	21.1	23.3
2011	Sklabiná	12.9	43.3	79.5	80.8	12.7	15.6	19.5	19.6
2012		37.4	19.1	57.8	152.7	11.5	16.7	20.1	21.9

RESULTS AND DISCUSSION

The average occurrence of *Fusarium* spp. in barley kernels in locality Hronovce was 2.14 %, a bit higher occurrence was recorded in locality Sklabiná – 3.57 %. Šudyová, Šliková (2011) found 95. 2 % contamination of wheat grains in 2006 and 64,3 % contamination of wheat grains in 2007. Our results showed lower incidence of *Fusarium* spp. Totally seven *Fusarium* species has been identified in barley kernels: *F. poae*, *F. equiseti*, *F. langsethiae*, *F. sporotrichoides*, *F. culmorum*, *F. avenaceum*, *F. tricinctum* (Figure 1 - Figure 4). *F. poae* achieved the highest frequency in both years. The prevalence of *F. poae* on wheat grains in Slovakia was also recorded in the works Hudec, Roháčik (2005), Mašková et al. (2009), Šudyová, Šliková (2011).

In locality Hronovce, five *Fusarium* species were isolated from the kernels: *F. poae*, *F. equiseti*, *F. langsethiae*, *F. sporotrichoides* and *F. culmorum* (Figure 1, Figure 2). *F. poae* was the predominant species with frequency of occurrence up to 4 %. *F. poae* was found only in samples collected by the end of the vegetation period. The second most frequent species was *F. equiseti* (3 %) species followed by *F. sporotrichioides* (2 %), *Fusarium langsethiae* and *F. graminearum* (Figure 1, Figure 2). The most diverse *Fusarium* spectrum was determined from samples collected during harvest. The highest relative density was represented by *F. poae* (50-100 %) and *F. equiseti* (100 %). *F. sporotrichoides*, *F. graminearum* and *F. langsethiae* had lowest relative density (<50 %).

In locality Sklabiná, six species of *Fusarium* species were identified from asymptomatic barley grains: *F. poae*, *F. avenaceum*, *F. equiseti*, *F. graminearum*, *F. sporotrichoides*, and *F. tricinctum*. In comparison with Hronovce there were not found *F. langsethiae* and *F. culmorum*, but three other species: *F. graminearum*, *F. tricinctum*, *F. avenaceum*. The predominant species was *F. poae* with frequency of occurrence from 1 to 4 %, followed by *F. graminearum* (3 %),

which have global distribution (Sarver et al., 2011). This species did not achieve a high incidence. The less frequent species were *F. equiseti*, *F. sporotrichoides*, *F. tricinctum*. *F. poae* achieved the highest relative density (>50 %) (Figure 3, Figure 4).

Each fungus in FHB disease system has somewhat different environmental requirements, which can, in part, explain why the frequencies of these species varies by location. *F. graminearum* grows well over a wide range of temperature up to 30 ºC and is associated with warmer regions of the world (Bottalico, Perrone, 2002). In our cases *F. graminearum* was observed in July – a month with the highest average temperature. Conversely, *F. poae* is a more efficient pathogen at lower temperatures (20ºC) and is found more frequently in temperate climates. In Slovakia, prevalent *Fusarium* species on wheat in the long period were *Fusarium culmorum* and *Fusarium graminearum*, depending on climate conditions (Šrobárová, Šrobár, 1982; Šrobárová 1995). Our results indicate change in the composition of *Fusarium* species over the years. Mašková et al. (2011) collected samples of winter wheat from 7 regions of Slovakia. They found out that the most frequent species was *F. poae*. These results are identical with our results on barley. Roháčik and Hudec (2005) also found the highest incidences of *F. poae* on winter wheat during the vegetation period of the years 1999, 2000, 2002 and 2003. Increased incidence of *F. poae* was observed also in France (Ioos et al., 2004). Xu et al. (2008) claimed that *F. poae* prefers dry and hot climatic conditions.

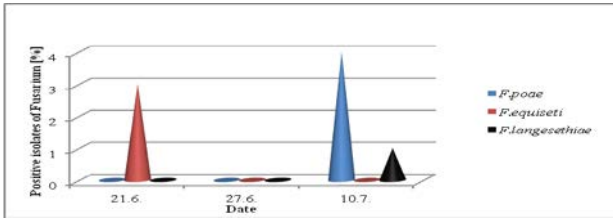

Figure 1: Frequency of occurrence of Fusarium species from barley asymptomatic grains in Hronovce, 2011

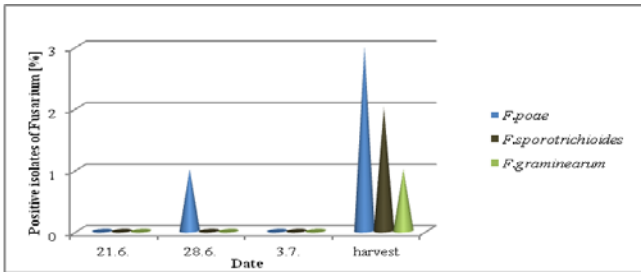

Figure 2: Frequency of occurrence of Fusarium species from barley asymptomatic grains in Hronovce, 2012

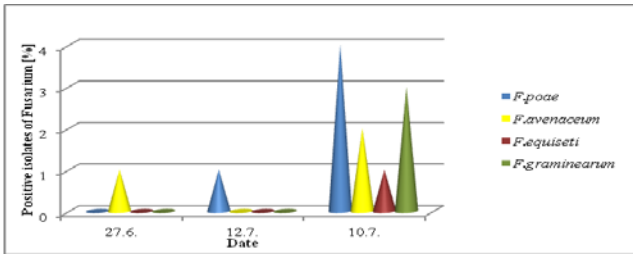

Figure 3: Frequency of occurrence of Fusarium species from barley asymptomatic grains in Sklabiná, 2011

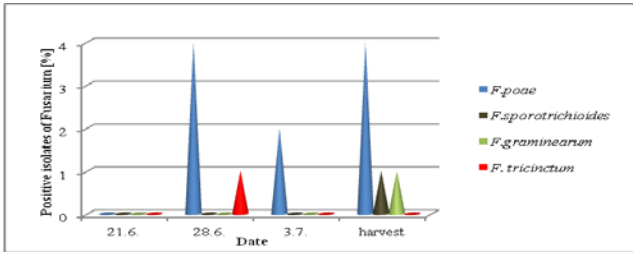

Figure 4: Frequency of occurrence of Fusarium species from barley asymptomatic grains in Sklabiná, 2011

Table 3: Relative density of Fusarium species on barley in Hronovce, 2011, 2012

Relative density (%)							
Year	**2011**			**2012**			
Date	**21.6.**	**27.6.**	**10.7.**	**21.6.**	**28.6.**	**3.7.**	**harvest**
F. poae	0	0	80	0	100	0	50
F. sporotrich.	0	0	0	0	0	0	33.33
F. graminearum	0	0	0	0	0	0	16.67
F. equiseti	100	0	0	0	0	0	0
F. langsethiae	0	0	20	0	0	0	0

Table 4: Relative density of Fusarium species on barley in Sklabiná, 2011, 2012

Relative density (%)							
Year	**2011**			**2012**			
Date	**27.6.**	**12.7.**	**10.7.**	**21.6.**	**28.6.**	**3.7.**	**harvest**
F. poae	0	100	40	0	80	100	66.67
F. sporotrich.	0	0	0	0	0	0	16.67
F. graminearum	0	0	30	0	0	0	16.67
F. equiseti	0	0	10	0	0	0	0
F. avenaceum	100	0	20	0	0	0	0
F. tricinctum	0	0	0	0	20	0	0

CONCLUSION

In Hronovce locality, the average infestation of *Fusarium* spp. on barley grains was 2.143 %. The highest average incidence of *Fusarium* spp. was observed in locality Sklabiná - 3.571 %. In total, the 7 *Fusarium* species were identified in barley grains: *F. poae*, *F. equiseti*, *F. langsethiae*, *F. sporotrichoides*, *F. culmorum*, *F. avenaceum*, *F. tricinctum*. *F. poae* achieved a higher frequency and relative density in both evaluated localities and both the years. *Fusarium graminearum* achieved relatively high frequency of occurrence, but low relative density. *Fusarium avenaceum* and *Fusarium equiseti* achieved high frequency of occurrence and relative density. Less frequent species - *F. tricinctum*, *F. sporotrichioides*, *F. langsethiae* achieved low relative density in population structure.Most scientists agree that management of FHB will require an integrated approach. The knowleage of spectrum of Fusarium in Slovakia and also in specific localities, understanding the epidemiology of the disease system is necessary for the success of predictive modeling system, as well as for integrative management of mycotoxins and disease. The composition of species did not change, only the dominance of species did.

REFERENCES

BODDU, J. – CHO, S. – KRUGER, W. M. – MUEHLBAUER, G. J., 2006: Transcriptome analysis of the barley – Fusarium graminearum interaction. In Mol Plant Microbe Interact, vol. 19, p. 407 417.

BOTTALICO, A. - PERRONE, G., 2002: Toxigenic species and mycotoxins associated with head blight in small-grain cereals in Europe. In *European Journal of Plant Pathology*, vol. 108, p. 611-624.

BURGESS, L. W. - NELSON, P. E. - TOUSSOUN, T. A. - FORBES, G. A. 1988. Distribution of *Fusarium* species in sections Roseum, Arthrosporiella, Gibbosum and Discolor recovered from grassland, pasture and pine nursey soils of Eastern Australia. In *Mycologia*, vol. 80, p. 815 –824.

DENSCHLAG, C. – VOGEL, R. F. – NIESSEN, L. -2012. Hyd5 gene-based detection of the major gushing-inducing Fusarium spp. in a loop-mediated isothermal amplification (LAMP) assay. In *Int J Food Microbiol*., vol. 156, p. 189-196.

DUIJNHOUWER, I. D. C. – GRASHOFF, C. – ANGELINO, S. A. G. F. 1993. Kernel filling and malting barley quality, Proc. Eur. Brew. Conv., Oslo, p. 121-128.

GARRETT, K. A. – DENDY, S. P. – FRANK, E. E. – ROUSE, M. N. , TRAVERS, S. E. 2006. Climate change effects on plant disease: genomes to ecosystems, In Annu. Rev. Phytopathol., vol. 44, p. 489-509.

GEDDES, J. – EUDES, F. – LAROCHE, A. – SELINGER, L. B. 2008. Differential expression of proteins in response to the interaction between the pathogen *Fusarium graminearum* and its host, *Hordeum vulgare*. In *Proteomics*, vol. 8, p. 545-554.

GONZÁLES, H. H. L. – PACIN, A. – RESNIK, S. L. – MARTÍNEZ, E. J. 1996. Deoxynivalenol and contamination mycoflora in freshly harvested Argantinean wheat in 1993. In *Mycopathologia*, vol. 135, p. 129-134.

HAIKARA, A. 1983. Malt and beer from barley artificially contaminated with *Fusarium* in the field. Proceedings of the European Brewery Convention Congress, London, IRL, Press: Oxford, p. 401-408.

HUDEC, K. - ROHÁČIK, T. 2005. Influence of Agro-enviromental factors on *Fusarium* infestation and population structure in wheat kernels. In *Ann Agric Environ Med*, vol. 12, no.1, p. 39-45.

IOOS, R. - BELHADJ, S. - MENEZ, M. 2004. Occurrence and distribution of *Microdochium nivale* and *Fusarium* species isolated from barley, durum and soft wheat grains in France from 2000 to 2002. In *Mycopathologia*, vol. 158, p. 351-362.

KLEEMOLA, T. – NAKARI-SETÄLÄ, T. – LINDER, M. – PENTTILÄ, M. – KOTAVIITA, E. – OLKKU, J. – HAIKIRA, A. 2001. Characterisation and detection of the gushing factors produced by fungi, Proceedings of the European Brewery Convention Congress, Budapest, Fachverlag Hans Carl: Nürnberg, Germany, CD ROM 2001, Contribution 12.

LESLIE, J. F. - SUMMERELL, B. A. 2006. The *Fusarium* Laboratory Manual. First edition. USA: Blackwell Publishing Proffesional, 387 p., ISBN- 13: 978-0-8138-1919-8.

LINKEŠ, V. - PESTÚN, V. - DŽATKO, M. 1996. Príručka pre používanie máp bonitovaných pôdno-ekologických jednotiek. 3. upr. vyd. Bratislava : VUPU, 1996. 104 p.

MAŠKOVÁ, Z. et al. 2009. Spektrum druhov rodu *Fusarium* izolovaných zo pšenice slovenského pôvodu a toxinogenitu vybraných kmeňov. In *Mykologické listy*, pp. 82-83, ISBN 978-80-254-6038-2.

MAŠKOVÁ, Z. - TANČINOVÁ, D. – LABUDA, R. – BARBORÁKOVÁ, Z. – DOVIČIČOVÁ, M. 2010. Species spectrum of the genus Fusarium isolated from Slovak wheat in season 2008. In Potravinárstvo, ročník 4, mimoriadne číslo, február/2010

MAŠKOVÁ, Z. - TANČINOVÁ, D. - BARBORÁKOVÁ, Z. - MOKRÝ, M. 2011. Frequented species of field fungi on wheat and their potential production of toxic metabolites. In *Potravinárstvo*, vol. 5, p.43-50.

MESTERHÁZY, Á. 2003. Control of *Fusarium* Head blight of Wheat by Fungicides. In: Leonard, K. J.- Bushnell, W. R. *Fusarium* Head Blight on Wheat and Barley, St. Paul: APS Press, The American Phytopathological Society, p. 84-120, ISBN 0-89054-302-X.

NELSON, P. E. - TOUSSOUN, T. A. - MARASAS, W. F. O. 1983. *Fusarium* species: An illustrated manual for identification. Pensylvania State University, University Park, USA.

OLIVEIRA, P. – ARENDT, E. K. 2012. The impact of Fusarium infection on brewing and beer quality. In 3rd International Young Scientists Symposium for the Brewing, Distilling and Malting Sectors. Nottingham, U. K., October, 2012.

OSBORNE, L. E. – STEIN, J. M. 2007. Epidemiology of *Fusarium* head blight on small-grain cereals, In *Int. Journal of Food Microbiology*, vol. 119, p. 103-108.

PARRY, D. W. – JENKINSON, P. – McLEAD, L. 1995. Fusarium ear blight (scab) in small grains cereals – a review. In *Plant Pathol*., vol. 44, p. 207-238.

SARVER, B. A. – WARD, T. J. – GALE, L. R. – BROZ, K. – KISTLER, H. C. – AOKI, T. – NICHOLSON, P. – CARTER, J. – O´DONELL, K. 2011. Novel *Fusarium* head blight pathogens from Nepal and Louisiana revealed by multilocus genealogical concordance. In *Fungal Genetics and Biology*, vol. 48, p. 1096-1107.

SCHAAFSMA, A. W. 2002. Economic changes imposed by mycotoxins in food grains: case study of deoxynivalenol in a winter wheat. In Mycotoxins and food safety. Edited by J. W. DeVries, M. W. Trucksess, and L. S. Jackson. Academic/Plenum Publishers, New York, p. 271-276.

SCHWARZ, P. – CASPER, H. – BARR, J. – MUSIAL, M. 1997. Impact of Fusarium head blight on the malting and brewing quality of barley. Proc 5th EU Fus. Semin., Szeget, vol. 25, p. 813-814.

ŠROBÁROVÁ, A. – ŠROBÁR, S. 1982. Characteristics of *Fusarium* species on wheat and their presence in different crop growing regions of Slovakia. In Sbornik UVTIZ, Ochrana rostlin, vol. 18, no. 1, pp. 27-34.

ŠROBÁROVÁ, A. 1995. The occurrence and biology of some *Fusarium* spp., on wheat in Slovakia. In Institute of Experimental Phytopathology and Entomology, Slovak Academy of Science, Ivanka pri Dunaji, 119 pp.

ŠUDYOVÁ, V. – ŠLIKOVÁ, S. 2011. Contamination of wheat grains with species of genera *Fusarium* in different localities of Slovakia in 2006-2008. In *Agriculture*, vol. 57, p. 110-117.

WEST, J. S. – HOLDGATE, S. – TOWNSEND, J. A. – EDWARDS, S. G. – JENNINGS, P. – FITT, B. D. L. 2012. Impacts of changing climate and agronomics factors on Fusarium ear blight of wheat in the UK. In Fungal Ecology, vol. 5, p. 53-61.

XU, X. M. – PARRY, D. W. – NICHOLSON, P. et al. 2005. Predominance and association of pathogenic fungi causing Fusarium ear blight in wheat in four European countries. In Eur J Plant Pathol, vol. 112, p.143-154.

XU, X. M. - NICHOLSON, P. - THOMSETT, M. A. - SIMPSON, D. - COOKE, B. M. - DOOHAN, F. M. - BRENNAN, J. - MONAGHAN, S. - MORETTI, A. - MULE, G. - HOMOK, L.- BEKI, E. - TATNELL, J. - RITIENI, A. - EDWARDS, S. G. 2008. Relationship between the fungal complex causing *Fusarium* head blight of wheat and environmental conditions. In *Phytopathology*, vol. 98, 2008, no. 1, p. 69 - 78.