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Some Aspects of the Distribution of *Fusarium* on Cereals of Russia

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Since 1995 the pathogens of genus *Fusarium* causing Root Rot, Snow Mold, *Fusarium* Head Blight of grain crops have been monitored in the Volga-Vyatka, Central, Central Chernozem and North Caucasian regions of the Russian Federation and in there were identified 15 *Fusarium* spp.: *F. culmorum, F. heterosporum, F. sporotrichioides, F. oxysporum, F. nivale, F. graminearum, F. avenaceum, F. gibbosum, F. sambucinum, F. moniliforme, F. semitectum, F. poae, F. lateritium, F. solani, F. redolens.* The high uniformity of *Fusarium* spp. has been revealed for the regions due to the susceptibility of crops to the soil-inhabiting micromycetes. Consistent long-term monitoring of the dynamics and frequency of occurrence of *Fusarium* spp. in a wide range of climatic areas has made it possible to characterize the state of the species of fungi in the regions and also to study their intraspecific and interspecific variability in toxicity and pathogenicity depended on biotic and abiotic factors. The prevalence of *Fusarium* spp. in the mycobiota has been determined by their plasticity and viability in the soil, on the weed roots and in the stubble of many crops and high competitive ability expressed pathogenicity and toxicity.

Keywords: cereals, fungal diseases, *Fusarium* spp., pathogenicity, toxicity, intraspecific and interspecific variability.

The distribution of facultative phytopathogenic fungi of the genus *Fusarium* in cereals has created threat to increase infectious background for the soil and the grain. The agents causing Root Rot, Snow Mold and *Fusarium* Head Blight have belonged to parasitic low specialized fungi founded in all regions of grain cultivation (Bilai, 1977; Gagkaeva et al., 2011). Pathogenicity of the *Fusarium* fungi has been determined due to their ability to produce hydrolytic enzymes and toxins (Pomeranz et al., 1990; Parry et al., 1995). For example, *F. graminearum, F. tricinctum* isolates have generated zearalenone, *F. moniliforme* and *F proliferatum* – fumonisin, *F moniliforme, F. acuminatum, F. avenaceum, F. oxysporum* – moniliformin (Ivanov et al., 2013). The mechanism of toxic action of fungi has been the ability to inhibit protein synthesis of living beings. Mycotoxins have been characterized by exhibiting strong anabolic and estrogenic effects (Toropova et al., 2013). Identified different levels of *Fusarium* toxicity have depended on the host plant and the cultivation conditions (Shakhnazarova et al., 2004).

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The study of the environmental effect on mycocenosis above-ground and underground parts of spring wheat have shown the annual dominance of *Fusarium* spp. along with the genera *Bipolaris* sp., *Alternaria* sp., *Penicillium* spp., *Cladosporium* spp., *Aspergillus* spp. The genus *Fusarium* has been characterized by significant species diversity and confinement to certain types of soils, sensitivity to fungicides (Burgess and Griffin, 1967).

The main factors of pathogen transmission have been able to contain the infection in soil, stubble and seed infected by the fungus.

Fungi of the genus *Fusarium* are capable of long saprotrophitic development in the soil. So the mycelium of *F. culmorum* has remained viable long time and has developed in the soil colonizing plant residues for the period to infect the plant (Tuterev, 2005; Kirichenko and Toropova, 2007).

The intensity of development and the prevalence of *Fusarium* spp. have depended on weather of vegetation period, genotypic and field resistance of grain cultivars, agronomical and technological methods of cultivation. Seeds have been transmitted to 60% of plant diseases (Toropova et al., 2011). Contamination of spring wheat seeds of *Fusarium* spp. is 3–5% annually. Infected seeds have got low germination rate, their viability has reduced (Toropova et al., 2010; Toropova et al., 2012).

The use of technology while minimizing the mechanical impact on soil and short crop rotations has led to expressive dynamics of root rot in agriculture of Russia (Wagacha and Muthomi, 2007; Toropova et al., 2015). Thus there were noted the highly toxic and invasive species of *F. sporotrichioides* (Sherb.), *F. poae* (Peck) Wr., *F. avenaceum* (Fr.) Sacc., *F. equiseti* (Corda) Sacc., *F. oxysporum* (Schlecht) in the forest-steppe of Western Siberia (Grishanova, 2014). Species of *F. culmorum* has dominated in colder areas of North and Central Russia. The *Fusarium* Head Blight is most active in Krasnodar territory, Kaliningrad region, Northern Russia (Leningrad region) and partly the Central Chernozem region – Belgorod, Vladimir, Ryazan, Tula and other areas favorable for cultivation of cereals. Contamination of grain by *Fusarium* spp. has made up around 5% in the Central and North-Western districts. A sharp increase in the infection intensity of seeds (up to 7%) in southern regions of the country (Krasnodar territory, Stavropol territory and the Rostov region) has contributed to epidemics of *Fusarium* in 2014 (Popov, 2015).

The species composition of the genus *Fusarium* may change depending on weather and cultural practices in the crop cultivation. The disease progress on the seeds has been noticed in wet years due to the hydrolytic breakdown of the carbohydrates has produced a favorable nutrition environment and reproduction of fungi (Toropova et al., 2010). *Fusarium* Head Blight is the disease typical for warm humid climate areas. So *F. graminearum* has caused the most famous epidemics in the southern part of Russia. The *Fusarium* spp. has been ecologically flexible and they are common for all grain-producing regions of Russia including the regions with insufficient moistening during the vegetation (Gagkaeva et al., 2011).

The goal of the work was the collation of data on species composition and frequency of occurrence of fungi of the genus *Fusarium* as well as to evaluate pathogenic and phytotoxic properties of strains of *Fusarium* spp. for the selection of the state collection for keeping and use in breeding studies as infectious material.

Materials and Methods

The infected material was sampled from plants of spring and winter wheat, spring barley and winter rye collected in 1995–2014 on the territory of the Volga-Vyatka, Central, Central Chernozem and North Caucasian regions of the Russian Federation. Mycological analysis of diseased samples of seeds and roots was carried out on a nutrient medium of čapek and 2% potato-dextrose agar according to the standard methods in a 5–10 replications (Dudka, 1982).

Isolates of *Fusarium* spp. were specified according to the morphology of colonies and conidia (Bilai, 1988). Phytopathological analysis of seed was conducted by the method of roll-examination by the V. A. Chulkina (Chulkina et al., 2009).

Pathogenicity and toxicity of *Fusarium* spp. isolates were studied in 2014–2015 in the ARRIP department using the method of biosample on seeds (Parfenova and Alekseeva, 1995). Pathogenicity of spore suspensions was determined as following. Petri dishes with potato–glucose agar were sown with pieces of mycelium using microbiological loop. Each strain was grown in 5 Petri dishes. Petri dishes were incubated in thermostat at 25 °C for 2 weeks. After incubation 10 ml of sterile tap water were added to each Petri dish, mycelium was washed off the agar surface with a scalpel and transferred to 250 ml Erlenmeyer flasks with a pipette. The flasks were shaken for 5 min and the spore suspension was filtered from the rest of mycelium through 2 layers of cloth and the volume was brought to 50 ml. Amount of spores in 1 ml of suspension was calculated in Garyaev camera. Ten seeds were placed in sterile Petri dishes on sterile filter paper and 6 ml of spore suspension were added into each dish. Tap water was used in control variants. Petri dishes with seeds were incubated for 5 days at room temperature and natural illumination. Further degree of seed germination was determined and length of coleoptiles and roots was measured. As the object-tester to determine the pathogenicity and toxicity of the *Fusarium* strains were used seedlings of the wheat cultivar Mironovskaya 808 susceptible to Root Rot pathogens.

The seeds of the wheat-tester were germinated in suspension of conidia (10^6) and the filtrate of culture fluid to detect the pathogenicity and toxicity of the fungus strains. Strains of pathogens were differentiated into four groups by the signs: non-pathogenic/non-toxic (inhibition of plant growth at 0–30%), slightly pathogenic/slightly toxic (inhibition at 31–50%), moderately pathogenic/moderately toxic (inhibition at 51–70%), pathogenic/toxic (inhibition of more than 70%). Pathogenicity and toxicity of fungus isolates were evaluated by the degree of inhibition of seed germination, growth of coleoptiles and the roots. The data of seed germination and growth of seedlings in water was used as a check.

All experiments were performed in three replicates. Statistical processing of results was performed using a modified program developed in the environment of Windows 98-based, Microsoft Excel (Sorokin, 2009).

Results and Discussion

Since 1995 in All-Russian Research Institute of Phytopathology there the pathogens of genus *Fusarium* causing Root Rot, Snow Mold, *Fusarium* Head Blight of grain crops have been monitored in the Volga-Vyatka, Central, Central Chernozem and North

Caucasian regions of the Russian Federation. There were identified 15 *Fusarium* spp.: *F. culmorum, F. heterosporum (Gibberella gordonii), F. sporotrichioides, F. oxysporum, F. nivale (Monographella nivalis), F. graminearum (G. zeae), F. avenaceum (G. avenacea), F. gibbosum (G. intricans), F. sambucinum (G. pulicaris), F. moniliforme (G. moniliformis), F. semitectum (F. incarnatum), F. poae, F. lateritium (G. baccata), F. solani (Nectria haematococca), F. redolens.*

The frequency of occurrence of *Fusarium* spp. has varied depending on climate of cultivation areas of grain crops, and weather conditions of the year, the localization of the pathogen on plants, previous crop, etc. However in the areas of cereal cropping the composition of *Fusarium* fungi was represented by the same species among which in the mycorrhiza of barley, wheat and rye there were most often identified the following 10 species: *F. culmorum, F. oxysporum, F. heterosporum, F. sporotrichioides, F. nivale, F. avenaceum, F. sambucinum, F. gibbosum, F. poae, F. solani* (Table 1). The incidence of *F. culmorum, F. heterosporum, F. sporotrichioides, F. oxysporum* together has accounted for more than 50% of all identified isolates of the fungi. Species such as *F. lateritium, F. semitectum, F. poae, F. solani, F. redolens* were met single.

Table 1

The mean frequency of occurrence of *Fusarium* spp. isolates in the major areas of wheat cultivation for the period 1995–2014, %

Species	Regions of cereal cultivation			
	Volga-Vyatka	Central	Central Chernozem	North Caucasus
F. culmorum	22.7	26.6	21.8	15.6
F. heterosporum	4.5	6.4	11.9	15.0
F. sporotrichioides	14.3	18.3	20.9	11.9
F. oxysporum	26.6	19.8	5.1	7.7
F. avenaceum	5.8	2.7	3.4	5.8
F. moniliforme	2.6	2.2	5.1	6.1
F. graminearum	0	1.2	17.5	26.4
F. poae	0	0.6	4.3	3.7
F. solani	0	1.4	3.4	1.5
F. semitectum	0	0.7	2.5	3.7
F. sambucinum	11.7	6.3		1.5
F. gibbosum		3.6		0.9
F. nivale	11.7	8.2	3.8	
F. lateritium		0.3		
F. redolens		1.4		
Total number of isolates	154 (100%)	671 (100%)	234 (100%)	326 (100%)

The isolates of *F. graminearum* were found more often in the southern regions of wheat cultivation. The frequency of occurrence of *F. graminearum* isolates in Central region has amounted to 1.2% and in the Central Chernozem and North Caucasus to 17.5% and 26.4%, respectively. Also, on the wheat there were noted the positive dynamics from North to South for *F. heterosporum*: from 4.5% in the Volga-Vyatka to 15.0% in the North Caucasus.

The incidence of *F. nivale* isolates from the Volga-Vyatka and Central regions in 2012 on rye (21.7–32.4%) was higher than on barley and wheat (from 10.7 to 14.7%) possibly due to the rye biology and habit (Table 2). Usually the winter rye has been cultivated in the northern parts of the country characterized long periods of moisture in autumn and spring, frequent thaws in winter provoking the *F. nivale* progress and as a result the roots and lower nodes of the stems of the crop have damaged.

It is known that the *Fusarium* infection accumulates in the soil and crop residues. There in the soil the fungus infects the primary roots of germinating seeds. During the growing season the infection (spores and hyphae) is transmitted by layers of plant colo-

Table 2

The incidence of *F. nivale* isolates on the roots of wheat, barley and rye in the Volga-Vyatka and Central regions in 2012, %

Origin	Wheat	Rye	Barley
Volga-Vyatka region	14.7	21.7	8.1
Central region	11.4	32.4	10.7

Table 3

The incidence of *Fusarium* spp. – the causative agents of Root Rot and *Fusarium* Head Blight of barley in the Central region in 2012–2014, %

Species	2012		2013		2014		Mean	
	roots	ears	roots	ears	roots	ears	roots	ears
F. culmorum	21.4	10.3	22.0	0	22.9	0	22.0	3.9
F. nivale	10.7	0	19.5	0	17.2	0	15.1	0
F. sambucinum	14.3	0	7.3	0	0	0	8.3	0
F. gibbosum	3.8	0	0	0	8.6	0	3.8	0
F. heterosporum	19.6	0	19.5	0	14.3	0	16.7	0
F. solani	5.4	0	2.4	0	5.7	0	4.5	0
F. sporotrichioides	8.9	41.4	14.6	27.3	14.3	76.0	12.1	48.7
F. oxysporum	12.5	27.6	9.8	36.4	17.1	12.0	12.9	25.0
F. poae	0	3.4	2.4	9.1	0	0	0.8	3.9
F. avenaceum	3.8	17.2	2.4	18.2	0	12.0	2.3	15.8
F. semitectum	0	0	0	9.1	0	0	0	2.6
Total isolates	56	29	41	22	35	25	132	76

nizing the leaves, spike and grains. The place of localization of *Fusarium* spp. and competition for food substrate affects the composition and frequency of their occurrence. Mycological researches of the affected barley plants collected in 2012–2014 in the Central region have been showed that the frequency of occurrence of *Fusarium* spp. selected from roots and ears was ambiguous (Table 3). Out of 208 isolates of fungi classified by the 10 *Fusarium* species there only *F. culmorum, F. sporotrichioides, F. oxysporum, F. avenaceum, F. poae* were common for the roots and ears of barley. *F. sporotrichioides* (48.7%), *F. oxysporum* (25.0%) and *F. avenaceum* (15.8%) were dominated on samples isolated from ears. The isolates of *F. culmorum* (22.0%), *F. nivale* (15.1%), *F. heterosporum* (16.7%) have met high frequency on the roots of the barley.

The most adapted pathogenic fungi survive under competitive conditions. Intraspecific variabilities of *Fusarium* spp. strains to pathogenicity and toxicity have been studied in 2011–2014 by biosample method – germination of wheat seeds (cult. Mironovskaya 808) treated spore suspensions and culture fluids.

There have not been revealed correlation between signs of toxicity and pathogenicity as a result of the comparison of wheat-tester seedlings treated spore suspensions and

Table 4

The incidence of toxicity and pathogenicity of 212 strains of *Fusarium* spp. isolated from cereals (on seedlings of the cultivar tester Mironovskaya 808, 2011–2014)

Species NP*		Pathogenicity, %				Toxicity, %			
		NP*	SP	MP	P	NT	ST	MT	T
Mean for 83 *Fusarium* strains isolated from barley	unit	32	12	16	23	2	4	12	65
	%	38.5	14.5	19.3	27.7	2.4	4.8	14.5	78.3
Mean for 129 *Fusarium* strains isolated from wheat	unit	37	45	32	15	8	25	51	45
	%	28.7	34.9	24.8	11.6	6.2	19.4	39.5	34.9
F. sporotrichiodes		13.3	0	33.3	53.3	0	0	18.7	81.3
F. culmorum		0	16.7	27.8	55.6	0	0	44.4	55.6
F. sambucinum		18.2	9.1	36.4	36.4	9.1	9.1	9.1	63.6
F. oxysporum		31.6	31.6	22.8	14.0	0	9.8	34.4	55.7
F. heterosporum		42.9	25.0	17.9	14.3	6.4	25.8	12.9	54.8
F. solani		66.7	22.2	11.1	0	0	0	55.6	44.4
F. graminearum		0	86.7	13.3	0	6.7	6.7	6.7	80.0
F. avenaceum		42.8	28.6	28.6	0	0	57.1	14.3	28.6
F. gibbosum		12.5	37.5	37.5	12.5	0	50.0	12.5	37.5

* NP/NT: non-pathogenic/non-toxic (inhibition of plant growth at 0–30%), SP/ST: slightly pathogenic/slightly toxic (inhibition at 31–50%), MP/MT: moderately pathogenic/moderately toxic (inhibition at 51–70%), P/T: pathogenic/toxic (inhibition of more than 70%)

culture fluids of 83 and 129 *Fusarium* strains isolated from barley and wheat, respectively. More than 50% of *Fusarium* strains have been showed low pathogenicity to the wheat-tester (Table 4). The total expression of pathogenic traits on tester seedlings was about the same as for the strains isolated from barley and wheat, and amounted to 47.0% and 36.4%, respectively. The majority of *Fusarium* strains had expressed the toxicity to the seedlings of the tester. The toxicity of the strains of fungi isolated from barley was 78.3% against 34.9% isolated from wheat. So the strains having high and moderate toxicity have been accounted for 74.4–92.8%.

The strains of the genus *Fusarium* have been largely varied according to the intensity of the pathogenicity and toxicity. There were noted minor differences in the studied traits of the *Fusarium* spp. Thus, there were revealed the predominance high pathogenic (from 36.4% to 55.6%) and toxic activity (from 55.6% to 81.3%) for *F. sporotrichiodes, F. culmorum, F. sambucinum* strains.

F. oxysporum and *F. heterosporum* strains have poorly inhibited the growth of seedling-testers treated spore suspensions, and they were mainly non-pathogenic (for 31.6 and 42.9%) and slightly pathogenic (25–31.6%). Moreover some strains of the species stimulated the growth of wheat seedlings by 15–30%. However, *F. oxysporum* and *F. heterosporum* have been showed high toxicity to the testers treated by culture fluids of the strains (54.8–55.7%).

Strains of *F. solani* have possessed contrasting properties: non-pathogenic were 66.7% of the strains, toxic (along with moderately toxic) – 100%.

Isolates of *F. gibbosum* and *F. avenaceum* were characterized by weak to moderate pathogenicity (87.5–100%) and relatively low toxicity (50–57.1%).

Most strains of *F. graminearum* have showed weak pathogenicity (86.7%) and high toxicity to the seedlings of testers (80%).

Strains of rare species of genus *Fusarium*, such as *F. redolens, F. verticiloides, F. tricinctum*, have produced moderate toxicity to seedlings of the test crops, but proved to be non-pathogenic, which may explain their insignificant component in the microbiota of Root Rot.

Conclusion

The composition of *Fusarium* pathogens in cereals was represented by widespread and rare species of these fungi. The results of the monitoring of fungi in stalks and roots of grain crops has been indicated relatively high uniformity of *Fusarium* spp. in the regions of the Russian Federation due to the susceptibility of crops to the soil-inhabiting micromycetes.

The frequency of occurrence of micromycetes has been stable for the roots and for the ears it has been determined by the weather during the growing season of plants.

The annual occurrence of *Fusarium* spp. on the ear and grain mainly has been associated with active reproduction of the fungus on the underground parts of plants.

Consistent long-term monitoring of the dynamics and frequency of occurrence of *Fusarium* spp. in a wide range of climatic areas there has made it possible to characterize the state of the species of fungi in the Russian regions and also to study their intraspecific and interspecific variability in toxicity and pathogenicity depending on biotic and abiotic factors.

The prevalence of *Fusarium* spp. have been determined by their plasticity and viability in the soil, on the weed roots and in the stubble of many crops and high competitive ability in the mycobiota of agriculture expressed pathogenic and toxic activity.

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