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

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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 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 was higher than in the barley grain samples and averaged 6089 × 10^{-3} and 2102 × 10^{-3} 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.) isolates from the grain harvested in the Amur region. Additionally, one atypical isolate, 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 among the analysed *F. culmorum* strains from remote regions was found; these strains were the 3-AcDON chemotype. After 140 years, we are still not very successful in controlling FHB if conditions are favourable for pathogen

development. Even at present, some of the grain harvest must be destroyed, as a high contamination of mycotoxins renders it unusable.

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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 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 was higher than in the barley grain samples and averaged 6089×10^{-3} and 2102×10^{-3} 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.) isolates from the grain harvested in the Amur region. Additionally, one atypical isolate, *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 among the analysed *F. culmorum* strains from remote regions was found; these strains were the 3-AcDON chemotype.

After 140 years, we are still not very successful in controlling FHB if conditions are favourable for pathogen development. Even at present, some of the grain harvest must be destroyed, as a 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 migrants associated the poisoning of people and animals with pink-coloured grain heads with black spots in their 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 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 aetiology, sent the affected plant material to famous Russian mycologists, and deposited specimens in herbaria. Thanks to this inquisitive individual, drawings of typical symptoms of the disease and pathogens were published (Fig. 1), and diseased grain heads are kept in the Herbarium LEP of our laboratory (the first specimens are dated 1912).

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

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 species and chemotype assays 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. According to previous studies 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 to Asian species within the *Fg* group (O'Donnell et al., 2004).

All species within the *Fg* complex are capable of producing type B trichothecenes, but based on the most activity produced by trichothecene metabolites, 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 a region that is often exposed to weather disasters, such as floods, which can lead to negative consequences insurmountable by human efforts and technological methods and cause 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 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.

Epidemics of FHB fuelled our interest in investigating this disease in the Russian Far East using available modern methods of research. 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.

Materials & Methods

Grain samples and climatic conditions of growth

The samples of spring wheat (nine samples) and barley (four samples) were harvested in different locations of the Amur region in the Russian Far East in the middle of August 2019. The collection of specimens at fields was approved by the Russian Science Foundation (project number: 19-76-30005).

The climatic conditions of 2019 were characterized by 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 under a stereomicroscope was conducted to reveal the presence of infected grains and fungal structures on seed surfaces, and photographs were taken under an Olympus BX53 microscope and an Olympus SZX16 stereomicroscope connected to a PROKYON camera (Jenoptik, Jena, Germany).

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 self-made potato sucrose agar medium (PSA) containing 1 mL/L of a mixture of antibiotics (HyClone ™, GE Healthcare Life Sciences, Wien, Austria) and 0.4 μL/L of Triton X-100 solution (Panreac, Barcelona, Spain) to reduce the linear growth of mycelial fungi. After 7 days of incubation in the dark at 24 °C, the number and the species composition of the fungi were counted and identified.

The taxonomic status of isolated fungi was determined according to the sum of their morphological features (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 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.

142 The total DNA from 200 mg of grain flour was isolated using the Genomic DNA Purification
143 Kit (Thermo Fisher Scientific, Vilnus, Lithuania) according the manufacturer's protocol. Using
144 the same kit, DNA was also isolated from the mycelium of *Fusarium* spp. strains cultivated on
145 PSA. DNA concentrations from the grain samples and fungal isolates were determined using a
146 Qubit 2.0 Fluorometer with a Quant-iT dsDNA HS Assay Kit (Thermo Fisher Scientific,
147 Waltham, MA, USA). Before the start of quantitative PCR (qPCR), the concentrations of all
148 DNA samples were aligned to 23–67 ng/μL.
149 In every total DNA sample extracted from grain flour, the DNA content of the *F.*
150 *graminearum* and *F. avenaceum* was evaluated by qPCR with TaqMan probes (Yli-Mattila et al.,
151 2008). The reaction was carried out in a 20-μL-volume mixture with 10 μL of a 2 × TaqM master
152 mix (AlkorBio, St. Petersburg, Russia), 300 nM of each primer, 100 nM of a fluorescent sample
153 (Evrogen, Moscow, Russia), and 2 μL of the corresponding DNA solution.
154 Additionally, the DNA content of 3-AcDON and 15-AcDON chemotypes of *F. graminearum*
155 was determined using qPCR with SYBR Green (Nielsen et al., 2012). The reaction was carried
156 out in a 20-μl-volume mixture with 4 μL of a 5 × qPCRmix-HS SYBR master mix (Evrogen,
157 Moscow, Russia), 500 nM of each primer, and 2 μL of the DNA solution.
158 All qPCR assays were run using the CFX 96 Real-Time System thermocycler (Bio-Rad,
159 Hercules, CA, USA). The DNA solutions of the *Fusarium* strains were diluted to 10 ng/μL and
160 used to construct calibration curves in subsequent dilutions of factors of 10 from 1 to 10^{-5} ng/μL.
161 Fold differences and standard errors were calculated from the Ct values, which were normalized
162 against the DNA of pure cultures of *Fusarium* spp. using the Bio-Rad CFX Manager 1.6
163 software package. DNA content was presented as the ratio of fungal DNA to total DNA in each
164 sample (pg/ng). The low quantification limit of 5×10^{-4} pg fungal DNA on 1 ng of total DNA was

established as the threshold value of DNA in a sample, which can be quantitatively determined with high precision. All samples were analysed 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.

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 security guard 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* fungi

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

187 related *Fusarium* strains with various geographic and substrate origins, the taxonomic status of
188 which requires appraisal, were included in the study (Table 2).

189 In order to assess the phylogenetic relationships between all the strains tested, fragments of
190 the translation elongation factor EF-1a (*TEF*), ammonium ligase gene (*URA*), reductase gene
191 (*RED*), and 3-O-acetyltransferase gene (*Tri101*) were used. Their amplification was carried out
192 using specific primers EF1/EF2, URA11/URA16, RED1d/RED2, and TRI1013E/TRI1015B,
193 respectively, according to the authors' protocols and instructions (O'Donnell et al., 2000, 2004,
194 2008).

195 The sequencing of the fragments was carried out on an ABI Prism 3500 sequencer (Applied
196 Biosystems, Hitachi, Japan) using the BigDye Terminator v3.1 cycle sequencing kit (Applied
197 Biosystems, USA).

198 The manual editing of the nucleotide sequences, obtaining consensus sequences of each
199 strain, and aligning were performed using the Vector NTI Advance 10 program (Thermo Fisher
200 Scientific) and MEGA X 10.2 program (Kumar et al., 2018). The Basic Local Alignment Search
201 Tool (BLAST) was used to perform similarity searches by comparing the consensus sequences
202 with other sequences in NCBI's GenBank database to identify the closest matching sequences
203 that were added to the alignment.

204 To address the phylogenetic relationships among taxa maximum likelihood (ML), maximum
205 parsimony (MP) analysis was conducted using the MEGA X 10.2 program as well as Bayesian
206 posterior probability (BP) by MrBayes v. 3.2.1 on the Armadillo 1.1 platform (Lord et al., 2012).
207 Nodal support was assessed by bootstrap analysis on 1,000 replicates. Sequence data were
208 deposited in GenBank.

The *Fusarium* spp. 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 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 pink-white colouration 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* fungi was the primary causal agent 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* fungi 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%). 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 fungi of the *Fusarium fujikuroi* species complex were lower.

The second group of occurrence in grain turned out to be *Alternaria* Nees fungi. Moreover, the infection of wheat grain with these fungi was almost two times lower (12.9%) than that of barley grain (21.5%). The representatives of *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.

Quantification of *Fusarium* biomass

The amount of *F. graminearum* DNA in grain flour was very high, averaging 4862×10^{-3} pg/ng (Table 3). In analysed 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 samples, the content of DON exceeded by up to 13 times 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) (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 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 and HT-2 toxins (18 and 58 ppb, respectively) produced by *F. sporotrichioides* were detected in two wheat 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 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 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. Traces of TEN were found in all samples (max 6.4 ppb).

Genotyping of *Fusarium* fungi

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 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 ML tree is presented in Figure 3 together with MP and BP values. 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 *Fusarium* strains isolated from Amur grain belonged to the clade with reference strain NRRL 5883 *F. graminearum* s. str. (Fig. 3). Among the analysed strains of *F. graminearum* s. str., nine strains were the 3-AcDON chemotype while 21 strains turned out to be the 15-AcDON chemotype.

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), which allows for accurately establishing its species affiliation. This one detected *F. vorosii* strain was 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.

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 most favourable conditions of extremely high humidity during floods in the Amur region in 2019.

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

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. However, in this situation in 2019, it no longer made any practical sense, since the infection rates for both wheat and barley were off 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, according to published information, the maximal content of DON in grain reached 10,000 ppb (Kononenko, 2005). Recently, a DON amount of 7,920 ppb was detected in wheat grain grown in southern Europe in 2017 (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 formed during the germination of cereal grains (Karlovsky, 2011). However, 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. However, there were no significant differences in the content of the trichothecene mycotoxin average between wheat and barley grains.

The morphological recognition 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 main pathogen damaging grain, in addition to *F. ussurianum* and *F. vorosii* (Yli-Mattila et al., 2009). Before the present study was conducted, we were confident that in the warmest and most humid conditions of 2019, we would identify a range of species of the Asian clade of the *Fg* group. Selecting freshly isolated fungi for analysis, we took cultures for 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 isolates from the Amur grains belonged to the *F. graminearum* s. str.

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* s. str. and to establish the quantitative presence of two different chemotypes. The occurrence of *F. graminearum* chemotypes contains regional differences and is analysed around the world where this pathogen is found (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.

347 According to our results, 30% of the analysed *F. graminearum* strains were the 3-AcDON
348 chemotype, while 70% of the strains were the 15-AcDON chemotype. Previously, the chemotype
349 analysis of the 105 *F. graminearum* strains collected in the Russian Far East in 1998–2006
350 revealed approximately equal occurrence of 3-AcDON (48%) and 15-AcDON (52%)
351 chemotypes (Yli-Mattila et al., 2009). The third chemotype of *F. graminearum* s. str. producing
352 nivalenol (NIV) has not yet been identified in Russia or China (Shen et al., 2012), although it is
353 known to be found in Europe (Pasquali et al., 2016).
354 In addition, we included in the analysis the *Fusarium* sp. strain MFG 60604 that was isolated
355 from wheat grain in the Western Siberia region (the Altai Krai); phenotypically, this strain was a
356 dubious representative of the *Fg* group. For this region, the occurrence of *F. graminearum* was
357 previously not typical, but in recent years, we have been identifying this pathogen in cereal
358 grains (Gagkaeva et al., 2019). The strain MFG 60604, isolated from wheat grain from West
359 Siberia, was clustered with the reference strain *F. vorosii* NRRL 45790 with high bootstrap
360 support (ML/MP/BP: 98/99/1.0), which allows for accurate establishment of its species
361 affiliation. This is the third *F. vorosii* strain found in the territory of Russia and the first one
362 identified in the Siberian region.
363 Previously, *F. vorosii* was detected in the wheat and barley grain grown in the Russian Far
364 East, and the strains belonged to the 15-AcDON chemotype (Yli-Mattila et al., 2009). In this
365 study, one detected *F. vorosii* strain was also determined to be a 15-AcDON chemotype.
366 However, among six *F. vorosii* strains originating from Korea, five were the NIV chemotype,
367 while only one was the 15-AcDON (Lee et al., 2016). The *F. vorosii* is also likely characterized
368 by the presence of the 3-AcDON chemotype, which was not found due to the small number of
369 analysed strains.

In addition, two strains of *F. culmorum* from the Western Siberia and Ural regions and one from the South European region of Russia were included in study. The high genetic similarity of analysed *F. culmorum* strains 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 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 single isolates 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 and the trichothecene chemotype of the *Fg* group on cereal crops are important to establish if any present or future shifts in populations occur. We expected the diversity of *Fusarium* species belonging to the *Fg* group in this territory to be higher than found in our study, since in the neighbouring countries of China and Japan, *F. asiaticum* are detected with high frequency on cereals (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 isolates were either 3-AcDON or NIV, with 3-AcDON being predominant.

Fusarium fungi 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* fungi than 140 years ago, we are still not very successful in controlling the diseases they cause on crops if conditions are favourable 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 detected maximum content of deoxynivalenol in wheat grains reached 13,141 ppb. Multilocus analysis revealed isolates belonging to *F. graminearum* s. str.

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Figure 1

The perithecia, spores of fungi and symptoms of *Fusarium* disease of cereals from the Far East of Russia, which were drawing and published in the book by N. A. Palchevsky (1891).

Figure 2

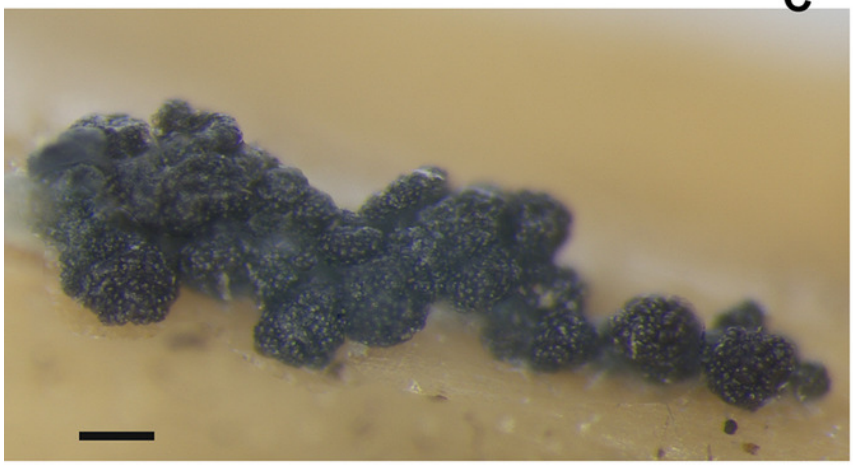
The diseased wheat (A) and barley grains (C); colony of *F. graminearum* on PSA, 10 days (B); macroconidia *F. graminearum* (D); the perithecia (E); expulsion of ascospores from the perithecia (F). Scale bars: D = 20 µm; E = 200 µm; F = 50 µm.

A

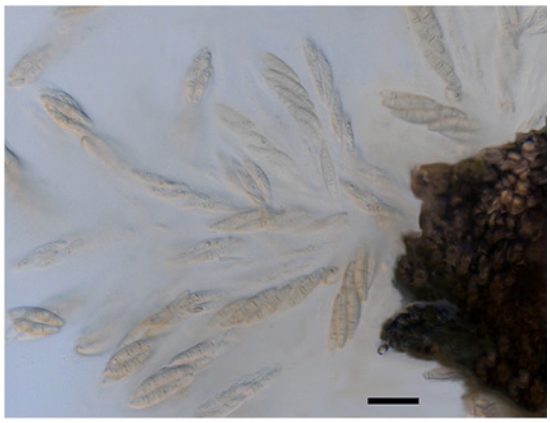
B

C

D



E



F

Figure 3

Maximum likelihood phylogenetic tree based on DNA sequence data from four loci (*EF-1a*, *URA*, *RED*, and *Tri101*) of *Fusarium* species.

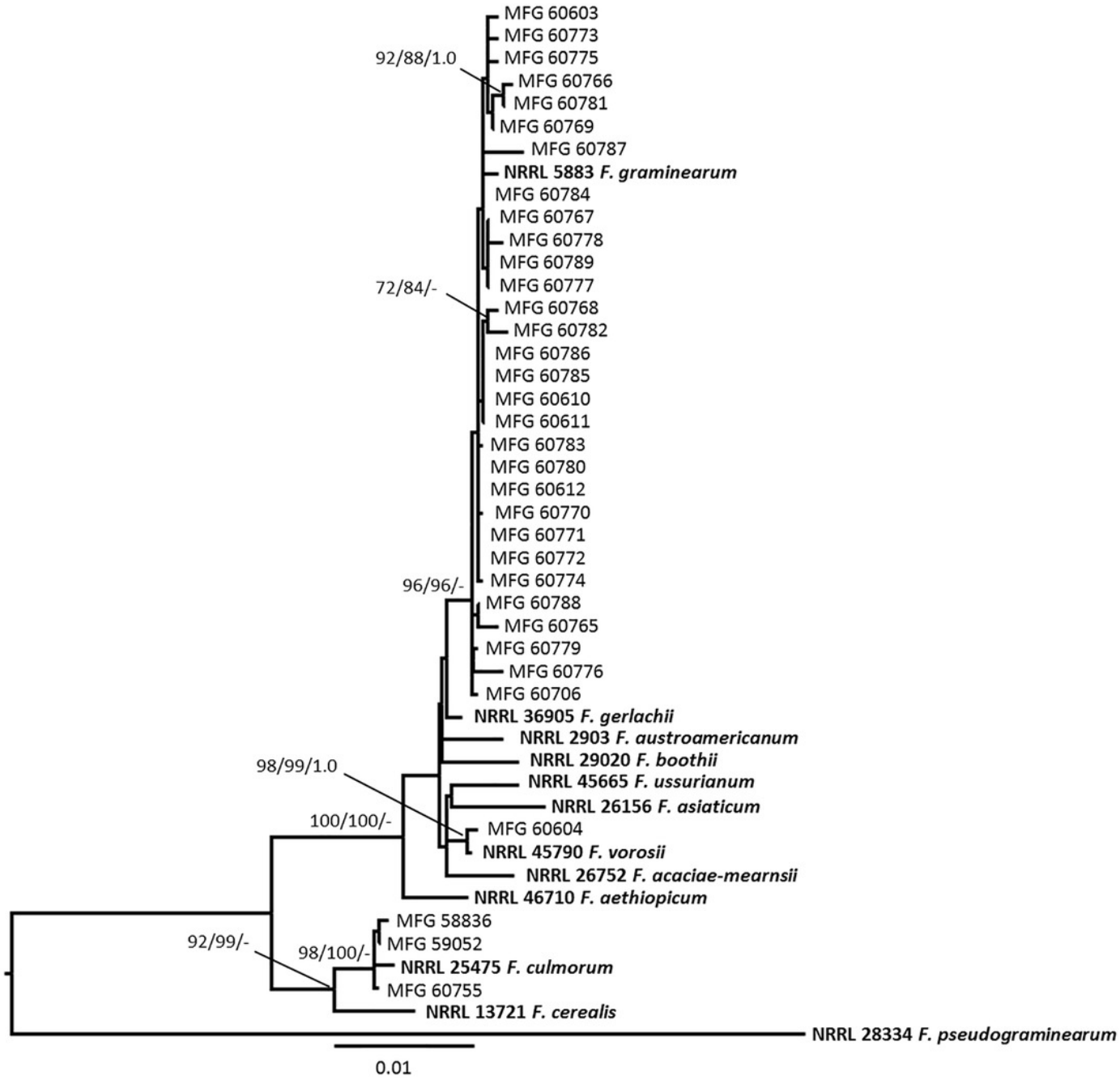


Table 1(on next page)

Climatic data during the growing season of 2019 in the Amur region (https://rp5.ru/)

Month	Average temperature, °C			Average humidity, %	Total rainfall, mm	Days with precipitation
	mean	min	max			
May	+12.0	−1.6	+27.5	56	17	19
June	+18.2	+7.2	+30.1	66	46	21
July	+21.1	+13.5	+30.2	83	300	27
August	+18.6	+10.6	+29.2	82	206	25

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Table 2(on next page)

Fusarium strains included in the study

[a] Reference strains whose nucleotide sequences were used in phylogenetic analysis. [b] Bold indicates the numbers of sequences obtained in this study.

Species	Strain	Host	Geographic original	Year	GenBank accession number				Chemo-type
					TEF	*URA*	*RED*	*Tri101*	
F. acaciae-mearnsii	NRRL 26752[a]	acacia	South Africa		AF212447	AF212705	AF212558	AF212594	
F. aethiopicum	NRRL 46710[a]	wheat	Ethiopia		FJ240296	FJ240274	FJ240252	FJ240339	
F. asiaticum	NRRL 26156[a]	wheat	China		AF212452	AF212710	AF212563	AF212599	
F. austroamericanum	NRRL 2903[a]		Brazil		AF212438	AF212696	AF212549	AF212585	
F. boothii	NRRL 29020[a]	corn	USA		AF212443	AF212701	AF212554	AF212590	

F. cerealis	NRRL 13721 [a]	potato	Poland		AF212464	AF212722	AF212575	AF212611	
F. culmorum	NRRL 25475 [a]	barley	Denmark		AF212463	AF212721	AF212574	AF212610	
F. culmorum	MFG 58836	wheat, grain	Russia, Omsk region	2015	**MW273182** [b]	**MW273250**	**MW273216**	**MW892041**	3-AcDON
F. culmorum	MFG 59052	wheat, grain	Russia, Krasnodar region	2017	**MW273183**	**MW273251**	**MW273217**	**MW892042**	3-AcDON
F. culmorum	MFG 60755	barley, grain	Russia, Tyumen region	2015	**MW273187**	**MW273255**	**MW273221**	**MW892043**	3-AcDON
F. gerlachii	NRRL	wheat	USA		DQ459742	DQ459776	DQ459793	DQ452409	

	36905[a]								
F. graminearum	NRRL 5883[a]	corn	USA		AF212455	AF212713	AF212566	AF212602	
F. graminearum	MFG 60765	wheat, grain	Russia, Amur region	2019	**MW273157**	**MW273225**	**MW273191**	**MW273259**	15-AcDON
F. graminearum	MFG 60766	wheat, grain	Russia, Amur region	2019	**MW273168**	**MW273236**	**MW273202**	**MW273270**	15-AcDON
F. graminearum	MFG 60767	wheat, grain	Russia, Amur region	2019	**MW273176**	**MW273244**	**MW273210**	**MW273278**	15-AcDON
F. graminearum	MFG 60768	wheat, grain	Russia, Amur region	2019	**MW273177**	**MW273245**	**MW273211**	**MW273279**	15-AcDON

F. graminearum	MFG 60769	wheat, grain	Russia, Amur region	2019	**MW273181**	**MW273249**	**MW273215**	**MW273283**	15-AcDON
F. graminearum	MFG 60770	wheat, grain	Russia, Amur region	2019	**MW273186**	**MW273254**	**MW273220**	**MW273286**	3-AcDON
F. graminearum	MFG 60771	wheat, grain	Russia, Amur region	2019	**MW273188**	**MW273256**	**MW273222**	**MW273287**	15-AcDON
F. graminearum	MFG 60772	wheat, grain	Russia, Amur region	2019	**MW273189**	**MW273257**	**MW273223**	**MW273288**	3-AcDON
F. graminearum	MFG 60773	wheat, grain	Russia, Amur region	2019	**MW273190**	**MW273258**	**MW273224**	**MW273289**	15-AcDON
F. graminearum	MFG	wheat,	Russia,	2019	**MW273158**	**MW273226**	**MW273192**	**MW273260**	15-

	60774	grain	Amur region						AcDON
F. graminearum	MFG 60775	wheat, grain	Russia, Amur region	2019	**MW273159**	**MW273227**	**MW273193**	**MW273261**	15-AcDON
F. graminearum	MFG 60776	wheat, grain	Russia, Amur region	2019	**MW273160**	**MW273228**	**MW273194**	**MW273262**	15-AcDON
F. graminearum	MFG 60777	wheat, grain	Russia, Amur region	2019	**MW273161**	**MW273229**	**MW273195**	**MW273263**	15-AcDON
F. graminearum	MFG 60778	wheat, grain	Russia, Amur region	2019	**MW273162**	**MW273230**	**MW273196**	**MW273264**	3-AcDON
F. graminearum	MFG 60779	wheat, grain	Russia, Amur	2019	**MW273163**	**MW273231**	**MW273197**	**MW273265**	15-AcDON

			region						
F. graminearum	MFG 60780	wheat, grain	Russia, Amur region	2019	**MW273164**	**MW273232**	**MW273198**	**MW273266**	3-AcDON
F. graminearum	MFG 60781	barley, grain	Russia, Amur region	2019	**MW273165**	**MW273233**	**MW273199**	**MW273267**	15-AcDON
F. graminearum	MFG 60782	barley, grain	Russia, Amur region	2019	**MW273166**	**MW273234**	**MW273200**	**MW273268**	3-AcDON
F. graminearum	MFG 60783	barley, grain	Russia, Amur region	2019	**MW273167**	**MW273235**	**MW273201**	**MW273269**	3-AcDON
F. graminearum	MFG 60784	barley, grain	Russia, Amur region	2019	**MW273169**	**MW273237**	**MW273203**	**MW273271**	3-AcDON

F. graminearum	MFG 60785	barley, grain	Russia, Amur region	2019	**MW273170**	**MW273238**	**MW273204**	**MW273272**	3-AcDON
F. graminearum	MFG 60786	barley, grain	Russia, Amur region	2019	**MW273171**	**MW273239**	**MW273205**	**MW273273**	15-AcDON
F. graminearum	MFG 60787	barley, grain	Russia, Amur region	2019	**MW273172**	**MW273240**	**MW273206**	**MW273274**	15-AcDON
F. graminearum	MFG 60788	barley, grain	Russia, Amur region	2019	**MW273173**	**MW273241**	**MW273207**	**MW273275**	15-AcDON
F. graminearum	MFG 60789	wheat, grain	Russia, Amur region	2019	**MW273174**	**MW273242**	**MW273208**	**MW273276**	15-AcDON
F. graminearum	MFG	barley,	Russia,	2019	**MW273175**	**MW273243**	**MW273209**	**MW273277**	15-

	60603	grain	Amur region						AcDON
F. graminearum	MFG 60612	wheat, grain	Russia, Kemerovo region	2019	**MW273178**	**MW273246**	**MW273212**	**MW273280**	3-AcDON
F. graminearum	MFG 60610	wheat, grain	Russia, Amur region	2019	**MW273179**	**MW273247**	**MW273213**	**MW273281**	15-AcDON
F. graminearum	MFG 60611	wheat, grain	Russia, Amur region	2019	**MW273180**	**MW273248**	**MW273214**	**MW273282**	15-AcDON
F. graminearum	MFG 60706	soybean, leaves	Russia, Amur region	2019	**MW273185**	**MW273253**	**MW273219**	**MW273285**	15-AcDON
F. pseudograminearu	NRRL 28334	*Medica go* sp.	South Africa		AF212470	AF212729	AF212580	AF212617	

m	[a]								
F. ussurianum	NRRL 45665 [a]	wheat, grain	Russia, Jewish autonomous region	2002	FJ240300	FJ240279	FJ240257	FJ240344	
F. vorosii	NRRL 45790 [a]	wheat, grain	Russia, Primorsky Krai	2006	FJ240302	FJ240281	FJ240259	FJ240346	
F. vorosii	MFG 60604	wheat, grain	Russia, Altay Krai	2018	**MW273184**	**MW273252**	**MW273218**	**MW273284**	15-AcDON

1

Table 3(on next page)

Infection of wheat and barley grain grown in the Amur region in the Russian Far East, 2019

Parameters		Samples of grain	
		Wheat	Barley
Grain infected with fungi	*Fusarium* spp.	**81.1** (58–98)	**80.5** (64–94)
on average (range), %	incl. *F. graminearum*	**68.0** (47–88)	**72.0** (61–92)
Content of mycotoxins	DON	**7,498** (3,207–13,343)	**5,390** (912–7,756)
on average (range), ppb	3-AcDON	**122** (27–293)	**131** (0–192)
	15-AcDON	**85.5** (23–179)	**93.5** (19–154)
	3-DON-glucoside	**1,011** (299–2,001)	**2,128** (98–3,803)
	ZEN	**1,153** (92–3,670)	**537** (111–928)
	MON	**70.2** (10–218)	**72.7** (5–207)
Amount of *Fusarium*	*F. graminearum*	**6,089** (2,658–11,342)	**2,102** (163–3,557)
DNA × 10^{-3} on average	3-AcDON genotype	**1,084** (395–2,007)	**508** (107–783)
(range), pg/ng	15-AcDON genotype	**1,708** (755–2,776)	**371** (101–713)
	F. avenaceum	**40** (6–97)	**13** (3–38)

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