Fusarium head blight in the Russian Far East: 140 years ~~of~~ after description of the 'drunken bread' problem~~problem~~

Tatiana Yu. Gagkaeva, Aleksandra S. Orina, Olga P. Gavrilova

Laboratory of Mycology and Phytopathology, All-Russian Institute of Plant Protection, St. Petersburg, Pushkin 196608, Russia

Corresponding Author:
Tatiana Yu. Gagkaeva
Podbelskogo shosse, 3, St. Petersburg, Pushkin 196608, Russia
Email address: t.gagkaeva@yahoo.com

Abstract

~~The Far East region of Russia is characterised by a monsoon climate that is very damp and warm during the summer.~~ The first appearance of Fusarium head blight (FHB)—and the beginning of scientific research of this disease—occurred ~~in on~~ the Far East region of Russia ~~this area~~ at the end of the 19th century.

In the summer of 2019, in the Amur region, which comprises 60–70% of grain production in the Russian Far East, flooding caused a state of emergency. ~~As a result, cereal yields were only partially saved and harvested.~~ The quality of wheat and barley grains grown under natural conditions of FHB outbreaks, including grain infection, fungal species composition, *F. graminearum* DNA content and their chemotypes, and the presence of various mycotoxins~~,~~ was studied.

Fusarium infection rates reached extremely high percentages, 51–98%, the majority of which were *F. graminearum* infections. The amount of *F. graminearum* DNA in wheat grain samples

Formatted: Font: Not Italic

was higher than in the barley grain samples and averaged 6.1 and 2.1 pg/ng, respectively. The content of deoxynivalenol (DON) in the wheat samples reached 13,343 ppb and in barley reached 7,755 ppb.

A multilocus genotyping assay was conducted on the partially sequenced fragments of the translation elongation factor EF-1a, ammonium ligase gene, reductase gene, and 3-O-acetyltransferase gene in 29 *Fusarium graminearum sensu lato* (s. lat.) strains from the grain harvested in the Amur region. ~~Additionally, one atypical strain, *F. graminearum* s. lat., from the Siberian region (wheat grain, 2018), and three *F. culmorum* strains from the Western Siberian, Ural, and South European regions of Russia were analysed.~~

All strains from the Far East region were characterized as *F. graminearum sensu stricto*; 70% were the 15-AcDON chemotype, while the other strains were the 3-AcDON chemotype. ~~One strain from Siberia belonged to *F. vorosii* and was characterized as the 15-AcDON chemotype. This is the first detection of this species outside the Far East region in Russia. High genetic similarity was found among the analysed *F. culmorum* strains from remote regions; these strains were the 3-AcDON chemotype.~~

According to the results, a~~A~~fter 140 years of study of FHB, we are still not very successful in controlling ~~FHB~~ this disease if conditions are favo~~u~~rable for pathogen development. Even at present, some of the grain harvested must be destroyed, as high contamination of mycotoxins renders it unusable.

Keywords: chemotype; deoxynivalenol; disease; DNA; epidemic; *Fusarium graminearum*; grain; multilocus genotyping; mycotoxins; Russian Far East.

Introduction

The first description of Fusarium head blight (FHB) within the territory of Russia was in the Far East in 1882 (Palchevsky, 1891; Voronin, 1890). This region is typically a monsoon climate with very damp and warm summers due to the influences of the Sea of Japan and the Pacific Ocean. The scientific investigation of this disease began at the end of the 19th century, but long before this, Chinese peasants and later Russian settlers related the poisoning of people and animals with pinkish grains and heads in the fields.

Between 1882 and 1914, epidemics of this disease in the Far East occurred almost every year (Naumov, 1916). Consumption of affected grain and straw caused numerous cases of food poisoning of people and farm animals. The initial signs and symptoms of the disease resemble those that can develop after drinking too much alcohol (including dizziness and headache, trembling hands, confusion, and vomiting) and thus ~~was~~ were named 'drunken bread'. The extensive research undertaken by Russian mycologists revealed that *Fusarium roseum* Link (*F. graminearum* Schwabe) with teleomorph stage *Gibberella saubinetii* Sacc. (*G. zeae* [Schwein.] Petch) was the principal cause of the disease (Jaczewski, 1904; Naumov, 1916; Voronin, 1890).

N.A. Palchevsky (1891), who lived in this territory and was one of the first to report the disease of grain crops, studied its ~~a~~etiology and deposited diseased grain head specimens in herbaria (kept in the Herbarium LEP of our laboratory, the first specimens are dated 1912). Thanks to this inquisitive individual, drawings of typical symptoms of the disease and pathogens were published (Fig. 1).

FHB was a persistent problem in the Far East during the 20th century (Abramov, 1938; Naumov, 1916) and continues to be today. High severities of FHB are reported nearly every year in the region. Mycological analyses of seed samples from 1998–2002 have shown a high level of

Formatted: Font: Not Italic

FHB-infected wheat and barley seed (23–32%). The most frequently isolated pathogen was *F. graminearum* (Gagkaeva et al., 2002; Ivaschenko et al., 2000).

Potential toxic effects of mycotoxins associated with FHB, particularly trichothecenes, which are secondary metabolites produced by *F. graminearum*, can result in numerous health problems after consumption of infected grain, flour, and processed products. *Fusarium* outbreaks are a concern because of loss of grain yield and quality and mycotoxin contamination.

The development of multilocus sequence typing (MLST) has facilitated the identification of species and chemotypes of the *F. graminearum* species (*Fg*) group (Ward et al., 2008). Among them, the ubiquitous *F. graminearum sensu lato* (s. lat.) includes at least 16 phylogenetic species (Aoki et al., 2012; O'Donnell et al., 2000, 2004, 2008) united into the *Fg* group. Based on MLST assays, several species of the *Fg* group, including *F. graminearum sensu stricto* (s. str.), *F. ussurianum* T. Aoki, Gagkaeva, Yli-Mattila, Kistler & O'Donnell, and *F. vorosii* B. Tóth, Varga, Starkey, O'Donnell, H. Suga & T. Aoki, were identified in the grain grown in the Russian Far East (Yli-Mattilla et al., 2009). A biogeographic hypothesis suggests that *F. vorosii*, *F. ussurianum*, and *F. asiaticum* O'Donnell, T. Aoki, Kistler & Geiser may be endemic Asian species within the *Fg* group (O'Donnell et al., 2004).

All species within the *Fg* group are capable of producing type B trichothecenes, but the activity of their formation is largely different. Three types of chemotypes have been identified among the strains: deoxynivalenol (DON) and 3-acetyldeoxynivalenol (3-AcDON), deoxynivalenol, and 15-acetyldeoxynivalenol (15-AcDON), and nivalenol and 4-acetyl-nivalenol (NIV) (Moss, Thrane, 2004; Ward et al., 2002).

High humidity and heavy rainfall stimulate the development of *F. graminearum* s. lat. in grain and, as a result, increase its contamination by DON (Aldred, Magan, 2004; Ramirez et al., 2006). The Russian Far East is often exposed to weather disasters, such as floods, which can lead to

negative consequences insurmountable by human efforts and technologies resulting in significant agricultural damage. In the summer of 2019, in the Amur region, which accounts for 60–70% of grain production in the Russian Far East, flooding after substantial rainfall has wreaks havoc with extensive damaged crops~~caused an emergency situation with grain crops~~. In this region, an emergency regime was established on July 25, 2019, and 'about 250,000 ha was flooded, which amounted to about 20% of the total cultivated area in the region' (TASS, 2019). As a result, the yield of cereals was only partially saved and harvested.

Despite the long history of the problem in the Far East, there is still no objective information on infection and mycotoxin contamination of harvested grain. Epidemics of FHB in the region fuelled our interest in investigating this disease using available modern methods of research.

Broad geographic surveys of *Fusarium* species on cereal crops are important to establish if any present shifts in populations occur in response to environmental change. We expected the diversity of *Fusarium* species belonging to the *Fg* group in this territory to be high~~,~~ since the conditions were very favorable for fungi and led to the disease epidemic. In addition, we assumed that *F. asiaticum* may appear in the complex of pathogens~~,~~ since in the neighbouring countries of China and Japan this species is detected on cereals with a high frequency (Gale et al., 2002; Láday et al., 2004; Qu et al., 2007; Suga et al., 2008). In China, where the problem of FHB is also acute, two species of the *Fg* group have been identified: *F. graminearum* s. str. and *F. asiaticum* (Qu et al., 2007). In the north of China, closest to the Amur Region, mostly *F. graminearum* was dominant, and all of the 15-AcDON chemotypes (Shen et al., 2012). But *F. asiaticum* was the predominant species in the Yangtze River Basin, and chemotypes of strains were either 3-AcDON or NIV, with 3-AcDON being predominant.

~~The aim of the study was to obtain objective information on the quality of grain grown under natural conditions of excessive moisture in the Amur region in 2019, including the species composition of fungi and the DNA content of pathogens and mycotoxins.~~

This~~e aim of this study was~~ study aimed to investigate (1) the natural *Fusarium* species occurrence and mycotoxin contamination of grain from the Amur region in the Far East in the most favorable conditions for pathogens and (2) provide the multilocus analysis of isolated strains of *F. graminearum* sensu lato to species and trichothecene genotype diversity.

Materials & Methods

Grain samples and climatic conditions of growth

~~In the mid-August, 2019, the samples of spring wheat (nine samples) and barley (four samples) were harvested from the different locations of the Amur region, Russian Far East.~~ In mid-August 2019, grain harvest samples were collected in various flood-rescued fields located in the Amur Region, the Russian Far East. These samples were spring wheat (nine samples of the most common Aryuna variety) and barley (four samples of the most common Acha variety). Collecting the representative sample from harvesting ~~The collection of specimens~~ at these locations was approved by the Russian Science Foundation (project number: 19-76-30005).

The weather in the summer period of 2019 was characterized by disastrous excessive moisture: the total rainfall in July and August was 2.2 and 1.7 times greater, respectively~~,~~ than the average means of long-term observations (according to https://rp5.ru/). In addition, the number of days with precipitation in these months was 50% and 39% more, respectively, than the average means of the climatic norm (Table 1).

Mycological analysis of grain

Microscopic examination was conducted to reveal the presence of infected grains and perithecia ~~fungal structures~~ on seed surfaces, and photographs were taken under ~~an~~ Olympus BX53 and Olympus SZX16 microscopes.

One hundred seeds per sample were chosen at random and surface disinfected by soaking in a 5% sodium hypochlorite solution for 1–2 min. Then the grains were washed with sterile water and put into Petri dishes on potato sucrose agar medium (PSA) containing 1 mL/L of an antibiotics solution (HyClone ™, Austria). Moreover, a commonly used detergent Triton X-100 (Panreac, Spain) which reduces the linear fungal growth (0.4 µL/L) was added. After 7-14 days of incubation in the dark at 24 °C, identification and demarcation of taxa were carried out (Gerlach, Nirenberg, 1982; Leslie, Summerell, 2006). The grain infection by the specific taxon of fungi was calculated as the ratio of the number of grains from which these fungi were isolated to the total number of ~~analysed~~ analyzed grains and expressed as the incidence percentage.

DNA extraction and quantification

The grain samples (20 g) were homogenized separately using sterilized grinding chambers of a batch mill Tube Mill Control (IKA, Königswinter, Germany). The grain flour was stored at −20 °C.

The total DNA from 200 mg of grain flour was isolated using the Genomic DNA Purification Kit (Thermo Fisher Scientific, Vilnius, Lithuania) according to the manufacturer's protocol and as previously described in Gagkaeva et al. (2019). Using the same kit, DNA was also isolated from the mycelium of *Fusarium* spp. strains cultivated on PSA. DNA concentrations from the grain samples and fungal strains were determined using a Qubit 2.0 Fluorometer with a Quant-iT dsDNA HS Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA). Before the start of quantitative PCR (qPCR), the concentrations of all DNA samples were normalized to 23–67 ng/µL.

In every total DNA sample extracted from grain flour, the DNA content of the *F. graminearum* and *F. avenaceum* was evaluated by qPCR with TaqMan probes (Yli-Mattila et al., 2008). The reaction was carried out in a 20-µL-volume mixture with 10 µL of a 2 × TaqM master mix (AlkorBio, St. Petersburg, Russia), 300 nM of each primer, 100 nM of a fluorescent sample (Evrogen, Moscow, Russia), and 2 µL of the corresponding DNA solution.

Additionally, the DNA content of 3-AcDON and 15-AcDON chemotypes of *F. graminearum* was determined using qPCR with SYBR Green (Nielsen et al., 2012). All qPCR assays were run using the CFX 96 Real-Time System thermocycler (Bio-Rad, Hercules, CA, USA). All samples were ~~analysed~~ analyzed at least twice.

Mycotoxin determination by HPLC-MS/MS

The HPLC-MS/MS multi-mycotoxin method was used to detect different fungal secondary metabolites. In the grain samples, 3-AcDON, 15-AcDON, alternariol (AOH), alternariol monomethyl ether (AME), beauvericin (BEA), DON, deoxynivalenol-3-glucoside (DON-3gl), diacetoxyscirpenol (DAS), fumonisins B1, B2, and B3, T-2 toxin, HT-2 toxin, T-2 triol, neosolaniol (NEO), fusarenone X, moniliformin (MON), nivalenol (NIV), tentoxin (TEN), tenuazonic acid (TeA), and zearalenone (ZEN) were ~~analysed~~analyzed.

The analysis of the mycotoxins was carried out following the described procedure (Malachová et al., 2014). Detection and quantification were performed with a QTrap 5500MS/MS system (Applied Biosystems, Foster City, CA, USA) equipped with a TurboV electrospray ionization (ESI) source and a 1290 series UHPLC system (Agilent Technologies, Waldbronn, Germany). Chromatographic separation was performed at 25 °C on a Gemini® C18-column, 150 × 4.6 mm i.d., with a 5-µm particle size, equipped with a C18 SecurityGuard cartridge, 4 × 3 mm i.d. (all from Phenomenex, Torrance, CA, USA). Elution was carried out in binary gradient mode. Both mobile phases contained 5 mM of ammonium acetate and were composed of

methanol/water/acetic acid ratios of 10:89:1 (v/v/v; eluent A) and 97:2:1 (v/v/v; eluent B), respectively. The recovery of mycotoxins from grain ranged from 79% to 105%.

Genotyping of *Fusarium* spp.

Among isolated fungi that were morphologically assigned to the *Fg* group (nearly 900), 29 monoconidial strains were randomly selected for further molecular analysis. Additionally, four related *Fusarium* strains with various geographic and substrate origins, the taxonomic status of which requires appraisal, were included in the study (Table 2).

~~In order t~~To assess the phylogenetic relationships between all the strains tested, fragments of the translation elongation factor EF-1a (*TEF*), ammonium ligase gene (*URA*), reductase gene (*RED*), and 3-O-acetyltransferase gene (*Tri101*) were used. Their amplification was carried out using specific primers EF1/EF2, URA11/URA16, RED1d/RED2, and TRI1013E/TRI1015B, respectively, according to the authors' protocols and instructions (O'Donnell et al., 2000, 2004, 2008).

The sequencing was carried out on an ABI Prism 3500 sequencer (Applied Biosystems, Hitachi, Japan) using the BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems, USA). To address the phylogenetic relationships among taxa maximum likelihood (ML), maximum parsimony (MP) analysis was conducted using the MEGA X 10.2 program (Kumar et al., 2018) as well as Bayesian posterior probability (BP) by MrBayes v. 3.2.1 on the Armadillo 1.1 platform (Lord et al., 2012). Nodal support was assessed by bootstrap analysis on 1,000 replicates. Sequence data were deposited in GenBank.

The *Fusarium* spp. a chemotype (3-AcDON, 15-AcDON, or NIV) was determined using PCR with primers Tri13P1/Tri13P2 according to the authors' protocols and instructions (Wang et al., 2008).

All tested *Fusarium* strains are maintained in the collection of the Laboratory of Mycology and Phytopathology at the All-Russian Institute of Plant Protection.

Statistical analysis

Data were ~~analysed~~ analyzed using Microsoft Office Excel 2010 (Microsoft, Redmond, WA, USA) and Statistica 10.0 (StatSoft, Tulsa, OK, USA). The significance of differences between mean values was estimated by ~~a~~ Tukey's test (95% confidence level).

Results

Detection of grain infection with fungi

Visual analysis of grain samples revealed the presence of various deformities, shrunken and with a pink-white colo~~u~~ration of grain heads in the amount of 5–42% (Fig. 2.). Due to prolonged wet weather, the salmon-orange conidia masses of the fungus and blue-black perithecia can be seen on the infected spikelet and glumes in barley. Most of the perithecia were mature, and when placed in a water drop, the ascospores with three septa appeared from asci.

The average germination of wheat grain was 25.1% (12–41%) and of barley grain was 55.3% (48–62%). Almost 100% infection by fungi of all grain samples was noted; often, different fungi were isolated from one grain.

Mycology analyses verified that infection by *Fusarium* spp. was the primary cause of damage in grains, and infection rates reached extremely high percentages (Table 3). Moreover, the proportion of *F. graminearum* s. lat. strains among all isolated *Fusarium* spp. averaged 83.7% in the wheat grain and 89.7% in the barley grain. *Fusarium sporotrichioides* Sherb. strains were detected in 61% of samples, but grain infection was low (1–4%) (the supplemental table). Among the isolated fungi, the occurrence of *F. avenaceum* (Fr.) Sacc., *F. anguioides* Sherb., *F.*

tricinctum (Corda) Sacc., *F. poae* (Peck) Wollenw., *F. cerealis* (Cooke) Sacc., *F. equiseti* (Corda) Sacc., *F. incarnatum* (Desm.) Sacc., and *F. heterosporum* Nees et T. Nees as well as four strains belonging to the *Fusarium fujikuroi* species complex were lower (the supplemental table).

Alternaria spp. were the second frequent genera isolated from the grains. Moreover, the infection of wheat grain with *Alternaria* spp. was almost two times lower (12.9%) than that of barley grain (21.5%). *Cladosporium* spp., *Clonostachys rosea* (Link: Fr.) Schroers, Samuels, Seifert & W. Gams, *Cochliobolus* spp., *Epicoccum nigrum* Link, and other fungi were also identified in the grain mycobiota (the supplemental table)**.**

Quantification of *Fusarium* biomass

The amount of *F. graminearum* DNA in grain flour was very high, averaging 4.9 pg/ng (Table 3). In ~~analysed~~ analyzed samples of wheat grain, the amount of *F. graminearum* DNA was higher than in the barley grain samples ($p = 0.032$). The amount of 3-AcDON *F. graminearum* DNA was on average 1.3–1.1 times higher than the content of 15-AcDON genotype DNA. *F. avenaceum* DNA was detected in all grain samples in an amount that was on average 160 times less than that of *F. graminearum* DNA.

Detection of mycotoxins

DON was found in all samples. The content of DON reached 13,343 ppb in wheat samples and 7,755 ppb in barley samples. In all ~~analysed~~ analyzed samples, the content of DON exceeded the maximum permissible limits (MPLs) in grain for food (700 ppb for wheat grain, 1,000 ppb for barley grain) and ~~for~~ fodder (1,000 ppb for cereal grain), by up to 13 times (TR TS 015/2011; TR TS 021/2011). The exception was one barley sample, in which the DON content was lower than the MPL: 911 ppb.

In addition, other type B trichothecene mycotoxins, 3-AcDON, 15-AcDON, and DON-3gl, were detected in the grain. Of the total content of trichothecenes, the share of DON in wheat grain was 86.5% and in barley grain was 69.5%.

The content of ZEN produced by *F. graminearum* in wheat grain (92–3,670 ppb) was on average 2.1 times higher than in barley grain (111–928 ppb).

Low contents of T-2 toxin (5 and 15 ppb) and HT-2 toxin (23 and 58 ppb) produced by *F. sporotrichioides* were detected in two barley grain samples.

The MON produced by *F. avenaceum* was detected in all samples in amounts up to 218 ppb without differences between crops. The mycotoxin BEA was detected in only two wheat samples in amounts up to 13 ppb. The fumonisins NEO, DAS, and fusarenone X produced by *Fusarium* fungi were not detected in the ~~analysed~~ analyzed grain samples.

The mycotoxin AOH produced by *Alternaria* fungi was detected in all grain samples in small amounts (8–49 ppb). Moreover, the content of this mycotoxin in barley grain, 11.7 (7.6–17.2), was significantly lower than in wheat grain, 29.0 (14.2–49.1) ($p = 0.032$). AME was found in all ~~analysed~~ analyzed grain samples except for two wheat samples in trace amounts. TeA was detected in all barley grains with a maximal level of 37.4 ppb and in 44% of wheat samples with a maximal level of 75.0 ppb (the supplemental table). Traces of TEN were found in all samples (max 6.4 ppb).

Genotyping of *Fusarium* spp.

Multilocus analyses of the *TEF*, *URA*, *RED*, and *Tri101* sequences were used to determine the genetic relationships among *Fusarium* strains. The dataset included 34 combined sequences of the ~~analysed~~ analyzed strains as well as the 12 reference sequences of *Fusarium* spp. belonging to the *Fg* group and consisted of a total of 2,941 characters (612 bp from the *TEF*, 558 bp from *URA*, 821 bp from *RED*, and 950 bp from *Tri101*). The sequence of the *F. pseudograminearum*

type strain NRRL 28334 was used as the outgroup. The resulting phylogenetic tree based on DNA sequence data of *Fusarium* species was ~~constracted~~ constructed (Fig. 3). Maximum likelihood and MP bootstrap support values greater than 70%, followed by Bayesian posterior probability scores greater than 0.95, are shown at the nodes.

The topology of phylogenetic trees constructed by different methods turned out to be similar and demonstrated the phylogenetic relationships between species established earlier (Aoki et al., 2012). Twenty-nine ~~analysed~~ analyzed *Fusarium* strains isolated from Amur grain belonged to the clade with reference strain NRRL 5883 *F. graminearum* s. str. (Fig. 3). Among the ~~analysed~~ analyzed strains of *F. graminearum* s. str., nine strains were the 3-AcDON chemotype while 21 strains turned out to be the 15-AcDON chemotype (Table 2).

From four doubtful *Fusarium* strains, one strain MFG 60604, isolated from wheat grain from the Altai Krai (Western Siberia), was clustered with the reference strain *F. vorosii* NRRL 45790 with high bootstrap support (ML/MP/BP: 99/99/1.0). Our phylogenetic analysis indicates that strain MFG 60604 is *F. vorosii* and it is determined as a 15-AcDON chemotype.

Three other doubtful strains, MFG 58836, MFG 59052, and MFG 60755, formed the clade with the reference strains *F. culmorum* NRRL 25475 with high bootstrap support (ML/MP: 98/100). All three *F. culmorum* strains were the 3-AcDON chemotype (Table 2).

Discussion

Despite the long history of the FHB problem in the Russian Far East, objective data on pathogen composition and content of mycotoxins in naturally infected grain is ~~clearly~~ under-published. ~~This was an intriguing study seeking to better understand which phylogenetic species and chemotypes prevailed on grain under the favourable conditions of extremely high humidity during floods in the Amur region in 2019.~~

The mycological analyses of grain from this region in 2019 revealed extremely high infection of grain with *Fusarium* spp.—up to 98%. The predominant cause of FHB was the *Fg* group, which accounted for 86% of all isolated *Fusarium* spp.

Interestingly, the amount of fungal DNA in the wheat grain was on average higher than in the barley grain, while the percentage of infected grains was the same. The revealed differences may be due to the abundance of fungal biomass concentrated on the surface of barley grains (husk, palea, pericarp), while the wheat grain is completely permeated with fungal hyphae. In general, in this situation in 2019, the infection rates for both wheat and barley were off the scale. In our opinion, the highest DON content detected in this study, in the amount of 13,343 ppb, exceeds the maximum amounts of this mycotoxin in grain previously detected in the Russian territory. During the outbreak of FHB in southern Europe in 1985–1991 the maximal content of DON in grain reached 10,000 ppb (Kononenko, 2005). Recently, in 2017, a DON amount of 7,920 ppb was detected in wheat grain grown in southern Europe (Kononenko et al., 2020).

The content of 3-AcDON in wheat and barley grain, as well as 15-AcDON, was similar and did not exceed 293 ppb. In the plant, DON can be present as a metabolite, DON-3gl, which is represents up to 46% of the total amount of DON in infected wheat and maize varieties (Berthiller et al. 2009). It has been shown that DON-3gl can be converted back to DON in mammals (Dall'Erta et al., 2013; Tucker et al., 2019). Therefore, DON-3gl is also frequently referred to as a masked mycotoxin. In our study, the maximum content of DON-3gl reached 3,803 ppb and was twice as high, on average, in barley grain than in wheat grain. The amounts of DON-3gl come to 13.5% and 39.5% of the total amounts of DON in infected wheat and barley samples, respectively. However, there were no significant differences in the content of the trichothecene mycotoxin average between wheat and barley grains.

Using morphology to accurately assess species limits for the *Fg* group is not reliable. ~~The latest study of the species composition of the *Fg* group in the Far East led to the understanding that *F. graminearum* s. str. is the dominant pathogen damaging grain, in addition to *F. ussurianum* and *F. vorosii* (Yli-Mattila et al., 2009).~~

Before this study, we hypothesized that in the extremely humid and warmest conditions of 2019, in the area where FHB outbreaks were observed for at least 140 years, ~~a number of~~several species of the Asian clade of the *Fg* group will be identified. Especially considering that earlier we have already found three species of the *Fg* group in this region: *F. graminearum* s. str., *F. ussurianum* and *F. vorosii* (Yli-Mattila et al., 2009). Selecting freshly isolated fungi for analysis, we took cultures for a detailed study, which included all the morphological diversity present within the limits possible for the *Fg* group (pigmentation, rate of formation of macroconidia, size, and shape). Multilocus phylogenetic analysis revealed that all strains from the Amur grains belonged to the *F. graminearum* s. str.

Molecular methods make it possible to reveal the intraspecific diversity of *F. graminearum* and to establish the quantitative presence of two different chemotypes. The *F. graminearum* strains are divided into 3-AcDON and 15-AcDON chemotypes depending on the prevailing ~~formation of a particular~~ acetylated form of DON (Alexander et al., 2011; Foround et al., 2019). ~~Molecular methods make it possible to reveal the intraspecific diversity of *F. graminearum* and to establish the quantitative presence of two different chemotypes.~~ Regional differences have been reported regarding ~~on~~ the occurrence of chemotypes within the *Fg* group (Foround et al., 2019; Pasquali et al., 2016). In our study, on average, the DNA content of the 3-AcDON and 15-AcDON fungus chemotypes in the grain was similar, but the DNA of the 15-AcDON chemotype in wheat grain was significantly higher (4.6 times) than in barley ($p = 0.014$), whereas the difference in DNA content of the 3-AcDON fungus chemotype in wheat and barley grain was

insignificant. It is not known whether the observed differences are related to ~~chemotype~~ chemotype-specific plant-host preferences. There may be a difference in pathogenicity between the 3- and 15-ADON chemotypes to wheat and barley (Foroud et al., 2019; Clear et al., 2013).

According to our results, 30% of the ~~analysed~~ analyzed *F. graminearum* strains were the 3-AcDON chemotype, while 70% of the strains were the 15-AcDON chemotype. Previously, the chemotype analysis of the 105 *F. graminearum* strains collected in the Russian Far East in 1998–2006 revealed the approximately equal occurrence of 3-AcDON (48%) and 15-AcDON (52%) chemotypes (Yli-Mattila et al., 2009). An increase in the 15-ADON chemotype has recently been shown in regions of Europe, where the 3-ADON chemotype was previously dominant, although many of the factors affecting their distribution are still unclear (Nielsen et al., 2012; Aamot et al., 2015; Pasquali et al., 2016; Foround et al., 2019). The third chemotype of *F. graminearum* s. str. producing nivalenol (NIV) has not yet been identified in Russia or China (Shen et al., 2012), although it is known to be found in Europe (Pasquali et al., 2016).

In our analysis, *Fusarium* sp. strain MFG 60604 was included that was isolated from wheat grain in the Western Siberia region (the Altai Krai); phenotypically, this strain was a dubious representative of the *Fg* group. In this region, the occurrence of *F. graminearum* was previously not typical, but in recent years, we have been identifying this pathogen in cereal grains (Gagkaeva et al., 2019). The strain MFG 60604, isolated from wheat grain from West Siberia, was clustered with the reference strain *F. vorosii* NRRL 45790 with high bootstrap support (ML/MP/BP: 98/99/1.0), which allows for accurate establishment of its species affiliation. A single strain (MFG 60604) identified as *F. vorosii* in this study, is the only third strain of *F. vorosii* found in Russia and the first one identified in the Siberian region. Previously identified strains of *F. vorosii* from the Russian Far East belonged to the 15-AcDON chemotype (Yli-Mattila et al., 2009) and so did the strain identified in this study. However, among six *F. vorosii*

strains originating from Korea, five were the NIV chemotype, while only one was the 15-AcDON (Lee et al., 2016). Among *F. vorosii*, no strains of the 3-AcDON chemotype have been identified, which, probably, were not detected due to the small number of strains of this species analyzed to date. In the limited surveys to date, strains of several species of the *Fg* group were found to represent only a single chemotype (Aoki et al., 2012).

Two strains of *F. culmorum* of closely related taxon to *Fg* group from the Western Siberia and Ural regions and one from the South European region of Russia were included in the study. The high genetic similarity of ~~analysed~~ analyzed *F. culmorum* strains collected from remote regions characterized by different climatic conditions (the distance between isolation points is about 2,500 km) is consistent with the previously shown information that *F. culmorum* is a single phylogenetic species with little or no differences between lineages, despite the geographic separation of genotypes (Obanor et al., 2010).

The studies ~~analysing~~ analyzing the occurrence of *F. culmorum* chemotypes in different regions, as a rule, show a significant excess of the occurrence of the DON chemotype compared to the NIV chemotype (Laraba et al., 2017; Pasquali et al., 2016; Scherm et al., 2012). Strains of the 15-AcDON chemotype typical for *F. graminearum* were not identified among the strains of *F. culmorum*. A previous analysis of a few strains of *F. culmorum* from the Russian territory has characterized them as the 3-AcDON chemotype (Yli-Mattila et al., 2009).

~~Broad geographic surveys of *Fusarium* species on cereal crops are important to establish if any present shifts in populations occur in response to environmental change. We expected the diversity of *Fusarium* species belonging to the *Fg* group in this territory to be higher than found in our study, since the conditions were very favorable for fungi and led to the disease epidemic. In addition, we assumed that *F. asiaticum* may appear in the complex of pathogens, since in the neighbouring countries of China and Japan this species is detected on cereals with a high~~

~~frequency (Gale et al., 2002; Láday et al., 2004; Qu et al., 2007; Suga et al., 2008). In China, where the problem of FHB is also acute, two species of the *Fg* group have been identified: *F. graminearum* s. str. and *F. asiaticum* (Qu et al., 2007). But in the north of China, mostly *F. graminearum* was dominant, and all of the 15-AcDON chemotypes (Shen et al., 2012). *F. asiaticum* was the predominant species in the Yangtze River Basin, and chemotypes of strains were either 3-AcDON or NIV, with 3-AcDON being predominant.~~

Fusarium spp. continue to pose a threat to farmers, destroying crops or dramatically reducing yields, as well as to animal and human health due to the production of mycotoxins. Even in our time, when we know much more about the nature of *Fusarium* spp. ~~than~~ then 140 years ago, we are still not very successful in controlling the diseases they cause on crops if conditions are favo~~u~~rable for the development of pathogens. Indeed, in the process of our study, it was shown in the mass media that although the grain was harvested with great difficulty, due to the significant contamination of the grain, part of the crop, 240 tons, had to be destroyed by fire.

Conclusions

The high prevalence of Fusarium head blight in cereal grains cultivated in the Far East is particularly alarming and strongly indicates the need for increased measures to prevent plant infection and improved food safety interventions. The maximum DON content in wheat grains reached 13,141 ppb in this study. ~~Multilocus~~ The multilocus sequence revealed that the majority of the strains used in this study belonged to *F. graminearum* s. str.

Formatted: Font: Not Italic

Acknowledgements

We are grateful to the managers of the Russian Branch of BASF company for their help with the grain sample collection and ~~The authors thank~~ Nadezhda N. Gogina from the All-Russian Scientific and Technological Institute of Poultry (Moscow region) for carrying out HPLC-MS/MS analysis.

References

Aamot HU, Ward TJ, Brodal G, Vrålstad T, Larsen GB, Klemsdal SS, Elameen A, Uhlig S, Hofgaard IS. 2015. Genetic and phenotypic diversity within the *Fusarium graminearum* species complex in Norway. European Journal of Plant Pathology **142:** 501–519 https://doi.org/10.1007/s10658-015-0629-4

Abramov IN. 1938. Diseases of agricultural plants on the Far East. Far Eastern State Regional Publishing House, Khabarovsk. (in Russian)

Aldred D, Magan N. 2004. Prevention strategies for trichothecenes. Toxicology Letters **153:**165–171 https://doi.org/10.1016/j.toxlet.2004.04.031

Alexander NJ, McCormick SP, Waalwijk C, van der Lee T, Proctor RH. 2011. The genetic basis for 3-ADON and 15-ADON trichothecene chemotypes in *Fusarium graminearum*. Fungal Genetics and Biology **48:**485–495 https://doi.org/10.1016/j.fgb.2011.01.003

Aoki T, Ward T, Kistler H, O'Donnell K. 2012. Systematics, phylogeny and trichothecene mycotoxin potential of Fusarium head blight cereal pathogens. Mycotoxins **62:**91–102 https://doi.org/10.2520/myco.62.91

Clear RM, Tucker JR, Gaba D, Patrick SK, Lee SJ, Demeke T, Tittlemier SA, Legge WG, Gräfenhan T. 2013. Deoxynivalenol levels and chemotype frequency in barley cultivars

450 inoculated with two chemotypes of *Fusarium graminearum*. Canadian Journal of Plant
451 Pathology **35:** 37–45 https://doi.org/10.1080/07060661.2012.751622
452 Dall'Erta A, Cirlini M, Dall'Asta M, Rio DD, Galaverna G. 2013. Masked mycotoxins are
453 efficiently hydrolyzed by human colonic microbiota releasing their aglycones. Chemical
454 Toxicology Research **26:**305–312. http://dx.doi.org/10.1021/tx300438c
455 Foround NA, Baines D, Gagkaeva TYu, Thakor N, Badea A., Steiner B., Bürstmayr M,
456 Bürstmayr H. 2019. Trichothecenes in cereal grains – an update. Toxins **11:**634
457 http://dx.doi.org/10.3390/toxins11110634
458 Gagkaeva T, Gavrilova O, Orina A, Lebedin Y, Shanin I, Petukhov P, Eremin S. 2019.
459 Analysis of toxigenic *Fusarium* species associated with wheat grain from three regions of Russia:
460 Volga, Ural, and West Siberia. Toxins **11:**252 https://doi.org/10.3390/toxins11050252
461 Gagkaeva TYu, Levitin MM, Zuev E, Terentjeva I. 2002. Evaluation of genetic resources
462 of wheat and barley from Far East of Russia for resistance to Fusarium head blight. Journal of
463 Applied Genetics **43A:**229–236
464 Gale LR, Chen L–F, Hernick CA, Takamura K, Kistler HC. 2002 Population analysis of
465 *Fusarium graminearum* from wheat fields in Eastern China. Phytopathology **92:**1315–1322
466 https://doi.org/10.1094/PHYTO200292121315
467 O'Donnell K, Ward TJ, Geiser DM, Kistler HC, Aoki T. 2004. Genealogical
468 concordance between the mating type locus and seven other nuclear genes supports formal
469 recognition of nine phylogenetically distinct species within the *Fusarium graminearum* clade.
470 Fungal Genetics and Biology **41**: 600–623 https://doi.org/10.1016/j.fgb.2004.03.003
471 Gerlach W, Nirenberg HI. 1982 The genus *Fusarium*—A Pictorial Atlas. In: *Mitteilungen*
472 *aus der Biologischen Bundesanstalt fur Land- und Forstwirtschaft, Berlin-Dahlem*;
473 Herausgegeben von der Biologischen Bundesanstalt für Land- und Forstwirtschaft, Berlin, 209

Ivashchenko VG, Shipilova NP, Levitin MM. 2000. Species composition of *Fusarium* fungi on cereals in the Asian part of Russia. Mikologia I Fitopatologia **34:**54–68 (In Russian)

Jaczewski AA. 1904. About drunken bread. Sheet for diseases and damages of cultivated and wild useful plants **11:**89–92 (In Russian)

Karlovsky P. 2011. Biological detoxification of the mycotoxin deoxynivalenol and its use in genetically engineered crops and feed additives. Applied Microbiology and Biotechnology **91:**491–504 https://doi.org/10.1007/s00253-011-3401-5

Kononenko GP. 2005. The system of mycotoxicological control of objects of veterinary, sanitary and environmental supervision. Theses of the doctoral dissertation, Moscow

Kononenko GP, Burkin AA, Zotova YeV. 2020. Mycotoxicological monitoring Part 3 Feedstuffs from raw grain processing. Veterinary Science Today **3:**213–219 https://doi.org/10.29326/2304-196X-2020-3-34-213-219

Kumar S, Stecher G, Li M, Knyaz C, Tamura K. 2018. MEGA X: Molecular evolutionary genetics analysis across computing platforms. Molecular Biology and Evolution **35:**1547–1549 https://doi.org/10.1093/molbev/msy096

Láday M, Juhász. Á, Mulé G, Moretti A, Szécsi A, Logrieco A. 2004. Mitochondrial DNA diversity and lineage determination of European isolates of *Fusarium graminearum* (*Gibberella zeae*). European Journal Plant Pathology **110:**545–550 https://doi.org/10.1023/B:EJPP0000032394391302c

Laraba I, Boureghda H, Abdallah N, Bouaicha O, Obanor F, Moretti A, Geiser DM, Kim H-S, McCormick SP, ProctorRH, KellyAC, Ward TJ, O'Donnell K. 2017. Population genetic structure and mycotoxin potential of the wheat crown rot and head blight pathogen *Fusarium culmorum* in Algeria. Fungal Genetics and Biology **103:**34–41 https://doi.org/10.1016/jfgb201704001

Lee T, Paek J-S, Lee K, Lee S, Choi J-H, Ham H, Hong S, Ryu J-G. 2016. Occurrence of toxigenic *Fusarium vorosii* among small grain cereals in Korea. Plant Pathology Journal **32:**407–413 https://doi.org/10.5423/PPJOA0520160123

Leslie JF, Summerell BA. 2006. The *Fusarium* laboratory manual. Blackwell Publishing, Ames https://doi.org/10.1002/9780470278376

Lord E, Leclercq M, Boc A, Diallo AB, Makarenkov V. 2012. Armadillo 11: An original workflow platform for designing and conducting phylogenetic analysis and simulations. PLoS ONE **7:**e29903 https://doi.org/10.1371/journalpone0029903

Malachová A, Sulyok M, Beltrán E, Berthiller F, Krska. R. 2014. Optimization and validation of a quantitative liquid chromatography–tandem mass spectrometric method covering 295 bacterial and fungal metabolites including all regulated mycotoxins in four model food matrices. Journal of ChromatographyA **1362:**145–156 https://doi.org/10.1016/jchroma201408037

Moss MO, Thrane U. 2004. *Fusarium* taxonomy with relation to trichothecene formation. Toxicology Letters **153:**23–28 https://doi.org/10.1016/jtoxlet200404021

Naumov NA. 1916. Drunken bread Observations under some species of genus *Fusarium* Bureau of Mycology and Phytopathology, Petrograd (In Russian)

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. International Journal of Food Microbiology **157:**384–392 https://doi.org/10.1016/jijfoodmicro201206010

O'Donnell K, Kistler HC, Tacke BK, Casper HH. 2000. Gene genealogies reveal global phylogeographic structure and reproductive isolation among lineages of *Fusarium graminearum*, the fungus causing wheat scab. PNAS **97:**7905–7910 https://doi.org/10.1073/pnas130193297

O'Donnell K, Ward TJ, Aberra D, Kistler HC, Aoki T, Orwig N, Kimura M, Bjornstad A, Klemsdal SS. 2008. Multilocus genotyping and molecular phylogenetics resolve a novel head blight pathogen within the *Fusarium graminearum* species complex from Ethiopia. Fungal Genetics and Biology **45:**1514–1522 https://doi.org/10.1016/jfgb200809002

O'Donnell K, Ward TJ, Geiser DM, Kistler HC, Aoki T. 2004. Genealogical concordance between the mating type locus and seven other nuclear genes supports formal recognition of nine phylogenetically distinct species within the *Fusarium graminearum* clade. Fungal Genetics and Biology **41:**600–623 https://doi.org/10.1016/jfgb200403003

Obanor F, Erginbas-Orakci G, Tunali B, Nicol JM, Chakraborty S. 2010. *Fusarium culmorum* is a single phylogenetic species based on multilocus sequence analysis. Fungal Biology **114:**753–765 https://doi.org/10.1016/jfunbio201007001

Palchevsky NA. 1891 Diseases of cultivated cereals of the South Ussuri region Public Benefit Partnership, Saint Petersburg (In Russian)

Pasquali M, Beyer M, Logrieco A, Audenaert K, Balmas V, Ryan Basler R, BoutignyA-L, Chrpová J, Czembor E, Gagkaeva T, González-Jaén MT, Hofgaard IS, Köycü ND, Hoffmann L, Lević J, Marin P, Miedaner T, Migheli Q, Moretti A, Müller MEH, Munaut F, Parikka P, Pallez-Barthel M, Piec J, Scauflaire J, Scherm B, Stanković S, Thrane U, Uhlig S, Vanheule A, Yli-Mattila T, Vogelgsang S. 2016. A European database of *Fusarium graminearum* and *F culmorum* trichothecene genotypes. Frontiers in Microbiology **7:**406 https://doi.org/10.3389/fmicb201600406

Qu B, Li HP, Zhang JB, Xu YB, Huang T, Wu AB, Zhao CS, Carter J, Nicholson P, Liao YC. 2007. Geographic distribution and genetic diversity of *Fusarium graminearum* and *Fusarium asiaticum* on wheat spikes throughout China. Plant Pathology **57:**15–24 https://doi.org/10.1111/j1365-3059200701711x

Ramirez ML, Chulze S, Magan N. 2006. Temperature and water activity effects on growth and temporal deoxynivalenol production by two Argentinean strains of *Fusarium graminearum* on irradiated wheat grain. International Journal of Food Microbiology **106:**291–296 https://doi.org/10.1016/jijfoodmicro200509004

Scherm B, Balmas V, Spanu F, Pani G, Delogu G, Pasquali M, Migheli Q. 2012. *Fusarium culmorum*: causal agent of foot and root rot and head blight on wheat. Molecular Plant Pathology **14:**1–19 https://doi.org/10.1111/mpp12011

Shen CM, Hu YC, Sun HY, Li W, Guo JH, Chen HG. 2012. Geographic distribution of trichothecene chemotypes of the *Fusarium graminearum* species complex in major winter wheat production areas of China. Plant Disease **96:**1172–1178 https://doi.org/10.1094/PDIS-11-11-0974-RE

Suga H, Karugia GW, Ward T, Gale LR, Tomimura K, Nakajima T, Miyasaka A, Koizumi S, Kageyama K, Hyakumachi M. 2008. Molecular characterization of the *Fusarium graminearum* species complex in Japan. Phytopathology **98:**159–166 https://doi.org/10.1094/PHYTO-98-2-0159

TASS, 2019. The flood damage in 2019 in the Amur region is estimated at more than 6 billion rubles. 16 Sept. 2019. https://tass.ru/ekonomika/6888626 (In Russian)

TR TS 015/2011 Technical regulation of the Customs Union 015/2011 "About grain safety" as amended on September 15, 2017 Supplement No 2 Available online: http://www.eurasiancommission.org/ru/act/texnreg/deptexreg/tr/Documents/ТР_зерно.pdf (In Russian) (accessed 7 May 2021)

TR TS 021/2011 Technical regulation of the Customs Union 021/2011 " About food safety" as amended on August 8, 2019 Supplement No 3 Available online:

http://www.eurasiancommission.org/ru/act/texnreg/deptexreg/tr/Documents/TR%20TS%20PisheVayaProd.pdf (In Russian) (accessed 7 May 2021)

Tucker JR, Badea A, Blagden R, Pleskach K, Tittlemier SA, Fernando WGD. 2019. Deoxynivalenol-3-glucoside content is highly associated with deoxynivalenol levels in two-row barley genotypes of importance to Canadian barley breeding programs. Toxins **11:**319 https://doi.org/10.3390/toxins11060319

Voronin MC. 1890. About drunken bread in the South Ussuri region. Botanical notes **3:**13–21 (In Russian)

Wang J, Li H, Qu B, Zhang J, Huang T, Chen F, Liao Y. 2008. Development of a generic PCR detection of 3-acetyldeoxynivalenol, 15-acetyldeoxynivalenol- and nivalenol-chemotypes of *Fusarium graminearum* clade. International Journal of Molecular Sciences **9:**2495–2504 https://doi.org/10.3390/ijms9122495

Ward TJ, Bielawski JP, Kistler HC, Sullivan E, O'Donnell K. 2002. Ancestral polymorphism and adaptive evolution in the trichothecene mycotoxin gene cluster of phytopathogenic *Fusarium.* PNAS **99:**9278–9283 https://doi.org/10.1073/pnas142307199

Ward TJ, Clear R, Rooney A, O'Donnell K, Gaba D, Patrick S, Starkey DE, Gilbert J, Geiser DM, Nowicki TW. 2008. An adaptive evolutionary shift in Fusarium head blight pathogen populations is driving the rapid spread of more toxigenic *Fusarium gramienarum* in North America. Fungal Genetics and Biology **45:**473–484 https://doi.org/10.1016/j.fgb.2007.10.003

Yli-Mattila T, GagkaevaT, Ward TJ, Aoki T, Kistler HC, O'Donnell K. 2009. A novel Asian clade within the *Fusarium graminearum* species complex includes a newly discovered cereal head blight pathogen from the Far East of Russia. Mycologia **101:**841–852 https://doi.org/10.3852/08-217

Yli-Mattila T, Paavanen-Huhtala S, Parikka P, Hietaniemi V, Jestoi M, 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* as compared to mycotoxin production in grains in Finland and Russia. Archives of Phytopathology and Plant Protection **41:**243–260 https://doi.org/10.1080/03235400600680659