BIULETYN INSTYTUTU HODOWLI I AKLIMATYZACJI ROŚLIN Nr 288 / 2020 : 41–45
E-ISSN: 2657–8913 DOI: 10.37317/biul-2020-0004

Komunikat
Short communication

Fusarium species and *Fusarium* mycotoxins in grain of barley in Poland in 2009 and 2010. Short communication

Gatunki *Fusarium* oraz toksyny fuzaryjne w ziarnie jęczmienia w Polsce w 2009 i 2010r. Komunikat

Tomasz Góral [1]✉, Piotr Ochodzki [1], Linda Kærgaard Nielsen [2], Dorota Walentyn-Góral [1]

[1] Department of Plant Pathology, Plant Breeding and Acclimatization Institute – National Research Institute, Radzików, 05-870 Błonie, Poland
[2] Sejet Plant Breeding, Nørremarksvej 67, 8700 Horsens
✉ e-mail: t.goral@ihar.edu.pl

Próby ziarna jęczmienia jarego ze zbiorów w 2009 i 2010r. zostały przeanalizowane pod kątem zawartości DNA gatunków *Fusarium* i toksyn fuzaryjnych (trichotecenów B). Próbki pochodziły z różnych pól z Radzikowa, w środkowej Polsce. Jakościowe i ilościowe oznaczanie gatunków *Fusarium* w ziarnie przeprowadzono techniką real-time PCR. Toksyny fuzaryjne w ziarnie analizowano metodą chromatografii gazowej. W ziarnie jęczmienia wykryto siedem gatunków *Fusarium*. Dominujące gatunki to *F. avenaceum*, *F. graminearum* i *F. poae*. Wykryto również występowanie *F. culmorum*, *F. langsethiae*, *F. sporotrichioides* i *F. tricinctum.* Stężenie trichotecenów B (deoksyniwalenolu, niwalenolu) w ziarnie było niskie. Najwyższy współczynnik korelacji deoksyniwalenol vs. DNA *Fusarium* stwierdzono dla *F. graminearum.* Jeśli chodzi o niwalenol, najwyższy był współczynnik korelacji z DNA *F. poae*.

Słowa kluczowe: DNA, *Fusarium*, jęczmień, real-time PCR, trichoteceny

Grain samples of spring barley from the 2009 and 2010 harvest were analysed for the content of DNA of *Fusarium* species and *Fusarium* toxins (type B trichothecenes). Samples originated from different fields in Radzików, Central Poland. Qualitative and quantitative determination of *Fusarium* species in the grain was performed using a real-time PCR. *Fusarium* toxins in the grain were analysed by gas chromatography. Seven *Fusarium* species were detected in barley grain. The dominating species were *F. avenaceum*, *F. graminearum* and *F. poae*. The presence of *F. culmorum*, *F. langsethiae*, *F. sporotrichioides* and *F. tricinctum* was also detected. The concentration of trichothecene toxins in grain (deoxynivalenol, nivalenol) was low. The highest correlation coefficient of deoxynivalenol vs. *Fusarium* DNA was found for *F. graminearum*. Regarding nivalenol, the highest correlation coefficient was with *F. poae* DNA.

Key words: barley, DNA, *Fusarium*, real-time PCR, trichothecenes

Introduction

Fusarium head blight (FHB) is a disease of cereals (including barley) caused by a complex of toxicogenic fungi of the genus *Fusarium* (Parry et al., 1995). The main species of this complex in Europe are *F. graminearum* and *F. culmorum*, identified as deoxynivalenol (DON), nivalenol (NIV) and zearalenone (ZEN) producers. However, other *Fusarium* species producing mycotoxins are also prevalent: *F. avenaceum* - moniliformin, enniatins and beauvericin (BEA) producer; *F. poae* - NIV, BEA producer. *F. langsethiae* and *F. sporotrichioides* - T-2 and HT-2 toxin producers, are also prevalent (Bottalico, 1998; Bottalico and Perrone, 2002; Jestoi et al., 2008; Vogelgsang et al., 2008; Somma et al., 2010). Because of the diversity of *Fusarium* species causing Fusarium head blight, monitoring of changes in the *Fusarium* population on wheat is important. The frequency of species infecting wheat is not stable and changes depending on the weather in a particular year. Large differences are also observed between different regions of wheat production in Europe. For example, other species are dominant in north-eastern Europe, as well as in the southwestern part of the continent (Bottalico, 1998; Bottalico and Perrone, 2002). Species compositions change over time, which is the results of global warming and changes in acreage of major cereal crops, i.e. an increase of maize area.

Barley is less infected by FHB compared to durum wheat or bread wheat (Langevin et al., 2009). However, its grain can also be contaminated with *Fusarium* toxins (Edwards, 2009; Malachova et al., 2010). Their presence (as well as the presence of *Fusarium* mycelium) is particularly important for malt barley, as it has a negative impact on beer quality (Havlova et al., 2006; Sarlin et al., 2007).

Data on barley contamination with *Fusarium* toxins or the frequency of *Fusarium* species infecting this cereal are much less available than for bread wheat. Hence, it would be interesting to find what the current situation in this field is. The aim of the present study was to determine the presence of *Fusarium* species and the content

of trichothecene type B mycotoxins in barley grain to compare species frequency with earlier reported data.

Material and methods

Five samples of spring barley grain from 2009 (2) and 2010 (3) were analysed. Samples were collected from two cultivars: 'Rufus' and 'Rubinek'. Barley was grown in five commercial fields near Radzików, Central Poland. Barley was harvested using a combine harvester. Ten subsamples weighing 1 kg were taken from the harvested grain and mixed thoroughly. Afterwards, a 1 kg grain sample was taken for further analysis. The collected samples were stored at –20°C before DNA and mycotoxin extraction. Qualitative and quantitative determinations of eight *Fusarium* species in the grain were performed by real-time PCR. The primers used were based on fungal TEF-1α gene sequences, designed by Nicolaisen et al. (2009), specific for the different *Fusarium* species: *F. avenaceum*, *F. culmorum*, *F. equiseti*, *F. poae*, *F. graminearum*, *F. langsethiae, F. sporotrichioide*s and *F. tricinctum*. The detailed methodology of DNA extraction and real-time PCR was described by Góral et. al (2019). The trichothecenes of group B - deoxynivalenol (DON), nivalenol (NIV) were quantified using gas chromatography techniques. The detailed methodology was described by Góral et. al (2019).

The original *Fusarium* DNA amount and toxin concentrations were transformed to logarithmic values in order to obtain a normal distribution for the variables. The relationships between the results for *Fusarium* DNA and *Fusarium* toxins were investigated by Pearson correlation tests. The correlation analyses were performed using Microsoft® Excel 2010/ XLSTAT©-Pro (Version 2013.4.07, Addinsoft, Inc., Brooklyn, NY, USA).

Results and discussion

Five samples of grain of spring barley collected from fields in Radzików, Central Poland, were analysed (Tab. 1). All samples contained DNA of *Fusarium* species at an average value of 11,287 pg of DNA per µg of wheat DNA (Tab. 1). The samples from 2010 were more contaminated with *Fusarium* than the samples from 2009 (17,669 pg/µg vs. 1,713 pg/µg), and the sample of cultivar 'Rubinek 10' contained the highest amount of *Fusarium* DNA (34,359 pg/µg). The lowest amount of DNA was detected in the sample of 'Rufus 09' (907 pg/µg).

Tabela 1
Table 1
Concentration of DNA of seven *Fusarium* species, and DON and NIV mycotoxins levels in grain of spring barley harvested in 2009 and 2010
Zawartość DNA siedmiu gatunków z rodzaju *Fusarium* oraz mykotoksyn DON i NIV w ziarnie jęczmienia jarego ze zbiorów w 2009 i 2010r.

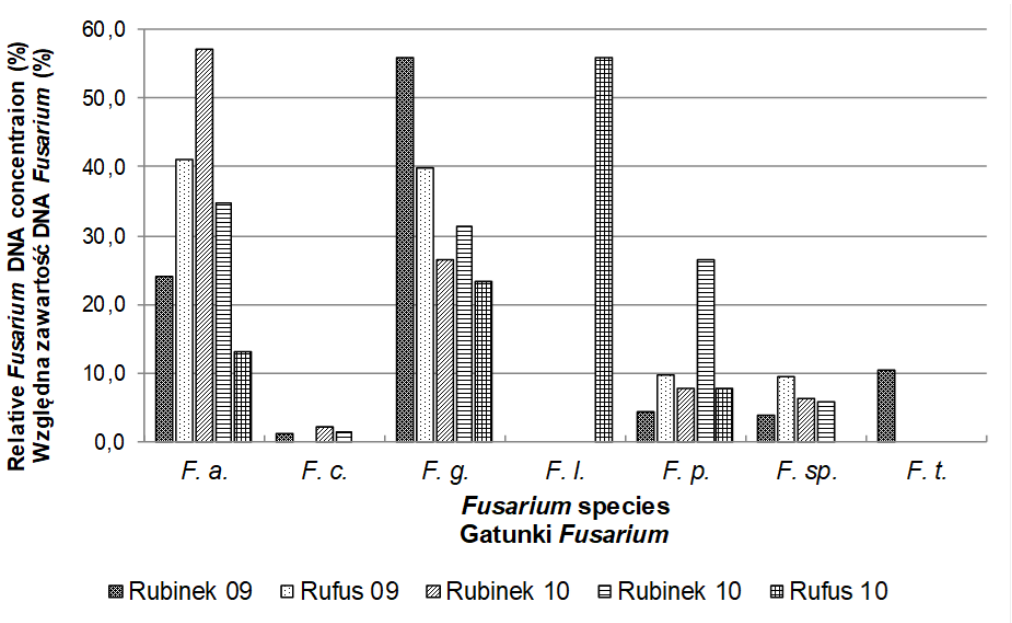
No. Lp.	Sample Próba	*Fusarium* DNA DNA *Fusarium* [pg/µg]							Mycotoxins Mykotoksyny [µg/kg]	
		F. a.	***F. c.***	***F. g.***	***F. l.***	***F. p.***	***F. sp.***	***F. t.***	**DON**	**NIV**
1	Rubinek 09	607	31	1407	0	112	97	266	202.0	0.0
2	Rufus 09	372	0	362	0	88	85	0	71.0	0.0
3	Rubinek 10	8159	303	3774	0	1117	916	0	113.3	57.5
4	Rubinek 10	11967	539	10755	0	9107	1991	0	226.1	100.7
5	Rufus 10	573	0	1022	2444	341	0	0	109.3	70.2
	Mean Średnia	**4336**	**174**	**3464**	**489**	**2153**	**618**	**53**	**144.3**	**45.7**

F. a. = F. avenaceum, F. c. = F. culmorum, F. g. = F. graminearum, F. l. = F. langsethiae, F. p. = F. poae, F. sp. = F. sporotrichioides, F. t. = F. tricinctum.

Seven *Fusarium* species were detected in the barley grain. Dominating species were *F. avenaceum* (4,336 pg/µg), *F. graminearum* (3,464 pg/µg) and *F. poae* (2,153 pg/µg) (Tab. 1, Fig. 1). These species were found in all samples. *Fusarium sporotrichioides* was found in four samples at an average DNA concentration of 618 pg/µg. *Fusarium culmorum* was present in three samples, but at a low concentration of 175 pg/µg. *Fusarium langsethiae* was found only in one sample ('Rufus' 10), but was the dominating species in this sample, and the DNA concentration amounted to 2,444 pg/µg. *Fusarium tricinctum* was also found in one sample ('Rubinek' 09) at 266 pg/µg.

The concentration of *Fusarium* DNA in barley grain in 2010 was higher than that in wheat grain in 2010 (Góral et al., 2019). The composition of *Fusarium* species infecting barley grain was similar to that of wheat, with *F. graminearum* prevailing over *F. culmorum* (Tomczak et al., 2002; Stępień and Chełkowski, 2010; Góral et al., 2019). According to Nielsen et al. (2014), in UK barley during the years 2007–2011, the dominating species were *F. poae*, *F. tricinctum* and *F. avenaceum*. *F. culmorum* and *F. graminearum* were less frequent. In Denmark in barley, the most frequent species in the period 2005 to 2007 were *F. avenaceum*, *F. langsethiae, F. culmorum*, *F. poae*, and *F. graminearum*, which were found in >85% of the samples (Nielsen et al., 2011). *F. tricinctum* was found in 67% of the samples, *F. sporotrichioides* in 15%, and *F. equiseti* in 2%. In wheat, the most frequent were *F. avenaceum*, *F. graminearum* and *F. culmorum.* Species composition in the above three countries seems to be similar. More species were involved in Fusarium head blight in barley than in wheat. Several species were also found in barley grain in northern USA (Salas et al., 1999). However, other than in Europe, *Fusarium graminearum* was the primary pathogen causing FHB epidemics and comprised from 62% to 64% of all *Fusarium* species isolated from infected kernels from 1994 to 1996. The authors also isolated *F. sporotrichioides, F. poae*, and *F. avenaceum* and stated that these species were involved in FHB infection, but to a limited extent. The above results show the effect of climatic conditions between northern Europe and the continental USA on *Fusarium* species in barley.

Fusarium langsethiae was found primarily in northern Europe on oat and barley (Yli-Mattila



F. a. = F. avenaceum, F. c. = F. culmorum, F. g. = F. graminearum, F. l. = F. langsethiae, F. p. = F. poae, F. sp. = F. sporotrichioides, F. t. = F. tricinctum.

Fig. 1. Relative concentration of DNA of seven *Fusarium* species in five samples of spring barley collected in 2009 and 2010.

Rys. 1. Względna zawartość DNA siedmiu gatunków z rodzaju *Fusarium* w pięciu próbach ziarna jęczmienia jarego ze zbiorów w 2009 i 2010r.

Tabela 2
Table 2

Coefficients of correlation between DNA concentration of three *Fusarium* species and amount of DON and NIV in spring barley grain.
Współczynniki korelacji pomiędzy zawartością DNA trzech gatunków *Fusarium* i zawartością DON i NIV w ziarnie jęczmienia jarego.

Variables Zmienne	*F. c.*	*F. g.*	*F. p.*	*Fusarium*	*F. c.* + *F. g.*	*F. c.* + *F. g.* + *F. p.*	DON
F. graminearum	**0,915**						
F. poae	0,758	**0,930**					
Fusarium	0,832	**0,972**	**0,963**				
DON	0,690	0,746	––	0,610	0,740	0,715	
NIV	0,428	0,698	0,820	0,843	0,696	0,724	0,232

F. c. = *F. culmorum*, *F. g.* = *F. graminearum*, *F. p.* = *F. poae*, DON = deoxynivalenol, NIV = nivalenol.

et al., 2008; Edwards et al., 2012). The occurrence of *F. langsethiae* on wheat in Poland was confirmed in 2008 (Łukanowski et al., 2008). This species was found mainly in northern Poland; however, it was present in some samples of wheat grain from Central Poland (Łukanowski and Sadowski, 2008). In 2009, *F. langsethiae* was found on wheat grain in the Netherlands, but at a low level (8% of the samples) (van der Fels-Klerx et al., 2012). Czaban et al. (2015) detected the presence of *F. langsethiae* in the years 2008–2010 in south-eastern Poland. However, this is the first report on the presence of *F. langsethiae* on barley in Poland.

The concentration of trichothecene toxins (DON, NIV) was low (Tab. 1) and was similar to that detected in naturally infected barley grain samples in the United Kingdom in 2002–2005 (Edwards, 2009) and 2007–2011 (Nielsen et al. 2014). Edwards (2010) found only one sample, which exceeded the legal limit for DON. Mycotoxin levels were also similar to that detected in barley in Poland in 1997 (Perkowski et al., 2003) and in the Czech Republic in he years 2001 and 2005 (Hajslova et al., 2007), but higher than that detected in the years 2005–2008 (Malachova et al., 2010).

The highest amount of DON was found in the sample 'Rubinek 10', which was the most *Fusarium* contaminated sample. In addition, this sample contained the highest amount of NIV and *F. poae* DNA, which is a producer of NIV (Stenglein, 2009). The highest correlation coefficient for DON vs. *Fusarium* was found for *F. graminearum* (Tab. 2). Regarding NIV, the highest correlation coefficient was with *F. poae* DNA concentration.

CONCLUSIONS

1. The dominating species in barley grain were *F. avenaceum*, *F. graminearum* and *F. poae*.
2. The presence of *F. culmorum*, *F. langsethiae*, *F. sporotrichioides* and *F. tricinctum* was also detected.
3. The concentration of deoxynivalenol and nivalenol was low.
4. The highest concentration of mycotoxins was found in the sample with the highest concentration of *Fusarium* DNA.

REFERENCES

Bottalico, A. 1998. *Fusarium* diseases of cereals: Species complex and related mycotoxin profiles, in Europe. J. Plant Pathol. 80: 85–103.

Bottalico, A., Perrone, G. 2002. Toxigenic *Fusarium* species and mycotoxins associated with head blight in small-grain cereals in Europe. Eur. J. Plant Pathol. 108: 611–624.

Czaban, J., Wróblewska, B., Sułek, A., Mikos, M., Boguszewska, E., Podolska, G., Nieróbca, A. 2015. Colonisation of winter wheat grain by *Fusarium* spp. and mycotoxin content as dependent on a wheat variety, crop rotation, a crop management system and weather conditions. Food Addit. Contam. Part A. Chem. Anal. Control. Expo. Risk Assess. 32: 799–807.

Edwards, S. G. 2009. *Fusarium* mycotoxin content of UK organic and conventional barley. Food. Addit. Contam. - Part A Chem. Anal. Control. Expo. Risk Assess. 26: 1185–1190

Edwards, S. G., Imathiu, S. M., Ray, R. V., Back, M., Hare, M. C. 2012. Molecular studies to identify the *Fusarium* species responsible for HT-2 and T-2 mycotoxins in UK oats. Int. J. Food Microbiol. 156: 168–175.

van der Fels-Klerx, H. J., de Rijk, T. C., Booij, C. J. H., Goedhart, P. W., Boers, E. A. M., Zhao, C., Waalwijk, C., Mol, H. G. J., van der Lee, T. A. J. 2012. Occurrence of Fusarium head blight species and *Fusarium* mycotoxins in winter wheat in the Netherlands in 2009. Food Addit. Contam. – Part A Chem. Anal. Control. Expo. Risk Assess. 29: 1716–1726.

Góral, T., Ochodzki, P., Nielsen, L. K., Walentyn-Góral, D. 2019. *Fusarium* species and *Fusarium* mycotoxins in grain of wheat in Poland in 2009 and 2010. Preprints. 2019090108.

Hajslova, J., Lancova, K., Sehnalova, M., Krplova, A., Zachariasova, M., Moravcova, H., Nedelnik, J., Markova, J., Ehrenbergerova, J. 2007. Occurrence of trichothecene mycotoxins in cereals harvested in the Czech Republic. Czech. J. Food. Sci. 25: 339–350.

Havlova, P., Lancova, K., Vanova, M., Havel, J., Hajslova J. 2006. The effect of fungicidal treatment on selected quality parameters of barley and malt. J. Agric. Food. Chem. 54: 1353–1360.

Jestoi, M. N., Paavanen-Huhtala, S., Parikka, P., Yli-Mattila, T. 2008. In vitro and in vivo mycotoxin production of *Fusarium* species isolated from Finnish grains. Arch. Phytopathol. Plant Prot. 41: 545–558.

Langevin, F., Eudes, F., and Comeau, A. 2004. Effect of trichothecenes produced by *Fusarium graminearum* during Fusarium head blight development in six cereal species. Eur. J. Plant Pathol. 110: 735–746.

Łacicowa, B., Kiecana, I. 1991. Fusariosis of spring barley cultivated in Lublin region. Mycotox. Res. 7(Suppl 2): 128.

Łukanowski, A., Lenc, L., Sadowski, C. 2008. First report on the occurrence of *Fusarium langsethiae* isolated from wheat kernels in Poland. Plant Dis. 92: 488–488.

Łukanowski, A., Sadowski, C. 2008. *Fusarium langsethiae* on kernels of winter wheat in Poland — Occurrence and mycotoxigenic abilities. Cereal Res. Commun. 36: 453–457.

Malachova, A., Cerkal, R., Ehrenbergerova, J., Dzuman, Z., Vaculova, K., and Hajslova, J. 2010. *Fusarium* mycotoxins in various barley cultivars and their transfer into malt. J. Sci. Food Agric. 90: 2495–2505.

Nicolaisen, M., Suproniene, S., Nielsen, L. K., Lazzaro, I., Spliid, N. H., Justesen, A. F. 2009. Real-time PCR for quantification of eleven individual *Fusarium* species in cereals. J. Microbiol. Methods. 76: 234–240.

Nielsen, L. K., Cook, D. J., Edwards, S. G., Ray, R. V. 2014. The prevalence and impact of Fusarium head blight pathogens and mycotoxins on malting barley quality in UK. Int. J. Food Microbiol. 179: 38–49.

Nielsen, L. K., Jensen, J. D., Nielsen, G. C., Jensen, J. E., Spliid, N. H., Thomsen, I. K., Justesen, A. F., Collinge, D. B., Jørgensen, L. N. 2011. Fusarium head blight of cereals in Denmark: species complex and related mycotoxins. Phytopathology 101: 960–969.

Parry, D. W., Jenkinson, P., McLeod, L. 1995. Fusarium ear blight (scab) in small grain cereals—a review. Plant Pathol. 44: 207–238.

Perkowski, J., Kiecana, I. & Kaczmarek, Z. 2003. Natural occurrence and distribution of *Fusarium* toxins in contaminated barley cultivars. European J. Plant Pathol. 109: 331–33

Salas, B., Steffenson, B. J., Casper, H. H., Tacke, B., Prom, L. K., Fetch, T. G., Schwarz, P. B. 1999. *Fusarium* species pathogenic to barley and their associated mycotoxins. Plant Dis. 83: 667–674.

Sarlin, T., Vilpola, A., Kotaviita, E., Olkku, J., Haikara, A. 2007. Fungal hydrophobins in the barley-to-beer chain. J. Inst. Brew. 113: 147–153

Somma, S., Alvarez, C., Ricci, V., Ferracane, L., Ritieni, A., Logrieco, A., Moretti, A. 2010. Trichothecene and beauvericin mycotoxin production and genetic variability in *Fusarium poae* isolated from wheat kernels from northern Italy. Food Addit. Contam. - Part A Chem. Anal. Control. Expo. Risk Assess. 27: 729–737.

Stenglein, S. A. 2009. *Fusarium poae*: A pathogen that needs more attention. J. Plant Pathol. 91: 25–36.

Stępień, Ł., Chełkowski, J. 2010. Fusarium head blight of wheat: pathogenic species and their mycotoxins. World Mycotoxin J. 3: 107–119.

Tomczak, M., Wiśniewska, H., Stępień, Ł., Kostecki, M., Chełkowski, J., Goliński, P. 2002. Deoxynivalenol, nivalenol and moniliformin in wheat samples with head blight (scab) symptoms in Poland (1998-2000). Eur. J. Plant Pathol. 108: 625–630.

Vogelgsang, S., Sulyok, M., Hecker, A., Jenny, E., Krska, R., Schuhmacher, R., Forrer, H. R. 2008. Toxigenicity and pathogenicity of *Fusarium poae* and *Fusarium avenaceum* on wheat. Eur. J. Plant Pathol. 122: 265–276.

Yli-Mattila, T., Paavanen-Huhtala, S., Jestoi, M., Parikka, P., Hietaniemi, V., Gagkaeva, T., Sarlin, T., Haikara, A., Laaksonen, S., Rizzo, A. 2008. Real-time PCR detection and quantification of *Fusarium poae, F. graminearum, F. sporotrichioides* and *F. langsethiae* in cereal grains in Finland and Russia. Arch. Phytopathol. Plant Prot. 41: 243–260.