

Black point in spring durum wheat under different environmental conditions

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The study of cereal seed mycocenosis is relevant in solving problems determining the production of healthy, environmentally friendly agricultural products. Black point of wheat grain is a disorder characterised by a brown-black discolouration at the embryo end of the kernel. It is found in all regions of durum wheat cultivation and it results in significant economic loss annually. We studied seven varieties of spring durum wheat that were grown in 2014-2019. The weather conditions of the growing season have a significant impact on the development of black point of durum wheat in the forest-steppe zone of the Altai Territory of Russia (South-Western Siberia). The defeat of seeds by a black point varied from 2.3 to 28.0 % on average for varieties. To obtain high-quality grain that is not affected by this disease, phytosanitary measures are required that reduce the infection of seeds with phytopathogens and limit the development of the disease during the growing season. Analysis of the pathogenic composition of fungal microorganisms in seeds of spring durum wheat, formed in different vegetations, revealed the presence of representatives of *Fusarium*, *Alternaria*, *Helminthosporium*, *Penicillium* and *Aspergillum* with a predominance of the first 3 genera. We found the negative effect of *Fusarium* fungi on seed germination. In the field conditions of the forest-steppe of the Altai Territory, the development of a black point in durum wheat grain is determined by the colonization of seeds with *Bipolaris sorokiniana*. No correlation was found between the incidence of black point and the infection of seeds by *Fusarium* and *Alternaria*.

Key words: durum wheat; black point; Disease Development Index (DDI); pathogenic fungi; genotype; environmental conditions

Introduction

Triticum durum Desf. is a major food crop in many parts of the world. The grain of this crop is a source of energy, carbohydrates, proteins, many vitamins, minerals, and other food components important in nutrition (Sissons et al., 2012). For example, durum wheat grains contain 8-20% protein, which is close to milk protein in nutritional value (McKeivith, 2004), which allows the use of grain products for baby and diet food. In durum wheat, a high content of carotenoids, B vitamins, E vitamin, lignin, phytosterol, which reduce the risk of cardiovascular and cancer diseases, were found (Graham & Welch, 2000; Liu, 2007). Certain properties of durum wheat grain (amber color, glassiness (transparency) and hardness) make it possible to produce high-quality pasta from it. The biological and nutritional value of soft wheat pasta is significantly inferior to durum wheat pasta. Therefore, the production of soft wheat pasta is prohibited in a number of European countries (Sissons et al., 2012). Semolina and cereals of the Poltavskaya and Artek brands are traditionally produced from durum wheat in Russia, and Bulgur and Kus-Kus cereal products are popular in Turkey, the Middle East and North Africa. In bakery, durum wheat flour is used as an improver. Processing enterprises make high demands on the quality of raw materials procured. In addition, with the expansion of export of durum wheat from Russia, the demand for raw materials meeting the requirements of the world market is inevitable.

One of the indicators of marketability of grain is its purity from black point. The black point is the darkening of the kernels, which most often covers the region of the embryo and grooves, is found in all regions of durum wheat cultivation. During grinding of durum wheat grain, black point gives a significant share of dark inclusions (specks). Pasta made from such grains acquires a dark shade and loses consumer qualities (Dexter & Matsuo, 1982; Vasilchuk, 2001). According to international standards, the development of the disease by more than 5% reduces the class and price of grain (Rees et al., 1984; Fernandez et al., 1994; Lehmensiek et al., 2004). The first description of the "black point" as a darkening of grain in barley, made in Germany, in the work of A. Zöbl. Since the beginning of the 20th century, work to describe and study the causes of the emergence of black point has been carried out everywhere where cereals were grown; these are Italy, Egypt, Morocco, Argentina, India, Canada, the USA, Germany, North Africa, Russia. The causes of the appearance of black pointed grain are most often associated with infection by *Alternaria*, *Helminthosporium*, *Fusarium* fungi and the activity of oxidative enzymes, in particular peroxidase (Fernandez et al., 1994; Conner et al., 1996; Mak et al., 2006; Kirichenko, 2008; Gannibal, 2014). Black point was identified as a problem in 1940, when the quality of wheat grain in Egypt decreased due to its strong development (El-Helaly, 1947). Currently, many researchers have noted that production in Russia of black point-free grain has become problematic (Gannibal, 2014; Koishybaev et al., 2014; Gaponov et al., 2016). When epiphytotic occur, the crop dies completely or becomes unsuitable for use due to poor quality, including an increased content of toxins, threatening the food security of regions and countries (Statler et al., 1975; Toropova et al., 2012).

The pathological process in agroecosystems is determined by the accumulation of phytopathogens in the soil, mycocenosis of seeds, genotypes of cultivated varieties, the climate of the region, and vegetation conditions. It has been established that the main agent in the development of black point in bread wheat in Western Siberia is *Bipolaris sorokiniana* (Sacc.) Shoem (syn *Helminthosporium sativum* Pammel, Ring et Bakke), a fungus that causes helminthosporiosis (root rot) in cereals (Kirichenko, 2008; Glinushkin et al., 2016; Barysheva et al., 2018). In Russia, the prevalence of this pathogen in agrocenoses of cereal crops reaches 82 % (Glinushkin et al., 2016). The pathogens of root rot retain their viability in the soil for more than 5 years, thus determining the conditions for their constant presence in the agroecosystem. The deterioration of the phytosanitary state of agrocenoses was facilitated by climate change, saturation of crop rotation with grain crops, the introduction of minimal and no-till technologies in agricultural practice, the expansion of areas under winter wheat (Wang et al., 2002; Hannibal, 2014; Koyshibaev et al., 2014; Gaponov et al., 2016).

Breeding and cultivation of resistant varieties are the most effective and environmentally friendly methods of protecting grain from pathogens (Toropova et al., 2012; Koishybaev et al., 2014). In early studies of the disease, it was revealed that durum wheat is more susceptible to black point than soft wheat (El-Helaly, 1947). We screened a collection of durum wheat in climatic conditions of the forest-steppe zone of the Altai Territory and revealed significant differences in susceptibility of durum wheat samples to black point. As a result of the study, we identified resistant genotypes. Highly resistant varieties formed the grain almost pure from black point under typical conditions for our region, and under conditions of strong epiphytotics they had a significant advantage over susceptible samples (Barysheva et al., 2016, 2018). A significant contribution to the formation of grain with a black point can make vegetation conditions. High humidity and temperature can stimulate the development of the black point and increase the darkening of the grain several times (Conner, 1989; Fernandes et al., 1994; Williamson, 1997; Kumar et al., 2002; Clarke et al., 2004; Mak et al., 2006; Moschini et al., 2006).

The aims of the study were to identify the diversity of fungi that colonize durum wheat seeds, as well as to assess the development and causes of the black point disease in the forest-steppe zone of the Altai Territory (South-Western Siberia of Russia) during growing seasons in 2015-2019.

Material and methods

Seven spring durum wheat varieties were grown in 2014-2019 on the experimental field of the Federal Altai Scientific Centre of Agro-BioTechnologies near Barnaul (15 km northwest of the center of Barnaul on the left bank of the Ob), the Altai Territory (South-Western Siberia), Russia (latitude 53° 25'14"N, longitude 83° 31'12"E) and harvested at maturity. Alejskaya, Oasis, Pamyati Yanchenko, Salyut Altaya, and Solnechnaya 573 were bred in Altai Research Institute of Agriculture (Barnaul), and Zhemchuzhina Sibiri and Omskij korund were created in Siberian Research Institute of Agriculture (Omsk). All varieties are included in the State Register of Breeding Achievements Permitted for Use (2019) and are approved for the production of durum wheat in the 10th region (Western Siberia of Russia). The cultivation technology of the studied varieties provided for the absence of fertilizers and fungicides. Seed sampling was carried out in accordance with standard procedures. The sample size of each genotype was 100 grains with 4 replicates. Based on visual assessment, mature seeds were categorised into black pointed grain and clean grain. The degree of seed damage by the disease was evaluated in points according to the scale developed by A. T. Tropova and interpreted by E. Yu. Toropova & A. A. Kirichenko (2012). Black pointed kernels were evaluated within 0.1-3 points in accordance with the criteria from very weak damage to strong one. The intensity of black point was evaluated by the Disease Development Index (*DDI*):

$$DDI = \frac{n \times 100}{N} \quad (\text{Chulkina et al., 2010}),$$

where *DDI* is the development of the disease, %; *N* is the total number of kernels; *n* is the number of grains affected by 1 point or more; **100** is percent conversion rate.

Assessment of germination and mycocenosis of seeds was carried out in the Microbiology Laboratory of Altai State University (Barnaul, Russia). We used nine durum wheat varieties grown in the field in 2016 and 2017 (Solnechnaya 573, Alejskaya, Salyut Altaya, Luch 25, Saratovskaya zolotistaya, Omskij korund, Omskij izumrud, Marina, Bezenchukskaya zolotistaya). Grain samples were prepared using wet filter paper rolls. Kernels were laid out on a wet strip of paper with 25 pcs. per each strip with 4 replications, then the paper was rolled up, placed vertically in a glass and moved in a Memmert climate chamber at a temperature of 20 ± 2°C for 7-8 days. Germination percentage was calculated 8 days after sowing (DAS). After seed germination, the rolls were unrolled, a piece of paper with an imprint of the developed microflora was cut out and cytological preparations were made. Microscopic analysis of cytological preparations was performed using an Olympus BX51 microscope. Using this method, the generic affiliation of fungi was determined.

To identify the species diversity of fungi, phytopathogens were isolated into pure cultures. The seeds were sterilized with KMnO₄ solution for 5 minutes, followed by several rinses in sterile distilled water and dried on filter paper. Surface sterilized kernel samples were aseptically placed into 9 cm diameter Petri plates with potato dextrose agar (PDA) and incubated at 25 ± 2°C for 21 days in darkness. Four Petri plates containing 10 kernels in each were repeated 4 times. The growing fungal colonies on the PDA plates were examined under the microscope. Screening of isolates was carried out in a laminar box by transferring individual conidium (in species with a large single conidium) or several conidia from one chain into Petri plates with PDA using a sterile needle. The transfer process was observed under a binocular. The fungi were subcultured on PDA Petri plates, and identified based on cultural characteristics and colony morphologies.

To study the influence of the factors "genotype" and "weather conditions" on the infection of durum wheat seeds with various pathogens we used analysis of variance (ANOVA). Means of each variety were compared by Student's t-test (P = 0.05). In addition, we calculated the correlation coefficients (*r*) between germination, *DDI* and colonization of seeds with various fungi. The statistical analyses were performed using the Microsoft Office Excel 2010 application software.

Results and discussion

It has been reported that more than 100 species of fungi can be isolated from wheat seeds. However, *Alternaria*, *Aspergillus*, *Chaetomium*, *Fusarium*, *Helminthosporium*, *Myrothecium*, *Nigrospora*, *Penicillium*, *Phoma*, *Rhizopus*, and *Stemphylium* were most often found in black-pointed grains (Rees et al., 1984; Conner et al., 1996; Kumar et al., 2002; Sisterna & Sarandon, 2005; Khulbe et al., 2011; Gannibal, 2014; Gaponov et al., 2016; Patel & Minipara, 2016). Unlike bread wheat, the development of this disease in durum wheat in Western Siberia is poorly studied. Most of the taxa that we isolated from durum wheat belonged to the group of pathogenic fungi, the share of which was 98%. Representatives of the genera *Bipolaris* Shoemaker (*Helminthosporium* Link), *Alternaria* Nees, and *Fusarium* Link predominated in the mycocenosis of seeds. Micromycetes of the genera *Penicillium* Link and *Aspergillum* were also found in some samples (Fig. 1). In many cases, phytopathogens were present simultaneously in the same grain, forming a mycobiotic pathogenic complex. *Fusarium* and *Alternaria* fungi turned out to be dominant; their share in seeds reached 36 and 35%, respectively. The relative abundance of the genus *Helminthosporium* in the seed pathocomplex was 27%.

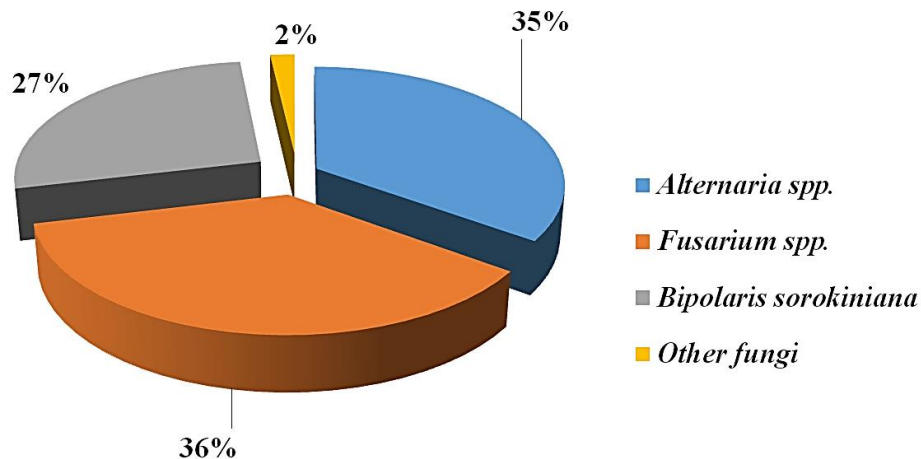


Fig. 1 Taxonomic composition and distribution of micromycetes in seeds of spring durum wheat in the forest-steppe of the Altai Territory of Russia

According to microscopic studies, in the pathocomplex of durum wheat seeds, the genus *Helminthosporium* is represented by one species of *Bipolaris sorokiniana*. Among the alternaria fungi, the small-spore species *Alternaria tenuissima* (Nees et T. Nees: Fies) Wiltshire, which is highly toxicogenic, predominated (Fig. 2). The toxicity of the culture filtrate of this species for chicken embryos has now been proven (Gannibal, 2014). In addition, it is known that his spores are one of the common allergens.



Fig. 2 Phytopathogens found in seeds of spring durum wheat in the forest-steppe of the Altai Territory of Russia: *Bipolaris sorokiniana* (left), *Alternaria tenuissima* (right).

We observed a significant variation in the frequency of occurrence of various phytopathogens among the studied genotypes, as evidenced by the data in Table 1. For example, the defeat of seeds by representatives of the genus *Fusarium* varied from 28 (Omskij korund) to 81 % (Marina) in 2016 and from 57 (Alejskaya) to 99 % (Luch 25) in 2017 reaching an average of 61.6 and 79.2 %, respectively. Alternariosis was detected in 55.7 and 52.3 % of seeds in 2016 and 2017, respectively. In this case, the percentage of seeds affected by *Alternaria* spp. varied from 48 (Bezenchukskaya zolotistaya) to 64 % (Salyut Altaya) in 2016 and from 41 (Alejskaya) to 65 % (Bezenchukskaya zolotistaya) in 2017. The defeat of the grain by the fungus *B. sorokiniana* during the field vegetation of 2016 was minimal in comparison with the other two pathogens and averaged 47.2 %. In 2017, the proportion of seeds with helminthosporious infection increased and on average for the studied varieties reached 69.9 %.

Table 1. The proportion of seeds (%) of spring durum wheat affected by various pathogens in the conditions of the forest-steppe of the Altai Territory of Russia.

Genotype	<i>Alternaria</i> spp.		<i>Bipolaris sorokiniana</i>		<i>Fusarium</i> spp.		Germination, %	
	Growing season							
	2016	2017	2016	2017	2016	2017	2016	2017
Solnechnaya 573	55	59**	37*	85**	76**	69*	37*	50**
Alejskaya	59**	41*	49	78**	73**	57*	36*	45
Salyut Altaya	64**	63**	41*	67	34*	73*	50**	63**
Luch 25	59**	62**	66**	70	62	99**	43	35*
Saratovskaya zolotistaya	52*	50	52**	39*	69**	91**	51**	34*
Omskij korund	55	47*	49	75**	28*	79	58**	36*
Omskij izumrud	58**	42*	40*	67	56*	70*	36*	49**
Marina	51*	42*	49	82**	81**	92**	45	34*
Bezenchukskaya zolotistaya	48*	65**	42*	66*	75**	83**	41*	47**
Average	55.7	52.3	47.2	69.9	61.6	79.2	44.1	43.5

Note: * – the value is significantly lower than average at $P < 0.05$; ** – the value is significantly higher than average at $P < 0.05$.

A two-way analysis of variance was performed to study the influence of the factors "genotype" and "weather conditions" on the infection of seeds with various pathogens. In a two-year experiment, seed colonization by *B. sorokiniana* fungi and species of the genus *Fusarium* was mainly determined by vegetation conditions. The share of the influence of the factor "weather conditions" was 90.1 % and 72.5 % when infected with *Helminthosporium* and *Fusarium* fungi, respectively. The factor "genotype" in both cases also turned out to be statistically significant, its influence on the presence of *B. sorokiniana* in the seeds was 3.9 %. The defeat of seeds by *Fusarium* spp. was determined by the genotype of the variety by 15.9 %. The influence of weather conditions was insignificant on the colonization of durum wheat grain by *Alternaria* species. Toklu et al. (2008) believes the differences in the black point incidences among bread wheat samples (in the range of 3 to 10 %) can be explained by the genotypic response of different varieties.

Germination of seeds of the studied varieties was rather low. The average values of this trait of grain formed under different vegetation conditions did not differ and amounted to 44.1 and 43.5 % in 2016 and 2017, respectively. A significant negative correlation was established between germination and colonization of seeds with fungi of the genus *Fusarium* ($r = -0.91$ in 2016 and $r = -0.62$ in 2017), which indicated a decrease in germination in the presence of this infection. It is interesting to note the positive correlation between the frequency of occurrence in the seeds of representatives of the genus *Alternaria* and the level of germination in 2016 ($r = +0.50$), which is consistent with the opinion of some authors who claim that these fungi settle on the largest grains. It is also suggested that, having penetrated inside the lodicules (flower films) *Alternaria* mycelium stimulates the vital activity of cells and tissues that bind the seed to the mother plant, and possibly lengthens the period of accumulation of nutrients by the grain. As a result, it is formed more complete and fulfilled. According to A.A. Kirichenko (2008), seeds infected with *B. sorokiniana* had low germination, and when colonizing with *Alternaria*, germination decreased only in immature seeds. Our study did not prove a reliable correlation between infection of grain with *B. sorokiniana* and the percentage of germination ($r = +0.23$). F. Toklu et al. (2008) reported that in all varieties of bread wheat, a black point had a negative effect on the percentage of germination. In addition, in all varieties, the infected grains were heavier than the normal ones. However, they noted that this disease did not affect the number of embryonic roots and the length of the coleoptile. Taking into account the reduced germination of seeds with a black point, the authors propose to increase the sowing rate in the field. Hudec (2007) found that fungal species of *Alternaria*, *Penicillium*, *Aspergillus* and *Fusarium* genera and *Cochliobolus sativus* were able to kill or reduce barley embryo vigor.

An analysis of the relationships between different representatives of the seed pathocomplex of durum wheat and the development of the black point revealed that this disease is caused mainly by the colonization of the grain with *B. sorokiniana* ($r = +0.43$). Other representatives of mycobiota that we identified are most likely responsible for the development of other pathogenic processes.

According to many authors, the cause of the black point in cereals was associated with extreme environmental conditions, such as heavy rain, high humidity, and extreme temperatures during grain filling (Conner, 1989; Williamson, 1997; Kumar et al., 2002; Clarke et al., 2004; Mak et al., 2006; Moschini et al., 2006; Khulbe et al., 2011). We studied the development of this disease in the grain of 7 varieties of spring durum wheat grown under the conditions of 6 field vegetation in the Altai Territory (Table 2). Two-way analysis of variance of the data revealed a significant influence of the factors "conditions of the year", "genotype", as well as their interaction on the variation of the trait. The shares of the influence of the factors amounted to 94, 4, and 2 %, respectively. The defeat of seeds by a black point was largely determined by the conditions of vegetation and varied from 2.3 to 28.0 % on average for varieties.

A minimal seed damage by black point was observed in 2015 (2.3 %), which did not exceed the permissible level. The variation among genotypes was from 1.9 (Salyut Altaya, Zhemchuzhina Sibiri) to 5.2 % (Alejskaya). In the next three vegetations, a strong development of black point occurred, exceeding the permissible limit by several times. Especially unfavorable growing seasons were 2016 and 2017, when the Disease Development Index (DDI) of genotypes averaged 22.9 and 28.0 %, respectively (Fig. 3).

It is known that the formation of grain with a black point increases significantly with increased humidity and temperature during the susceptibility of wheat to infections. This period lasts from the end of flowering to the middle of dough stage (Conner, 1987). In our zone, this period usually falls on July. Meteorological data during the grain filling period in the forest-steppe zone of our region are presented in Table 3. According to the data, in the vegetation when the disease developed most actively, precipitation fell twice as normal. So in July 2016 and 2017, 116.3 and 138.0 mm of precipitation fell. While the long-term average value was 64.0 mm. The average daily temperature in July in 2017 was 1 °C higher than the climatic norm, and in 2016, on the contrary, it was lower than the norm. We believe that this fact caused the increase in grain with a black point.

Table 2. The development of black point (DDI) in durum wheat grain in the forest-steppe of the Altai Territory of Russia, %.

Genotype	Growing season						Average
	2014	2015	2016	2017	2018	2019	
Salyut Altaya	1.4	1.9	26.2	24.2	7.0	6.3	11.2
Pamyati Yanchenko	1.6	2.1	24.2	27.9	12.8	7.9	12.8
Solnechnaya 573	3.4	2.1	26.0	26.0	9.9	7.8	12.5
Omskij korund	5.3	3.9	20.4	26.6	9.8	11.5	12.9
Zhemchuzhina Sibiri	7.8	1.9	24.7	27.9	13.8	7.2	13.9
Alejskaya	4.8	5.2	23.8	35.3	13.8	11.5	15.7
Oazis	4.4	2.6	15.2	28.1	6.5	7.8	10.8
Average	4.1	2.3	22.9	28.0	10.8	8.6	-

**Fig. 3** Seeds of spring durum wheat obtained in 2015 (left) and in 2017 (right) in the field conditions of the forest-steppe of the Altai Territory of Russia**Table 3.** Hydrothermal conditions in July (according to Altai Center for Hydrometeorology and Environmental Monitoring (Barnaul, Nauchnyj gorodok).

Parameter	Growing season						Long-term average
	2014	2015	2016	2017	2018	2019	
The average daily temperature, °C	20.1	20.3	20.8	18.8	18.8	19.9	19.9
Precipitation, mm	107.6	63.6	116.3	138.0	41.3	42.3	64.0

The revealed significant differences among the varieties in their ability to resist infection in typical weather conditions ensured the formation of a grain that was unaffected or slightly affected by a black point. So in 2015, the DDI for all studied samples, except Alejskaya, did not exceed 5%. However, under typical conditions of the hydrothermal regime in July 2019 (Table 2), we were not able to obtain high-quality seeds. We assume that this is due to the high infection rate of the seed. In annual crops, 75.1 % of fungal pathogens are known to be transmitted through seeds and planting material (Toropova et al., 2012). In our study, seeds sown in 2019 were collected in the field in 2018. As a result, in 2019, the grain of all varieties exceeded the threshold of harmfulness for the development of black point. Probably, the cause of the development of the disease in 2019 was the high infection rate of seeds obtained in the previous growing season.

Conclusion

The weather conditions of the growing season have a significant impact on the development of black point of durum wheat in the forest-steppe zone of the Altai Territory. To obtain high-quality grain that is not affected by this disease, phytosanitary measures are required that reduce the infection of seeds with phytopathogens and limit the development of the disease during the growing season. Analysis of the pathogenic composition of fungal microorganisms in seeds of spring durum wheat, formed in different vegetations, revealed the presence of representatives of *Fusarium*, *Alternaria*, *Helminthosporium*, *Penicillium* and *Aspergillum* with a predominance of the first 3 genera. We found the negative effect of *Fusarium* fungi on seed germination. In the field conditions of the forest-steppe of the Altai Territory, the development of a black point in a durum wheat grain is determined by the colonization of seeds with *B. sorokiniana*. No correlation was found between the incidence of black point and the infection of seeds by *Fusarium* and *Alternaria*.

Conflict of interest

The authors declare that they have no conflict of interests.

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