Diversity of soil micromycetes in winter wheat crops of the Volgograd region according to different predecessors

N I Budinkov¹, V A Poplevina², S N Mihaleva¹, G F Yarcev¹,³ and A G Goncharov¹

¹All-Russian Research Institute of Phytopathology, Moscow region, Odintsovo district, pos. Bolshie Vyazemy, st. Institute, ow. 5, 143050, Russia; ²Moscow State University M.V. Lomonosov, Moscow, Leninskie Gory 1, bld. 12, 119234, Russia. ³Orenburg SAU, Orenburg region, Orenburg, Chelyuskintsev street, 18

E-mail: oranzar@yandex.ru

Abstract. The dynamics of the quantitative and qualitative composition of the soil microbiota of the rhizosphere and rhizoplane of plants during the growing season of winter wheat after the predecessors: winter wheat, spring wheat, chickpea and flax in 2017-2018 was studied during the period of spring and autumn tillering, on the farms of the Volgograd region. The conducted field and laboratory studies made it possible to identify the predominant representatives of the microbiota. According to the research results, it was revealed that almost all samples were dominated by pathogenic fungi: Fusarium solani and Fusarium moniliforme, saprotrophic mucoral and non-spurulating fungi; Fusarium avenaceum was less common. Also, almost all samples contained the bacteria Pseudomonas spp., and Bacillus spp. Highly pathogenic fungi F. moniliforme were more often detected after cereal spike predecessors, less often after chickpea; F. oxysporum - mainly after chickpea and flax. Chickpeas and flax, with adequate control of pathogenic microbiota on the subsequent culture, are better predecessors for winter wheat in the Volgograd region than winter wheat and spring crops. Depending on the characteristics of the year of research, as well as the season of sampling, specificity in the formation of mushroom consortia was observed in the experimental fields. Thus, in the fall of 2018, previously unseen pathogenic fungi Fusarium sambucinum, F. gibbosum, F. sporotrichiella, an antagonist fungus Gliocladium virens were isolated from the soil. In some samples, the fungi Penicillium spp., in particular, P. purpurogenum, were present, which, due to the secreted phytotoxins, can inhibit the growth and development of plants. The toxin-forming fungus Aspergillus niger has also been isolated. A significant part of micromycetes isolated from soil samples are pathogenic for cultivated plants, including winter wheat. There were very few representatives of micromycetes-antagonists Trichoderma viride, Gliocladium virens in the soil, probably not enough to resist the accumulation of pathogens.

1. Introduction
Stable production of cereals, legumes and oilseeds, the realization of the potential of plants are the key problems of the crop growing industry in the Volgograd region of the Russian Federation. Unstable yields in the region are often caused by the cultivation of crops without strict adherence to scientifically
based technological recommendations; untimely varietal renewal and varietal change, unbalanced mineral nutrition, adequate systems of plant protection against biotic factors are not always applied.

Wheat, one of the most important cereals and the main food crop in the world [1]. Wheat is cultivated in more than 80 countries.

Feed grain is the basis of the fodder base for animal husbandry. Wheat grain contains from 11 to 20% protein, 63 - 74% starch, about 2% fat, the same amount of fiber and ash. When keeping and feeding livestock, green fodder, bran, and straw, which are obtained from the cultivation of grain crops, are also widely used [2].

When growing winter wheat, increased requirements are imposed on predecessor crops. In this work, we considered the role of the precursor in the accumulation or suppression of pathogens in the soil and rhizosphere of winter wheat plants. The development of most dangerous microorganisms and pests is associated with the root layer of the soil. Pathogenic microbiota accumulates here on plants and plant residues of predecessors, therefore it is important to observe crop rotation and conduct regular monitoring of pathogens [3, 4].

The purpose of the presented work: to study the qualitative and quantitative composition of the dominant microorganisms in field soil samples, the rhizosphere part of growing plants of winter wheat during spring and autumn tillering after different predecessors; compare the results obtained and identify the precursor cultures most favorable for the preservation and accumulation of useful soil microbiota, suppression of pathogens.

2. Methods and materials

The study was carried out on soil material collected from the fields of winter wheat farms in the Volgograd region, in the laboratory of the Federal State Budgetary Scientific Institution All-Russian Research Institute of Phytopathology in 2017–2018. The soil was selected in spring and autumn, in fields with different predecessors: winter and spring wheat, flax and chickpeas.

Microbiological analysis of the soil and rhizosphere in the laboratory of the Federal State Budgetary Scientific Institution All-Russian Research Institute of Phytopathology were carried out according to the technique using the artificial nutrient medium of Czapek.

Small lumps of soil were applied to the nutrient medium in order to take into account their colonization by microorganisms of fungal and bacterial nature [5-7].

The analysis of the species composition of the microbiota released from soil samples was carried out on the 7th day of incubation. The species of micromycete colonies formed on the nutrient medium was determined by the morphology of the sporulation organs (conidiophores, conidia, asci, spores, etc.) under a microscope.

Based on the analysis results, the occurrence of soil micromycete species was assessed. The number of colonies of microorganisms of different species isolated in vitro was counted, then the proportion (in%) of each isolated microorganism was calculated relative to the number of soil applications on the nutrient medium; the proportion of samples with colonization by one or another microorganism or a group of them was also calculated [5-7].

The dominant species of isolated microorganisms and their ratio, taking into account the seasonality and the predecessor, were identified.

3. Results and discussion

From spring soil samples of 2017, saprotrophic fungi of the genus Mucor were isolated, with a colonization level of 8.4 - 19.1%, the antagonist fungus Trichoderma viride 0 - 6.7%, as well as non-spurring fungi Mycelia sterilia 9.0 - 21.8 %. The largest amount of cenotically relatively neutral mucor and non-spurulating fungi was found in the soil under winter wheat after the precursor “flax”, and a lesser amount after the semi-steam predecessor. For spring wheat, saprotrophic fungi were isolated less than for winter wheat. The smallest number of mukors was noted for the "chickpea" predecessor; at the same time, there were more Mycelia sterilia fungi for chickpeas than for winter and spring wheat. With regard to winter wheat, the occurrence of mucor and non-spurling fungi in the soil under winter
wheat turned out to be higher in our experimental fields than in spring wheat. With the antagonist
fungus *Trichoderma*, the situation with the predecessors "winter" and "spring" wheat was the opposite
- with respect to chickpeas and flax, this mushroom was practically not detected. Despite the advertised
hyper antagonistic properties, trichoderma was rarely found in field agrocenoses.

From the group of phytotoxicant fungi, penicillus fungi were most often found under winter wheat
for chickpeas and winter wheat. Their occurrence for spring wheat did not exceed 6.8% in 2017; they
were not identified for flax. Aspergillus was found in a relatively small number of samples, and only
for spring wheat (Table 1).

The occurrence of fungi of varying degrees of pathogenicity in the root layer of the soil was
ambiguous: *Fusarium solani* was isolated with a colonization frequency of 64.9 - 80.3%, *F. avenaceum*
0.0 - 5.3%, *F. moniliforme* 17.0 - 31.1%. In addition to fungi, the presence of saprotrophic and
pathogenic bacteria *Pantoea agglomerans* 0.0 - 3.3%, *Pseudomonas syringae* 0.0 - 3.3%, saprotrophic
bacteria *Bacillus spp.* 15.0 - 70.0%, *Pseudomonas spp.* 0.0 - 10.0%, individual strains of the genus of
radiant bacteria *Actinomyces* 0.0 - 5.0% (Table 1).

In the spring samples of 2018, saprotrophic fungi were found: mucorous with colonization of
17.5%, *Trichoderma viride* 0.0 - 43.0%, non-spurulating fungi *Mycelia sterilia* 0.0 - 15.0%, pathogenic
- four species from the genus *Fusarium*: *F. solani* with colonization 92.5 - 97.5%, *F. avenaceum* 0.0 -
2.5%, *F. moniliforme* 1.0 - 30.0%, *F. oxysporum* 0.0 - 2.5%. Fungi of the genus *P. purpurogenum* 5.0
- 22.5%, *Aspergillus niger* with a colonization of 0.0 - 7.5% were isolated from mold toxin-forming
agents. In addition to fungi, bacterial strains *Bacillus spp.* have been identified. 60.0 - 78.0%,
*Pseudomonas spp.* 13.0 - 25.0%, *Erwinia spp.* 0.0 - 2.7% (Table 2).

There were no differences in the occurrence of saprotrophic mucor fungi in the soil under winter
wheat for different predecessors (2018) - it was at the level of 17.0%. The maximum occurrence of the
fungus-antagonist *Trichoderma* was noted for the predecessor "flax", 19.8% less - for winter wheat;
the third in this ranking was chickpeas. For spring wheat, *Trichoderma* was not detected in the
surveyed fields. The opposite pattern of occurrence according to these predecessors was observed for
the non-spurulating fungi *Mycelia sterilia*. The maximum of penicilli was observed after the cereal
predecessors, the minimum - after the chickpea. Aspergillus was not distinguished here for winter
wheat and chickpea; an insignificant amount of these toxic fungi was noted for spring wheat and three
times more for flax.

The differences between the predecessors for *F. solani* between the studied variants were within the
experimental error, although a slight decrease in the occurrence in the form of a trend was noted for
winter wheat.

*F. moniliforme*, a dangerous pathogen for cereals, was most often found in the soil after winter wheat
29.6% and spring wheat 30.3%, in much smaller quantities after dicotyledonous predecessors - chickpea
13.2% and flax 13.7%.

The occurrence [7] of pathogenic fungi *F. avenaceum* and *F. oxysporum* was either low or zero. It
should be noted that *F. oxysporum*, in the spring of 2018, was able to isolate only 2.4% of the “chickpea”
precursor, where it is a very dangerous pathogen and can accumulate in the absence of timely protective
measures in the plant biomass.

The minimum of saprotrophic bacteria from the genus *Pseudomonas* was noted after spring wheat, maximum after flax; the values of the occurrence of these bacteria after winter wheat and chickpea had intermediate values. Extreme differences in the incidence of bacilli were observed between winter and spring wheat. The occurrence of pathogenic bacteria *Erwinia* was either insignificant (for winter wheat and chickpea), or zero for spring wheat and flax (Table 2).
The growing season preceding the autumn tillering with high microbiological activity due to high summer temperatures and a continuous abundance of processed organic matter left its mark on the occurrence of soil and rhizosphere microorganisms during the autumn tillering of winter wheat.

In the autumn samples of 2018, fungi of the genus *Fusarium* were represented by species: *F. solani* with colonization 80.3 - 94.9%, *F. oxysporum* 0.0 - 5.3%, *F. avenaceum* 7.2 - 27.8%, *F. moniliforme* 0.0 - 17.7%, *F. sambucinum* 0.0 - 2.6%, *F. gibbosum* 5.2 - 13.4%, *F. sporotrichiella* 0.0 - 2.5% (Table 3). In addition to fungi, bacterial strains of saprotrophic bacteria *Pseudomonas spp.* were identified. 5.2 - 13.3%, *Bacillus spp.* 68.4 - 83.3%. No pathogenic bacteria were found.

There were no noticeable differences in the occurrence of mucor fungi for different predecessors. The maximum of the antagonist fungus (*Gliocladium virens*) was observed for the “chickpea” predecessor, where nonsorulatory fungi were practically absent - a small amount of them was isolated only from the cereal predecessors. For micromycetes of different levels of pathogenicity, the picture was ambiguous. The maximum of the most common moderately pathogenic fungus *F. solani* was observed in the fields for chickpeas 94.9%, for both grain predecessors it was slightly less. The opposite picture was observed for highly pathogenic *F. moniliforme* - the maximum for spring wheat

| Season | Predecessor | Mu | Tric | My | Peni | Asp | Fus | Fus | Fus | Fus | Pse | Bac | Erw |
|--------|-------------|----|------|----|------|-----|-----|-----|-----|-----|-----|-----|-----|
| Spring | Winter wheat | 176,0 | 23,1 | 10,0 | 22,4 | 0,0 | 92,5 | 29,6 | 0,0 | 0,0 | 20,1 | 60,2 | 2,7 |
| Spring wheat | 175,0 | 0,0 | 15,2 | 22,5 | 2,3 | 97,1 | 30,3 | 2,5 | 0,0 | 12,7 | 78,6 | 0,0 |
| Flax | 174,0 | 43,1 | 0,0 | 12,6 | 7,5 | 97,4 | 13,7 | 0,0 | 0,0 | 25,2 | 64,8 | 0,0 |
| HCP0.05 | 2,9 | 3,4 | 1,3 | 2,5 | 0,0 | 14,9 | 2,2 | 0,1 | 0,1 | 3,8 | 9,9 | 0,2 |

Table 2. Microbial colonization of soil in winter wheat fields by micromycetes during spring tillering (%). 2018
was 17.7%, slightly less for winter wheat, 15%, and the absence was for chickpeas. *F. avenaceum* for winter wheat and chickpea was approximately equal, while for spring wheat this fungus was three times less. *F. oxysporum* was isolated only by chickpea, a very favorable host plant for this pathogen. The occurrence of *F. sambucinum* and *F. sporotrichiella* was low and they were found only on cereals. The greatest amount of *F. gibbosum* was isolated for chickpeas, significantly less for spring wheat, and even less for winter wheat.

Pathogenic bacteria were not found in the fields in this experiment. The maximum of saprotrophic pseudomonads was isolated from the soil for winter wheat, and the minimum for spring wheat. The highest occurrence of bacilli was noted for chickpeas, the lowest for winter wheat (Table 3).

In addition to fungi, the saprotrophic bacteria *Pseudomonas* spp. Have been identified. 0.0–53.2%, *Bacillus* spp. 77.3-100.0%; pathogenic bacteria *Pseudomonas syringae* 2.3-7.8%, *Pantoea agglomerans* 0.0-13.4%, *Erwinia* spp. 0.0-5.5%.

**Table 3.** Microbial colonization of soil in winter wheat fields by micromycetes during autumn tillering (%). 2018

| Seasion | Predecessor | Mucor spp. | Glis dioclum | Mycelia sterilia | Fusarium avenaceum | Fusarium moniliforme | Fusarium oxysporum | *Pseudomonas* spp. | Bacillus spp. |
|---------|-------------|-------------|-------------|-----------------|--------------------|---------------------|-------------------|------------------|--------------|
| Winter wheat | 2018 spring | 17.6 | 23.3 | 10.0 | 92.5 | 29.6 | 0.0* | 0.0 | 20.1 |
| | 2018 autumn | 30.2 | 2.5 | 2.4 | 80.3 | 15.0 | 25.1 | 0.0 | 13.3 |
| Spring cereals | 2018 spring | 17.5 | 0.0 | 15.2 | 97.1 | 30.3 | 2.5 | 0.0 | 12.7 |

Evaluation by years and seasons of the occurrence of the dominant microorganisms of field agrocenoses of the Volgodgrad region showed the following results (Table 4)

The minimum occurrence of mucoral fungi in the soil on winter wheat crops for winter wheat was noted in the spring of 2018, then there was an increase in this indicator, which remained in the fall of 2018.

Mushrooms of the genus Mucor spp. are natural inhabitants of the soil; they do not cause any damage to developing cultivated plants, but with the appearance of excess moisture, a decrease in plant immunity due to other pathogens, they can manifest themselves in the form of active dense mold on the grain, the root zone of plants, on organs and tissues during the growing season and during storage in granaries and elevators [6].

**Table 4.** Annual and seasonal dynamics of the dominant soil microorganisms in the fields of winter wheat for the predecessors "winter wheat", "spring cereals", "chickpeas". 2018-2019

| Predecessor | Year | Season | Mucor spp. | Tricho derma viride, Glioclum virens | Mycelia sterilia | Fusarium avenaceum | Fusarium moniliforme | Fusarium oxysporum | *Pseudomonas* spp. | *Bacillus* spp. | *Pseudomonas* syringae |
|-------------|------|--------|------------|-----------------------------------|-----------------|-------------------|---------------------|-------------------|-------------------|-------------------|-------------------|
| Winter wheat | 2018 | spring | 17.6 | 23.3 | 10.0 | 92.5 | 29.6 | 0.0* | 0.0 | 20.1 | 60.2 | 0.0 |
| | 2018 | autumn | 30.2 | 2.5 | 2.4 | 80.3 | 15.0 | 25.1 | 0.0 | 13.3 | 68.4 | 0.0 |
| Spring cereals | 2018 | spring | 17.5 | 0.0 | 15.2 | 97.1 | 30.3 | 2.5 | 0.0 | 12.7 | 78.6 | 0.0 |
Mushrooms-antagonists of *Trichoderma* and *Gliocladium* were found in maximum quantities on the soil of winter wheat fields in the winter spring of 2018. The same trend was noted for the legume predecessor, but with less contrast. On spring wheat, these micromycetes in 2018 were not found. *Gliocladium virens* and *Trichoderma viride* are saprotrophic antagonistic representatives of soil biota, suppressors. For the purpose of using their suppressive properties in fields, fungal biological preparations are created [8, 10]. Species of the genus *Trichoderma* are active producers of the cellulase enzyme and are capable of deep destruction of both plant cell walls and individual difficult-to-digest plant polysaccharides: cellulose, hemicellulose, pectin to monomeric forms. At present, the phenol oxidases of micromycetes are being actively studied in connection with the significant role of these enzymes in the biodegradation of lignin [11, 12].

*Mycelia sterilia* nonsporulatory fungi did not show the regularity of seasonal occurrence characteristic of all predecessors. So for winter wheat, their maximum number was found in the spring of 2018. For spring cereals in 2018, from spring to autumn, a sharp decrease in the occurrence of *Mycelia sterilia* was also noted.

Fungi of the genus *Fusarium* have a wide variety of enzymes for survival and cause severe diseases in various organisms. Most representatives of fungi of the genus *Fusarium* are phytopathogens that also cause mycoses and toxicoses in humans and warm-blooded animals [13, 14].

Wheat diseases caused by phytopathogenic fungi lead to a loss of up to 30-40% of the yield [15]. The most common fungi in field agrocenoses are *Fusarium solani* [16]. In our experiments, the occurrence of *F. solani* was consistently high for all predecessors, but in autumn this micromycete was isolated slightly less than in spring (Table 4). Probably, in spring, the ecological niche for its accumulation was larger than in autumn. In this regard, it is necessary to monitor and timely protect grain crops from toxin-forming phytopathogens [17, 18].

In our experiments, the incidence of saprotrophic pseudomonads in all cases decreased from spring to autumn, while the incidence of bacilli increased. These species play the role of suppressors, occupying a certain ecological niche, making it inaccessible to other species. Probably, the seasonal volume of bacterial niches is quite constant, and if there is an increase in the occurrence of one group of species, and in parallel, the occurrence of another decreases. In autumn, there is a huge niche of plant residues in the fields, unprotected by immunity, and more saprotrophic forms of bacteria - bacilli - progress on it.

### 4. Conclusion

As a result of the field and laboratory studies, the dynamics of the most common fungi accompanying winter wheat in the soil and rhizosphere in different seasons was analyzed according to the predecessors "winter wheat", "spring wheat", "chickpea", "flax". All samples were dominated by saprotrophic micromycetes *Mucor spp.*, *Mycelia sterilia*, pathogenic - *Fusarium solani*, *Fusarium moniliforme*, *F. avenaceum*, *F. oxysporum*, saprotrophic bacteria from the genera *Pseudomonas* and *Bacillus*. Fungi from the genera *Trichoderma*, *Gliocladium*, *Torula*, *Fusarium sambucinum*, and *F. Gibbosum* were found less frequently.

Very few saprotrophic representatives of the antagonistic fungal microflora of *Trichoderma* and *Gliocladium* were isolated and they could hardly withstand the accumulation of pathogens in the experimental fields. Possibly, the saprotrophic bacteria from the genera *Bacillus* and *Pseudomonas* play an important role in suppressors.
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