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Features of the fungal spectrum in the air environment in the Rostov region according to the results aeropalynologic monitoring 2019

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ABSTRACT

BACKGROUND: In recent decades, there has been an epidemic growth of allergic diseases, in which fungi along with other allergens significantly play a role in their etiology. Spores of a number of micromycetes are present in the air. Aeropalynology environmental monitoring enables examination of the composition of airborne microorganisms, their dynamics, and role in the formation of allergic diseases. The Rostov region has climatic and geographical features that affect the qualitative and quantitative compositions of the fungal spectrum in the air environment.

AIM: This study aimed to investigate the composition and features of the fungal spectrum of the air environment in Rostov-on-Don, to assess the dynamics of the concentration of fungal spores during the monitoring period (March to October), and to make a calendar of plant pollination and fungal spore production for this region.

MATERIALS AND METHODS: In this longitudinal, observational, single-center study, aeroallergens were detected using a volumetric Burkard trap. Identification of plant pollen and fungal spores was performed by microscopy of colored slides obtained from a sticky tape covered with a special mixture.

RESULTS: Results of aeropalynological monitoring in 2019 in the air environment of Rostov-on-Don revealed the presence of pollen taxa and fungal spores, represented by the mold fungi *Cladosporium herbarum* and *Alternaria alternata* in high increasing concentrations. Their dynamics were recorded throughout the observation period (March to October). The specific weight of fungal sensitization in patients with seasonal allergic rhinitis was 11.6%.

CONCLUSIONS: This study identifies the regional features of the fungal spectrum of air allergens, and a calendar of dusting and sporulation was compiled for the city of Rostov-on-Don.

Keywords: aeropalynological monitoring; fungal spores; plant pollen; fungal allergy; seasonal allergic rhinitis

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Особенности грибкового спектра воздушной среды в Ростовской области по результатам аэропалинологического мониторинга 2019 года

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АННОТАЦИЯ

ОБОСНОВАНИЕ. Последние десятилетия характеризуются эпидемическим ростом аллергических заболеваний, в этиологии которых наряду с прочими аллергенами значительная роль принадлежит грибковым. Споры ряда микромицетов содержатся в том числе в воздушной среде. Аэропалинологический мониторинг окружающей среды позволяет изучить состав аэроаллергенов, их динамику и роль в формировании аллергопатологии. Ростовская область имеет климатогеографические особенности, которые отражаются на качественном и количественном составе грибкового спектра воздушной среды.

ЦЕЛЬ — изучить доминирующий состав и особенности спороношения преобладающего грибкового спектра воздушной среды в г. Ростове-на-Дону; исследовать динамику концентрации пыльцевых зёрен и спор наиболее часто встречаемых аскомицетов в течение периода мониторинга (март-октябрь 2019 г.); составить календарь пыления растений и спороношения доминирующих грибов для данного региона.
МАТЕРИАЛ И МЕТОДЫ. Одномоментное обсервационное (одноцентровое) исследование длительностью 8 мес, в котором аэропалинологический мониторинг проводили с использованием волюметрического пыльцевого уловителя VPPS 2000, установленного на высоте 15 м от поверхности земли. Подсчёт данных выполняли по стандартной международной методике подготовки, окраски и подсчёта слайдов. Идентификацию пыльцы растений и спор грибов проводили методом микроскопии окрашенных предметных стёкол, полученных с липкой ленты, покрытой специальной смесью. При идентификации использовали атласы спор грибов для аэробиологических исследований. Подсчёт спор плесневых грибов и пыльцы в образце проводили тремя непрерывными трансектами, параллельными продольной оси препарата. Результаты подсчётов пересчитаны на единицу объёма воздуха и представлены как число пыльцевых зёрен в 1 м^3. Методом ImmunoCap (Phadia IDM ImmunoCap-100) в сыворотке пациентов с сезонным аллергическим ринитом определяли IgE к аллергенам амброзии, полыни, мари, альтернарии.
РЕЗУЛЬТАТЫ. В результате аэропалинологического мониторинга в Ростов-на-Дону в течение 8 мес 2019 г. в воздушной среде наряду с пыльцевыми таксонами обнаружены споры грибов рода *Cladosporium* и *Alternaria*, зафиксировано изменение их концентраций на протяжении всего периода наблюдения (март-октябрь) с тенденцией к росту. Наряду с этим у пациентов с сезонным аллергическим ринитом был определён удельный вес сенсибилизации к грибам *Alternaria alternata* (11,6%) путём выявления аллерген специфических IgE в сыворотке крови методом ImmunoCap.
ЗАКЛЮЧЕНИЕ. Выявлены особенности доминирующего грибкового спектра аэроаллергенов воздушной среды, составлен календарь пыления и спороношения для г. Ростова-на-Дону и Ростовской области.

Ключевые слова: аэропалинологический мониторинг; споры грибов; пыльца растений; грибковая аллергия; сезонный аллергический ринит

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Background

Mold and yeast fungi play a significant role in the etiology of allergic diseases. Concurrently, the increase in the incidence of allergy to micromycetes coincides with the global trend of increasing allergic diseases [1, 2].

Fungi or *Mycota* represent an independent kingdom of approximately 1.5 million species [3]. Of these, only 80,000 species have been studied so far [3]. Nearly 150 species are thought to be primarily pathogenic to humans and animals, and 350 species conditionally pathogenic [3]. Among the pathogenic species, *Trichophyton*, *Epidermophyton,* and *Microsporum* have the highest sensitizing power [3]. An allergy thereto is followed by the primary fungal disease associated with the same species. Moreover, several nonpathogenic fungi with their spores being detected in the air may predispose one to bronchial asthma, allergic rhinitis, allergic dermatoses, and allergic gastrointestinal lesions [4].

Since the 1990s, molecular methods for studying fungi have been actively introduced, which led to global changes in the structure of their taxonomy, whereby ascomycetes are one of the most extensive classes. They are able to form sexual spores (ascospores) in a special sacular cell (ascoma). In addition, the modern taxonomy of fungi is based on genosystematics, morphology, and features of the life cycle. Some classifications of fungi are based on various characteristics [5]. Most frequently, the compilation is based on the morphology of fungi and the methods of their reproduction. Thus, based on morphology, fungi are divided into two groups: yeast fungi (consisting of individual cells and reproducing by division and budding) and mold fungi (hyphalic or mycelial fungi—multicellular organisms that are characterized by the presence of mycelium and reproduce through spores and fragmentation of hyphae, i.e., outgrowths 3–4 μm wide) [5].

The reproduction of fungi depends largely on climatic, meteorological, and specific environmental features [6, 7]. For example, the spread of the spores of many fungi may depend on the level of atmospheric humidity: Showers, fogs, and damp nights often contribute to the release of spores. In addition, the so-called dry dispersion, i.e., dispersion of spores by air movement, is common to several fungi [6]. In this case, the concentration of spores in the air increases depending on the air velocity and decreases during rains [6]. Under conditions of high temperature, increased wind speed, wind turbulence, and convection, the number of fungal spores (potential allergens) increases exponentially [6]. This applies, in particular, to representatives of the *Alternaria alternata* and *Cladosporium herbarum* mold fungi [6, 7]. Various physical factors may also influence the spore formation process. For example, some fungi depend on the intensity and duration of daylight. In particular, the mold *C. herbarum* requires an interval in the dark to form spores [6]. Studies show that the spores of the *Cladosporium* genus are much more common within the daytime, but in a warm and dry climate, spores of the *Alternaria* genus may dominate [6]. These fungi prefer moist and warm places for their habitat. They are easily found in soil, decaying wood and leaves, and silage and are quite common in human dwellings. Among those of fungi circulating in the air with pronounced allergenic properties, the spores of the *Cladosporium*, *Penicillium*, *Alternaria*, *Aspergillus*, *Botrytis*, and *Fusarium* genera should be noted. However, *Cladosporium* and *Alternaria* spores are of particular interest due to their dominance.

The influence of changing climate on the intensity of sporulation and the corresponding increase in the prevalence of allergopathology are of great interest. Evidently, climate and weather affect the amount of fungal spores in the atmosphere, and both their number and species of origin are diverse, as proved by many published studies on the subject worldwide [7]. However, only a few authors have studied the effect of climate change on this factor. A study from New England (USA) showed an earlier onset of the season (2–4 weeks) of atmospheric mold spores and a higher number of spores after the El Niño event [8]. J. Corden and W. Millington [9] studied the concentration of *Alternaria* spores in Derby (Great Britain) over several years (1970–1998) and found that the concentration of spores increased with an increase in local temperature. A similar association was found, with an earlier onset and longer spore season. Other studies showed that *A. alternata,* which was grown at elevated CO_2 levels, produced approximately thrice as much spores and twice as much major protein [10]. These studies prove that climate change may affect the concentration of fungal spores in the atmosphere, the onset and duration of sporulation seasons, and the allergenicity of spores, which potentially increases the risk of allergies and asthma. Moreover, the number of fungal spores in the air is subject to seasonal fluctuations, ranging from minimum in winter to peak in late summer and early autumn [11].

Cladosporium fungi, along with other micromycetes (*Mycosphaerella* and *Venturia*), are saprophytes and parasites of several vegetables. The number of *C. herbarum* and *Cladosporium cladosporioides* spores in bioaerosol within daytime hours reaches 5,000–15,000 spores/m^3, and that of the other species (*Cladosporium macrocarpum* and *Cladosporium sphaerospermum*) is far less [6].

According to different authors, on dry hot days during summer and autumn, *Alternaria* spores are found in the air at a concentration of 500–1,000 spores/m^3 [6, 11]. Of these, those of *A. alternata* (*Alternaria tenuis*) and *Alternaria tenuissima* are most common [6].

Numerous studies conducted in different geographic regions have shown that fungi of the *Aspergillus, Alternaria,* and *Cladosporium* genera provoke allergic reactions the most [6]. Along with *Penicillium*, they are the most frequently found genera in ambient air worldwide [1, 12]. The spread of micromycete spores in ambient air depends directly on a particular area's climatic factors such as changes in temperature, acidity, relative humidity, and times of year and day. The taxonomic and biological composition and concentration of mold spores may vary in various geographic regions.

In view of the above, studies on sporulation and the effect of an increase in the concentration of mold spores in the atmospheric air on the development of allergopathology resulting from fungal sensitization in various regions of Russia, i.e., regions with different climates, are of great interest.

The Rostov region possesses its own climatic and geographic features that determine the composition and concentration of aeroallergens in the air. This region is located in the southern part of the East European Plain, partially in the North Caucasus region. In the north, it borders with the Central Russian Upland, and in the west with the eastern part of the Donetsk Ridge. One of the largest rivers in Europe, the Don, flows within the territory of the region; Tsimlyansk Reservoir and the Seversky Donets and Manych rivers (navigable tributaries of the Don) are located here. The climate in the Rostov region is moderately continental with cloudy and windy winters and dry, hot, and windy summers. Dust storms are typical in the southeastern parts of the region: up to 20–25 days per year and in some years up to 60 days [13]. The duration of sunshine is intensive, specifically 2,050–2,150 h per year [13]. When characterizing the typical meteorological features of the region, it should be noted that the average annual precipitation is 424 mm [13]; relative humidity is inversely related to temperature, with maximum values (85%–90%) occurring in the winter months and minimum values (48%–60%) in the summer [13].

AIM: The aims of this study were to investigate the composition and dynamics of the concentration of pollen grains and spores of the most common ascomycetes (*Cladosporium* and *Alternaria*) in the air of the city of Rostov-on-Don and the Rostov region during the monitoring period from March to October, 2019, and compile a single calendar of plant dusting [14] and fungal sporulation for these regions.

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Materials and methods

Study design

A cross-sectional observational (single-center) study (monitoring of airborne allergens) was conducted for eight months (March–November, 2019).

Methods for recording outcomes

Standard international technique with Hirst-type pollen traps (Burkard VPPS 2000 volumetric trap), capable of registering particles from 5 to 100 μm, was used for aeropalinological monitoring [15]. The air intake rate was 10 l/min, or 14.4 m^3/day, which was comparable to the respiration rate of an adult [14]. The pollen trap drum had a clock mechanism, adjusted to any time interval within one week when necessary, which ensured continuous operation for the trap impactor without additional control for any period within one week [14]. Determination of concentration and identification of fungal spores (according to their morphological structure) were conducted by microscopic (Lomo Mikmed-6 microscope, Russia) examination of stained preparations (slides), which were obtained from adhesive tape (covered with a mixture of Vaseline and paraffin) and removed from the trap impactor drum. Microscopy with the use of Lomo Mikmed-6 enabled the observation of objects by dark field and phase contrast methods, under both polarized and fluorescent lights. Parallely, the information was recorded as a digital photo. The impactor was mounted 15 m above ground level [15]. The slides were processed at the Department of Soil Science and Assessment of Land Resources, Modern Microscopy Center, Southern Federal University. Subsequently, the concentration of fungal spores in 1 m^3 was calculated. Allergen-specific IgE was determined by the ImmunoCap method (Phadia IDM ImmunoCap-100, Sweden) in the blood serum of patients with seasonal allergic rhinitis due to allergens of trees, meadow and weed grasses, and alternaria.

Statistical analysis

Mathematical data processing and plotting of graphs and diagrams were performed in Microsoft Excel 2019. The "moving average" method was applied to the dusting period data (long-term observations for seven days) to account for both hardware errors (power outages and unforeseen trap malfunction) and subjective errors. Monitoring of the quality of slide preparation, work performance, and calendar construction was accomplished by E. E. Severova. The dusting calendar was built using the AeRobiology software package (http://rstudio-pubs-static.s3.amazonaws.com/487049_df18e86409664a2bb89f2b6c62f8feb0.html).

Results

Aeropalynological monitoring revealed that the dusting season lasted for at least eight months (March–October) in Rostov-on-Don. In 2019, a total of 24 taxa from the region's dominant plant groups (pollen grains of trees, cereals, and weeds) were registered in the air basin of the region [14]. Among the weeds, the dominant and "quarantine" plants for the region were ragweed and, to a lesser degree, wormwood [14]. Increasing concentrations of mold spores dominated by the *Alternaria* and *Cladosporium* genera were also found (Fig. 1).

C. herbarum is a typical species of the genus *Cladosporium*. The microscopic spores of *C. herbarum*, both conidiospores and conidia, form long and typically branching chains. Conidiophores are bundled or densely crowded (or probably solitary), mostly erect, and septate and with only one or two branches (if not nonbranching). They are elliptical or oblong, and their ends are rounded (see Fig. 1). Coloration is pale or medium olive-brown, frequently dark colored, and brownish. Closer to the apex, they are knotty or dentate. Conidia are formed by the holoblastic type; they differ in shape, size, and number of septa. Young conidia are always smooth, colorless, and unicellular. As they mature, their shell becomes spiky, and septa appear in many species. Their colors may vary from white-olive to brown. As shown in the photos (see Fig. 1), conidia are almost spherical. They may be oblong or cylindrical and are mostly rounded, truncated (in some cases pointed at the ends), and olive or light brown. Frequently, branching chains are formed. The size of the unicellular conidia is 5.5–3.0 μm × 3.8–6.0 μm [3]. These airborne spores are most common in sum-

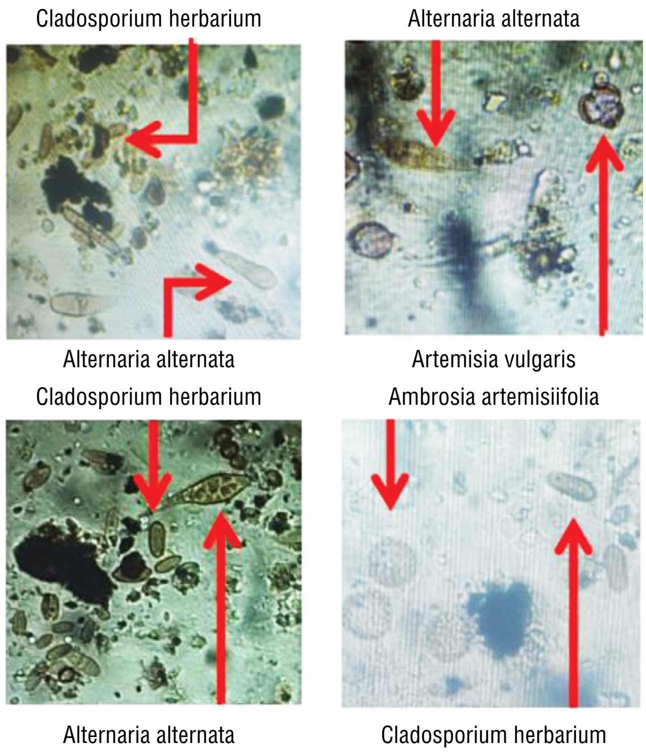

Fig. 1. Spores of mold fungi *Alternaria*, *Cladosporium* and pollen grains of ragweed (*Ambrosia artemisiifolia*), common sagebrush (*Artemisia vulgaris*) (microscope LOMO-Mikmed-6; ×400). Personal photo of the authors.

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mer (peak concentrations) and autumn; may be found indoors or on organic materials in the soil; and show themselves as a plant parasite. Optimal conditions for their development include temperatures between +18°C and +28°C, with maximum growth temperatures between +28°C and +32°C at pH 6 [6, 11]. The formation of conidia is higher in humid conditions compared with that in dry conditions.

According to the literature [3–6], *Alternaria* alternata (the best known representative of mold fungi in nature) is considered one of the most common aeroallergens in Europe. It reproduces through mycelial particles (vegetative body of the fungus) and spores. Morphologically, it is characterized by a mycelium (yellowish green or yellow-brown), conidia (multicellular and brown colored with transverse and longitudinal septa; see Fig. 1), and a variety of ovoid or spindle-shaped forms (see Fig. 1). Conidia typically form easily disintegrating chains (see Fig. 1). The sizes are 20–63 μm × 9–18 μm. Optimal conditions for sporulation include temperatures from +25 to +30°C, windiness, high humidity, and fertile soil [3, 11].

The results of monitoring aeroallergens through observation over months in 2019 in Rostov-on-Don revealed specific dynamics of the concentration of mold fungi sporulation (spore units in 1 m^3, U/m^3) (Fig. 2) and plant pollen (pollen grain in 1 m^3, PG/m^3) in the air of the region [14]. There was a wide variation in the concentrations of pollen grains and mold spores. Moreover, the concentration of pollen grains was significantly less pronounced compared with that of mold spores.

The maximum concentrations of mold spores for each month of observation are presented below, which facilitates the ability to trace concentration peaks under certain weather conditions and pollen concentrations. To begin, in March, the maximum monthly concentrations of *Cladosporium* and *Alternaria* spores reached 3,526 U/m^3 and 20 U/m^3, respectively, as indicated in a previously published article [14]. In April, the peak concentrations of *Cladosporium* and *Alternaria* fungal spores continued to increase, which amounted to 7,328 U/m^3 and 237 U/m^3, respectively, and in May, the maximum concentrations of these 2 species were 24,510 U/m^3 and 618 U/m^3, respectively. Furthermore, in June, high and increasing maximum concentrations of *Cladosporium* and *Alternaria* spores were still observed, i.e., 18,227 U/m^3 and 569 U/m^3, respectively. In July, the same fungal spores were registered in the maximum concentrations for the entire observation period—32,461 U/m^3 and 1,556 U/m^3, respectively (as of July 31, 2019), whereas in August, the concentrations of *Cladosporium* and *Alternaria* spores were consistently high—27,744 U/m^3 and 982 U/m^3, respectively. In addition, an interesting phenomenon was recorded: On the day of the maximum ragweed concentration (393 PG/m^3 as of August 19, 2019), another sharp rise in the concentrations of *Cladosporium* and *Alternaria* spores was observed, i.e., 21,069 U/m^3 and 721 U/m^3, respectively (see Fig. 2). It appeared to be associated with extreme weather conditions on this particular day (thunderstorm, hurricane, and rain).

Data on the hourly distribution within a day will be presented in another paper.

In September, a spike in the maximum concentrations of *Alternaria* spores (380 U/m^3 as of September 18, 2019) was recorded (Fig. 3), which coincided (according

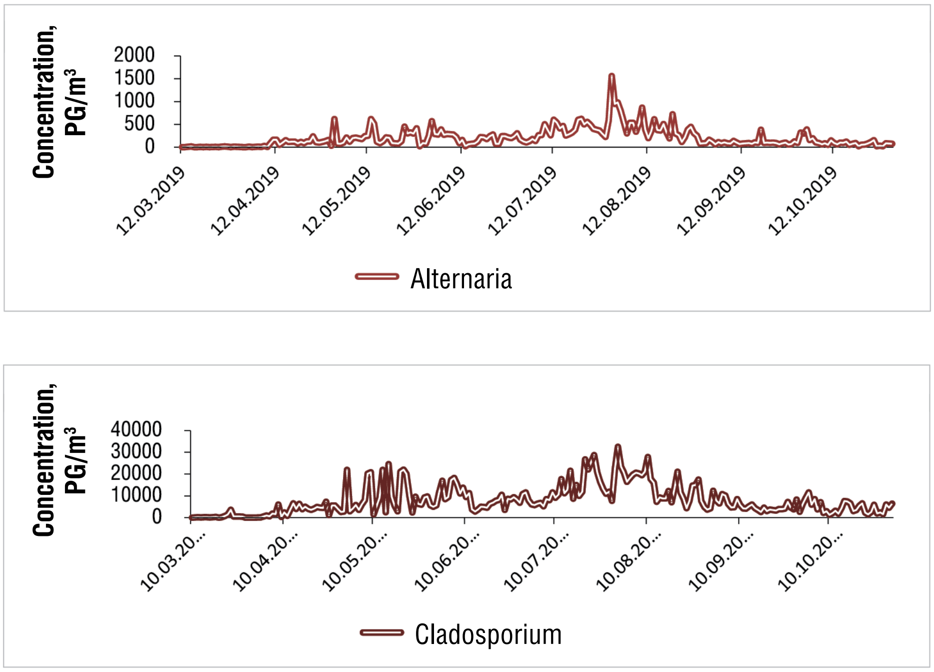

Fig. 2. Concentration of mold spores (spores/м^3) in the atmospheric air during the monitoring period (March-October, 2019).

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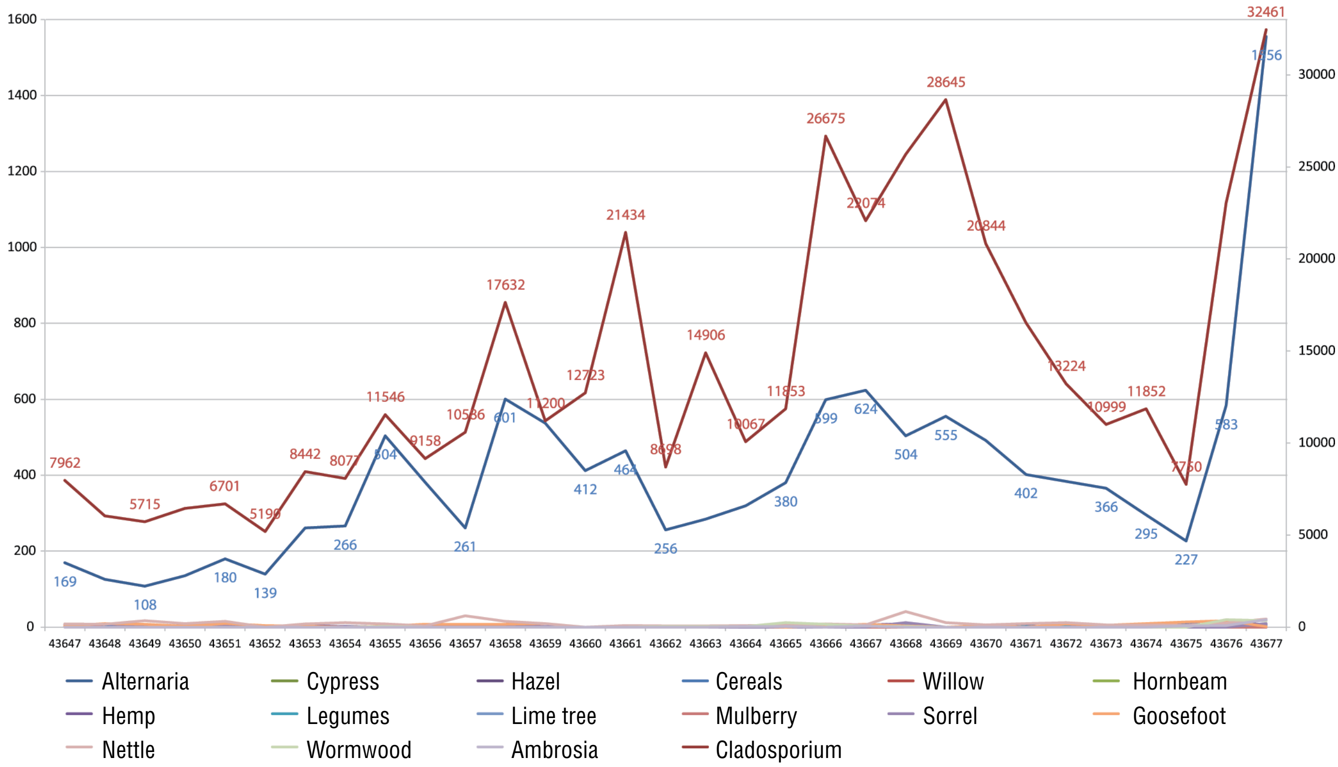
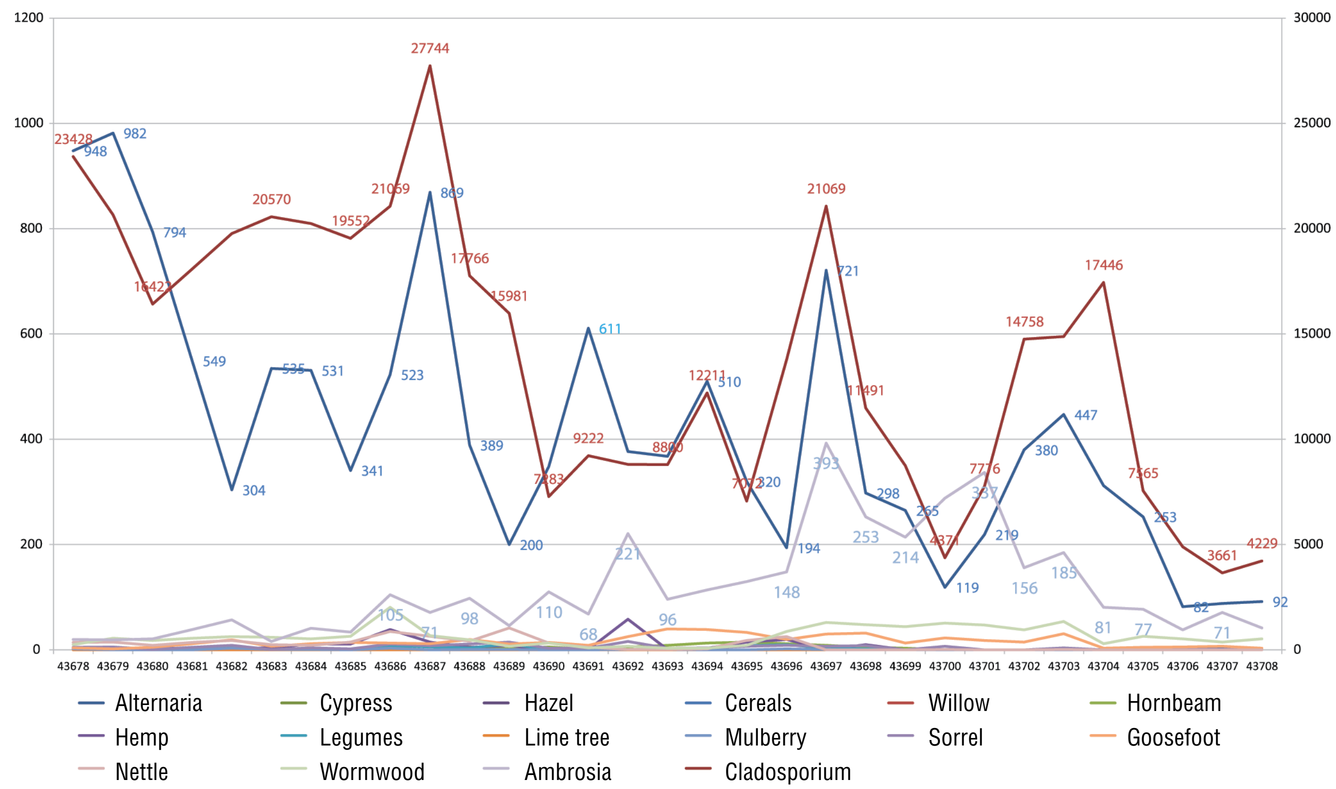
July, 2019



August, 2019



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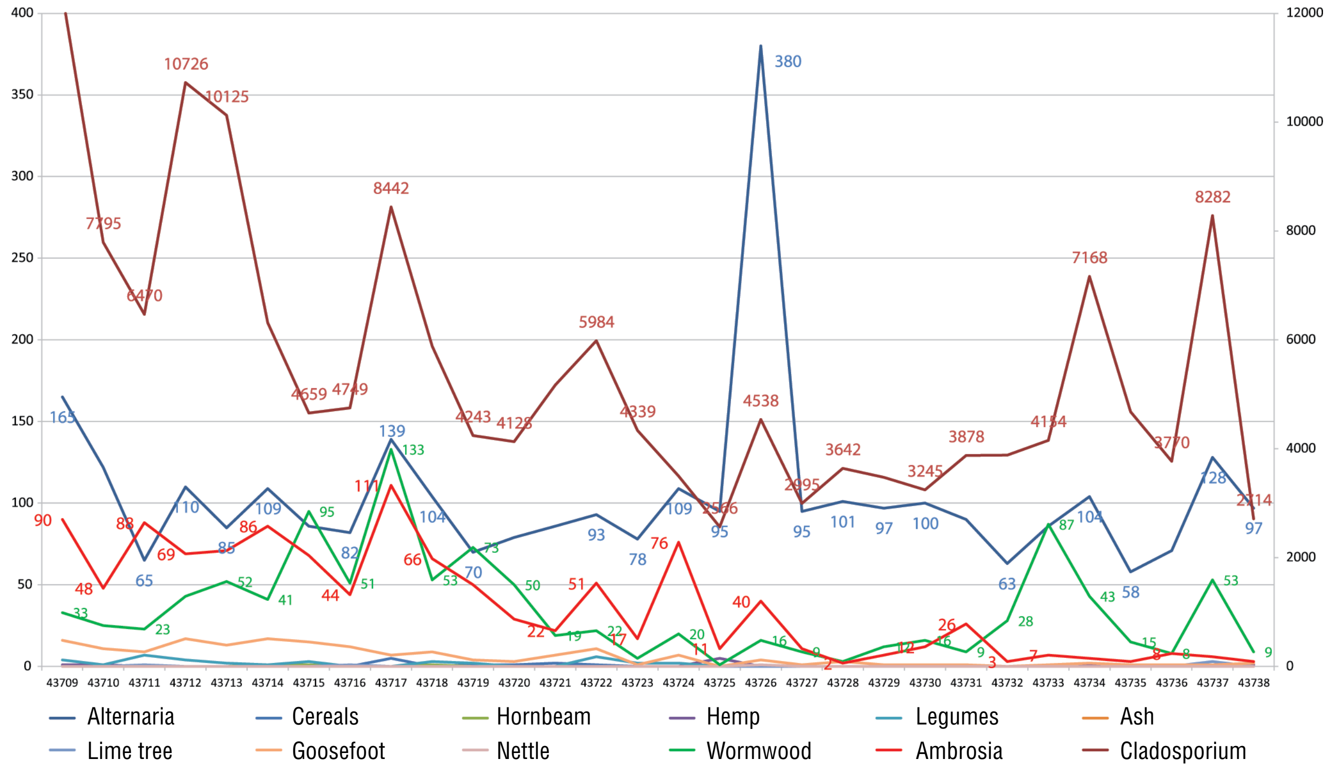
September, 2019



October, 2019

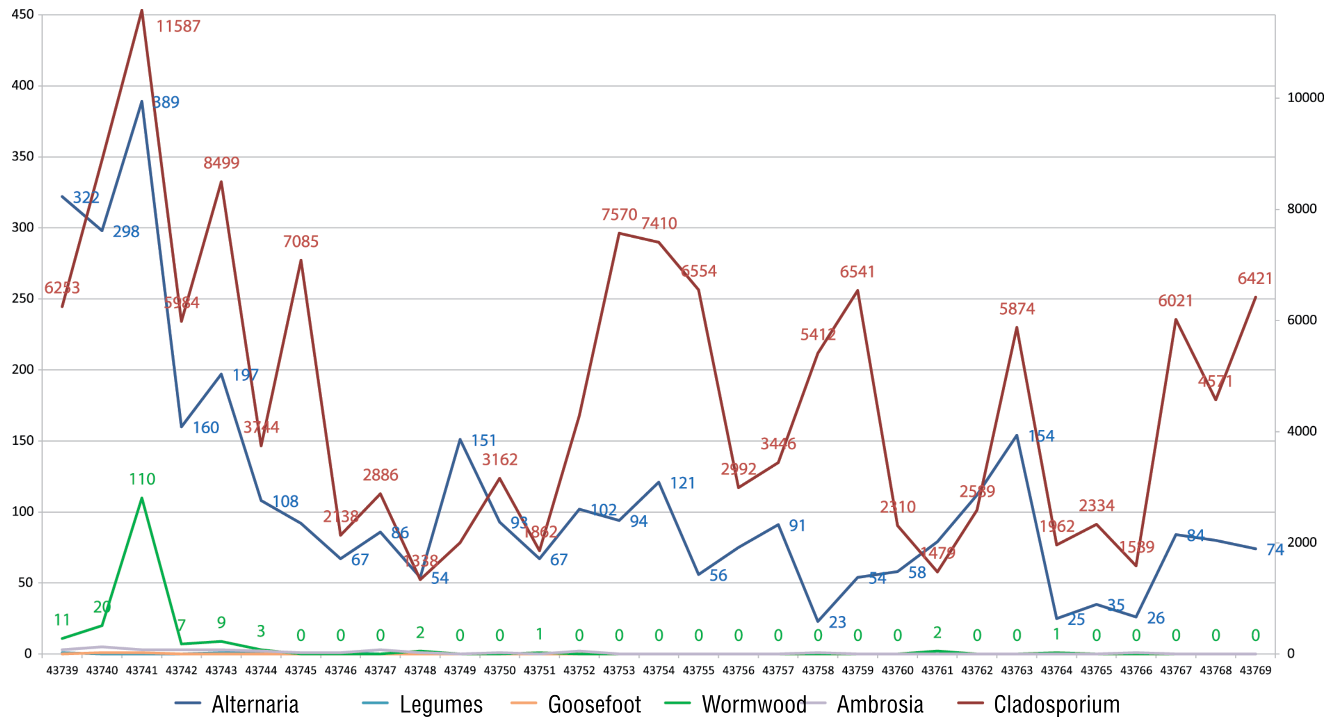


Fig. 3. Concentrations of pollen of individual taxa and fungal spores in atmospheric air in July, August, September and October 2019 in Rostov-on-Don.

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to weather stations) with rain in the evening hours, an increase in humidity to 78%, and a westerly wind. It is planned to involve detailed weather observation data and conduct a regression analysis to prove that this rise in concentration is associated with an increase in humidity. Then, in October, a jump in the concentration of wormwood to 110 PG/m^3 and a decrease in the concentration of ragweed pollen grains (to single PG/m^3) were observed (as of October 3, 2019). Interesting is the fact of coincidence in recording the concentration peaks of wormwood pollen and *Cladosporium* and *Alternaria* spores on this day (11,587 U/m^3 and 389 U/m^3, respectively), which was related with thunderstorm rain and a sharp change in wind direction to the east. Such abrupt changes in the concentration of several very different taxa at a time may be associated with the known phenomenon of "thunderstorm asthma," as well as to their long-range transport. In this regard, it will also be interesting to analyze the change in the wind rose and assess the probability of long-range transport from neighboring or distant regions.

High concentrations of fungal spores (*Alternaria* and *Cladosporium*) with a gradual increase in their concentration were observed within the entire monitoring period. Thus, there was approximately a tenfold increase in the concentrations of *Alternaria* and *Cladosporium* spores from March to April—342 and 8,967 spores per 1 m^3 in March and 2,620 and 98,935 spores per 1 m^3 in April, respectively. However, the concentrations of *Alternaria* and *Cladosporium* spores were almost unchanged in May and June, i.e., 6,760 and 6,474 spores per 1 m^3 and 308,495 and 274,449 spores per 1 m^3, respectively.

In July–August, a sharp increase in *Alternaria* and *Cladosporium* concentrations with the onset of weed blooming was observed, i.e., 12,370 and 434,153 spores per 1 m^3, respectively, whereas from September, the same steep decrease in spore concentration started—up to 3,175 and 162,631 spores per 1 m^3, respectively. In November, the presence of *Alternaria* spores (1,226 U/m^3) and *Cladosporium* spores (94,539 U/m^3) was also found. Total concentrations of mold spores for the entire monitoring period were 49,340 U/m^3 for *Alternaria* and 1,958,249 U/m^3 for *Cladosporium* (Fig. 4); i.e., monthly total concentrations that characterize the seasons and each month in terms of the atmospheric load with mold spores have been indicated.

According to the literature [5], diagnostic fungal extract-based allergens available in Europe have a low sensitivity. For example, a positive reaction to *Cladosporium* mycelium extract (commercial allergen) is noted in 12%–65% of the examined patients, which is associated with difficulty in standardization of raw materials (micromycete culture) and possible mutations at the cultivation stage [16]. Unfortunately, there are currently no diagnostic fungal allergens for skin testing available in Russia. Therefore, until the appearance of standardized registered allergens of molds, determination of the true frequency of mycogenic allergy using skin allergy testing is not possible.

In Russia, blood tests to detect allergen-specific immunoglobulin E (asIgE) for various fungal allergens are used to diagnose fungal sensitization. Recently, component-resolved diagnosis of allergic diseases has become widespread.

A clinical, simple, randomized, cross-sectional, and observational study was conducted to identify the sensitization spectrum in patients with seasonal allergic rhinitis. Inclusion criteria were as follows: patients with allergic

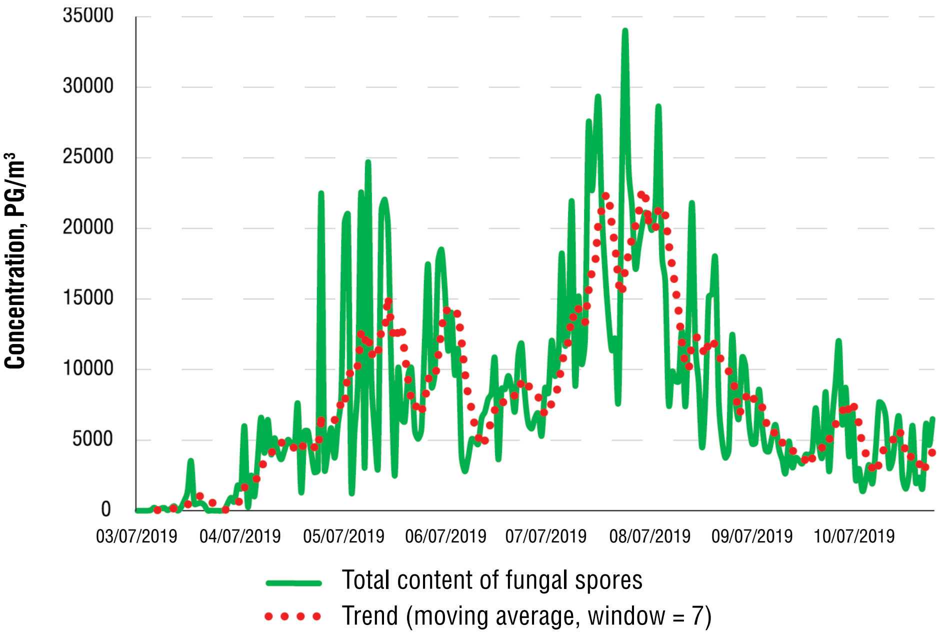

Fig. 4. The total content of fungal spores for the observation period (March-October) 2019 in Rostov-on-Don.

rhinitis, including men and women ($n = 671$) aged 18–65 years. No exclusion criteria were used. The history-taking process elucidated that 361 patients (53.8%) experienced seasonal allergic rhinitis, 231 patients (34.4%) had perennial allergic rhinitis, and 79 patients (11.7%) demonstrated no detectable sensitization. Molecular allergy diagnostics using ImmunoCap-100 (together with the AllergoDon Medical Center) was performed to verify the etiological diagnosis. Values of asIgE ⩾ 0.35 kU/l were considered positive. Based on the medical history of each patient, the identified sensitization was clinically relevant. The following results were obtained: Among patients with seasonal allergic rhinitis, patients with sensitization to ragweed (w230, nAmb a 1; $n = 85$ [13.0%]), alternaria (m229, rAlt a 1; $n = 78$ [11.6%]), and wormwood (w231, nArt v 1; $n = 60$ [8.9%]) prevailed; sensitization to meadow grasses (g213, rPhl p 1 / rPhl p 5b; $n = 51$ [7.6%]) ranked fourth, whereas sensitization to birch (t215, rBet v 1; $n = 24$ [3,6%]) placed fifth. Cross-reactivity was found in some cases: to meadow grasses (g214, rPhl p 7 / rPhl p 12; $n = 6$ [0.9%]) and birch (t213, rBet v 2 / rBet v 4; $n = 2$ [0.3%]). Component-resolved diagnosis to *C. herbarum* (Cla h 8, Cla h 6, and Cla h 3) was not conducted. However, the data of our aeropalynological monitoring, which revealed extremely high levels of *Cladosporium* concentrations, necessitate studying *C. herbarum* allergen and its influence on the occurrence and course of allergic rhinitis and bronchial asthma. Moreover, the established cross-reactivity between *A. alternata* and *C.Vherbarum* due to the presence of homologous allergens (Alt a 1 and Cla h 3) increases the relevance of this problem, since Alt a 1 (the main *a* allergen) is associated with the development of bronchial asthma. In this study, the pronounced clinical symptoms of allergic rhinitis in 11.7% of patients may have been due to the absence of a cause-significant allergen (including *C. herbarum*) and the need to include nasal provocative tests in clinical practice with the aim of excluding or confirming local allergic rhinitis.

The present article demonstrates the specific weight of fungal allergens in the structure of etiologically significant allergens in allergic rhinitis and the value of studying the sporulation of mold fungi in the ambient air and their influence on the development and course of allergic rhinitis and bronchial asthma.

Based on the aeropalynological monitoring results, the dusting and sporulation calendar for Rostov-on-Don and the Rostov region (plant palynology data are described in the corresponding article [14]) was updated for the first time in 47 years. For this purpose, 24 taxa, with their pollen having allergenic properties and prevailing in the air within the territory of Rostov-on-Don and the Rostov region [14], were selected, and the obtained data of recorded mold fungi sporulation (previously not reflected in dusting calendars) were included (Fig. 5), which undeniably contributed to the work of clinical allergists.

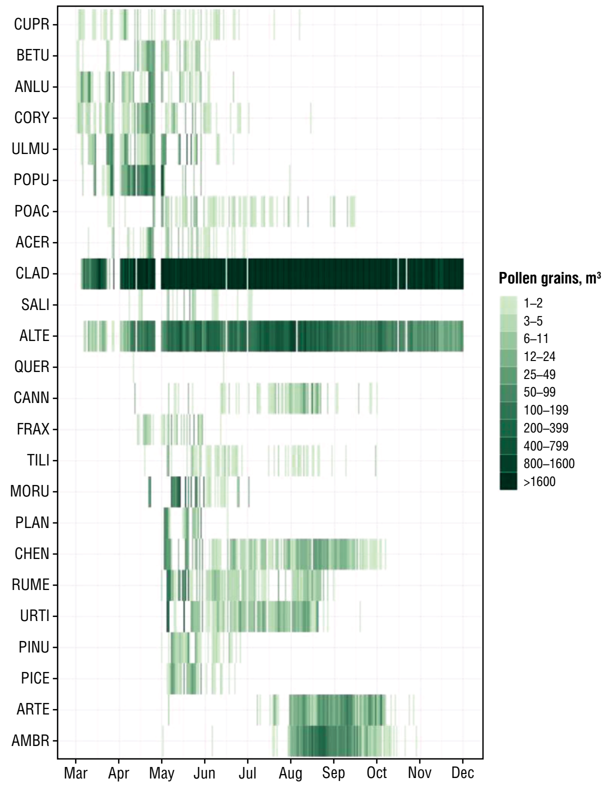

Fig. 5. The calendar dusting of plants* and sporulation of fungi** for Rostov-on-Don, 2019.
*Birch (Betula), Poplar (Populus), Pine (Pinus), Spruce (Picea), Maple (Acer), Hazel (Corylus), Alder (Alnus), Willow (Salix), Ash (Fraxinus), Elm (Ulmus), Linden (Tilia), Oak (Quercus), Hornbeam (Carpinus), Mulberry (Morus), Cypress (Cupressaceae), Cereals (Poaceae), legumes (Fabaceae), plantain (Plantago), sorrel (Rumex), nettle (Urtica), hemp (Cannabis), haze family (Chenopodiaceae), Ambrosia (Ambrosia), wormwood (Artemisia); **Alternaria (Alternaria), Cladosporium (Cladosporium).

Discussion

C. herbarum is a typical species of the genus *Cladosporium,* with its airborne spores most common in summer (peak concentrations) and autumn. These spores may be found indoors or on organic materials in the soil and manifest as a plant parasite. The organisms grow optimally at temperatures between +18°C and +28°C, with maximum growth temperatures between +28°C and +32°C at pH 6 [6, 11]. Spore formation is higher in humid conditions compared with that in dry conditions. *C. herbarum* spores are easily dispersed in air currents, which enhance their effect as a fungal respiratory allergen, being one of the major fungal causes of allergic rhinitis and bronchial asthma in the western hemisphere [4].

Over 60 *C. herbarum* antigens have been identified; of these, 36 antigens were associated with blood serum IgE isotype antibodies of patients [17]. Currently, the following allergenic molecules of *C. herbarum* are described: Cla h 1, Cla h 2, and Cla h 3 (aldehyde dehydrogenase), Cla h 4 (commonly known as Cla h 5 [ribosomal ribonucleic acid {RNA} P2]), Cla h 6 (enolase), Cla h 7 (YCP4 protein), Cla h 8 (mannitol dehydrogenase), Cla h 9 (vacuolar serine protease), Cla h 10 (aldehyde dehydrogenase), Cla h 12 (ribosomal RNA P1), Cla h abH (alpha/beta hydrolase), Cla h8 CSP (cold shock protein), Cla h GST (glutathione-S-transferase), Cla h HCh1 (hydrophobin), Cla h HSP70 (heat shock protein 70), Cla h NTF2 (nuclear transport factor 2), and Cla h TCTP (translationally controlled tumor protein or histamine-releasing factor). Moreover, the above-mentioned Cla h 5 (ribosomal P2 protein, previously designated as Cla h 4) shows 60% aminoacidic sequence homology with other ribosomal P2 proteins [17]. The predominant major *Cladosporium* allergen was identified after the determination of nicotinamide adenine dinucleotide phosphate-dependent mannitol dehydrogenase (Cla h 8), which was revealed by isotype IgE antibodies in 57% of *Cladosporium*-sensitized patients [18].

Alternaria spores may be carried by the wind for several hundreds of kilometers and, therefore, detected both in the ambient air and in house dust [4]. The source of *A. alternata* spores is considered to be rotting plant parts, birds' nests, seeds of cereal crops, and soil [4]. Optimal conditions for sporulation include temperatures of +25 to +30°C, windiness, increased humidity, and fertile soil [3, 11]. These features explain the seasonal peak in the concentration of fungal spores in the air during leaf fall (late summer and early autumn). *A. alternata* allergens are detected in high concentrations in bioaerosols from enterprises that process agricultural products (wood and grain) or fur and may be detected in the air of greenhouses [4]. In addition, pulmonary fungal infections and localized skin infections, e.g., in patients who receive long-term glucocorticosteroid therapy and especially in those with immunodeficiency, can be a source of the allergen [4]. Sensitization to *A. alternata* antigens has been shown to be associated with the development and severity of bronchial asthma, allergic rhinitis, and atopic dermatitis [4]. At present, 11 allergens of *A. alternata* extract are described, with Alt a 1 and Alt a 2 identified as species-specific (major) *A. alternata* allergens and the other as cross-reactive [19]. Alt a 1 is the main major allergen that reacts with the IgE serum of over 90% of patients sensitized to *Alternaria* [19], whereas Alt a 2 (aldehyde dehydrogenase) is determined by IgE antibodies in 61% of people allergic to *Alternaria* [19]. Minor *A. alternata* allergens, such as Alt a 3 (heat shock protein), Alt a 4 and Alt a 5 (enolase, formerly known as Alt a 11), Alt a 6 (highly conserved fungal enolase allergens), Alt a 7, Alt a 10, Alt a 12, Alt a 70kD, and Alt a NTF2 (nuclear transport factor 2) may be found as well [20]. Thus, Alt a 1 and Alt a 6 are important components of *A. alternata* allergens in view of their ability to induce primary sensitization (Alt a 1) or explain cross-reactivity (Alt a 6) [20].

Cross-reactivity is observed between *Alternaria* and *Cladosporium* due to the existence of homologous allergens (Alt a 10 and Cla h 3), aldehyde dehydrogenase (Alt a 6 and Cla h 4), ribosomal RNA P2 (Alt a 7 and Cla h 5), *Saccharomyces cerevisiae* protein (YCP4), Alt a 11, and Cla h 6 (enolase, major allergen) [21]. In addition, enolase from *S. cerevisiae* (baker's yeast) has been shown to have high cross-reactivity with other fungal enolases, particularly *C. herbarum*, *A. alternata*, *Candida albicans*, and *Aspergillus fumigates* [22], whereas latex enolase from Hev b 9 is characterized by cross-reactivity with *C. herbarum* and *A. alternata* enolases [17, 23], which must be considered in the etiological verification of the allergic disease.

The coincidence of the peak of wormwood concentration on October 3, 2019, with the peak concentration of fungal spores on this day (11,587 U/m^3 of *Cladosporium* and 389 U/m^3 of *Alternaria*) and the coincidence of this circumstance with the thunderstorm rain that had passed a few hours before are consistent with the results of several studies in children and adults, which revealed that the increase in the number of spores in the atmosphere was associated with a significant rise in the number of patients with asthma symptoms and emergency care visits [24, 25]. Allergenic protein concentrations increase with thunderstorm allergy, and therefore, it is planned to analyze the available hourly dusting/sporulation results for that day and study the possibility of long-range transport of pollen and spores, given existing evidence that increased mold spore concentrations may play a role in thunderstorm asthma, a phenomenon characterized by a dramatically high number of hospital admissions for asthma patients after thunderstorms. In particular, a Canadian study showed that the number of fungal spores doubled and the number of asthma emergencies in children increased by >15% per day during thunderstorms, while the concentration of pollen and other air pollutants remained constant [26]. These studies suggest that airborne mold may also be a risk factor for the development and exacerbation of asthma. On this basis, it was appropriate to demonstrate the above-mentioned clinical noncomparative observational study on identifying the sensitization spectrum in patients with seasonal allergic rhinitis, which exposes the possible specific weight of fungal allergens (*Cladosporium* and *Alternaria*) in the structure of causally important allergens in allergic rhinitis. This work once more confirmed the importance of studying the sporulation of mold fungi in the ambient air and their role in the development and course of allergic rhinitis and bronchial asthma.

Findings

Fungal allergens play an important role in the development of the spectrum of sensitization in patients with allergopathology. Fungal reproduction greatly depends on climatic, geographical, meteorological, and specific environmental features. Aeropalinological monitoring, which was conducted in Rostov-on-Don (March–October, 2019) revealed the following features:

1. Presence of mold spores in the ambient air was recorded within the entire observation period. The main

spores that were detected in the air were fungi of the *Cladosporium* and *Alternaria* genera. The sporulation of these mold fungi already observed in March, after the snow cover melted, refutes the statements found in the available literature on the beginning of formation of *Cladosporium* and *Alternaria* spores in the region since May [3, 5]. The recorded levels of *Cladosporium* concentrations exceeded those indicated in many literature sources by a factor of 2 [3, 5].

2. Sporulation of mold fungi of the *Cladosporium* and *Alternaria* genera observed within the entire period of plant vegetation in the region may have resulted from respiratory allergy in patients of the Rostov region with the peak of clinical manifestations in July–September.
3. The synchronous period of plant blooming and micromycete sporulation increased the antigenic load, which contributed to aggravation of sensitization and development of allergopathology in the population of the Rostov region with transformation into more severe forms of the disease and promoted the expansion of causal allergens and mixed sensitization [14].
4. Since sensitization by mold fungi is a powerful risk factor for severe allergic rhinitis and bronchial asthma and *C. herbarum* volatile spores dominate in the atmosphere, which was revealed by aeropalynological monitoring, it is recommended to further study *C. herbarum* allergen and its influence on the occurrence and course of allergic rhinitis and bronchial asthma.
5. For the purpose of a differential diagnostic approach and etiotropic selection of allergenic immunotherapy, it is imperative to detect sensitization to mold fungi, for which it is required to amend the standards for providing medical care to the population of the Rostov region.
6. Nasal provocative tests should be performed to exclude/confirm local allergic rhinitis when studying the sensitization spectrum in patients with allergic rhinitis.
7. Revealing the results of sporulation within a continuous calendar year, including November–February, is of interest.

Conclusions

Thus, this study was the initial stage of dynamic observation of the qualitative and quantitative composition of the regional airborne allergens, including the dominant fungal allergens in the ambient air of the region, which are represented by the *Cladosporium* and *Alternaria* genera. The calendar of plant dusting and sporulation of *Cladosporium* and *Alternaria* fungi was updated for Rostov-on-Don and the Rostov region. This calendar, which was compiled for the region in 2019, will become one of the preventive tools of practical allergology, which will allow both patients to plan their rhythm of life, thereby independently forecasting the course of pollen and fungal allergies, and doctors to reduce the number of allergy exacerbations and ambulance calls based on the knowledge of the hourly assessment of pollen and fungal spore concentration with parallel monitoring of weather conditions.

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