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Qualitative changes of rye grain and flour after *Fusarium* spp. contamination evaluated by standard methods and system Mixolab

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Abstract

The effect of *Fusarium* spp. infestation on rheological quality of rye flour in different growing systems (organic and conventional) was measured by the rheological system Mixolab. The content of mycotoxins (DON, D3G, 3-ADON and ZON) and standard technological quality parameters of grain were determined as well. Significantly worse technological and rheological parameters representing starch quality were determined for rye artificially inoculated by *Fusarium* spp. in both growing systems compared to rye with natural *Fusarium* spp. contamination. A statistically significant negative correlation, indicating deteriorated rheological quality, was discovered between the content of mycotoxins and falling number (−0.69* for DON), and also between mycotoxin content and a rate of starch gelatinization, represented by Mixolab parameter C3 (−0.57* for DON, −0.86** for 3-ADON and −0.87** for ZON). Despite the visible shifts of Mixolab curves of samples from the organic and conventional growing system, the Mixolab characteristics were statistically comparable. Distinct differences of Mixolab curves, presumably caused by the weather conditions, were found between the monitored years.

Key words: growing system, Mixolab, mycotoxins, *Secale cereale*.

Introduction

Rye (*Secale cereale* L.) is an undemanding crop grown mainly in mountainous and foothill areas. Nowadays there is a growing demand for this crop because it is a particularly important source of dietary fibre and bioactive compounds such as alkyresorcinols, lignans, phenolic acids, phytosterols, tocopherols, tocotrienols and folates (Rye and health, 2002). Its positive effect is not only evident in lower cholesterol levels; rye also influences other mechanisms, which are reflected in reduced blood pressure and an improved sugar metabolism. Lignans secoisolariciresinol and matairesinol, present in the outer layers of rye grains have a positive effect against cancers of the prostate, colon and breast (Petr et al., 2008).

Like other small grain cereals, rye grain also can be infected by *Fusarium* head blight (FHB) – a fungal disease caused by pathogenic fungi *Fusarium* spp. and has become a serious danger to the worldwide grain industry. This infection can result in the yield loss and a reduction of the end-use quality (Gilbert, Haber, 2013) as the pathogen destroys starch granules, storage proteins and cell walls, and subsequently affects the quality of dough properties (Lancova et al., 2008). FHB also leads to the accumulation of mycotoxins in grain, which are stable low-molecular secondary metabolites produced during the fungal infection process (Bailey et al., 2007). Despite contradictory reports in the literature concerning the close correlations between FHB and mycotoxin content, it is overall accepted that the accumulation of mycotoxins in kernels would also require successful infection (Smith et al., 2004). Moreover, in the case of a very strong infectious pressure to cereals, induced by an artificial inoculation, it is possible to presume, that also the content of mycotoxins will be high and exceed the hygienic limit which was, for the most detected mycotoxins deoxynivalenol (DON) and zearalenone (ZON), set to 1250 and 100 μg kg^{-1} for unprocessed cereals and 750 and 75 μg kg^{-1} for cereal flours, respectively (European Commission, 2006).

The negative effects of a *Fusarium* infection on the baking quality of wheat have already been found (Horvat et al., 2015) but there are not many studies on the changes in baking quality of rye grain and flour after *Fusarium* contamination. However, the processors of rye are sometimes confronted with problems arising from abnormal behaviour of dough made from rye flour. These problems may occur, as with other cereals grains, due to contamination by *Fusarium* spp. Different rheological analysis, e.g., farinograph, extenzograph, amylograph or mixograph, are used to evaluate baking properties of cereals (Dobraszczyk, Morgenstern, 2003). The new apparatus Mixolab, accepted as the ICC standard method No. 173, enables evaluation of physical dough properties such as dough stability or weakening and starch characteristics in one measurement by intense mixing and controlled heating of the kneader to 90°C and ensuing cooling to 50°C (Rosell et al., 2007). Important researches on predicting the bread and cookie baking quality from different wheat flours have been carried out (Cato, Mills, 2008) but studies about a capability of the system Mixolab to predict rheological parameters of other cereals with various grade of fungi infestation are almost not available.

The study was focused on the detection of qualitative changes in rye grain and flour with a different intensity of *Fusarium* spp. contamination from two different growing systems, using standard methods for technological quality determination and rheological evaluation by the system Mixolab.

Materials and methods

Material. Winter rye (*Secale cereale* L.) two cultivars (‘Dankowskie Diament’ and ‘Askari’) from the precision field plot trial, conducted in the years 2010–2011 and 2011–2012 at the experimental station of the Department of Crop Production of the Czech University of Life Sciences in Uhříněves (295 m a.s.l., average annual temperature 8.4°C, average sum of precipitation 575 mm) were used for the evaluation of the effect of *Fusarium* head blight (FHB) infestation on the *Fusarium* mycotoxin (DON, D3G, 3-ADON and ZON) content in grain, the standard technological quality parameters of grain and the rheological parameters of dough. Field plot trials were carried out by the method of randomized blocks in three replications; an average size of an experimental plot was 12 m^2. The precision field experiments were conducted in an organic farming system on an experimental area, certified for organic farming and also in comparison with a conventional system using herbicide treatment and nitrogen fertilization – 120 kg ha^{-1} N, applied in two doses – 60 kg ha^{-1} N after overwintering and 60 kg ha^{-1} N at the beginning of shooting. Treatments with natural *Fusarium* spp. contamination were evaluated in both growing systems; to ensure a higher level of *Fusarium* infestation artificial inoculation of flowering ears was used, too.

Artificial inoculation. The isolates of *F. culmorum* and *F. graminearum* used for the artificial inoculation were obtained from the mycological collection of the Crop Research Institute in Prague and cultivated on sterile wheat grains. Wheat grains with the cultures of *F. culmorum* and *F. graminearum* were put into a vessel with water and shaken for 15 min in a laboratory shaker to release the spores into water. The obtained suspension was filtered through a piece of the gauze. Then artificial inoculation was made using the suspension of *F. culmorum* and *F. graminearum* spores in the ratio of 1:1, 10^7 of spores ml^{-1} (Bürker chamber was used for the verification of inoculums density), 2 l of suspension per experimental plot (12 m^2). The suspension was dosed with a hand sprayer at the beginning of the rye flowering. Harvested grain samples were used for *Fusarium* mycotoxin determination.

Fusarium mycotoxin determination. For the isolation of analytes from grain we used a modified QuEChERS (quick, easy, cheap, effective, rugged and safe) procedure (Petr et al., 2008). It is the multiresidue determination of wide range of mycotoxins applicable within different matrices (cereals, feed and others), based on the extraction of analytes with acetonitrile: water and further purification of the extract consists of the division between the two phases by means of inorganic salts (NaCl, $MgSO_4$). Analytes were transported into the upper acetonitrile layer while the polar co-extract matrix (e.g., sugars or amino acids) remained in the aqueous phase (Džuman et al., 2014). The ultra-high performance liquid chromatograph Acquity UPLC System (“Waters”, USA) coupled with the tandem mass spectrometer LCT Premier XE (“Waters”, USA) with analyzer TOFMS was used for the identification and detection of analytes. Wider scale of *Fusarium* mycotoxins was determined – deoxynivalenol (DON) and its conjugated forms, zearalenone (ZON) and its metabolits, enniatins, and so forth but some of them only in trace amount. Only the mycotoxins, which were detected most frequently in our rye grain samples – DON, deoxynivalenol-3-β-D-glucoside (D3G), 3-acetyldeoxynivalenol (3-ADON) and ZON – are discussed in this paper.

Standard technological quality parameters of grain. Rye grain samples were grinded using standard laboratory grinder (sieve with apertures in average 0.8 mm) and obtained meal was used for determination of standard baking quality parameters. Crude protein content of grain according to the Kjeldahl method (ISO 20483; ICC Standard No. 105/2), falling number (ISO 3093), volume weight (ISO 7971-2) and thousand kernel weight were determined.

Rheological parameters of flour. Protein and starch characteristics of rye flour: dough development, weakening of the protein, starch gelatinization, diastatics activity, anti-stalling effect, were determined by the apparatus Mixolab (“Chopin, Tripette et Renaud”, France) according to the Mixolab protocol Chopin+ (Mixolab applications handbook, 2008). Valuated flour was obtained by milling the cereal grain samples on a Bühler mill automat MLU 202 (Bühler Ltd., Switzerland). Traditional rye bread flour – type T930 with ash content in flour dry matter ca 0.93% and flour yield ca 31% (mix of only two fractions of reduction flour) was used.

A typical Mixolab curve is separated into five stages represented by five (C1–C5) points (Fig. 1). The two first stages correspond to the rheological characteristics of proteins – stability, elasticity and water absorption, whereas the other stages relate mainly to the starch and amylolytic activity. Evaluated characteristics from the measured Mixolab curve are: C1 (Nm) marks maximum torque during mixing, used to determine water absorption; C1 (min) time required to achieve the maximum torque; C2 (Nm) measures the weakening of the protein based on the mechanical work and the increasing temperature; C3 (Nm) indicates the rate of starch gelatinization; C4 (Nm) represents the stability of the hot-formed gel; C5 (Nm) expresses starch retrogradation during the cooling period; difference C1–C2 represents the protein network strength under the increasing heating; difference C3–C4 shows diastatic activity and relates to falling number; difference C5–C4 correlates with the anti-stalling effects, represents the shelf life of end products; DS indicates the stability of the dough before weakening (Mixolab Applications Handbook, 2008).

Results were statistically evaluated by the analysis of variance (*ANOVA*) and the correlation analysis with the statistical significance expression on the level α = 0.05 and α = 0.01 in the *Statistica 9.0 CZ* (StatSoft Inc., 2009).

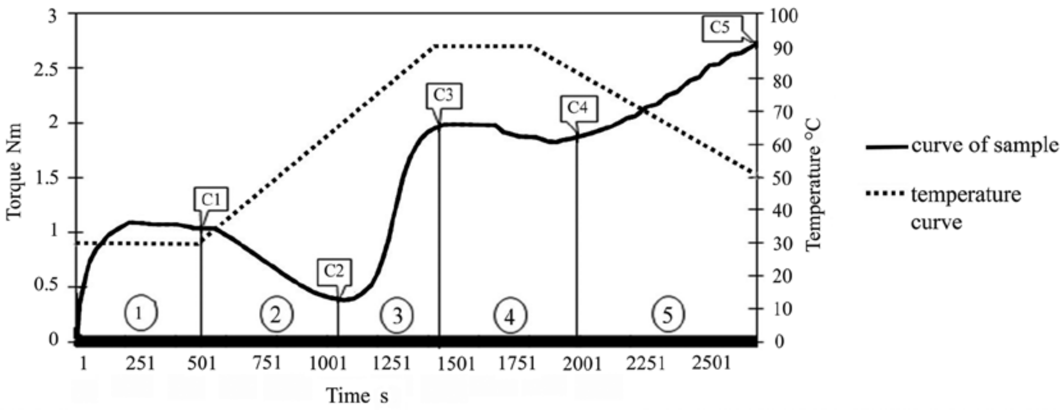

Figure 1. Standard Mixolab curve

Results and discussion

Content of mycotoxins and standard technological quality parameters of grain. The average content of *Fusarium* mycotoxins evaluated in rye grain is summarized in Table 1, where distinctive differences especially between the treatments with or without artificial *Fusarium* inoculation are visible. The correlation between the *Fusarium* spp. infection level and mycotoxin content do not have to be always significant (Garcia et al., 2009). However, it is assumed that by using a strong artificial inoculation it is possible to emphasize this correlation. This was confirmed by the significant differences in the evaluated mycotoxin content between treatments with natural contamination and artificial *Fusarium* spp. inoculation in both growing systems. Differences between organic and conventional growing systems within each of them were statistically insignificant; however, the treatments growing in the organic system showed a better resistance against *Fusarium* spp. inoculation. This higher resistance was evident mainly for results of DON content after artificial inoculation (7942 μg kg^{-1} in the organic system compared to 12324 μg kg^{-1} in conventional system).

Table 1. Mycotoxin content (μg kg^{-1}) in rye grain with *Fusarium* spp. inoculation and natural contamination, in organic and conventional growing system (average data of both cultivars)

Treatment	Growing system	DON	D3G	3-ADON	ZON
Artificial inoculation	organic	7 495.2 b	3 455.2 b	182.3 ab	759.2 ab
	conventional	12 324.8 b	3 043.9 b	285.7 b	1164.9 b
Natural contamination	organic	1 929.5 a	481.7 a	62.4 a	296.7 a
	conventional	2 155.6 a	492.3 a	87.4 a	315.3 a

Note. DON – deoxynivalenol, D3G – deoxynivalenol-3-β-D-glucoside, 3-ADON – 3-acetyldeoxynivalenol, ZON – zearalenone; values with different letter combinations are statistically significant at $p \leq 0.05$ (probability of error max. 5%).

There are inconsistent findings about the effect of *Fusarium* spp. contamination on the protein content in grain. Some papers indicate an increase in protein content (Boyacioğlu, Hettiarachchy, 1995), but other studies suggest a subtle decrease (Gärtner et al., 2008). Our results for rye showed in Table 2 imply a slight increase in the protein content indicating quality changes of the protein. Lower values of falling number found in the artificially inoculated treatments were probably caused by the degradation of starch due to the invoked higher enzyme activity in kernels (Hareland, 2003).

Table 2. Technological characteristics of rye grain with *Fusarium* spp. inoculation and natural contamination, in organic and conventional growing system (average data of both cultivars)

Treatment	Growing system	Crude protein %	Falling number s	Volume weight kg hl^{-1}	Thousand kernel weight g
Artificial inoculation	organic	10.9 ab	171.3 a	64.6 ab	31.3 a
	conventional	11.8 b	146.5 a	62.3 a	31.9 a
Natural contamination	organic	10.1 a	207.0 a	68.1 b	35.3 b
	conventional	11.2 ab	194.8 a	67.0 b	36.0 b

Note. Values with different letter combinations are statistically significant at $p \leq 0.05$ (probability of error max. 5%).

These conclusions were corroborated by the correlations between the mycotoxin content and technological characteristics of rye flour for the rye cultivars in both growing systems (Table 3).

Positive correlations were found between the content of DON and the protein content in rye grain likely due to low volume weight and thousand kernel weight. The significant negative correlation was determined between the DON, 3-ADON and ZON content and the values of falling number indicating grain starch damage (correlation: −0.69*, −0.87** and −0.81**). The negative effect of the *Fusarium* infection on starch granules has already been published (Wang et al., 2005).

Table 3. Correlation between rye flour technological parameters and the mycotoxin content (μg kg^{-1})

	DON	D3G	3-ADON	ZON
Crude protein %	0.49	0.15	0.16	−0.01
Falling number s	−0.69*	−0.33	−0.87**	−0.81**
Volume weight kg hl^{-1}	−0.32	−0.39	−0.04	0.04
Thousand kernel weight g	−0.26	−0.52*	0.07	0.18

Note. DON – deoxynivalenol, D3G – deoxynivalenol-3-β-D-glucoside, 3-ADON – 3-acetyldeoxynivalenol, ZON – zearalenone; * – statistically significant at $p \leq 0.05$, ** – at $p \leq 0.01$.

Mixolab. Unfortunately, there are almost no published studies concerning the rheological behaviour of rye flour measured by the system Mixolab therefore it is not possible to compare our results with those of other studies.

The averaged characteristics for both rye cultivars with artificial and natural *Fusarium* spp. contamination are shown in Table 4. Compared with the available published results for wheat cultivars (Chen et al., 2013) there was found a significantly lower time of dough development (C1) that increased slightly for the artificially inoculated rye. This very low development time is in contrast with the results of Banu et al. (2010), who detected longer development time after addition of rye to wheat flour. The reason for this disagreement could be that in our study we measured separated rye flour with different physical properties. Values of the protein weakening represented by C2 values were on a rather lower level by comparison with wheat flour (Banu et al., 2011). The negative effect of *Fusarium* inoculation was again reflected on a higher protein weakening, based on the mechanical work and an increasing temperature, represented by the characteristic C1–C2. The better results in this protein part of the Mixolab curve were achieved for the conventional growing system.

Table 4. Average Mixolab characteristics of rye flour from treatments with *Fusarium* spp. inoculation and natural contamination, in organic and conventional growing system (average data of both cultivars)

Treatment	Growing system	C1 (min)	C2 (Nm)	C3 (Nm)	C4 (Nm)	C5 (Nm)	C1–C2 (Nm)	C3–C4 (Nm)	C5–C4 (Nm)	DS (min)
Artificial inoculation	organic	0.93 b	0.31 a	2.44 a	1.15 a	1.33 a	0.80 b	1.29 a	0.19 a	2.13 b
	conventional	0.81 ab	0.35 a	2.46 a	1.08 a	1.55 a	0.76 b	1.38 a	0.46 a	1.78 ab
Natural contamination	organic	0.52 a	0.40 ab	3.07 a	1.51 a	2.01 a	0.68 ab	1.56 a	0.49 a	1.31 ab
	conventional	0.75 ab	0.52 b	2.86 a	1.25 a	1.68 a	0.56 a	1.61 a	0.50 a	1.19 a

Note. C1 – time required for maximum torque during mixing, C2 – protein weakening, C3 – starch gelatinization, C4 – stability of gel, C5 – starch retrogradation, C1–C2 – fall of protein strength, C3–C4 – diastatic activity, C5–C4 – anti-stalling effect, DS – time of dough stability before weakening; values with different letter combinations are statistically significant at $p \leq 0.05$ (probability of error max. 5%).

With regard to the starch characteristics of the second part of the Mixolab curve, there were confirmed no statistically significant differences between the individual growing systems within the same treatment. Despite the shifts of individual curves (Fig. 2) resulting starch parameters for the various types of treatment were statistically insignificant but there is a visible distinct effect of the year. The values in the starch part of Mixolab curves are much lower for all treatments in 2011 than in 2012, indicating more damaged starch grains. These results correspond to lower falling number values in 2011 (101–162 s) compared with values in 2012 (163–285 s). This big difference was probably caused by heavy rainfall in July 2011 (166 mm), which was twice as high as in the same period in 2012. The effect of wet conditions on starch grains was mentioned also in other studies (Beckles, Thitisaksakul, 2013).

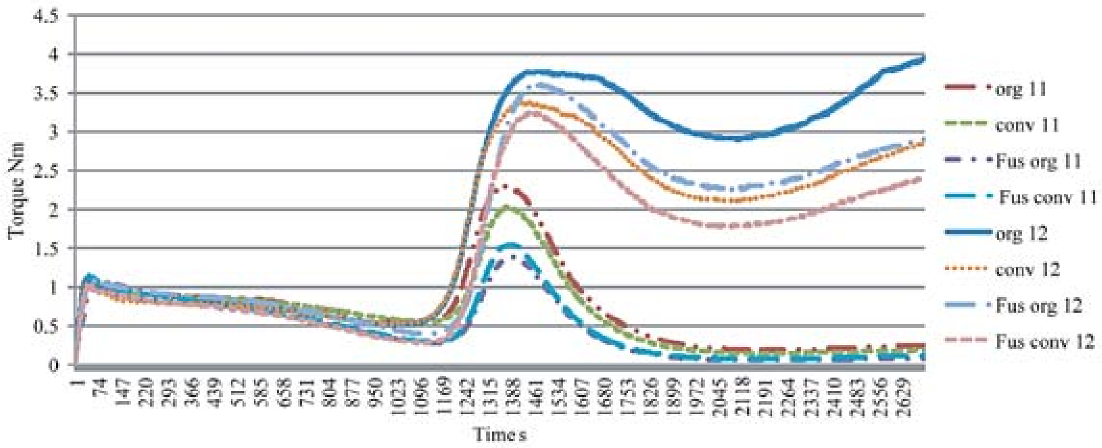

Figure 2. Mixolab curve of rye cultivar 'Askari' with *Fusarium* spp. (Fus) inoculation and natural contamination, in organic (org) and conventional (conv) growing system, in 2011 and 2012

The above mentioned findings were also confirmed by the correlation between mycotoxin content indicating a *Fusarium* spp. infection and Mixolab parameters (Table 5). Significant negative correlations were found not only for the parameters relating to the quality of starch but worse protein quality was also visible (−0.57* between C2 and DON content, 0.58* between C1–C2 and DON content).

Table 5. Correlation between Mixolab parameters and the mycotoxin content (μg kg^{-1}) for the rye cultivars in both growing systems

	DON	D3G	3-ADON	ZON
C1 (min)	0.46	0.49	0.54*	0.55*
C2 (Nm)	−0.53*	−0.46	−0.46	−0.41
C3 (Nm)	−0.57*	−0.31	−0.86**	−0.88**
C4 (Nm)	−0.42	−0.18	−0.73**	−0.77**
C5 (Nm)	−0.41	−0.21	−0.72**	−0.76**
C1–C2 (Nm)	0.58*	0.54*	0.53*	0.44
C3–C4 (Nm)	−0.23	−0.26	−0.10	−0.02
C5–C4 (Nm)	−0.36	−0.27	−0.65*	−0.70*
DS (min)	0.45	0.74**	0.56*	0.65*

C1 – time required for maximum torque during mixing, C2 – protein weakening, C3 – starch gelatinization, C4 – stability of gel, C5 – starch retrogradation, C1–C2 – fall of protein strength, C3–C4 – diastatic activity, C5–C4 – anti-stalling effect, DS – time of dough stability before weakening; DON – deoxynivalenol, D3G – deoxynivalenol-3-β-D-glucoside, 3-ADON – 3-acetyldeoxynivalenol, ZON – zearalenone; statistically significant for $p \leq 0.05$ (*) and $p \leq 0.01$ (**)

Conclusion

The negative effect of *Fusarium* spp. contamination on the quality of rye (*Secale cereale* L.) grain and flour was confirmed not only by the standard methods for technological quality determination but also by the rheological system Mixolab. This system reflected a good sensivity to the quality changes evoked by *Fusarium* spp. infection. The significantly worsened rheological quality and the negative effect especially on the starch part of the Mixolab curves were observed in rye artificially inoculated by *Fusarium* spp. compared to rye with natural *Fusarium* spp. contamination. No significant statistical differences were determined for both growing systems. There was only a visible contrast between the monitored years.

Our results confirmed that some of the problems related to the rheological properties of flour during processing, which regularly occur in some years and in some areas may be caused by *Fusarium* spp. contamination. However, if the *Fusarium* spp. contamination is not so high and the content of mycotoxins in the kernels does not exceed the allowed hygienic limit, this flour can be used (after modification of the processing technology, if necessary).

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Kokybiniai pokyčiai rugių grūduose ir miltuose po užkrėtimo *Fusarium* spp., nustatyti standartiniais metodais ir taikant sistemą Mixolab

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Santrauka

Taikant reologinę sistemą *Mixolab*, buvo tirta rugių grūdų užkrėtimo *Fusarium* spp. įtaka miltų reologinei kokybei, juos auginant ekologinėje ir tradicinėje sistemose. Buvo nustatyti mikotoksinų (DON, D3G, 3-ADON ir ZON) kiekiai ir standartiniai technologiniai grūdų kokybės rodikliai. Esmingai prastesni technologiniai ir reologiniai rodikliai, parodantys krakmolo kokybę, buvo nustatyti *Fusarium* spp. dirbtinai užkrėstiems rugiams abiejose auginimo sistemose, palyginus su *Fusarium* spp. natūraliai užsikrėtusiais rugiais. Esminė neigiama koreliacija, rodanti prastėjančią miltų reologinę kokybę, buvo nustatyta tarp mikotoksinų kiekio ir kritimo skaičiaus (−0,69* mikotoksinui DON), taip pat tarp mikotoksinų kiekio ir krakmolo geliacijos greičio, kurį rodo *Mixolab* parametras C3 (−0,57* mikotoksinui DON, −0,86** – 3-ADON ir −0,87** – ZON). Nepaisant ekologinės ir tradicinės sistemos mėginių *Mixolab* kreivėse matomų nuokrypių, *Mixolab* nustatytos savybės buvo statistiškai panašios. Ryškūs *Mixolab* kreivių skirtumai, galimai sukelti oro sąlygų, buvo nustatyti tarp tirtų metų.

Reikšminiai žodžiai: auginimo sistema, mikotoksinai, *Mixolab*, *Secale cereale*.