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INVESTIGATION OF SPECIES COMPOSITION OF THE FUNGI OF THE *FUSARIUM* GENUS AND THE RESISTANCE OF THE CHORNOBYL RADIO-MUTANTS TO FUSARIUM HEAD BLIGHT FOR THE PURPOSES OF WINTER WHEAT BREEDING IN THE FOREST-STEPPE OF UKRAINE

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Aim. To investigate the field resistance of collection spelt-like samples of the Chornobyl radio-mutants (RM) of winter wheat to Fusarium head blight and to determine the species composition of the fungi of the *Fusarium* genus in the Forest-Steppe of Ukraine. **Methods.** The resistance (in scores) of ten RM-samples of winter wheat to Fusarium head blight was determined in field conditions by visual inspection of plants using standard methods (Trybel et al, 2010). To determine the infection rate of the seeds, the biological method of seed germination in the rolls of filtration paper was used. DNA was extracted using the reaction kit AGROSORB NK (Agrogen Novo, Ukraine), according to the manufacturer’s instructions. The molecular identification of the species composition of the fungi of the *Fusarium* genus was conducted with the commercial test systems “Fuzarioz zlakiv” (AgroDiagnostica, RU) according to the manufacturer’s instructions, using the real-time PCR. **Results.** The results of evaluating the field resistance of ten spelt-like RM-samples of winter wheat to Fusarium head blight are presented. The species composition of the Fusarium fungi as of 2016–2018 in the Forest-Steppe of Ukraine, namely in Kyiv, Cherkasy, Sumy, Vinnytsia, Chernivtsi, Poltava, Kharkiv, Ternopil, and Khmelnytsky regions is presented. To study the species composition of the Fusarium fungi, 639 seed samples of winter wheat were taken from the farms located in the territory of nine regions in the Forest-Steppe zone. The results of molecular identification of the infection rate in the seed material demonstrate the presence of seven out of eight fungi species under investigation: *F. avenaceum, F. culmorum, F. graminearum, F. langsethiae, F. poae, F. sporotrichioides, F. tricinctum* during the years of studies. Yet, *F. culmorum*, *F. langsethiae* were not identified in 2017, and *F. cerealis* was not found during the study years, in 2016–2018. It should be noted that the most frequent incidence was found for *F. graminearum, F. avenaceum* and *F. tricinctum,* which was within the range of 25.8–44.0 %, and according to (Zhdanova, 2002) it refers to the group of frequently dispersed species. The results of the PCR diagnostics of the species composition of *Fusarium* fungi in Kyiv region, where field studies were conducted to evaluate the resistance of collection spelt-like Chornobyl radio-mutants in 2016–2018 (Bila Tserkva Breeding Research Station (BTBRS) of the Institute of Bioenergy Crops and Sugar Beet of the NAAS (Mala Vilshanka village, Bila Tserkva district, Kyiv region) demonstrated the spreading of six species of *Fusarium* fungi*: F. graminearum, F. avenaceum*, *F. sporotrichioides*, *F. poae, F. tricinctum*, *F. culmorum,* among which high aggressiveness to the wheat spikelets was observed in *F. avenaceum, F. culmorum, F. graminearum;* medium aggressiveness – in *F. poae,* and low aggressiveness – in *F. sporotrichioides*, *F. tricinctum.* On the background of the obtained species diversity of the disease agent, we evaluated the field resistance in ten spelt-like RM-samples of winter wheat to Fusarium head blight, whose resistance fluctuated within 7.4–8.4 scores in the study years. Under the climatic conditions most favorable for the disease development, in 2016 (from the time of spring vegetation restoration on March 01, to full ripeness of grain on July 09, there was 404.2 mm of precipitation which exceeded the perennial indices by 175 %) the samples under investigation: RM-1, RM-3, RM-4, RM-5, RM-6, RM-9, RM-10 exceeded the resistance of the standard variety, Lisova Pisnia, – 7.0 scores. **Conclusions.** The field and laboratory studies related to the species composition and geographic distribution of the *Fusarium* fungi give grounds for the assumption of the presence of an expansive species complex of Fusarium fungi in the territory of nine regions in the Forest-Steppe of Ukraine: *F. avenaceum, F. culmorum, F. graminearum, F. langsethiae, F. poae, F. sporotrichioides, F. tricinctum.* A close association between the biology of the group of *Fusarium* fungi and weather conditions of their environment was used by us to investigate the resistance of ten collection spelt-like samples of the Chornobyl radio-mutants in 2016–2018 and to isolate five RM-samples of winter wheat (RM-9, RM-5, RM-6, RM-10, RM-4), which had higher resistance to Fusarium head blight than the standard variety, Lisova Pisnia, on average for three years of studies. It should be noted that in 2016, under weather conditions favorable for pathogen development, RM-3 and RM-4 were found to be reliably more resistant compared to the standard. The five spelt-like RM-samples of winter wheat (RM-9, RM-5, RM-6, RM-10, RM-4) with high resistance indices on average for the years of studies (2016–2018) are introduced by us to the breeding programs as the initial material to obtain varieties, resistant to Fusarium head blight.

Key words: spelt-like RM-samples, wheat, resistance, *Fusarium*, PCR.

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INTRODUCTION

In recent years, the global community has frequently raised the issue of global safety of food products, which could be improved via the intensification and implementation of modern technologies in plant cultivation, the enhanced level of efficiency in using assortment of varieties and, as a result, the decrease of the negative impact on the environment (Miroshnychenko et al, 2014).

The phenotype of the variety is formed due to the interaction between the genotype and the environment. The imperfect agriculture and global climatic changes promote the increase in the harmfulness of weeds, diseases, and pests, which aggravates the phytosanitary state of agrophytocenoses (Mykhalska et al, 2019; Sanin et al, 2019). Among the biotic factors, decreasing the potential performance of the variety, the leading role is played by harmful organisms (Tsyliuryk, 2019), including fungal disease agents. Once spread, fungal diseases decrease the grain performance considerably along with the deterioration of food products, produced from the infected materials (Mykhalska et al, 2019; Foround et al, 2016).

The most harmful organisms on awned cereals are fungi of the *Fusarium* genus, whose manifestations may be diverse – wheat crown rot, root rots, head or ear blight, etc. (Gencheva et al, 2020). In recent years, the harmfulness of Fusarium head blight and the accumulation of inoculum of disease agents in the agrophytocenoses of Ukraine and the world has been increasing rapidly and becoming global. Fusarium head blight causes the infection of grain in the spike, which results in annual 20–30 % losses of harvest and in the epiphytotic years – up to 45–73 % (Sanin et al, 2019; Liew et al, 2018; Murashko et al, 2021).

The wheat spikes are infected with Fusarium head blight during the phases of blossoming-kernel ripening. The characteristic symptoms of spike infection are the occurrence of watery brown spots at the base of the spike glume and the early aging of some spikes. Under the conditions favorable for the development of the disease, a layer of mycelium and spores of the fungus appear on the spike glume; its color depends on *Fusarium* species. From the surface glume, the infection spreads along the tissues of the generative organs. Also, the macroconidia and chlamydospores may penetrate the spike along with the drops of rain and dew and promote the infection of Fusarium head blight (Scherm et al, 2013; Vughan et al, 2016). The specificity of Fusarium head blight is its particular etiology – the participation of several species of *Fusarium spp.* in the pathogenic process (Burkyn et al, 2015; Oryna et al, 2017).

The infection of wheat spikes and grain with fungi species of *Fusarium* genus has a negative effect on the energy of seed germination, seedling, thousand-grain weight, chemical and technological properties of grain, and the products of processing plant residues into the feeds for cattle (Foround et al, 2016; Gagkaeva et al, 2013; Scherm et al, 2013).

Also, the fungi of *Fusarium* genus are capable of producing mycotoxins – secondary metabolites of microscopic fungi with expressed toxic properties, which are considered to be among the most harmful substances for the health of humans and animals in recent half a century. The products of processing cereals, including wheat, are the main sources of Fusarium mycotoxins.

In addition to causing direct diseases, mycotoxins can promote delayed negative effects on living organisms: teratogenic, embryotoxic, carcinogenic, mutagenic. Some metabolites are the reason for food poisoning with different degrees of complications for humans and animals (Kaminska et al, 2020). Some of the most widespread and harmful mycotoxins of *Fusarium* fungi, which get accumulated in winter wheat grain, are deoxynivalenol, T-2 toxin, zearalenone, and aflatoxin. They are notable for their immunosuppressive effect and no sensibilization of the organism (Miedaner et al, 2021). Their specificity lies in the ability to have a negative impact in minimal doses (Mykhalska et al, 2019; Foround et al, 2016; Nesic, 2018). The contamination of agrophytocenoses with mycotoxins decreases the global crop production and animal products considerably (Liew et al, 2018; Alshannaq et al, 2017).

The breeding of genetically resistant material is the most effective and well-known method of crop protection in the world (Morgun et al, 2016; Mykhalska et al, 2019; Kovalyshyna et al, 2017). Therefore, it is rather urgent now to search for and investigate the sources of resistance for the purpose of creating new varieties of winter wheat.

The aim of our studies was to determine the species composition of *Fusarium* fungi in the territory of nine regions of the Forest-Steppe of Ukraine and to evaluate the field resistance of ten spelt-like collection samples of the Chornobyl radio-mutants (RM) of winter wheat (*Triticum aestivum* L.) to Fusarium head blight.

MATERIALS AND METHODS

The studies were conducted in 2016–2018. The species composition of *Fusarium* fungi in the grain was identified in the laboratory conditions of the Bila Tserkva Diagnostic Center of "Syngenta" LLC (26/74, Liudmyla Pavlichenko Str., Bila Tserkva, Ukraine). To determine the grain infection, 639 samples of seed material of soft winter wheat were obtained from the farms in nine regions (Kyiv, Cherkasy, Sumy, Vinnytsia, Chernivtsi, Poltava, Kharkiv, Ternopil, Khmelnytsky) in the Forest-Steppe of Ukraine. For further determination of the infection rate, the grain was germinated in wet filtration paper at 25 °C for seven days. The seeds with evident signs of Fusarium head blight infection were selected for further molecular identification of the species composition of *Fusarium* fungi using real-time PCR. For the purposes of molecular-biological studies, DNA was individually isolated from 639 samples of seed material of winter wheat.

The isolation of total DNA from the infected grain was conducted using the commercial test kit "AGRO-SORB NK" (Agrogen Novo, Ukraine), according to the manufacturer's instructions. The DNA concentration from the obtained samples was determined using the spectrophotometer "NanoDrop 1000" (Thermo Fisher Scientific, USA) by the optic density at $\lambda = 260$ nm, and the purity of the preparation was determined by the ratio of A260/A280 and A260/A230.

The identification of the species composition of *Fusarium* fungi in the investigated samples involved the use of commercial test systems for PCR-amplification of DNA of phytopathogens in real time, "Fuzarioz zlakiv" (AgroDiagnostica, RU) according to the manufacturer's instruction. PCR-amplification was conducted with the Real-time PCR cycler "CFX 96 TOUCH" (Bio-Rad Laboratories Ltd., USA). The results of PCR-RT were automatically registered and interpreted using the software Bio-Rad CFX Manager 3.1 (Bio-Rad Laboratories, Inc., USA).

In the conditions of the scientifically substantiated crop rotation of the Bila Tserkva Breeding Research Station (BTBRS) of the Institute of Bioenergy Crops and Sugar Beet of the NAAS (Mala Vilshanka village, Bila Tserkva district, Kyiv region), we studied the resistance of spelt-like samples of winter wheat from the Bila Tserkva collection of the Chornobyl radio-mutants (samples RM1-10) (Fig. 1–2).

The collection originates from the wheat plants, selected in 1987 under the guidance of D.M. Hrodzinsky, O.D. Kolomiets, P.K. Shkvarnikov, and M.F. Batyhin in the agricultural fields in the 20-km Chornobyl exclusion zone and for two vegetation periods – in the field, sown in 1986 and self-sown field of 1987, which were under chronic radiation on all the stages of organogenesis. As of the time of the Chornobyl catastrophe, the following varieties of soft winter wheat were cultivated in a radius of 20 km from the ruined reactor: Bilotserkivska 47, Myronivska 808, Poliska 70, Kyianka. In 1988, 239 samples in the form of kernels of individually thrashed spikes were transferred to the BTBRS for further studies. The mutated forms are re-sown by the pedigree method; the backcrosses, reciprocal, and analyzing crosses of the obtained mutants with initial varieties are conducted. As a result of non-stable mutagen-

Fig. 1. The spelt-like spike of soft winter wheat (RM-9)

Fig. 2. The heads of the spelt-like spike (RM-9) after threshing

esis, the spectrum of mutations has got extended from generation into generation for 34 years. At present, the collection covers about three thousand mutants and the obtained wide spectrum of mutations: with a different degree of spike sterility manifestation, squarehead, spelt with crack duramen of the spike and aggravated threshing, dwarfs with the plant height of 25 cm and high plants of over 150 cm, with twisted stem under the spike, and complicated shooting of the spike from the leaf sheath, with two spikes on the stem, and many other plant forms, ugly in their morphology, which are usually not present in nature. There were also systemic mutations with the features of other hexaploid species: *Triticum spelta*, *Triticum compactum*, *Triticum Vavilovi* (Amagai et al, 2017).

The investigated samples RM-1-10 were obtained as a result of chronic radiation in the field of 1986 and the self-sown field of 1987 in agrophytocenoses of the agricultural farms in the 20 km Chornobyl exclusion zone. After the specialists of BTBRS received morphologically different forms from the initial varieties (1988), every year they re-sow them for 26 generations (1988–2014) by the pedigree method. At the start of the breeding work, the corresponding codes depending on the origin were obtained (Table 1).

Table 1. The origin and morphological description of spelt-like RM-samples of winter wheat from the Bila Tserkva collection of the Chornobyl radio-mutants

Code	Investigated RM-sample	Morphological description
	Bilotserkivska 47	
756/89	RM-1	*T. spelta* L. awnless spelt
	RM-2	*T. spelta* L. awnless spelt
	RM-3	*T. spelta* L. awned spelt
	RM-4	*T. spelta* L. awned spelt
20006/89	RM-5	*T. spelta* L. awned spelt
	RM-6	*T. spelta* L. awned spelt
20038/89	RM-7	*T. spelta* L. awnless spelt
	RM-8	*T. spelta* L. awnless spelt
	RM-9	*T. spelta* L. awnless spelt
	Poliska 70	
20157/89	RM-10	awnless speltoid

Table 2. The evaluation scale for the resistance of wheat to Fusarium head blight

Resistance scores	Visual symptoms of spike disease
9–8	No damage, or insignificant yellowing of the spikelet, brown staining of glumes of some spikelets or kernels
7–6	Damage is scattered along the entire spike or local
5–4	Moderate damage in the form of brown staining of some spikelets, or 1/3–1/2 of the spike
3–2	About 2/3 of the entire spike is affected
1	The entire spike is affected

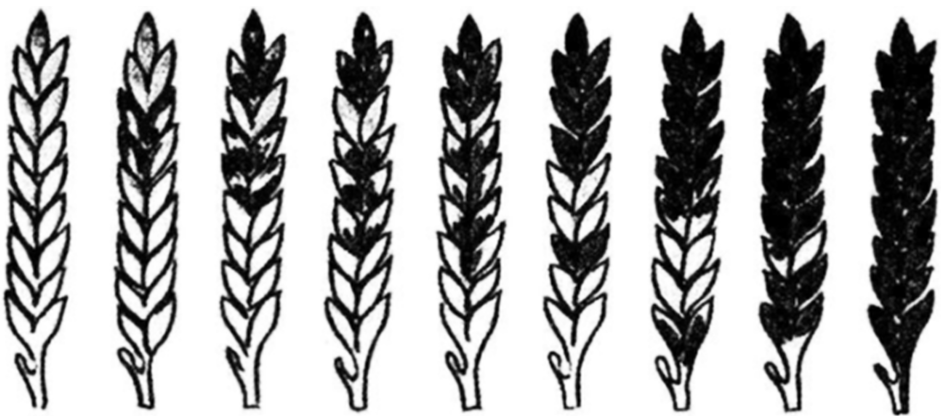

Fig. 3. The degree of damage intensity to wheat spikes by Fusarium head blight

After obtaining constant forms (2014), RM-1-10 samples were sown in the control nursery to study their biological, morphological, and economically valuable traits.

The Chornobyl radio-mutants (10 samples: RM1-10) were sown in the control nursery of the scientifically substantiated crop rotation of the BTBRS. The fungicidal treatment was not used during the crop vegeta-

Table 3. The precipitation and air temperature from the time of spring vegetation restoration until the complete ripeness of soft winter wheat grain in 2016–2018

Decade	Precipitation, mm				Air temperature, °C			
	2016	2017	2018	Perennial data	2016	2017	2018	Perennial data
March								
I	2.8	–	–	9.0	5.9	5.6	–	< 2.0
II	12.4	12.2	–	9.0	2.8	4.1	–	< 0.3
III	22.6	4.2	–	12.0	4.6	7.6	–	3.1
April								
I	3.6	40.0	1.5	14.0	12.3	11.6	10.3	7.0
II	52.9	15.3	1.3	17.0	13.8	4.5	13.8	7.8
III	4.5	2.8	5.3	16.0	10.9	11.8	15.7	10.4
May								
I	45.2	14.6	3.7	16.0	13.8	13.6	20.4	13.5
II	66.7	9.8	19.1	12.0	12.4	12.8	15.9	15.3
III	57.8	16.1	–	18.0	16.8	18.3	18.8	15.8
June								
I	22.7	10.2	2.2	23.0	15.3	18.8	19.4	17.3
II	50.1	14.3	23.3	27.0	19.4	18.8	21.9	17.4
III	32.2	17.9	33.2	23.0	23.5	21.6	19.1	18.7
July								
I	30.7	5.5	30.0	35.0	19.3	19.0	18.8	18.5
II	–	9.7	21.3	24.0	–	20.1	20.5	19.4
HTC								
–	–	–	–	–	2.33	0.86	0.66	1.27

tion; all the registrations were done with the natural infectious background. The area of the experimental plot for one sample is 10 sq.m., there were three repeats, and the number of plants of each sample, selected for the analysis (N) – 75 it., the ones selected in the period of 71–85 stages of development (BBCH Growth Stage Keys for cereals). The predecessor was a pea, the seeds were sown in the terms, optimal for the cereal, using the grain seeder C3-3.6. For comparison, the standard variety for the Forest-Steppe of Ukraine, Lisova Pisnia, was used, which is considered to be resistant to Fusarium head blight according to the data of the originator and the state variety trial. The resistance of plants to Fusarium head blight was evaluated in the period of maximal development of the disease using the 9-point-scale (Table 2) (Fig. 3) (Trybel et al, 2010).

The hydrothermal coefficient of Selyaninov was used for a comprehensive evaluation of the humidity conditions (Opalko et al, 2017).

In 2016–2018, the weather conditions were notable for considerable fluctuation of indices, which affected the growth and development of winter wheat and the investigated pathogen in particular. The conditions of 2016 were more favorable for the development of Fusarium head blight. There were 404.2 mm of precipitation from the time of spring vegetation restoration (March 01) to the complete ripeness of grain (July 09). The amount of precipitation in 2017 and 2018 was less for a similar period of 2016 by 241.3 and 276.1 mm respectively. In 2016, the hydrothermal coefficient up to the complete ripeness of grain was 2.33. In 2017 and 2018, the restoration of wheat vegetation occurred on March 06 and April 04, respectively. In 2017, the complete ripeness of grain was registered on July 12, and in 2018 – on July 14, according to the hydrothermal coefficient of Selyaninov, 0.86 and 0.66, respectively, from the time of air temperature transition every 10 °C until the completion of wheat ontogenesis (Table 3).

The incidence of the fungi of *Fusarium spp.* in the experimental samples was calculated by the formula:

$$C = A \times 100/B\ (\%),$$

where C – the incidence, %; A – the number of samples, where this species was found; B – the total number of the investigated samples (Beznosko et al, 2022).

If the registered frequency of microscopic fungi of one species was found to be over 50 %, this species was considered dominant, 30–50 % – rather widespread, 10 % and less – a rare species (Zhdanova, 2002).

RESULTS

To understand the species diversity of the disease agent, we conducted the monitoring of eight species of fungi of the *Fusarium* genus in the territory of nine regions (Kyiv, Cherkasy, Sumy, Vinnytsia, Chernivtsi, Poltava, Kharkiv, Ternopil, Khmelnytsky) in the Forest-Steppe of Ukraine.

The results of the molecular study demonstrate the presence of the following species of Fusarium fungi on the seed material of soft winter wheat, selected in the agrophytocenoses of the Forest-Steppe of Ukraine in 2016–2018*: F. avenaceum, F. culmorum, F. graminearum, F. langsethiae, F. poae, F. sporotrichioides, F. tricinctum.* The species of *F. cerealis* was not found during the studies, and in 2017 there were no *F. culmorum* and *F. langsethiae* (Table 4).

The molecular identification of the species of *Fusarium* fungi demonstrated that in the population of the fungi, on average for the years of studies, the most frequently observed fungus was *F. graminearum* which is especially dangerous due to its ability to form mycotoxins DON-deoxynivalenol and ZEA-zearalenone (Perczak A et al, 2019). *F. avenaceum* and *F. tricinctum* produce MON-moniliformin, which is lethal for mammals and birds. The average incidence of these agents during the years of studies was 25.3, 36.5, and 16.7, respectively.

It should be noted that in the territory of Kyiv region, where the resistance of spelt-like samples of the Chornobyl radio-mutants was evaluated, in addition to the abovementioned species of *F. graminearum* and *F. avenaceum,* in 2016, *F. sporotrichioides* (13.3 %) – a producer of highly toxic T-2 toxin and *F. poae* with the share of 6.7 %, mainly forming trichothecenes of B group, were identified. In the following years, the ratio of species changed considerably, and we found *F. tricinctum* with a frequency of 40.0 % in 2017 and 11.6 % (2018) and in 2018 *F. culmorum* (5.8 %) – a producer of trichothecenes, was found. In 2017–2018, *F. sporotrichioides* and *F. langsethiae* were not found.

It was determined that under conditions, most favorable for the development of Fusarium head blight in 2016, when HTC coefficient during the spring-summer vegetation of winter wheat in the studies was 1.97, and the sum of air temperature indices was 170.8 °C, seven investigated samples (RM-1, RM-3, RM-4, RM-5, RM-6, RM-9, RM-10) exceeded the standard variety in terms of resistance (7.0 scores) (Table 5). Yet, only RM-3 and RM-4 were reliably resistant. RM-7 sample

Table 4. The incidence of *Fusarium* fungi in the seed material of winter wheat in the territory of nine regions of the Forest-Steppe of Ukraine (the incidence, % from the total number of cases)

Years	Species							
	Fusarium avenaceum	*Fusarium cerealis*	*Fusarium culmorum*	*Fusarium graminearum*	*Fusarium langsethiae*	*Fusarium poae*	*Fusarium sporotrichioides*	*Fusarium tricinctum*
Kyiv								
2016	33.4	0	0	46.6	0	6.7	13.3	0
2017	25.0	0	0	25.0	0	10.0	0	40.0
2018	47.4	0	5.8	29.4	0	5.8	0	11.6
Cherkasy								
2016	0	0	0	100.0	0	0	0	0
2017	37.5	0	0	12.5	0	0	0	50.0
2018	27.3	0	0	9.0	0	36.4	27.3	0
Sumy								
2016	33.4	0	66.6	0	0	0	0	0
2017	27.3	0	0	18.2	0	9.1	9.1	36.3
2018	80.0	0	0	20.0	0	0	0	0
Vinnytsia								
2016	n/s*	n/s	n/s	n/s	n/s	n/s	n/s	n/s
2017	24.5	0	0	35.0	0	0	16.0	24.5
2018	75.0	0	0	12.5	0	0	0	12.5
Chernivtsi								
2016	n/s	n/s	n/s	n/s	n/s	n/s	n/s	n/s
2017	100	0	0	0	0	0	0	0
2018	50.0	0	0	0	0	0	0	50.0
Poltava								
2016	0	0	10.0	30.0	0	20.0	30.0	10.0
2017	50.0	0	0	0	0	0	0	50.0
2018	43.6	0	0	25.0	6.3	12.5	6.3	6.3
Kharkiv								
2016	25.0	0	0	41.6	0	16.7	19.7	0
2017	58.4	0	0	0	0	0	0	41.6
2018	50.0	0	0	0	0	0	50.0	0
Ternopil								
2016	5.0	0	5.0	20.0	10.0	20.0	20.0	20.0
2017	0	0	0	100	0	0	0	0
2018	33.4	0	0	33.4	0	0	0	33.2
Khmelnytsky								
2016	10.0	0	10.0	10.0	20.0	10.0	20.0	20.0
2017	50.0	0	0	50.0	0	0	0	0
2018	26.7	0	0	13.4	0	40.0	6.6	13.3
Average								
	36.5		3.9	25.3	1.4	7.5	8.7	16.7

Note. n/s* – not studied.

Table 5. The resistance of collection spelt-like RM samples of winter wheat to Fusarium head blight (by 9-point scale)

Name of selective sample	Morphological description	Resistance scores			Average score for years
		2016	2017	2018	
RM-1	*T. spelta* L. awnless spelt	7.3	8.0	9.0	8.1
RM-2	*T. spelta* L. awnless spelt	6.3	8.6	9.0	7.9
RM-3	*T. spelta* L. awned spelt	7.6	8.0	8.0	7.8
RM-4	*T. spelta* L. awned spelt	8.0	8.6	8.0	8.2
RM-5	*T. spelta* L. awned spelt	7.3	9.0	8.6	8.3
RM-6	*T. spelta* L. awned spelt	7.3	8.6	9.0	8.3
RM-7	*T. spelta* L. awnless spelt	6.0	8.6	8.0	7.5
RM-8	*T. spelta* L.	6.3	8.0	8.0	7.4
RM-9	*T. spelta* L. awnless spelt	7.3	9.0	9.0	8.4
RM-10	Awnless speltoid	7.3	8.6	9.0	8.3
Lisova Pisnia (St)	*T. aestivum* L. awned	7.0	8.6	8.6	8.1
Average		7.1	8.5	8.5	
$HIP_{0.05}$		0.55	0.84	0.62	0.67

had resistance at the level of 6.0 scores, which was the lowest index in 2016. In 2017–2018, in dry phases of the formation and ripening of kernel, Fusarium head blight was less evident. For instance, the resistance of Lisova Pisnia variety was 8.6 scores. In 2017, five samples were at the level of the standard, and two (RM-5, RM-9) exceeded it, having 9 scores. The highest resistance (9 scores) in 2018 had samples RM-1, RM-2, RM-6, RM-9, RM-10. In 2018, the least resistant samples (8 scores) were RM-3, RM-4, RM-7, RM-8 (Table 5).

DISCUSSION

Ukraine is a part of the region of intense agricultural production in Europe. The cultivation of quality agroproducts depends on weather conditions during the period of plant vegetation. At present, global climatic changes on the planet and the effect of unfavorable environmental factors become more tangible and determinative for a country on the global agrarian market (Batsmanova et al, 2014).

Noteworthy are fluctuating weather conditions, observed in recent years, when each phase of crop vegetation is affected by non-favorable conditions – from drought in autumn to rapid temperature changes in winter, and, as a result, unstable snow cover, which results in the change of the phytopathological situation in the fields of winter cereal (Vozhehova et al, 2018).

Perennial studies determined that the damage to the spike and grain by Fusarium head blight occurs under meteorological conditions, favorable for the development of micromycetes, the sensitivity of a host plant, and the presence of a virulent pathogen (Battilani et al, 2016; Moretti et al, 2019). The intensity of Fusarium head blight depends directly on the amount of precipitation in the phases of blossoming–kernel ripening of wheat. Under an excessive amount of precipitation, the damage is serious, and under dry conditions, it is less significant (Kovalyshyna et al, 2017).

Environmental conditions have a considerable effect on the species composition of *Fusarium* fungi. Every particular species has its specific distribution and its niche in the agrocenosis (Xu et al, 2009; Gruber-Dorninger et al, 2017; Medina et al, 2015).

The rapid development of Fusarium head blight of cereals and contamination of food products with fusariotoxins is a problem which has become global now (Sanin et al, 2019; Liew et al, 2018). The wide distribution of *Fusarium* fungi, their flexibility, and scientifically grounded facts of major hazards, coming from mycotoxins for the health of humans and animals, trigger a considerable interest of the scientific community in Fusarium head blight. The scientists' efforts are directed at the study of the life cycle of *Fusarium* fungi, and the search for ways of effective control of their number in global agrophytocenoses (Gencheva et al, 2020).

A widespread distribution of some species and local distribution of others, constant outbreaks of epiphytoties in some regions and the insignificant isolated manifestation of diseases in others demonstrate different ecological needs of Fusarium fungi within the genus. Frequently, an average sample of grain contains 10–15 species of fungi of the *Fusarium genus.*

Yet, simultaneous domination of up to four species of fungi is possible in some territories. Some species are notable for narrow specialization and geographic limitations (Zhang X-X et al, 2015). The species diversity of the population of *Fusarium* fungi on the seed material of winter wheat in 2016–2018 in the territory of Kyiv, Cherkasy, Sumy, Vinnytsia, Chernivtsi, Poltava, Kharkiv, Ternopil, and Khmelnytsky regions is rather rich (Table 2). The presence of seven species (*F. avenaceum, F. culmorum, F. graminearum, F. langsethiae, F. poae, F. sporotrichioides, F. tricinctum.)* out of eight species under investigation may be explained by a large territory and environmental conditions. In Kyiv region, the Right-Bank Forest-Steppe of Ukraine, where field experiments were launched for the evaluation of the resistance of spelt-like samples of the Chornobyl radio-mutants, in 2016 under high moisture provision (Table 1) the presence of four species was found: *F. graminearum, F. avenaceum, F. sporotrichioides,* and *F. poae.* During the following years (2017–2018), in the vegetation restoration-complete ripeness period, the least amount of precipitation was noted (Table 1), which in 2017–2018 promoted the occurrence of *F. tricinctum* and in 2018 – *F. culmorum.* Yet, during these three years of studies, *F. graminearum, F. avenaceum, F. poae* were present in the agrophytocenoses of Kyiv region. It was noted that the group of cereals is affected by a large number of species of *Fusarium* genus, the most widespread being: *F. graminearum, F. culmorum, F. sporotrichioides, F. avenaceum. F. nivale, F. poae,* and *F. sambucinum,* among which Fusarium head blight is most frequently induced by *F. graminearum, F. culmorum* and *F. avenaceum.* It should be noted that a considerable amount of *F. graminearum* prevailed in warm climate, whereas the representatives of species *F. culmorum, F. avenaceum* were more notable for dry and cool conditions of development (Beukes et al, 2017; Harris et al, 2016; Shen et al, 2012; Mykhalska et al, 2019; Hrytsev et al, 2021). It was found that *F. poae* and *F. langsethiae* did not manifest visible symptoms of being affected, and the rest of the abovementioned agents had massive or possible manifestations (Table 6) (Kotowicz et al, 2015; Beukes et al, 2017).

Among the most widespread species of *Fusarium* fungi, high aggressiveness to the spikes of cereals is shown by *F. avenaceum, F. culmorum, F. graminearum;* medium aggressiveness – *F. poae, F. verticillioides,* and low aggressiveness – by all the rest of species (Kotowicz et al, 2015; Beukes et al, 2017).

Table 6. The main species of fungi of the *Fusarium* genus and their ability to manifest visible damage symptoms (according to the data of Kotowicz et al, 2015; Beukes et al, 2017)

Species	Presence of damage symptoms	
	on the spike	on the kernel
F. graminearum	++	++
F. culmorum	++	++
F. sporotrichioides	+	–
F. poa	–	–
F. langsethiae	–	–
F. avenaceum	++	+
F. tricinctum	+	–
F. verticillioides	++	+

Note. ++ – massive manifestation; + – possible manifestation; – no manifestation.

Fusarium head blight is one of the types of infecting wheat plants by the fungi of *Fusarium* genus; according to different data, this disease may result in a sharp decrease in grain harvest and quality (Malihipour et al, 2017). The character of the disease agent's infecting a plant allows for investigating field resistance depending on the species composition of the pathogen in the agrophytocenosis. The obtained results of the evaluation of field resistance of spelt-like RM samples (Table 5) confirm the presence of selective RM-samples with high resistance to Fusarium head blight. Under favorable climatic conditions of 2016 and the presence of *F. graminearum* and *F. avenaceum,* aggressive to the spike of cereals, we isolated samples RM-3 and RM-4, reliably more resistant compared to the standard. During this period, the resistance of the standard variety Lisova Pisnia was at the level of seven scores, which was under the indices of samples RM-1, RM-5, RM-6, RM-9, RM-10 by 0.3 scores. The high resistance of spelt-like RM samples, confirmed by the studies, will be used in the selection work of the BTBRS. The analysis of the scientific literature demonstrates that the use of genetic resistance is considered to be the most effective and ecologically safe measure of controlling the diseases of crops (Morgun et al, 2016; Mykhalska et al, 2019; Kovalyshyna et al, 2017).

CONCLUSIONS

The analysis of the literature data on the geographic distribution, species composition, and the character of damage done by the fungi of the *Fusarium* genus, confirms the global distribution and harmfulness, caused for cereals by the species complex of Fusarium fungi. Field and laboratory studies of species diversity in the territory of nine regions in the Forest-Steppe zone confirm the presence of the following species of *Fusarium* fungi in the agrobiocenoses: *F. avenaceum, F. culmorum, F. graminearum, F. langsethiae, F. poae, F. sporotrichioides, F. tricinctum.* The dependence of Fusarium fungi on the environmental conditions is evident in the results of the studies on the species ratio in conditions of the Right-Bank Forest-Steppe of Kyiv region. In 2016, high indices of HTC and the sum of temperatures promoted the distribution of *F. graminearum* – 46.6 % and in the following years, 2017–2018, in the same period, the amount of precipitation was lower by 241.3 and 276.1 mm respectively, which provoked the occurrence of more drought-stable *F. culmorum* in 2018 – 5.8 % and *F. avenaceum* in 2017 – 25 %, and in 2018 – 47.4 %. A close association between the biology of the group population of *Fusarium* fungi and weather conditions and specific character of the manifestation of visual traits of their damage was used by us to investigate the resistance of ten collection spelt-like samples of the Chornobyl radio-mutants to Fusarium head blight in 2016–2018 and to isolate five RM-samples of winter wheat (RM-9, RM-5, RM-6, RM-10, RM-4), which had higher resistance to Fusarium head blight than the standard variety, Lisova Pisnia, on average for three years of studies. The abovementioned RM-samples were introduced by us into the breeding programs of Bila Tserkva BRS to create the varieties, resistant to Fusarium head blight.

Our studies confirmed the need for permanent monitoring of the dynamics of distribution for *Fusarium* fungi and the search for the initial material, resistant to one of the most harmful diseases of cereals – Fusarium head blight.

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Дослідження видового складу грибів роду *Fusarium* і стійкості Чорнобильських радіомутантів до фузаріозу колосу для селекції пшениці озимої в Лісостепу України

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Мета. Дослідити польову стійкість колекційних спельтоподібних зразків Чорнобильських радіомутантів (RM) пшениці озимої до фузаріозу колосу та визначити видовий склад грибів роду Fusarium на території Лісостепу України. **Методи.** Стійкість (у балах) 10 RM-зразків пшениці озимої до фузаріозу колосу визначали в польових умовах методом візуальної оцінки рослин за загальноприйнятими методиками (Trybel et al, 2010). Для визначення зараженості насіння використовували біологічний метод пророщування насіння у рулонах фільтрувального паперу. Екстракцію ДНК проводили за використання набору реактивів «АГРОСОРБ НК» («Агроген Ново», Україна) згідно інструкції виробника. Молекулярну ідентифікацію видового складу грибів роду *Fusarium* проводили за допомогою комерційних тест-систем «Фузаріоз злаків» («АгроДіагностика», росія) згідно інструкції виробника, методом ПЛР (полімеразна ланцюгова реакція) в реальному часі. **Результати.** Представлені результати оцінки польової стійкості спельтоподібних 10 RM-зразків пшениці озимої до фузаріозу колосу. Видовий склад фузарієвих грибів у 2016–2018 рр. на території Лісостепу України, а саме Київській, Черкаській, Сумській, Вінницькій, Чернівецькій, Полтавській, Харківській, Тернопільській та Хмельницькій областях. Для дослідження видового складу грибів роду *Fusarium* було відібрано 639 зразків насіннєвого матеріалу пшениці озимої з господарств, розташованих на території 9-ти областей Лісостепової зони. Результати молекулярної ідентифікації ураженості насіннєвого матеріалу свідчать про наявність семи з восьми досліджуваних видів грибів: *F. avenaceum, F. culmorum, F. graminearum, F. langsethiae, F. poae, F. sporotrichioides, F. tricinctum* у роки досліджень. При цьому *F. culmorum*, *F. langsethiae* не ідентифіковано у 2017 р., а *F. cerealis* не виявлено в роки досліджень – 2016–2018 рр. Слід зазначити, що найчастіше зустрічалися *F. graminearum, F. avenaceum* та *F. tricinctum,* частота трапляння яких була в межах 25,8–44,0 %, що за Zhdanova (2002) відноситься до групи часто поширених видів. Результати ПЛР діагностики видового різноманіття грибів роду *Fusarium* на території Київської області, де були проведені польові дослідження оцінки стійкості колекційних спельтоподібних зразків чорнобильських радіомутантів у 2016–2018 рр. засвідчили поширення 6-ти видів грибів роду *Fusarium, F. graminearum, F. avenaceum*, *F. sporotrichioides*, *F. poae, F.* t*ricinctum*, *F. culmorum,* серед яких високу агресивність до колосу зернових мають – *F. avenaceum, F. culmorum, F. graminearum;* середню – *F. poae,* та низьку *F. sporotrichioides*, *F. tricinctum.* На фоні отриманого видового різноманіття збудника було проведено оцінку польової стійкості у 10-ти спельтоподібних RM-зразків пшениці озимої до фузаріозу колосу, стійкість яких, у середньому за роки досліджень коливалася в межах 7,4–8,4 бали. За найбільш сприятливих кліматичних умов для розвитку хвороби в 2016 р. (від часу відновлення весняної вегетації 01,03 до повної стиглості зерна 09,07 випало 404,2 мм опадів, що на 175 % перевищувало багаторічні показники) досліджувані зразки: RM-1, RM-3, RM-4, RM-5, RM-6, RM-9, RM-10 перевищили стійкість сорту-стандарту Лісова Пісня – 7,0 балів. **Висновки**. Проведені польові та лабораторні дослідження стосовно видового складу та географічного розповсюдження грибів роду *Fusarium* дають підставу стверджувати про наявність широкого видового комплексу фузарієвих грибів на території 9-ти областей Лісостепової зони України: *F. avenaceum, F. culmorum, F. graminearum, F. langsethiae, F. poae, F. sporotrichioides, F. tricinctum.* Тісний зв'язок біології групи грибів роду *Fusarium* з погодними умовами середовища існування дав змогу дослідити стійкість 10-ти колекційних спельтоподібних зразків чорнобильських радіомутантів у 2016–2018 рр. та виділити п'ять RM-зразків пшениці озимої (RM-9, RM-5, RM-6, RM-10, RM-4), що мали вищу стійкість до фузаріозу колосу за стандарт Лісова Пісня, в середньому за три роки досліджень. Слід зазначити, що в 2016 р. за сприятливих погодних умов для розвитку патогена, достовірно стійкішими в порівнянні зі стандартом були RM-3, RM-4. П'ять вищезазначених спельтоподібних RM-зразки пшениці озимої (RM-9, RM-5, RM-6, RM-10, RM-4), що мали високі показники стійкості в середньому за роки досліджень (2016–2018 рр.), залучені нами в селекційні програми, як вихідний матеріал для створення стійких до фузаріозу колосу сортів.

Ключові слова: спельтоподібні RM-зразки, пшениця, стійкість, *Fusarium,* ПЛР.

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