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The effects of nitrogen rates and intercropping on the occurrence of *Fusarium* spp. on barley kernels

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Abstract. The aim of the field experiments was to compare the effect of nitrogen rates and intercropping on the occurrence of *Fusarium* spp. in barley kernels. The experiments were performed in Central Estonia (58°33´N, 25°34´E) in 2009 and 2010. The composition of fungi in spring barley kernels was found through isolation and subsequent sequence analyses of the internal transcribed spacer (ITS) region and morphological features. During the study, 13 species of micro-fungi were identified in the grain samples. The most common species of fungi found in barley were *Fusarium avenaceum, Fusarium poae*, as well as *Phoma pinodella*. The compositions of pathogenic fungi on Estonian barley kernels were affected by the level of nitrogen fertilization and growing on barley-pea intercropping. The study showed tendencies that barley-pea intercropping had fewer occurrences of *Fusarium* species than sole barley.

Key words: *Fusarium* spp., spring barley, nitrogen rates, intercropping

INTRODUCTION

The occurrence of *Fusarium* fungi in small grain crops causes poor quality of the grain and reduction of the yield. The fungal infection of the grain leads to shrunken kernels and contamination with toxic compounds like mycotoxins. The most common disease of cereals is the Fusarium head blight (FHB) caused mainly by five toxic pathogenic species, like *Fusarium graminearum*, *Fusarium culmorum*, *Fusarium poae*, *Fusarium sporotrichioides* and *Fusarium avenaceum* (Champeil et al., 2004; Terzi et al., 2014). The species have been thoroughly studied and investigated worldwide (Wagacha & Muthomi, 2007; Xu et al., 2007; Fernandez et al., 2008; Miedaner et al., 2008; Yli-Mattila, 2010; Fredlund et al., 2013). Moreover, the earlier studies in Estonia have found that the most common diseases on cereals are leaf blights and root rot, following snow mould and FHB caused by *Microdochium nivale*, *Fusarium culmorum*, *Fusarium avenaceum*, *Fusarium sporotrichiella*, *Fusarium solani*, *Fusarium verticillioides* and *Fusarium oxysporum* (Lõiveke et al., 2004, 2008; Lõiveke, 2008).

The spread of pathogenic soil fungus *Fusarium* spp. is affected by various factors, like agro-meteorological conditions and agronomic practices. Weather conditions like warm or cool temperatures, low or high humidity and rare or intense precipitation during anthesis and maturity of cereals all strongly influence the occurrence of various *Fusarium* strains in different geographical locations (West et al., 2011; Bernhoft et al., 2012; Parikka et al., 2012; Popovski & Celar, 2013). Agronomic practices in the field have also an impact on the diversity and spread of *Fusarium* and other pathogenic fungi on grain. The results of previous studies have found that the preceding crops, fertilization, use of pesticides, crop variety, tillage and cultivation have an effect on the mycobiota in soil, plant and grain. In addition, there are reports that maize as preceding crop, cereals rich rotation, zero or minimal soil tillage and use of glyphosate favoured the spread of *Fusarium* fungi on cereals (Fernandez et al., 2008; Fernandez et al., 2009; Wegulo et al., 2015).

A good method of controlling plant diseases and *Fusarium* spp. is reasonable use of fertilizers in integrated plant production system. Moreover, nitrogen is the most important nutrient affecting disease development in cereal crops (Dordas, 2008). Hauggaard-Nielsen et al. (2008) summarized in the results of their study that intercropping of cereal with pulse improved the use of natural soil nitrogen and reduced the need for mineral nitrogen fertilizers. Cereal mixture with pulse also reduced incidence of the disease in the range of 20–40%. The effect of N fertilizers on occurrence of *Fusarium* spp. is not clear. Van der Burgt et al. (2011) and Krnjaja et al. (2015) reported that, enhancing the rate of N fertilization did not promote the infection of *Fusarium* in grain. On the other hand, Lemmens et al. (2004) and Suproniene et al. (2011) found that high rate of nitrogen increases occurrence of *Fusarium* fungi on grain. Still, little is known how intercropping of cereal with pulse affects the occurrence of *Fusarium* spp.

The aim of the current study was to compare *Fusarium* species composition in spring barley kernels under different rates of mineral nitrogen fertilizer and in pea-barley intercropping.

MATERIALS AND METHODS

The field experiment treatment

The field experiments were conducted in 2009 and 2010 on a *Podzoluvisol* (*PD*) (FAO, ISSS, ISRIC. 1998) in Central Estonia (58°33´N, 25°34´E). The experiment was laid out in randomized plots with design of four replicates. Plot size was 2.5 x 10 m. Winter wheat was the previous crop. In the autumn the experimental plots were ploughed. Barley variety 'Anni' and pea variety 'Clarissa' were grown in the experiments. Mineral complex fertilizer (Skalsa N5–$P_2O_5$10–K_2O25, dose of fertilizer 250 kg ha^{-1} and nitrogen amount 12.5 kg ha^{-1}) was sown together with the seeds for all the treatment plots. The seeds were sown in first decade of May. The ammonium nitrate fertilizer was added as top dressing in the beginning of tillering (GS 21).

The rates of nitrogen (N) were as follows:

1) barley with added ammonium nitrate (dose of fertilizer 312.5 kg ha^{-1}) – N_{120} kg ha^{-1};

2) barley with added ammonium nitrate (dose of fertilizer 138 kg ha^{-1}) – N_{60} kg ha^{-1};
3) barley without added ammonium nitrate – N_{40};
4) barley-pea intercrops without ammonium nitrate – BP.

We presumed that the soil of barley-pea intercrop contains naturally around 40–50 kg of free N per ha (Freyer, 2003). The sowing rate of the sole barley was 550 seeds per m^2 and in the intercrop 120 barley and 80 pea seeds per m^2. Herbicide Butoxone (active ingredient MCPB 400 g l^{-1}) was used for weed control in dosage of 3.8 l ha^{-1}, and no fungicides were used for disease control. After harvesting in the end of August the seeds were dried to 14% of standard moisture, sorted and a 1.5 kg sample was taken from each treatment. Samples were stored at –4 °C until laboratory analysis.

The isolation and identification of fungi

For isolation and identification of fungi the surface of barley grains were sterilized for 5 minutes with 1% of sodium hypochlorite (NaOCl) and rinsed twice with sterile distilled water. According to Abildgren et al. (1987), the 100 kernels were placed in Petri dishes containing CZID medium (35 g of Czapek Dox broth, 15 g of Agar, 1 ml of trace solution, 1 ml of dichloran, 1 ml of Chloramphenicol in 1 L of MQ water, autoclaved at 121 °C for 15 min). The plates were incubated at 20 °C in the dark (8 h) and in the light (16 h) cycle. After seven days of incubation, the fungi were transferred to Potato Dextrose Agar (PDA: 39 g of Potato Dextrose Agar, 1 mL of trace solution, 1 L of MQ water, autoclaved at 121 °C for 15 min) and the inoculated Petri dishes were kept in the dark for 14 days at 28 °C. Fungi determined morphologically accordingly to Samson et al. (2002) and Leslie and Summerell (2006). After incubation the DNA of all fungi was purified with the Ultra Clean Microbiol DNA Isolation Kit (MOBio Inc.CA) according to the manufacturer´s protocol. The internal transcribed spacer (ITS) region of the rDNA gene (S) was amplified using fungal universal primers: ITS1 (5´TCCGTAGGTGAACCTGCGG) and ITS4 (5´TCCTCCGCTTATTGATATGC) (White et al. 1990). The thermal cycler profile was used with denaturation at 98 °C for 5 min; followed by 30 cycles of amplification with denaturation at 98 °C for 1 min, primer annealing at 52 °C for 1 min, extension at 72 °C for 5 min, followed by cooling at 4 °C. Next, the 35 µl of PCR product was taken and cleaned further with Qiaquik PCR Purification Kit (Qiagen). The Sample Submission guide of EUROFINS MWG Operon was followed to prepare DNA to sequence the PCR products. The obtained DNA sequences were analysed using the UNITE database (https://unite.ut.ee) (Koljalg et al., 2013). The BLAST searching system at GenBank sequence database http://blast.ncbi.nlm.nih.gov/ was used to identify the fungi strains.

Weather conditions

The weather conditions during vegetation period (from May to August) in 2009 and 2010 were different (Table 1). In 2009, the average temperature for four-month period was 2.4 °C degrees cooler than in 2010. The overall amount of precipitation over the years did not differ significantly, being 332.9 mm in 2009 and 316.2 mm in 2010. However, the amount of precipitation during the vegetation period distributed unevenly. In 2009, June was rainy (sum of precipitation 85.3 mm) and July extremely rainy (sum of precipitation 135.6 mm). In 2010, July was dry (sum of precipitation 42.8), but August was very rainy (sum of precipitation 143.2 mm).

Table 1. Weather conditions in 2009 and 2010 in Central Estonia

		Day of rainfall		Sum of precipitation, mm		Average air temperature, °C	
Month	Decade	2009	2010	2009	2010	2009	2010
May	I	3	7	5.2	38.2	10.5	7.3
	II	2	4	6.0	4.8	9.9	17.2
	III	3	5	5.6	9.8	13.9	12.5
		8	**16**	**16.8**	**52.8**	**11.4**	**12.3**
June	I	4	3	48.7	18.6	11.3	13.9
	II	9	6	35.6	27.4	13.1	13.8
	III	2	2	1.0	31.4	17.3	16.3
		15	**11**	**85.3**	**77.4**	**13.9**	**14.7**
July	I	5	3	46.2	16.0	16.1	20.6
	II	6	3	47.6	9.2	17.9	23.6
	III	8	4	41.8	17.6	17.4	22.8
		19	**10**	**135.6**	**42.8**	**17.1**	**22.3**
August	I	5	3	20.6	10.6	17.0	21.4
	II	7	6	56.6	68.4	15.2	19.8
	III	4	7	18.0	64.2	15.0	14.6
		16	**16**	**95.2**	**143.2**	**15.7**	**18.6**
Total/average		**58**	**53**	**332.9**	**316.2**	**14.6**	**17.0**

The statistical analysis

The frequency of occurrence isolated fungal species calculated as number of isolated species divided total number of isolated species. The percentage occurrence of fungal species was found as the number of kernels in what species occurred divided on total number of kernels multiplied by 100. Analysis of variance (ANOVA, SAS 2002) used for determining effects of nitrogen rates and intercropping on the occurrence of *Fusarium* spp. on the barley kernels. The significance threshold was set at $p < 0.05$.

RESULTS AND DISCUSSION

During the study in total 85 isolates and 12 species of fungi were identified in the spring barley kernels, while the occurrence of *Fusarium* fungi was appreciable different in both years of the study (Table 2). The seven fungal genera were identified in 2009 and two fungal genera in 2010. In 2009, *Fusarium* spp. were isolated from 38% of kernels, whereas the occurrence of other pathogenic fungi was 62%. While in 2010, *Fusarium* was the common genus (96%) isolated from barley kernels. Our results confirm the findings of several researchers: that the weather factors have a significant effect on the occurrence of *Fusarium* spp. and other pathogenic fungi in kernels (Blandino et al., 2008; Parikka et al., 2012; West et al., 2012). Research of Blandino et al. (2008) showed that the climatic factors influenced the presence of fungal species; moreover, the rate of nitrogen application had an effect on fungal infection in maize kernels. Parikka et al. (2012) suggested that warm weather with more rainfall near harvest increases the spread of *Fusarium* spp., while high temperature, dryness and intense rainfall cause plant stress and favour of *Fusarium* infection on cereals in Northern Europe. West et al. (2012) concluded that the weather conditions together with the effect of agronomic practices influenced the interaction of pathogenic fungal species. Consequently, our study confirmed that warmer vegetation period and rainy harvesting season as it was in 2010 favoured the contamination of kernels with *Fusarium* fungi. On the contrary, in 2009, the moderate air temperatures with high rainfalls in July increased the occurrence of other pathogenic fungi that may suppress *Fusarium* fungi.

Table 2. Frequency of occurrence (%) of isolated fungal species from spring barley kernels in 2009 and 2010

Fungal species	Frequency (%)		
	2009	2010	Mean
Arthrinium sacchari	2.8	2.0	2.4
Alternaria infectoria	2.8	0.0	1.2
Epicoccum nigrum	8.3	0.0	3.5
Fusarium avenaceum	11.1	51.0	34.1
Fusarium equiseti	16.7	2.0	8.2
Fusarium oxysporum	0.0	6.1	3.5
Fusarium poae	2.8	16.3	10.6
Fusarium sporotrichioides	0.0	10.2	5.9
Fusarium tricinctum	5.6	10.2	8.2
Microdochium bolleyi	13.9	2.0	7.1
Phoma pinodella	22.2	0.0	9.4
Parastagonospora nodorum	13.9	0.0	5.9

Unlike the weather condition, the impact of nitrogen on the occurrence of *Fusarium* and other pathogenic fungi was less obvious. However, we still found that the effects of N rates and intercropping were statistically significant. In this study, the occurrence of micro-fungi on the barley kernels was higher at N_{120} and N_{40} compared to N_{60} rate and intercropping treatment (Fig. 1). The frequency of occurrence of micro-fungi on the barley kernels in the intercropping (BP) treatment was 1.8 times lower than in N_{40} and 1.6 times lower than in N_{120} treatment. Several researchers concluded that high rates of nitrogen fertilizer application increased *Fusarium* infection level in grain (Suproniene et al., 2012; Lemmens et al., 2004, van der Burgt et al., 2011). Lemmens et al. (2004) explained that increasing N input changes the plant canopy density, which in turn influences microclimatic conditions in plant-soil environment and delays the flowering period, therefore creating favourable conditions for infection. Probably the low or high rate of nitrogen leads the plants to stress that makes them more susceptible to *Fusarium* spp. and other pathogenic micro-fungi (Blandino et al., 2008). The highest occurrence of micro-fungi in the rate of N40 was probably due to the stress caused by nutrient deficiency; also, the plant height (unpublished data) was lower than in other treatments. It may be caused by the fact that the macro-conidia from Fusarium spp. can splash-disperse as high as 40–60 cm vertically from the source (Jenkinson & Parry, 1994; Lemmens et al., 2004), thereby contaminating the barley kernels. Review article by Dordas (2008) discusses the effect of N on contamination of pathogen depending on the type of fungi. It says that although high N application increases the severity of infestation by obligate parasitic fungi (such as Puccinia spp. and other diseases), it also decreased the infestation by facultative fungi (such as Alternaria spp., Fusarium spp.) In addition to interactions, repression and competitions are held between the different microorganisms, where the host plant responds with a complex of biochemical reactions, thereby becoming more susceptible or resistant to the pathogens (Dordas, 2008). The characteristic of plant density can also influence a distribution of *Fusarium* and other fungi whereas in tight plant spacing humid conditions could remain for longer than in a sparser intercrop. In this study, we found tendencies that the high occurrence of *Fusarium* fungi corresponds with low rate of the N_{40}, followed the top dressing treatment rates of the N_{120} and N_{60}.

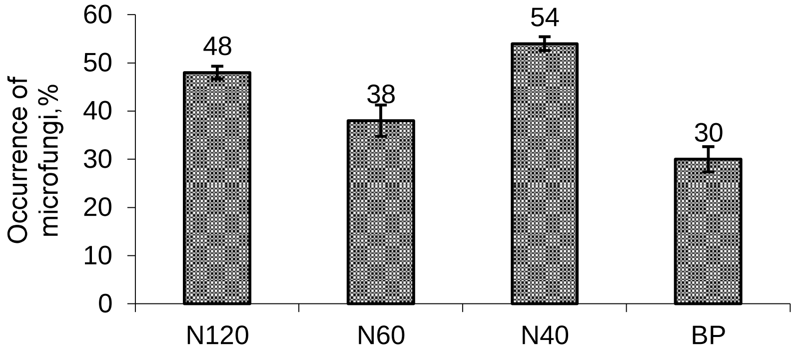

Figure 1. The average frequency of occurrence of micro-fungi in barley cropped under different nitrogen levels and barley-pea (BP) intercropping in 2009 and 2010.

Our study revealed that *Fusarium avenaceum* and *Fusarium poae* were the most common fungal species isolated from barley kernels (Table 3). *Phoma pinodella* and *Microdochium bolleyi* had high frequency of other isolated fungi. *Fusarium lateritium* dominated in kernels from all the different rates of nitrogen and also in intercropping. *Fusarium poae* was detected in the kernels from the mineral nitrogen rate and the rate of N_{40} but not in intercropping barley kernels. *Fusarium sporotrichioides* identified only in kernels from the nitrogen rate of N_{120} and N_{40}, but not in N_{60} and intercropping kernels. In barley kernels from intercropping treatment we identified only *Fusarium lateritium* compared at the rates of N_{120}, N_{60} and N_{40}. Other identified micro-fungi also showed interesting tendencies. The pathogenic fungi *Parastagonospora nodorum* occurred mainly in the rate of N_{120} in barley kernels. It was statistically significant that pathogenic fungus *Phoma pinodella* dominated only in kernels from intercrop. However, our results showed that the intercropping of cereal with pea might be an option to decrease the infestation of barley grain with *Fusarium* fungi. In addition, the occurrence of micro-fungi on grain decreased in moderate mineral nitrogen application as N_{60} compare with high rate and very low rate of nitrogen.

In this study the species *Fusarium avenaceum* dominated in the kernels from all treatments. The *Fusarium* species like *Fusarium poae* and *Fusarium sporotrichioides* were modestly represented in top dressing mineral nitrogen type and N_{40} but not in intercropping.

Results from this study showed tendencies that in the barley kernels from intercropping treatment, *Fusarium* species occurred less than in mineral nitrogen top dressing fertilization treatment. Likewise van der Burght et al. (2011) found significant positive effect of top dressing on the FHB infected seeds. We consider that compared to the intercropping of barley with pea the top dressing at sole barley favourably affected the grain contamination by *Fusarium* and in low fertilization rate as N_{40} caused stress in plants, thereby making them more susceptible to the *Fusarium* and other pathogenic fungi. Hauggaard-Nielsen et al. (2001) explained that in intercropping the barley is capable to take more soil mineral nitrogen in contrast to sole barley. This explains why barley mixed with pea does not starve from lack of nitrogen like barley in the N_{40} treatment. Thus, lower occurrence of *Fusarium* spp. in intercropped kernels compared with other types of treatments may be due to moderate supply of nitrogen as well as sparse plant density in this treatment.

Table 3. The probability of occurrence of fungi species in barley cropped under different rates of nitrogen and barley-pea (BP) intercropping in 2009 and 2010

Species	N_{120}	N_{60}	N_{40}	BP
Arthrinium sacchari	ns	no	ns	no
Alternaria infectoria	ns	ns	ns	no
Epicoccum nigrum	ns	no	ns	no
Fusarium avenaceum	0.004	0.001	0.002	0.006
Fusarium equiseti	ns	ns	ns	ns
Fusarium oxysporum	ns	no	ns	no
Fusarium poae	0.004	0.05	0.04	no
Fusarium sporotrichioides	0.05	no	0.004	no
Fusarium tricinctum	ns	no	ns	ns
Microdochium bolleyi	ns	ns	ns	no
Phoma pinodella	no	no	no	0.002
Parastagonospora nodorum	0.001	no	ns	no

no – not occurred

ns – not statistically significant at the $p < 0.05$

CONCLUSIONS

Based on the results of study we found tendencies that the higher air temperature and rainy weather during the maturity of barley favourably influenced the infestation ofkernels by *Fusarium* spp. The moderate temperatures and more than average precipitation in 2009 were favourable conditions for *Phoma pinodella*, *Microdochium bolleyi* and other mould. Frequency of occurrence of *Fusarium* was 36% in 2009. In warmer and rainy 2010 *Fusarium* species were most abundant (frequency of occurrence of *Fusarium* 96%) fungi in the barley.

The barley-pea intercropping and investigated rates of mineral nitrogen in barley influenced the occurrence of *Fusarium* and other micro-fungi differently.

The *Fusarium avenaceum* was dominant in all treatments. Experiments revealed tendencies that *Fusarium poae* and *Fusarium sporotrichioides* were mainly represented in the mineral fertilizer top dressing treatments. The frequency of occurrence of micro-fungi in the intercropping treatment was lower as mineral nitrogen fertilizer treatments.

Fusarium spp previously not been investigated in kernels from intercropping and nd under two years experiments not enough to make definitive conclusions. We found interesting tendencies, but more experiments need for final conclusions.

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REFERENCES

Abildgren, M.P., Lund, F., Thrane, U. & Elmholt, S. 1987. Czapek-Dox agar containing iprodione and dicloran as a selective medium for the isolation of *Fusarium* species. *Letters in Applied Microbiology* **5**, 83–6.

Bernhoft, A., Torp, M., Clasen, P-E., Løes A-K. & Kristoffersen, A.B. 2012. Influence of agronomic and climatic factors on *Fusarium* infestation and mycotoxin contamination of cereals in Norway. *Food Additives and Contaminants* Part A **29**, 1129–1140.

Blandino, M., Reyneri, A. & Vanara, F. 2008. Influence of nitrogen fertilization on mycotoxin contamination of maize kernels. *Crop Protection* **27**, 222–230.

Champeil, A., Fourbet, J.F., Doré, T. & Rossignol, L. 2004. Influence of cropping system on Fusarium head blight and mycotoxin levels in winter wheat. *Crop Protection* **23**, 531–537.

Dordas, C. 2008. Role of nutrients in controlling plant diseases in sustainable agriculture. A review. *Agronomy for Sustainable Development* **28**, 33–46.

FAO, ISSS, ISRIC.1998. World reference base for soil resources. *World Soil Resources Report*. Rome, 84.

Fernandez, M.R., Huber, D., Basnyat, P. & Zentner, R.P. 2008. Impact of agronomic practices on populations of *Fusarium* and other fungi in cereal and noncereal crop residues on the Canadian Prairies. *Soil and Tillage Research* **100**(1–2), 60–71.

Fernandez, M.R., Zentner, R.P., Basnyat, P., Gehl, D., Selles, F. & Huber, D. 2009. Glyphosate associations with cereals diseases caused by *Fusarium* spp. in the Canadian Praires. *European Journal of Agronomy* **31**, 133–143.

Fredlund, E., Gidlund, A., Sulyok, M., Börjesson, T., Krska, R., Olesen, M. & Lindblad, M. 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.

Freyer, B. 2003. Fruchtfolgen.Konventionell.Integriert.Biologisch.Verlag Eugen Ulmer.

Hauggaard-Nielsen, H., Ambus, P. & Jensen, E.S. 2001. Interspecific competition N use and interference with weeds in pea-barley intercropping. *Field Crops Research* **70**, 101–109.

Hauggaard-Nielsen, H., Jørnsgaard, B., Kinane, J. & Jensen, E.S. 2008. Grain-legume cereal intercropping: The practical application of diversity, competition and facilitation in arable and organic cropping system. *Renewable Agriculture and Food Systems* **23**, 3–2.

Jenkinson, P. & Parry, D.W. 1994. Splash dispersal of conidia of *Fusarium culmorum* and *Fusarium avenaceum*. *Mycological Research* **98**, 506–510.

Koljalg, U., Nilsson, R.H., Abarenkov, K., Tedersoo, L, Taylor, A.FS, Bahram, M., Bates, S., Bruns, T.M., Douglas, B., Drenkhan, T., Eberhardt, U., Daeñas, M.,Grenenc, T., Griffith, G.W., Hartmann, M., Kirk, P.M., Kohout, P., Larsson, E., Lindahl, B.D., Lücking, R., Martin, M.P., Matheny, B.P.,Nguyen, N.H., Niskanen, T., Oja, J., Peay, K., Peintner, U., Peterson, M., Poldmaa, K., Saag, L., Saar, J., Schüβler, A., Scott, J., Senes-Guerro, C., Smith, M.E., Suija, A., Taylor, D.L., Telleria, M.T., Weiss, M. & Larsson, K-H. 2013. Towards a unified paradigm for sequence - based identification of fungi. *Molecular Ecology* **22**, 5271–5277.

Krnjaja, V., Mandić, V., Lević, J., Stanković, S. & Petrović, T. 2015. Influence of N-fertilization on Fusarium head blight and mycotoxin levels in winter wheat. *Crop Protection* **67**, 251–256.

Lemmens, M., Haim, K., Lew, H. & Ruckenbauer, P. 2004. The Effect of Nitrogen Fertilization on Fusarium Head Blight Development and Deoxynivalenol Contamination in Wheat. *Journal of Phytopathology* **152**, 1–8.

Leslie, J.F. & Summerell, B. A. 2006. The *Fusarium* Laboratory Manual. Blackwell Publishing.

Lõiveke, H., Ilumäe, E. & Laitamm, H. 2004. Microfungi in grain and grain feeds and their potential toxicity. *Agronomy Research* **2**, 195–205.

Lõiveke, H. 2008.Teraviljadefusarioosid Eestis. Summary 5–6.
Lõiveke, H., Ilumäe, E. & Akk, E. 2008. Microbiology and safety of grain. *Journal of Agricultural Science* **19**(2), 38–44. (in Estonian, abstract in English).
Miedaner, T., Cumagun, J.R. & Chakraborty, S. 2008. Population Genetics of Three Important Head Blight Pathogens *Fusarium graminearum*, *F. pseudograminearum* and *F. culmorum*. *Journal of Phytopathology* **156**, 129–139.
Parikka, P., Hakal, K. & Tiilikkala, K. 2012. Expected shifts in *Fusarium* species' composition on cereal grain in Northern Europe due to climate change. *Food Additives and Contaminants* Part A **29**, 1543–1555.
Popovski, S. & Celar, F.A. 2013. The impact of agro-environmental factors on the infection of cereals with *Fusarium* species and mycotoxin production-a review. *Acta Agriculturae Slovenica* **101**, 105–116.
Samson, R.A., Haekstra, E.S., Frisvad, J.C. & Filtenberg, O. 2002. Introduction to Food- and Airborne Fungi. Sixth edition. Centraalbureau Voor Schimmelcultures – Utrecht.
Suproniene, S., Mankevičiene, A., Kadziene, G., Feiziene, D., Feiza, V., Semaskiene, R. & Dabkevicius, Z. 2011. The effect of different tillage-fertilization practices on the mycoflora of wheat grains. *Agricultural and Food Science* **20**, 312–326.
Suproniene, S., Mankevičiene, A., Kadziene, G., Kačergius, A., Feiza, V., Freiziene, D., Semaškiene, R., Dabkevičius, Z. & Tamošiūnas, K. 2012. The impact of tillage and fertilization on *Fusarium* infestation and mycotoxin production in wheat grain. *Žemdirnyste=Agriculture* **99**, 265–272.
Terzi, V., Tumino, G., Stanca, AM. & Morcia, C. 2014. Reducing the incidence of cereal head infection and mycotoxins in small grain cereal species. *Journal of Cereal Science*, 284–293.
Van der Burgt, G.J.H.M., Timmermanns, B.G.H., Scholberg, J.M.S. & Osman, A.M. 2011. *Fusarium* head blight and deoxynivalenol contamination in wheat as affected by nitrogen fertilization. *Wageningen Journal of Life Sciences* **58**, 123–129.
Wagacha, J.M. & Muthomi, J.W. 2007. *Fusarium culmorum*: Infection process, mechanisms of mycotoxin production and their role in pathogenesis in wheat. *Crop Protection* **26**, 877–885.
Wegulo, S.N., Baenziger, P.S., Nopsa, J.H., Bockus, W.W. & Hallen-Adams, H. 2015. Management of *Fusarium* of head blight of wheat and barley. *Crop Protection* **73**, 100–107.
West, J.S., Holdgate, S., Townsend, J.A., Edwards, S.G., Jennings, P. & Fitt, B.D.L. 2012. Impacts of changing climate and agronomic factors on fusarium ear blight of wheat in the UK. *Fungal Ecology* **5**, 53–61.
Xu, X., Nicholson, P. & Ritieni, A. 2007. Effects of fungal interactions among *Fusarium* head blight pathogens in disease development and mycotoxin accumulation. International *Journal of Food Microbiology* **119**, 67–71.
Yli-Mattila, T. 2010. Ecology and Evolution of toxigenic *Fusarium* species in cereals in northern Europe and Asia. *Journal of Plant Pathology* **92**(1), 7–18.