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FACTORS AFFECTING *Alternaria* APPEARANCE IN GRAINS IN EUROPEAN RUSSIA

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Abstract

Infection of cereal seeds induced by fungi of the genus *Alternaria* is very common. There are different opinions about the damage of *Alternaria* spp. probably because of the fact that these fungi are not a homogenous group. It is rational and available at this moment to identify *Alternaria* fungi isolated from the grain samples up to the section level only. Members of two sections, *Alternaria* and *Infectoriae*, are the most widespread. Our work was aimed to reveal impact of a number of factors on *Alternaria* infection rate in cereal grain using relatively extensive data. We studied significance of the crop, its cultivar, predecessor, region, district, season, infestation by other *Alternaria* species and by fungal species of some another genera. During this research, 422 grain samples of wheat, barley, sorghum and maize collected in 2010-2012 in seven regions of European part of Russia were analyzed. The majority of samples represented wheat grain from Stavropolskii Kray and Krasnodarskii Kray (southern Russia regions). Meteorological factors played the principal role in infection. A district in different years and neighbouring districts during one growing season were characterized by highly divergent (p < 0,001) *Alternaria* infection rate of wheat and barley grain. In *Alternaria*, infection rate ranged from 7.0 to 71.5 % for sect. *Alternaria*, and from 8.6 to 74.0 % for sect. *Infectoriae*. Grain of all wheat and barley cultivars were similarly greatly infected by *Alternaria* fungi (p was equal to 0.6-0.9 for section *Alternaria* and 0.1-0.5 for section *Infectoriae*). As it was previously shown, rye and oat usually are infected as much as wheat and barley. Maize has several tough and thick husk leaves around the cob that likely protect the grain against *Alternaria* infection. We did not observe a significant impact of predecessors (maize, sunflower, sugar beet, winter wheat and fallow) on the infection rate in the wheat grain samples. Correlation of *A.* sect. *Alternaria* infection of wheat and barley grain with appearance of *Bipolaris sorokiniana* and *Pyrenophora* spp. was negative and moderate (r = –0.69 and –0.61, respectively). The same pattern was found for coinfection of *Alternaria* sect. *Infectoriae* with *Bipolaris sorokiniana* (r = –0.64) and *Pyrenophora* spp. (r = –0.61). However, species of *Alternaria* sect. *Alternaria* did not significantly affect infestation of wheat and barley by *Alternaria* sect. *Infectoriae* fungi, and vice versa. Difference between germinability of seeds infected by *Alternaria* and those free from *Alternaria* fungi was statistically insignificant. On average, the fungal contamination of germinable and ungerminable seeds differed by –1.6 % within *Alternaria* sect. and by +2.1 % within *Infectoriae* sect.

Keywords: *Alternaria alternata, A. tenuissima, A. infectoria*, grain infection rate, germinability, wheat, barley

The fungi of the *Alternaria* genus can be found in plant seeds, including crops, very often and everywhere. The contamination of grain with these micromycetes in some cases reaches almost 100%; it may adversely affect the quality of seeds, food, and feed grain [1]. The study of cereal *Alternaria* blights have been conducted in Russia for more than 100 years, but it is still relevant. The conflicting views on the etiology and harmfulness of diseases, traditionally associated with the fungi of the *Alternaria* genus, exist. For example, in early works, it was reported that the seeds infected with *Alternaria* are well developed, fertile, and give rise to healthy plants [2-3]. Further on, different descriptions of the internal infection of seeds that promoted the development of root rot, weak the sprouts or leading to the death of plants, appeared [4, 5].

One of the most probable causes of conflicting data is the diversity of fungi of the *Alternaria* genus, which is not always taken into account adequately. Among the species of *Alternaria* found in the grain, saprotrophic and weakly phytopathogenic ones, the species that produce phyto- and mycotoxins, and do not synthesize them, exist [1]. Despite the obvious need for correct identification, it is often carried out either inaccurately (only to the genus) or with errors due to some objective difficulties. In general, there are more than 50 specific epithets of the *Alternaria* genus, denoting fungi related to the contamination of cereals, but only about 10 specific names are legitimate and used [6]. The so-called small-sporous species mentioned most often are *A. alternata*, *A. arborescens*, *A. tenuissima* and other species of the *A. infectoria* geni group [7-9]. The *Alternaria* genus, including about 300 species, has been divided into 27 sections recently [10-12] as a result of a thorough revision. *A. alternata*, *A. arborescens*, and *A. tenuissima* species are similar phylogenetically and placed in one *Alternaria* section, featuring in a total of about 60 species [13], many of which are so similar in molecular genetic markers that they should be combined into 10-15 species obviously [12, 14]. The species of *A. infectoria* are in the *Infectoriae* section. Within the sections, species are differentiated by morphological features primarily, which remains controversial [15, 16]. In other words, only differentiation of grain infecting *Alternaria* spp. on the representatives of the *Alternaria* and *Infectoriae* sections can be considered a sustainable one. In solving most applied tasks, identification to the section is sufficient, as the sections combine species that are generally similar in their environmental properties [11]. The species of the *Alternaria* and *Infectoriae* sections are typical components of the grain microbiota on all continents where crops are grown [1, 8, 17, 18]. In the European Russia in wheat, the representatives of both sections are found with about equal frequency (the infection rate for *A. tenuissima* is of 2-56%, for the species of the *A. infectoria* complex 2 to 72%). In the Far East, *A. tenuissima* was almost the only species in wheat representing the genus (29-76%) [1, 18].

The harm caused by these fungi is connected with contamination by mycotoxins, dangerous to humans and animals [19, 20]. The species of *Alternaria* can synthesize toxic metabolites. The spectrum of myco- and phytotoxins secreted by them is almost identical and depends more on the strain than on the species of fungus [9, 21]. Most of *A. tenuissima* strains isolated from cereals and other hosts produce several toxins of different chemical nature in different combinations [7, 9, 22]: alternariol, alternariol monomethyl ether, altenuene, tentoxin, tenuazonic acid, altertoxins I, II, and III. Some isolates of the *Infectoriae* section can produce the same toxins as the species of the *Alternaria* section but in small quantities [23, 24]. Most of the section species are characterized by metabolites which are the structural analogs of several mycotoxins [9, 25, 26], but the biological activity of these substances has not been studied.

Great practical importance of grain Alternaria blight determines the necessity of studying the influence of the external conditions on the infection rate of grain with fungi of the *Alternaria* genus. It should be taken into account that depending on the environmental conditions, representatives of different *Alternaria* sections may have different effects on grain quality. There is not so much information about the influence of different factors on the contamination of grain by the *Alternaria* species. Thus, the role of abiotic factors contributing to the development of the black embryo was reported. The appearance of such an embryo and Alternaria blight of grain are associated with the exposure to similar abiotic factors in the period of flowering—ripening, e.g. heavy precipitation, long-term dew, abundant watering, high air humidity [27-31], to a lesser extent extreme temperatures [32, 33]. Many studies have suggested that the black embryo is also caused by the *Alternaria* species, but they have not been identified, and such a relationship has not been shown, so the interpretation of the results is difficult. Most often, the correlation between symptoms and *Alternaria* contamination is absent [34].

One can make a few assumptions (null hypotheses) about what phenomena should affect the frequency of occurrence of *Alternaria* spp. in cereal crops seeds. It is obvious that weather and climatic factors can influence the development of grain Alternaria blight. In this case, the difference in weather conditions in different areas during one season and in one area in case of the change of seasons should lead to different average contamination in different regions in one year and in one area in different years. All of the above-mentioned *Alternaria* species are not a substrate-specialized; therefore, the species and variety of plants should not affect the contamination. Maize can be a single exception which has dense cob shells, which can prevent the penetration of the *Alternaria* infection in caryopsis. As the source of infection is the remains of any plants, including weeds and wild vegetation, the forecrop and crop rotation should not affect the development of Alternaria blight.

The question of the impact of another species of the same genus or other genera on the contamination with one of the *Alternaria* species is of particular interest. The fungi of the *Alternaria* genus penetrate only into the surface layers of the seed and occupy it not completely, and the presence of several strains and species of *Alternaria* and other fungi in different parts of the seed seems quite probable.

This paper presents the first results of the hypothesis tests that the average grain contamination by the *Alternaria* species does not differ significantly for different crops, varieties, and forecrops, but is different when comparing different regions and seasons.

The work objective is to determine the relationship between the contamination of cereal crops seeds by the representatives of two sections of the *Alternaria* genus and germination, plant species, growing conditions in the European part of Russia.

Techniques. The grains of wheat, barley, sorgo, and maize (422 samples from the harvest of 2010-2012) were received from 7 regions of the European part of Russia for the analysis. Most of the samples were wheat seeds from the South of the country — from Stavropol and Krasnodar Krai (2010, 2011 and 2012) (31, 53, 38 and 65, 64, 72 samples, respectively). In these regions, grain was selected in 22-28 areas.

Grain contamination with *Alternaria* spp. in the regions was compared at 48 samples of barley from 6 regions and 161 samples of wheat from 8 regions, in the regions − at the samples from Stavropol and Krasnodar Krai. Comparison by areas was performed in 3-fold repetition (harvest 2010-2012), by regions only once (harvest 2012). The selection of every year contains 16-72 samples of wheat of different varieties from 5-15 areas of each region. The area was represented by at least 3 samples from different farms.

The influence of conditions during the year was assessed on 72 wheat samples from 6 areas of Stavropol and Krasnodar Krai (harvest of 2010-2012, 3-6 samples from every area in one year).

To compare contamination of crops, 48 samples of barley and 76 samples of wheat (harvest 2012) from 6 regions of Russia, 5 samples of sorgo from

Samara Region (2012), 5 samples of maize from Lipetsk Region (2012) and 6 samples of maize from Krasnodar Krai (2011) were used. The influence of the variety was studied on 131 samples of wheat (harvest 2011 and 2012), representing 16 varieties from different areas of Stavropol and Krasnodar Krai.

During the study of the forecrop role, 74 samples of wheat of harvest 2010 from Krasnodar and Stavropol Krai were evaluated, grown after maize and sunflower (both regions), sugar beet (Krasnodar Krai), winter wheat, and fallow (Stavropol Krai).

The frequency of joint infection by the representatives of different *Alternaria* sections was determined using 235 samples of wheat and barley of harvest 2012 obtained from different regions. To assess the relationship between the contaminations with various dark-colored hyphomycetes, the same sets of grain 48 samples of barley harvest 2012 from 6 regions were used where infection by *Alternaria* species of *Alternaria* section and *Infectoriae* section was 3-80% and 0-50%, respectively, infection by *Bipolaris sorokiniana* was 0-66%, and by *Pyrenophora* spp. 0-37%.

The correlation of germination after contamination, considering it separately for viable and not viable seeds, was examined at 11 samples of 8 varieties of wheat of harvest 2011 from 6 districts of the Stavropol Krai.

Before all the tests, the seeds, for surface sterilization, were placed in 1% sodium hypochlorite solution for 2 min with constant stirring, rinsed with sterile water three times and transferred in the Petri dishes (10 seeds per a dish 90 mm in diameter) with potato-sucrose agar (PSA) [35], in which 0.002% Triton X-100 was added to slow the growth of fungi. Cultures were incubated during 7-10 days at 22 °C. Fungi of *Alternaria*, *Pyrenophora*, and *Bipolaris* genera were identified by direct scanning of colonies and by sporulation (the top-light microscope Stemi 2000C, Carl Zeiss AG, Germany, ×50 magnification). In case of impossibility of unambiguous identification, the colonies were individually deselected in Petri dishes with potato-carrot agar (PCA) [6] and incubated under 12/12 h photoperiod (the fluorescent lamps of 1000-2000 lux), identifying the habitus of sporulation and morphology of conidia [6, 35].

Mean values (*M*), mean errors (±SEM), statistical significance (p) by Fisher criterion (*F*-criterion) and the correlation coefficient (*r*) were calculated with Statistica 6.1 (StatSoft, Inc., USA).

Results. For further averaging and comparison, 9 selections were formed from the common pool (Table 1). Every selection (grain of one crop/crop variety of one year in one region/area) included 3-11 samples.

1. Characterization of cereal crop seed saplings used in assessing the impact of various factors on the contamination by fungi of *Alternaria* genus

Factor	Crop	Year	*N*	Sampling with regard to: regions	areas	cultivars
Region	Wheat	2012	161	8		
	Barley	2012	48	6		
Area	Wheat	2010	31	1 (Stavropol Krai)	16	24
		2011	53		18	25
		2012	38		11	25
		2010	65	1 (Krasnodar Krai)	24	26
		2011	64		16	22
		2012	72		15	28
Season	Wheat	2010-2012	72	2 (Stavropol and Krasnodar Krai)	6	
Crop	Wheat	2012	76	6		
	Barley	2012	48	6		
	Sorgo	2012	5	1 (Samara Region)		
	Maize	2012	5	1 (Lipetsk Region)		
	Maize	2011	6	1 (Krasnodar Krai)		

Continued Table 1

Variety	Wheat	2011	25	1 (Stavropol Krai)		5
		2012	9			3
		2011	43	1 (Krasnodar Krai)		6
		2012	55			11
Forecrop	Wheat	2010	65	1 (Stavropol Krai)		
			31	1 (Krasnodar Krai)		
Alternaria species interinfluence	Wheat, barley	2012	235	10		
Bipolaris and *Pyrenophora* species influence	Barley	2012	48	6		
Germination	Wheat	2011	11	1 (Stavropol Krai)	6	8
Total sampling	Wheat, barley, sorgo, maize	2010-2012	422	7	> 60	

N o t e. *N* — the number of samples. Gaps in the table mean that these characteristics were not essential for the experiment and were not taken into account during selection.

By the example of 8 regions, it was shown that regional features significantly affect the contamination of wheat and barley grain by the *Alternaria* species (Tables 2, 3). At the same time, the contamination by the representatives of different sections may vary several times depending on the region.

2. Average contamination of wheat and barley seeds with fungi of the *Alternaria* genus in different regions of the European part of Russia (harvest 2012)

Region	Wheat					Barley				
	N	Alt	±SEM	Inf	±SEM	*N*	Alt	±SEM	Inf	±SEM
Stavropol Krai	38	46.0	2.5	30.6	2.0	11	49.9	8.0	23.5	5.0
Krasnodar Krai	79	59.0	1.4	24.6	1.2					
Lipetsk Region	13	31.4	3.0	44.2	2.5	9	64.7	6.0	22.7	2.0
Kursk Region	10	38.8	4.4	38.2	2.7	9	39.1	6.5	17.1	3.6
Voronezh Region	6	47.7	9.0	38.2	3.8	8	34.9	4.1	35.4	4.5
The Republic of North Ossetia	6	46.2	9.2	5.5	2.7					
Leningrad Region	5	13.0	4.3	10.4	4.6	5	12.2	2.2	18.6	3.7
Tambov Region	4	37.0	5.8	42.3	4.1	6	37.7	7.7	29.7	8.0

N o t e. N — the number of samples; Alt — mean contamination (*M*) by *Alternaria* section species; Inf — mean contamination (*M*) by *Infectoriae* section species; ±SEM — error of the mean. Gaps in the table mean the absence of data.

3. Statistical significance (p) **of the combination of factors affecting the contamination of wheat and barley by fungi of *Alternaria* genus in Stavropol and Krasnodar Krai in different years**

Factor	Sample formation parameter	*Alternaria* section	*Infectoriae* section
Region	Wheat	< 0.001	< 0.001
	Barley	0.001	0.027
Area	Stavropol Krai, 2010	0.927	0.487
	Krasnodar Krai, 2010	0.748	0.917
	Stavropol Krai, 2011	< 0.001	< 0.001
	Krasnodar Krai, 2011	0.041	0.122
	Stavropol Krai, 2012	< 0.001	< 0.001
	Krasnodar Krai, 2012	< 0.001	< 0.001
Season (year)		< 0.001	0.012
Crop (wheat/barley)		0.975	0.304
Wheat cultivar	Stavropol Krai, 2011	0.872	0.109
	Krasnodar Krai, 2011	0.678	0.508
	Stavropol Krai, 2012	0.806	0.515
	Krasnodar Krai, 2012	0.595	0.272
Forecrop	Stavropol Krai, 2010	0.699	0.506
	Krasnodar Krai, 2010	0.493	0.723
Contamination:			
Bipolaris sorokiniana		< 0.001	< 0.001
Pyrenophora spp.		< 0.001	< 0.001
Alternaria section species		–	0.090
Infectoriae section species		0.090	–
Germination	Crop (wheat)	0.846	0.637

N o t e. Dashes in the table indicate that the selection included samples of different years from several regions.

Despite the significant fluctuations from year to year, when comparing the indicators for Stavropol and Krasnodar Krai, it is clear that, at average, in the first case, the contamination by the *Alternaria* section members is lower, and by *Infectoriae* section is higher. The exception was in 2010 when the contamination of wheat with the species of the *Alternaria* section in both regions was low and did not differ significantly.

4. Average contamination (%) of wheat seeds by fungi of *Alternaria* genus in some areas of Stavropol and Krasnodar Krai in the years of examination

Area	*Alternaria* section			*Infectoriae* section		
	2010	2011	2012	2010	2011	2012
			Stavropol Krai			
Georgievskii	15.3	30.7	31.8	43.3	56.7	20.0
Izobilnenskii	14.3	50.0	48.0	34.7	42.8	44.3
Novoaleksandrovskii	13.8	60.2	52.5	25.3	31.7	33.3
Predgornii	15.0	37.8	70.0	32.7	46.8	24.0
Average per Stavropol Krai	15.1	47.4	46.0	34.2	42.6	30.6
±SEM	1.4	2.0	2.5	3.0	2.1	2.0
min	10.3	17.3	22.3	25.3	29.4	14.2
max	15.3	60.2	70.0	43.3	74.0	45.0
			Krasnodar Krai			
Kanevskii	9.0	52.2	52.0	20.8	48.5	33.0
Novokubanskii	16.5	56.5	58.3	19.8	29.3	31.0
Average per Krasnodar Krai	12.1	58.3	59.1	24.3	35.2	23.8
±SEM	0.9	1.5	1.4	1.6	1.1	1.2
min	7.0	17.3	38.8	14.0	29.4	8.6
max	19.0	60.2	71.5	28.3	74.0	38.0

5. Average contamination (%) of seeds by fungi of *Alternaria* genus in Stavropol and Krasnodar Krai for different wheat cultivars in the years of examination

Variety	*N* (*n*)	*Alternaria* section		*Infectoriae* section	
		M	±SEM	*M*	±SEM
		Stavropol Krai, 2011			
Batko	6 (6)	50.0	5.4	44.7	4.5
Nota	6 (5)	45.8	5.6	47.1	4.1
Tanya	7 (4)	53.7	3.8	34.3	4.4
Evklid	3 (3)	50.4	7.9	28.5	2.2
Yunona	3 (3)	48.6	9.7	43.0	9.2
		Stavropol Krai, 2012			
Batko	3 (2)	44.7	5.2	41.7	6.3
Viktoria Odesskaya	3 (3)	50.7	9.7	21.7	9.2
Grom	3 (3)	49.7	4.1	31.7	9.2
		Krasnodar Krai, 2011			
Gratsiya	4 (4)	56.3	4.1	36.9	3.2
Irishka	7 (6)	65.3	3.8	29.0	1.2
Krasnodarskaya 99	3 (3)	53.1	3.2	39.1	3.2
Moskvich	12 (7)	61.3	2.7	33.7	4.4
Sila	4 (3)	59.3	4.3	33.4	2.0
Tanya	13 (10)	62.0	6.5	34.5	4.7
		Krasnodar Krai, 2012			
Batko	4 (3)	63.8	5.7	29.0	6.1
Vassa	11 (6)	61.5	2.1	18.1	3.1
Grom	4 (4)	51.8	3.6	25.5	3.5
Delta	3 (2)	71.3	3.7	32.7	1.9
Irishka	3 (3)	49.3	9.8	16.3	8.2
Krasnodarskaya 99	3 (3)	57.3	6.7	16.7	2.7
Lebed	4 (4)	56.8	5.4	25.5	4.3
Liga	4 (3)	62.8	6.8	21.8	6.7
Moskvich	3 (3)	64.3	6.7	30.3	3.8
Nota	9 (6)	58.4	5.3	26.3	3.7
Tanya	7 (5)	58.9	5.9	28.3	4.4

Note. *N* — the number of samples, *n* — the number of areas.

The contamination of wheat seeds with the *Alternaria* genus species by areas varied in different years (Table 4). In Stavropol and Krasnodar Krai, the difference for the species of both sections in the areas was unreliable in 2010, but reliable in 2012 (see Table 3). In 2011, the influence of the area as a factor was statistically insignificant only for the *Infectoriae* section members in Krasnodar Krai. Area features were not reproduced from year to year. So, in one area, this figure could be one of the highest in the region for 2 years, but it decreased in the 3rd year. In other words, contamination depends more on the weather conditions than on climatic or other physical and geographical factors.

A survey in 6 areas of two regions within 3 years (see Table 4) showes that the average contamination by species of *Alternaria* section in the area may vary almost 6 times over the years. The impact of the year, as expected, is significant (see Table 3). The contamination of seeds by the members of *Infectoriae* section varied less, 2.3-fold, but the difference was statistically significant.

The comparison of wheat and barley seed contamination by *Alternaria* genus fungi in 6 regions showed no significant difference (see Tables 2, 3). Maize grains were affected much less than others. The contamination of samples from Krasnodar Krai (6 pcs., 2011) and Lipetsk Region (5 pcs., 2012) by the species of *Alternaria* section was 0.2-0.4% (the *Infectoriae* section species were not revealed). At the same time, wheat infection in the same regions in 2011 and 2012 reached 32-59 and 24-44%, respectively. The contamination of 5 samples of grain sorgo from Samara Region was 29.2% at average for *Alternaria* section, and 0.6% for *Infectoriae* section.

The differences in wheat varieties by contamination by both *Alternaria* sections were insignificant in 2011 and 2012 (Tables 3, 5).

6. Average contamination (%) **of wheat seeds by fungi of *Alternaria* genus with different forecrops** (harvest 2010)

Forecrop	N	*Alternaria* section		*Infectoriae* section	
		M	±SEM	M	±SEM
		Krasnodar Krai			
Maize	21	13.4	1.8	25.5	3.4
Sunflower	24	10.9	1.3	23.5	2.6
Sugarbeet	7	11.3	2.2	27.4	3.6
Others	13	12.5	1.5	22.2	2.9
		Stavropol Krai			
Maize	3	15.0	1.1	24.0	4.0
Sunflower	3	11.7	2.0	31.7	1.2
Fallow	7	18.4	3.6	40.0	6.8
Winter wheat	9	16.3	3.1	38.4	6.3
Others	9	14.5	2.7	37.1	4.4

Note. N — the number of samples.

Contamination of wheat by *Alternaria* species does not significantly depend on the forecrop (Table 6). The comparison of the samples of wheat harvest 2010, grown after maize, sunflower, and other forecrops, does not reveal a significant difference for *Alternaria* and *Infectoriae* sections and in both Krasnodar and Stavropol Krai (see Table 3).

In case of high contamination with fungi of the *Alternaria* genus and other dark-colored hyphomycetes, their joint growth from one seed was often observed. The relationship of *Alternaria* infection with the presence of *Bipolaris sorokiniana* and *Pyrenophora* spp. was negative and moderate, $r = -0.69$ and -0.61, respectively, for *Alternaria* section, and $r = -0.64$ and -0.61 for *Infectoriae* section, at low significance levels (see Table 3). In wheat and barley, the fungi of *Alternaria* genus (*Alternaria* section) and *Fusarium* genus (*F. langsethiae*, *F. tricinctum*, and *F. graminearum*) were found in the same caryopsis. Sometimes, the intensive growth of *Alternaria* and the weak growth of *Fusarium* from the same caryopsis was observed. However, in most cases, *Fusarium* fungi grew faster and suppressed the growth of *Alternaria*, often covering the colony with its mycelium (reliable identification of *Alternaria* was often impossible). In exceptional cases, the colonies of *Fusarium* sp. and *Alternaria* of *Infectoriae* section or *Fusarium* sp. and two *Alternaria* isolates from different sections grew from the same caryopsis simultaneously.

Two *Alternaria* isolates, belonging to different sections, were separated from some grains. If one imagines that these species do not compete with each other, being in the same seed, the frequency of their joint meeting should be equal to the product of the frequency of occurrence of each. However, the species of *Alternaria* section usually start growth and sporulation earlier and do it more intensively than the species of the *Infectoriae* section. Therefore, in some cases of the joint growth, the colonies of the *Infectoriae* species are probably unnoticed. In other words, the use of traditional assessment methods makes the observed rate of co-infection slightly lower than it is expected in theory. For 235 samples of wheat and barley (harvest 2012) from different regions, the product of the frequency of occurrence of the representatives of every section separately was calculated. The expected frequency of co-infection was 2 times lower than the actual proportion of seeds with co-infection (0.12 vs. 0.06). To understand whether there is an antagonistic effect, two correlation coefficients were calculated. For the expected and the actual percentage of the seeds with joint infection, it was high ($r = 0.81$, $p < 0.001$), and the relationship between the infection with the species of sections A*lternaria* and *Infectoriae* was weak ($r = 0.31$, $p > 0.090$). It does not confirm the positive or negative influence of the representatives of different sections on each other.

The relationship of germination and contamination of *Alternaria* spp. turned out to be unreliable (see Table 3). At average, the contamination of germinated and non-germinated seeds differed by –1.6% for the representatives of *Alternaria* section and by +2.1% for *Infectoriae* section, which was less than the standard error of the average.

The group of small-sporous species of *Alternaria* is cosmopolitical. They are found on all continents, on various plant substrates, in natural and agrophytocenoses, in the street and indoor air [36]. These fungi are common on cereals in different parts of the world. The species of *Alternaria* section infect grain often and everywhere [1] and are very plastic eurybiontic fungi. The species of *Infectoriae* section are also common in grain in many regions of the planet, but almost never occur, for example, in the South of the Far East. Our data show that in the European part of Russia, the types of both sections are extremely common in all regions. The average contamination varies from region to region and from year to year. Within the boundaries of the region in different areas, this indicator may vary greatly or may be similar in relation to a particular year. The differences between the areas of seed contamination proved to be significant only in 2011 and 2012. The reason is that in 2010, the average contamination with fungi of the *Alternaria* genus was lower than in subsequent years. This was especially true for the *Alternaria* section (the figure in 2010 in the two most studied regions is about 3 times lower than in 2011 and 2012). The absence of significant differences in 2010 might also be associated with a smaller number of the analyzed samples from Stavropol Krai. These trends indicate that the leading factors affecting the contamination of grain by *Alternaria* are the weather conditions.

Different crops were contaminated with small-sporous *Alternaria* almost equally. Wheat and barley were infected greatly. Previously, it was shown that rye and oat are equally susceptible to the infection [1, 37]. Maize was infected in the least degree. Resistant cultivars were not revealed. Resistance to some necrotrophic weakly specialized species, if detected, has a quantitative character. In the case of widely specialized *Alternaria* species, which do not actually infect the seed but only colonize its covers, the detection of resistant varieties is highly improbable. These fungi can develop and persist on any plant residues and many plants, including weeds. It means that the sources of inoculum are abundant in almost all phytocenoses. Despite the relatively large size, the *Alternaria* spores can spread in the air. Therefore, a large number of *Alternaria* conidia are found almost everywhere in the boreal non-arid zone. Forecrops and crop rotation, as expected, have little effect on the *Alternaria* contamination due to aerogenicity rather than the soil origin of the infection and because almost any plant residues, including those outside the field, could be a sufficiently productive source of spores.

In the review by F. Culshaw et al. [38], the facts demonstrating the lack of influence of *Alternaria* spp. on the size and germination of caryopsis are considered. The authors have also shown the lack of relationship between the infection rate and laboratory germinating capacity. It is known that the infection occurs after flowering, and the embryo is not affected by the *Alternaria* mycelium. The saprotrophic nature of the *Alternaria* species associated with cereal crops is manifested in the fact that only the seed coat is affected. Therefore, the embryo non-infected by *Alternaria* can germinate and, under favorable conditions for the plant, will not be infected by these fungi growing from the seed coat.

Specific weather conditions (temperature, humidity, precipitation) during the vegetation season and some peculiarities of the agricultural technology (crop density, irrigation, fungicides) should be included in the number of underinvestigated factors affecting the spread of fungi of the *Alternaria* genus in grain crops. The influence of the *Alternaria* species on field germination under different weather and edaphic parameters is still an important issue.

Thus, regardless of the variety, wheat, barley, rye, and oat grains are about equally infected with fungi of *Alternaria* genus from *Alternaria* and *Infectoriae* sections. Maize is infected in a less degree. The significant influence of the forecrop (maize, sunflower, sugar beet, fallow, winter wheat) on the proportion of the infected seeds of wheat is not found. Weather and climatic conditions play a decisive role in grain contamination. We revealed negative correlation between the contamination of barley grain by *Alternaria* species and some other fungi (*Bipolaris sorokiniana* and *Pyrenophora* spp.) and the lack of a reliable relationship between the contamination of wheat and barley seeds by species from both sections. The inconsistency of the results of previous studies may be the consequence of incorrect attributing species (with regard at least the section).

REFERENCES

1. Gannibal F.B. *Mikologiya i fitopatologiya*, 2008, 42(4): 359-368 (in Russ.).
2. Ziling M.K. V knige: *Bolezni zernovykh kul'tur* /Pod redaktsiei K.E. Murashkinskogo [In: Diseases of cereals. K.E. Murashkinskii (ed.)]. Omsk, 1932: 15-39 (in Russ.)
3. Geshele E.E. *Metodicheskoe rukovodstvo po fitopatologicheskoi otsenke zernovykh kul'tur* [Methodological guidelines for the phytopathological evaluation of cereals]. Odessa, 1971 (in Russ.)
4. Chulkina V.A. V sbornike: *Kornevye gnili khlebnykh zlakov i mery bor'by s nimi* [In: Root rot of cereals and measures to combat them]. Moscow, 1970: 109-112 (in Russ.)
5. Shpokauskene O.Yu. *Trudy AN LitSSR. Ser. V. Biologicheskie nauki*, 1984, 4(88): 53-59 (in Russ.)
6. Simmons E. G. *Alternaria. An Identification Manual.* CBS, Utrecht, 2007.
7. Andersen B., Thrane U. Differentiation of *Alternaria infectoria* and *Alternaria alternata* based on morphology, metabolite profiles, and cultural characteristics. *Can. J. Microbiol.,* 1996, 42: 685-689 (doi: 10.1139/m96-093).
8. Andersen B., Thrane U., Svendsen A., Rasmussen I.A. Associated field mycobiota on malt barley. *Can. J. Bot.*, 1996, 74(6): 854-858 (doi: 10.1139/b96-106).
9. Andersen B., Krøger E., Roberts R.G. Chemical and morphological segregation of *Alternaria arborescens*, *A. infectoria* and *A. tenuissima* species-group. *Mycol. Res.*, 2002, 106(2): 170-182 (doi: 10.1017/S0953756201005263).
10. Lawrence D.P., Gannibal Ph.B., Peever T.L., Pryor B.M. The Sections of *Alternaria*: Formalizing species-group concepts. *Mycologia*, 2013, 105(3): 530-546 (doi: 10.3852/12-249).
11. Lawrence D.P., Rotondo F., Gannibal Ph.B. Biodiversity and taxonomy of the pleomorphic genus *Alternaria*. *Mycol. Prog.*, 2016, 15(1): art.3 (doi: 10.1007/s11557-015-1144-x).
12. Woudenberg J.H.C., Seidl M.F., Groenewald J.Z., de Vries M., Stielow J.B., Thomma B.P.H.J., Crous P.W. *Alternaria* section *Alternaria*: Species, formae speciales or pathotypes? *Stud. Mycol.*, 2015, 82: 1-21 (doi: 10.1016/j.simyco.2015.07.001).
13. Gannibal Ph.B. Distribution of *Alternaria* species among sections. 2. Section *Alternaria*. *Myco-*

taxon, 2015, 130(4): 941-949 (doi: 10.5248/130.941).
14. Andrew M., Peever T.L., Pryor B.M. An expanded multilocus phylogeny does not resolve morphological species within the small-spored *Alternaria* species complex. *Mycologia*, 2009, 101(1): 95-109 (doi: 10.3852/08-135).
15. Andersen B., Sørensen E.L., Nielsen K.F., van den Ende B.G., de Hoog S. A polyphasic approach to the taxonomy of the *Alternaria infectoria* species—group. *Fungal Genet. Biol.*, 2009, 46: 642-656 (doi: 10.1016/j.fgb.2009.05.005).
16. Mercado Vergnes D., Renard M.-E., Duveiller E., Maraite H. Identification of *Alternaria* spp. on wheat by pathogenicity assays and sequencing. *Plant Pathol.*, 2006, 55: 485-493 (doi: 10.1111/j.1365-3059.2006.01391.x).
17. Webley D.J., Jackson K.L. Mycotoxins in cereals — a comparison between North America, Europe and Australia. *Proc. Australian Postharvest Technical Conference (Canberra, 26-29 May 1998)*. CSIRO Entomology, Canberra, 1998: 63-66.
18. Gagkaeva T.Yu., Gannibal F.B., Gavrilova O.P. *Zashchita i karantin rastenii*, 2012, 1: 37-41 (in Russ.)
19. Magan N., Cayley G.R., Lacey L. Effect of water activity and temperature on mycotoxin production by *Alternaria alternata* in culture and on wheat grain. *Appl. Environ. Microb.*, 1984, 47(5): 1113-1117.
20. Logrieco A., Bottalico A., Solfrizzo M., Müle G. Incidence of *Alternaria* species in grains from Mediterranean countries and their ability to produce mycotoxins. *Mycologia*, 1990, 82(4): 501-505 (doi: 10.2307/3760022).
21. Coulombe R.A. *Alternaria* toxins. In: *Mycotoxins and phytoalexins*. R.P. Sharma, D.K. Salunkhe (eds.). CPC Press, Boca Raton, 1991: 425-433.
22. Andersen B., Krøger E., Roberts R.G. Chemical and morphological segregation of *Alternaria alternata*, *A. gaisen* and *A. longipes*. *Mycol. Res.*, 2001, 105(3): 291-299 (doi: 10.1017/S0953756201003446).
23. Oviedo M.S., Sturm M.E., Reynoso M.M., Chulze S.N., Ramirez M.L. Toxigenic profile and AFLP variability of *Alternaria alternata* and *Alternaria infectoria* occurring on wheat. *Braz. J. Microbiol.*, 2013, 44(2): 447-455 (doi: 10.1590/S1517-83822013000200017).
24. Kahl S.M., Ulrich A., Kirichenko A.A., Müller M.E. Phenotypic and phylogenetic segregation of *Alternaria infectoria* from small-spored *Alternaria* species isolated from wheat in Germany and Russia. *J. Appl. Microbiol.*, 2015, 119(6): 1637-1650 (doi: 10.1111/jam.12951).
25. Larsen T.O., Perry N.B., Andersen B. Infectopyrone, a potential mycotoxin from *Alternaria infectoria*. *Tetrahedron Letters*, 2003, 44: 4511-4513 (doi: 10.1002/chin.200338207).
26. Christensen K.B., Van Klink J.W., Weavers R.T., Larsen T.O., Andersen B., Phipps R.K. Novel chemotaxonomic markers of the *Alternaria infectoria* species-group. *J. Agric. Food Chem.*, 2005, 53(24): 9431-9435 (doi: 10.1021/jf0513213).
27. Svetov V.G. *Mikologiya i fitopatologiya*, 1989, 23(5): 471-473 (in Russ.)
28. Clarke M.P., Gooding M.J., Jones S.A. The effects of irrigation, nitrogen fertilizer and grain size on Hagberg falling number, specific weight and black point of winter wheat. *J. Sci. Food Agr.*, 2004, 84: 227-236 (doi: 10.1002/jsfa.1657).
29. Conner R.L. Influence of irrigation and precipitation on incidence of black point in soft white spring wheat. *Can. J. Plant Pathol.*, 1989, 11(4): 388-392 (doi: 10.1080/07060668909501085).
30. Conner R.L., Davidson J.G.N. Resistance in wheat to black point caused by *Alternaria alternata* and *Cochliobolus sativus*. *Can. J. Plant Pathol.*, 1988, 68: 351-359 (doi: 10.4141/cjps88-046).
31. Southwell R.J., Brown J.F., Wong P.T.W. Effect of inoculum density, stage of plant growth and dew period on the incidence of black point caused by *Alternaria alternata* in durum wheat. *Ann. Appl. Biol.*, 1980, 96(1): 29-35 (doi: 10.1111/j.1744-7348.1980.tb04765.x).
32. Fernandez M.R., Clarke J.M., DePauw R.M., Irvine R.B., Knox R.E. Black point and red smudge in irrigated durum wheat in southern Saskatchewan in 1990-1992. *Can. J. Plant Pathol.*, 1994, 16: 221-227 (doi: 10.1080/07060669409500757).
33. Fernandez M.R., Clarke J.M., DePauw, R.M., Irvine R.B., Knox R.E. Black point reaction of durum and common wheat cultivars grown under irrigation in southern Saskatchewan. *Plant Dis.*, 2000, 84(8): 892-894 (doi: 10.1094/PDIS.2000.84.8.892).
34. Mak Y., Willows R.D., Roberts T.H., Wrigley C.W., Sharp P.J., Copeland L. Black Point is associated with reduced levels of stress, disease- and defense-related proteins in wheat grain. *Mol. Plant Pathol.*, 2006, 7(3): 177-189 (doi: 10.1111/j.1364-3703.2006.00330.x).
35. Samson R.A., Hoekstra E.S., Frisvad J.C., Filtenborg O. *Introduction to food and airborne fungi*. Centraalbureau voor Schimmelcultures, Utrecht, 2002.
36. Rodriguez-Rajo F.J., Iglesias I., Jato V. Variation assessment of airborne *Alternaria* and *Cladosporium* spores at different bioclimatical conditions. *Mycol. Res.*, 2005, 109(4): 497-507 (doi: 10.1017/S0953756204001777).
37. Gavrilova O.P., Gannibal F.B., Gagkaeva T.Yu. *Fusarium* and *Alternaria* fungi in grain of oats grown in the North-Western Russia regarding cultivar specificity. *Sel'skokhozyaistvennaya biologiya* [*Agricultural Biology*], 2016, 51(1): 111-118 (doi: 10.15389/agrobiology.2016.1.111eng).
38. Culshaw F., Cook R.J., Magan N., Evans E.J. *Blackpoint of wheat. HGCA Research Review No. 7.* Home Grown Cereals Authority, London, 1988.