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Fusarium AND *Alternaria* FUNGI IN GRAIN OF OATS GROWN IN THE NORTH-WESTERN RUSSIA REGARDING CULTIVAR SPECIFICITY

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Abstract

Oat (*Avena sativa* L.) is widely cultivated in a moist, cool climate and it is important crop particularly in Northern Europe. The most general usage of oat is for livestock feed. However, oat consumption as human food has recently increased, perhaps due to its reported health benefits. The abundance and species composition of the microbiota are the important factors in determining the quality of the grain. *Fusarium* fungi produce toxic metabolic products called mycotoxins. Mycotoxin contamination of food commodities can affect both human and animal health. Species of the genus *Alternaria* have rather less significance as the pathogens of cereals and sources of grain contamination. The aim of this study was to evaluate the natural infestation by *Fusarium* and *Alternaria* fungi of oat grain harvested in 2014 in the northwestern Russia. Asymptomatic seeds of 56 oat samples harvested in five provinces of the northwestern Russia (7 samples from Arkhangelsk and Pskov provinces, 20 samples from Vologda Province, 12 samples from Leningrad province and 10 samples from Novgorod Province) were assayed for the presence of filamentous fungi. Grain samples (except for a few unnamed) belonged to 14 varieties (Adamo, Argamak, Borets, Borrus, Krechet, Lev, LOS 3, Skakun, Scorpion, Teremok, Fukhs, Chernigovskiy, Chernigovskiy 83 and Yakov). Seeds were surface sterilized and placed on Petri dishes with Potato-dextrose agar (PDA). The resulting fungal colonies from each kernel were isolated and identified based on cultural and morphological features. Fungal contamination and the percentage of species belong to *Fusarium* or *Alternaria* fungi (%) were calculated in each sample. Mycological analysis revealed presence of different species of fungi belonging to the genera *Alternaria*, *Aspergillus*, *Bipolaris*, *Cladosporium*, *Epicoccum*, *Fusarium*, *Penicillium*, and others. The main representatives of mycobiota were *Fusarium* and *Alternaria* fungi. Infection of *Fusarium* fungi was detected in 88.9 % analyzed oat samples (average grain infestation in different regions ranged from 6.1 to 18.7 %, the maximum value was 64.0 %). *Alternaria* fungi were found in 91.0 % grain samples (average infestation ranged 1.5 to 48.0 %, the maximum value was 85.0 %). Among *Fusarium* fungi the *F. poae*, *F. sporotrichioides* and *F. langsethiae* which belong to trichothecene-producing species were detected with the highest frequency. A number of species, such as *F. anguioides*, *F. avenaceum*, *F. graminearum*, *F. incarnatum*, *F. subglutinans*, and *F. tricinctum*, were low-frequent. For the first time *F. langsethiae* was found on the territory of Arkhangelsk Province which is the most northern border of this fungus areal in Russia. The high positive correlation between portion of grains damaged by *F. langsethiae* and accumulation of T-2/HT-2 toxins was found. Fungi of *Alternaria* genus were mainly presented by toxin-producing species *A. tenuissima* and *A. arborescens* (66-86 % of the total number of *Alternaria* isolates). Cultivars Lev, Adamo, Yakov, Krechet were the most infected by *Fusarium* (an average infestation rate was more than 20 %). The significantly higher grain infection by *Alternaria* fungi was detected in cultivar Lev in comparison with another analyzed oat cultivars. The obtained data are in line with earlier reported results of toxicological analysis (A.A. Burkin et al., 2015) in which the presence of mycotoxins in the grain from all provinces of the northwestern Russia was revealed, particularly T-2/HT-2 toxins were found in 60.7 % of samples, deoxynivalenol was detected in 62.5 % and alternariol was presented in 29.0 % of the tested oat samples.

Keywords: oats, cultivars, infection, mycotoxins, *Fusarium* and *Alternaria* fungi.

Currently, the research of oat grain infestation with toxin-producing fungi species is particularly relevant, since the demand for this crop as a source of raw material for food products, including baby and dietetic food has increased considerably [1]. Therefore, more attention is paid to the safety of oat-containong products.

The abundance and species composition of the microbiota are the important factors in determining grain quality. *Fusarium* fungi produce toxic secondary metabolites that can adversely affect the health of cereal product consumers. Currently, the maximum permissible concentrations (MPC) of deoxynivalenol (DON) trichothecene fusarium toxins of 700-1000 μg/kg, T-2/HT-2 toxins (T-2/HT-2) of 100 μg/kg, and zearalenone (ZEN) of 200-1000 μg/kg [2] are set for cereals and a variety of processed products in Russia.

Annual mycotoxicological analysis showed a high level of *Fusarium* fungi infestation and grain contamination with fusarium toxins. It averaged 17.0 % in the northwest of Russia in 2008. T-2/HT-2 and DON mycotoxines were found in 46 and 47 % of samples, respectively, and their maximum concentration was 182 and 2505 μg/kg [3]. Studies performed in Norway indicate that oats are significantly more susceptible to *Fusarium* infection than wheat. In addition, trichothecene mycotoxins producers, the *F. poae* (Peck) Wollenw. and *F. langsethiae* Torp et Nirenberg fungi, were also more frequent in oats than in wheat and barley [4]. Within 10 years of observations, Finland, Norway and Sweden researchers have shown that the permissible content of DON (1750 μg/kg) was exceeded in 3-28 % of the samples of grain used for food, depending on the region [5]. However, they failed to identify the meteorological factors and agricultural practices that affect the increase in mycotoxin content in cereals. In 2010-2011, analysis of oat samples from three regions of Sweden demonstrated that the majority of samples (90-100 %) were infected with *F. poae*, *F. langsethiae* and *F. avenaceum* (Fr.) Sacc. A positive correlation of not less than $r = 0.52$ at a 99 % significance level between the presence of *Fusarium* species and the amount of fusarium toxines in grain [6] was identified. The study of 98 grain crop samples of oat grown in 2009-2011 in Poland indicate a high prevalence of T-2/HT-2, nivalenol (NIV), DON and ZEN in the grains and derived products. According to the authors, the greatest risk is caused by the maximum content of NIV of 655 μg/kg [7].

Grain infestation with *Alternaria* and contamination with mycotoxin produced by the fungi of this group has also been reported [8-14]. *Alternaria* species are rather less significant as cereal pathogens and the source of grain contamination compared to *Fusarium* [15]. Toxicity of secondary metabolites formed by some species of this genus is investigated actively [16, 17]. Alternariol (AOL) is one of the most common and hazardous *Alternaria* spp. secondary metabolites [18, 19].

The first report on fusarium toxines found in oat grains from several regions of Russia was published in 2009 [20]. High incidence of *Fusarium* and *Alternaria* on cereals, widespread cultivation of oats in the regions with temperate climate and its extensive use in the production of feed and food suggest the need for the quality control of raw grain material and for the improvement of mycotoxicosis prevention measures.

The purpose of this study was to evaluate oat grain infestation by *Fusarium* and *Alternaria* fungi considering the varietal characteristics, and to search for the correlations between the intensity of grain infestation by dominant fungi species and mycotoxin accumulation.

Techniques. In 2014, 56 oat seed samples harvested in five provinces of the northwestern Russia were studied for the presence of fungi infestation of which 7 samples were from the Arkhangelsk regions, 7 samples were from Pskov regions, 20 samples were from the Vologda region, 12 samples were from the Leningrad region, and 10 samples were from the Novgorod region.

Except for a few not attributed to a certain variety and unnamed, the grain samples belonged to 14 varieties: Adamo (n = 1), Argamak (n = 2), Borets (n = 2), Borrus (n = 15), Krechet (n = 1), Lev (n = 9), LOS 3 (n = 2), Skakun (n = 4), Scorpion (n = 1), Teremok (n = 1), Fukhs (n = 4), Chernigovskiy (n = 2), Chernigovskiy 83 (n = 1), and Yakov (n = 4).

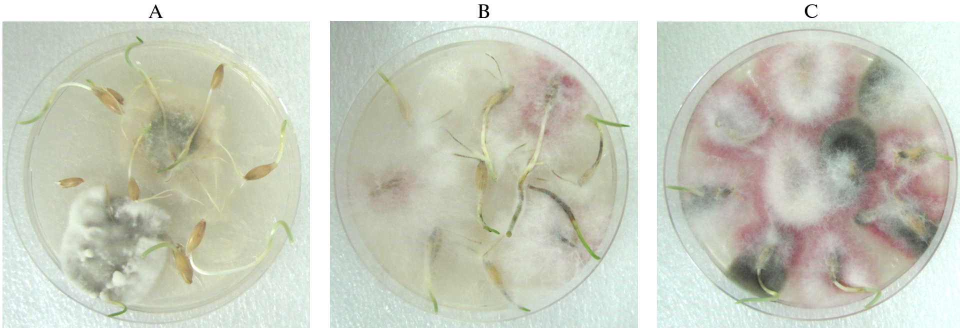
To study sample infestation, at least 100 grains of a pooled sample were surface sterilized with 70 % ethanol and placed on potato-dextrose agar (PDA); the fungi colonies grown from grains were registered in 10-14 days [21]. Fungi species was attributed using the keys [22-24]. Grain sample infestation with fungi (%) was calculated as the portion of grains infested with certain fungal species or genus to the total number of grains analyzed. Proportions of particular species (%) in the *Fusarium* or *Alternaria* pathogen complexes were determined in each sample as the ratio of grains infected with particular fungi species to the number of grains infected by all species of the genus.

Statistical processing, including calculation of correlation coefficients was performed using Microsoft Excel 2010 and Statistica v. 6 software.

Results. Among the studied samples of oats grown in the Northwest region, the largest group was represented by variety Borrus (Germany) with the proportion of 32 %. This variety has been regionalized in Russia since 1982 and is still in demand from the agricultural manufacturers due to its high yield and tolerance to diseases. Lev (Nemchinovka Moscow Agricultural Research Institute) with 18 % was the next most common variety. Skakun, Fukhs, and Yakov varieties were more rare, and their portions not exceeded 8 %.

Mycological analysis of oat infestation showed the presence of different fungal species of the genera *Alternaria*, *Aspergillus*, *Bipolaris*, *Cladosporium*, *Epicoccum*, *Fusarium*, *Penicillium*, etc. *Fusarium* and *Alternaria* species were most common among the micromycetes identified (Fig.).

A B C



***Fusarium*, *Alternaria*, *Bipolaris*, *Epicoccum* infection in oat grains** (surface sterilized, 10 days culture on potato-dextrose agar): A — Borrus variety (Leningrad region), B — Skakun variety (Arkhangelsk region), C — Lev variety (Pskov region) (2014).

***Fusarium* and *Alternaria* fungi infection in oats grains in the North-West district regions**

Region	*Fusarium*			*Alternaria*		
	portion of infested samples, %	grain infestation, %		portion of infested samples, %	grain infestation, %	
		average ($M \pm m$)	min-max		average ($M \pm m$)	min-max
Arkhangelsk region	71.4	9.3±10.8	2-27	71.4	1.5±1.4	1-3
Vologda region	100	18.7±14.8	2-64	100	48.0±13.0	24-70
Leningrad region	83.3	6.1±8.5	1-26	91.6	23.7±20.2	1-78
Novgorod region	90.0	17.5±17.6	5-40	80.0	24.1±23.3	1-57
Pskov region	100	8.7±10.3	1-29	100	42.8±34.6	14-85
Total for the region	88.9	12.1±14.5	1-64	91.1	32.0±24.0	1-85

Fusarium infection was found in 88.9 % samples analyzed. Average grain infestation in the North-West district regions ranged from 6.1 to 18.7 %, the maximum value was 64.0 %. *Alternaria* fungi were found in 91.0 % grain samples studied. Average infestation ranged 1.5 to 48.0 % in the regions, the maximum value was 85.0 % (Table).

Analysis of *Fusarium* species composition demonstrated predominance of *F. poae* fungi on oat grains from all the studied areas. Its portion in the fusarium complex was 73.9 % in the Arkhangelsk region, 80.5 % in the Vologda region, 47.8 % in the Leningrad region, 88.0 % in the Novgorod region, and 59.0 % in the Pskov region. The relatively weak *F. poae* pathogen is usually localized in the flower film and does not penetrate deep inside the grain. However, a high level of this fungus infestation reduces both forage and seed grain quality. Usually, *F. poae* is frequent in oats [25]. It is capable of producing the NIV trichothecene metabolite with highly toxic properties, but its MPC in the grain has not been standardized. *F. sporotrichioides* Sherb. and *F. langsethiae* species were the next ones in occurrence. *F. langsethiae* was detected for the first time in the territory of the Arkhangelsk region. All isolates of this species were obtained from the cereals grown in the Velsk region, bordering with the Vologda region, where *F. langsethiae* is a typical representative of fusaric fungi in the grain [26]. Currently, it covers the entire area of the European part of Russia, which requires careful monitoring of T-2/HT-2 in this area harvest.

Besides these species, *F. anguioides* Sherb., *F. avenaceum*, *F. graminearum* Schwabe, *F. incarnatum* (Desm.) Sacc., *F. subglutinans* (Wollenw. et Reinking) P.E. Nelson, Toussoun et Marasas, *F. tricinctum* (Corda) Sacc. were found at a low frequency in the North-West.

Compared to *Fusarium*, *Alternaria* species were more common. Toxin producing *A. tenuissima* (Nees et T. Nees: Fr.) Wiltshire was the predominant species, infectious *A. arborescens* E.G. Simmons. was less common. The species of the *A. infectoria* complex were low frequent. This species composition and the degree of contamination have also been detected in the cereals in the European part of Russia in previous years [27-29]. Toward the south of the Arkhangelsk region, average *Alternaria* infestation varied depending on the region, but reached high values everywhere (57-85 %). In most cases, the proportion of *A. tenuissima* and *A. arborescens* was 66-86 % of the total count of *Alternaria* isolates.

Lev, Adamo, Yakov, and Krechet were the most *Fusarium*-infected varieties with an average infestation rate of more than 20 %, therefore they were attributed to the group of highly susceptible oat varieties cultivated in the northwest of Russia. A significantly higher rate of *Alternaria* infestation was observed in variety Lev compared to other varieties analyzed ($p \le 0.05$).

Identification of different amounts of mycotoxins in oat grains in the samples from all areas has been reported earlier [10]. Thus, T-2/HT-2 toxines produced by *Fusarium* fungi were found in 60.7 % samples. MPC exceeding was recorded for these mycotoxins in five grain samples from the Vologda (Vologda, Gryazovets, Totem districts), Leningrad (Lomonosov district), and Novgorod regions (Shimsk district). According to our data, *F. langsethiae* was most responsible for grain T-2/HT-2 contamination. Correlation between *F. langsethae* grain infestation and the content of these mycotoxins was positive with *r* amounted 0.7 ($p \le 0.05$). No significant relationship between the presence of *F. sporotrichioides* — a widespread T-2/HT-2 producer — and the amount of mycotoxins in the grain has been found. The DON mycotoxin was detected in 62.5 % of the analyzed oat samples [10], and the MPC excess was recorded in two samples only, i.e. in Borrus (Khvoininsk district, the Novgorod region) and Skakun varieties (Ust'yansk district, the Arkhangelsk region) at 1159 и 1990 μg/kg, respectively. *F. graminearum* and *F. culmorum* were the main DON producers.

F. graminearum fungus which has been previously considered as a typical pathogen of cereals cultivated in warm and humid climate was recently first identified by us in the north-west Russia [30]. The problem of the emergence of dangerous pathogens and their adaptation to new areas is of particular importance due to global warming [31]. This once again confirms the need to conduct a thorough seed phyto expertise to prevent the introduction of pathogenic organisms uncharacteristic for the seeds into new regions.

A total of 29 % of these oat samples were found to contain AOL [10]. A considerable amount of this metabolite was identified in the grains from the Novgorod (Khvoininsk district) and Pskov (Pechera district) regions — 1159 and 1545 μg/kg, respectively. No significant relationship between AOL contamination and *Alternaria* grain infestation has been found neither for individual species, nor for the species taken together.

Fungal growth and mycotoxin production proceed at different intensities and largely depend on many factors (i.e. fungus species and strain, host plant genotype, infection timing and environmental conditions). Furthermore, coexistence of various microorganisms, including toxin producing ones, on the same nutrient-rich substrate involves various types of interaction. In addition to the direct competition for nutrients and living space, the interaction of organisms may result in the changes in metabolic activity and affect mycotoxin production [32, 33]. Evaluation of the mutual effects of mycobiotal components on the quality of the grain is the priority direction of modern research. Close monitoring of the species composition of pathogens in crops makes it possible to track the emergence of new dangerous fungal toxin producing species and prevent a decrease in the quality of feed and food.

Thus, we have estimated *Fusarium* and *Alternaria* infestation of 56 oat grain samples (*Avena sativa* L.) in 14 varieties (Adamo, Argamak, Borets, Borrus, Krechet, Lev, LOS 3, Skakun, Scorpion, Teremok, Fukhs, Chernigovskiy, Chernigovskiy 83 and Yakov, as well as uncertain and unnamed ones) from five regions (Arkhangelsk, Pskov, Vologda, Leningrad, and Novgorod regions). We calculated sample fungal infestation and the portions of particular species (%) in the *Fusarium* or *Alternaria* pathogen complex for every sample. Mycological analysis revealed the presence of different fungal species belonging to the genera *Alternaria*, *Aspergillus*, *Bipolaris*, *Cladosporium*, *Epicoccum*, *Fusarium*, *Penicillium*, etc. *Fusarium* and *Alternaria* fungi appeared to be most common. *Fusarium* infection was detected in 88.9 % analyzed oat samples (with average grain infestation ranged from 6.1 to 18.7 %, and the maximum value of 64.0 %), and *Alternaria* fungi were found in 91.0 % grain samples (with 1.5 to 48.0 % infection, and the maximum value of 85.0 %). Three *Fusarium* species, the *F. poae*, *F. sporotrichioides* and *F. langsethiae*, which are the producers of trichothecene mycotoxins were the most high frequent *Fusarium* fungi species. *F. anguioides*, *F. avenaceum*, *F. graminearum*, *F. incarnatum*, *F. subglutinans*, and *F. tricinctum* were identified as low frequent in the North-West region. *F. langsethiae* was first found in the territory of the Arkhangelsk region, the most northern border of this fungus habitat in Russia. A high positive correlation between *F. langsethiae* detection and accumulation of T-2/HT-2 toxins in grains was found. *Alternaria* fungi were mainly presented by toxin producing species *A. tenuissima* and *A. arborescens* (66-86 % of the total number of *Alternaria* isolates). Lev, Adamo, Yakov, and Krechet varieties were the most infected by *Fusarium* (average infestation rate of more than 20 %). A significantly higher *Alternaria* infection in variety Lev compared to other oat varieties was observed. Evaluation of the mutual effects of mycobiotal components on the quality of the grain should be considered the priority of modern research. To track the emergence of new dangerous fungal toxin producing species and prevent a decrease in the quality of feed and food, close monitoring of the species composition of pathogens in crops is required.

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