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EXPERIMENTAL ARTICLES

Seasonal Dynamic of the Numbers of Epiphytic Yeasts

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Abstract—The numbers of epiphytic yeasts on the leaves and flowers of 25 plant species throughout their vegetation period was determined. The numbers of yeasts on the leaves were found to change regularly throughout the year. The average dynamics for all of the plant species investigated included an increase in yeast numbers during spring and summer with the maximum in late autumn and early winter. The character of the yeasts' dynamics depends on the ecological characteristics of the plants and the duration of the ontogenesis of their leaves and flowers. Three types of dynamics of epiphytic yeasts were revealed: year-round with an increase in autumn–winter, year-round without visible changes, and seasonal with a terminal increase for annual plants.

Key words: yeasts, phyllosphere, epiphytic microorganisms, seasonal dynamics.

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Yeasts constitute a considerable part of the microbial population which is always present on the surface of living plant leaves. Plant exudates (secretions of living plants, which contain simple sugars, organic acids, and other easily utilized compounds) are the main source of nutrients for epiphytic yeasts. By consumption of these exudates, the epiphytic yeasts can, in turn, stimulate plant metabolism. Some epiphytic yeasts can act as biocontrol agents, excreting compounds that suppress growth of phytopathogenic microorganisms. Thus, epiphytic yeasts and higher plants form a symbiotic system, which can serve as a model for a number of fundamental issues of ecology and evolution.

The amount of exudates available to epiphytic microorganisms depends on a number of factors. Such features as the cuticle thickness, the number of stoma, and other anatomical features of the leaves (the presence of trichomes, glandular fuzz, and extrafloral nectaries), which determine the availability of water and nutrients for epiphytic yeasts, vary in plants of different ecological and taxonomic groups and necessarily affect yeast numbers. Moreover, the development of the vegetative and generative organs of each plant follows a specific pattern. The amount of exudates produced depends on the growth stage of leaves, flowers, or fruits. The exudates have a direct effect on the formation of the structure of epiphytic yeast communities. All these processes develop against a background of seasonal changes in hydrothermal conditions.

Thus, the abundance and taxonomic composition of the epiphytic yeast communities can be expected to vary greatly during the year, and these changes may be different for various plant species. However, dependable published data confirming this phenomenon are virtually absent. Attempts to study the seasonal changes in the numbers of epiphytic yeasts have been undertaken in some works [1–5]. However, the analyses were performed several times a year and their number was insufficient to reveal reliable long-term changes.

In order to investigate this problem in more detail, multiple analyses of the plants yeast populations are required, performed throughout the period of formation, development, and dying off of the vegetative and generative structures; the ecological characteristics of the plants should be considered. This investigation was performed by us in 2001–2005. Some of the results concerning the dynamics of the epiphytic yeast communities on individual plant species have been presented in our previous publications [6, 7]. In the present work, the data on the seasonal dynamics of yeast numbers on 25 plant species in Moscow and Moscow oblast are summarized.

MATERIALS AND METHODS

The research was carried out in Moscow (Losinyi Ostrov National Park and Izmailovo Park) and in the vicinity of the Burtsevo village (Shakhovskoi raion, Moscow oblast). Plant samples were collected in two types of biogeocenoses: mixed fir–birch forest and secondary after-forest meadow on mid-loamy sod-podzolic soils. A number of plant species in the anthropogenic zone within the Moscow State University campus were also investigated. As the objects of investigation, 25 plant species were chosen (Table); these include a variety of forms and ecological groups. The duration of

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Characteristics of the plant species investigated

Species	Life form*	Ecological group**	Pollination type***	Biotope
Acer negundo	T	M	I	Forest
Aesculus hippocastanum	T	M	W	City
Ajuga reptans	G	M	W	Forest
Alchemilla vulgaris	G	M	W	Forest
Asarum europaeum	G	X	W	Forest
Betula verrucosa	T	M	I	Forest
Carex pilosa	G	X	I	Forest
Equisetum sylvaticum	G	M	–	Forest
Ficaria verna	G	H	W	Forest
Hypericum perforatum	G	M	W	Meadow
Impatiens glandulifera	G	H	W	Meadow
Impatiens noli-tangere	G	H	W	Meadow
Impatiens parviflora	G	H	W	Meadow
Larix decidua	T	M	I	Forest
Oxalis acetosella	G	H	W	Forest
Picea abies	T	X	I	Forest
Plantago major	G	M	W	Meadow
Populus alba	T	M	I	City
Quercus robur	T	M	I	Forest
Ranunculus repens	G	M	W	Meadow
Sambucus racemosa	B	M	W	Forest
Syringa vulgaris	B	M	W	City
Tanacetum vulgare	G	M	W	Meadow
Taraxacum officinale	G	M	W	Meadow
Tilia cordata	T	M	W	Forest

Notes: * G, grasses; B, bushes; T, trees.
** H, hygrophytes; M, mesophytes; X, xerophytes.
*** I, insect-pollinated; W, wind-pollinated.

the vegetative period of their phyllosphere varies from four to twelve months and more.

The leaf samples were collected two to three times a week throughout the period of vegetation, from the rudimentary bud leaves to the decaying leaves in the mulch. During winter, the samples of evergreen species were collected from under the snow. In the case of flowers, the research included buds, the time of active blossoming, wilting, and fruit formation. Fruit samples were also analyzed from the formation period until their complete decomposition. The samples were analyzed on the day of sampling or within two to three days. Over 7000 samples of plant substrates were analyzed.

For the quantification of yeasts, the leaves, flowers, and fruits were cut with scissors; five to ten samples (0.1–0.5 g) were selected. Sterile water was added to the samples in order to produce 1 : 50 dilutions; these were then vortexed for three min. Malt agar acidified with lactic acid to pH 4–4.5 in order to suppress bacterial growth was used as the nutrient medium. Each sample was inoculated in two repeats. After incubation at room temperature for five to seven days, the colonies were counted under a dissection microscope. The total numbers of yeasts expressed in CFU per gram of dry matter (CFU/g) were determined for each sample.

RESULTS AND DISCUSSION

General patterns in the dynamics of the numbers of epiphytic yeasts. The average total number of epiphytic yeasts on plant leaves was 10^4 CFU/g; it varied from 10^3 to 10^5 CFU/g, depending on plant species. However, the numbers varied significantly throughout the year (from 10^2 to 10^7 CFU/g). The changes in the average number of epiphytic yeasts during the year, averaged for all the plant species, were regular (Fig. 1). The lowest number of yeasts on leaves (approx.

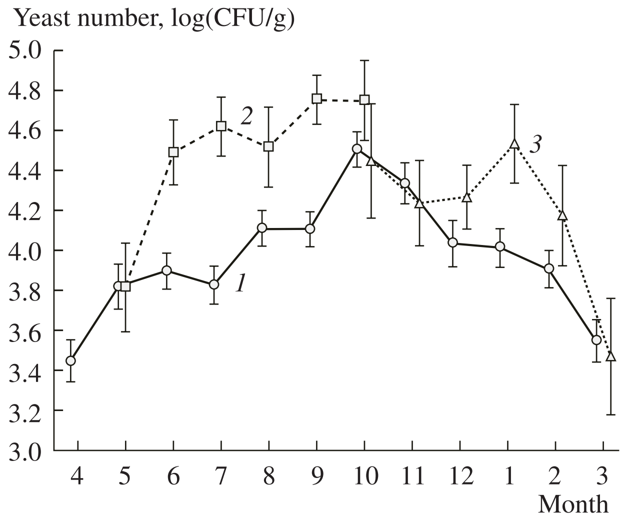

Fig. 1. Average monthly numbers of epiphytic yeasts (average values for all the investigated plant species) on various plant substrates during the year: leaves (*1*), flowers (*2*), and litter (*3*). Signs indicate monthly averages; vertical lines, standard deviation.

10^3 CFU/g) was recorded in spring. The average number of epiphytic yeasts increased gradually and peaked in autumn (over 10^4 CFU/g). In the entomophilic flowers, which produce nectar with a high content of simple sugars, the number of yeasts was highest. The average number of yeasts on flowers increased sharply in May, remained high throughout the summer, and reached the maximum in the plants which blossom in autumn. On the remaining leaves of the evergreen plants, the number of yeasts commenced to decrease in January and reached the minimum value by spring. A certain increase in yeast numbers was observed in the litterfall and in fresh plant debris in January, under snow; afterwards, the numbers decreased and reached the minimum in March–April.

These are the most general patterns of the dynamics of yeast numbers on leaves and flowers. However, significant variations in the character of this dynamics were observed for different plant species, their ecological characteristics, and place of growth. These will be discussed below in more detail.

Dynamics of the numbers of epiphytic yeasts on various plant species. The yearly dynamics of the epiphytic yeasts was found to be closely related to the properties of their ontogenesis and the ecological characteristics of the plants. Three types were specified (Fig. 2).

1. On the leaves of hygrophytes and mesophytes that spend winter with green leaves (for example, *Oxalis acetosella*, *Ajuga reptans*), the number of yeasts increased gradually during the vegetation period, peaked early in winter, after the snow cover established, and decreased again in spring. This dynamic is probably the result of morphophysiological changes in the lamina. In spring, young leaves are known to have an undamaged, well-developed cuticle, which protects them from infection by pathogenic microorganisms [8]. This dense cuticle decreases the amount of surface exudates and therefore limits the growth of the saprotrophic microbial complex. Moreover, extrafloral nectaries, another source of nutrients for the epiphytes, are underdeveloped at this time. In autumn, the leaves undergo subsenile and senile stages of development. The cuticle loses its integrity, and the amount of simple sugars available to yeasts increases sharply. This is probably the main cause of the increase in the numbers of epiphytic yeasts. The pool of easily available compounds becomes exhausted by spring, resulting in decreased yeast numbers.

All the deciduous trees investigated (*Betula verrucosa*, *Quercus robur*, and *Tilia cordata*) exhibited the same dynamics of yeast numbers on their leaves. In this case, however, the period of autumn–winter is represented by forest litter, from tree waste in late autumn until the complete leaf decomposition the next spring.

2. In the case of xerophytic evergreen grasses and the evergreen trees with the xerophytic type of the lamina, the number of epiphytic yeasts did not vary reliably during the year. Some xerophytes (*Asarum europaeum*, *Carex pilosa*, and *Picea abies*) were analyzed for 1.5–2 years. No reliable changes in the average monthly numbers of yeasts were detected during this period. The value exhibited insignificant oscillations around the average and practically did not depend on the season or on the weather conditions during the particular year of investigation.

The absence of pronounced seasonal variation in the number of epiphytes is probably the result of such ecological features of these plants as their thick cuticle (and, consequently, low surface exudation) and low number of stomatic cells; in xerophytes, the latter are often submerged into the leaf tissues.

3. A sharp increase in the number of epiphytic yeasts in the end of vegetation was revealed in all the investigated species with leaves which complete the ontogenetic cycle before winter. For instance, the ephemer *Ficaria verna*, in which all the phyllosphere development occurs during April–June, exhibited this pattern. A sharp increase in yeast numbers was also revealed for three touch-me-not species; the leaves of these plants die off in autumn, after the first morning frost. The plants which retain their leaves until the first snow (the leaves die off during late autumn–early winter), also exhibited an increase in yeast numbers immediately before withering away. Examples of such plants are *Equisetum sylvaticum* and *Plantago major* among grasses and *Larix decidua* among trees.

Thus, in the case of the plants with the phyllosphere development terminating before winter, the number of yeasts increases gradually throughout the vegetative period; it usually increases sharply at the end of vegetation, immediately before the leaves die off. The

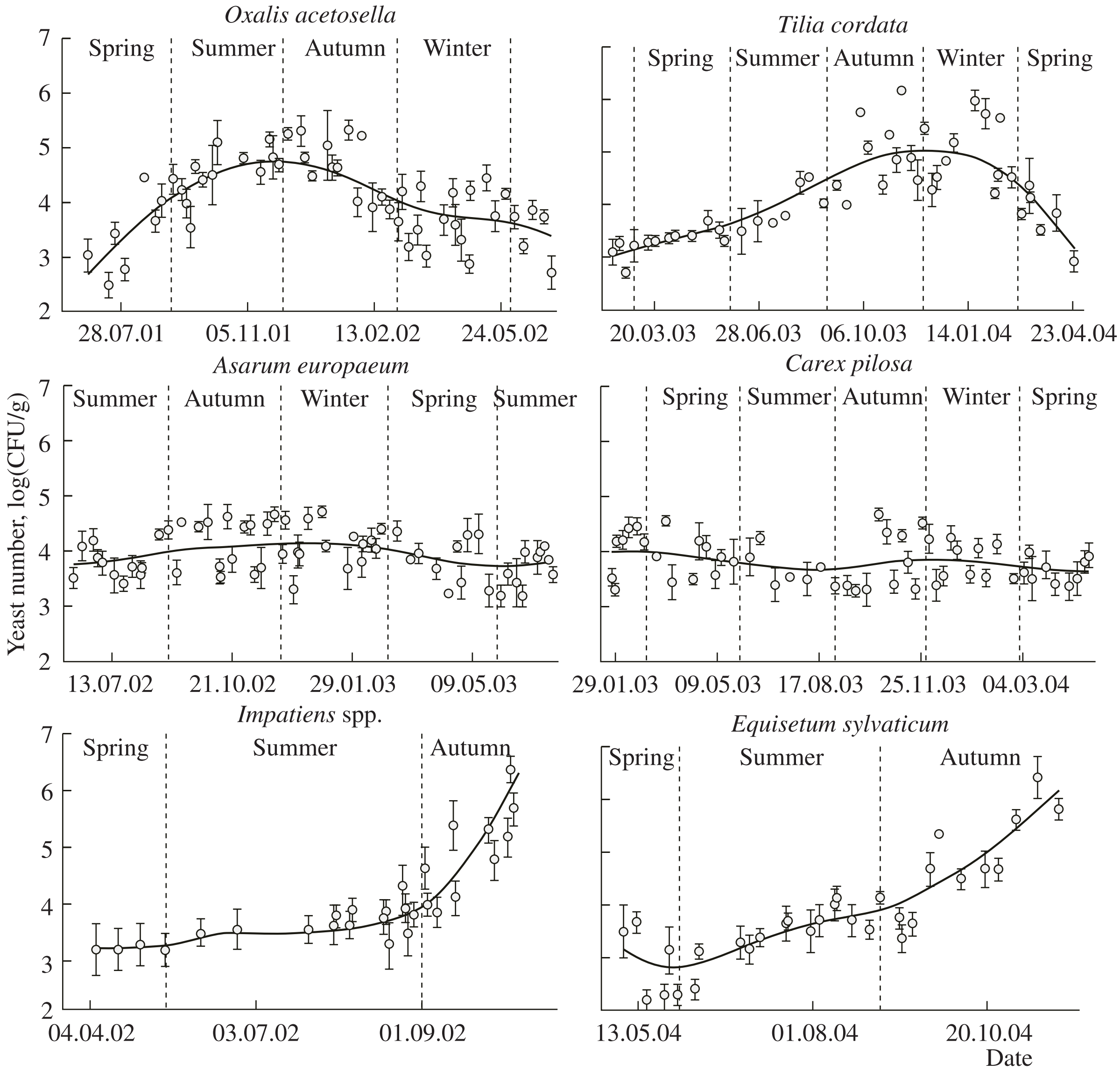

Fig. 2. Examples of the seasonal dynamics of yeast numbers on leaves of various plants. Signs indicate averages for each analysis; vertical lines, standard deviation; and smooth lines, median evening-out.

growth of epiphytic yeasts is terminated only by the rapid decomposition of the substrate.

The dynamics of the numbers of epiphytic yeasts was therefore closely related to the ontogenesis of the plants and to its ecological classification with respect to water (hygrophytes, mesophytes, or xerophytes). When averaged for all the plant species analyzed, the pattern of yeast number dynamics was the same for all the groups: increase during summer, maximum in late autumn, and decrease during winter (in the litter or on the leaves of evergreen plants). The extent of the autumnal maximum, however, depended on the ecological group to which the plant belonged. The maximum was the most pronounced in the phyllosphere of hygrophytes and the least pronounced in the phyllosphere of mesophytes. In the case of xerophytes, neither the season nor the structure of the lamina and its ontogenetic changes had noticeable effects on the number of yeast cells. This number remained almost constant; it was also the lowest on average (Fig. 3).

The investigated plant species comprised both wind-pollinated and insect-pollinated (entomophilic) ones; the numbers of epiphytic yeasts on the members of these groups were significantly different. In the forest, the average number of yeasts during the vegetation period on the wind-pollinated plants (0.13×10^4 CFU/g) was significantly lower than on the entomophilic ones (2.84×10^4 CFU/g). On the meadow, the number of yeasts on the plants of both types (3.16×10^4 CFU/g) did not differ significantly. This finding can possibly be explained by the fact that on the meadow the plants grow close to each other and their mutual semination with yeasts is

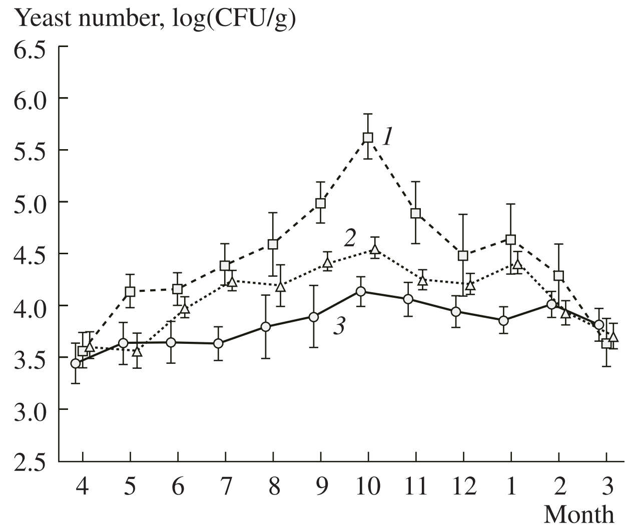

Fig. 3. Average monthly values of yeast numbers on the leaves of the plants of ecological groups differing in respect to water: hygrophytes (*1*), mesophytes (*2*), and xerophytes (*3*).

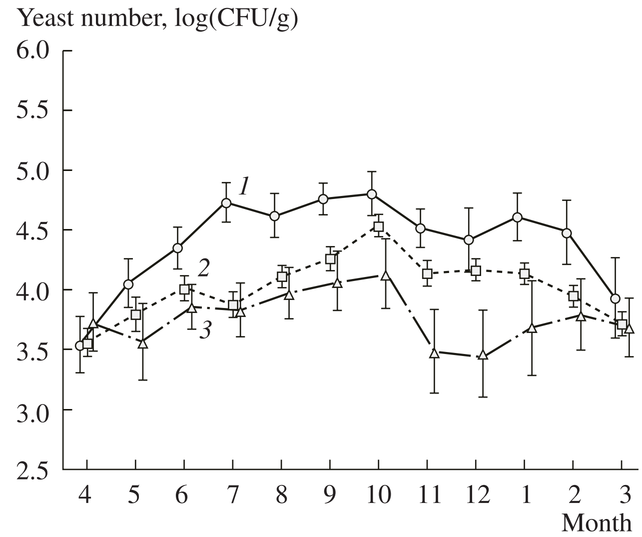

Fig. 4. Average monthly values of yeast numbers on the leaves of the plants from different biogeocenoses: meadow (*1*), forest (*2*), and city (*3*).

possible. In the forest, the spatial isolation of individual plants is more pronounced; it can contribute to the more pronounced differences between plant types. We have previously reported higher numbers of epiphytic yeasts and lower spatial variations in the desert biogeocenoses with varying degrees of plant coverage [9].

The number of epiphytic yeasts also depended on the life form of a plant. In the phyllosphere of grasses, the average number of yeasts during the vegetation period was 2.54×10^4 CFU/g, while in the phyllosphere of trees, it was 0.44×10^4 CFU/g. During the year, the average monthly values for trees and grasses changed according to similar patterns. The lower number of yeasts on tree leaves can be explained by the absence of hygrophytes among this group. Moreover, the number of yeasts on grasses can be higher due to the closely located substrates (primarily entomophilic flowers, which are densely populated by yeasts).

The numbers of epiphytic yeasts in various types of phytocenoses. Comparison of the average numbers of epiphytic yeasts in various types of phytocenoses (independent of the plant species) revealed significant differences; the pattern of changes, however, was similar in all phytocenoses. The highest average number of epiphytic yeasts throughout the period of investigation was recorded in the phyllosphere of meadow plants; it was appreciably lower on the leaves of forest plants, and lower still on the leaves of urban plants (Fig. 4).

The higher numbers of yeasts on meadow plants may be the result of the fact that most meadow plants are entomophilic; their nectar-bearing flowers are visited by insects, which are efficient agents of yeast dissemination [10]. Moreover, in an open meadow environment, varied plant species grow densely and are subject to wind; mutual dissemination by yeasts and other microorganisms is therefore more pronounced.

Anthropogenic factors have an evident negative effect on the development of epiphytic yeasts. The number of epiphytic mycelial fungi has been shown to be substantially lower in urban conditions, compared to natural environments [11].

Effect of specific habitats. For two plant species (*Dactylis glomerata* and *Taraxacum officinale*), the seasonal dynamics of epiphytic yeast numbers was monitored in two similar meadow biogeocenoses separated by approximately 150 km (Losinyi Ostrov and the vicinity of the Burtsevo village).

The overall trend of the yeast numbers in the phyllosphere was the same for both sites: the numbers increased gradually throughout the vegetation period. The average monthly values, however, apart from the plant species and season, also depended on the specific location (Fig. 5). In Losinyi Ostrov, both species exhibited a certain increase in July; no such increase was observed for the plants collected in the vicinity of the Burtsevo village. Thus, certain factors which do not depend on the plant species, determine some characteristics of the yeast number dynamics. These factors can be termed the effects of specific sites. They probably include the microclimate, local development of insects (the main vector for dissemination of epiphytic yeasts), etc. Since such factors are numerous and include some irreproducible ones, the specific characteristics of each experimental site can hardly be accounted for. Each habitat is therefore unique and always differs from any other in at least some respects.

Thus, our investigation has revealed that the number of epiphytic yeasts on plants varies significantly

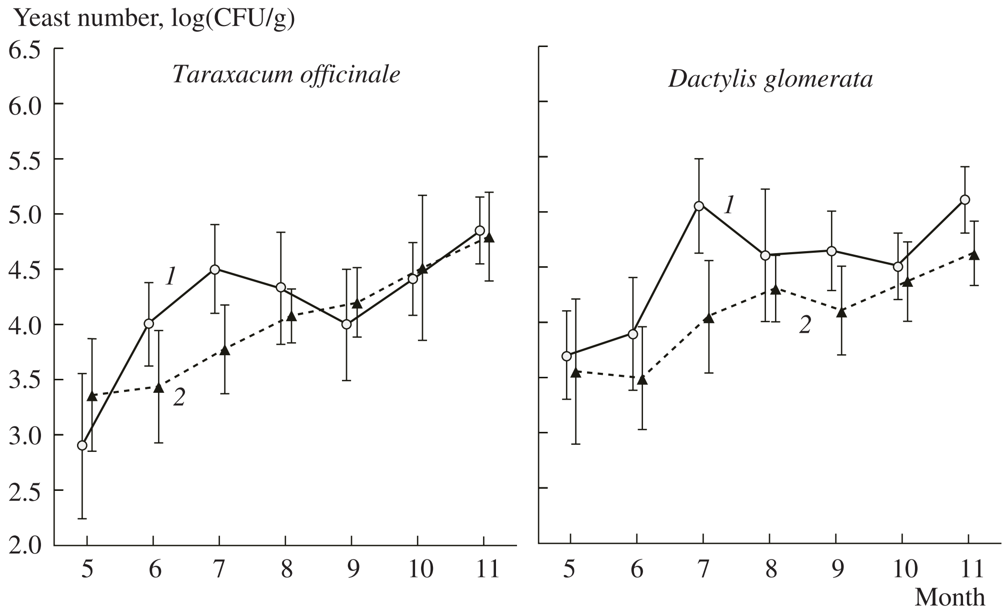

Fig. 5. Dynamics of the numbers of epiphytic yeasts in different sites: Losinyi Ostrov (*1*) and Burtsevo village (*2*).

depending on a number of factors and changes over the year in a regular manner. The dynamics of the numbers of epiphytes depends primarily on the ecological characteristics of the plants and on the duration of ontogenesis of their aerial organs. The number of epiphytic microorganisms depends primarily on the amount and composition of plant exudates, rather than on the hydrothermal conditions as such. These facts should be considered in the monitoring research of microbial biodiversity and in the development of the biocontrol methods for phytopathogenic microorganisms.

The data obtained in this work on the dynamics of the taxonomic structure of epiphytic yeast communities will be discussed in our subsequent publications.

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