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To the study of saprotrophic micoromycetes complexes associated with wild and cultivated vines of grapes in the Western Ciscaucasia (Russia)

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Abstract. The assessment of the diversity of fungic communities and the study of the bioindicational significance of mycological parameters is relevant for the biomonitoring of ampelocenoses. We studied the quantitative and qualitative composition of saprotrophic micromycetes communities associated with annual shoots of wild and cultivated grape plants. Studies have shown that the number of saprotrophic micromycetes associated with an annual vine is different depending on the place of sampling and ranges from 232.9 ... 3203.2 thousand CFU / g dry matter on wild grapes and within 118.9 ... 344.1 thousand CFU/g dry matter on cultivated grapes. The highest density indicators of fungal populations - 1119.7 and 3203.2 CFU/g dry matter - were recorded on a vine from forest ecotopes, the lowest indicator of 118.9 CFU/g dry matter was recorded on a vine from ampelocenosis, On wild vines, major share in the studied of fungic communities was occupied by hyphal or mold fungi, on average their share was 95.1%, while yeast occupied 4.2%, and yeast-like fungi - 0.7% of the complex. On cultivated vines, hyphal fungi also occupied the largest part in the structure of fungic communities, but their share was 1.7 times less than on wild plants. Yeast (26.5%) and yeast-like fungi (18.4%) took much larger share.

1. Introduction

In recent years, in agricultural and forest phytopathology and mycology, there has been a growing research interest in studying fungi role in the processes of biota functioning, modeling the evolutionary development of taxonomic units, revealing the influence of anthropogenic and technogenic factors on the distribution and biology of fungi, information about the biodiversity and variability of the properties of fungi is accumulating [1-4]. An important role of these organisms in ecosystems was formed in the process of their co-evolution with plants as two integral components - autotrophs and heterotrophs. Fungi are capable of assimilating various ecological niches and by their ecological plasticity they stand out among all living organisms [5, 6].

There is no need to prove that fungi, along with perennial grape plants, are the most important components of ampelocenoses. The phylloplane of grape plants is colonized by a large number and varied populations of micromycetes of various functional orientations. Changes currently taking place

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in the biosphere as a result of active anthropogenic activity and climatic changes are increasingly affecting the habitat of fungal organisms [7-10]. The processes accompanying the anthropogenic and abiotic transformation of fungic communities in perennial agrocenoses (vineyards) stimulate adaptation genesis of fungi, contributing to the emergence of more aggressive biotypes among typical phytopathogenic dominants, as well as the emergence of new harmful mycopathogens [11, 12].

The strategy of modern adaptive agriculture emphasizes the important role of the biocenotic approach in the development of ecologized and biologized technologies for protecting grapes from diseases. Understanding the impact of anthropogenic interference on fungic diversity in ampelocenoses is of paramount importance for conserving renewable resources and increasing the phytosanitary resilience of biosystems. In addition, the experience of studying the change in the diversity of fungal communities and the variability of fungi under conditions of technogenic load of different levels and quality gives an idea of the wide possibilities of their usage in assessing the quality of natural environments [13]. Therefore, the problem of assessing the diversity of fungal communities, studying the bioindication significance of mycological indicators is relevant for the biomonitoring of ampelocenoses.

The aim of the research was to study the quantitative and qualitative composition of communities of saprotrophic micromycetes associated with annual shoots of wild and cultivated grape plants.

2. Materials and methods

The studies were carried out in 2020 in various regions of Krasnodar region (Western Ciscaucasia). The objects of the study were wild-growing grape plants from the natural conditions of forest ecotopes of the state reserve "Utrish" (Anapsky district) and Krymsky district, as well as cultivated grape plants of grapes of 4 varieties of different genotypes, cultivated in the plantations of the AO farming company "South" (Temryuk district). Grape varieties:

- Pervenec Magaracha is a complex interspecific hybrid Vitis vinifera convar. pontica Negr. subconvar. georgica-caspica x (V. vinifera x (V. vinifera + V. riparia + V. rupestris));
- Bianka is a complex interspecific hybrid (V. vinifera + V. labrusca + V. riparia + V. rupestris + V. berlandieri + V. aestivalis + V. cinerea) x V. vinifera;
- Kober 5 BB rootstock variety V. berlandieri + V. riparia;
- Merlo V. vinifera convar occidentalis subconvar. Gallica.

The climate type of Temryuk region (Taman Peninsula) is moderately continental, warm, arid. The average annual air temperature is $+ 11.1 \degree$ C. The frost-free period lasts 193 - 233 days. Average annual precipitation is 400-450 mm. About a third of their annual rate falls on summer, 112 days a year - precipitation is less than 0.1 mm. The land forms of the Taman Peninsula are flat-ridge-hilly, formed by low (up to 150-160 m) ridges with gentle slopes and slightly concave valleys. The soils of Taman are classified as southern chernozems. They are characterized by low humus content (2.5 - 3%), significant thickness (100-130 cm) and the presence of carbonates in the form of mold in the layer 40-45 cm and deeper. Weak and medium alkalinity is due to the presence of calcium bicarbonate in the solution.

The climate type of the territory of the "Utrish" reserve can be defined as Mediterranean with the influence of the climate of temperate latitudes [14]. The average annual air temperature is - 12.1 - 12.2 ° C, the average temperature in January is 2 - 3 ° C, in July - 23.3 - 23.5 ° C. The average annual precipitation is in the range of 570 - 700 mm. The altitude above sea level in the study area at the Shirokaya Shchel tract (inter-ridge depression) ranges from 40 m to 100 m.

The climate of the Crimean region is moderately continental. The height above sea level ranges from 300 to 800 m. The land forms are heavily indented, hilly upland of the north and north-western slopes of the Caucasus Range. The total annual precipitation is 657 mm (for the growing season - 250-355 mm). The average annual air temperature is $10.6 \degree$ C. The main soil variety is merged chernozems, thick, dark gray forest and podzolized soils of loamy texture, soddy-calcareous soils are found [15].

Five populations of wild grapes growing not very far from each other (400-600 m between plants) were found and investigated in Shirokaya Shchel. Two populations were studied in Krymsky district. In

total, the assessment of the complexes of saprotrophic micromycetes was carried out on 7 samples of wild-growing vines and 4 samples from agrocenoses, the ecological and geographical characteristics of which are presented below (table 1).

Table 1. Ecological and geographical characteristics of wild and cultivated grapes habitats.

| N⁰ | The studied | l samples | | Terrain | Slope | Slope | Vegetation | Soil type |
|----|--|---------------|---|--------------------------------|--------------------|----------|-------------------|------------------------------|
| | Location where the grape samples were taken | sample no. | sample name | | steepness, deg. | exposure | type | |
| 1 | Krymsky district "Forest" | 1 | Red forest, slope | On the mountain | 35 | SE | forest | typical chernozem |
| | | 2 | Red forest, stream | flatlands near the brook | | | forest | leached chernozem |
| 2 | "Utrish" reserve Shirokaya Shchel | 3 | Wide crevice 1 | Low- mountain | 5 | SE | Oak-ash forest | Brown carbonated stony |
| | | 4 | Wide crevice 2 | Low- mountain | | SE | | |
| | | 5 | Wide crevice 3 | Low- mountain | | SE | | |
| | | 6 | Wide crevice 4 | Low- mountain | | SE | | |
| | | 7 | Wide crevice 5 | Low- mountain | | SE | | |
| 3 | AO farming company "South"(Taman Peninsula) | 8 | Commercial vineyard (grape variety Pervenec Magaracha) | flatlands | - | - | vineyard | southern chernozem |
| | | 9 | Commercial vineyard (grape variety Bianka) | flatlands | - | - | | southern chernozem |
| | | 10 | Commercial vineyard (grape variety Kober 5 BB) | flatlands | - | - | | southern chernozem |
| | | 11 | Commercial vineyard (grape variety Merlo) | flatlands | - | - | | southern chernozem |

The vegetation of the locations from which the vine samples were taken for analysis differed markedly among themselves (table 2).

| N⁰ | Location | Tree | layer | Sł | nrub layer | Herbaceous layer | | |
|----|--|---|---|---|---|---|--------------------------------|--|
| | where the grape samples | S | | | | | | |
| | were taken | - | | | | | | |
| 1 | Krymsky | Quercus robur | English | Cornus | European | Viola odorata L. | sweet | |
| | district "Forest" | L. Fraxinus | oak Euronean | mas L. Cornus | cornel Southern | | violet | |
| | Forest | excelsior L. | European ash | australis C.A.Mey, Thelycrani | gaiter-tree | Lithospermum purpurocaerule um (L.) Holub | pigeonw eed | |
| | | а · | Г | a australis | N 11 | F 1 1' | 1 | |
| | | Carpinus betulus L. | European hornbeam | C. monogina Jacg. | Mayblossom | Euphorbia amygdaloides L. | wood spurge | |
| | | Acer | English | C. | Pentagynianh | Geum | Europea | |
| | | campestre L. | field maple | pentagyna Waldst. et Kit. | awhorn | urbanum L. | n avens | |
| | | Acer tataricum L. | Tartarian maple | Corylus avellana L. | nutwood | Geranium robertinum L. | Robert's crane's- bill | |
| | | Pyrus caucasica | Pyrus communis | Berberis vulgaris L. | European barberry | Galium aparine L. | cleavers | |
| | | Fed. Malus orientalis Uglitzk | oriental apple tree | Sambucus nigra L. | European elder | Hesperis matronalis L. | damewo rt | |
| | | Ulmus L. | Ulmus glabra | Viburnum opulus L. Rosa L. Rubus caesius L. Prunus cerasifera | European dogwood rosehip European dewberry cherry plum | | | |
| | | | | Ehrh. Prunus spinosa L. | blackthorn | | | |
| | | | | Euonymus europaeus L. | louseberry | | | |
| | | | | Humulus lupulus L. | wild hop | | | |
| 2 | "Utrish" reserve Shirokaya Shchel | Fraxinus excelsior L. Carpinus orientalis Mill. | European ash oriental hornbeam | Cornus mas L. Sambucus nigra L | European cornel European elder | Clematis vitalba L. Galium aparine L. | traveller's joy cleavers | |

Table 2. Phytocenotic features of grape growing places.

| | Quercus | stalkless | Staphylea | European | Astragalus | white |
|-----------------------|-------------------------|--------------------|------------------------------|----------------------|--------------------------------------|----------------|
| | petraca | flowered | pinnata L | bladdemut | utriger Pall. | locoweed |
| | (Mattuschka) Lindl. | oak | | | | |
| | Salix alba L. | European willow | Sorbus torminalis (L.) | wild service tree | Carex acutiformis Ehrh. | sedge grass |
| | | | Crantz. | | Potentilla taurica Willd. | potentilla |
| | | | | | Alyssym obtusifolium Stev.exDC | allison |
| AO farming | Vitis vinifera | grape- | Rubus | European | Convolvulus | European |
| company "South"(Ta | L. | vine | fruticosus L. | blackberry | arvensis L. | glorybind |
| man Peninsula) | Juglans nigra L. | American walnut | Prunus spinosa L. | blackthorn | Sonchus arvensis L. | dindle |
| , | Gleditsia | black | Physocarp | Viburnifolio | Cynanchum | swallow- |
| | triacanthos L. | locust | us opulifolius (L.) | us spirea | acutum L. | wort |
| | | | Maxim. | | | |
| | Prunus armeniaca L. | apricot | Tamarix gallica L. | tamarix | Amaranthus retroflexus L. | amaranth |
| | Robinia pseudoacacia | false acacia | | | | |
| | L. | | | | | |

Vine samples were taken in October-November and represented one-year lignified shoots without visible damage / lesions, 20-25 cm in size (2-3 eyes from the shoot base). Selection of biosamples of wild-growing vines was carried out by route-reconnaissance method, cultural vines - using route-based surveys of industrial vineyards.

The number and structure of micromycetes complexes associated with one-year-old vine were determined by sowing a diluted suspension on a solid nutrient medium. To do this, 5 samples of annual vines were taken in each variant of the experiment, washed with water, then they were ground in a laboratory mill, the resulting mixture was thoroughly mixed and an average sample weighing 10 g was prepared. Before each use, the mill was thoroughly washed. The prepared sample was introduced into a sterile 250 ml flask with 100 ml of sterile water. Then the flask with the samples was placed on a DSR-2800P laboratory shaker for 15 min. For seeding, a 1:10 dilution suspension was used. The resulting suspension was plated 0.3 ml in Petri dishes on potato sucrose agar (PSA) in 3 replicates and evenly distributed with a spatula. All operations were performed under aseptic conditions. The seeded dishes were incubated in a thermostat at 25 ° C for 7-10 days. Laboratory glassware, accessories and culture media were sterilized in an autoclave at Psig. = 1 atm. during one hour. To inhibit the growth of bacteria, antibiotics, streptomycin sulfate (20 mg / l) and levomycin (20 mg / l), were added to the nutrient medium. In addition, samples of biosamples weighing 10 g were taken according to the variants of the experiment to determine the absolutely dry plant mass. This indicator was necessary to calculate the propagules of micromycetes per 1 g of dry matter. The calculation of the quantitative content of propagules was carried out according to the formula (1):

$$K = \frac{(60 \cdot n)}{a \cdot b \cdot c} \tag{1}$$

where:

K - CFU in 1 g of dry matter;

60 - suspension volume, ml.;

n is the number of colonies, pcs;

a - number of seeded Petri dishes in one version, pcs. (a = 3);

b is the volume of the suspension inoculated into one cup, ml. (b = 0.3);

c - weight of absolutely dry plant mass, g.

The grown micromycetes were microscoped at a magnification of x1350. A quantitative account of the colonies of filamentous fungi, yeast and yeast-like fungi was made. The species of fungi was determined according to domestic and foreign keys [16-20] using a Micros MC 20. The data were statistically processed using Microsoft Excel 2000, the mean and standard deviation were calculated.

3. Results and discussion

Studies have shown that the number of saprotrophic micromycetes associated with an annual vine is different depending on the place of sampling and ranges from 232.9 ... 3203.2 thousand CFU / g dry matter on wild grapes and within 118.9 ... 344.1 thousand CFU / g dry matter on cultivated grapes (figure 1).

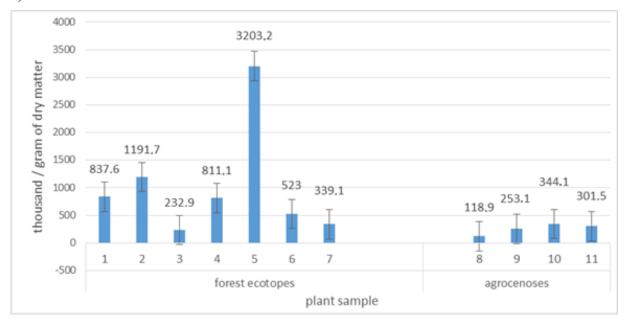


Figure 1. Total number of saprotrophic micromycetes in complexes associated with grapes (annual vine), Western Ciscaucasia, 2020.

The highest density indicators of fungal populations - 1119.7 and 3203.2 CFU / g dry matter - were recorded on a vine from forest ecotopes, the lowest indicator of 118.9 CFU / g dry matter was recorded on a vine from ampelocenosis, the determining factor here was humidity of samples.

The structure of saprotrophic micromycetes associated with annual vines was significantly different between wild and cultivated grapes. On wild vines, the main share in the studied fungal communities was occupied by hyphae or mold fungi, on average their share was 95.1%, while yeast occupied 4.2%, and yeast-like fungi occupied 0.7% of the complex (table 3).

On cultivated vines, hyphal fungi also occupied the largest part in the structure of fungal communities, but their share was 1.7 times less than on wild plants. A much larger share was occupied by yeast (26.5%) and yeast-like fungi (18.4%) (table 4).

| Sample no. | Sample name | In % of the total number of micromycetes | | | | | |
|------------|--------------------------------------|--|-------|------------|--|--|--|
| | | Hyphal (mouldy) | Yeast | Yeast-like | | | |
| 1 | Krymsky district «Forest», slope | 95.9 | 3.7 | 0.4 | | | |
| 2 | Krymsky district «Forest», stream | 93.7 | 5.9 | 0.4 | | | |
| 3 | Wide crevice 1 | 90.8 | 8.1 | 1.1 | | | |
| 4 | Wide crevice 2 | 94.2 | 4.2 | 1.6 | | | |
| 5 | Wide crevice 3 | 99.8 | 0.2 | 0.0 | | | |
| 6 | Wide crevice 4 | 96.9 | 3.1 | 0.0 | | | |
| 7 | Wide crevice 5 | 94.2 | 4.2 | 1.6 | | | |
| | Average | 95.1 | 4.2 | 0.7 | | | |

Table 3. Structure of saprotrophic micromycete complexes associated with an annual grape vine by morphological features, forest ecosystems, Western Ciscaucasia, october-november, 2020.

Table 4. Structure of saprotrophic micromycete complexes associated with an annual grape vine by morphological features, ampelocenoses, Western Ciscaucasia, october-november, 2020.

| Sample no. | Sample name | In % of the total number of micromycetes | | | | |
|------------|---|--|-------|------------|--|--|
| | | Hyphal (mouldy) | Yeast | Yeast-like | | |
| 8 | Commercial vineyard (grape variety Pervenec Magaracha) | 36.3 | 42.0 | 21.7 | | |
| 9 | Commercial vineyard (grape variety Bianka) | 31.5 | 29.5 | 39.0 | | |
| 10 | Commercial vineyard (grape variety Kober 5 BB) | 99.5 | 0.0 | 0.5 | | |
| 11 | Commercial vineyard (grape variety Merlo) | 53.0 | 34.7 | 12.3 | | |
| | Average | 55.1 | 26.5 | 18.4 | | |

Analysis of fungal communities on an annual vine of grapes growing in cenoses with different anthropogenic load revealed the following ratio of taxa (tables 5, 6).

Table 5. Taxonomic structure of hyphal fungi associated with an annual vine of wild grapes in % of their number, 2020.

| sam ple no. | sample name | Phoma sp. | Botryodi plodia sp. | Penic illium sp. | Alterna ria sp. | Clados poriu m sp. | Fusari um sp. | Aspergi llus niger | Coryne um sp. | Sterile myceli um |
|-------------------|---|--------------|---------------------------|------------------------|--------------------|--------------------------|------------------|--------------------------|------------------|-------------------------|
| 1 | Krymsky district «Forest», slope | 59.4 | 33.7 | 0.0 | 1.0 | 1.3 | 0.0 | 0.0 | 4.0 | 0.0 |

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| 2 | Krymsky district «Forest», stream | 34.1 | 52.6 | 0.0 | 0.3 | 7.9 | 0.2 | 0.3 | 3.7 | 0.1 |
|---|--|------|------|------|-----|------|------|------|-----|-----|
| 3 | Wide crevice 1 | 86.0 | 7.0 | 0.0 | 1.3 | 10.0 | 0.3 | 0.0 | 0.7 | 6.0 |
| 4 | Wide crevice 2 | 23.8 | 58.1 | 0.2 | 3.9 | 13.8 | 0.0 | 0.0 | 0.0 | 0.0 |
| 5 | Wide crevice 3 | 70.6 | 22.1 | 0.0 | 0.4 | 3.4 | 0.0 | 0.0 | 1.7 | 0.0 |
| 6 | Wide crevice 4 | 79.4 | 15.4 | 0.0 | 1.3 | 3.6 | 0.0 | 0.0 | 0.0 | 0.0 |
| 7 | Wide crevice 5 | 62.6 | 21.4 | 0.0 | 1.6 | 8.4 | 0.0 | 0.0 | 2.3 | 0.4 |
| A | Average | 59.3 | 30.0 | 0.03 | 1.3 | 6.8 | 0.07 | 0.04 | 1.8 | 0.8 |

Table 6. Taxonomic structure of hyphal fungi associated with the annual vine of cultivated grapes in % of their number, 2020.

| Samp le no. | Sample name | Penicilli um sp. | Alterna ria sp. | Cladosp orium sp. | Fusarium sp. | Aspergill us niger | Sterile myceliu m |
|----------------|--|---------------------|--------------------|-------------------------|-----------------|-----------------------|-------------------------|
| 8 | Commercial vineyard (grape variety Pervenec Magaracha) | 7.8 | 11.4 | 55.6 | 3.1 | 16.7 | 5.4 |
| 9 | Commercial vineyard (grape variety Bianka) | 0.0 | 0.9 | 96.8 | 0.8 | 1.5 | 0.0 |
| 10 | Commercial vineyard (grape variety Kober 5 BB) | 0.0 | 0.8 | 97.1 | 1.3 | 0.8 | 0.0 |
| 11 | Commercial vineyard (grape variety Merlo) | 6.3 | 1.8 | 86.6 | 3.2 | 2.1 | 0.0 |
| | Average | 3.5 | 3.7 | 84.0 | 2.1 | 5.3 | 1.4 |

An analysis of all hyphal fungi taxa list that were found during the study of the taxonomic composition of fungal complexes of grapevine from different ecotopes made it possible to determine the frequency of occurrence and the frequency of dominance of different genera of micromycetes in the studied econiche - on an annual vine. Of the 8 fungal taxa identified by us, the first place in frequency of occurrence on wild-growing grapes was taken by the fungi Phoma sp., Botryodiplodia sp., Cladosporium sp., Alternaria sp. (100%), second place - Coryneum sp. (70%). The next group of micromycetes with a frequency of occurrence of 14% consisted of: Penicillium sp., Fusarium sp. and Aspergillus niger Tiegh. Of the 5 identified taxa of hyphal fungi associated with annual vine from industrial plantations of grapes, Cladosporium sp. Aspergillus niger, Alternaria sp., Fusarium sp. (100%), in second place is Penicillium sp. (fifty %).

Micromycetes of the genus Phoma Sacc were found as a dominant in forest ecosystems on an annual vine. Fungi of the genus Botryodiplodia Sacc were noted as subdominants on the wild vine. The identified fungi of other genera Cladosporium Link, Alternaria Nees, and Coryneum Nees should also be noted. Fungi from the genera Penicillium Link, Fusarium Link, and the species Aspergillus niger

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Tiegh were isolated as minor components. When studying the complexes of saprotrophic micromycetes associated with annual vine in ampelocenoses, fungi of the genus Cladosporium Link were distinguished as the dominant species. Note also the species Aspergillus niger and fungi from the genera Fusarium Link Alternaria Nees and Penicillium Link. In both groups of biosamples of grapes, Alternaria fungi characteristic of the phyllosphere of various plants were identified as common species.

4. Conclusion

Thus, the analysis of the taxonomic structure of fungal communities in ecosystems with different anthropogenic load made it possible to identify both general patterns and some differences. For both studied groups of biosamples of annual grape vine, a monodominant structure of micromycete complexes was revealed, but differences in the composition of dominants were noted. It should also be noted that the differences in the number and ratio of the taxa of fungi in the studied biosamples are probably related to different anthropogenic loads (pesticide treatments, artificial formations, etc.). The results obtained show the expediency of further research on the study of the diversity of fungic communities and the identification of the bioindication significance of mycological parameters in ampelocenoses.

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