

# BLACK POINT OF WINTER WHEAT SEEDS

**Goal.** To study infection of seeds of winter wheat varieties by «black point» and to establish the phytopathogenic composition of pathogens in the Right Bank Forest-Steppe of Ukraine. **Methods.** Laboratory — macroscopic analysis was performed according to DSTU 4138-2002, phytopathogenic composition was identified using nutrient medium, statistical analysis of the obtained data — calculation of the degree of pathogen severity, HIP. **Results.** A collection of 27 varieties of soft winter wheat was studied to determine the degree of seed infection. The highest percentage of infection was found in varieties Vodogray Bilotserkivsky (Ukraine), Adele (Russia), Balitus (Austria), Viglanka (Slovakia), Sefeg-2 (Azerbaijan). The least susceptible to the disease are varieties Daria (Croatia), Bodycek (France), Gratsia Bilotserkivska (Ukraine, Bila Tserkva DSS), Azano (Sweden), Gospodarka (Ukraine), Kozyr (Ukraine), Morozko (Russia), the number of grains with signs of darkening in the embryonic zone for these varieties did not exceed 1%. The sowing qualities of the diseased seeds were determined. The species composition of phytopathogens that cause the disease has been established. The phytopathogenic complex of seeds with the black point included fungi of the genera *Alternaria*, *Fusarium*, *Curvularia*, *Bipolaris*, *Aspergillus*, *Acremoniella*, *Stemphillium*, *Sordaria* and *Epicoccum*. Most often, the seeds were colonized by fungi of the genus *Alternaria* Nees. Their share in the years of research was 76.6—83.1%. **Conclusions.** The infection of seeds of winter wheat varieties by black point under conditions of the Right-Bank Forest-Steppe of Ukraine in 2018—2019 ranged from 0 to 19.8% depending on the variety and year of research. The phytopathogenic complex included 13 species of fungi from 9 genera: *Alternaria*, *Fusarium*, *Curvularia*, *Bipolaris*, *Aspergillus*, *Acremoniella*, *Stemphillium*, *Sordaria*, *Epicoccum*. The part of *Alternaria* spp. in the years of research was over 75%, the most common species were *A. tenuissima* and *A. infectoria*. The

percentage of species from other genera was insignificant and did not exceed 4.6%.

## black point; winter wheat; phytopathogenic complex; *Alternaria*

Scientists from most countries where these crops have been grown for the last century have studied the cause of the appearance of cereals on seeds, especially wheat, and darkening of the embryo. Scientists from Australia, India, Argentina, Egypt, Lithuania, the United States, Canada, Russia, and others have shown great interest in this issue in the last decade. Their research is primarily aimed at studying the patterns of appearance of the dark color of the embryo depending on weather conditions, varietal characteristics and species composition of phytopathogens that cause its appearance.

Thus, the level of grain contamination of Egyptian wheat varieties ranged from 0,29% to 64%. The most susceptible to damage were wheat varieties Sakha 8 and Sakha 93, in which the number of affected embryos reached 64% and 63%, respectively. More significant damage was observed in soft wheat varieties, durum wheat grain was affected by no more than 1,3%. [1]. In a study of Pakistani varieties, it was found that the highest level of infection was in the variety Punjad — 32.0%, in Mehran-89 — 26.0%, Fareed-08 — 23.7% [2]. Canadian scientists have proven the influence of genetic characteristics of the variety on the level of infection of grain *Cochliobolus sativus* and *Alternaria* sp. with artificial infection [3].

Evaluation of the susceptibility of 58 varieties of spring durum



wheat to the manifestation of black point in the Altai Territory of Russia found that the average grain damage by black germ in 2014—2015 was 9.50%, varying in genotypes from 2,75 to 30,00%. The most affected varieties and lines were Lilek, Bezenchukskaya 205, 12S2-24, Bezenchukskaya 209, Bezenchukskaya steppe, Bezenchukskaya 210, Luchistaya, 12S1-14, Tverdnyia, G1734, Pamyati Chekhovich, Saratovskaya Zolotistaya, Bezenchukskaya Zolotistaya. The lowest degree of damage was in the varieties: Salute Altai, Memory of Yanchenko, Altai amber, Solnechnaya 573, Angel, Omsk emerald, 1480d-2, Ray 25, Kharkov 46, Don Elegy, Orenburg 10, Divide [4].

In Ukraine, similar studies were also conducted in Polissya. The stability of 33 wheat samples from different countries was studied. The highest percentage of grains with black point was observed in the varieties Valencia (19%), Tsarivna (13.8) and Lugastar (12.8%). The highest resistance was shown by the Vyshivanka variety and Chinese samples (Zhongsi 1048 (D-227), Zhongsi 1258 (D-226) [5].

Most studies in the world have found that the main reason for the appearance of dark-colored germ in wheat is the penetration of fungi of the genus *Alternaria* into the germ zone during grain filling. Studies by Chinese scientists have shown that the most common genera of species of fungi that cause the appearance of black embryo are *Alternaria* (frequency of 56.7%), *Bipolaris* (16.1%) and *Fusarium* (6.0%). The frequency of detection of fungi from the genera *Curvularia*, *Aspergillus*, *Cladosporium*, *Exserohilum*, *Epicoccum*, *Nigrospora*, *Penicillium* and *Ulocladium* ranged from 0.8 to 4.8% [6]. Fungi of the genus *Alternaria* (49.9%), to a lesser extent *Aspergillus* (26.5),



*Drechslera* (11%), *Fusarium* (7.9%), *Cladosporium* (3.8%) and *Curvularia* were mainly found in Pakistan. 0.7%) [6].

When studying seeds with a dark germ, a careful examination of the grains did not reveal any damage to the surface, which would improve the conditions for the penetration of fungi. *B. sorokiniana* and fungi of the genus *Alternaria* were isolated equally from apparently healthy grain and with signs of black point. Therefore, according to Mohsen Khani et al. The appearance of darkening was most likely the result of the plant's physiological response to environmental conditions during grain ripening [7].

Chinese scientists in vitro managed to obtain darkening in the area of the germ of wheat seeds, keeping it in a solution of catechol with the addition of H<sub>2</sub>O<sub>2</sub>. When the extract of the fungus *B. sorokiniana* was added to the medium, the degree of darkening increased. According to them, the cause of this phenomenon is the fermentation process with the accumulation of phenolic compounds in the grain, in particular in the embryonic zone enhanced by the presence of the fungus *B. sorokiniana* [8].

Contradictory data are found in the literature on the effect of embryo darkening on sowing qualities. Thus, Poonam Rani and Anita Singh from India conducted a study on the effect of the degree of darkening of the grain in the germ zone on its germination. It was found that seed germination where the darkening is more than 1/2 germ reduces germination by 75 percent or more depending on the variety [9].

Analysis of literature sources in recent years indicates the need for such research in Ukraine. The role of dark grain color in the germ zone has not yet been fully studied. The analysis of local varieties will allow to assess their resistance — susceptibility to damage by pathogens of black embryo and to develop measures to reduce the development of this disease during grain maturation.

**Materials and methods of research.**

To study the degree of damage to the seeds of winter wheat black point was used a collection of varieties from the National Center for Plant Genetic Resources of Ukraine Institute of Plant Breeding V.Ya. Yuryeva. The collection was sown during 2018—2019 at the research site of

the Institute of Plant Protection of NAAS in the Research and Production Department of the Institute of Physiology and Genetics of NASU, p. Glevakha, Vasylykiv district of Kyiv region. Macroscopic analysis for the detection of seeds with signs of dark color in the area of the embryo was performed according to DSTU 4138-2002 [10].

Selected seeds with a black point were sown on a selective nutrient agar. Since a significant proportion of fungi that cause black germ belong to the genus *Alternaria* used potato carrot agar (PCA). Incubation was performed at a temperature of 22—25°C under fluorescent lamps. Identification of the species composition of phytopathogens from seeds with black point was performed 10—14 days after sowing. Species affiliation of fungi of the genus *Alternaria* was determined by several parameters: morphological and cultural features, sporulation habit and size and structure of conidia [11]. Other species were identified by morphological features of conidia using a microscope according to reference books [12, 13, 14].

Sowing suitability of seeds with black germ was determined according to DSTU 4138-2002 [10]. The seeds were sown between layers of filter paper in the plant and germinated at a temperature of 20°C. Germination energy was determined on the 4-th, and germination on the 8-th day after sowing, the development of phytopathogens was observed on the 10-th day. The obtained research results were statistically processed in the Excel program.

**Results and discussion.** In 2018—2019, the degree of damage to soft winter wheat seeds by the black point in the conditions of the Right-Bank Forest-Steppe of Ukraine was studied. 27

varieties were analyzed, of which 15 — local selection, others — from Croatia, France, Sweden, Russia, Slovakia, Azerbaijan and Austria.

The degree of damage to varieties in 2018 ranged from 0 to 10.74%, and in 2019 from 0 to 19.8% depending on the variety and conditions of the year. The average number of grains with signs of black point was 3.12% in 2018 and 3.42% of the analyzed seeds in 2019, respectively (Table 1). Most varieties had a low degree of black embryo damage.

The highest percentage of seeds with signs of black germ in 2018—2019 was observed in varieties Pyshna (Ukraine, IR) — 4.68%,

**Table 1. Defeat of seeds of winter wheat varieties by black germ (Kyiv region, 2018—2019)**

№	Variety name	Origin	Blakc point seeds, %		
			2018	2019	average
1	Daria	Croatia	0.5	0	0.25
2	Bodycek	France	0.72	0	0.36
3	Grace of Bila Tsercva	Ukraine	0	0.89	0.45
4	Azano	Sweden	0.48	0.63	0.6
5	Hospodarka	Ukraine	1.18	0	0.6
6	Kozyr	Ukraine	1.24	0	0.6
7	Morozko	Russia	0.96	0.34	0.7
8	Donera	Russia	1.5	0.56	1.0
9	Klad	Ukraine	1.64	0.48	1.0
10	Prydniprovskya	Ukraine	0.7	2.27	1.1
11	Argument	Ukraine	1.26	1.42	1.3
12	Vozdvygenka	Ukraine	1.68	1.57	1.6
13	Sotnycia	Ukraine	3.26	1.15	2.2
14	Sich	Ukraine	2.28	2.18	2.2
15	Korovayna	Ukraine	3.16	1.44	2.3
16	Perlyna Polissya	Ukraine	1.4	3.37	2.4
17	Kesaria podiliska	Ukraine	5.1	3.56	5.0
18	Vyd	Russia	3.32	2.99	3.2
19	Kubok	Ukraine	4.94	1.38	3.16
20	Tabor	Russia	2.56	3.95	3.3
21	Pushna	Ukraine	4.66	4.7	4.7
22	Donna	Russia	7.74	2.2	4.9
23	Adel	Russia	5.68	8.07	6.9
24	Vodogray of Bila Tsercva	Ukraine	5.1	8.82	6.9
25	Viglanka	Slovenia	4.98	14.4	9.7
26	Balitus	Austria	3.62	18.1	10.9
27	Sefeg-2	Azerbaijan	10.74	19.8	15.3
Average			3.12	3.42	3.27
LSD <sub>05</sub> by factor «grade»			6.0		
LSD <sub>05</sub> by factor «year»			1.6		

Vodogray Bila Tserkva (Ukraine, BC DSS) — 6.9%, Adele (Russia, Krasnodar Research Institute of agricultural) — 6.9%, Balitus (Austria) — 10.9%, Viglanka (Slovakia) — 9%, Sefeg-2 (Azerbaijan) — 15.3%. The least susceptible to the disease were varieties Daria (Croatia) — 0.25%, Bodycek (France) — 0.36%, Grace Bila Tserkva (Ukraine, Bila Tserkva DSS) — 0.45%, Azano (Sweden), Gospodarka (Ukraine, IFRiG) and Kozyr (Ukraine, SGI) by 0.6%, Morozko (Russia, Krasnodar Research Institute of agricultural) — 0.7%.

From the analyzed collection, the seeds of the varieties that were most affected by the black germ during the years of research were selected: Pyshna, Caesarea Podilska, Vodogray Bila Tserkva, Adele, Viglanca, Sefeg-2. The influence of black point seed damage on sowing qualities of these varieties was studied. As a result of research it was found that the germination energy and germination of these varieties in the years of research ranged from 90 to 100% depending on the variety and year of research (Table 2). The seeds had good germination rates and therefore the effect of darkening in the embryonic zone did not show a significant effect on germination.

Analysis of seeds with signs of black embryo for pathogenic microflora revealed significant damage by pathogens of fungal etiology and bacterial infection. The phytopathogenic complex included 13 species of fungi from 9 genera. Thus, fungi from the genera *Alternaria*, *Fusarium*, *Curvularia*, *Bipolaris*, *Aspergillus*, *Acremonia*, *Stemphillium*, *Sordaria* and *Epicoccum* were found (Fig. 1).

The most common fungi were of the genus *Alternaria*, which accounted for 76.5% in 2018 and 83.1% in 2019. In 2018, two species of the genus *Alternaria* — *Alternaria tenuissima* — 37.5% and *Alternaria infectoria* — 39% were identified on wheat grain with black germ. In 2019, 4 species were identified — *Alternaria tenuissima* — 41.5%, *Alternaria infectoria* — 35.4%, *Alternaria alternata* — 2.3% and *Alternaria sp.* — 3.8%.

The share of other species in 2018 was 23.5%, most often of which were isolated fungi from the genera *Fusarium* 3.1%, *Stemphillium* 4.7%, *Sordaria* 7.8% and

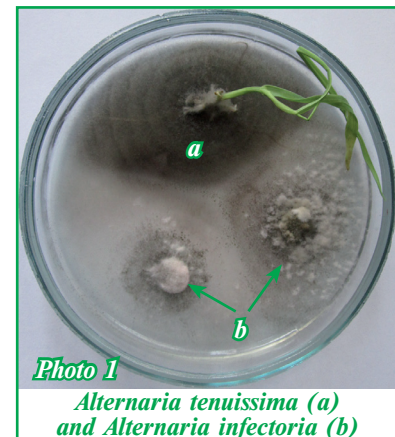
**Table 2. Laboratory germination of winter wheat seeds with signs of black point harvest 2018–2019**

№	Variety name	Germination energy, %		Similarity, %	
		2018	2019	2018	2019
1	Pushna	90	100	90	100
2	Kesaria Podilska	95	95	100	95
3	Vodogray of Bila Tserkva	90	100	100	100
4	Adel	95	100	95	100
5	Viglanca	100	95	95	95
6	Sefeg-2	100	100	100	100

species that did not form sporulation 6.3%. In 2019, fungi from the genera *Aspergillus* 4.6%, *Sordaria* 3.8%, *Epicoccum* 2.3%, *Stemphillium* 1.5%, *Acremonia* 0.8%, *Fusarium* 0.8%, *Bipolaris* 1.5% and bacterial infection 1.5%, the share of which was 17%.

Detected fungi have different etiologies, so the species *Alternaria*, *Fusarium*, *Bipolaris* can cause disease in plants and are pathogens of seeds, which often affect sowing qualities and cause the accumulation of mycotoxins. The species *Curvularia*, *Aspergillus*, *Acremonia*, *Stemphillium*, *Sordaria* and *Epicoccum* act as saprophytes and usually settle on dead tissues or plant remains.

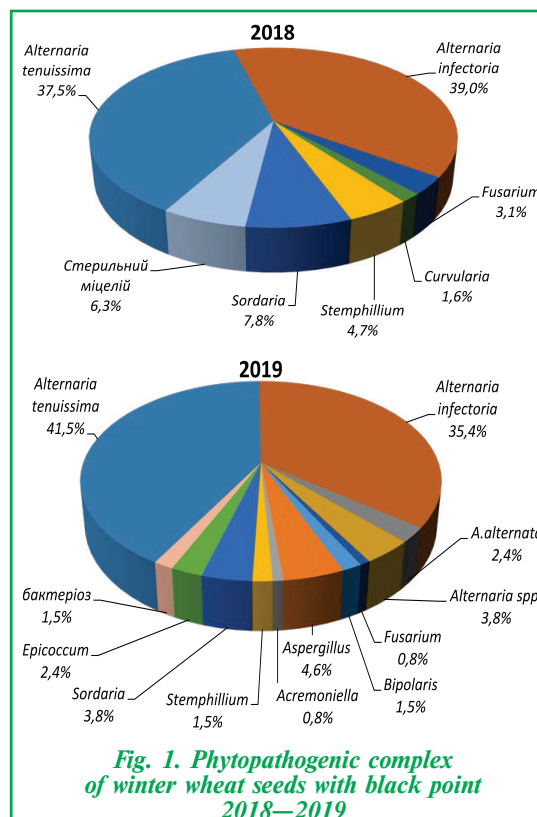
Fungi of the genus *Alternaria*, most often found on the seeds of major cereals. The seeds usually affect small-spore species *Alternaria tenuissima* and a complex of species *Alternaria infectoria* (Photo 1),



**Photo 1**  
*Alternaria tenuissima* (a)  
and *Alternaria infectoria* (b)

but there are other members of this genus — *A. alternata*, *A. avenicola*, *A. arborescens*, etc. Species of this genus are saprophytes and parasites with necrotrophic type of food. During the growing season, they are able to produce several generations. Colonies from light gray to dark olive color are formed on the nutrient agar. Conidia are multicellular, dark-colored inverted-club-shaped with transverse and longitudinal membranes, single or grouped in chains of acropetal type of different length. The main systematic features are the shape and structure of conidia and the habit of sporulation [11, 13].

Fungi of the genera *Stemphillium* and *Sordaria* were usually found in the complex (Photo 2). Most species of the genus *Stemphillium* are saprophytes, sometimes facultative parasites. Colonies of this fungus are dark, felt, mycelium submerged or partially superficial. Conidiophores solitary, in the form of lateral short or elongated branches, straight or tortuous, septate. Conidia are usually solitary, oval, round or ovoid, inverted mace-like or almost round olive-brown, with a smooth surface, sometimes warty or prickly, with transverse and longitudinal membranes,



**Fig. 1. Phytopathogenic complex of winter wheat seeds with black point 2018–2019**

with one or more constrictions, with a scar (Photo 3) [13, 14]

Fungi of the genus *Sordaria* are representatives of coprophilous fungi, usually do not have conidial sporulation. Form dark brown or black pear-shaped perithecia (Photo 4). Bags are cylindrical, contain 8 dark green or dark brown ellipsoidal ascospores [12].

The complex of *Aspergillus* species on the surface of the seed acts as a saprotroph, but in case of non-compliance with storage conditions or under unfavorable germination conditions can cause seed mold. Manifested in the form of powdery heads of different colors with a mass of spherical spores located on the filaments. Species of this genus are highly toxin-forming and in the process of their vital activity produce a number of poisonous substances — aflatoxins, ochratoxins, fumizinin, trichothecins, and others. Metabolites that form these fungi cause diseases of the internal organs of animals and humans by exhibiting neurotoxic, hepatotoxic and nephrotoxic effects [15].

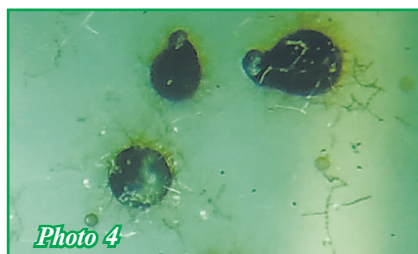
Often the main causative agent of the black point is the species *Bipolaris sorociniana*, which is a member of the genus *Bipolaris* (Photo 5). The penetration of the mycelium of this fungus under the shell of the embryo causes its darkening. The degree of darkening depends on the depth of the mycelium. Grain affected by this fungus is usually thin, underdeveloped has low germination rates. This pathogen affects more than 90 species of cereals. In addition to seeds, it can infect leaves in the form of dark brown spots and the basal part of the stem, causing the development of root rot [13, 15].

Colonies of the fungus of the genus *Curvularia* are dark (gray, brown or almost black), velvety, immersed in the substrate. Conidia solitary, irregularly shaped, often curved, mace-like, ellipsoidal, broadly spindle-shaped, have three or more membranes. They mostly cause black spotting in cereals, but can affect seeds [13].

*Epicoccum* colonies were rarely detected in samples. On the surface of the medium, they formed a sporology with dark-colored spherical unicellular warty conidia on short conidiophores (Photo 6). Usually pathogens of this genus are the causative agents of leaf spot on corn and

other plants can cause the destruction of wood [13].

Another species that is quite rare in our samples of wheat seeds was *Acremoniella atra*. This species affects a wide range of plants, causing root rot and leading to significant



crop losses. On the environment it is shown in the form of air fluffy mycelium, at first light, in due course brown. Forms single unicellular globular light brown conidia with double shell [13].

The amount of grain with a dark-colored germ is regulated by DSTU 3768: 2019. Depending on the seed class, its number should not exceed 8% in durum wheat of grades 1—3 and 30% in durum wheat, and in durum wheat seeds they should be absent altogether. Therefore, selection on this basis, especially in durum wheat is of great importance [10].

## CONCLUSIONS

The degree of damage to winter wheat seeds black point in the zone of the Right Bank Forest-Steppe of Ukraine in 2018—2019 was studied. The highest percentage of seeds with black point (6.9—15.3%) was found in varieties Vodogray Bila Tserkva (Ukraine, BC DSS) — 6.9%, Adele (Russia, Krasnodar Research Institute of agricultural), Balitus (Austria), Viglanka (Slovakia), Sefeg-2 (Azerbaijan).

The least susceptible to the disease are Daria (Croatia), Bodycek (France), Grace Bila Tserkva (Ukraine, Bila Tserkva DSS), Azano (Sweden), Gospodarka (Ukraine, IFRIG) and Kozyr (Ukraine, SGI), Morozko (Russia, Krasnodar) NDISG, the number of grains with signs of darkening in the embryonic zone did not exceed 1% in the years of research.

Phytopathogenic complex of winter wheat seeds with signs of darkening in the germ zone in the Right Bank Forest-Steppe of Ukraine in 2018—2019 included 13 species of fungi from 9 genera: *Alternaria*, *Fusarium*, *Curvularia*, *Bipolaris*, *Aspergillus*, *Acremoniella*, and *Stemphylidium*.

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#### Чорний зародок насіння пшениці озимої

**Мета.** Дослідити ураження насіння сортів пшениці озимої чорним зародком та встановити фітопатогенний склад збудників захворювання в умовах Київської області (Правобережний Лісостеп України). **Методи.** Лабораторні — макроскопічний аналіз виконували згідно з ДСТУ 4138-2002; склад фітопатогенів ідентифікували з використанням живильного середовища. Статистичний — аналіз одержаних даних, розрахунок ступеня ураження збудниками, НІР. **Результати.** Для виявлення ступеня ураження насіння чорним зародком досліджено колекцію з 27-ми сортів пшениці озимої м'якої. Найбільше ураженнями були сорти Водограй білоцерківський (Україна), Адель (Росія), Valitus (Австрія), Viglanka (Словаччина), Sefeg-2 (Азербайджан) — ураження понад 6% в середньому за роки досліджень. Найменш сприйнятливі до захворювання сорти Daria (Хорватія), Vodusek (Франція), Грація білоцерківська (Україна), Білоцерківська ДСС), Azapo (Швеція), Господарка (Україна), Козир (Україна), Морозко (Росія). Кількість зерен з ознаками потемніння в зоні зарodka у них не перевищувала 1%. Визначено посівні якості ураженого хворобою насіння. Встановлено видовий склад фітопатогенів, які спричинюють прояв захворювання. Ідентифіковано гриби з родів *Alternaria*, *Fusarium*, *Curvularia*, *Bipolaris*, *Aspergillus*, *Acremonia*, *Stemphillium*, *Sordaria* та *Epicoccum*. Найчастіше насіння колонізували гриби з роду *Alternaria* Nees. Їх частка в роки досліджень становила 76,6—83,1%. **Висновки.** Ураження насіння сортів пшениці озимої чорним зародком в умовах Київської області (Правобережний Лісостеп України) у 2018—2019 рр. варіювало від 0 до 19,8% залежно від сорту та року досліджень. Фітопатогенний комплекс включав 13 видів грибів з 9-ти родів. Частка грибів з роду *Alternaria* у роки досліджень становила понад 75%, найчастіше виявляли види *A. tenuissima* (Kunze) Wiltshire та

*A. infectoria* E.G. Simmons. Частка видів з інших родів була незначною і не перевищувала 4,6%.

**чорний зародок; пшениця озима; фітопатогенний комплекс; *Alternaria***

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#### Чорний зародок насіння пшениці озимої

**Цель.** Изучить пораженность семян различных сортов пшеницы озимой черным зародышем и установить фитопатогенный состав возбудителей заболевания в условиях Правобережной Лесостепи Украины. **Методы.** Лабораторные — макроскопический анализ выполняли согласно ДСТУ 4138-2002; состав фитопатогенов идентифицировали с использованием питательной среды. Статистический — анализ полученных данных, расчет пораженности семян возбудителями, расчет НСР. **Результаты.** Для выявления поражённости семян черным зародышем исследовали коллекцию из 27-ми сортов пшеницы мягкой озимой. Наибольший процент поражения обнаружен у сортов Водограй белоцерковский (Украина), Адель (Россия), Valitus (Австрия), Viglanka (Словакия), Sefeg-2 (Азербайджан). Наименее восприимчивыми к заболеванию отмечены сорта Daria (Хорватия), Vodusek (Франция), Грация белоцерковская (Украина, Белоцерковская ДСС), Azapo (Швеция), Хозяйка (Украина), Козыр (Украина), Морозко (Россия). Количество зерен с признаками потемнения в зоне зарodka у этих сортов не превышало 1%. Определены посевные качества пораженного болезнью семян. Установлен видовой состав фитопатогенов, вызывающих проявление заболевания. Фитопатогенный комплекс семян с черным зародышем включал грибы из родов *Alternaria*, *Fusarium*, *Curvularia*, *Bipolaris*, *Aspergillus*, *Acremonia*, *Stemphillium*, *Sordaria* и *Epicoccum*. Чаще всего семена колонизировали грибы из рода *Alternaria* Nees. Их часть составила 76,6—83,1%. **Выводы.** Поражение семян сортов пшеницы озимой черным зародышем в условиях Правобережной Лесостепи Украины в 2018—2019 гг. варьировало от 0 до 19,8% в зависимости от сорта и года исследований. Фитопатогенный комплекс включал 13 видов грибков с 9-ти родов: *Alternaria*, *Fusarium*, *Curvularia*, *Bipolaris*, *Aspergillus*, *Acremonia*, *Stemphillium*, *Sordaria*, *Epicoccum*. Часть грибков из рода *Alternaria* в годы исследований составляла более 75%, преобладали виды *A. tenuissima* и *A. infectoria*. Количество видов из других родов было незначительным и не превышало 4,6%.

**чорний зародок; пшениця озима; фітопатогенний комплекс; *Alternaria***

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