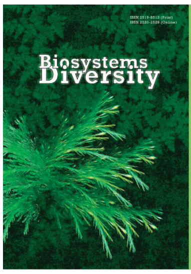

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Distribution of species of Fusarium and Alternaria genera on cereals in Ukraine

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In recent years phytosanitary situation in agrophytocenooses has worsened, and damage caused by diseases increased. In the conditions of field experiments in the vegetative seasons of 2015–2018 the dissemination of *Fusarium* and *Alternaria* spp., and composition of *Fusarium* species in Ukraine were studied. The selection of plant samples was carried out twice – at the beginning of vegetation and before entering into wintering. In autumn the samples of winter wheat with the symptoms of root rot from more than 150 fields from different oblasts of Ukraine were selected. Later these samples were analyzed using PCR method in real time to determine the species composition of *Fusarium* genus. In the analysis of grain material from different regions of Ukraine we determined high level of *Fusarium* infection. Fusariosis of grain were observed in several areas in more than 50% of samples from all regions of county. In all oblasts of Ukraine, the high level of *Alternaria* infection were identified also. It is found out that the main sources of the root rot are *F. tricinctum*, *F. graminearum*, *F. poae* and *F. avenaceum*. The role of *F. avenaceum*, as the pathogen of root rot was at the level of *F. graminearum* and *F. sporotrichioides* in most regions of Ukraine. The studies reveiled that the dominant species in the grain material were *F. tricinctum* and *F. graminearum*, presence of which exceeded 20–25%. Quite often *F. poae* and *F. sporotrichioides* was observed, presence of which in grain equaled 15–20%. In the Central, Eastern and Southern regions, the dominant species was *F. avenaceum* with a level of identification 15–23%. *F. culmorum*, *F. langsethiae* were within 5–7%. The species composition of fusariosis in the grain and the species composition of pathogens that cause fusariosis root rot in general are similar. Fusariosis becomes one of the most common and harmful diseases of grain crops, and cause no less harm than smut diseases. The fact that their danger continues to grow is explained by adverse weather conditions (alternation of wet and arid periods) and reduction of general level of cultivation: distribution of corn fields, decrease of expenses on tillage, and low-quality pesticides, often generics. It should be noted that the best precursors for cereal crops, for example, soy bean, is also vulnerable to fusariosis. Therefore, performing protective measures at preparing of grain for sowing and in the period of vegetation of the crop will contribute to total reduction of the levels of infection of agrophytocenoses with *Fusarium* species and potential damage to the cultivated plants. The study revealed that species of fungi belonging to the *Alternaria* and *Fusarium* genera infect wheat grain in the yields of each year. The results of data analysis of the genera showed antagonistic interaction between them. Perhaps, particularly at such negative interaction in the vegetative season of 2018, during, practically continuous warm rains in the "grain belt" of Ukraine during the generative period of development of wheat, the grain was infected with *Alternaria* spp. at moderate presence of *Fusarium* species. These patterns in the interaction of *Alternaria* and *Fusarium* spp. are expedient to be taken into account during development of systems of control of fusariosis using fungicides. Absence of contamination of grains with mycotoxins of *Fusarium* species does not reduce the levels of danger for crops posed by probable increase in the levels of presence of *Alternaria* mycotoxins – alternariol, alternariol monomethyl ether, tenuazonic acid, etc. Certainly, the results of the vegetation season of 2018 year and the large-scale alternariosis infication require clarifications to the current normative documentation. The conducted researches are important for improvement of protection systems for crops in intensive technologies of cultivation.

Keywords: Wheat; Diseases; fusariosis; fungicide; phytosanitary situation; wheat grain.

Introduction

Increasing the efficiency of crop production is closely linked to the introduction of modern cultivation technologies (Godfray et al., 2010; Morgun et al., 2010; Schwartau et al., 2016). However, the decline of the conditions for the cultivation of grain crops due to reduced crop rotation and, consequently, the increase in the severity of weeds, diseases and pests, leads to a complication of the phytosanitary situation. Losses from damage by harmful organisms can be very large. Many pathogens can independently destroy most of the crop's yield. The presence of several harmful objects almost always poses a threat of losing 30–60% of the future harvest. In addition, not only the quantity of crop reduces, but its quality is deteriorating considerably. In this case, the deterioration can be so significant that the grain becomes unsuitable for both food and fodder purposes. Grain crops can be affected by more than 350 species of various harmful organisms. But on most cultivated area, the main threat to crop, especially in the initial phases of the development, in the autumn-winter period, are pathogens of fungal infections. In recent years, the area of crops affected by fungal diseases is increasing.

Widespreadness, connection with the soil, lack of narrow specialization in the damage to host plants cause the constant presence of fungi of cereals, pathogens of numerous diseases, in agrocenoses. As a result of infection, the yield losses of cereals can reach 5.6% to 50%, and under epiphytotic conditions, yield loss in separate fields exceeds 70% (Retman et al., 2011).

In recent years, the proportion of grain affected by smut fungi (*Tilletia caries*, *Ustilago nuda*, *Ustilago tritici*) has increased. This was due to moisture and moderately warm weather during the flowering period of cereals. In some areas, more than 15% of plants were affected. However, these pathogens can be controlled with high efficacy using triazole derivatives as fungicidal preparations (Gagkaeva et al., 2012; Schwartau et al., 2016). Significant spread over the recent years became helminthosporioses. The source of infection is seeds and vegetative remains, and the spread is aerogenic and causes manifestations in the form of leaf spots, in particular: the net-like and dark brown spots which dominated in the Forest-steppe and the Steppe and partly in Polissya. In many regions, the symptoms of the disease were recorded at the beginning of the tillering season in conditions of warm humid weather at air humidity of 95–97% and temperatures of 15 °C and higher. These conditions were extremely favorable for the infection of plants. Special protective measures against helminthosporiosis are carried out for economic reasons in small areas. The disease progresses very quickly and reaches the maximum development in the phase of milky-waxy ripening, affecting the seeds of the new harvest. Treatment with fungicides after visual manifestations of the disease is of low efficiency.

Rains, long periods of humidation of plants, fogs in the period of ripening of the crops, prolonged harvesting period contribute to infecting of seeds of grain crops with saprophytic fungi: *Cladosporium* and *Alternaria* spp. On ears and aging leaves, olive-black, velvet, dense layer is formed in the form of scales. At first glance, stalks ears infected by smut disease, seemingly covered with teliospores of smut fungi, therefore, in practice, the disease is mistakenly considered "common smut". The causative agent of that is an imperfect fungus *Cladosporium herbarum*. The pathogen mycelium is placed near the surface of the tissue of the plant, on which olive-black conidiophores with conidia develop in the form of bundles. Symptoms of the infectioning with *Alternaria* are similar to those described above. The causative agent of this disease is an imperfect fungus *Alternaria temus*. Infectioning of scales of ears does not cause significant damage to plants. However, the infected grain, although is well ripened according to external parameters, is physiologically underdeveloped, has a reduced energy of germination and sprouting. Plants which grow from infected seeds have slow growth and development. *Alternaria* spp. infection of cereals at epiphytotic level was observed in Ukraine in 2002 (Gannibal, 2014), as well as in 2018.

In recent years, fungi of the *Fusarium*, *Bipolaris*, and *Drechslera* genera are very common on barley seeds, one of the leading crops among spring and winter cereals by the area of planting and yield. On the grain of barley, oats and spring wheat, the fusariosis complex is represented by *F. culmorum*, *F. oxysporum*, *F. avenaceum*, *F. graminearum*, *F. heterosporum*. However, are most commonthe three first species (Schwartau et al., 2016; Drehval et al., 2018; Hrytsev et al., 2018).

Along with fusariosis of ear, helminthosporium and smut diseases, especially dangerous for crops are root rot diseases. They become one of the most widespread and harmful diseases of grain crops, being no less harmful than smut infections and other dangerous diseases. The fact that their harmfulness continues to grow is due to unfavorable weather conditions (alternation of wet and dry periods) and a decrease in the overall level of cultivation.

Losses from fungal diseases of wheat are quite high. Thus, the decline in yield from the fusariosis of the ear can reach up to 0.2 t/ha, and losses from septoriosis – up to 0.6 t/ha. The classic approach – the use of fungicides – is the least effective compared with the economic feasibility of using other groups of pesticides and agrochemicals – herbicides, insecticides and fertilizers. This may be related both to the rapid emergence of resistant strains of pathogens, and the limited control of organisms with a complex life cycle, with both sexual and asexual forms, using fungicides synthesized 20–40 years ago. Currently, the Ukrainian market offers a fairly wide range of different domestic and foreign fungicides different in mechanisms of action. However, among the long list of them, only a few can be considered highly effective and selective drugs against the diseases described above.

The level of contamination of agrophytocenoses by inoculum of *Fusarium* pathogens is global in nature. Relatively low levels of disease control by existing preparations of protection (agrotechnical and chemical) direct the efforts of geneticists and breeders to create *Fusarium*-resistant varieties and hybrids of cultivated plant. However, as the results of industrial experiments in all soil-climatic regions of Ukraine indicate, only innovative genetic and biotechnological achievements and the introduction of varieties / hybrids of cereals resistant to fusariosis does not provide the proper level of disease control and the possibility of obtainning high-quality grain. Therefore, in developing effective control measures of infestioning of grain crops with fusariosis, attention should be paid to all components of the system of cultivating cultivated plants, pointed by a number of authors (Retman et al., 2010, 2011; Gagkaeva et al., 2011). Separate preparations of controlling fusariosis have insufficient efficacy and are unlikely to provide an adequate level of disease control. Therefore, the achievement of high and high-quality cereal crops is possible through the complex application of various strategies against the disease: the breeding of resistant varieties / hybrids, agrotechnical methods, first of all, the return of the horticulture of the country to a biologically based crop rotation and the introduction of the required fertilizer doses, and the use of highly effective fungicides that provide a high level of yield preservation, reducing the risk of accumulation of mycotoxins, ensuring high quality grains and economic expediency of grain production.

Fusariosis of ears of cereals and maize are highly harmful diseases that annually reduce the grain yield in Ukraine and cause contamination of the harvest with mycotoxins harmful to humans and animals. In Ukraine, fusarium root rot caused by pathogens of the *Fusarium* genus is also harmful. Considering significant economic losses from these diseases and the health risks of the warm-blooded, in most countries the level of mycotoxins in the grain is regulated at a rather low level, ppb and lower.

As in classical studies (Bilay, 1955, 1977), and in recent studies (Retman et al., 2008, 2011; Gagkaeva et al., 2011; Schwartau et al., 2016) it is noted that infecton with *Fusarium* species, three conditions are necessary: the presence of virulent pathogen, vulnerability of host plants to the pathogens and favorable environmental conditions. The variability of each of the factors affects the level of disease.

To determine the distribution of *Fusarium* and *Alternaria* spp. and effective control of them in 2015–2018, we conducted studies to determine the presence of the species in the regions of Ukraine.

Materials and methods

The research was carried out in field and laboratory conditions, fulfilling aseptic measures. In the autumn, samples of plant material of winter wheat with symptoms of root rot disease from over 150 fields from different oblasts of Ukraine were selected. The selection was carried out twice – at the beginning of the vegetation and before entering the winter. Samples from more than 150 fields from all oblasts of Ukraine were selected to determine the infection on the ear. Infection of the seeds was determined during sprouting in rolls of filter paper at a temperature of 23 ºC at a constant humidity of 70%. On day 10th, the percentage of plants attacked by different species of *Alternaria* and *Fusarium* was determined, the source of infection of which was seeds. Later microscopic analysis of pathogens was performed using an optical microscope.

Plants that had characteristic signs of fusariosis lesions were later analyzed using PCR analysis to determine the species composition of fungi of the *Fusarium* genus (Wiese, 1987; Wilson et al., 2004; Xu et al., 2005). The total fungal genomic DNA was extracted using the Agrosorb NK kit (LLC Agrogen Novo, Ukraine). The quantity and purity of extracted DNA were measured using a spectrophotometer Nano Drop 1000 (Thermo Fisher Scientific, USA). Purity DNA preparations have expected at A260/A280 ratio and DNA concentration is estimated by measuring the absorbance at 260 nm. Two readings were taken for each sample. Subsequently, sterilized deionized water was added to a final concentration of 10 ng per 1 μL.

To confirm the correct identification of the *F. graminearum*, *F. culmorum*, *F. sporotrichioides*, *F. langsethiae*, *F. poae*, *F. avenaceum*, *F. tricinctum* and *F. cerealis* we used a commercial test system (LLC Agro Diagnostika, Russia). Real-time PCR was performed using a Bio-Rad CFX96 Real-Time PCR Detection System (Bio-Rad Laboratories Ltd., USA). Bio-Rad CFX Manager 3.1 (Bio-Rad Laboratories, Inc.) was used to manage all real-time PCR analyses on the CFX96 Touch instrument. The molecular identification of the species composition of fungi of *Fusarium* genus was carried out using a set of reagents for PCR-amplification of DNA phytopathogens in real time using the PCR method according to the manufacturer's instructions (LLC AgroDiagnostika, Russia).

Sterile deionized water (ddH_2O) was used as a negative control to test for the presence of contamination in PCR reagents. was used to control the DNA isolation and determine the inhibition of PCR reaction (Fig. 1).

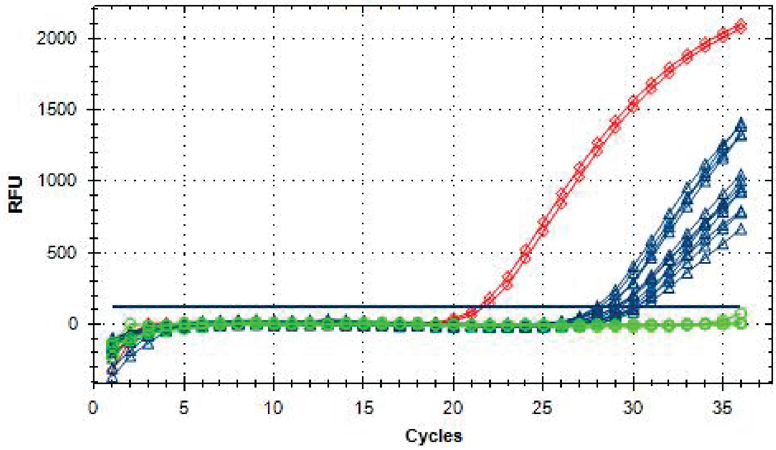

Fig. 1. The represent the amplification curves associated with a sample infected of *Fusarium graminearum* by PCR in real time: in the reaction samples of winter wheat No. 5–19 were used – marked with blue, red – positive control, green – negative control

Data from field assays were subjected to analysis of variance (ANOVA), means compared by Tukey's test ($P < 0.05$).

Results

In the analysis of grain material from different regions of Ukraine, a high level of defeat by fusariosis infection has been established. It is shown (Table 1) that grain fusariosis is identified in a number of areas in more than 50% of samples from all surveyed batches. Practically it is impossible to meet the level of infectious agents of the genus *Fusarium* below 20% in the "grain belt" of Ukraine. In all regions of Ukraine, a high level of damage was identified as an alternative. It should be noted that in the experiments the seed material was analyzed, in which in most cases a higher level of crop protection was implemented, including 2–3-fold treatment with fungicides, than when growing fodder grain.

In the analysis of grain material from different regions of Ukraine, a high level of fusariosis infection was determined. It is shown (Table 1) that grain fusariosis is identified in a number of oblasts in more than 50% of samples of all examined stores of grain. It is practically impossible to observe the level of infectious agents of the *Fusarium* genus below 20% in the "grain belt" of Ukraine. In all oblasts of Ukraine, a high level of alternariosis was determined. It should be noted that in the experiments, the seed material was analyzed, in which in most cases a higher level of crop protection was implemented, including 2–3-times processing with fungicides, than when growing fodder grain.

According to the results of an analysis of the infection of grains with fungi of *Fusarium* and *Alternaria* spp. genera in regions of the country, it was shown that when the contamination of grain with fusariosis increases, the laboratory sprouting decreases somewhat – almost to 90% of the values close to 100%.

Infection with *Alternaria* was significantly more frequent and did not affect the laboratory sprouting of the seeds ($r = 0.08$). By contrast, with the increase in levels of grain damage caused by fusariosis, the sprouting of winter wheat seeds significantly decreased ($r = –0.73$).

A high level of infection of grain of wheat by fusariosis in different regions of Ukraine was observed during 2015–2017 years (Table 2). The trend of high levels of infection persisted for several years of research, regardless of the variability of the weather conditions of the growing seasons. The level of infection in the years of observation was, on average, 20–30% in all regions of Ukraine. In 2016, when the conditions for the development of the fungus were more favorable in the West and North of Ukraine, it was found that the infection rate reached 50–60%.

Most species of the *Fusarium* spp. genus are oligophagous which can parasitize in several cultures, including soybeans and other legumes. However, these crops are among the best precursors for cereals. According to surveys of soybeans in almost all Oblasts, the infection rate of seeds reached 20% (Table 3). Therefore, although legumes are considered to be a good precursor to cereals, unlike maize and sunflower, they can simultaneously be an additional source of infection regarding *Fusarium* species.

Table 1
Infection of winter wheat with *Alternaria* and *Fusarium* spp. in the regions of Ukraine in 2015 (%, n = 150)

Oblasts of Ukraine	*Alternaria* spp.	*Fusarium* spp.	Seed germination
Chernihiv	33.1^{a}	4.9^{a}	97.5^{a}
Kherson	35.0^{b}	7.0^{b}	98.5^{a}
Vinnytsia	25.5^{c}	16.4^{c}	96.0^{a}
Donetsk	37.9^{d}	19.5^{d}	97.2^{a}
Lugansk	46.1^{e}	20.0^{d}	96.4^{a}
Nikolaev	33.9^{a}	22.7^{d}	95.2^{b}
Odesa	58.5^{f}	22.9^{d}	97.0^{a}
Dnipropetrovsk	44.8^{e}	24.5^{e}	97.0^{a}
Zaporizhia	57.4^{f}	24.8^{e}	97.6^{a}
Poltava	49.6^{g}	26.4^{e}	97.3^{a}
Kharkiv	50.1^{g}	26.4^{e}	96.5^{a}
Kyivska	26.2^{h}	27.3^{f}	97.0^{a}
Cherkassy	41.7^{e}	29.6^{f}	97.1^{a}
Khmelnitsky	53.9^{g}	37.8^{g}	97.7^{a}
Zhytomyr	58.2^{f}	38.3^{g}	97.3^{a}
Sumy	44.6^{e}	38.4^{g}	95.6^{b}
Ternopil	43.2^{e}	44.2^{i}	95.3^{b}
Lviv	45.8^{e}	53.4^{j}	91.3^{c}
Ivano-Frankivsk	40.8^{d}	54.3^{j}	90.0^{c}

Table 2
The infestation of winter wheat by *Fusarium* spp. in the regions of Ukraine (2015–2017, %, n = 150)

Regions of Ukraine	2015	2016	2017
Center	34^{a*}	27^{a}	24^{a}
North	28^{b}	64^{b}	33^{b}
South	22^{c}	31^{a}	22^{a}
East	23^{c}	36^{c}	32^{b}
West	33^{a}	53^{d}	33^{b}

Notes: * – see Table 1; Center – Vinnytsia, Kirovograd, Poltava, Cherkassy oblasts; North – Zhytomyr, Kyivska, Chernihiv, Sumy oblasts; South – Zaporizhia, Dnipropetrovsk, Kherson, Odesa, Nikolaev oblasts; East – Kharkiv, Donetsk, Lugansk oblasts; West – Lviv, Ternopil, Ivano-Frankivsk, Khmelnitsky, Rivne, Volyn oblasts.

Regarding the species composition of pathogens of the *Fusarium* genus, found on the seed material, it was found that the dominant species are *F. tricinctum* and *F. graminearum* (Table 5), the presence of which exceeded 20–25%. Although, if we take separate regions of Ukraine, then a number of differences should be noted. In the West, one can distinguish four main species. In addition to the two above-mentioned, *F. poae* and *F. sporotrichioides* were quite common, the presence of which in the grain was 15–20%. In the central, eastern and southern regions, the dominant species was *F. avenaceum* with occurence level of

15–23%. Other species, such as *F. culmorum*, *F. langsethiae* and others, were found to be much less common – within the range of 5–7%.

The species composition of pathogens found on grain material in 2018 was somewhat different. It has been established that the dominant species were *F. avenaceum*, *F. tricinctum*, *F. graminearum* and *F. poae* (Table 5), and their presence was most observed in the western and eastern regions. The main dominant species there were *F. poae*, *F. avenaceum*, and *F. graminearum*. In the north of Ukraine, there were two dominant species – *F. avenaceum* which was observed in large numbers (about 83%), and *F. graminearum*. In the southern oblasts, *F. avenaceum*, *F. tricinctum*, and *F. graminearum* were fairly frequent, the presence of which in the grain was determined at levels of 36%, 36% and 21% respectively. In the central regions, the dominant species was *F. avenaceum* with a level of occurrence equaling about 52%. Other species, such as *F. culmorum*, *F. langsethiae*, and *F. sporotrichioides* were found much rarer – within 0–10%.

Table 3
Infection rate of soybean seeds *Fusarium* spp.
in the regions of Ukraine in 2015 (%, n = 120)

Oblasts of Ukraine	Fusariosis infection rate,%
Odesa	8.7^{a*}
Kherson	16.9^{b}
Chernihiv	18.6^{c}
Sumy	18.7^{c}
Kharkiv	19.3^{c}
Zhytomyr	19.7^{c}
Rivne	19.9^{c}
Vinnytsia	20.0^{c}
Chernivtsi	20.7^{d}
Cherkassy	20.8^{d}
Ivano-Frankivsk	20.8^{d}
Poltava	21.3^{d}
Kyivska	22.6d
Khmelnitsk	24.3e

Note: * – see Table 1.

Table 4
Identifying different types of fungi of the *Fusarium* genus on the grain in the regions of Ukraine (%, average over 2015–2017, n = 150)

Regions of Ukraine	*F. graminearum*	*F. culmorum*	*F. sporotrichioides*	*F. langsethiae*	*F. poae*	*F. avenaceum*	*F. tricinctum*	*F. cerealis*
Center	23^{a}	8^{a}	8^{a}	0^{a}	13^{a}	23^{a}	26^{a}	0^{a}
North	20^{a}	4^{b}	12^{b}	4^{b}	8^{b}	20a	24^{a}	8^{b}
South	14^{b}	7^{a}	7^{a}	7^{c}	14^{a}	14^{b}	29^{a}	7^{b}
East	23^{a}	0^{c}	15^{b}	8^{c}	15^{a}	15^{b}	23^{a}	0^{a}
West	27^{a}	0^{c}	13^{b}	0^{a}	20^{c}	7^{c}	27^{a}	7^{b}

Note: see Table 2.

Table 5
The presence of *Fusarium* species
in grain of winter wheat in Ukraine in 2018 (%, n = 120)

Regions of Ukraine	*F. graminearum*	*F. culmorum*	*F. sporotrichioides*	*F. langsethiae*	*F. poae*	*F. avenaceum*	*F. tricinctum*
Center	21^{b}	2^{a}	2^{a}	2^{a}	8^{b}	52^{b}	13^{b}
North	17^{a}	–**	–	–	–	83^{c}	–
South	22^{b}	–	8^{b}	–	–	35^{d}	35^{c}
East	10^{c}	–	10^{b}	10^{b}	40^{c}	20^{e}	10^{d}
West	16^{a}	–	3^{a}	–	33^{d}	30^{f}	18^{a}
Ukraine	18^{a}	1^{a*}	3^{a}	2^{a}	17^{a}	43^{a}	16^{a}

Notes: * – see Table 2; ** – the species of the fungus are not identified.

In the case of root rot, their main sources were first of all, almost equally (15–25%), *F. tricinctum*, *F. graminearum*, *F. poae*, and *F. avenaceum*. Moreover, the role of the latter species, as a causative agent of root rot, was at the level of the same as of *F. graminearum* in most regions of Ukraine, as well as *F. sporotrichioides* (Table 6).

Table 6
Distribution of types of pathogens of *Fusarium* root rot
in different regions of Ukraine, average for 2015–2017 (%, n = 150)

Regions of Ukraine	*F. graminearum*	*F. culmorum*	*F. sporotrichioides*	*F. langsethiae*	*F. poae*	*F. avenaceum*	*F. tricinctum*
Center	24^{a*}	9^{a}	14^{a}	2^{a}	18^{a}	19^{a}	14^{a}
North	36^{b}	2^{b}	20^{b}	3^{a}	12^{b}	23^{a}	4^{b}
South	20^{a}	4^{b}	14^{a}	12^{b}	20^{a}	21^{a}	9^{b}
East	10^{c}	2^{b}	24^{b}	0^{a}	15^{a}	28^{b}	21^{c}
West	30^{b}	4^{b}	17^{a}	9^{b}	15^{a}	12^{c}	12^{a}

Note: * – see Table 2.

According to the alternariosis epiphytotia of 2018 in Ukraine, the sharp decrease in the presence of pathogens of root rot diseases – *F. culmorum*, *F. sporotrichioides*, and a slight decrease in the presence of *F. graminearum* was observed in the plantations of cereals (Table 7). At the same time, the levels of infection of plants with *F. avenaceum* increased. These changes in the *Fusarium* species composition could be explained by the absence of high levels of infection of plants season with *Fusarium* root rot during the growing in the 2018 season, as well as contamination of the grain with mycotoxins produced by the species of *Fusarium*.

Table 7
Distribution of types of pathogens of Fusarium root rot
in the regions of Ukraine in 2018 (%, n = 150)

Regions of Ukraine	*F. graminearum*	*F. culmorum*	*F. sporotrichioides*	*F. langsethiae*	*F. poae*	*F. avenaceum*	*F. tricinctum*
Center	18^{a*}	1^{a}	3^{a}	2^{a}	17^{a}	43^{a}	16^{a}
North	21^{a}	2^{a}	2^{a}	2^{a}	8^{b}	52^{b}	13^{a}
South	17^{a}	0	0	0	0	83^{c}	0
East	10^{b}	0	10^{b}	10^{b}	40^{c}	20^{d}	10^{b}
West	21^{a}	0	7^{b}	0	0	36^{a}	36^{c}

Note: * – see Table 2.

Discussion

Traditionally it is believed that the main object of control of the infection in seeds is loose smut disease. However, loose smut infections in recent years are rare enough due to high levels of inhibition of the development of *Tilletia caries*, *Ustilago nuda*, *Ustilago tritici* in modern grain cereal cultivation technologies. First of all, this is related to the fact that grain of cereals are recommended to be treated with fungicidal pesticides, practically all active substances of which are of systemic actions (mainly groups of triazoles). They are highly effective in controlling pathogens of loose smut diseases both on the surface and inside the grain. However, with regard to other sources of infection that can be transmitted with seeds, high levels of control of pathogens can not be achieved. *Fusarium* and *Alternaria* spp. refer to widespread colonizers of wheat philosphere. Certain species are highly pathogenic and produce numerous mycotoxins. Number of spores of *Fusarium* spp. and levels of their production were higher in regions with a more humid and cooler micro-climate under planting. *Alternaria* species, on the other hand, showed an opposite tendency towards genetic variability, and release of spores not correlated with any of the indicators of microclimatic conditions and was determined as more uniform in the fields (Schiro et al., 2018).

Alternaria alternata is one of the most studied fungi today due to the effects on plants and high allergenicity for humans. The species *Alternaria* coexist with the *Fusarium* species, especially on plant remains. *Alternaria alternata* and *Fusarium oxysporum* produce highly active volatile sesquiterpenes and under varying conditions of growth – high and low levels of nutrition, monoculture or coexistence, *F. oxysporum* has higher number of volatile organic compounds than *A. alternata* (Weikl, et al., 2016). These differences in the production of secondary volatile metabolites are likely to have caused active spread of alternoses of crops during growing the season 2018 at a sharp change in weather conditions favorable for the development of *Fusarium* species.

Previously, it has been shown that species of fungi belonging to the *Alternaria* and *Fusarium* genera infect wheat grains of practically every year yield. Distribution of *Alternaria* and *Fusarium* spp. significantly differed in the samples of grain of reduced quality compared to the samples of high-quality grain. *Alternaria* spp. was more often found in samples of high-quality grain, particularly due to the presence of *A. infectoria*, which is most often isolated and is the most common species of genus. *Fusarium* spp. was predominant in samples of low-quality grain. The most commonly identified species of *Fusarium* were *F. avenaceum*, *F. poae*, *F. culmorum* and *F. tricinctum*. Other important toxinogenic species of *Fusarium* were *F. graminearum*, and *F. equiseti*. The levels of infection with *F. graminearum*, and *F. culmorum* were determined to be significantly higher in samples of reduced-quality grain. The presence of these species showed a negative interaction between *F. graminearum* and *Alternaria* spp., as well as between *F. graminearum* and another *Fusarium* spp. (Kosiaka et al., 2004). It is likely that precisely because of such negative interaction during the vegetative season of 2018 in the conditions of practically continuous warm rains in the "grain belt" of Ukraine during the generative period of the development of wheat, the grain of the crops was infected with an alternariosis at moderate presence of *Fusarium* species. These dependencies in the interaction of *Alternaria* and *Fusarium* spp. should be taken into account when developing systems of control of fusariosis using fungicides. The absence of contamination of the grain with mycotoxins of the *Fusarium* species does not reduce the levels of danger of crops as a result of increase in the levels of presence of mycotoxins of *Alternaria* – alternariol, monomethylether of alternariol, tenouzonic acid, and the like.

Although the levels of accumulation of *Alternaria* mycotoxins, in contrast to mycotoxins of *Fusarium*, are generally low, occasional high levels of contamination with ennyatine and tenuazonic acids can occur. *In vitro* studies have revealed that the genotoxic effect of enniatines A, A_1, B_1, bovicin, moniliformine, alternariol, monomethyl ether of alternariol, altertoxins, stemphyltoxin III. In addition, in vitro studies showed the immunomodulatory activity of the newest mycotoxins and the danger to reproductive health of human posed by alternariol, and enniatin B. To update the risk assessment conducted by the European Food Safety Authority, the immunomodulatory effect of most new toxins and danger for human is noted; this requires additional data on the individual and combined effects of these pollutants on the reproductive and immune systems of both humans and animals. Given the new information on the proliferation of tenuazonic acid, its full avaiibility in peroral form of administration, low total clearance in pigs and broiler chickens, and limited data on toxicity, it is impossible to completely exclude the risk to human health. In addition, a number of less known *Alternaria* toxins, especially genotoxic alterotoxins and stemphyloxin III (Fraeyman et al., 2017) should also be included in the risk assessment.

Of course, the results of the growing season in 2018 and large-scale damage to grain caused *Alternaria* require further research and, possibly, refinements in the state regulatory documentation (DSTU, State Standards of Ukraine, 3768-2010 Wheat, Specifications).

High levels of infection of crops with fusariosis indicate that the system of protection against pathogens of the disease is currently imperfect and differs in terms of the effectiveness of protection of winter wheat, in particular from the abovementioned fungal diseases. Fusariosis control system requires more efficient solutions and new approaches, and this is a significant potential for increasing the productivity of the crop, since the affected grain has lower crop quality (Table 1) and can not fully disclose its genetic potential during planting.

According to DSTU 3768-2010, percentage of grains affected by fusariosis should not exceed 0.3–0.5%, depending on the grain class. When conducting research, we found the level of infection at the level of 20–30%, and even 50–60%. Of course, it is necessary to distinguish between infected grain and affected grain. When acffected, the fungus penetrates directly into the grain itself, in its aleuronium layer, destroying the proteins with the release of NH_3 and toxins. Grain affected by *Fusarium* spp. often looks hollow and loses sowing qualities of seeds, and bread made from this grain was called "drunk bread". When making fodder from such grain, mycotoxins can be dangerous also for domestic animals.

At infecting the grain, the fungus is on the shell of the grain or in the pericarp. In this case, we do not observe such severe consequences of poisoning, however, with high probability we can expect a high damage to seedlings with root and pre-root rot diseases. It should be noted that in testing for laboratory sprouting the infected grain did not differ from the noninfected and met the standards for sowing material. According to the data obtained, we see that even when infecting *Fusarium* agents at 40–60%, the laboratory sprouting of the seeds is generally satisfactory. However, at intering the soil, the young seedlings can already be affected by root rot. Separate mentioning needs to made about the appearance of *F. langsethiae* as a relatively new species on wheat, since it was most often identified earlier on barley and oat. This species is similar in the biology and morphology to *F. poae*, but differs by a high production of dangerous mycotoxins HT-2 and T-2 (Grabowski et al., 2012; Cuperlovic-Culf et al., 2016; Figueroa et al., 2017). Its appearance on wheat and, in general, in sufficient quantities is a dangerous tendency towards increase in the levels of contamination of the grain with mycotoxins. It should be noted, despite the fact that in the *F. langsethiae* is not common on maize, as a source of infection with root rot, it belongs to the main group of harmful species.

Summarizing the above, one can conclude that fusariosis is one of the most important problems among the complex of diseases in the grain production of Ukraine. Almost every third-fourth grains obtained in Ukraine are affected by fungi of the *Fusarium* genus, which poses a potential threat of infection with root rot, which results in lowering the density of sowing, yield, tolerance to cold over wintering. Another no less dangerous aspect of the *Fusarium* pathogens is affecting quality of grain. In addition to weight loss and, as a consequence, yield, infected grains are dangerous for use as food due to contamination with mycotoxins. Therefore, the control of these pathogens should be given at least as much attention as controlling pathogens of wheat loose smut diseases.

According to the level of distribution of this group of fungi and taking into account practically complete seed treatment and annual increase of fungicidal treatments, there is a question of using more effective preparations that have high levels of efficiency and selectivity in controlling this infection. It is important to consider the fact that the source of fusariosis as a disease are different species of *Fusarium* spp. with different biology, ability to infection in different conditions, and which differentiate different mycotoxins (Schwartau et al., 2016; Sanin et al., 2019).

Conclusions

Thus, the species composition of the fusariosis pathogens in the grain and the species composition of the pathogens causing *Fusarium* root rot are similar in general features. Fusariosis in Ukraine become one of the most widespread and harmful diseases of grain crops, being no less harmful than wheat smut diseases. The fact that their danger continues to grow due to unfavorable weather conditions (alternation of wet and dry periods) and a decrease in the overall level of cultivation: the spread of corn fields, lower costs on soil cultivation and low quality of generic pesticides. It should be noted that the best precursors for grain crops, in particular soybeans, are also affected by fusariosis. Therefore, taking to account protective measures, both in preparation for the sowing of grain and during the growing season of the crop, will contribute to the overall reduction of the levels of infection with agrophytocenoses by *Fusarium* species and the potential damage to cultivated plants and cereals.

It was determined that species of fungi belonging to the *Alternaria* and *Fusarium* genera infect wheat grain actually in yeald each year. The results of the analysis of the presence of these genera showed antagonistic interaction between them. It is likely that under such negative interaction in the growing season of 2018, in conditions of almost continuous warm rains in the "grain belt" of Ukraine during generative period of the development of wheat, grain was infected by alternariosis at moderate presence of *Fusarium* species. These dependencies in the interaction of *Alternaria* and *Fusarium* spp. should be taken into account over the development of fusariosis control systems using fungicides. Absence of contamination of grain with mycotoxins of *Fusarium* species does not reduce the level of danger for cereals by possible increase of the levels of presence of mycotoxins produced by *Alternaria* spp. – alternariol, monomethylether of alternariol ether, tenuazonic acid, etc. Of course, the results of the 2018 growing season and the massive infection of grain with alternarisosis require making adjustments to state regulatory documents.

Global experience shows that only biologically-grounded protection of cereals, which includes the introduction of disease-resistant varieties, the protection of crops from sowing to harvest, namely the control of diseases, pests and weeds, as well as the introduction of nutrition systems that increase resistance of plants to pathogens of diseases, makes it possible to produce crops higher than 10 t/ha. For another approach, one can rely on the "traditional" 30 quintal/ha, and this is under favorable conditions.

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