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PHYTOPATHOLOGY

# Distribution of Fungi from the Genus *Fusarium* Link. on Cereals

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**Abstract**—The species composition of fungi from the genus *Fusarium* was studied, and the frequency of occurrence on cereals was determined. Populations of *Fusarium* spp. were represented by widespread species *F. culmorum*, *F. heterosporum*, *F. sporotrichioides*, *F. oxysporum*, *F. nivale*, *F. graminearum*, *F. avenaceum*, *F. gibbosum*, *F. sambucinum*, *F. moniliforme*, etc. The dynamics and frequency of occurrence of *Fusarium* spp. has made it possible to characterize the state of the species of fungi in a wide range of climatic areas of cereal cultivation in the Russian regions. The prevalence of the genus *Fusarium* was caused by the cultivars' susceptibility to micromycetes. The frequency of occurrence of *Fusarium* species isolated from roots was steady, and, on the ears, it depended on the influence of weather conditions during the growing season of plants. The study of the intraspecific variability of fungi from the genus *Fusarium* has revealed a significant difference in signs of toxicity and pathogenicity depending on biotic and abiotic factors. More than 50% of strains from the genus *Fusarium* possessed low pathogenicity but had severe toxicity to wheat seedlings. Most strains of *F. sporotrichiodes*, *F. culmorum*, and *F. sambucinum* were highly pathogenic (from 36.4 to 55.6%) and toxic (from 55.6 to 81.3%). High adaptability to preservation in the soil and on the roots of weeds and crop residues of most crops expressing pathogenic and toxic activity was conductive to the widespread *Fusarium* spp.

*Keywords:* cereals, root rot, *Fusarium*, isolate, interspecific variability, pathogenicity, toxicity, frequency of occurrence

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## INTRODUCTION

In recent years, the crop loss from an increase in the proportion of grain contaminated with fungi that cause different types of molds—fusarium head blight, root rot, and snow mold—are increasing. The relevance of the study of fungi with a similar etiology is associated with their danger to human and animal health [1–3]. Monoculture of cereals, farming rotation with short rotations, high rates of fertilization into the soil, and other methods of intensification of plant cultivation introduced without the necessary scientific elaboration have caused an expansion of the area of the harmfulness of phytopathogenic fungi in Russia and have increased the growth of phytosanitary problems, respectively [4–6].

Complexes of species of toxin-forming fungi from the genus *Fusarium*, *Bipolaris* Shoemaker (*Helminthosporium* Link), *Alternaria* Nees, *Penicillium* Link, *Cladosporium* spp, *Aspergillus* spp., etc. pose the greatest danger. In the process of production, storage, and sale of grain, the losses from the damage by these fungi and their mycotoxins account for 80% of total loss of grain [7]. The pathogenic properties of fungi of the genus *Fusarium* are contingent upon its ability to accumulate in infected plants and grain secondary toxic metabolites–mycotoxins (fusariotoxins) [8–10]. The level of toxinogenesis in fungi from this genus depends on the host plant and the conditions of its cultivation. The greatest damage is observed when plants were affected at the stage of flowering and at the milky stage. That is why it is recommended to monitor fusarium head blight by exploring the crops in the initial stage of milky ripeness [11]. According to E.Yu. Toropova, in western and southern Siberia in recent years, the frequency of occurrence of fungi from the genus *Fusarium* has significantly increased in samples of infected grain. However, recently, *Bipolaris sorokiniana* was the most common fungi [12]. Significant changes in the species composition of micromycetes were detected on oat and barley in the north of the Nonblack Earth Region [13]. In fungi from this species, the level of toxin production depends on the host plant and the conditions for its cultivation. In particular, in affected samples of barley, the fungi of the species *Bipolaris sorokiniana* was isolated with a higher frequency (up to 60%), and the species of the genus *Fusarium* were more often in samples of oat (up to 90%). The main cause of fusarium head blight is *F. graminearum*, which is most active in areas with a warm and humid climate: Krasnodar krai, Stavropol krai, Rostov oblast, and the Central Black Earth Region. Frequent epiphytoties of *F. graminearum* con-

**Table 1.** Origin of samples of cereals harvested for mycological studies and affected by root rot agents

| Region: oblast | Year of collection of plant samples | Number of isolates of fungi isolated from plants | | |
|---|---|---|---|---|
| | | wheat | barley | rye |
| Central region: Moscow oblast (Ruza, Odintsovo, Kolomna, and Naro-Fominsky raions), Tula oblast, Bryansk oblast, Ryazan oblast, and Vladimir oblast | 2003–2016 | 671 | 1476 | 225 |
| North-Caucasian region: Krasnodar krai, Stavropol krai, Rostov oblast | 2007, 2010, 2011, 2015–2016 | 326 | – | – |
| Volga region: Saratov oblast, Volgograd oblast, Republic of Tatarstan | 2006 | 150 | 736 | – |
| Ural region: Orenburg oblast (Adamovsky, Pervolotsky, Tashlinsky, Orenburg raions) | 2013, 2014 | 750 | – | – |
| Volga-Vyatka region: Kirov oblast (Falyansky, Urzhum raions) | 2003, 2014–2016 | 154 | 308 | 69 |

tribute to a heavy increase in crop losses in the southern regions of the country [14, 15].

Species from the genus *Fusarium* are ecologically plastic, so these fungi are common in all areas of cereal cultivation in Russia, including regions with low humidity during the vegetation period. On the background of contamination by fungi from this genus, the screening of varieties of cereals allows us to identify and select disease–resistant samples, which is important for obtaining grain products without mycotoxins.

The purpose of this work was a determination of the species composition of micromycetes, their prevalence, and degree of disease development in cereals in selected regions of the Russian Federation and the selection of strains of *Fusarium* spp. for use as an infectious material in the screening of varieties for resistance to disease.

## MATERIALS AND METHODS

*Tríticum* L., *Hordeum* L. and *Secale cereal* L. infected with root rot agents, fusarium mold, and fusarium head blight collected in from 2003 to 2016 on crops in five grain regions of Russia—Central region (Moscow oblast, Tula oblast, Vladimir oblast, Bryansk oblast, Ryazan oblast), North Caucasus region (Krasnodar krai, Stavropol krai, Rostov oblast), Volga region (Saratov oblast, Volgograd oblast, Republic of Tatarstan), Urals region (Orenburg oblast), and Volgo-Vyatsky region (Kirov oblast)—served as material for research (Table 1).

Mycological studies were conducted by the staff members of the Department of Mycology and Immunity of the All-Russia Scientific Research Institute of Phytopathology (ARRIP, Bolshye Vyazemy, Moscow oblast) in the years of collection of samples. As a reference, we used plants of wheat, barley, or rye in the phase of the milky ripeness (at least ten pieces) with signs of infection of root rot, fusarium mold, and fusarium head blight and picked with roots at one point of the field. From 100 to 150 segments of roots and near-the-ground nodes of the samples prewashed from soil using current water and surface sterilized with 70% ethanol were used for mycological studies of underground parts of infected plants. We also sterilized samples with an infected surface of the leafs or with an infected grain. Then, segments of roots, nodes of leaves from 5 to 7 mm in size, or caryopsides were laid out by 5 or 6 pieces in a petri dish with Czapek's medium or with 2% Potato Dextrose Agar Medium in sterile conditions.

The analysis of the species composition of mycobiota was carried out after from 10 to 14 days of incubation during microscopy of conidia, conidiophores, and asci of fungus isolates. The species affiliation of micromycete colonies was established using identification guides [16, 17]. The frequency of occurrence of the isolated microorganisms was calculated by the number of colonies of each isolated fungus relative to the number of applications to the nutrient medium and expressed as a percentage. The intensity of the contamination of plants and the prevalence rate of plants disease (%) were evaluated in the tillering period and in the full-ripe stage. The density of planting (pcs/m$^2$) and crop productivity in hundreds of kilograms per hectare were taken into account at the end of the vegetation period.

## RESULTS AND DISCUSSION

According to the results of the mycological analysis, 10000 isolates of fungi entered into the pure culture from roots, leaves, and heads. On the basis of species affiliation, samples were assigned to 15 species of the genus *Fusarium*: *F. culmorum*, *F. heterosporum* (*Gibberella gordonii*), *F. sporotrichioides*, *F. oxysporum*, *F. nivale* (*Monographella nivalis*), *F. graminearum* (*G. zeae*), *F. avenaceum* (*G. avenacea*), *F. gibbosum*

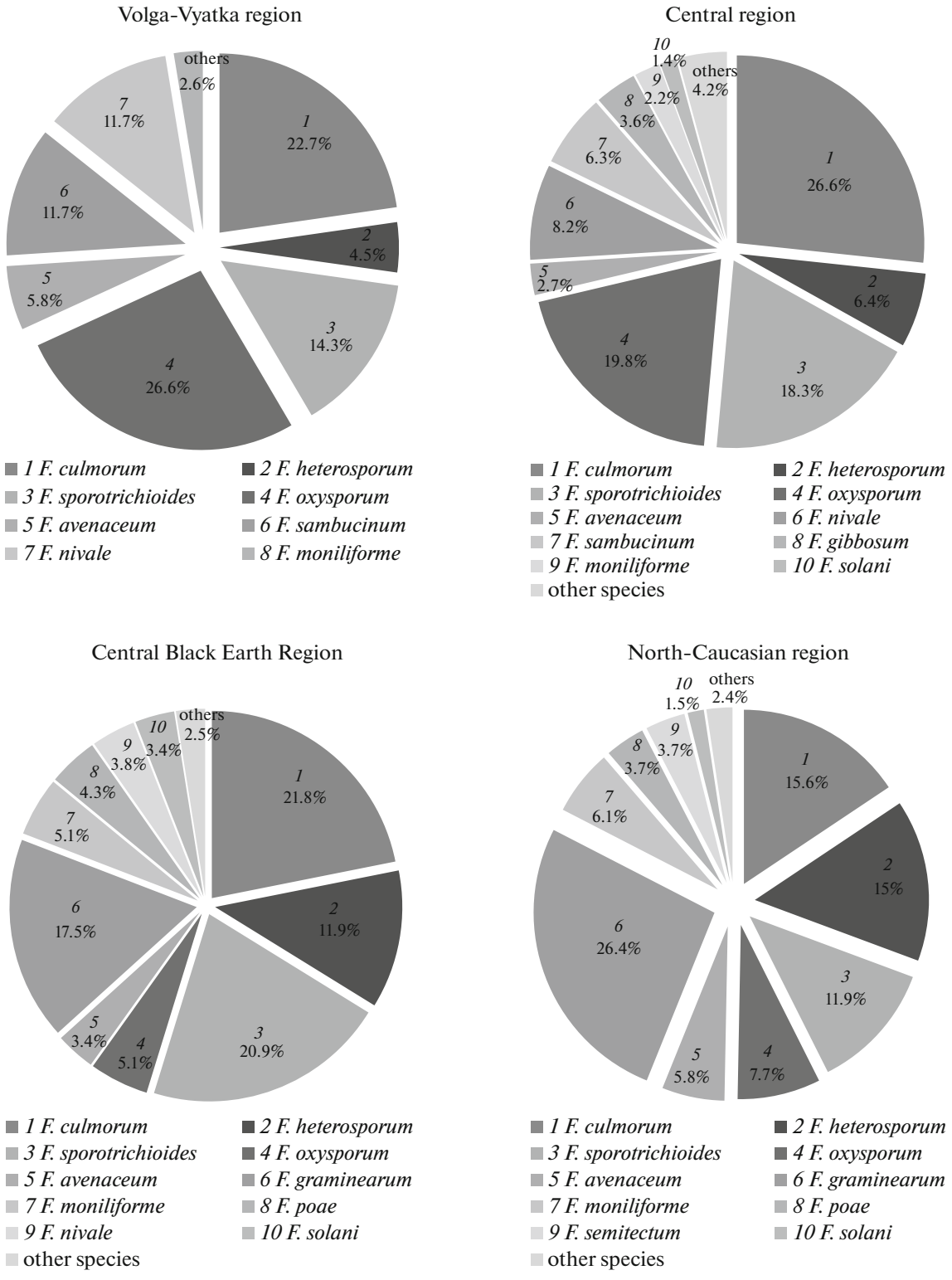


**Fig. 1.** Average frequency of occurrence of (%) of *Fusarium* spp. in the main areas of cultivation of cereals, from 1995 to 2016.

(*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 fungi from the genus *Fusarium* varies with climatic zones of cultivation of cereals and the weather conditions of the year, the location of the pathogen on plants, the preceding

**Table 2.** Frequency of occurrence of isolates of *Fusarium* spp., isolated from roots and heads scales of Zazersky 85 barley variety (Odintsovo raion, Moscow oblast), from 2012 to 2014.

| Taxon | Average incidence of *Fusarium* species in % | | | | | |
|---|---|---|---|---|---|---|
| | roots | | | head | | |
| | 2012 | 2013 | 2014 | 2012 | 2013 | 2014 |
| *F. culmorum* | 21.4 | 22.0 | 22.9 | 8.0 | 10.3 | 9.1 |
| *F. heterosporum* | 19.6 | 19.5 | 14.3 | 8.0 | 6.9 | 4.5 |
| *F. sporotrichioides* | 8.9 | 14.6 6 | 14.3 | 40.0 | 34.5 | 27.3 |
| *F. oxysporum* | 12.5 | 9.8 | 17.1 | 16.0 | 20.7 | 22.8 |
| *F .nivale* | 10.7 | 17.1 | 17.2 | 0 | 0 | 0 |
| *F. avenaceum* | 3.8 | 2.4 | 0 | 16.0 | 17.2 | 18.2 |
| *F. gibbosum* | 3.8 | 0 | 8.6 | 0 | 6.9 | 0 |
| *F. sambucinum* | 14.3 | 7.3 | 0 | 12.0 | 0 | 0 |
| *F. solani* | 5.4 | 4.8 | 5.7 | 0 | 0 | 0 |
| *F. poae* | 0 | 2.4 | 0 | 0 | 3.4 | 9.1 |
| *F. semitectum* | 0 | 0 | 0 | 0 | 0 | 9.1 |
| Total number of isolates | 56 | 41 | 35 | 25 | 29 | 22 |

culture, and other factors. However, in the territory of cultivation of cereals, the composition of the fungi from this genus was represented by the same species. In mycorrhiza of barley, wheat, and winter rye, the following ten species of fungi were most often found: *F. culmorum*, *F. oxysporum*, *F. heterosporum*, *F. sporotrichioides*, *F. nivale*, *F. avenaceum*, *F. sambucinum*, *F. gibbosum*, *F. poae*, *F. solani*. The occurrence of *F. culmorum*, *F. heterosporum*, *F. sporotrichioides*, and *F. oxysporum* combined was more than 50% of all identified isolates of fungi. Some species, such as *F. lateritium*, *F. semitectum*, *F. poae*, *F. solani*, and *F. edolens*, were isolated in pure culture in single quantities. Isolates of *F. graminearum* were more common in the southern wheat-growing area. The frequency of occurrence of isolates of *F. graminearum* in the Central Region was 1.2%, while that in the Central Black Earth and North Caucasus was 17.5 and 26.4%, respectively (Fig. 1). From north to south, positive dynamics was observed on wheat for species *F. heterosporum*—from 4.5% in the Volga-Vyatka to 15.0% in the North Caucasus region.

The frequency of occurrence of isolates of *F. nivale* was dependent on the biological characteristics of cereals. Thus, in 2011 in Odintsovo raion of Moscow oblast, the frequency of occurrence of *F. nivale* was 3.3% for spring barley and 5.6% for spring wheat. On the samples of the roots of winter grains, including rye collected in Moscow oblast and Kirov oblast in the second and third decades of June 2011, the proportion of *F. nivale* isolates reached 18.9 and 28.3%, respectively. In 2012, the results of a study of the species composition of fungi from the genus *Fusarium* collected in the Volga-Vyatka and Central regions showed that the frequency of occurrence of *F. nivale* on winter rye (from 21.7 to 32.4%) was higher than in barley and wheat (from 8.1 to 14.7%). This is perhaps due to the conditions of growth and the biology of culture. As a rule, for the areas of cultivation of winter rye (more northern regions of the country), the long periods of moistening in the autumn and spring periods, frequent thaws in winter are characteristic. That provokes the fungoid growth and, consequently, the asphyxiation of the roots and lower nodes of the stems.

It is known that the initial fungi contamination of the primary roots of the germinating seed occurs in the soil from autumn. In the soil layer, infection persists in the form of resting structures on stubble plant residues during the winter. During the process of vegetation in the spring and summer, infection spreads through the spores and hyphae on the tiers of plants and colonizes the leaves, heads, and grains. Place of localization of species from the genus *Fusarium* and competition of fungi for nutrition affect the species composition and the frequency of occurrence of studied fungi. This probably affects the specific organotropic confinement of species. According to the data of mycological research of affected plants of Zazersky 85 variety of barley harvested in 2012–2014 on crops of the Zakharovo farm in the Odintsovo raion of Moscow oblast, the frequency of occurrence of species from the genus *Fusarium* isolated from roots and heads of barley was not the same (Table 2). The results indicate the presence of a certain organotropic specialization of the species from this genus on the vegetative plant. These conclusions were confirmed during the analysis of affected plant samples collected in the Central Region in 2012–2016 (Fig. 2). Among 215 isolates belonging to ten taxa, *F. culmorum*, *F. sporotrichioides*, *F. oxysporum*, *F. avenaceum*, and *F. poae* are common on the

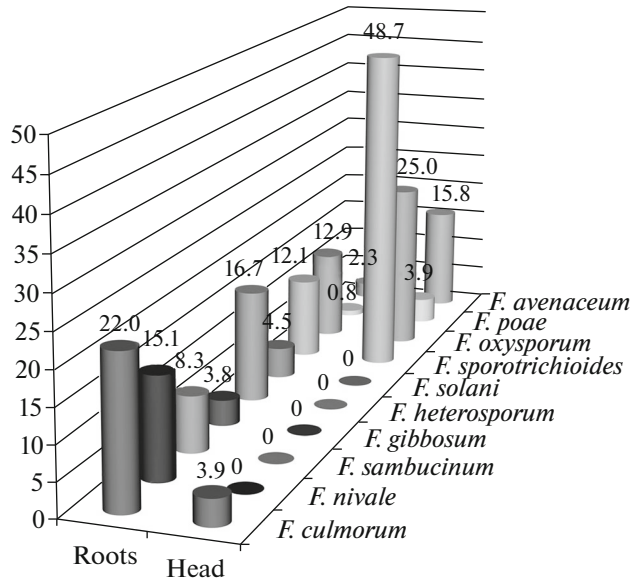


**Fig. 2.** Average frequency of occurrence of *Fusarium* spp., which are the causative agents of root rot and fusarium head blight on barley in the Central region in %, from 2012 to 2014.

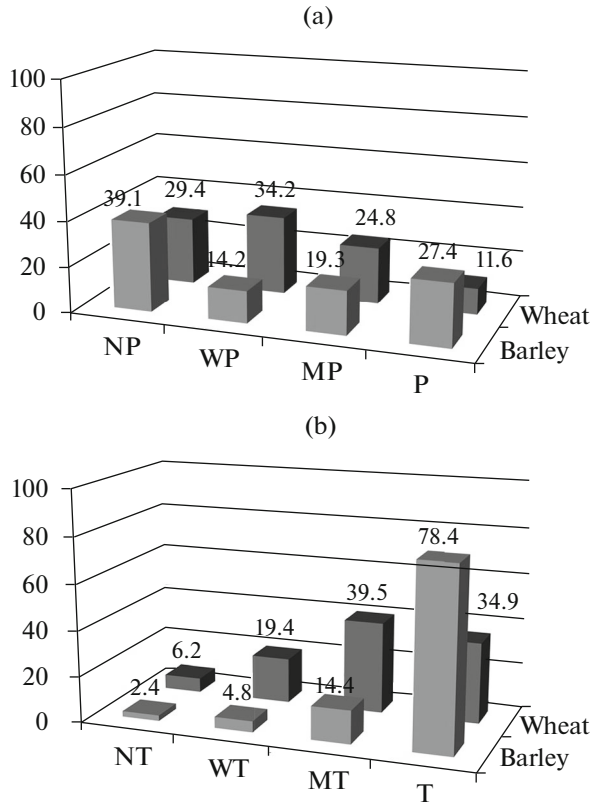


**Fig. 3.** Degree of (a) pathogenicity and (b) toxicity of strains of *Fusarium* spp. isolated from barley and wheat, %; NP—nonpathogenic; NT—nontoxic; WP—weakly pathogenic; WT—weakly toxic; MP—moderately pathogenic; MT—moderately toxic; P—pathogenic; T—toxic.

roots and scales of the head. *F. sporotrichioides* (up to 48.7%), *F. oxysporum* (25.0%), and *F. avenaceum* (15.8%) prevailed on scales of the head. On the roots of barley, *F. culmorum* (22.0%), *F. nivale* (15.1%), and *F. heterosporum* (16.7%) were found with high frequency.

It is known that, in a competitive environment, the most adapted to the environment and pathogenic fungi survive. Intraspecific variability based on pathogenicity and toxicity of *Fusarium* spp. were studied by the bioassay method on seedlings of test plants (Mironovskaya 808 wheat variety) treated with spore suspensions and culture liquids. There is no correlation between the signs of pathogenicity and toxicity during the analysis of the reaction of shoots of test plants to treatment with 83 and 129 strains isolated from barley and wheat, respectively. More than 50% of strains from the genus *Fusarium* had a low level of pathogenicity to the test variety (Fig. 3). On the seeds of the test variety, the total manifestation of pathogenic features was approximately the same for strains isolated from barley and wheat, and they amounted to 46.7 and 36.4%, respectively. At the same time, most strains from the genus *Fusarium* had a pronounced toxicity to shoots of the test variety. On the basis of this, it is possible to isolate strains obtained from barley among which the toxicity to the testers was 78.4 vs. 34.9% for strains isolated from wheat. In total, strains with high and medium toxicity were from 74.4 to 92.8%.

Strains from the genus *Fusarium* largely varied by intensity of signs of pathogenicity and toxicity. Insignificant differences between species of this genus are noted on the studied traits. Strains with a high pathogenic (from 36.4 to 55.6%) and toxic (from 55.6 to 81.3%) activity prevailed among the species *F. sporotrichiodes*, *F. culmorum*, and *F. sambucinum*. Some strains from these species did not suppress seedling development and stimulated their growth by 15–30% compared to the control. However, *F. oxysporum* and *F. heterosporum* showed high toxicity to shoots of test plants treated with culture liquids of strains of these species (from 54.8 to 55.7%). In the studied properties, *F. solani* varied: 66.7% of the strains were nonpathogenic and 100% were toxic (together with moderately toxic) strains. *F. gibbosum* and *F. avenaceum* were characterized by mild and moderate pathogenicity (from 87.5 to 100%) and relatively low toxicity (from 50 to 57.1%). Most strains of *F. graminearum* showed both low and moderate pathogenicity (86.7%) and high toxicity to seedlings of tests plants (80%). Strains belonging to the low-frequency species *F. redolens*, *F. verticiloides*, and *F. tricinchtum* also had moderate toxicity to germs of test cultures. That may explain their insignificant quantity in the mycobiota of root rot.

Thus, the composition of *Fusarium* pathogens on cereals is represented by widespread and low-fre-

quency species of fungi. At the same time, the uniformity of type of *Fusarium* spp. was noted both in the cultures and in the regions of the Russian Federation due to the susceptibility of agrocenoses to soil-forming micromycetes. The constant presence of micromycetes is noted on the roots. The damage of leafs and heads depends on weather conditions during the vegetative period of plants. Sequential long-term observations for the dynamics and frequency of occurrence of *Fusarium* spp. in a wide range of climatic zones of cultivation of cereals allowed us not only to characterize the state of these species of fungi in the regions of Russia but also to study their intraspecies and interspecific variability in toxicity and pathogenicity depending on biotic and abiotic factors. Extension of *Fusarium* spp. was caused by their plasticity and viability in the soil, on the roots of weedage and on afterharvesting residues of many crops, and high competitive ability in the mycobiotic agrocenosis expressed by pathogenic and toxic activity.

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