

INFLUENCE OF GENOTYPE

on representativeness of *Alternaria* sp. inside the seeds of winter wheat

Goal. To establish the influence of the genotype of winter wheat on the presence of *Alternaria* sp. in the seeds of winter wheat under different growing conditions, for different periods of time, among varieties from different original institutions of Ukrainian and foreign selection. **Methods.** Field — cultivation of wheat varieties in the Forest-Steppe of Ukraine; organizational — collection of seed samples; laboratory — analysis of the mycoflora of winter wheat seeds by biological method on the PGA, determination of *Alternaria* fungi on the environment of the PCA; analytical and mathematical — analysis of the obtained results and their statistical comparison. **Results.** The study of the influence of the variety on the isolation of *Alternaria* fungi for their cultivation in different years for the period from 2011 to 2020 and in different growing conditions proved the existence of a significant difference in their total amount and number of individual species, especially dominant. Differences were noted in the presence of *Alternaria* sp. inside the seeds grown in two climatic zones (Polissya and Forest-Steppe), on farms and in experimental fields. Three-year observation of the species of these fungi on two varieties from one originator showed their different isolation: both the presence of certain representatives and the number of their isolation. Analysis of 9 and 17 genotypes of wheat of Ukrainian and foreign selection during 2019 and 2020 also showed a different picture of the population of *Alternaria* fungi. **Conclusions.** Significant influence of genotype on the selection of *Alternaria* sp. was installed. Based on several years of studying the population of winter wheat seeds with *Alternaria* fungi, genotypes with a smaller presence were identified: in 2013–2015 — Gordovyta variety, in 2019–2020 — Emil, Pylypivka. It should be borne in mind that fewer alternative fungi reduce the presence of their mycotoxins, but increase the number of other fungi in the mycoflora, sometimes more dangerous.

T. ROZHKOVA

Sumy National Agrarian University,
160, H. Kondratieva str., Sumy,
40021, Ukraine,
e-mail: rozhkova8@gmail.com

winter wheat; variety; *Alternaria* sp.; percentage of isolation

Alternaria Nees 1816 — fungi that are an integral part of the wheat mycoflora in the world and in Ukraine. Mankind did not fully understand their role in the seed. But the predominance of these fungi among other seed endophytes in plants of *Triticum aestivum* L. has been proven [1]. They have a negative effect on wheat, causing alternariosis of spike, leaf spot and is one of the causes of black point of seeds. With the participation of plant pathogenesis, *Alternaria* fungi are able to produce phytotoxins. They are also known to have a negative effect on human and animal health — the production of mycotoxins. *Alternaria* sp. capable of producing more than 30 mycotoxins [2]. In connection with the revision of the taxonomy of the genus [3] and the production of these secondary metabolites at the beginning of the XXI century, researchers are interested in *Alternaria* sp. increased. Modern world research is related to the issues of taxonomy [4], species distribution and discovery of new [5], their biological activity [6], the presence of seeds of different crops in the mycoflora [7], mechanisms of plant pathogenicity [8], study of spectrum and the amount of mycotoxins [9], etc.

In Ukraine, most studies of *Alternaria* fungi are devoted to the study of them as components of the fungal complex of seeds of cereals, especially wheat. As factors influencing the infection of seeds of *Alternaria* sp. consider the variety [10, 11], vegetation conditions of a particular year

[12], place of cultivation [13], protective measures and other elements of wheat cultivation technology [12].

The study of genotype culture for the selection of *Alternaria* fungi in most cases does not take into account a multifaceted approach: a small number of varieties for analysis, short study period (1–2 years), analysis of varieties of one originator, conclusions based on examination of samples grown only in scientific institutions.

The aim of the research was to establish the influence of the genotype of winter wheat on the presence of *Alternaria* sp. in the seeds of winter wheat under different growing conditions, for different periods of time, among varieties from different institutions-originators of Ukrainian and foreign selections.

Materials and research methods. Samples of wheat seeds were obtained both from farms of Sumy region and grown in the conditions of ESPC SNAU (Educational and Scientific Production Complex of Sumy National Agrarian University). Seed mycoflora was determined by biological methods in the laboratory [14]. Before analysis, the seed was washed for one hour under running water. Then it was kept in a 1% solution of potassium permanganate for 1–2 minutes for surface disinfection. Then the seeds were dried on filter paper and decomposed into Petri dishes on PGA (potato-glucose agar). The dishes were placed in a thermostat, where colonies and seeds germinated at a temperature of 22–24°C for 7 days. Each sample contained 200 pcs. seeds. Species of *Alternaria* were determined on a special medium (potato-carrot agar) by sporulation habit and morphology of conidia and conidiophores, features of colony formation [15, 16].

Results and discussion. The amount of *Alternaria* fungi inside the seeds of winter wheat depending on the variety was studied. Long-term observation was carried out on samples

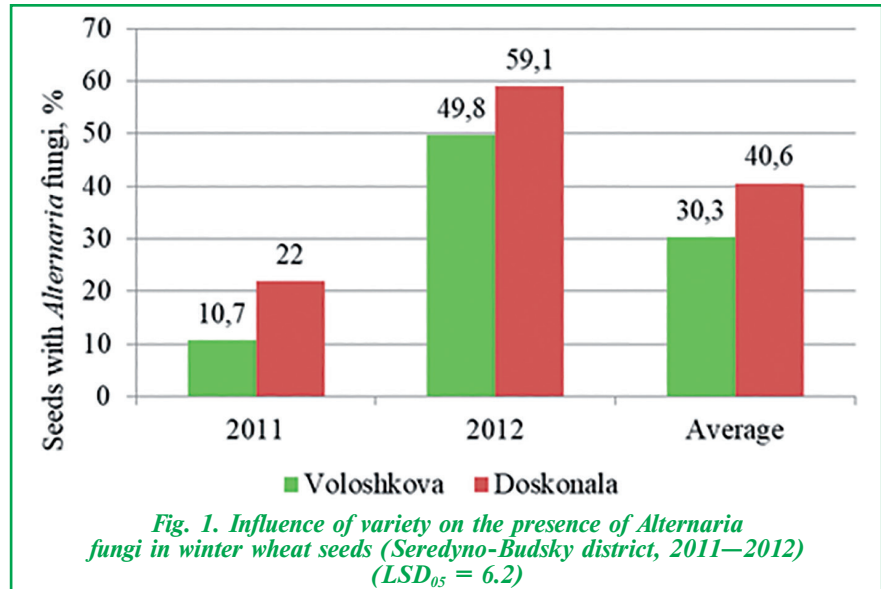
grown in different climatic zones, administrative districts, farms of various forms of ownership. The total number of fungi and the number of individual species were compared.

The study of the amount of seeds with *Alternaria* fungi of two varieties of wheat in the Polissya area for two years showed a significant difference in this indicator (Fig. 1).

The varieties differed in the content of these fungi in the seeds during two years of research: in 2011 the Voloshkova variety had almost twice as many seeds with *Alternaria* fungi as the Doskonala variety. In 2012, the difference between the varieties was 10.7%.

Subsequent comparisons of the number of *Alternaria* fungi were made in the Forest-Steppe zone. Analyzed 4 Ukrainian varieties: Voloshkova selection Myronivka Institute of Wheat named after V.M. Remeslo of NAASU (MIW), Favorytka and Zolotokolosa — MIW and the Institute of Plant Physiology and Genetics of NASU (IPPG); Rozkishna — the Plant Production Institute named after V.Ya. Yuriev NAASU (PPI). The study of grain from the farm of Krasnopil district of Sumy region for two years showed the selection of two species of the genus *Alternaria*: *A. tenuissima* and *A. alternata* (Table 1).

In 2012 and 2013, there was a significant difference in the selection of both individual species of *Alternaria* fungi and their combined amount from different varieties of winter wheat. Although in general, all varieties have a significant amount of these fungi. In 2012, the largest number of *Alternaria* fungi was isolated on



the Voloshkova variety. In 2013, they also exceeded the number of other varieties, but only 1.6% of the variety Rozkishna. The average amount of *Alternaria* sp. was higher in the first year of the study (64.8%), in the second year it was — 58.9%.

The study of the origin of the varieties made it possible to understand that the MIW selection varieties have a similar genotype: Favoritka and Zolotokolosa (contain similar genoplasms): the first variety is Krymka, Ukrainka 0246, Myronivska 808 and wheat-rye translocations (1BL / 1RS), the second — Ukrainka 0246, Krymka and wheat-rye translocations (1AL / 1RS) [17]. Close to them is the variety Rozkishna (Odeska 162 / Kolosysta) [18], Odeska 162 is a direct descendant of the direct line Myronivska 808, and Kolosysta — indirect through the Russian

wheat Donskaya semi-dwarf and Severodonskaya [17]. The Voloshkova variety was created by selecting winter forms from spring wheat of the Flambard variety, sown for the winter [19]. Perhaps this fact explains the difference in the population of *Alternaria* fungi seeds of this variety.

During 2013–2015, the dependence of the isolation of *Alternaria* fungi on the genotype of the culture during cultivation in the experimental field of SNAU was observed (Table 2).

It was found that the variety significantly affected the number of seeds from which the colonies of *Alternaria* sp. The smallest amount of *Alternaria* fungi was noted on the Hordovyta variety, which was obtained by crossing Krymchanka / Albatros odeskyy. The latter is a descendant of Ukrainka 0246 wheat through its ancestor Mayak variety and great-ancestor Dniprovskaya 521 [17]. Variety Poliska 90 (descendant of Bezostoy 1) differed from others by the largest amount of *Alternaria* sp. during the first two years. In 2015, the highest rate of isolation of *Alternaria* fungi was observed in the variety Rozkishna.

A three-year study of the content of fungi of the genus *Alternaria* was conducted on two varieties of Ukrainian selection from one originator — MIW: Voloshkova and Bohdana (Fig. 2). Voloshkova variety comes from Flambard, it was registered in 2008. [19] The Bohdana variety was created by mutagenesis from the Donetsk variety 48 [17], and it was registered in 2006.

Different presence of *Alternaria*

Table 1. The presence of *Alternaria* sp. inside the seeds of winter wheat of Ukrainian varieties («Zlak», 2012–2013)

Variety	The presence of <i>Alternaria</i> sp. inside the seeds, %		Whole seeds with <i>Alternaria</i> fungi, %
	<i>A. tenuissima</i>	<i>A. alternata</i>	
2012			
Voloshkova	29.7	46.7	76.4
Favorytka	55.2	2.6	57.8
Rozkishna	56.5	4.7	61.2
Zolotokolosa	63.6	—*	63.6
LSD ₀₅	2.7	1.7	3.4
2013			
Voloshkova	64.8	—	64.8
Favorytka	50.7	1.2	51.9
Rozkishna	62.3	0.9	63.2
Zolotokolosa	50.1	5.7	55.8
LSD ₀₅	3.1	0.8	2.2

Note: —* — colonies of this species did not germinate from the seeds of winter wheat

sp. was observed on two genotypes for three years. In 2017, the maximum amount of species was identified: on the Voloshkova variety — three, on Bohdana — four. *A. alternata* dominated the Voloshkova variety (46.2%), and *A. arborescens* (28.9%) dominated Bohdana. In 2018 and 2019, the prevalence of one of these species was noted, but the presence of different varieties of others. In 2018, another species of *A. infectoria* (12.8%) was isolated on Voloshkova, and *A. avenicola* (23.1%) on Bohdana. In 2020, two species were identified on the Voloshkova variety, and only one on the Bohdana variety with the maximum amount of its isolation in three years of research (62.7%).

In 2019, foreign (German) were involved in the study of the influence of varieties. The species composition of *Alternaria* fungi on different genotypes was studied (Table 3).

Among the seeds grown in 2019, three species of *Alternaria* were identified: *A. arborescens*, *A. avenicola* and *A. infectoria*. *A. arborescens* dominated on all studied varieties. There was a significant difference in its presence inside the seeds of different varieties. Of the five varieties, two species of *Alternaria* were isolated, and of the four, one. *A. avenicola* was isolated from four varieties with a small percentage of presence among other fungi. *A. infectoria* was identified only on the Svitanok Myronivsky variety. The maximum amount of *Alternaria* fungi was isolated from Ukrainian varieties: the smallest — from the variety Pylypivka, the largest — from the variety Podolyanka. A smaller number of this genus than the Ukrainian ones (except for Pylypivka) were determined from the German varieties. But a small amount of *Alternaria* fungi can lead to more undesirable consequences. Usually *Alternaria* sp., which are contained inside wheat seeds, in Ukraine do not have a negative impact on seed germination, unlike other fungi. But we observed all the internal microflora of the seeds and noted the germination of fungal colonies, which delayed the germination of wheat. Dominance of *Aureobasidium pullulans* (de Bary & Luwenthal) (54.3% of all fungal colonies) and a high percentage of *Nigrospora* sp. (20.4%) was observed in 2019 on the variety Pylypivka. The latter influenced the development of seedlings and their roots. On the

Table 2. Isolation of *Alternaria* sp. from seeds of winter wheat varieties of Ukrainian selection (SNAU, 2013–2015)

Variety	Originator, year of registration	Seeds with <i>Alternaria</i> sp.,%			Average,%
		2013	2014	2015	
Hordovyta	PPI, 2010	24	33	30	29
Sonechko	IPPG and MIW, 2009	47	52	55	51
Poliska 90	Institute of Agriculture NAASU (hereinafter referred to as IA), 1994	56	60	48	55
Rozkishna	PPI, 2009	51	55	60	55
LSD ₀₅		5.9	4.5	5.3	5.7

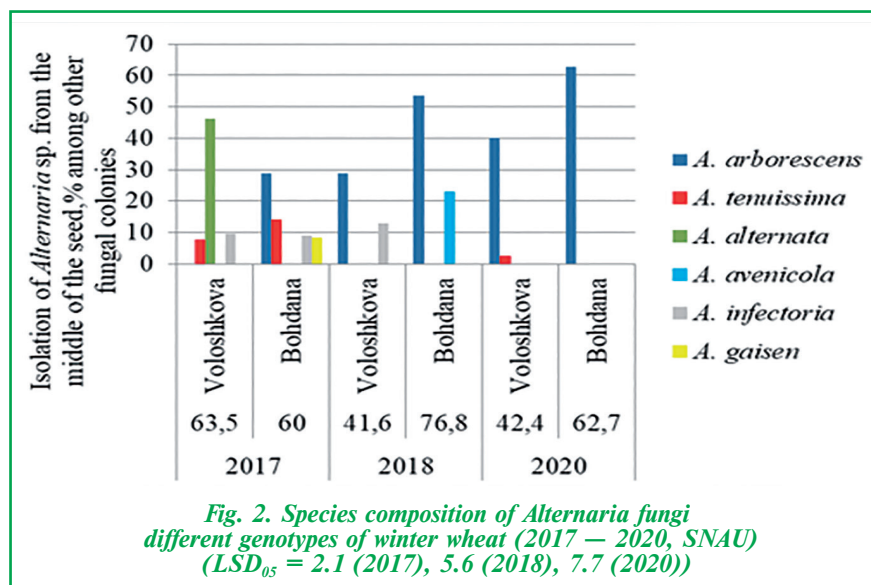


Fig. 2. Species composition of *Alternaria* fungi different genotypes of winter wheat (2017 – 2020, SNAU) (LSD₀₅ = 2.1 (2017), 5.6 (2018), 7.7 (2020))

German variety Emil was dominated by *Nigrospora oryzae* (Berkeley et Broome) Petch. (37.2%).

In 2020, we expanded the range of originators and countries to study the impact of different varieties on the isolation of *Alternaria* fungi (Table 4).

In 2020, four species of *Alternaria* fungi were identified from the studied varieties. One species dominated — *A. arborescens*, which isolated one of most varieties. Only the seeds of two

varieties (Pylypivka and Vyshivanka) contained 3 species of fungi each, and the Chinese line Zhongsi 1048 and the variety Voloshkova — 2. The smallest amount of *Alternaria* fungi was isolated from the variety Valencia, and the largest — from the varieties Vyshivanka and Ronin. The small amount of *Alternaria* sp. in 2020, it was compensated by a greater presence of *A. pullulans* (63.9%) in the seeds of the Valencia variety,

Table 3. Isolation of *Alternaria* sp. from winter wheat seeds of Ukrainian and German selections (SNAU, 2019)

Variety, year of registration	Originator	The number of <i>Alternaria</i> sp. from the middle of the seed,% among other fungal colonies			Total, %
		<i>A. arborescens</i>	<i>A. avenicola</i>	<i>A. infectoria</i>	
Bohdana, 2006	MIW	29.4	3.5	—	32.9
Svitanok Myronivsky, 2014		37.4	—	2.5	39.9
Podolyanka, 2003		40.3	—	—	40.3
Zdobna, 2016	PPI	35.5	0.9	—	36.4
Krayevyd, 2013	IA	32.8	0.8	—	33.6
Pylypivka, 2011	BGI–NCSSVR*	14.8	—	—	14.8
Lira Odeska, 2013		32.3	—	—	32.3
Ronin, 2017	KWS SAAT SE & Co. KGaA	22.6	2.8	—	25.4
Emil, 2017		20.7	—	—	20.7
LSD ₀₅		2	We did not count		1.1

Note: BGI–NCSSVR* — Breeding and Genetic Institute — National Center for Seed Science and Variety Research

and the Emil variety — by a significant presence of *Mucor mucedo* L. (19.2%) and *N. oryzae* (18.3%). The Valencia variety was obtained by a hybrid combination of Yermak / Demetra by treatment with NMU 0.005% [20]. Yermak variety took 4th place with the lowest isolation of *Alternaria* fungi.

Study of the presence of *Alternaria* sp. most often demonstrates different isolation of them from different varieties: in Pakistan on 12 varieties the range of their selection was 22–47.5 [21]; in central Ukraine, a study of 18 varieties of MIW showed fluctuations in *Alternaria* fungi depending on the variety in 2013 in the range of 12.5–65%, in 2014 — 20–64% [11]; in the Central Black Earth Region of Russia, out of 23 varieties, these fungi were isolated in the range of 6–68% [22]; the analysis of 15 varieties and lines of winter soft wheat of the competitive variety test of the selection of the Povolzhskiy RIBS of the 2018 harvest also showed different fungal population of wheat seeds (6–19%) [23].

CONCLUSIONS

The study of the influence of the variety on the isolation of *Alter-*

naria fungi for their cultivation in different years for the period from 2011 to 2020 and in different growing conditions showed a significant difference in their total amount and number of individual species, especially dominant.

Differences in the presence of *Alternaria* sp. inside seeds grown in two climatic zones (Polissya and Forest-Steppe), on farms and in experimental fields, were shown. During 2011–2012, a significant difference was found between 2 varieties from the Polissya zone in the amount of seeds that contained *Alternaria* fungi. The percentage of seeds with fungi of the genus *Alternaria*, grown in the Forest-Steppe of the 2012–2013 harvest, differed the most on the Voloshkova variety, which has the greatest pedigree difference. A three-year analysis (2013–2015) of the influence of varieties from different originators on the percentage of seeds with *Alternaria* fungi showed a significant difference in this indicator.

Further studies of the species composition depending on the genotype of the culture also showed significant differences in the number of colonies of both individual species and the total number of *Alter-*

naria fungi. Three-year observation (2017–2020) of the species of these fungi on two varieties from one originator showed their different isolation: both the presence of certain representatives and the number of their selection. A total of 6 species of *Alternaria* fungi were identified: *A. tenuissima*, *A. alternata*, *A. infectoria*, *A. gaisen*, *A. arborescens* and *A. avenicola*. The percentage of the latter was determined not only by the genotype of the culture, but also by the growing season. The most common was *A. arborescens*.

Expansion of originators and involvement in the study of varieties of foreign selection in 2019 and 2020 also showed a different picture of the presence of *Alternaria* fungi (especially the dominant species — *A. arborescens*) from seeds of different varieties.

Based on several years of studying the population of winter wheat seeds *Alternaria* sp. identified genotypes with a smaller presence of these fungi: for 2013–2015 — Hordovita variety, for 2019–2020 — Emil, Pylypivka. But it is necessary to take into account the fact that a smaller number of *Alternaria* fungi reduces the presence of their mycotoxins and leads to an increase in the number of other fungi in the mycoflora, sometimes more dangerous.

Table 4. Isolation of *Alternaria* sp. from winter wheat seeds of varieties and lines of Ukrainian and foreign selection (SNAU, 2020)

Variety, line	Originator, country	Isolation of <i>Alternaria</i> sp. from the middle of the seed, %				Total, %
		<i>A. arborescens</i>	<i>A. avenicola</i>	<i>A. alternata</i>	<i>A. tenuissima</i>	
Bohdana	MIW, Ukraine	62.7	—*	—	—	62.7
Voloshkova		40	—	—	2.4	42.4
Svitanok Myronivsky		33.3	—	—	—	33.3
Podolyanka		56.4	—	—	—	56.4
Vyshyvanka		48.2	16.3	5.7	—	70.2
Valensiya		8.2	—	—	—	8.2
Pylypivka	BGI-NCSSVR, Ukraine	28.9	2.3	1.6	—	32.8
Lira Odeska		55.8	—	—	—	55.8
Antonivka		50	—	—	—	50
Luhastar	LLC «Seeds of Luhansk region», Ukraine	56.4	—	—	—	56.4
Zhongsi 1258 (D 226)	Academy of Agriculture Sciences, Dingxi, China	24.2	—	—	—	24.2
Zhongsi 1048 (D 227)		44.9	6.1	—	—	51
Yermak	RRIC*, Russia	27.4	—	—	—	27.4
Krasota	KRIA**, Russia	46.2	—	—	—	46.2
Lupus	Saatbau Linz, Austria	49.1	—	—	—	49.1
Ronin	KWS SAAT SE & Co. KGaA, Germany	71.1	—	—	—	71.1
Emil		22.5	—	—	—	22.5
LSD ₀₅		1.2	He We did not count			1

Note: RRIC* — All-Russian Research Institute of Cereals named after I.G. Kalinenko, KRIA** — Krasnodar Research Institute of Agriculture named after P.P. Lukyanenko

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Рожкова Т.О.

Сумський національний аграрний університет, вул. Г. Кондратьєва, 160, м. Суми, 40021, Україна
e-mail: rozhkova8@gmail.com

Вплив генотипу на репрезентативність *Alternaria* sp. усередині насіння пшениці озимої

Мета. Встановити вплив генотипу пшениці озимої на наявність *Alternaria* sp. у насінні пшениці озимої за різних умов вирощування культури, за різний проміжок часу, серед сортів від різних установ-оригінацій української та зарубіжної селекції. **Методи.** Польові — вирощування сортів пшениці в умовах Лісостепу України; організаційні — збір зразків насіння; лабораторні — аналіз мікофлори насіння пшениці озимої біологічним методом на КМА, визначення альтернарієвих грибів на середовищі КМА; аналітичні та математичні — аналіз одержаних результатів та їх статистичне порівняння. **Результати.** Вивчення впливу сорту на виділення альтернарієвих грибів за їх вирощування у різні роки за період 2011—2020 рр. та в різних умовах довело наявність істотної різниці в їх загальній кількості та чисельності окремих видів, особливо домінуючих. Виявлено відмінності присутності *Alternaria* sp. усередині насіння, вирощеного у двох природно-кліматичних зонах (Полісся та Лісостеп), у фермерських господарствах та на дослідних полях. Трирічне спостереження за видами цих грибів на двох сортах від одного оригінатора продемонструвало їхнє різне виділення: як присутності певних представників, так і кількості їх виділення. Аналіз 9-ти генотипів пшениці української та 17-ти іноземної селекції впродовж 2019 та 2020 років продемонструвало різну картину заселення альтернарієвими грибами. **Висновки.** Встановлено істотний вплив генотипу на виділення *Alternaria* sp. На основі кількох років вивчення заселеності

насіння пшениці озимої альтернарієвими грибами визначили генотипи з меншою їх присутністю: за 2013—2015 рр. — сорт Гордовита, за 2019—2020 рр. — Еміл, Пилітівка. Необхідно врахувати той факт, що менша кількість альтернарієвих грибів зменшує наявність їх мікотоксинів, але призводить до збільшення кількості інших грибів у мікофлорі, інколи більш небезпечних.

пшениця озима; сорт; *Alternaria* sp.; відсоток виділення

Рожкова Т.А.

Сумской национальной аграрный университет, ул. Г. Кондратьева, 160, г. Сумы, 40021, Украина
e-mail: rozhkova8@gmail.com

Влияние генотипа на репрезентативность *Alternaria* sp. внутри семян пшеницы озимой

Цель. Установить влияние генотипа пшеницы озимой на наличие *Alternaria* sp. в семенах пшеницы озимой при разных условиях выращивания культуры, в различный промежуток времени, среди сортов от разных учреждений-оригинаторов украинской и зарубежной селекций. **Методы.** Полевые — выращивание сортов пшеницы в условиях Лесостепи Украины; организационные — сбор образцов семян; лабораторные — анализ микрофлоры семян пшеницы озимой биологическим методом на КМА, определение альтернариевых грибов на среде КМА; аналитические и математические — анализ полученных результатов и их статистическое сравнение. **Результаты.** Изучено влияние сорта на выделение альтернариевых грибов при их выращивании в разные годы за период с 2011 до 2020 г. В различных условиях выращивания доказано наличие существенной разницы в их общем количестве и численности отдельных видов, особенно доминирующих. Было отмечено различие в присутствии *Alternaria* sp. внутри семян, выращенных в двух природно-климатических зонах (Полесье и Лесостеп), в фермерских хозяйствах и на опытных полях. Трёхлетнее наблюдение за видами этих грибов на двух сортах от одного оригинатора продемонстрировало их разное выделение: как в присутствии определенных представителей, так и количестве их выделение. Анализ 9-ти и 17-ти генотипов пшеницы украинской и иностранной селекций в течение 2019 и 2020 годов продемонстрировал также различную картину заселения альтернариевыми грибами. **Выводы.** Установлено существенное влияние генотипа на выделение *Alternaria* sp. По данным нескольких лет изучения заселенности семян пшеницы озимой альтернариевыми грибами определили генотипы с меньшим их присутствием: в период 2013—2015 гг. — сорт Гордовита, 2019—2020 гг. — Эмил, Пыльтивка. Необходимо учесть тот факт, что меньшее количество альтернариевых грибов уменьшает наличие их микотоксинов, но приводит к увеличению количества других грибов в микрофлоре, иногда более опасных.

пшениця озима; сорт; *Alternaria* sp.; процент виділення

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