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Formation of the Population of Micromycetes in the Leaf Microbiome of Cereal Cultures Using Different Plant Cultivation Technologies

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ABSTRACT

The selection of cereal crops varieties as a factor in the regulation of the phytopathogenic microbiome in agrocenoses is an actual direction of the research. Cultivation of such varieties leads to a decrease in the level of biological pollution in agrocenoses and increases the quality as well as safety of agricultural products in agroecosystems. Therefore, the influence of the environmental factors (including abiotic, biotic, anthropogenic, and other) on the formation of micromycete populations in the leaf microbiome of grain crops using different plant cultivation technologies has been thoroughly studied earlier. The results of the selection the plant varieties by the indicators of influence on their population density, the frequency of the occurrence, and the intensity of the micromycete sporulation, were presented in this article. Vegetative organs of plants of the cereal crops (including the oats of Parliamentsky variety, Tembre variety, and spring barley Salomi and Sebastian varieties) were selected in the following phases: tillering, stem stage of growth, and earing. It was determined that using the traditional and organic technologies of plant cultivation in the leaf microbiome of Tembre oats and Salomi variety spring barley, the population density, the frequency of the occurrence of micromycete species, and their sporulation intensity were significantly lower compared to the plants of Parliamentsky oat and Sebastian spring barley. This shows that the cultivation of the cereal crops varieties capable of restraining the formation of micromycete on an ecologically safe level will result in a decrease in the level of biological pollution of agrocenoses and increase the biosafety of plants.

Keywords: ecological risk, biosafety, biological pollution, population density, the frequency of the occurrence, the intensity of the micromycete sporulation, the phytopathogenic micromycetes.

INTRODUCTION

The interaction of the populations of the phytopathogenic microorganisms with cereal crop plants leads to the formation of a phytopathogenic microbiome, which is a factor of biological pollution in agrocenoses [Van Montagu 2020; O'Brien 2017]. Excessive usage of chemical pesticides, as well as the usage of resistant, genetically homogeneous varieties, and some changes in soil and climatic conditions, lead to the expansion of specie diversity and increased destructive activity of phytopathogenic microorganisms, as well as to formation of their resistant forms with increased aggressiveness. This contributes to the emergence of some ecological risks in agro-ecosystems and the reduction of biosafety in the production the plant products of cereal grain crops. Therefore, more and more attention is being paid to identifying the reasons for the disruption of the natural relationship between the plant and the pathogen [Lapin et al. 2013; Tack et al. 2013] as well as the study of mechanisms and factors that substantially slow down the formation of the number of phytopathogenic microorganisms in the agrocenoses of cereal grain crops [Köhl et al. 2019].

The production of high-quality and safe grain crop products requires solving a number the problems caused by the interaction of the populations of phytopathogenic fungi with plants of different genetic origins in the agrocenoses of Ukraine [Parfenyuk et al. 2016; Parfenyuk 2017; Beznosko et al. 2021; Reinhold-Hurek et al. 2015]. The global warming, especially in the winter months, causes the expansion of the range of pathogens in the areas where they did not occur before. Under the conditions of sufficient humidity, the populations of micromycetes of the *Fusarium* spp. genus take a dominant position in the agrocenoses of cereal grain crops (as the causative agents of fusariosis). Sharp changes in driness and humidity led to intensive reproduction of the populations of *Alternaria* spp. micromycetes and their rapid spread in the agrocenoses of cereal grain crops.

Many studies have a purpose of researching soil and climatic conditions during the growing season, which is an important factor in regulating the number of populations of harmful organisms based on the wide use of natural resources [Rejeb et al. 2014; Shvartau et al. 2017; Vozhehova et al. 2018]. Changes in soil and climatic conditions as well as the intensive use of chemical protection agents led to the spread of micromycete populations and the accumulation of their infectious structures on the vegetative organs of plants [Beznosko et al. 2022; Lamichhane 2017] It is a known fact that a resistant variety, especially the one created by genetic modification, is a powerful factor in the directed selection in the populations of microorganisms, and a susceptible variety is a factor in the growth of their populations [Ngoune et al. 2020; Beznosko et al. 2022b; Petrenkova et al. 2016]. They affect the qualitative and quantitative indicators of the phytopathogenic background to a great extent, which in turn significantly worsens the conditions of agrophytocenoses and also deteriorates the biological safety of agroecosystems [Dermenko 2016; Mostovyak et al. 2020]. Therefore, it is important to study the formation of micromycete populations on the vegetative organs of plants of cereal grain crops under the conditions of using different cultivation technologies, taking into account the soil and climatic conditions.

The density of micromycete population is an important indicator of ecological assessment of the vegetative organs of plants. It enables to determine the number of colony-forming units in plant materials under the influence of some environmental factors. It is a known fact that this number is an important indicator of the characteristics of the population of biological microorganisms. A change in the number of the original population or a delay in its growth can be an indicator of the assessment of the variety as a factor of the environmental risk. The analysis of the frequency of the species occurrence in the microbiome of the vegetative organs of plants allows establishing the dominant species and their abundance in the agrocenoses of cereal grain crops. The intensity of the propagative and resting spores formation in phytopathogenic micromycetes of the vegetative organs of cereal grain crop varieties is an ecological indicator of the culling of varieties that are able to stimulate the development of pathogens or the selection of those that, on the contrary, are able to restrain their development [Barratt et al. 2018; Ternovy et al. 2018; Hardoim et al. 2015]. Therefore, the study of the formation of the micromycete populations in the leaf microbiome of cereal grain crops is a priority area of scientific research. The evaluation of plant varieties as a factor in the regulation of the phytopathogenic microbiome in the agrocenoses of cereal grain crops will ensure a decrease in the level of biological pollution and increase the quality as well as safety of plant products.

MATERIALS AND METHODS

The research was conducted on the basis of the laboratory of biocontrol of agroecosystems and organic production of the Institute of Agroecology and Nature Management of the National Academy of Sciences (2020–2022). The formation of the micromycete population in the leaf microbiome of Parliamentsky and Tembre oat varieties, Salomi and Sebastian spring barley, under the conditions of traditional and organic technology growing plant was studied. The vegetative organs of plants of cereal crops were selected in the phases: tillering, stem extension stage of growth, and earing in the fields of the Skvirsk Research Station of Organic Production of the Institute of Scientific Research of the National Academy of Sciences of the Russian Academy of Sciences in accordance with the generally accepted methods [Korniychuk 2015].

It is a known fact that the ontogenesis of cereal crops as well as the spread and development of diseases are significantly influenced by

temperature and rainfall. The hydrothermal coefficient (HTC) is an integrated indicator of these factors. The values of HTC recorded during the growing season of cereal cropsin the years of the study are presented in Table 1.

Under the conditions of the traditional cultivation technology, various chemical fungicides were used, and under the conditions of organic technology, plant protection agents were not used (Table 2).

The density of micromycete population in the leaf microbiome of plants was determined using the method of dilution and surface sowing of the suspension on Capek nutrient medium. The number of micromycetes was specified in colony-forming units (CFU) per 1 g of dry leaf, and determined according to DSTU 7847:2015 [DSTU 2016].

The rate of occurrence (%) of micromycete species was determined using the formula [Sessitsch et al. 2021]:

$$A = \frac{B \times 100\%}{C} \quad (1)$$

where: A is the frequency of occurrence of the species;
B is the number of the samples in which the species were detected;
C is the total number of the selected species.

The identification of the isolates of microscopic fungi to genus and species was carried out on a biological microscope DN-200D using some determinants [Guaro et al. 2012, Koval et al. 2016, Colin et al. 2013, Marin-Felix 2017, Gostinčar 2020, Ruytinx 2021] and using the MycoBank online database. The indicator of the intensity of the micromycetes sporulation was determined by counting macro- and microconidia in the Goryaev-Tom chamber according to the formula:

$$N = (a \times 1000/h \times S) \times n \quad (2)$$

where: N – the number of cells in one ml of suspension;
a – the average number of cells in a lattice square;
h – the camera depth (0.1 mm);
S – the area of the grid square (0.04 mm^2);
n – the dilution of the initial suspension.

One-way analysis of variance (ANOVA, Tukey's test) was used for statistical processing of the experimental data. The difference between control and experimental indicators was considered significant when the probability of the difference was $P<0.05$.

RESULTS AND DISCUSSION

The density of micromycete population in the leaf microbiome of oat plants

According to the research carried out under the conditions of using traditional plant cultivation technology, it was determined that in the oat leaf microbiome, the density of micromycete population ranged from 0.45 to 5.6 thousand CFU/g of green plant mass (Figure 1). The studied indicator differs significantly, depending on the climatic conditions of the year of the study, namely: high air temperature and significant amount of precipitation.

In the tillering phase, the density of the population in the leaf microbiome of Tembr oat variety ranged from 0.8 to 1.1 thousand CFU/g of the green plant mass. At the same time, in the leaf microbiome of Parliamentsky variety, this indicator was in the range of 1.5 to 2.1 thousand CFU/g of the green plant mass. In the stem of growth phase, the density of micromycete population increased and ranged from 1.8 to 2.9 on oat leaves of both varieties. During the earing phase, the density of micromycete population increased by 2–2.5 times, which indicates a change in weather conditions at the end of the growing season during the years of the study. Also, the introduction of

Table 1. The value of HTC recorded during the growing season of 2020–2022.

Year	Month						Average the meaning of HTC
	April	May	June	July	August	September	
2020	1.2	1.8	1.0	0.8	0.7	0.5	**1.0**
2021	0.8	2.0	1.6	0.9	1.0	0.6	**1.3**
2022	0.6	1.7	0.9	0.6	0.3	0.4	**0.7**

Note: HTC ≥1 – sufficient hydration; HTC 0.8–1.0 – moderate hydration; HTC 0.6–0.7 – insufficient hydration.

Table 2. The protection scheme of cereal grain crops against diseases in the conditions of different cultivation technologies

Cultivation technologies	Period of using fungicide	The name of the preparation	Active substance	Rate of consumption
Traditional	pre-sowing treatment seed	Vitavax 200 FF (fungicide)	Karboxin: 200 g/l Tiram: 200 g/l	3.0 l/t
	tillering	Granstar Gold 75 (FMC) (herbicide)	Tribenuron-methyl: 562.5 g/kg Thifensulfuron-methyl: 187.5 g/kg	25 r/ra
Organic	Without fertilizers and fungicides			

chemical plant protection agents had a significant impact on the growth of the micromycete population, which contributed to the rapid reproduction of micromycetes in response to unfavorable conditions for the existence of species. It should be noted that the variety Parliamentsky, owing to physiological biochemical substances, is able to stimulate the formation of micromycete populations, which contributes to the accumulation of infectious structures in the oat leaf microbiome. Compared with using the traditional technology of plant cultivation, with the organic technology of growing oat plants, the density population of micromycetes in the leaf microbiome increased as the crop aged and ranged from 0.5 to 3.6 thousand CFU/g of green plant mass (Figure 2).

During the tillering phase, the population of micromycetes in the leaf microbiome of Tembre variety ranged from 0.5 to 0.8 thousand CFU/g of green plant mass. At the same time, in the leaf microbiome of Parliamentsky variety, this indicator ranged from 0.8 to 1.1 thousand CFU/g of green plant mass. In the stem of growth phase, the density of micromycete population increased significantly in the leaf microbiome of Parliamentsky variety and reached 2.9 thousand CFU/g of green plant mass, while this indicator was 2 times lower on Tembre oat variety. The highest population density was characterized by the earing phase, where it increased 1.5 times in the leaf microbiome of Tembre and Parliamentsky oat varieties, and ranged from 1.8 to 3.6 thousand CFU/g of green plant mass. It should be noted that the population density in the leaf microbiome of Tembre variety is almost 50% lower than in the leaves of the variety Parliamentsky. This shows that the plants are able to influence the formation of micromycete population density in the oat leaf microbiome in different ways.

The species spectrum of micromycetes in the leaf microbiome of oat plants

According to the conducted laboratory studies, it was found that 18 species of micromycetes including *F. sporotrichiella, F. graminerum, F. oxysporum, F. incarnatum, F. culmorum, F. verticillioides, D. avenae, A. alternata, A. infectoria, R. nigricans, A. flavus, C. herbarum, T. roseum, H. avenae, S. avenae, A. avenae, P. avenae, P. notatum,* parasitized in the leaf microbiome of the Parliamentsky variety using the traditional technology of cultivation, and this phenomenon was characterized by different frequencies of occurrence, ranging from 10 to 70%. At the same time, 12

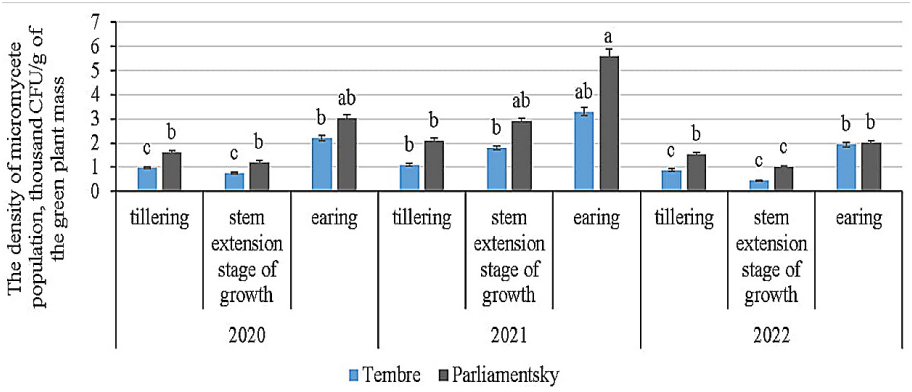


Figure 1. The density of micromycete population in the leaf microbiome of different oat varieties using traditional cultivation technology: x ± SD, Tukey's test, n = 5 replicates); a, b, c – statistically significant differences in the number of microorganisms ($P < 0.05$)

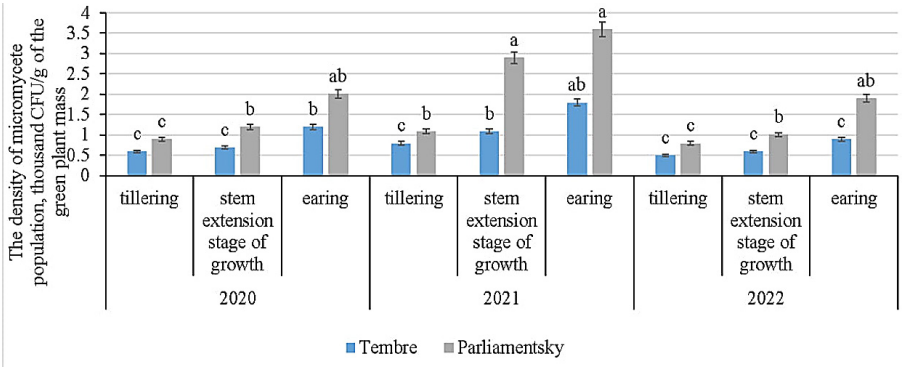


Figure 2. The density of micromycete population in the leaf microbiome of different oat varieties using organic cultivation technology: x ± SD, Tukey's test, n = 5 replicates; a, b, c – statistically significant differences in the number of microorganisms (P < 0.05)

species of micromycetes, such as: *F. oxysporum, F. verticillioides, F. incarnatum, A. flavus, A. alternata, D. avenae, R. nigricans, C. herbarum, H. avenae, S. avenae, P. avenae, A. avenae* were identified in the leaf microbiome of Tembre variety. Their frequency of occurrence ranged from 10 to 60% (Figure 3).

The leaf microbiome of the oats of Parliamentsky variety was dominated by 5 species of micromycetes, namely *D. avenae, A. alternata, A. infectoria, F. oxysporum, F. incarnatum.* Their frequency of the occurrence varied from 55 to 70%. Some common species included micromycetes like *F. verticillioides, F. culmorum,, F. sporotrichiella, F. graminerum, R. nigricans, C. herbarum, S. avenae, A. avenae, H. avenae,* with the frequency of occurrence varying from 23 to 45%. Rare species included micromycetes like *P. avenae, P. notatum, A. flavus, T. roseum* with the frequency of occurrence reaching 18%. At the same time, the leaf microbiome of oats of Tembre variety was dominated by the following 4 species of micromycetes: *D. avenae, A. alternata, F. oxysporum, F. incarnatum,* and their frequency of occurrence reached 60%. The common species included the following micromycetes: *R. nigricans, S. avenae, F. verticillioides, A. avenae.* Their frequency of occurrence ranged from 22 to 28%. Moreover, 4 rare species of micromycetes, *H. avenae, A. flavus, C. herbarum, P. avenae,* were identified, and their frequency of occurrence reached 20%.

In comparison with the traditional technology of plant cultivation using organic growing technology, over the years of research, the spectrum of micromycetes in the leaf microbiome oat was more diverse, but with a lower frequency of occurrence of species (Figure 4).

Namely, in the leaf microbiome of Parliamentsky variety, 19 species of micromycetes: *F. sporotrichiella, F. graminerum, F. oxysporum, F. incarnatum, F. culmorum, F. verticillioides, D. avenae, A. alternata, A. infectoria, R. nigricans, A. flavus, A. niger, C. herbarum, T. roseum, H. avenae, S. avenae, A. avenae, P. avenae, P. notatum,* were found as parasites, with the frequency of the occurrence varying from 8 to 45%. In the leaf microbiome of Tembre oat variety, 15 species of micromycetes like *F. oxysporum, F. verticillioides, F. incarnatum, F. culmorum, A. flavus, A. alternata, D. avenae, R. nigricans, C. herbarum, H. avenae, S. avenae, P. avenae, A. avenae, T. harzianum ma T. Viride,* with the frequency of the occurrence of 10–45%, were identified. In the leaf microbiome of oats of Parliamentsky variety, the common species included micromycete: *D. avenae, A. alternata, A. infectoria, R. nigricans F. graminerum, F. oxysporum* with the frequency of occurrence varying from 35 to 45%. Other identified micromycetes included some rare species like *F. sporotrichiella, F. incarnatum, F. culmorum, F. verticillioides, A. flavus, A. niger, C. herbarum, T. roseum, H. avenae, S. avenae, A. avenae, P. avenae, P. notatum* with the frequency of occurrence reaching 18%. At the same time, the micromycetes species like *T. harzianum, T. viride R. nigricans, H. avenae, D. avenae, A. alternate, F. incarnatum, F. verticillioides, F. culmorum, F. oxysporum* were common in the leaf microbiome of Tembre variety oats. Their frequency of occurrence ranged from 25 to 45%. Also, 5 rare species of micromycetes: *F. culmorum, P. avenae, S. avenae, A. flavus, C. herbarum,* where their frequency of occurrence reached 20% were identified there. It should be noted that the antagonistic fungi of the genus were characterized

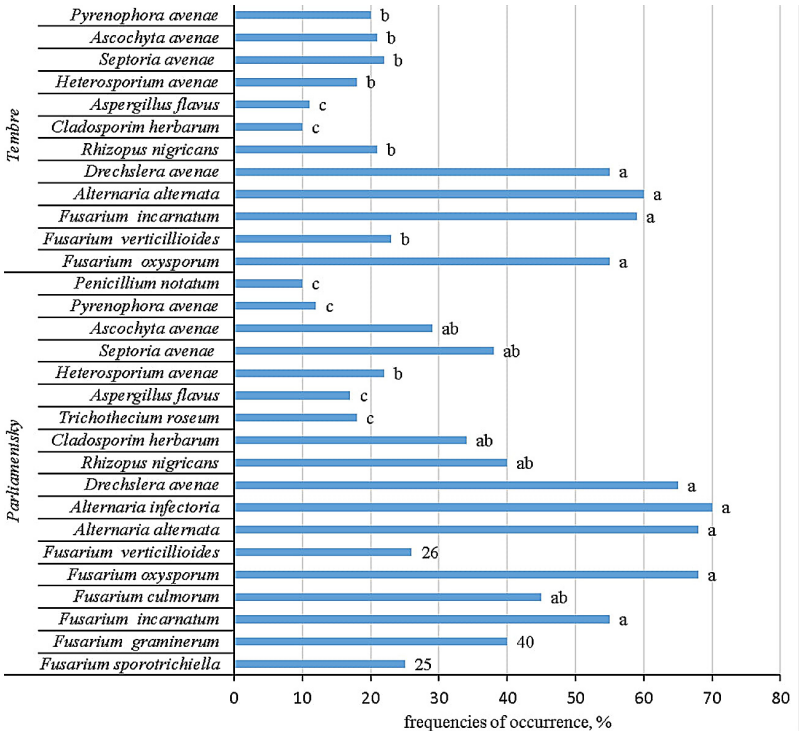


Figure 3. The species spectrum of micromycete populations in the leaf microbiome of different varieties oat using traditional cultivation technology: x ± SD, Tukey's test, n = 5 replicates; a, b, c – statistically significant differences in the number of microorganisms ($P < 0.05$)

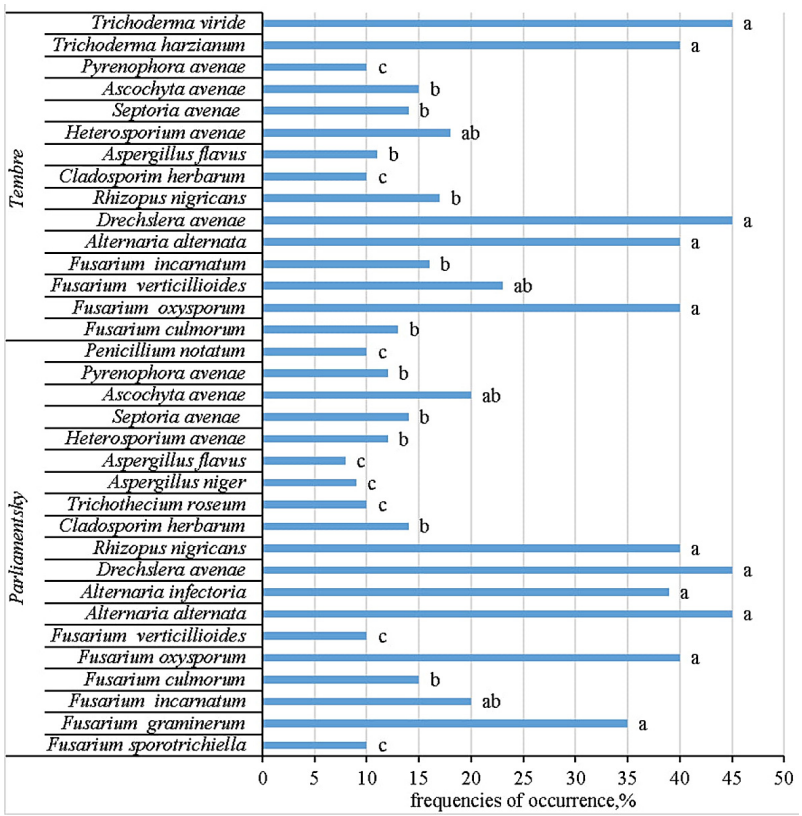


Figure 4. The species spectrum of micromycete populations in the leaf microbiome of different oat varieties using organic cultivation technology: x ± SD, Tukey's test, n = 5 replicates; a, b, c – statistically significant differences in the number of microorganisms ($P < 0.05$)

by a high occurrence of *Trichoderma spp (T. harzianum ma T. viride),* which reached 45%, in terms of using the organic technology of plant cultivation, in the leaf microbiome of Tembr variety in addition to phytopathogenic micromycetes. Simultanously, only the following phytopathogenic micromycetes: *F. oxysporum, D. avenae, A. alternata, A. infectoria,* with the frequency of the occurrence varying from 35 to 45% prevailed in the leaf microbiome of Parliamentsky variety.

Therefore, such climatic conditions, as an abiotic factor, namely an increase in air temperature, frequent droughts, rare but abundant rains, etc., which changed depending on the year of the study, significantly influenced the formation of micromycete populations in the oat leaf microbiome. Plant cultivation technologies, as an anthropogenic factor, significantly influenced the spectrum of species and their frequency of occurrence in the leaf microbiome of the oats of different varieties.

In turn, using the organic technology of plant cultivation, the spectrum of micromycete populations turned out to be more diverse, but with a lower frequency of occurrence of species compared to using the traditional technology of plant cultivation. Also, such varieties of oat plants, as a biotic factor, owing to the physiological substances of plants, are able to restrain the spread of micromycete populations in the leaf microbiome of plants or stimulate them.

The intensity of micromycete sporulation in the leaf microbiome of oat plants

In the course of the laboratory studies, it was found that while using traditional cultivation technology, the spectrum of micromycetes in the leaf microbiome of oats of various varieties was characterized by a high sporulation index, especially in the earing phase, which ranged from 1.1 to 7.2 million units/ml (Fig. 5a).

As it is indicated in Figure 5, in the leaf microbiome of Parliamentsky oats, the micromycetes of the genera named *Fusarium spp., Alternaria spp., Drechslera spp*. were characterized by the highest sporulation intensity, which ranged from 6.3 to 7.2 million units/ml. Simultaneously, the same indicator of Tembre variety was 50% lower. This clearly demonstrates the role of the variety as a biotic factor in the regulation of phytopathogenic micromycetes in the microbiome of the vegetative organs of plants.

While using the organic technology of plant cultivation, in the leaf microbiome of oats of the Parliamentsky variety, micromycetes of the following genera, *Fusarium spp., Alternaria spp., Drechslera spp*., were characterized by a high intensity of sporulation, which ranged from 1.8 to 2.1 million units/ml (Fig. 5b). This is 1.5 times lower than while using the traditional technology of plant cultivation. At the same time, in the leaf microbiome of Tembre variety, the intensity of sporulation of micromycetes, in the earing phase, ranged from 0.6 to 1.7 million units/ml. This gives a reason to believe that oat plants of different breeding origins are able to significantly influence the intensity of sporulation of dominant micromycetes. In the leaf microbiome of Tembre variety oats, antagonistic fungi of the genus *Trichoderma* spp. were characterized by a high intensity of sporulation, which reached 3.9 million units/ml. These micromycetes are able to quickly spread and occupy the entire habitat, displacing other pathogens.

Therefore, researching the intensity of micromycete sporulation in oat agrocenoses under the influence of different cultivation technologies, it was found that not all dominant micromycetes sporulated intensively, which is due to varietal characteristics of plants. It should be noted that the diversity of micromycete species was significantly higher in terms of using organic cultivation technology than under the circumstances of using traditional cultivation. At the same time, the frequency of occurrence and the intensity of of micromycete sporulation under the conditions of using organic technology significantly decreased (2–3.5 times) compared to the figures obtained while using traditional technology. This shows that crop cultivation technologies are one of the main influencing factors on the formation of populations in the agrocenoses of cereal grain crops.

The density of micromycete population in the leaf microbiome of spring barley plants

During the years of research using the traditional technology of plant cultivation, the density of micromycete population in the leaf microbiome of spring barley of both varieties increased significantly during the earing phase and ranged from 7.8 to 22.3 thousand CFU/g of green plant mass (Figure 6).

In the phase of stem of growth, the density of micromycete population in the leaves of both

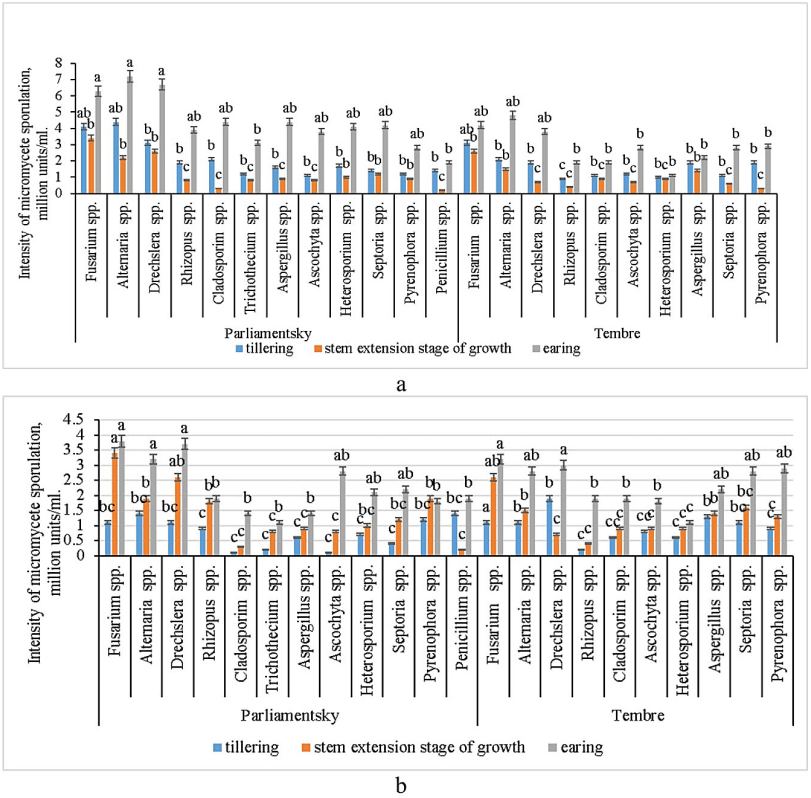


Figure 5. The intensity of micromycete sporulation in the leaf microbiome of different varieties of oat using different cultivation technologies (a – traditional; b – organic): x ± SD, Tukey's test, n = 5 replicates; a, b, c – statistically significant differences in the number of microorganisms (P < 0.05)

varieties of spring barley decreased and ranged from 3.2 to 7.4 thousand CFU/g of green plant mass. This can be explained by the fact that using fungicides in the tillering phase reduced the density of micromycetes in the stem of growth phase.

Using the organic cultivation technology, the density of the population of micromycetes in the leaf microbiome of plants spring barley of both varieties was 50% lower compared to the traditional technology and increased during the growing season from the tillering phase to the earing phase (Figure 7).

In the leaf microbiome of spring barley of Sebastian variety, the density of micromycete populations ranged from 4.9 to 15.1 thousand CFU/g of the green mass. At the same time, in the leaf microbiome of Salomi variety, the density of micromycete population ranged from 3.6 to 10.8 thousand CFU/g of the green mass. This indicates that spring barley plants of Salomi variety restrain the development of micromycete population density at an ecologically safe level compared to the plants of Sebastian variety, which significantly stimulates the development of phytopathogenic micromycetes and can cause biological contamination of agrocenoses of the grain crops.

Therefore, in terms of using organic cultivation technology, an increase in the density of micromycete population in the leaf microbiome of both varieties was observed from the tillering phase to the earing phase, which indicates the absence of pesticide pressure on the cereal agrocenosis. Salomi variety plants significantly restrained the development of the population of micromycetes growing on vegetative organs compared to the plants of Sebastian variety.

The species spectrum of micromycetes in the leaf microbiome of spring barley plants

During the years of research using the traditional technology of plant cultivation, 14 species of micromycetes, namely *F. graminerum, F. oxysporum, F. moniliforme, F. gibbosum, F. avenaceum, D. sorociniana, D. teres, A. alternate, A. niger, R. serealis, P. herpotrichoides, S. nodorum, A. flavus, P. notatum,* parasitized in the leaf microbiome of Sebastian variety, which were characterized by a different frequency of occurrence

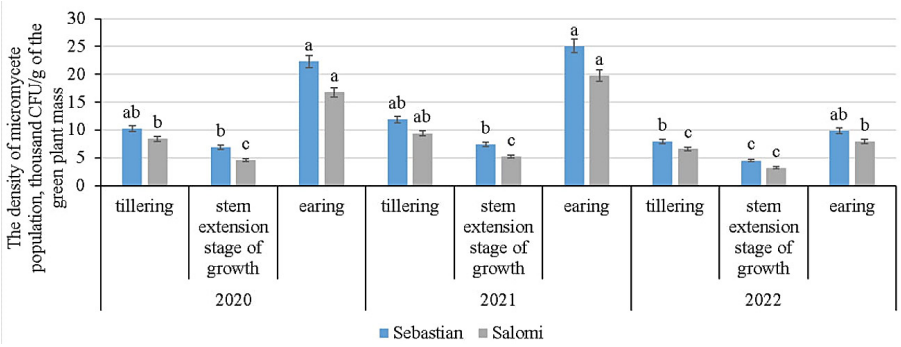


Figure 6. The density of micromycete population in the leaf microbiome of different varieties of spring barley using traditional cultivation technology: x ± SD, Tukey's test, n = 5 replicates; a, b, c – statistically significant differences in the number of microorganisms (P < 0.05)

from 11 to 86%. At the same time, 13 species of micromycetes, such as *F. graminerum, F. oxysporum, F. gibbosum, F. avenaceum, D. sorociniana, D. teres, A. alternate, R. serealis, P. herpotrichoides, S. nodorum, A. flavus, A. niger, P. notatum* were identified in the leaf microbiome of Salomi variety (Figure 8)

The leaf microbiome of Sebastian variety of was dominated by the following 6 species of micromycetes: *D. sorociniana, F. avenaceum, F. oxysporum, F. graminerum, D. teres, A. alternate,* the frequency of their occurrence varied from 65 to 86%. Common species included the following micromycetes *A. niger, R. serealis, P. herpotrichoides, S. nodorum, F. moniliforme, F. gibbosum,* with a frequency of occurrence of 28–48%. Also, in the leaf microbiome of Sebastian spring barley, two rare species *A. flavus* and *P. notatum* were found, with a frequency of occurrence up to 20%. At the same time, the leaf microbiome of spring barley Salomi was dominated by 8 species of micromycetes including *T. harzianum, F. avenaceum, D. sorociniana, D. teres, A. alternate, F. moniliforme, F. gibbosum, F. oxysporum,* with the frequency of occurrence varying from 53 to 80%. In addition, 5 rare species of micromycetes (*P. notatum, S. nodorum, A. flavus, R. serealis, and R. secalis*) were identified, their frequency of occurrence did not exceed 20%.

Under the conditions of organic cultivation technology, in the leaf microbiome of spring barley of both varieties, the spectrum of micromycetes was the most diverse compared to the traditional plant cultivation technology, but the frequency of occurrence of species was lower and ranged from 11 to 70% (Figure 9).

The leaf microbiome of spring barley the variety of Sebastian was parasitized by 19 species of micromycetes, among them were found: the dominant species – *T. harzianu, D. sorociniana, D. teres, D. graminea, A. alternate, F. oxysporum, F. avenaceum, F. graminerum,* with the frequency of occurrence of 58–70%; the common species are *R. secalis, R. serealis, P. herpotrichoides, S. nodorum, F. moniliforme, F. gibbosum, A. niger, F. sporotrichella, F. solani,* the frequency of occurrence of 23–48%, and the rare ones are *A. flavus, P. notatum* (16–19%) (Figure 9). At the same time, in the leaf microbiome of Salomi variety were parasitized by the following 16

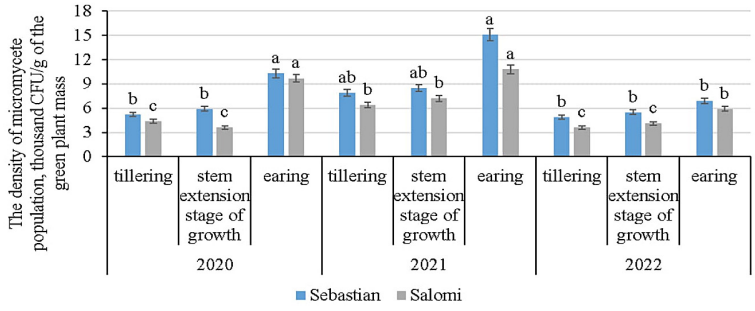


Figure 7. The density of micromycete population in the leaf microbiome of different varieties of spring barley using organic cultivation technology: x ± SD, Tukey's test, n = 5 replicates; a, b, c – statistically significant differences in the number of microorganisms (P < 0.05)

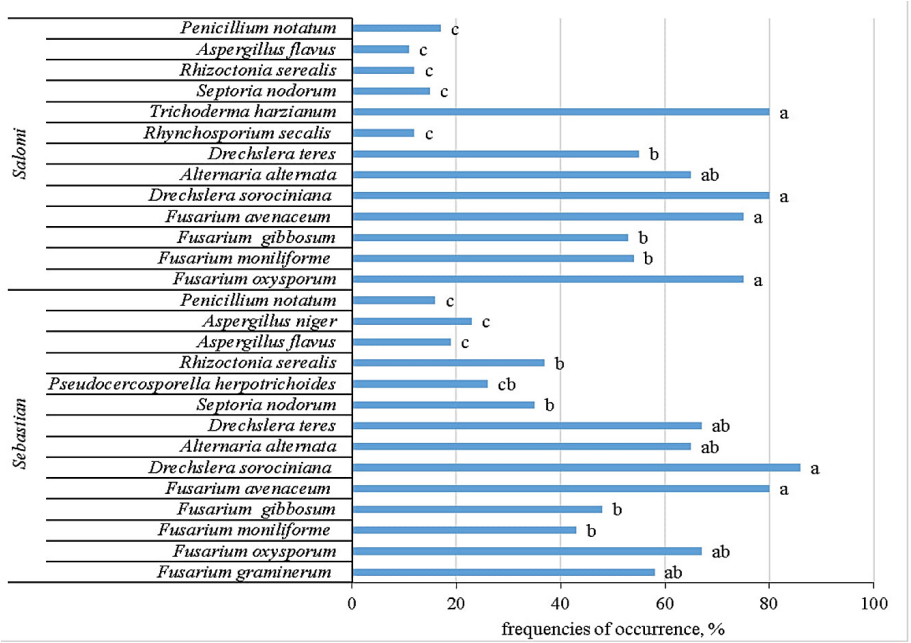


Figure 8. The species spectrum of micromycete populations in the leaf microbiome of different varieties of spring barley using traditional cultivation technology: x ± SD, Tukey's test, n = 5 replicates; a, b, c – statistically significant differences in the number of microorganisms (P < 0.05)

species of micromycetes: *T. harzianum, D. sorociniana, D. teres, D. graminea A. alternate, F. oxysporum, F. avenaceum, F. moniliforme, F. gibbosum, F. graminerum R. secalis, A. flavus*, *P. notatum, P. herpotrichoides, S. nodorum, R. serealis* the frequency of occurrence varied from 11 to 65%. The dominant species of micromycetes included: *T. harzianum, D. sorociniana, D. teres, D. graminea A. alternate, F. oxysporum, F. avenaceum, F. moniliforme, F. gibbosum,* the frequency of occurrence reached up to 65%. The common species included the micromycete *F. Graminerum,* the frequency of occurrence reached up to 35%. Also, 6 rare species of micromycetes: *R. secalis, A. flavus*, *P. notatum P. herpotrichoides, S. nodorum, R. Secali*s were identified, with their frequency of occurrence ranged from 11 to 17% (Figure 9).

Using the traditional technology of plant cultivation in the leaf microbiome of the studied varieties of the spring barley, the index of species biodiversity was less than the one while using the conditions of organic cultivation technology, but the frequency of the occurrence was significantly higher, which indicates greater competition between species.

The plants of spring barley of Salomi variety, with their biologically active substances, significantly restrained the development of micromycete populations in the leaf microbiome compared to plants of the Sebastian variety using both of the cultivation technologies.

Intensity of micromycete sporulation in the leaf microbiome of spring barley plants

Laboratory studies showed that in the leaf microbiome of spring barley using different cultivation technologies, the intensity sporulation of dominant micromycetes varied from 0.2 to 22.5 million units/ml (Figure 10-a,b).

While using the traditional cultivation technology, in the leaf microbiome of Sebastian spring barley micromycetes of the following genera *Fusarium spp., Drechslera spp*., *Alternaria spp.* та *Septoria spp*. *and Septoria spp*., were characterized by a high intensity of the sporulation, and their indicator ranged from 17.7 to 22.5 million units/ml. At the same time, in the leaf microbiome of the variety Salomi micromycetes of the genus *Fusarium spp., Drechslera spp*. were characterized by a high intensity of the sporulation, which ranged from 13.6 to 14.6 million units/ml (Fig. 10-a). This shows that plants of spring barley, due to varietal characteristics, are able to influence micromycete populations and their reproductive capacity in different ways.

While using the organic technology of cultivation in the leaf microbiome of Sebastian variety, the intensity of the sporulation of micromycetes

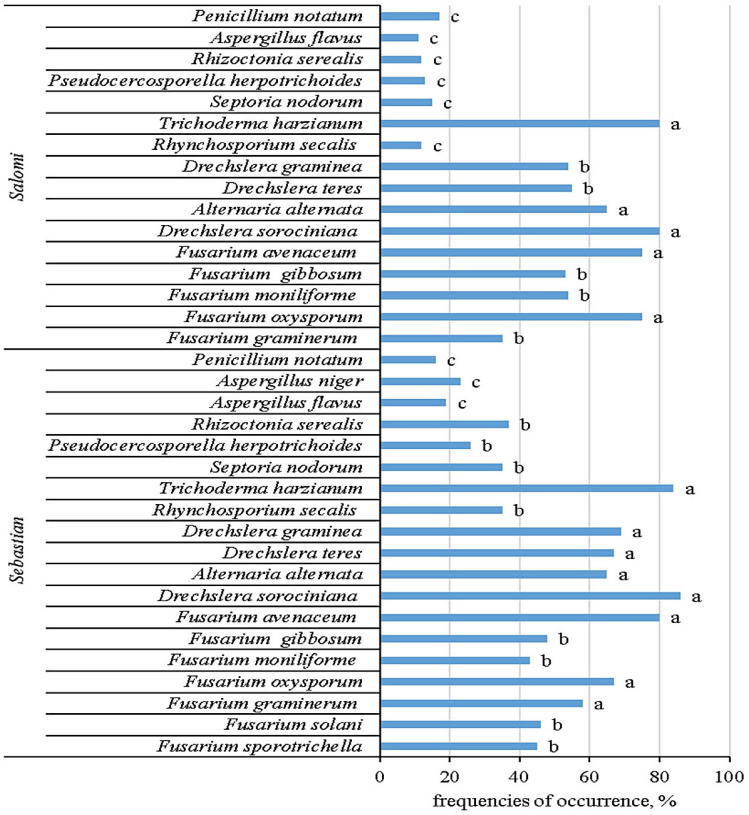
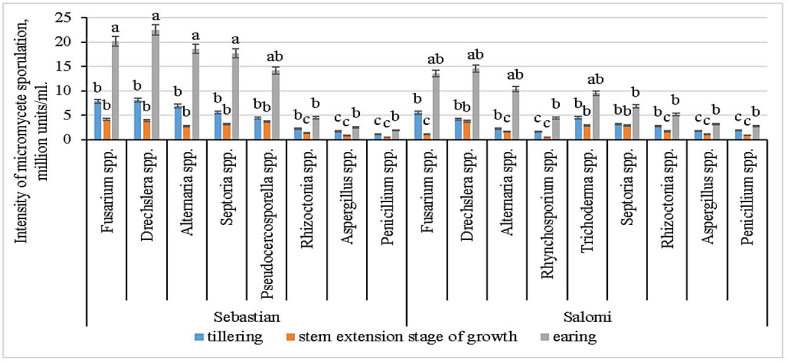
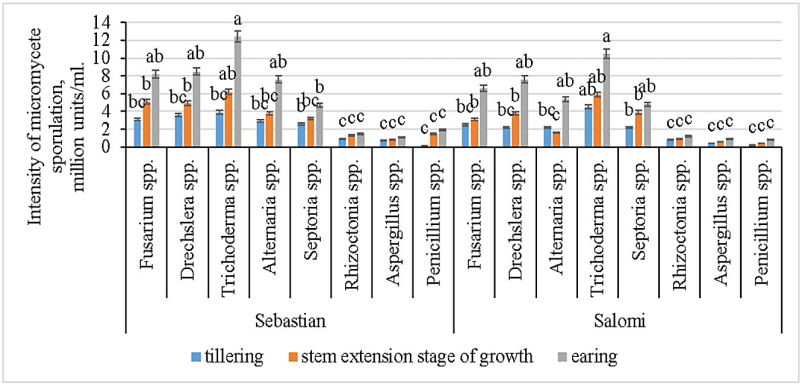


Figure 9. The species spectrum of micromycete populations in the leaf microbiome of different varieties of spring barley using organic cultivation technology: x ± SD, Tukey's test, n = 5 replicates; a, b, c – statistically significant differences in the number of microorganisms ($P < 0.05$)



a



b

Figure 10. The intensity of micromycete sporulation in the leaf microbiome of spring barley plants using different cultivation technologies (a – traditional; b – organic): x ± SD, Tukey's test, n = 5 replicates; a, b, c – statistically significant differences in the number of microorganisms ($P < 0.05$)

of the genera named *Fusarium spp., Drechslera spp*., *Alternaria spp.* and *Septoria spp*., was 2–3 times lower compared to the one recorded while using traditional cultivation technology, and it amounted to 4.7–8.5 million units/ml. In the leaf microbiome of Salomi variety, the intensity of the sporulation of the specified genera of micromycetes ranged from 2.4 to 6.6 million units/ml. It should be noted that the micromycete genus *Trichoderma spp*. is characterized by a high intensity of the sporulation (10.5–12.4 million units/ml.) (Figure. 10-b). The plants of Sebastian variety had a significant effect on increasing the frequency of the occurrence of species density, the population, and the sporulation intensity compared to Salomi variety, which was characterized by lower indices when evaluating these indicators. Regardless of the growing season and the introduction of certain preparation, the trend was maintained regarding the varietal characteristics of the plants. Physiological and biological features of the plants of Sebastian variety stimulated the development of micromycete populations in the leaf microbiome of spring barley, while plants of Salomi variety restrained it.

CONCLUSIONS

The analysis of the frequency of the species occurrence in the leaf microbiome of plants under the conditions of using different technologies of the plant cultivation allows singling out the dominant species and revealing the intensity of their distribution in the agrocenoses of cereal grain crops. Phytopathogenic fungi in the leaf microbiome of cereal crops using the traditional growing technology are characterized by a high frequency of the occurrence of the following micromycetes *F. oxysporum, A. alternata*. At the same time, using organic cultivation technology, the antagonistic fungi of the species *T. harzianum*, *T. viride*, prevailed, which competed among the phytopathogenic mycobiota.

The indicators such as the population density and the sporulation intensity of micromycetes characterize the ability to form and accumulate infectious structures in the leaf microbiome of the plants. Regardless of abiotic (temperature, humidity) and anthropogenic (cultivation technology) factors, the population density and sporulation intensity of micromycetes was significantly lower in the leaf microbiome of oat of Tembre variety and spring barley of Salomi variety compared to the oat plants of Parliamentsky variety and spring barley of Sebastian variety, which increased by 2–4 times.

Therefore, the assessment of the formation of the micromycete populations in the leaf microbiome of cereal crops according to such indicators as the population density, the frequency of the species occurrence and their sporulation intensity under the conditions of using different cultivation technologies is an important ecological criterion. This will make it possible to characterize the variety as a factor in regulating the number of phytopathogenic micromycetes in agrocenoses of cereal crops.

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