Different grain grinding methods affect detection of *Fusarium graminearum* DNA and mycotoxins

Tapani YLI-MATTILA[1], Sari RÄMÖ[2], Taha HUSSIEN[1,4], Marion RAUVOLA[5], Veli HIETANIEMI[3] and Jukka KAITARANTA[5]

[1] Molecular Plant Biology, Department of Biochemistry, University of Turku, FI-20014 Turku, Finland
[2] Natural Resources Institute Finland (Luke), Management and Production of Renewable Resources, FI-31600 Jokioinen, Finland
[3] Natural Resources Institute Finland (Luke), Bio-Based Business and Industry, FI-31600 Jokioinen, Finland
[4] Mycotoxins Lab, Department of Toxins and Contaminants, National Research Center, Cairo, Egypt
[5] Turku University of Applied Sciences, FI-20520 Turku, Finland

Summary. *Fusarium graminearum* is the most important deoxynivalenol (DON) producing species in northern Europe and Asia. The highest DON levels in Finland have been found in oat grain, and DNA levels of *F. graminearum* are in agreement with DON levels, when DON is measured by accredited GC-MS. In addition to DON, 3ADON is present in Finnish grain samples. Large variations in DON content and amounts of *F. graminearum* DNA, and poor coefficient of determination (R^2) between these, have been detected in oat grain when the *RIDA®QUICK* SCAN kit results for DON content have been used. This study confirmed that the coefficient of determination was usually less when DNA or DON were extracted from oat flour, which was not ground with 0.8 mm or 1 mm sieves. DON levels obtained with the Rida®Quick method were usually higher than those obtained with accredited GC-MS in Finnish oat, barley and wheat samples. The homogenization of the oat flour by sieving is therefore likely to be connected to the variations in DON detection. Amounts of *F. graminearum* DNA and DON close to legislative limits should be reconfirmed with accredited quantitative analyses.

Key words: oat, barley, wheat, mycotoxins, qPCR.

Introduction

Fusarium head blight (FHB) was first described in Europe in England in 1884 (Parry *et al.*, 1995). By 1924, FHB was already found in England, Russia, Sweden, France, Italy, Germany, Holland and Norway. According to Sundheim *et al.* (2013), the first *F. graminearum* isolate in Norway was found from oat in 1911, while in Finland the fungus was reported in 1932 (Rainio, 1932). At present, FHB is common in all European countries producing wheat, maize, and other cereals such as barley and oat.

Fusarium graminearum sensu stricto, together with other closely related species of the *F. graminearum* species complex, is the most common cause of FHB in wheat, barley, oat and other small grain cereals in most parts of the world. The main species of *F. graminearum* species complex in Europe is *F. graminearum sensu stricto*, which is also the most important deoxynivalenol (DON) producer in most countries in Europe (Pasquali *et al.*, 2016; Yli-Mattila and Gagkaeva, 2016).

Fusarium graminearum DNA levels in oat are associated with DON levels, when DON is measured by gas chromatography-mass spectrometry (GC-MS). When the Rida® Quick Lateral Flow test results for DON content were compared to DNA levels of *F. graminearum*, the relationships between the two were much less distinct. The homogenization of the oat flour during grinding of grain and passage through a 1 mm sieve is likely to be connected to this variation (Yli-Mattila *et al.*, 2013).

In the Nordic countries, high levels of *F. graminearum* and DON have been found especially in oat grain,

Corresponding author: T. Yli-Mattila
E-mail: tymat@utu.fi

while in north-western Russia *F. graminearum* was found only after 2003 (Yli-Mattila *et al.*, 2009; Yli-Mattila and Gagkaeva, 2010; Fredlund *et al.*, 2013; Sundheim *et al.*, 2013). High grain infestation levels of *F. graminearum* and amounts of DON have caused serious losses in germination at many instances e.g. in Norway (Kimen Seed Laboratory, 2009). Increased *F. graminearum* and DON levels may be associated with climate change (van der Fels-Klerx *et al.*, 2012). These effects have led the requirement for seed imports into Norway.

Recently, *F. graminearum* has been spreading northward in Europe (e.g., Waalwijk *et al.*, 2003; Nicholson *et al.* 2003; Stepien *et al.*, 2008; Nielsen *et al.*, 2011; Talas *et al.*, 2011; Somma *et al.*, 2014; Suproniene *et al.*, 2010, 2016) displacing the closely related *F. culmorum*. This shift may be due to changing agricultural practices, climate change and increased maize cropping. In Finland, *F. graminearum* was reported in cereals in 1932 (Rainio, 1932) and in the 1960s (Uoti and Ylimäki, 1974), and this fungus has been present since then (Ylimäki *et al.*, 1979; Eskola *et al.*, 2001). In north-western Russia *F. graminearum* was found more recently (Yli-Mattila *et al.*, 2009). *Fusarium graminearum* is also present in Norway, Sweden and Estonia (Yli-Mattila *et al.*, 2011). According to Sundheim *et al.* (2013) and Aamot et al. (2015), *F. graminearum* has become the dominant DON-producing species in Norway since 2000. Also in Finland, *F. graminearum* is now the main DON-producer in cereals (Yli-Mattila, 2010, Hietaniemi et al., 2016)

The 3ADON chemotype of *F. graminearum* is dominant in most northern areas, while the 15ADON chemotype is predominating in Central and southern Europe (Pasquali *et al.*, 2016). The north-western Russian isolates belong to the 3ADON chemotype, like Finnish isolates and unlike most isolates from southern Russia. This suggests that the north-western Russian *F. graminearum* populations may have originated from Finland, while the southern Russian populations may be more closely related to the central and southern European 15ADON populations, as suggested by Yli-Mattila (2010).

Several immunological methods have been developed for estimation of DON levels in routine analyses carried out by food laboratories. However, antibodies may show cross-reactivity with analogous compounds. Thus, DON can be overestimated, with 15ADON, 3ADON, DON-3β-glucoside and other DON derivatives, which are masking compounds formed by plant detoxification activity (Lemmens *et al.*, 2005; Audenaert *et al.*, 2013) , while matrix effects can lead to over- or under-estimation of mycotoxins (Aamot *et al.*, 2012).

The aim of the present study was to investigate DON levels in Finnish cereal grain, obtained with accredited GC-MS and Rida®Quick methods, to compare these with *F. graminearum* DNA levels, and also measure effects of grain grinding methods on DON and *F. graminearum* DNA levels.

Materials and methods

Fusarium isolate

The single spore isolate G243 of F. graminearum was used as the standard in qPCR assays, as described by Yli-Mattila *et al.* (2008, 2011).

Mycotoxin analyses

Oat grain samples (21 samples) of the year 2013 were a part of the total number of samples analyzed for DON, 3ADON, 15ADON and other trichothecenes at MTT Agrifood Research Finland (Natural Resources Institute Finland, FI-31600 Jokioinen, Finland). These were selected from 125 oat samples, so that in 19 samples had DON levels greater than 1,750 ppb, one had 1,100 ppb DON and one had less than 200 ppb. In addition, the DON levels in 20 oat samples from the year 2013 were estimated using Rida® Quick Scan mycotoxin tests (R-Biopharm AG). These samples were selected so that 15 contained more than 1,750 pbb of DON and five samples had less than 1,750 ppb. At MTT, the trichothecenes were extracted and analyzed with an accredited GC-MS method, as described by Hietaniemi *et al.* (2004) and Yli-Mattila *et al.* (2011). The grain flour was produced using a Falling number hammer mill KT-120 (Koneteollisuus Oy) using a 1 mm sieve in order to obtain homogenous samples and to achieve good repeatability in mycotoxin and DNA measurements. The grain samples from a major milling company were used for Rida®Quick measurement of DON and they were ground using a separate mill without a sieving procedure prior to toxin measurement.

DNA extraction

At the University of Turku, the 21 oat grain samples from MTT and the 20 samples from the milling company used for mycotoxin analyses were ground

for DNA extraction using a mill (Krups KM75 Coffee Grinder) without sieving as described by Yli-Mattila *et al.* (2013). DNA was extracted from ground grain samples and from the *F. graminearum* isolate G243 standard using the GenElute™ Plant Genomic DNA kit (Sigma-Aldrich), as described by Yli-Mattila *et al.*, (2008, 2009, 2013).

Quantitative PCR

The TMFg12 primers and probe have been designed for the *F. graminearum* specific RAPD-PCR product (Yli-Mattila *et al.*, 2008). An IQTM5 Real-Time PCR Detection System (Bio-Rad) was used for running qPCR samples. Amounts of *Fusarium* species DNA in grain samples were determined as total DNA quantified by a Qubit fluorometer (Invitrogen), as described by Yli-Mattila *et al.* (2011).

Rida®Quick DON analyses

Oat (30), wheat (20) and barley (20) samples from different locations in Finland were analyzed using the Rida®Quick DON method at Turku University of Applied Sciences (TUAS), and the results were compared with the accredited GC-MS method described above. The reproducibility of the method was tested with six parallel samples from the three cereals, with three different DON levels. Ground oat samples (12) were also obtained from an industrial laboratory (Laboratory 1), and 12 ground wheat samples were obtained from a second industrial laboratory (Laboratory 2).

Grain samples for comparing the two methods for measuring DON levels were ground using three mills. In Laboratory 1 they were ground using laboratory mill KT 30 (Koneteollisuus OY) without sieving, while in the Laboratory 2 the samples were ground by laboratory mill 3100 Falling number with a sieve of 0.8 mm. At MTT, a laboratory Falling number hammer mill with a 1 mm sieve was used as described above.

Each ground grain sample (1 g) was placed in a Falcon tube and 15 ml of Rida®Quick DON extraction buffer was added. The mixture was manually shaken for 3 min before centrifugation for 10 min (3,500 g). The clear supernatant (100 μL) was pipetted onto the application area of the test strip and the result was read after 5 min.

Statistical analyses

R^2 (coefficient of determination), regression slope and *P* (significance of regression slope) were calculated using the SigmaPlot version 12.0 (SPSS Inc.). The original DNA and toxin concentrations were transformed to logarithmic values $[1 + \lg (x)]$ to obtain normal distribution for these parameters. Samples giving concentrations less than 0.5 mg kg^{-1} or greater than 5.5 mg kg^{-1} with Rida®Quick were excluded from the analyses, because these values were outside the linear part of the detection.

The reproducibility between amounts of DON estimated with Rida®Quick in oat grain samples was tested using three samples with DON levels of 1.4, 1.7 and 2.1 mg kg^{-1} (n = 6, Table 1). Commercial standard (Check sample Deoxynivalenol in wheat. Romer Labs. 1.431 ± 0.2566 mg kg^{-1}) was also used. In barley, the DON levels were 0.81, 1.30 and 1.60 mg kg^{-1}, while in wheat they were 0.79, 1.00 and 1.40 mg kg^{-1}. Rida®Quick results were compared to accredited GC-MS results using regression analysis, and paired t-tests were applied to reveal the 95 % significance level. Relative standard deviation (RSD %) was also calculated.

Table 1. The reproducibility of DON detection results (mean ± SE of six DON extractions and DON measurements for each ground grain sample obtained from MTT) in oat, barley and wheat grain samples with three different DON contents.

Grain	MTT result (mg kg^{-1})	Rida®Quick result (mg kg^{-1} ± SE)	RSD %
Oat	1.40	1.69 ± 0.04	5.27
	1.70	1.98 ± 0.07	8.51
	2.10	2.32 ± 0.09	9.87
Barley	0.81	1.80 ± 0.16	22.3
	1.30	2.30 ± 0.12	12.7
	1.60	2.32 ± 0.150	15.8
Wheat	0.79	1.32 ± 0.06	22.6
	1.00	1.59 ± 0.15	12.0
	1.40	2.11 ± 0.10	10.3

Results

In the reproducibility analysis between the Rida®Quick and GC-MS detection methods, most results gave RSD percentages equal to or less than 20, especially when the DON levels were close to or above the allowed limit (Table 1).
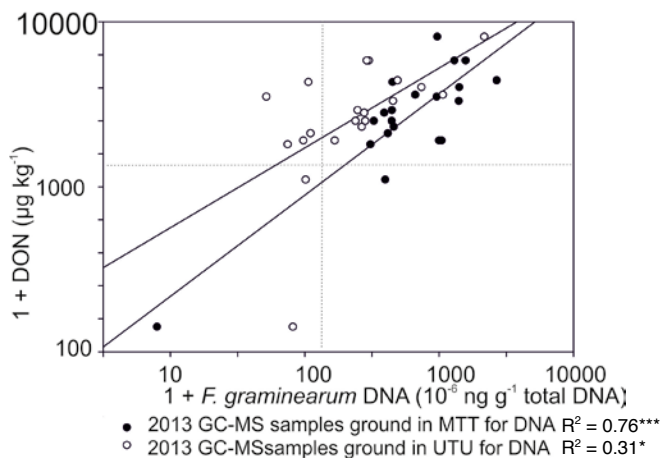
The correlation between *F. graminearum* DNA and DON levels in Finnish oat samples from the year 2013 was greater ($R^2 = 0.76$), when the DNA was extracted and measured from grain samples ground at MTT using a mill with a sieve. When the *F. graminearum* DNA was measured from the same grain samples ground at the University of Turku, using a method lacking the sieve, the correlation was less ($R^2 = 0.31$, Figure 1). The R^2 value from 20 oat samples from a food company was 0.71, which was also less than for grain samples ground at MTT (Figure 2).

There was a good correlation between the DON levels obtained from 30 oat, 20 barley and 21 spring wheat samples using accredited GC-MS and the Rida®Quick method (Figure 3). Pearson correlation values were 0.935, 0.931 and 0.935, respectively, for oat, barley and spring wheat samples. R^2 values were 0.88, 0.80 and 0.89, respectively, for the oat,

barley and spring wheat samples. However, paired t-tests (t-values 5.694, 7.854 and 3.544, respectively, for the three grain types) showed differences at the 95% significance level between the Rida®Quick and GC-MS results in all three cereals. This was mainly due to the greater DON values obtained using Rida®Quick method, including seven false positive oat and five false positive barley and wheat samples with Rida®Quick. 3ADON levels were measured in oat samples and they were usually about 10–20% of the DON levels.

The reproducibility of DON levels is shown in Table 1. Commercial wheat standard (Romer Labs 1.431 mg kg⁻¹) resulted on average 2.11 mg kg⁻¹ with RSD 21 %.

When the DON levels from oat grain samples ground at MTT and analyzed with the accredited GC-MS were compared to those obtained from the same samples in two industrial laboratories and TUAS with the Rida®Quick method, the correlations were high (R^2 usually greater than 0.89; Figure 4), although DON amounts obtained with GC-MS were usually less in oat, wheat and barley samples (Table 1). The correlation was also good, when DON levels from wheat grain samples ground in Laboratory 2 with a mill having a sieve were compared with the Rida®Quick method in the three laboratories ($R^2 >$


Figure 1. Correlation between log *Fusarium graminearum* DNA and log deoxynivalenol (DON) concentration in Finnish oat grain samples in 2013. In all samples DON was measured at MTT using accredited GC-MS. DNA was measured either from grain samples ground, at MTT, in a mill with a sieve or from the same grain samples ground with a mill without a sieve, at UTU. Regression slopes and R2 (coefficient of determination) values are shown. * and *** indicate statistically significant (*P*<0.05 or 0.001).
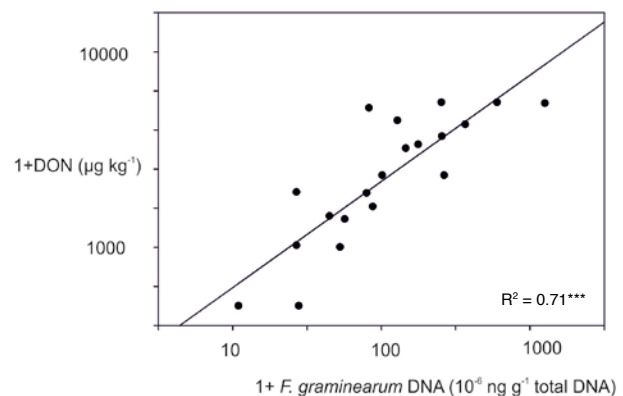

Figure 2. Correlation between log *Fusarium graminearum* DNA and log deoxynivalenol (DON) concentrations in Finnish oat grain samples in 2013. DON level was estimated using the RIDA® QUICK SCAN. Oat samples were ground by a milling company for DON analysis, or by Turku University without sieving for DNA extraction. Regression slopes and R2 (coefficient of determination) values are shown. *** indicates statistically significant (*P*<0.001)
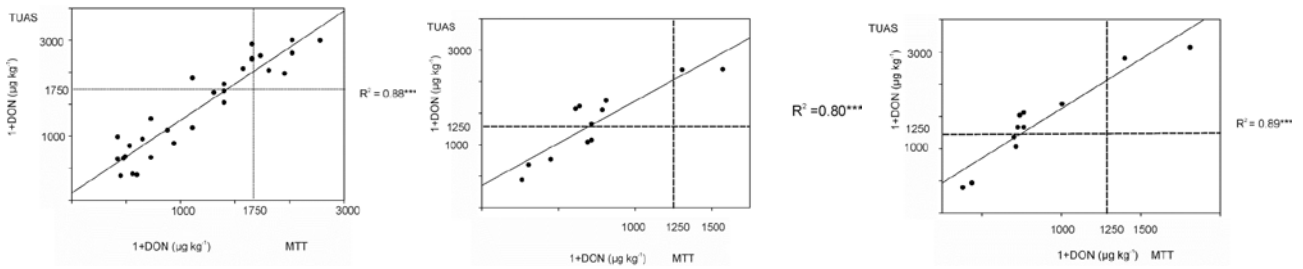
Figure 3. Correlations between log deoxynivalenol (DON) concentrations in Finnish oat (a), barley (b) and wheat (c) grain samples in 2013, estimated using the RIDA® QUICK SCAN compared with to log DON concentrations obtained at MTT by using GC-MS. The samples were ground using the same mill at MTT for DON estimation, with sieving. Regression slopes and R2 (coefficient of determination) values are shown. *** indicates statistically significant ($P<0.001$).
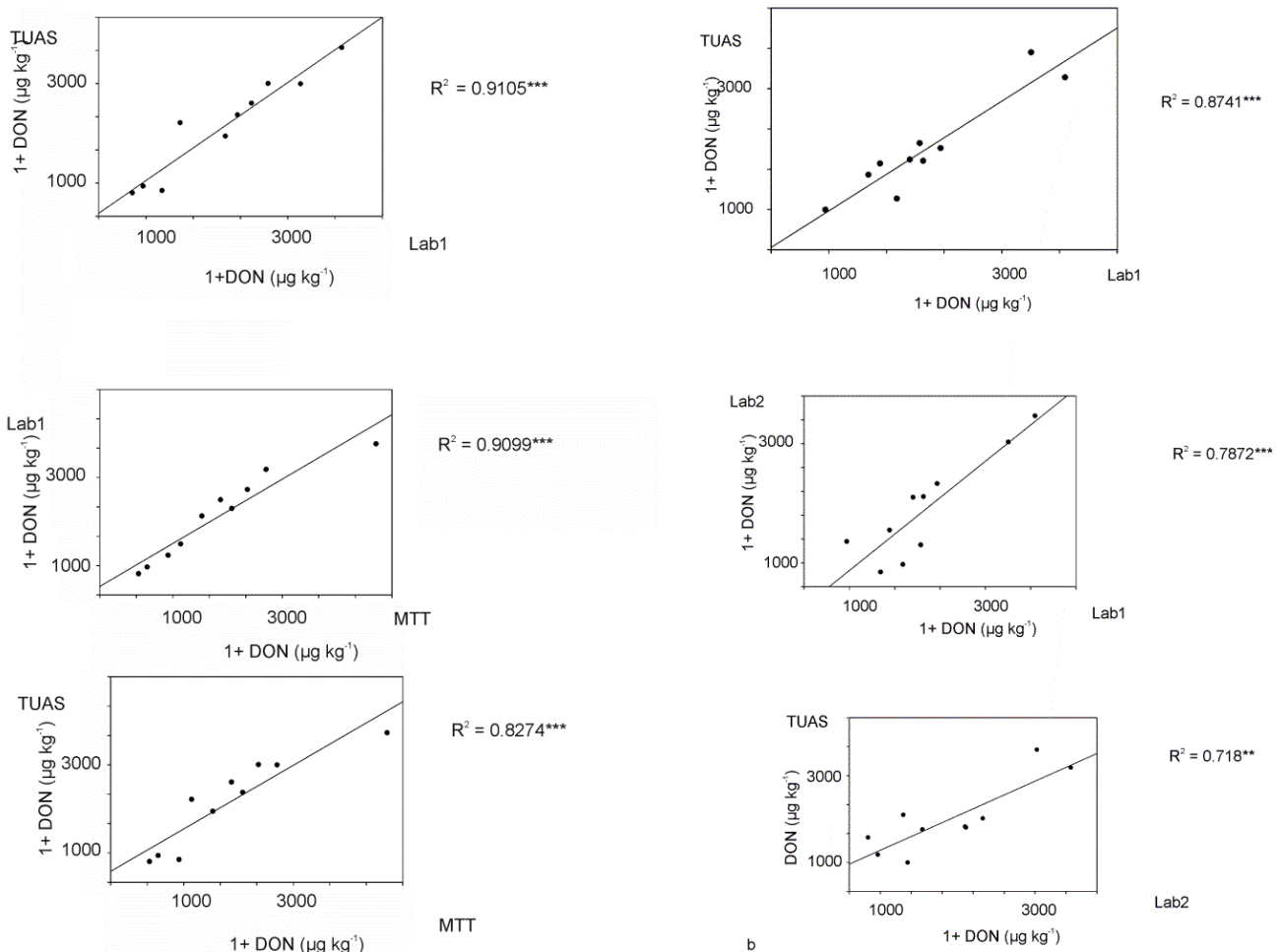
Figure 4. Correlations between log DON concentrations in oat grain processed at two milling company laboratories (Lab 1 and Lab 2), compared with DON concentrations measured at TUAS and MTT. The grain samples were ground at MTT (a) in a mill with a sieve, or at Lab 1 (b) in a mill without a sieve. *** and ** indicate statistically significant ($P<0.001$ or 0.01).
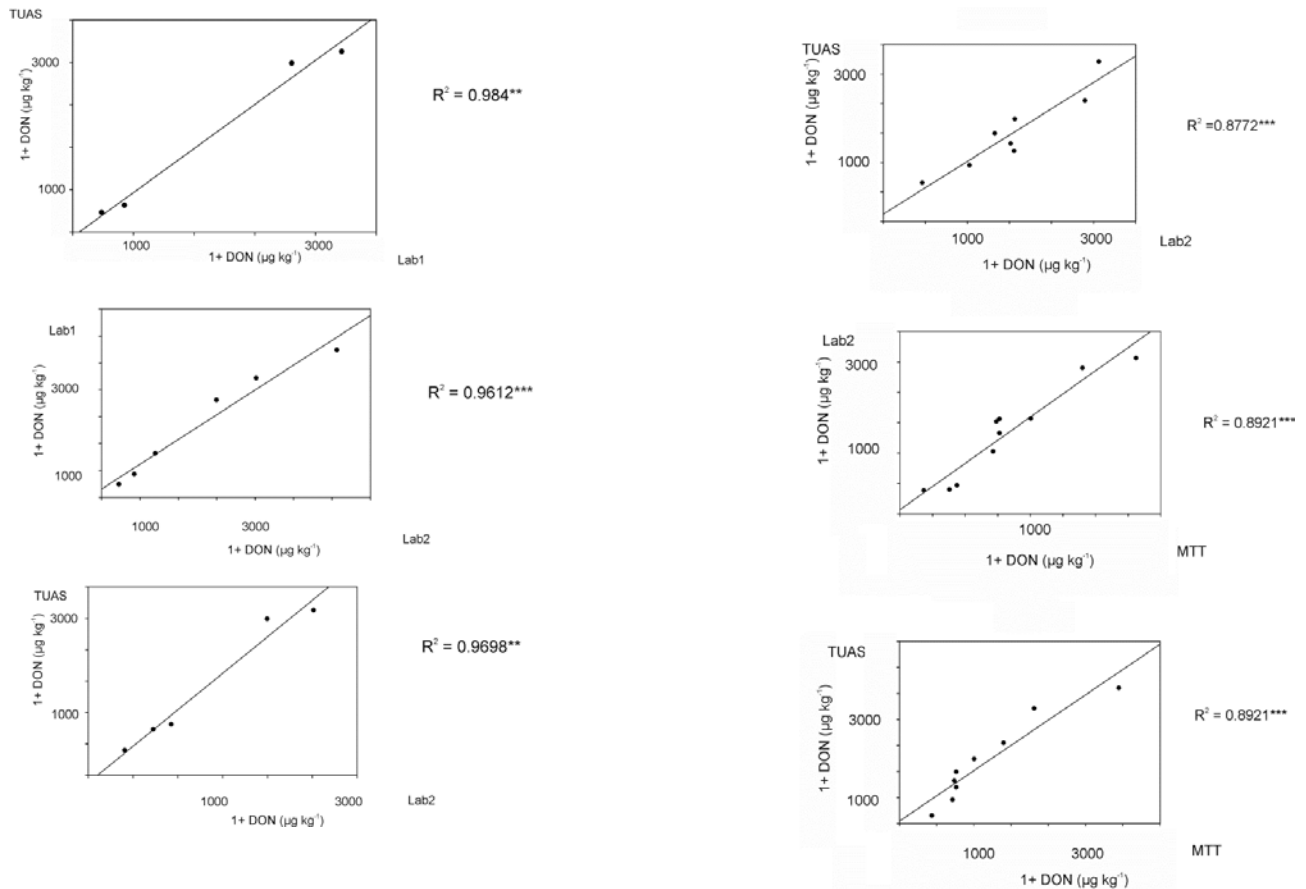
Figure 5. Correlations between log DON concentrations in wheat grain samples ground at Lab 2 (a) and MTT (b), both in mills with sieves. *** indicates statistically significant (*P*<0.001 or <0.01).

0.9, Figure 5). In contrast, when DON levels from oat grain samples ground in Laboratory 1 with a mill without a sieve were compared with Rida®Quick method in the three laboratories, the correlations were less correlated (usually < 0.8, Figure 5).

Discussion

Our results indicate that the semiquantitative Rida®Quick method for DON detection in most cases met the requirements of the Commission Regulation (EC) 401/2006 for DON determination, since the RSD percentage values were equal to or less than 20.

DNA levels of *F. graminearum* in oat grains were, in all cases, in agreement with DON levels when DON was measured by accredited GC-MS. When the Rida®Quick DON kit results were compared

to DNA levels of *F. graminearum*, the variation was much greater. Securing the homogenization of the oat flour using a 1 mm sieve as compared to grinding without a sieve was associated with the variations of DON and *F. graminearum* DNA levels. These results are in agreement with previous results (Yli-Mattila *et al.*, 2013; Hietaniemi *et al.*, 2016).

More false positive results were also found in all three cereals when using the Rida®Quick method as compared to accredited GC-MS detection. This was due to the generally higher DON levels obtained with the Rida®Quick method. The false positive results may have been at least partially due to 3ADON found detected in oat samples. Excessively high values of DON have also been previously detected using some rapid immunological tests (e.g. Aamot *et al.*, 2012).

The DON levels obtained with the Rida®Quick method were usually greater than those obtained

with the accredited GC-MS method, in all three cereals. However, the semiquantitative Rida®Quick DON method used here met, in most cases, the requirements of the Commission regulation (EC) 401/2006 for a quantitative DON determination. This should show RSD % equal or less than 20. But with low DON levels in barley and wheat, the RSD % was above 20%.

For consumers, low DON amounts guarantee that the DON levels are below the EU limit, but for farmers they mean that their crops may be rejected due to false positive DON values. We conclude that both *RIDA® QUICK* SCAN and qPCR can be applied to screening for detection of high DON levels in cereal grain samples, but the Rida®Quick assays indicating DON contents close to the legislative limits should be repeated and, if necessary, reconfirmed with an accredited quantitative analysis. In future it is important that assay results obtained from different rapid immunoassay kits used by food industries are compared to those obtained using accredited chemical analyses.

Acknowledgements

Literature cited

Aamot H.U., I.S. Hofgaard, G. Brodal, O., Elen, M. Jestoi and S.S. Klemsdal, 2012. Evaluation of rapid test kits for quantification of deoxynivalenol in naturally contaminated oat and wheat. *World Mycotoxin Journal* 5, 329–350.

Aamot H.U., T.J. Ward, G. Brodal, T. Vrålstad, G.B. Larsen, S.S. Klemsdal, A. Elameen, S. Uhlig and I.S. Hofgaard, 2015. Genetic and phenotypic diversity within the *Fusarium graminearum* species complex in Norway. *European Journal of Plant Pathology* 142, 501–519

Audenaert K., M. De Boevre, A. Vanheule, J. Callewaert, B. Bekaert, M. Höfte, S. De Saeger, G. Haesaert, 2013. Mycotoxin glucosylation in commercial wheat varieties: Impact on resistance to *Fusarium graminearum* under laboratory and field conditions. *Food Control* 34, 756–762

Eskola M., P. Parikka and A. Rizzo, 2001. Trichothecenes, ochratoxin A and zearalenone contamination and *Fusarium* infection in Finnish cereal samples in 1998. *Food Additives and Contaminants* 18, 707–718.

Fredlund E., A. Gidlund, M. Sulyok, T. Börjesson, R. Krska, M. Olsen and M. Lindblad 2013. Deoxynivalenol and other

selected *Fusarium* toxins in Swedish oats - Occurrence and correlation to specific *Fusarium* species. *International Journal of Food Microbiology* 167, 276–283.

Hietaniemi V., M. Kontturi, S. Rämö, M. Eurola, A. Kangas, M. Niskanen and M. Saastamoinen, 2004. Contents of trichothecenes in oat during official variety, organic cultivation and nitrogen fertilization trials in Finland. *Agricultural and Food Science* 13, 54–67.

Hietaniemi V., S. Rämö, T. Yli-Mattila, M. Jestoi, S. Peltonen, M. Kartio, E. Sieviläinen, T. Koivisto and P. Parikka, 2016. Updated survey of the *Fusarium* species and toxins in Finnish cereal grains. *Food Additives and Contaminants* 33, 831–848.

Lemmens M., U. Scholz, F. Berthiller, C. Dall'Asta, A. Koutnik, R. Schuhmacher, G. Adam, H. Buerstmayr, A. Mesterházy and R.Krska, 2005. The ability to detoxify the mycotoxin deoxynivalenol colocalizes with a major quantitative trait locus for Fusarium head blight resistance in wheat. *Molecular Plant Microbe Interact*ion 18, 1318–1324.

Nicholson P., E. Chandler, R.C. Draeger, N.E. Gosman, D.R. Simpson, M. Thomsett and A.H. Wilson 2003. Molecular tools to study epidemiology and toxicology of Fusarium head blight of cereals. *European Journal of Plant Pathology* 109, 691–703.

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

Parry D.W., P. Jenkins and L. McLeod, 1995. *Fusarium* ear blight (scab) in small grain cereals – a review. *Plant Pathology* 44, 207–238.

Pasquali M., M. Beyer, A. Logrieco, K. Audenaert, V. Balmas, R. Basler, A-L. Boutigny, J. Chrpová, E. Czembor, T. Gagkaeva, M.T. González-Jaén, I.S. Hofgaard, N.D. Köycü, L. Hoffmann, J. Levic, P. Marin, T. Miedaner, Q. Migheli, A. Moretti, M.E.H. Müller, F. Munaut, P. Parikka, M. Pallez-Barthel, J. Piec, J. Scauflaire, B. Scherm, S. Stankovic, U. Thrane, S. Uhlig, A. Vanheule, T. Yli-Mattila and S. Vogelgsang, 2016. A European database of *Fusarium graminearum* and *F. culmorum* trichothecene genotypes. *Frontiers in Microbiology* 7, 406.

Somma S., A.L. Petruzzella, A.F. Logrieco, G. Meca, O.S. Cacciola and A. Moretti, A. 2014. Phylogenetic analyses of *Fusarium graminearum* strains from cereals in Italy, and characterisation of their molecular and chemical chemotypes. *Crop & Pasture Science* 65, 52–60

Stepien L., D. Popiel, G. Koczyk and J. Chelkowsky 2008. Wheat-infecting *Fusarium* species in Poland – their chemotypes and frequencies revealed by PCR assay. *Journal of Applied Genetics* 49, 433–441.

Suproniene S., A.F. Justesen, M. Nicolaisen, A. Mankeviciene, Z. Dabkevicius, R. Semaskiene and A. Leistrumait, 2010. Distribution of trichothecene and zearalenone producing *Fusarium* species in grain of different cereal species and cultivars grown under organic farming conditions in Lithuania. *Annals of Agricultural and Environmental Medicine* 17, 73–80.

Suproniene S., S. Sakalauskas, K. Sturmbriene, R. Zvirdauskiene and P. Svegzda 2016. Variances in trichothecene

chemotype distribution in Lithuanian wheat grain and within pure culture *Fusarium graminearum* isolated from the same grain samples. *European Journal of Plant Pathology* 144, 371–381.

Sundheim L., G. Brodal, I.S. Hofgaard and T. Rafoss, T, 2013. Temporal variation of mycotoxin producing fungi in Norwegian cereals. *Microorganisms* 1, 188–198.

Talas F., Parzies, H.K., Miedaner, T. 2011. Diversity in genetic structure and chemotype composition of *Fusarium graminearum* sensu stricto populations causing wheat head blight in individual fields in Germany. *European Journal of Plant Pathology* 131, 38–48.

Uoti J. and A. Ylimäki 1974. The occurrence of *Fusarium* species in cereal grain in Finland. *Annales Agriculturae Fenniae* 13, 5–17.

Van der Fels-Klerx H.J., S. Klemsdal, V. Hietaniemi, M. Lindblad, E. Iannou-Kakouri and E.D. Van Asselt 2012. Mycotoxin contamination of cereal grain commodities in relation to climate in North West Europe. *Food Additives and Contaminants* Part A. 29, 1581–1592.

Waalwijk C., P. Kastelein, I de Vries, Z. Kerenyi, T. van der Lee, T. Hesselink, J. Kohl. and G. Kema, 2003. Major changes in *Fusarium* spp. in the Netherlands. *European Journal of Plant Pathology* 109, 743–754.

Ylimäki A., H. Koponen, E-L. Hintikka, M. Nummi, M-L. Niku-Paavola, T. Ilus and T.M. Enari, 1979. Mycoflora and occurrence of *Fusarium* toxins in Finnish grain. Technical Research Centre of Finland, *Materials and Processing Technology publication 21*, Valtion Painatuskeskus, Helsinki, 1–28.

Yli-Mattila T., 2010. Ecology and evolution of toxigenic *Fusarium* species in cereals in northern Europe and Asia. *Journal of Plant Pathology* 92, 7–18.

Yli-Mattila T. and T. Gagkaeva, 2010. Molecular chemotyping of *Fusarium graminearum*, *F. culmorum* and *F. cerealis* isolates from Finland and Russia. In: *Molecular Identification of Fungi.* (Y. Gherbawy, K. Voigt, ed.), Springer Verlag, 159–177.

Yli-Mattila T. and T. Yu Gagkaeva, 2016. *Fusarium* Toxins in Cereals in Northern Europe and Asia. In: *Fungi: Applications and Management Strategies* (S.H. Deshmukh, J.K Misra, J.P. Tewari, P. Tamas, ed.), CRC Press, Taylor & Francis Group, 293–317.

Yli-Mattila T., S. Paavanen-Huhtala, P. Parikka, V. Hietaniemi, M. Jestoi, T. Gagkaeva, T. Sarlin, A. Haikara, S. Laaksonen and A. Rizzo, 2008. Real-time PCR detection and quantification of *Fusarium poae*, *F. graminearum*, *F. sporotrichioides* and *F. langsethiae* as compared to mycotoxin production in grains in Finland and Russia. *Archives of Phytopathology and Plant Protection* 41, 243–260.

Yli-Mattila T., P. Parikka, T. Lahtinen, S. Ramo, M. Kokkonen, A. Rizzo, M. Jestoi and V. Hietaniemi, 2009. In: *Fusarium DNA levels in Finnish cereal grains, Current Advances in Molecular Mycology* (Y. Gherbawy, R.L. Mach, M. Rai, ed.), Nova Science Publishers, Inc., New York, USA, 107–138.

Yli-Mattila T., S. Rämö, R.Tanner, H. Loiveke and V. Hietaniemi, 2011. *Fusarium* DNA levels as compared to mycotoxin levels in Finnish and Estonian grain samples. *Plant Breeding and Seed Science* 64, 131–140.

Yli-Mattila T., S. Rämö, T. Hussien, A.L. Carlobos-Lopez and C.J.R. Cumagun, 2013. Molecular quantification and genetic diversity of toxigenic *Fusarium* species in northern Europe as compared to those in southern Europe. *Microorganisms* 1, 162–174.