Journal of Plant Pathology
https://doi.org/10.1007/s42161-022-01233-9

ORIGINAL ARTICLE

Comparison of biomass and deoxynivalenol production of northern European and southern European *Fusarium graminearum* isolates in the infection of wheat and oat grains

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Received: 21 July 2021 / Accepted: 29 August 2022

Abstract
The 3ADON chemotype of *Fusarium graminearum* predominates in northern Europe, whereas the 15ADON chemotype is predominant in central and southern Europe. Therefore, it has been suggested that there are two *F. graminearum* populations in Europe, which may have been specialized to different host plants. The aim of the present work was to test this hypothesis by comparing southern European isolates (15ADON chemotype) from southern Russia and northern European isolates (3ADON chemotype) from Finland in the infection of grains in wheat cultivar Wellamo and oat cultivar Venla. *F. graminearum* biomass levels were measured by TaqMan (2018) and SYBR Green (2019) qPCR, while DON levels were measured by chromatographic methods. Most of the qPCR and DON results are supporting the hypothesis that in *F. graminearum* the 15ADON isolates from southern Russia are more specialized to wheat than the 3ADON isolates from Finland. In oat, there were not as clear differences between the 15ADON and 3ADON isolates, but in 2018 higher *F. graminearum* DNA levels and in 2019 higher DON and *F. graminearum* DNA levels were found in oat samples inoculated with 3ADON isolates. Our results are in line with literature according to which *F. graminearum* DNA and DON levels are also highest in oat in northern Europe, while in southern Europe they are highest in wheat and maize.

Keywords *Fusarium graminearum* · Chemotypes · 3ADON · 15ADON · Oat · Wheat

Introduction

Fusarium head blight (FHB) was first described in England in 1884 (Parry et al. 1995). By 1924, FHB was already found in England, Russia, Sweden, France, Italy, Germany, Netherlands, 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).

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 (Goswami and Kistler 2004; Aoki et al. 2012). 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 Europe (Pasquali et al. 2016; Yli-Mattila and Gagkaeva 2016). *F. graminearum* infects oat, and the highest *F. graminearum* DNA and DON levels in Finland have been found in oat grains (Yli-Mattila et al. 2009, 2013, 2017).

In the Nordic countries, high levels of *F. graminearum* and DON have been found mainly in oat grain, while in southern Europe the highest levels have been found in wheat and maize (Yli-Mattila et al. 2009; Yli-Mattila and Gagkaeva 2010; Sundheim et al. 2013; Fredlund et al. 2013). There is a good correlation between *F. graminearum* DNA and DON levels in wheat, barley, oat, and maize (Sarlin et al. 2006; Fredlund et al. 2008; Yli-Mattila et al. 2009; Nicolaisen et al. 2009).

Fusarium graminearum produces trichothecene mycotoxins DON, nivalenol (NIV) and their acetylated derivatives 3-acetyl-deoxynivalenol (3ADON), 15-acetyl-deoxynivalenol

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Published online: 06 October 2022

(15ADON), and 4-acetyl-nivalenol (fusarenon X). According to the major trichothecene produced, strains and populations of *F. graminearum* are assigned to chemotypes designated 3ADON, 15ADON, and NIV. An extensive overview of the distribution of these chemotypes in Europe is available (Pasquali et al. 2016). It must be mentioned, however, that the chemotypes listed in this work were mostly determined mainly by PCR tests, which are not always reliable as compared to the chemotype determined by chemical analysis (Pasquali et al. 2011; Villafana et al. 2020). Also in the present work the chemotypes were determined by PCR tests.

In central and southern Europe, 15ADON chemotype has been predominating, while the 3ADON chemotype has been rarely found in Germany, England, Poland, Hungary, Luxembourg, and Italy. NIV chemotype of *F. graminearum* is rare or missing in most countries of Europe (Talas et al. 2011; Pasquali et al. 2016), but in western Europe it is frequently found (Waalwijk et al. 2003; Jennings et al. 2004). The southern European population of *F. graminearum* has been spreading northward in Europe (Stepien et al. 2008; Nielsen et al. 2012) displacing the closely related *F. culmorum.* The overview by Pasquali et al. (2016) summarized these results.

It has been suggested that the two main populations of *F. graminearum* in Europe possess distinct chemotypes (Yli-Mattila 2010; Yli-Mattila and Gagkaeva 2010; Yli-Mattila et al. 2013). The northern European population possess the 3ADON chemotype, as shown for *F. graminearum* populations in Finland, Norway, Sweden, north-western Russia, Russian Far East, northern Japan, and some parts of Canada (Ward et al. 2008; Yli-Mattila and Gagkaeva 2010; Talas et al. 2011, Mert-Türk and Gencer 2013). 3ADON isolates of *F. graminearum* and *F. culmorum* inhibit the growth of wheat seedlings more strongly and cause more necrotic lesions than 15ADON isolates (Yli-Mattila and Gagkaeva 2010). This agrees with the higher DON production of 3ADON isolates (Ward et al. 2002; Yli-Mattila and Gagkaeva 2010; Talas et al. 2011). *F. graminearum* isolates of the 3ADON chemotype from Norway grew faster than isolates of 15ADON chemotype (Aamot et al. 2015). The northern European population has recently been spreading from Finland to northwestern Russia (Yli-Mattila and Gagkaeva 2010) or it has been there already before World War II, when some parts of northwestern Russia belonged to Finland and *F. graminearum* was reported there (Rainio 1932).

According to Aamot et al. (2015), the 15ADON chemotype of *F. graminearum* was rare in Norway. In contrast to southern European countries, Norwegian populations of *F. graminearum* were dominated by 3ADON chemotype. In Denmark, the 3ADON chemotype of *F. culmorum* was predominating in wheat in 1957–1960 and 1997–2000, while in 1965–1968 the 3ADON chemotype of *F. graminearum* was predominating in wheat (Nielsen et al. 2011, 2012). After 2003, the 15ADON chemotype of *F. graminearum* has been the most common chemotype in wheat in Denmark. In barley the 3ADON chemotype of *F. culmorum* has been predominating all the time, but in 1965–1968 the 3ADON chemotype of *F. graminearum* was also found and after 2005, the 15ADON chemotype of *F. graminearum* was also found in barley. Oat is the only cereal in which the 3ADON chemotype of *F. graminearum* remains dominant in Denmark, although the 3ADON chemotype of *F. culmorum* and the 15ADON chemotype of *F. graminearum* are also present.

While the 3ADON chemotype of *F. graminearum* are rarely found in wheat in southern Europe, oat grains in northern Europe are infected with the 3ADON chemotype and only 3ADON chemotype has been found in oat in Europe (Pasquali et al. 2016). It has therefore been suggested that the two *F. graminearum* populations in Europe may have specialized to different host plants (Yli-Mattila et al. 2013).

The aim of the present work was to test this hypothesis by comparing grain weight and the biomass of southern Russian 15ADON isolates and Finnish 3ADON isolates after artificial infection of wheat and oat field plots for two years. During the second year the effect of the artificial inoculation on DON level as well as other *Fusarium* species and their mycotoxins were also investigated.

Materials and methods

The experimental field for wheat and oat

The experimental field was located in the Southwest Finland region (Marttila local place, 60.63050209 N, 22.98930788 E). In 2018–2019 years spring wheat cultivar Wellamo and oat cultivar Venla were used (Table 1). In 2018 spring wheat (400 kg ha^{--1}, 1000 seed weight 36.5 g, germination % 53, which makes about 580 germinating seeds m^{-2}) and oat (210 kg ha^{-1}, 1000 seed weight 40.0 g, germination % 80, which makes about 420 germinating seeds m^{-2}) were sown on May 10 and they got 81 kg nitrogen/ha. On June 23, weeds were treated by MCPA (2-methyl-4-chlorophenoxyacetic acid, 500 g/ha; (FARM-MCPA, Lantmännen Agro, Helsinki, Finland). In 2019 spring, wheat (240 kg/ha, 1000 seed weight 31.8 g, germination % 95, which makes about 716 germinating seeds m^{-2}) and oat (215 kg ha^{-1}, 1000 seed weight 36.5 g, germination % 97, which makes about 570 germinating seeds m^{-2}) were sown on April 25 and fertilized with 81 kg/ha nitrogen. Wheat seeds were coated with Celest Formula M (200 ml/100 kg seeds) containing fludioxonil (25 g/l). On June 1, weeds were treated with MCPA (250 g/ha) and Basagran SG (800 g/ha; Berner, Helsinki, Finland), containing 870/kg Bentazon. The sowing machine was Tume KL 2500 H SC, which is 2.5 m wide. The experimental field was surrounded by a timothy field.

Table 1 Rainfall (mm) in Marttila and conditions for the experiment with artificial inoculation of wheat cv. Wellamo and oat cv. Venta in 2018 and 2019

Date	2018	2019
	Sowingon May 9:	Sowing on April 25:
May, 21	irrigation 1300 l water/200 m^2	-
Total (mm) in May	0 (after sowing)	38 (after sowing)
June, 1	irrigation 600 l water/200 m^2	
Total (mm) in June	33	22
July, 3	-	artificial inoculation
July, 5	artificial inoculation (wheat)	
July, 6	artificial inoculation (oat)	-
Total (mm) in July	47	49
August, 14–15	harvesting	
August, 21–22		harvesting
Total (mm) in August	23	53
Total amount of rain (mm)	103	162

Fusarium graminearum isolates

Nine *F. graminearum* strains of the northern European population from south-western Finland and ten *F. graminearum* strains of the southern European population from southern Russia (Table 2) were used. All isolates were single-spore isolates. All southern Russian isolates and seven Finnish isolates were from wheat, while three Finnish isolates were from oat. Species identification was confirmed by *F. graminearum*-specific qPCR primers TMFg12f/R and probe TMFg12p (Yli-Mattila et al. 2008). Chemotyping with primers Tri13,f/Tri13,r and GzTri7,f1/GzTri7,r1 as described by Yli-Mattila and Gagkaeva (2010) and with qPCR primers 3ADONf/r and 15ADONf/r (Nielsen et al. 2012) showed that all northern European isolates belonged to 3ADON chemotype and all southern European strains belonged to 15ADON chemotype. PCR reactions were carried out on a CFX96 Real-Time System thermal cycler (BioRad, USA).

For the production of inoculum, the strains were cultivated on potato dextrose agar (PDA; Scharlau Chemie S.A., Barcelona, Spain) and SNA minimal medium (Gerlach and Nirenberg 1982) on Petri dishes at 25 °C. Macroconidia formation was also induced by white and UVA light (cycles of 12-h light/12-h darkness) from fluorescence tubes as described by Yli-Mattila et al. (2009). More conidia were obtained on SNA than PDA medium. The Petri dishes of each isolate were washed with 25 ml of sterile water and after that the conidia concentration was counted with haemocytometer and the inoculum was diluted to final concentration. The strains are maintained in the collection of the Laboratory of Mycology and Phytopathology (All-Russian Institute of Plant Protection, St. Petersburg, Russia).

Field experiment plots

Oat and wheat field plots (0.5 m^2 in 2018 and 1 m^2 in 2019) were artificially inoculated with *F. graminearum* strains in the middle of anthesis (Table 1,). Each plot was inoculated by spraying with 100 ml of spore suspension and the surrounding plots were protected by plates. The concentration of conidia in 15ADON isolates was about 10^5 spores/ml for each isolate in 2018. In 3ADON isolates, it was more difficult to obtain high amounts of conidia, especially on PDA medium. That is why the concentration used for inoculation with 3ADON isolates was about 5. x 10^4 spores/ml for each isolate in 2018. In 2019, the concentration of conidia was $1–1.5 \times 10^4$/ml for all isolates. The timetable of the

Table 2 Finnish 3ADON and Russian 15ADON *Fusarium graminearum* isolates used in field experiments. All Finnish strains were from south-western (SW) Finland, while Russian strains were from Krasnodar and Stavropol regions in southern Russia

№	MFG strain	Origin	Plant	Year	Chemotype
1	59064	SW Finland	wheat	2017	3ADON
2	59065	SW Finland	wheat	2017	3ADON
3	59066	SW Finland	wheat	2017	3ADON
4	59067	SW Finland	wheat	2017	3ADON
5	59068	SW Finland	wheat	2017	3ADON
6	59069	SW Finland	wheat	2017	3ADON
7	59111	SW Finland	oat	2017	3ADON
8	59112	SW Finland	oat	2017	3ADON
10	59114	SW Finland	oat	2017	3ADON
11	58566	Krasnodar krai	wheat	2014	15ADON
12	59060	Krasnodar krai	wheat	2017	15ADON
13	58703	Krasnodar krai	wheat	2014	15ADON
14	58769	Stavropol krai	wheat	2015	15ADON
15	58772	Stavropol krai	wheat	2015	15ADON
16	58775	Stavropol krai	wheat	2014	15ADON
17	58777	Stavropol krai	wheat	2014	15ADON
18	58580	Krasnodar krai	wheat	2014	15ADON
19	58890	Krasnodar krai	wheat	2016	15ADON
20	58570	Krasnodar krai	wheat	2014	15ADON

experiments and the amount of rain are shown in Table 1. Every second plot was inoculated by a 3ADON (isolates 1–10) and a 5ADON (isolates 11–20) isolate and we had three replicates per each isolate in all experiments. The experimental designing was similar to that used by Gagkaeva et al. (2013) instead of a completely randomized system, which is more difficult to follow in field conditions.

The field containing seedlings was 2.5 m wide. So, at least one side of each plot was not against another plot. The distance between the plots was about 20 cm. In 2018, wheat ears and oat panicles were harvested manually, and grains were separated from the dried ears and panicles. In 2019, the Minibatt sample harvester (Agricultural Supply Services, Gloucester, UK) was used for harvesting grains. In both years, grains (100–200 g per sample) were dried to the moisture of about 14% by ventilation and temperature of about 25˚C soon after harvesting to stop the growth of *F. graminearum*.

DNA extraction for qPCR

In 2018 grain samples were ground by using a laboratory falling number hammer mill with a 1 mm sieve as described before (Yli-Mattila et al. 2017). DNA was extracted from 100 mg ground grain and from the standard *F. graminearum* isolate Fg8 using the GenElute™ Plant Genomic DNA kit (Sigma-Aldrich). Total DNA for TaqMan qPCR was quantified by a Qubit fluorometer (Invitrogen, Carlsbad, USA), as described by Yli-Mattila et al. (2011).

In 2019, ground grain samples from Turku were sent to Goettingen, where they were finely ground in reciprocal ball mill (M400, Retsch, Germany) with WC-sphears for 2 min at full power to increase the efficiency of DNA extraction. DNA was extracted and its quality tested as described by Brandfass and Karlovsky (2006). For SYBR Green qPCR quantified standard-DNA of *F. graminearum* and other *Fusarium* species was mixed with DNA extracted from uncontaminated wheat or oat flour to imitate matrix effects. A dilution series from 400 fg to 100 pg of *F. graminearum* DNA with three-fold dilution series and two replicates was produced. The concentration of *F. graminearum* DNA extracted from pure fungal cultures for SYBR Green qPCR was determined by densitometry after agarose electrophoresis, using DNA of bacteriophage Lambda as a standard.

Quantitative PCR

In 2018 TaqMan qPCR was performed at the University of Turku by using an iQTM5 Real-time PCR detection system (Bio-Rad) as described by Yli-Mattila et al. (2009, 2013). The Finnish isolate Fg8 of *F. graminearum* was used as standard in qPCR assays, as described by Yli-Mattila et al. (2013, 2017). Two replicas of each standard and sample were used in each TaqMan qPCR assay. *F. graminearum* DNA was measured as fungal DNA per total DNA.

In 2019 SYBR Green qPCR for *F. graminearum* and *F. culmorum* was performed at the University of Goettingen by using a real-time PCR thermocycler CFX 384 (Biorad, Rüdigheim, Germany) as described by Brandfass and Karlovsky (2008), SYBR Green qPCR assay for *F. poae*-DNA was conducted as described by Alisaac et al. (2019) and SYBR Green qPCR assay for *F. proliferatum* DNA as described by Nutz et al. (2011). Two replicas of each standard were used in each SYBR Green assay. The amount of fungal DNA per gram of dry flour was used as a measure of fungal colonization.

Mycotoxin extraction and quantification with HPLC

Grain samples for mycotoxin extraction were ground at the University of Turku by using the same laboratory falling number hammer mill with a 1 mm sieve, which was used for qPCR samples as described before (Yli-Mattila et al. 2017). Mycotoxins were extracted from 4 g flour into 40 mL acetonitrile/water/acetic acid (84/15/1 (v/v/v). The mixture was vortexed, shaken overnight, and centrifuged at 4750 rpm. One mL of the supernatant was transferred to a 2-mL Eppendorf tube and dried at 40 °C. Dried samples were resuspended in 1 mL methanol/water (20/80 (v/v)) for HPLC analysis as described by Beule et al. (2019). Quantification of mycotoxins was performed on an Agilent 1290 Infinity II HPLC system linked to an Agilent 6460 QQQ (Agilent Technologies, Waldbronn, Germany). Details concerning limits of detection (LODs) and limits of quantification (LOQs) are listed in (Table S1). NIV, diacetoxyscirpenol (DAS), DON, 3ADON, 15ADON, BEA and enniatin standards were from Merck (Darmstadt, Germany), while fusaric acid HT-2 and T-2 standards were from Enzo Life Sciences (Lörrach, Germany), fusarin X from AppliChem (Darmstadt, Germany) and zearalenone (ZEA) was from Romer Labs (Tulln, Austria). Recovery % varied in wheat between 53 (enniatin B) to 117 (T-2 toxin), while in oat recovery % were lower and varied between 10 (enniatin A) and BEA (78).

Statistical analyses

Statistics was performed using the statistical graph pad prism 5. One-way analysis of variance (ANOVA) was analysed by non-parametric Kruskal–Wallis test. Data are expressed as mean ± SE (standard error of the mean). Values of $p < 0.05$ were considered to be significant. R^2 (coefficient of determination) and regression slope were calculated using the SigmaPlot version 14.0 (SPSS Inc.). The original DNA and DON concentrations were transformed to logarithmic values $\lg[1+(x)]$ to obtain normal distribution for these parameters.

Results

Weather conditions

Experimental plots received more rainfall (162 mm) during the growing season in 2019 as compared to that in 2018 (103 mm) (Table 1). Especially the beginning of the growing season in 2018 was dry and the first rain (12 mm) after sowing came only on June 19. The plots were irrigated twice after sowing to increase the germination rate and support the growth of the seedlings. In May and June 2018, young wheat and oat plants suffered from visible drought symptoms and some parts of the experimental field could not be used for artificial inoculation, because seeds did not germinate at all: high temperatures in May further increased the drought stress. In June, the temperatures dropped to normal, but in July and August, the temperatures were much higher than is common for the regions. Weather data of the growing seasons 2018 and 2019 from Jokioinen weather station, which is situated 30 km from Marttila, is shown in Fig. S1. In 2018 it was raining during anthesis before artificial inoculation on July 3rd 22 mm and on July 4th 8 mm. In 2019 it was raining during anthesis before artificial inoculation on July 1st 3 mm and after artificial inoculation on July 5th 26 mm.

Infection progress

No visual symptoms of infection were found in the ears and panicles of the plants during the growing season. Also, the one kernel weight did not show significant differences between grains harvested from inoculated and un-inoculated plots for both wheat and oat in 2018 and 2019, although in wheat and oat controls the kernel weights in 2018 were somewhat higher than in inoculated ones (Tables S2 and S3).

The wheat seeds used in the year 2018 were older and had lower germination rate than those used in 2019. Therefore, more wheat seeds were sown in 2018 (see Materials and Methods). But even after irrigating, the germination rate in 2018 was lower in many parts of the experimental field than in 2019. Due to the low germination rate in 2018, we could use only half of the area for experimental plots, which resulted in smaller experimental plots (0.5 m^2) in 2018 as compared to 2019 (1 m^2).

Fungal biomass in grains

In wheat cultivar Wellamo *F. graminearum* DNA levels were significantly higher in the samples inoculated with 15ADON isolates as compared to wheat samples inoculated with 3ADON isolates and controls in 2018 and 2019 (Fig. 1a, b). Also in wheat samples inoculated with 3ADON isolates *F. graminearum* DNA levels were significantly higher than in controls in 2018 (Fig. 1a), but not in 2019 (Fig. 1b).

In oat cultivar Venla, *F. graminearum* DNA levels were significantly higher in samples inoculated with 3ADON isolates as compared to controls in 2018 and 2019, while there were no significant differences between samples inoculated with 15ADON isolates and controls (Figs. 1a, b). In 2019, there was also a significant difference between oat samples inoculated with 3ADON and 15ADON isolates. No *F. graminearum* DNA was detected in oat samples inoculated with 15ADON isolates and in control samples in 2019 (Fig. 1b).

Fusarium culmorum DNA was only found in oat samples and there were no significant differences between inoculated samples and controls, although *F. culmorum* DNA levels were lower in inoculated samples (Fig. S2a). *Fusarium poae* DNA levels were higher in oat samples; while in wheat *F. poae* DNA levels were significantly higher in inoculated samples as compared to controls (Figure S2b). *Fusarium proliferatum* DNA was not detected in any grain sample.

Mycotoxins

DON levels were clearly higher in controls and inoculated samples in oat as compared to wheat (Fig. 2a). In oat DON levels after inoculation with 3ADON isolates were more than ten times higher as compared to wheat, while after the inoculation with 15ADON isolates DON levels were only about twice higher as compared to wheat. DON levels were significantly higher in oat samples artificially inoculated with 3ADON isolates as compared to controls, while in wheat DON levels were significantly higher in samples artificially inoculated with 15ADON isolates as compared to controls. This was in both cases mainly due to a couple of isolates producing high levels of DON either in oat or wheat. 3ADON was detected only from two samples containing the highest levels of 3ADON isolate DNA. DON levels were also higher in wheat samples inoculated with 15ADON isolates as compared to those inoculated with 3ADON isolates and in oat samples inoculated with 3ADON isolates as compared to those inoculated with 15ADON isolates, but the differences were not significant. The correlation between *F. graminearum* DNA and DON levels in 2019 was higher in oat ($R^2 = 0.54$) than in wheat ($R^2 = 0.26$).

Nivalenol, BEA and T-2 toxin were only found in oat. The highest NIV level was higher (1.02 mg/kg) than the highest DON level (0.81 mg/kg) in oat. Enniatins were found both in wheat and oat and enniatin levels were significantly higher in wheat samples, which were artificially inoculated with 15ADON isolates as compared to controls (Fig. 2b). There was only a slight corelation in oats between *F. culmorum* DNA and DON levels ($R^2 = 0.07$) and *F. poae* DNA and NIV levels ($R^2 = 0.01$). Other mycotoxins are shown in Table S1.

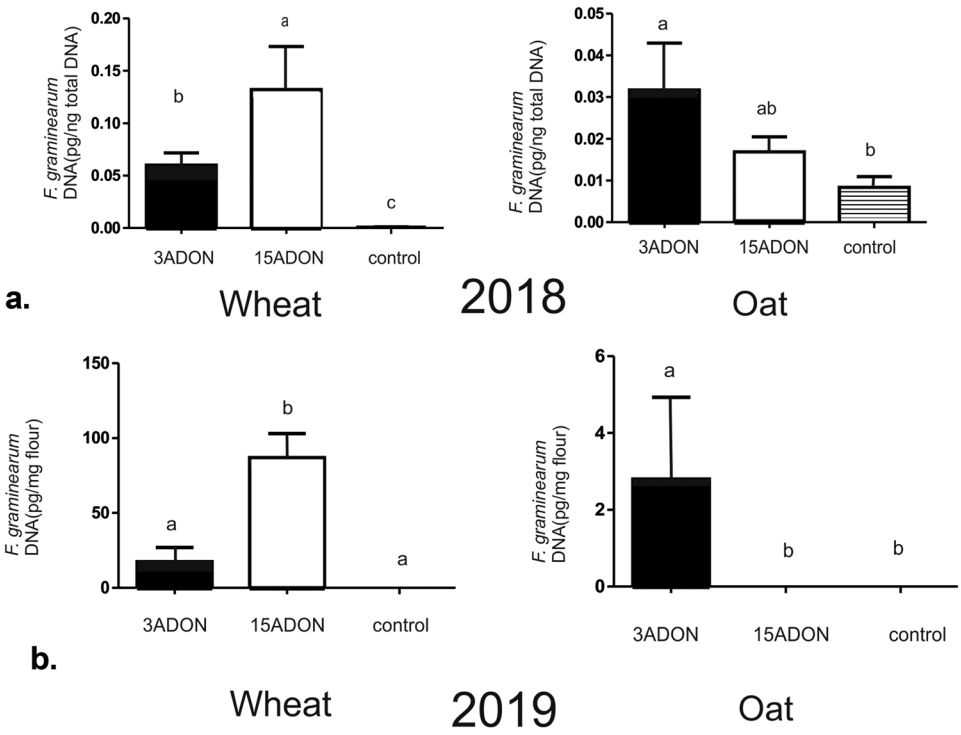

Fig. 1 **a** *Fusarium graminearum* DNA levels (pg DNA/ng ± SE total DNA) in 2018 in wheat and oat samples inoculated with 3ADON and 15ADON isolates and controls by the TaqMan qPCR method. **b** *Fusarium graminearum* DNA levels (pg DNA/mg ± SE flour) in wheat and oats samples inoculated with 3ADON and 15ADON isolates and controls in 2019 by the SYBR Green qPCR method. Different letters above the column are showing significant differences ($p < 0.05$) between the means of the samples and controls. Means with the same letter do not differ significantly

Discussion

This is the first investigation, in which the effect of artificial inoculation of different chemotypes of *F. graminearum* has been investigated in oat. *Fusarium graminearum* DNA and DON levels were more sensitive in measuring the effects of artificial inoculation than grain weight levels in the present work, No clear differences were found in wheat ears and oat panicles after artificial inoculation.

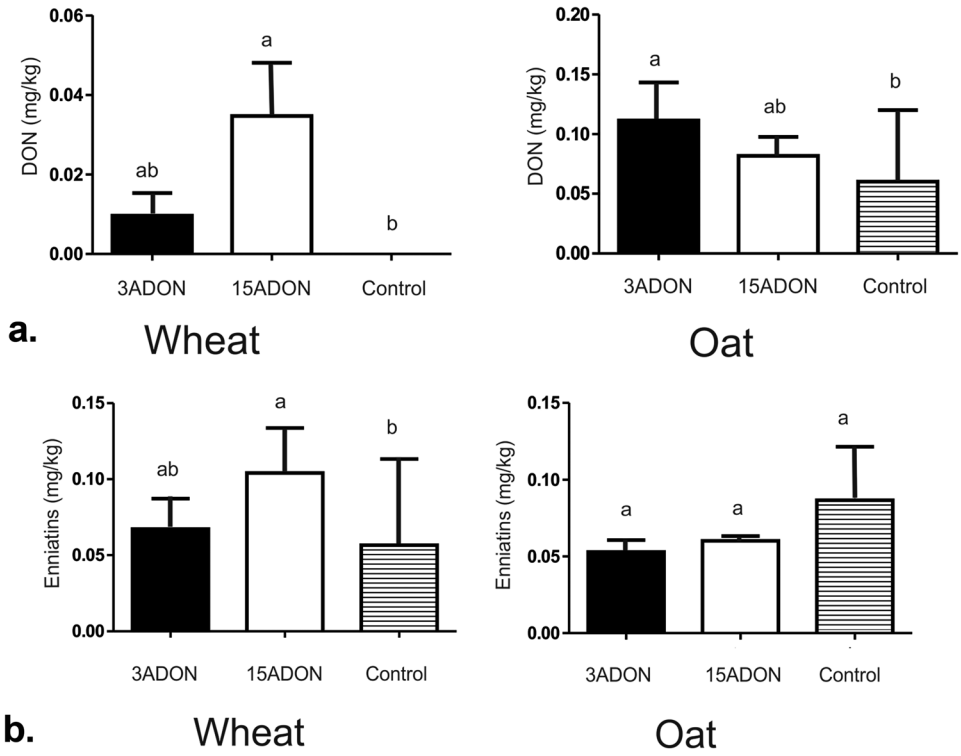

Fig. 2 DON (**a**) and enniatin (**b**) levels (mg/kg ± SE) in wheat and oats samples inoculated with 3ADON and 15ADON isolates and controls in 2019. Different letters above the column are showing significant differences ($p < 0.05$) between the means of the samples and controls. Means with the same letter do not differ significantly

In artificially contaminated grain samples, *F. graminearum* DNA levels were significantly higher in wheat plots inoculated with 15ADON isolates in both years, while in oat *F. graminearum* DNA levels were significantly higher in oat samples inoculated with 3ADON isolates only in 2019. But also in 2018 the difference between oat samples inoculated with 3ADON isolates and controls was evident, although the difference between oat samples inoculated with 15ADON isolates and controls was not significant.

No *F. graminearum* DNA could be detected in oat samples inoculated with 15ADON isolates and controls in 2019. This may be due differences in DNA extraction during the two years and to the fact that in 2018 the results were counted per total DNA and in 2019 per dry weight. Other reasons for these differences might be random variation within the grain samples due to uneven distribution of infected grains or problems in homogenization of ground samples as has been shown by (Yli-Mattila et al. 2017).

DNA levels of *F. culmorum* and *F. poae* were clearly higher in oat samples than in wheat plots. Both of them were also present in controls of oat plots due to natural contamination. This is in accordance with previous qPCR results in Finland (Yli-Mattila et al. 2009). *Fusarium culmorum* is able to produce DON, but there was no correlation between *F. culmorum* DNA and DON levels. *Fusarium poae* DNA levels were also significantly higher in artificially inoculated wheat samples. *Fusarium proliferatum*, which is able to produce fumonisin, was not detected in grains, but Gagkaeva and Yli-Mattila (2020) found another fumonisin producer *F. verticillioides* in winter wheat straw in Finland in July 2019. The most common *Fusarium* species in spring wheat and oat in Finland is usually *F. avenaceum* (Yli-Mattila et al. 2009), which produces enniatins. There is usually a strong correlation between *F. avenaceum* DNA and enniatin levels (Yli-Mattila et al. 2009. In the present work, enniatin levels were even slightly favoured by *F. graminearum* infection caused by 15ADON isolates in wheat.

Fusarium poae is also producing enniatins and it is the main producer of NIV in Finland (Jestoi et al.2008), but there was only a slight correlation between *F. poae* DNA and NIV levels in oat and no correlation between *F. poae* DNA and enniatin levels in the present work. NIV was only detected in oat and *F. poae* DNA levels were also higher in oat than in wheat.

The differences of the present work in wheat and oat in DON production as compared to controls and in *F. graminearum* DNA levels between European 3DON and 15ADON isolates are similar to those found by von der Ohe et al. (2010) and Gilbert et al. (2010) in DON production between the two chemotypes in wheat, although American 3ADON isolates are more effective DON producers than 15ADON isolates in wheat. So, North American 3ADON and 15ADON populations probably differ from European 3ADON and 15ADON populations. According to preliminary variable number tandem repeat analysis results (Yli-Mattila et al. 2018, 2019, T. Yli-Mattila, manuscript in preparation), the southern Russian 15ADON population is more closely related to Russian Far East 15ADON/3ADON and North American 3ADON population than to northern European 3ADON population or North American 15ADON population. Also the lack of significant differences in kernel weights between the plots inoculated with different chemotypes in wheat and oat are in agreement with the lack of significant differences in FHB indices and aggressiveness between the 3ADON and 15ADON chemotypes in wheat in these previous works. The clearly higher DON production of Finnish and north-western Russian 3ADON isolates as compared to southern Russian 15ADON isolates on potato sucrose agar medium (Yli-Mattila and Gagkaeva 2010) shows that European 3ADON isolates of *F. graminearum* have potential to produce more DON than 15ADON isolates on certain growth media. Further work is needed to confirm, if the differences in DON production between the European chemotypes are also significant in oat and how well the Finnish/north-western Russian and southern Russian *F. graminearum* isolates of the present work represent northern and southern European populations.

The results of the present work are similar to those found between closely related *Microdochium nivale* var. *nivale* and *Microdochium nivale* var. *majus* isolates by Diamond and Cooke (1997) and Simpson et al. (2000). Diamond and Cooke (1997) could not find a clear host specialisation by using detached leaf method, but Simpson et al. (2000) could find that *M. nivale* var. *nivale* isolates were more pathogenic to rye, while *M. nivale* var. *majus* isolates were more pathogenic to wheat and oat by using qPCR.

The increase of DON levels in oat samples inoculated with 3ADON isolates and in wheat samples inoculated with 15ADON isolates was mainly due to two of isolates producing high levels of DON either in oat (3ADON isolates) or wheat (15ADON isolates). High NIV levels were also measured in oat. 3ADON was detected only in two samples containing the highest levels of 3ADON isolate DNA and DON. Trace amounts of beauvericin (BEA) and T-2 toxin were only found in oat.

Thus, most of the results are supporting the hypothesis that in *F. graminearum* the 15ADON isolates from southern Russian population are more specialized to wheat, while many results also indicate that 3ADON isolates from Finland are more specialized to oat. This may be due to the fact that oat has been the most important crop in northern Europe, while wheat has been more important in southern Europe. *F. graminearum* DNA and DON levels are also highest in oat in northern Europe, while in southern Europe they are highest in wheat and maize (Pasquali et al. 2016). The flowering and harvesting conditions are also important for DON production. In Finland *F. graminearum*

DNA and DON levels are higher in spring wheat than in winter wheat, while in southern Europe *F. graminearum* DNA, DON levels are higher in winter wheat and spring wheat is less important than in Finland.

In Canada, the genome of one Canadian 3ADON isolate has been compared to one Canadian 15ADON isolate (Walkowiak et al. 2015). They found about 150,000 different SNPs in these two isolates representing Canadian 3ADON and 15ADON population. According to Laurent et al. (2017) ca. 150,000 SNPs seem to be a typical variation between genomes of distal isolates belonging to different populations. Northern European 3ADON and southern European 15ADON populations are, however, probably different from North American 3ADON and 15ADON populations of *F. graminearum*. It may be that these *F. graminearum* populations originate from different ancestral hosts of wild grasses (Kelly and Ward 2018).

In future, it should be investigated if the differences found between Finnish 3ADON isolates and southern Russian 15ADON isolates in spring wheat cultivar Wellamo and oat cultivar Venla are present also in other wheat and oat cultivars after artificial inoculation with other northern European 3ADON and southern European 15ADON isolates from different parts of northern and Southern Europe. Point inoculation (Hautsalo 2020) might be more effective in comparing the isolates than the inoculation by spraying, which was used in the present work. In addition, it should be studied, which genes are associated with host specificity in 3ADON and 15ADON isolates in the early biotrophic infection phase in wheat and oat. Sperschneider et al. (2015) found such genes in wheat and according to them three cell wall degrading enzymes are involved in host adaptation.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s42161-022-01233-9.

Acknowledgements This research was funded by Finnish Cultural Foundation personal grant of Tapani Yli-Mattila for three years on February 2. 2017 and travelling grants 324397 and 324819 of the Academy of Finland to Tapani Yli-Mattila. Taha Hussien was financially supported by Egyptian mission department and Asmaa Abbas by The Centre for International Mobility, Finland (CIMO).

The authors would like to thank Prof. Petr Karlovsky and Dr. Mohammad Alhussein for their valuable help in mycotoxin and qPCR analyses in Goettingen and their comments on the manuscript, Dr. Tatiana Gagkaeva for giving us *Fusarium* strains, comments on the manuscript, and participating in field experiments and finally the internship trainee Verda Kutuk for her assistance in field experiments. We are also grateful for The Natural Research Institute of Finland and Boreal Plant Breeding Ltd. for providing us wheat and oat seeds for our experiments.

Funding Open Access funding provided by University of Turku (UTU) including Turku University Central Hospital. This research was funded by Finnish Cultural Foundation personal grants 00171173, 00182381, and 00192433 of Tapani Yli-Mattila for three years on February 2. 2017 and travelling grants 324397 and 324819 of the Academy of Finland to Tapani Yli-Mattila. Taha Hussien was financially supported by Egyptian mission department and Asmaa Abbas by The Centre for International Mobility, Finland (CIMO).

Availability of data and material Data that supports the findings of this study are available from the corresponding author on reasonable request.

Code availability Not applicable.

Declarations

Conflicts of interest The authors have no conflict of interest to declare.

References

Aamot HU, Ward TJ, Brodal G, Vrålstad T, Larse GB, Klemsdal SS, Elameen A, Uhlig S, Hofgaard IS (2015) Genetic and phenotypic diversity within the *Fusarium graminearum* species complex in Norway. Eur J Plant Pathol 14:1–19

Alisaac E, Behmann J, Rathgeb A, Karlovsky P, Dehne H-W, Mahlein A-K (2019) Assessment of *Fusarium* infection and mycotoxin contamination of wheat kernels and flour using hyperspectral imaging. Toxins 11:556. https://doi.org/10.3390/toxins11100556

Aoki T, Ward TJ, Kistler HC, O'Donnell K (2012) Systematics, phylogeny and trichothecene mycotoxin potential of *Fusarium* head blight cereal pathogens. Mycotoxins 62:91–102

Beule L, Lehtsaar E, Rathgeb A, Karlovsky P (2019) Crop Diseases and Mycotoxin Accumulation in Temperate Agroforestry Systems. Sustainability 11:2925

Brandfass C, Karlovsky P (2006) Simultaneous detection of *Fusarium culmorum* and *F graminearum* in plant material by duplex PCR with melting curve analysis. BMC Microbiol 6:4

Brandfass C, Karlovsky P (2008) Upscaled CTAB-based DNA extraction and real-time PCR assays for *Fusarium culmorum* and *F. graminearum* DNA in plant material with reduced sampling error. Int J Mol Sci 9:2306–2321

Diamond H, Cooke BM (1997) Host specialisation in *Microdochium nivale* on cereals. Cer Res Comm 25:533–538

Fredlund E, Gidlund A, Olsen M, Börjesson T, Spliid NHH, Simonsson M (2008) Method evaluation of Fusarium DNA extraction from mycelia and wheat for down-stream real-time PCR quantification and correlation to mycotoxin levels. J Microbiol Methods 73(1):33–40. S0167701208000262. https://doi.org/10.1016/j.mimet.2008.01.007

Fredlund E, Gidlund A, Sulyok M, Börjesson T, Krska R, Olsen M, Lindblad M (2013) Deoxynivalenol and other selected *Fusarium* toxins in Swedish oats—Occurrence and correlation to specific *Fusarium* species. Int J Food Microbiol 167:276–283

Gagkaeva T, Gavrilova O, Yli-Mattila T, Loskutov I (2013) Sources of resistance to *Fusarium* head blight in VIR oat collection. Euphytica 191:355–364

Gagkaeva TY, Yli-Mattila T (2020) Emergence of *Fusarium* verticillioides in Finland. Eur J Plant Pathol 158:1051–1057

Gerlach W, Nirenberg H (1982) The genus *Fusarium*: a pictorial atlas. Mitteil. biol. Bundesanstalt für Land- und Forstwirtschaft Berlin-Dahlem 209: 1–406. Parul Parey: Berlin

Gilbert J, Clear RM, Ward TJ, Gaba D, Tekauz A, Turkington TK, Woods SM, Nowicki T, O'Donnell K, (2010) Relative aggressiveness and production of 3- or 15-acetyldeoxynivalenol and deoxynivalenol by *Fusarium graminearum* in spring wheat. Can J Plant Pathol 32:146–152

Goswami RS, Kistler HC (2004) Heading for disaster: *Fusarium graminearum* on cereal crops. Mol Plant Pathol 5:515–525

Hautsalo J (2020) Phenotyping Fusarium head blight resistance in oats having low deoxynivalenol content. Dissertationes schola doctoralis scientiae circumiectalis, alimentariae, biologicae, universitatis helsinkiensis 21:51

Jennings P, Coates ME, Walsh K, Turner JA, Nicholson P (2004) Determination of deoxynivalenol- and nivalenol-producing chemotypes of *Fusarium graminearum* isolates from wheat crops in England and Wales. Plant Pathol 5:643–652

Jestoi M, Paavanen-Huhtala S, Parikka P, Yli-Mattila T (2008) *In vitro* and *in vivo* mycotoxin production of *Fusarium* isolated from Finnish grains. Arch Phytopathol Plant Prot 4:545–558

Kelly SC, Ward TJ (2018) Population genomics of *Fusarium graminearum* reveals signatures of divergent evolution within a major cereal pathogen. PLoS ONE 13(3):e0194616

Laurent B, Moinard M, Spataro C, Ponts N, Barreau C et al (2017) Landscape of genomic diversity and host adaptation in *Fusarium graminearum*. BMC Genomics 18:203

Mert-Türk F, Gencer R (2013) Distribution of the 3-AcDON, 15-AcDON and NIV chemotypes of *Fusarium culmorum* in the North-West of Turkey. Plant Prot Sci 49:57–64

Nicolaisen M, Supronienė S, Nielsen LN, Lazzaro I, Spliid NH, Justesen AF (2009) Real-time PCR for quantification of eleven individual *Fusarium* species in cereals. J Microbiol Meth 76:234–240

Nielsen LK, Jensen JD, Nielsen GC, Jensen JE, Spliid NH, Thomsen IK, Justesen AF, Collinge DB, Jorgensen LN (2011) *Fusarium* head blight of cereals in Denmark: Species complex and related mycotoxins. Phytopathology 101:960–969

Nielsen LK, Jensen JD, Rodríguez A, Jørgensen LN, Justesen AF (2012) TRI12 based quantitative real-time PCR assays reveal the distribution of trichothecene genotypes of *F. graminearum* and *F. culmorum* isolates in Danish small grain cereals. Int J Food Microbiol 157:384–392

Nutz S, Doell K, Karlovsky P (2011) Determination of the LOQ in real-time PCR by receiver operating characteristic curve analysis: application to qPCR assays for *Fusarium verticillioides* and *F. proliferatum*. Anal Bioanal Chem 401:717–726

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

Pasquali M, Beyer M, Bohn T, Hoffmann L (2011) Comparative analysis of genetic chemotyping methods for *Fusarium*: Tri13 polymorphism does not discriminate between 3- and 15-acetylated deoxynivalenol chemotypes in *Fusarium graminearum*. J Phytopathol 159:700–704

Pasquali M, Beyer M, Logrieco A, Audenaert K, Balmas V, Basler R, Boutigny AL, Chrpova J, Czembor E, Gagkaeva T, González-Jaén MT, Hofgaard IS, Köycü ND, Hoffmann L, Levic J, Marin P, Miedaner T, Migheli Q, Moretti A, Müller MEH, Munaut F, Parikka P, Pallez-Barthel M, Piec J, Scauflaire J, Scherm B, Stankovic S, Thrane U, Uhlig S, Vanheule A, Yli-Mattila T, Vogelgsang S (2016) A European database of *Fusarium graminearum* and *F. culmorum* trichothecene genotypes. Front Microbiol 7:406

Rainio AJ (1932) Punahome *Fusarium* roseum Link. - Gibberella saubinetii (Mont.) Sacc. ja sen aiheuttamat myrkytykset kaurassa. Valt Maatal Koetoim, Julk 50:1–45 (in Finnish, abstract in German)

Sarlin T, Yli-Mattila T, Jestoi M, Rizzo A, Paavanen-Huhtala S, Haikara A (2006) Real-time PCR for quantification of toxigenic Fusarium species in barley and malt. Eur J Plant Pathol 114(4):371–380. https://doi.org/10.1007/s10658-006-0001-9

Simpson DR, Rezanoor HN, Parry DW, Nicholson P (2000) Evidence for differential host preference in *Microdochium nivale var. majus* and *Microdochium nivale var. nivale*. Plant Pathol 49(2):261–268

Sperschneider J, Gardiner DM, Thatcher LF, Lyons R, Singh KB, Manners JM et al (2015) Genome-wide analysis in three *Fusarium* pathogens identifies rapidly evolving chromosomes and genes associated with pathogenicity. Genome Biol Evol 7:1613–1627

Stepien L, Popiel D, Koczyk G (2008) Chelkowsky, J Wheat-infecting *Fusarium* species in Poland—Their chemotypes and frequencies revealed by PCR assay. J Appl Genet 49:433–441

Sundheim L, Brodal G, Hofgaard IS, Rafoss T (2013) Temporal variation of mycotoxin producing fungi in Norwegian cereals. Microorganisms 1:188–198

Talas F, Parzies HK, 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. Eur J Plant Pathol 131:39–48

von der Ohe C, Gauthier V, Tamburic-Ilincic L, Brule-Babel A, Fernando W, Clear R, Ward TJ, Miedaner T (2010) A comparison of aggressiveness and deoxynivalenol production between Canadian *Fusarium graminearum* isolates with 3-acetyl and 15-acetyldeoxynivalenol chemotypes in field-grown spring wheat. Eur J Plant Pathol 127:407–417

Villafana RT, Ramdass AC, Rampersad SN (2020) TRI Genotyping and chemotyping: A balance of power. Toxins 12:64. https://doi.org/10.3390/toxins12020064

Waalwijk C, Kastelein P, de Vries I, Kerényi Z, van der Lee T, Hesselink T, Köhl J, Kema G (2003) Major changes in *Fusarium spp*. in the Netherlands. Eur J Plant Pathol 109:743–754

Walkowiak S, Bonner CT, Wang L, Blackwell B, Rowland O (2015) Subramaniam, R. Intraspecies Interaction of *Fusarium graminearum* Contributes to Reduced Toxin Production and Virulence. Mol Plant Microbe Interact 28:1256–1267

Ward TJ, Bielawski JP, Kistler HC, Sullivan E, O'Donnell K (2002) Ancestral polymorphism and adaptive evolution in the trichothecene mycotoxingene cluster o phytopathogenic Fusarium. Proc Natl Acad Sci USA 99:9278–9283. https://doi.org/10.1073/pnas.142307199

Ward TJ, Clear R, Rooney A, O'Donnell K, Gaba D, Patrick S, Starkey D, Gilbert J, Geiser D, Nowicki T (2008) An adaptive evolutionary shift in *Fusarium* head blight pathogen populations is driving the rapid spread of more toxigenic *Fusarium graminearum* in North America. Fungal Genet Biol 45:473–484

Yli-Mattila T (2010) Ecology and evolution of toxigenic Fusarium species in cereals in northern Europe and Asia. J Plant Pathol 92:7–18

Yli-Mattila T, Gagkaeva T (2010) Molecular chemotyping of *Fusarium graminearum*, *F. culmorum* and *F. cerealis* isolates from Finland and Russia. In: Gherbawy Y, Voigt K (eds) Molecular identification of fungi, Springer Verlag, Berlin, Heidelberg, New York, Germany, pp 159–177

Yli-Mattila T, Gagkaeva TY (2016) *Fusarium* toxins in cereals in northern Europe and Asia. In: Deshmukh SK, Misra JK, Tewari JP, Papp T (eds) Fungi Applications and Management Strategies, CRC Press., Boca Raton, London, New York, USA, pp 293–317

Yli-Mattila T, Gagkaeva T, Aamot H, Ward T (2018) *Fusarium graminearum* diversity in Finland, Norway and Russia. 14th European *Fusarium* seminar, Tulln, Austria, April 8–11, 2018.- Abstract book, 30

Yli-Mattila T, Gagkaeva T, Aamot H, Ward T (2019) *Fusarium graminearum* molecular chemotypes in Finland, Norway and Russia. – Nordic Baltic *Fusarium* seminar in Esbjerg in Denmark April 2–4, 2019. Book of abstracts, 12

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

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

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

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

Yli-Mattila T, Rämö S, Hussien T, Rauvola M, Hietaniemi V, Kaitaranta J (2017) Different grain grinding methods affect detection of *Fusarium graminearum* DNA and mycotoxins. Phytopathol Mediterr 56:167–174