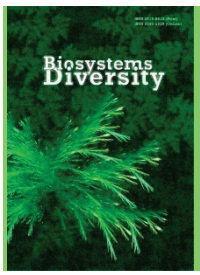

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Features of the seasonal dynamics of airborne fungal spore concentrations in Ukraine

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Fungi represent a distinct kingdom of ubiquitous living beings. Most other organisms are constantly in contact with elements of the fungal body. In particular, the widespread vegetation of fungi in the environment, their sporulation and the further spread of spores and mycelia by air currents make the contact of fungal particles with human respiratory organs inevitable. This can lead to the development of sensitization and allergies. Therefore, the aim of the presented study was to determine the taxonomy of airborne fungal spores, the qualitative and quantitative changes in this composition at different times of the year compared with previous sporulation seasons, as well as the prevention of allergic diseases. The study of fungal sporulation was carried out from 2011 to 2021 by volumetric method using Burkard trap of a Hirst type in a 24-hour mode (from February to November). The device was installed on the roof of the chemical building of the National Pirogov Memorial Medical University, Vinnytsya. Reading of the microscopic slides obtained from air samples was carried out using light microscopes with a magnification of 400 and 1000 by the method of 12 vertical transects. It was found that spores of various taxonomic groups of fungi, namely, of Asco- and Basidiomycetes were observed throughout the growing season from March to October with the highest concentrations in mid and late summer. Thus, in June, July and August, peak concentrations of spores of the fungi *Ganoderma*, *Coprinus*, *Cladosporium* were observed; in July, September and October – of Ustilaginales; from July to November – of *Alternaria*, *Epicoccum*; in October – of different Basidiospores, and also *Periconia*, *Stemphylium* and Uredinales; in September – of *Agrocybe*. Among all studied micromycetes, concentrations of *Cladosporium* spores were the highest. However, significant sporulation persisted even in the autumn period, which made fungal spores a specific causative agent of airborne respiratory diseases at this time, especially against the background of low pollen concentrations. Climate change can lead to modification in the length of the fungal growing season. This information should be taken into account when predicting the symptoms of seasonal allergies in the population.

Keywords: airborne fungi; aeroallergens; sporulation season; allergic diseases; climate changes.

Introduction

Fungi constitute a distinct kingdom of living organisms, representatives of which are ubiquitous in nature (Bilous et al., 2018). Therefore, contact with elements of the fungal body in most other living organisms occurs constantly. In particular, the widespread vegetation of fungi in the environment, their sporulation and the further spread of spores and mycelium by air currents make the contact of fungal particles with human respiratory organs inevitable (Pashley & Wardlaw, 2021; Anees-Hill et al., 2022). Because of their ubiquity and ability to affect the health of plants, animals and humans, fungi are of practical interest to the fields of ecology, parasitology, botany, zoology and medicine.

The fragments of fungi are present both indoors and outdoors all year round. Their concentrations vary depending on meteorological parameters and the location of the emission sources (Hanson et al., 2022; Martinez-Bracero et al., 2022). In general, fungi can grow in extreme conditions: under high / low temperatures, high concentrations of salts / sugars, under low acidity as well as under low oxygen levels. In nature, fungi most often play the role of saprophytes. They can also be commensals, parasites and symbiotics. In ecological chains, fungi are reductants (Charalampopoulos et al., 2022). The main contribution of these microorganisms to nature is the decomposition of organic materials (McLaughlin & Spatafora, 2014).

Useful properties of fungi have been utilized by mankind for a long time. Many species of *Aspergillus*, *Penicillium* have been of great economic, biotechnological, medical and social importance (McLaughlin & Spatafora, 2014; Tsang et al., 2018).

On the other hand, fungi are well known for their harmful effects on agricultural crops. Throughout human history, they have been known for their ability to cause plant diseases. Such fungi cause significant economic losses and negatively impact plants with the release of toxic substances that can also affect human health.

Moulds such as *Alternaria*, *Aspergillus*, *Cladosporium*, *Penicillium* and others can cause food spoilage (Tsang et al., 2018). They cause a decrease in the yield of food crops before and after harvest. Many species that spoil food are also mycotoxin producers (Mousavi et al., 2016; Martinez-Bracero et al., 2022).

Some of them are infectious agents and cause disease in humans. The most well-known pathogenic species is *Aspergillus fumigatus* (Seyedmousavi et al., 2015; Rudramurthy et al., 2019; Gnat et al., 2021). This is the etiological factor for most cases of aspergillosis (Gnat et al., 2021). Some fungi can cause allergic symptoms, which occur after a contact with a significant amount of fungal spores entering the respiratory tract and due to prior sensitization of a potential patient to fungal allergens (Crameri et al., 2014; Fukutomi & Taniguchi, 2015; Rekalova & Petrenko, 2018; Li et al., 2019). In particular, allergens of microscopic fungi (micromycetes), the concentrations of which change during the growing season, can act as etiological factors of seasonal allergies (Priyamvada et al., 2017).

Aeroallergens can cause skin, respiratory, and conjunctival reactions in sensitized individuals. For example, they are the factor in the development of allergic rhinoconjunctivitis and bronchial asthma (Crameri et al., 2014; Brożek et al., 2017; Rekalova & Petrenko, 2018).

Both pathogenic fungi that cause invasive diseases, and opportunistic fungi that grow on the surface of the skin and mucous membranes can trigger allergic symptoms. Fungal spores or, more rarely, parts of the mycelium are causally significant in the development of disease (Zuberbier et al., 2018).

Nevertheless, among airborne allergens, fungal spores are the least studied. In particular, unlike the pollination calendar of plants, which is

approximately known to allergists, the periods of fungal sporulation and their systematic composition in the air remain poorly known. At the same time, the concentration of spores of microscopic fungi in the atmosphere can be several tens or even hundreds of times higher than the concentration of pollen grains (Burks et al., 2020). Therefore, fungal spores are biological particles that are most often found in the air, which makes them the predominant component of bioaerosols having a great impact on the ecology, economy and health of living organisms. The economic and social consequences of such impacts can be reduced through accurate and timely forecasting of airborne spore concentrations.

Aerobiological monitoring serves this purpose. In Ukraine, long-term observations, which include the monitoring of fungal spores, are carried out in Vinnytsia, Zaporizhzhia, Lviv, Ivano-Frankivsk and Kyiv. The network of monitoring stations does not cover the entire territory of Ukraine. In addition, fungal spores are monitored on a permanent basis only by the stations in Vinnytsia and Zaporizhzhia. Therefore, the issue of assessing fungal air pollution in the context of the prevention of allergic diseases remains relevant.

The purpose of the presented study was to determine the taxonomy of airborne fungal spores, the qualitative and quantitative changes in this composition at different times of the year compared with previous sporulation seasons, as well as the prevention of allergic diseases.

Materials and methods

The study of fungal sporulation patterns was conducted on the basis of the Educational Scientific and Research Laboratory for the Study of Allergenic Factors of the Environment (LSAFE) of National Pirogov Memorial Medical University, Vinnytsya.

Based on the application of the volumetric aerobiological method, air samples were taken by a Burkard trap of a Hirst type in a 24-hour mode during the years 2011–2021 from February to November. The device was set on the roof of the chemical building of the National Pirogov Memorial Medical University, Vinnytsya in accordance with the requirements of the European Aerobiological Society (Galan, 2012).

A sample of Melinex tape with a length of 345 mm was wound on the drum, which was controlled by a timing mechanism and one revolution in the device was made during one week. Before sampling, the "Melinex" tape was covered with a sticky substance, which was a solution of glycerin, gelatin and phenol. After one week's exposure, the tape was divided into 7 equal fragments corresponding to 1 day of observation. One microscopic sample was made from each fragment, which was fixed on a glass slide with a glycerin-gelatin mixture. For better identification of fungal spores, the samples were stained with the same mixture with the addition of basic fuchsin.

Reading of the obtained microspecimens, which provided for fungal spores counting, was carried out using light microscopes with magnifications of × 400 and × 1000 by the method of 12 vertical transects. This made it possible to obtain values of both hourly and daily concentrations of fungal spores in a cubic meter of air.

The taxonomy of the collected fungal spores was determined according to the scientific literature (Smith, 1984, 1986; Kagen et al., 2004–2005).

Tools of the descriptive statistics of the European Aeroallergen Network (EAN) were used for the general characteristics of individual spore seasons, which made it possible to determine the start, duration, end of the season as well as the peak concentrations of fungal spores during the sporulation seasons. The determination of the amount of spores collected during the season of individual categories, as well as the arithmetic mean (x) and standard deviation (SD), was carried out using the Excel program.

A threshold of 100 spores/m^3 was established to determine the clinically significant concentrations of fungal spores of most taxa, which trigger allergic symptoms, except for *Cladosporium*. This value corresponds to that described in the literature, namely, for *Alternaria* (Sofiev & Bergmann, 2013). For *Cladosporium*, according to the same source, the threshold was set at 2,500 spores/m^3, due to the lower allergenicity of this spore type and its presence in the air in the highest concentrations. Both season timing and timing for concenreations over the settled threshold are indicated on the graphics corresponding to the description of every spore type in the Results section.

Results

The study showed that the total concentrations of most spores were the highest in the middle and at the end of summer. In autumn, though, when little pollen was observed, the sporulation of most fungi was also characterized by relatively high concentrations (Fig. 1).

In terms of the sporulation of fungi of individual genera and orders, the following seasonal dynamics were observed for fungi of the Division Basidiomycota of the Classes Ustilagomycetes, Agaricomycetes, Urediniomycetes, which usually vegetate in autumn.

The fungal season of *Ustilago* in 2011–2021 was recorded from the end of February to November (Fig. 2, Table 1). The highest levels of *Ustilago* spores were observed in the second fornight of July and in September and October. The average season length was 201.1 days and ranged from 70 days in 2018 to 247 days in 2020 and 2021.

Table 1
Characteristics of the *Ustilago* sporulation in 2011–2021

Year	Characteristics of the sporulation season							
	the start of the season, date	the end of the season, date	duration of the season, days	the day of peak sporulation	max, spores/m^3 (peak value)	Σ, spore/m^3/season	number of days with concentration >100 spores/m^3	the number of intervals in spore registration
2011	17.03	13.10	210	24.06	547.0	14453.0	36	0
2012	22.03	21.10	213	22.09	296.9	15692.3	53	0
2013	12.03	18.10	220	01.09	307.4	10421.1	25	0
2014	26.02	09.10	225	26.02	254.9	5508.1	3	2
2015	01.03	04.06	91	01.03	162.4	999.2	1	5
2016	04.03	22.10	232	15.10	309.3	2821.3	3	2
2017	26.02	19.10	235	01.03	216.7	5020.7	6	2
2018	28.06	06.09	70	09.08	166.1	2937.4	10	1
2019	12.03	20.10	222	14.04	2158.6	51802.2	162	4
2020	26.02	30.10	247	11.10	932.7	29174.5	102	2
2021	28.02	02.11	247	28.06	872.9	20228.1	63	6
Average for 2011–2021 (x ± SD)	–	–	201.1 ± 61.0	–	565.9 ± 592.6	14459.8 ± 15102.0	42.2 ± 50.9	–

The average peak value of the airborne spore concentration during this period was 565.9 spores/m^3. Among the highest ones were the peak values of *Ustilago* spore concentrations observed in 2011 and 2021 at the end of June with values of 547.0 spores/m^3 and 872.9 spores/m^3, respectively, as well as in October 2020 (932.7 spores/m^3). The lowest peak value for this type of fungi was recorded in March 2015 being 162.4 spores/m^3. The highest peak value for the entire observation period was registered in April 2019 and was 2,158.6 spores/m^3. The average total number of collected spores was 14,459.8 spores/m^3/season. At the same time, the seasonal amount of spores varied from 999.2 spores/m^3/season in 2015 to 51,802.2 spores/m^3/season in 2019. The average seasonal number of days with a clinically significant concentration of more than 100 spores/m^3 was 42.2 ± 50.9 days.

The intervals in *Ustilago* spores' recording were as follows: in 2021, 6 intervals were observed, in 2015 – 5 intervals, in 2019 – 4 intervals, in 2014, 2016, 2017 and 2020 – 2 intervals each, in 2018 – 1 interval. In 2011–2013, *Ustilago* spores were observed continuously without any gaps throughout the season (Table 1).

The *Coprinus* season in 2011–2021 began in March and lasted until November (Fig. 3, Table 2). The highest concentrations of *Coprinus* spores were observed from mid-May to mid-August. The average length of the season was 161.5 days and ranged from 61 days in 2018 to 229 days in 2016.

The average peak value during this period was 1,139.4 spores/m^3. For almost the entire period of observation, the peak concentrations recorded for *Coprinus* were significant. So, in 2018, the peak was

registered in July with a value of 1858.0 spores/m^3. In 2011, 2013, 2019 and 2021, peaks were observed in June with values of 1,145.0 spores/m^3, 1,374.1 spores/m^3, 1,196.3 spores/m^3, 1,172.8 spores/m^3, respectively. In general, the peak values for this fungi species ranged from the lowest amount of 71.6 spores/m^3 in May 2015 to the highest – of 2,462.4 spores/m^3 in October 2020.

The average total number of spores collected was 27,662.9 spores/m^3/season. At the same time, the seasonal amount of spores varied from 1,217.2 spores/m^3/season in 2015 to 66,426.9 spores/m^3/season in 2020. The average number of days with a concentration of more than 100 spores/m^3 was 75.9 ± 43.1 days. The number of intervals during the registration of *Coprinus* spores for the monitoring period was as follows: 6 intervals were observed in 2021, 5 intervals – in 2015, 4 intervals – in 2019, 2 intervals each – in 2014, 2016, 2017 and 2020 years. 1 interval was registered in 2018. In 2011–2013, *Coprinus* spores were observed without gaps throughout the season continuously (Table 2).

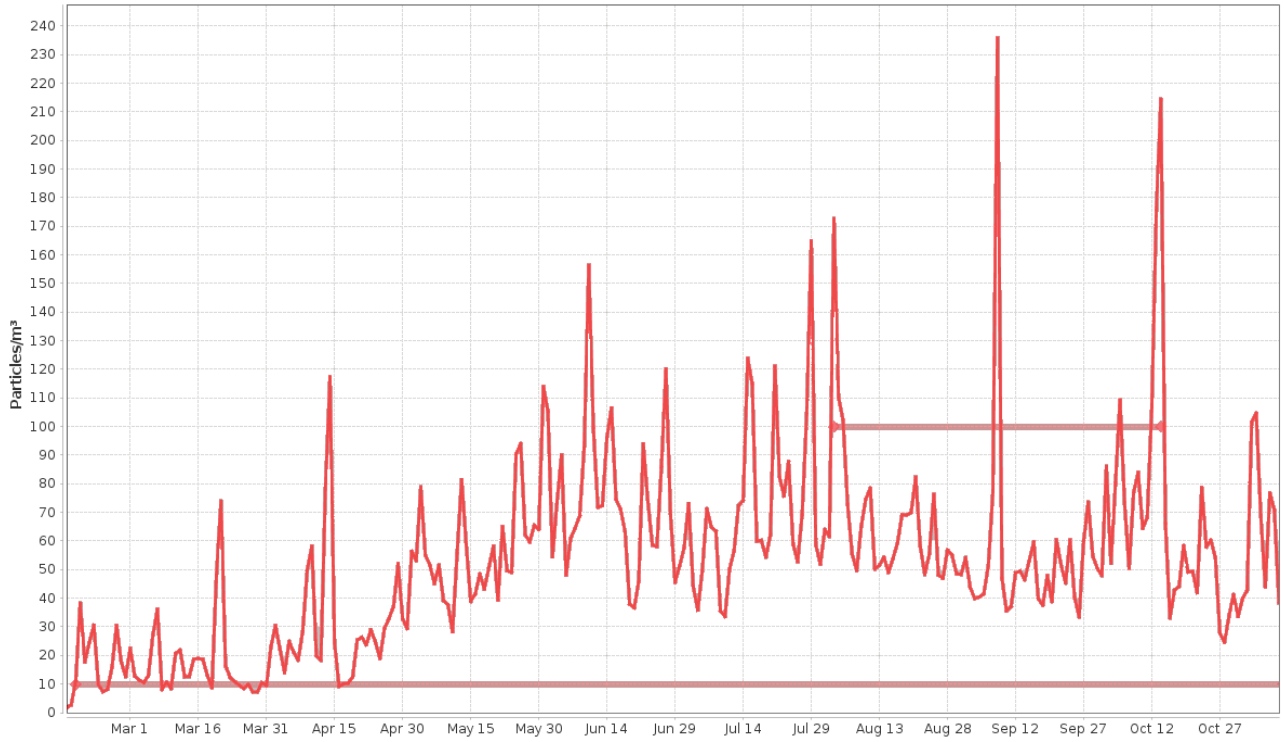

Fig. 1. The average seasonal dynamics of *Agrocybe*, *Alternaria*, Ascospores, Basidiospores, *Epicoccum*, *Fusarium*, *Ganoderma*, *Helminthosporium*, *Leptosphaeria*, *Periconia*, Pithomyces, *Pleospora*, *Puccinia*, *Stemphylium*, Uredinales, *Ustilago* in Ukraine during 2011–2021

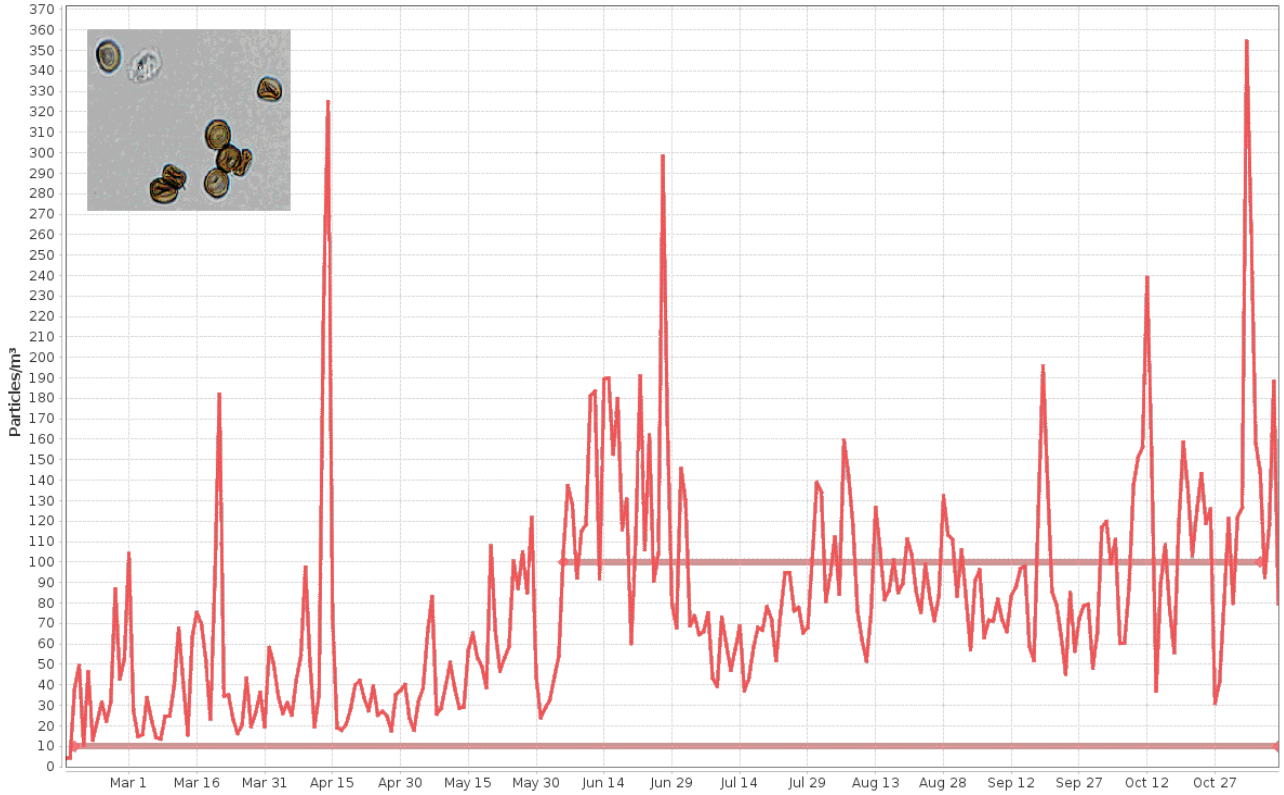

Fig. 2. The average seasonal dynamics of *Ustilago* during 2011–2021

The *Agrocybe* season in 2011–2021 was recorded from March to the end of October (Fig. 4, Table 3). The most intensive period of *Agrocybe* sporulation was registered in September. The average seasonal period of these spores registration was 116.6 days and varied from 42 days in 2018 to 188 days in 2011 and 2014.

The average peak value of the concentration of spores in the air during this period was 123.6 spores/m^3. Among the highest were the peak concentrations of *Agrocybe* spores, which were recorded in August 2011 with a value of 110.0 spores/m^3, in October 2012 – with a value of 117.9 spores/m^3, in September 2017 – with a value of 165.4 spores/m^3.

Table 2
Characteristics of the Coprinus sporulation in 2011–2021

Year	Characteristics of the sporulation season							
	the start of the season, date	the end of the season, date	duration of the season, days	the day of peak sporulation	max, spores/m^3 (peak value)	Σ, spore/m^3/season	number of days with concentration >100 spores/m^3	the number of intervals in spore registration
2011	04.05	01.10	150	20.06	1145.0	27046.0	77	0
2012	22.04	23.10	184	16.08	911.1	23114.3	82	0
2013	11.05	12.10	154	09.06	1374.1	28369.2	90	0
2014	17.04	14.09	150	26.05	544.4	10436.8	32	2
2015	26.03	03.06	69	08.05	71.6	1217.2	0	5
2016	04.03	19.10	229	30.06	724.1	10845.4	29	2
2017	11.04	23.10	195	19.10	1074.1	27088.1	91	2
2018	27.06	27.08	61	21.07	1858.0	25310.0	57	1
2019	20.03	20.10	214	09.06	1196.3	42562.5	125	4
2020	21.03	01.11	225	11.10	2462.4	66426.9	140	2
2021	01.05	23.09	145	22.06	1172.8	41875.6	112	6
Average for 2011–2021 (x ± SD)	–	–	161.5 ± 56.8	–	1139.4 ± 636.3	27662.9 ± 17980.6	75.9 ± 43.1	–

The lowest peak value was recorded in May 2014 and was 16.1 spores/m^3. The highest peak value for the entire observation period was registered in September 2021 and was 608.0 spores/m^3.

The average total number of spores collected was 1,021.4 spores/m^3/season. At the same time, the amount of spores per season varied from 87.0 spores/m^3/season in 2019 to 5,789.4 spores/m^3/season in 2021.

The average seasonal number of days with a clinically significant concentration of more than 100 spores/m^3 was 1.7 ± 4.1 days. The intervals in spores registration were changed as follows: in 2021, 6 intervals were observed, in 2015 – 5 intervals, in 2019 – 4 intervals, in 2014, 2016, 2017 and 2020 – 2 intervals each. In 2018, 1 interval was observed. In 2011–2013, *Agrocybe* spores were recorded continuously throughout the season, without gaps (Table 3).

The Uredinales season in 2011–2021 was recorded from the end of February to the end of October (Fig. 5, Table 4). Maximum levels of Uredinales spores were recorded from mid-May to mid-October. The average length of the season for these fungi was 173.4 days. It ranged from 61 days in 2018 to 233 days in 2021.

The average peak concentration of spores during this period was 99.6 spores/m^3. The following peak concentrations were noticed during the period of observation: in August 2012 the peak had a value of 119.1 spores/m^3, in October 2013 – 153.1 spores/m^3, in June 2020 – 147.5 spores/m^3. The lowest peak value for Uredinales was recorded in March 2015 with a value of 19.1 spores/m^3 and the highest peak value was recorded in August 2019 with a value of 169.7 spores/m^3.

The average total number of spores collected was 2,468.9 spores/m^3/season. At the same time, the seasonal amount of spores varied from 98.7 spores/m^3/season in 2015 to 3,910.2 spores/m^3/season in 2019.

The average number of days with a clinically significant concentration of Uredinales spores was 1.1 ± 1.5 day.

The number of intervals during the registration of these fungal spores for the monitoring period was as follows: 6 intervals were noted in 2021, 5 intervals – in 2015, 4 intervals – in 2019; 2 intervals in each 2014, 2016, 2017 and 2020 years, one interval – in the year 2018. In 2011–2013, spores of Uredinales fungi were observed continuously throughout the season, without gaps (Table 4).

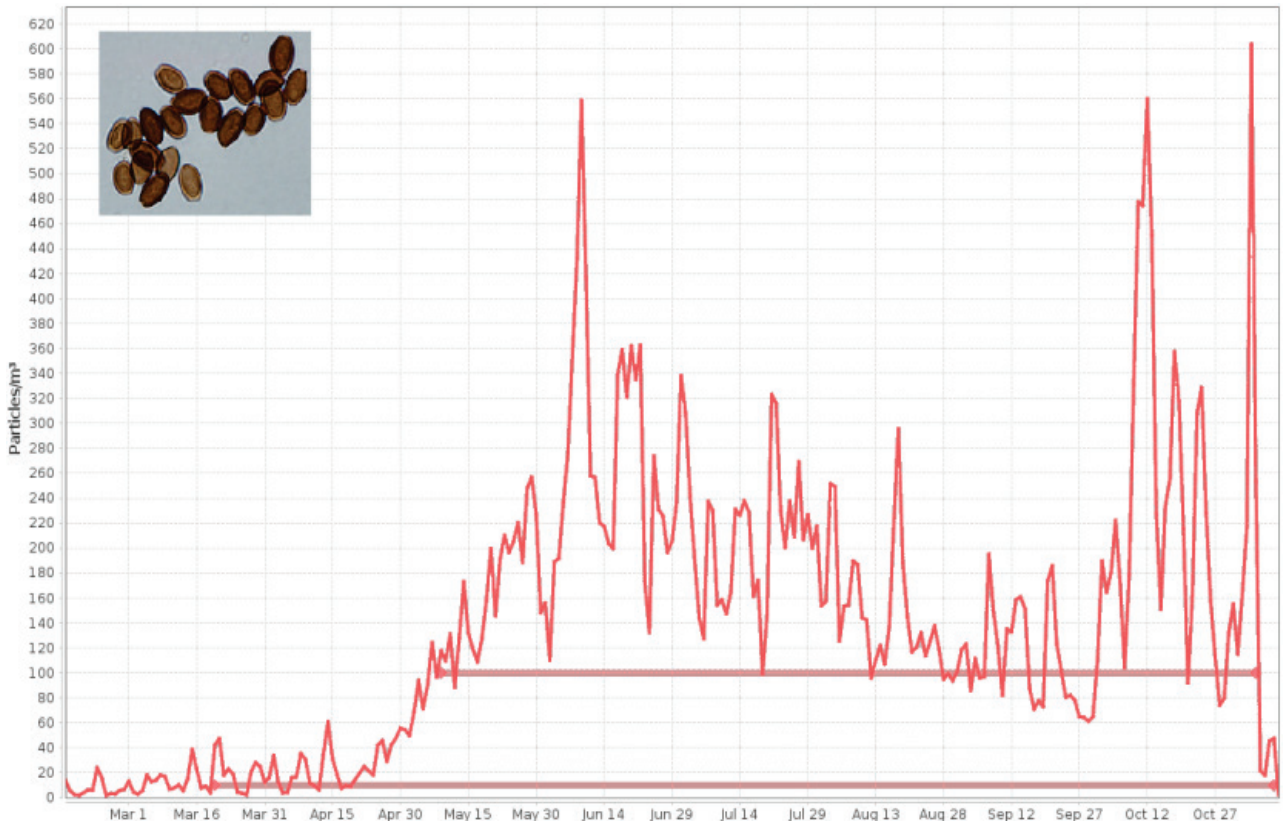

Fig. 3. The average seasonal dynamics of *Coprinus* during 2011–2021

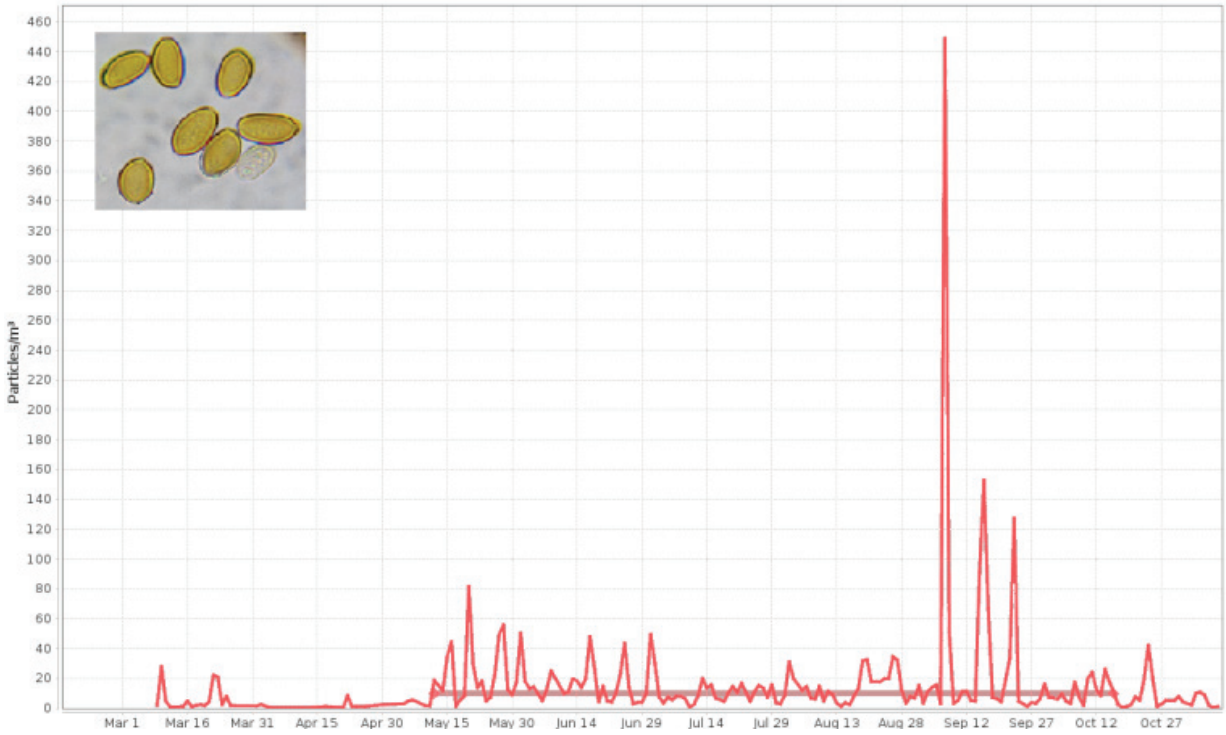

Fig. 4. The average seasonal dynamics of *Agrocybe* during 2011–2021

Table 3
Characteristics of the Agrocybe sporulation in 2011–2021

Year	Characteristics of the sporulation season							
	the start of the season, date	the end of the season, date	duration of the season, days	the day of peak sporulation	max, spores/m^3 (peak value)	Σ, spore/m^3/season	number of days with concentration >100 spores/m^3	the number of intervals in spore registration
2011	10.03	14.09	188	19.08	110.0	825.0	1	0
2012	05.05	23.10	171	23.10	117.9	1652.8	2	0
2013	20.05	07.10	140	29.07	38.9	447.4	0	0
2014	24.03	28.09	188	25.05	16.1	200.7	0	2
2015	19.03	06.06	79	22.03	22.2	124.7	0	5
2016	10.07	01.10	83	21.08	49.4	500.6	0	2
2017	07.07	08.10	93	16.09	165.4	707.2	1	2
2018	28.06	09.08	42	02.08	143.2	718.5	1	1
2019	12.03	16.05	65	16.05	61.7	87.0	0	4
2020	06.07	14.10	100	14.07	26.6	181.6	0	2
2021	19.05	30.09	134	23.09	608.0	5789.4	14	6
Average for 2011–2021 (x ± SD)	–	–	116.6 ± 50.6	–	123.6 ± 168.7	1021.4 ± 1643.7	1.7 ± 4.1	–

Table 4
Characteristics of the Uredinales sporulation in 2011–2021

Year	Characteristics of the sporulation season							
	the start of the season, date	the end of the season, date	duration of the season, days	the day of peak sporulation	max, spores/m^3 (peak value)	Σ, spore/m^3/season	number of days with concentration >100 spores/m^3	the number of intervals in spore registration
2011	14.04	11.10	180	09.10	99.0	3331.0	0	0
2012	25.03	04.10	193	16.08	119.1	3879.9	3	0
2013	16.03	18.10	216	18.10	153.1	3846.6	3	0
2014	06.03	02.10	210	28.07	77.2	2429.6	0	2
2015	04.03	06.06	94	18.03	19.1	98.7	0	5
2016	01.07	30.10	121	01.07	76.6	1325.4	0	2
2017	26.03	12.10	200	30.07	88.9	2485.3	0	2
2018	05.07	04.09	61	04.09	101.9	1346.2	1	1
2019	04.04	27.09	176	21.08	169.7	3910.2	1	4
2020	22.02	02.10	223	11.06	147.5	2813.3	4	2
2021	06.03	25.10	233	22.06	43.2	1691.4	0	6
Average for 2011–2021 (x±SD)	–	–	173.4 ± 56.6	–	99.6 ± 46.1	2468.9 ± 1249.8	1.1 ± 1.5	–

Ganoderma spores were recorded in 2011–2021 from mid-March to mid-October (Fig. 6, Table 5). The highest levels of these fungal spores were observed during all summer months, September and early October. The average length of the season was 118.3 days. It ranged from 72 days in 2018 to 167 days in 2017.

The average peak value for *Ganoderma* during this period was 136.8 spores/m^3. During the observation period, peak concentrations of *Ganoderma* spores were noted in 2012 and 2020 in August with values of 204.3 spores/m^3 and 243.8 spores/m^3, respectively. In 2021, the peak was registered in September with a value of 206.8 spores/m^3. In general, the peak value for this fungal species ranged from the lowest peak of 7.4 spores/m^3 in March 2015 to the highest of 308.0 spores/m^3 in August 2019.

The average total number of collected spores was 4,083.5 spores/m^3/season. At the same time, the seasonal sum of spores varied from 43.2 spores/m^3/season in 2015 to 11,699.9 spores/m^3/season in 2019.

Clinically significant concentrations of *Ganoderma* spores were observed, on average, for 8.9 ± 14.5 days.

The number of intervals during the registration of *Ganoderma* spores for the monitoring period was as follows: in 2021, 6 intervals were observed, in 2015 – 5 intervals, in 2019 – 4 intervals, in 2014, 2016, 2017 and 2020 – 2 intervals, in 2018 – 1 interval. In 2011–2013, *Ganoderma* spores were observed continuously throughout the season, without any gaps (Table 5).

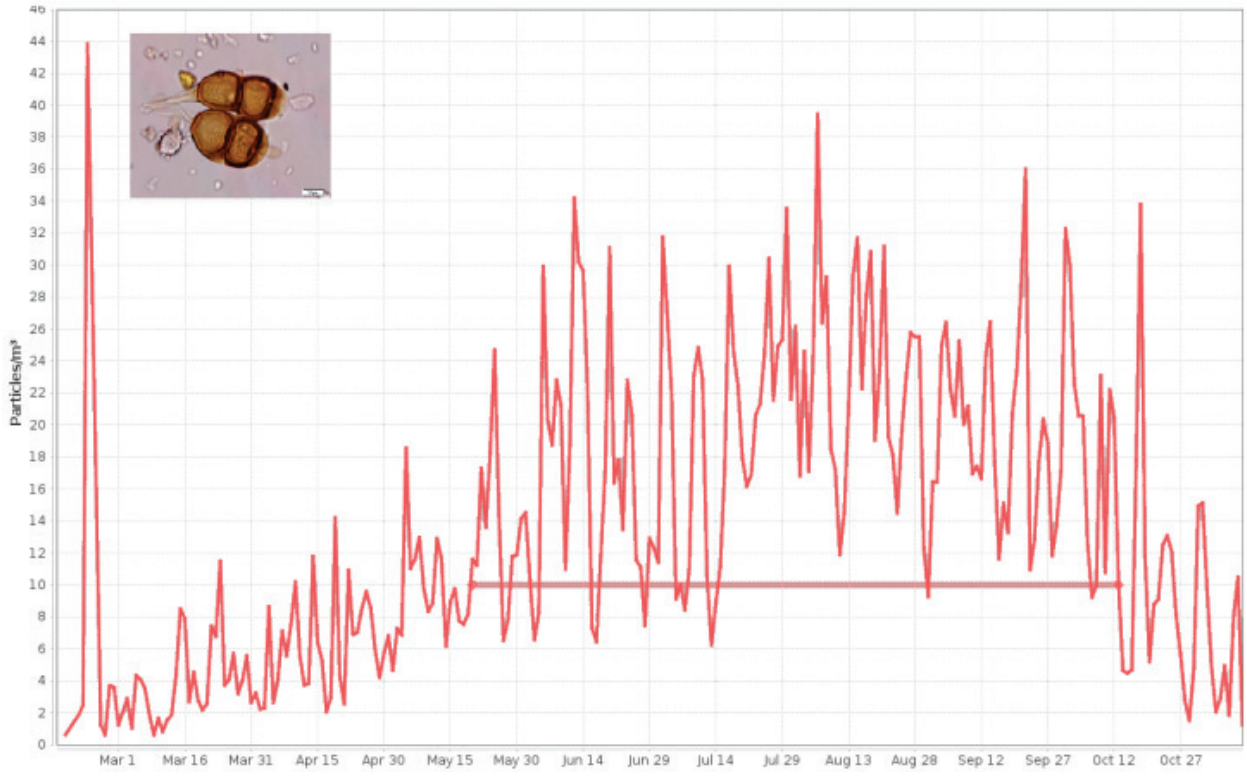

Fig. 5. The average seasonal dynamics of Uredinales during 2011–2021

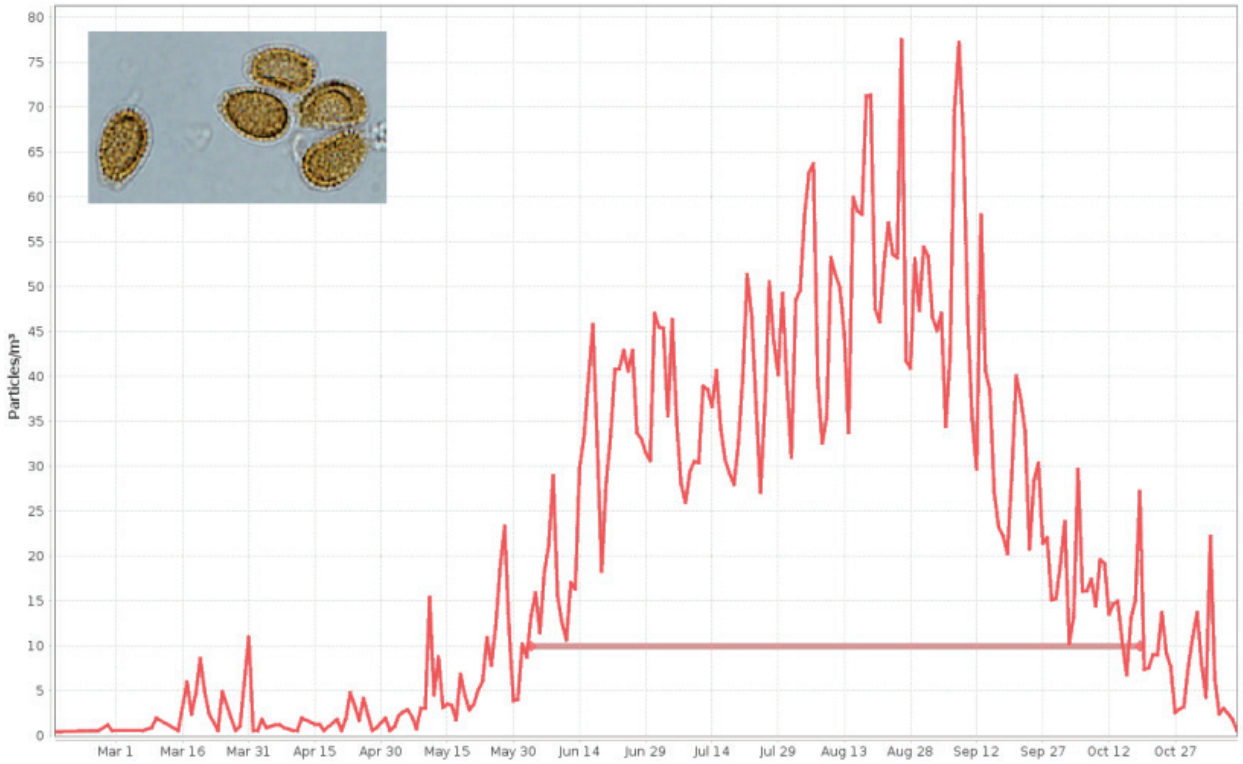

Fig. 6. The average seasonal dynamics of *Ganoderma* during 2011–2021

Unclassified basidiospores, which were combined into the Basidiospores category, were recorded in 2011–2021 from the end of February to November (Fig. 7, Table 6). Maximum levels of Basidiospores were observed in October. Though, in 2014 and 2018, this type of spore was not registered. The average season duration was 92.3 days. It ranged from 1 day in 2013 and 2015 to 240 days in 2021.

The average peak value for Basidiospores during this period was 1,822.3 spores/m^3. During the observation period, the highest peak concentrations of Basidiospores were recorded in 2017, 2019 and 2021 in April with values of 59.9, 1,282.7 and 600.6 spores/m^3, respectively. The peak for this type of fungi ranged from the lowest value of 1.2 spores/m^3 in May 2015 to the highest – for 14,394.6 spores/m^3 in October 2020. The average total number of collected spores was 11,766.9 spores/m^3/season. At the same time, the seasonal amount varied from 2.5 spores/m^3/season in 2015 to 62,333.2 spores/m^3/season in 2020.

The average number of days with a clinically significant concentration of more than 100 spores/m^3 was 26.4 ± 40.6 spores/m^3. The number of intervals during the registration of Basidiospores during the monitoring

period changed as follows: 6 intervals were observed in 2021, 5 intervals in 2015, 4 intervals in 2019, 2 intervals in 2016, 2017 and 2020 each. In 2011–2013, Basidiospores were observed continuously throughout the season, without any gaps (Table 6).

Table 5
Characteristics of the *Ganoderma* sporulation in 2011–2021

Year	Characteristics of the sporulation season							
	the start of the season, date	the end of the season, date	duration of the season, days	the day of peak sporulation	max, spores/m^3 (peak value)	Σ, spore/m^3/season	number of days with concentration >100 spores/m^3	the number of intervals in spore registration
2011	24.04	03.10	162	23.08	149.0	4825.0	5	0
2012	15.05	10.10	148	16.08	204.3	6520.4	10	0
2013	30.05	22.09	115	08.09	153.1	3619.9	3	0
2014	23.05	14.09	114	31.08	55.0	1396.2	0	2
2015	18.03	06.06	80	19.03	7.4	43.2	0	5
2016	30.06	06.10	98	01.09	59.3	1243.2	0	2
2017	05.05	19.10	167	19.10	69.1	1654.9	0	2
2018	27.06	07.09	72	21.07	48.8	1051.1	0	1
2019	26.05	18.09	115	26.08	308.0	11699.9	47	4
2020	13.06	19.10	118	09.08	243.8	5685.6	10	2
2021	03.06	23.09	112	07.09	206.8	7179.5	23	6
Average for 2011–2021 (x ± SD)	–	–	118.3 ± 30.4	–	136.8 ± 96.1	4083.5 ± 3515.3	8.9 ± 14.5	–

Fungi of the Division Ascomycota of the Class Dothideomycetes were characterized by the following seasonal dynamics.

The season of *Cladosporium* in 2011–2021 lasted from March to the end of October (Fig. 8, Table 7). *Cladosporium* spores were the most recorded in summer and their concentrations were the highest among all studied micromycetes. The average length of the season was 184.9 days. It varied from 61 days in 2018 to 228 days in 2016.

Table 6
Characteristics of Basidiospores seasons of 2011–2021

Year	Characteristics of the sporulation season							
	the start of the season, date	the end of the season, date	duration of the season, days	the day of peak sporulation	max, spores/m^3 (peak value)	Σ, spore/m^3/season	number of days with concentration >100 spores/m^3	the number of intervals in spore registration
2011	16.03	23.03	7	16.03	2.0	4.0	0	0
2012	03.04	13.09	132	13.09	49.4	84.0	0	0
2013	28.05	29.05	1	29.05	7.4	8.6	0	0
2015	28.05	29.05	1	29.05	1.2	2.5	0	5
2016	04.03	16.03	12	16.03	3.1	10.5	0	2
2017	28.02	15.05	76	03.04	59.9	1336.4	0	2
2019	20.03	25.09	189	14.04	1282.7	22503.8	65	4
2020	13.05	02.11	173	13.10	14394.6	62333.2	99	2
2021	25.02	23.10	240	23.04	600.6	19619.4	74	6
Average for 2011–2021 (x ± SD)	–	–	92.3 ± 93.5	–	1822.3 ± 4734.6	11766.9 ± 21013.1	26.4 ± 40.6	–

The average peak value of the *Cladosporium* during observation period was 12,190.4 spores/m^3. For almost the entire period of observation, the peak concentrations observed for *Cladosporium* were significant. So, in July 2011, the peak was registered with a value of 16,491.0 spores/m^3, in June 2019 – with a value of 20,059.4 spores/m^3, in August 2021 – with a value of 16,289.6 spores/m^3. The peak value for this fungal species ranged from the lowest peak of 366.7 spores/m^3 in April 2015 to the highest – of 35,937.3 spores/m^3 in July 2020.

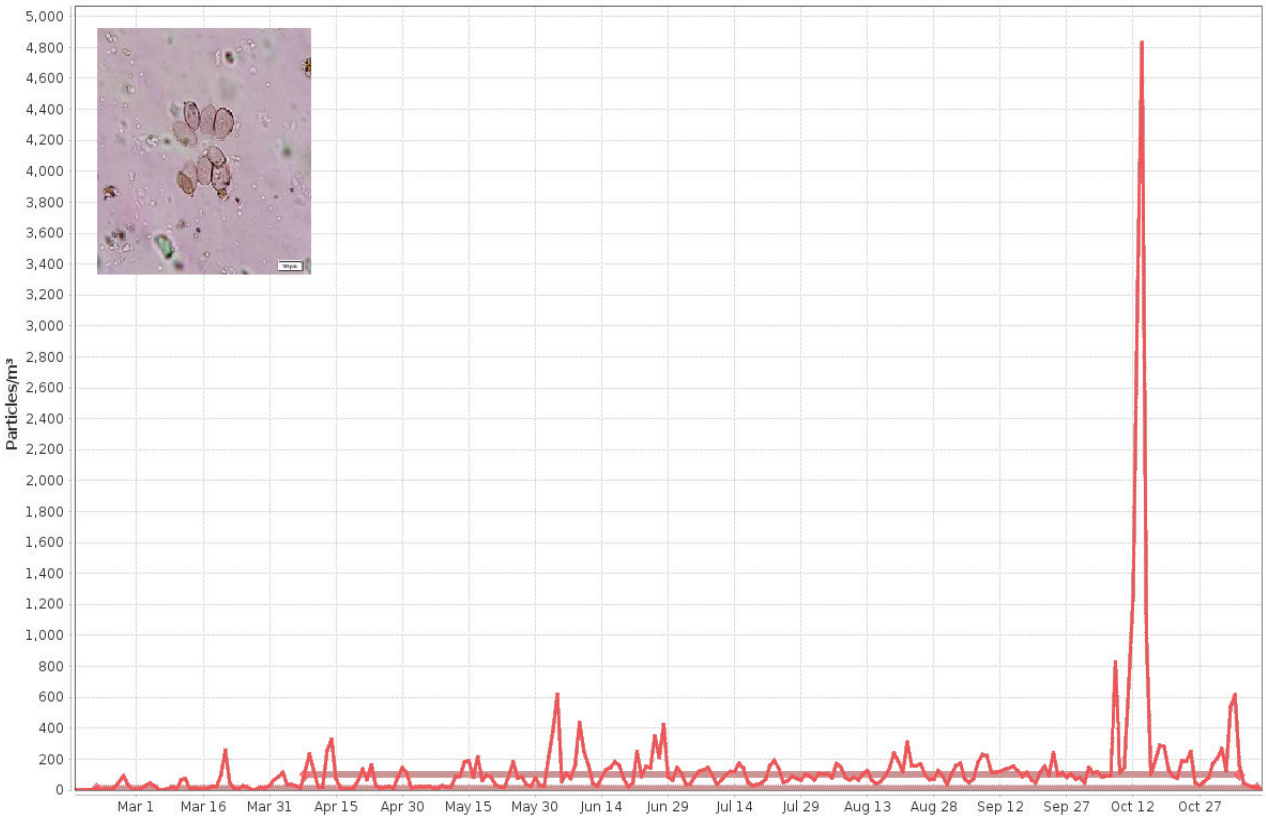

Fig. 7. The average seasonal dynamics of Basidiospores during 2011–2021

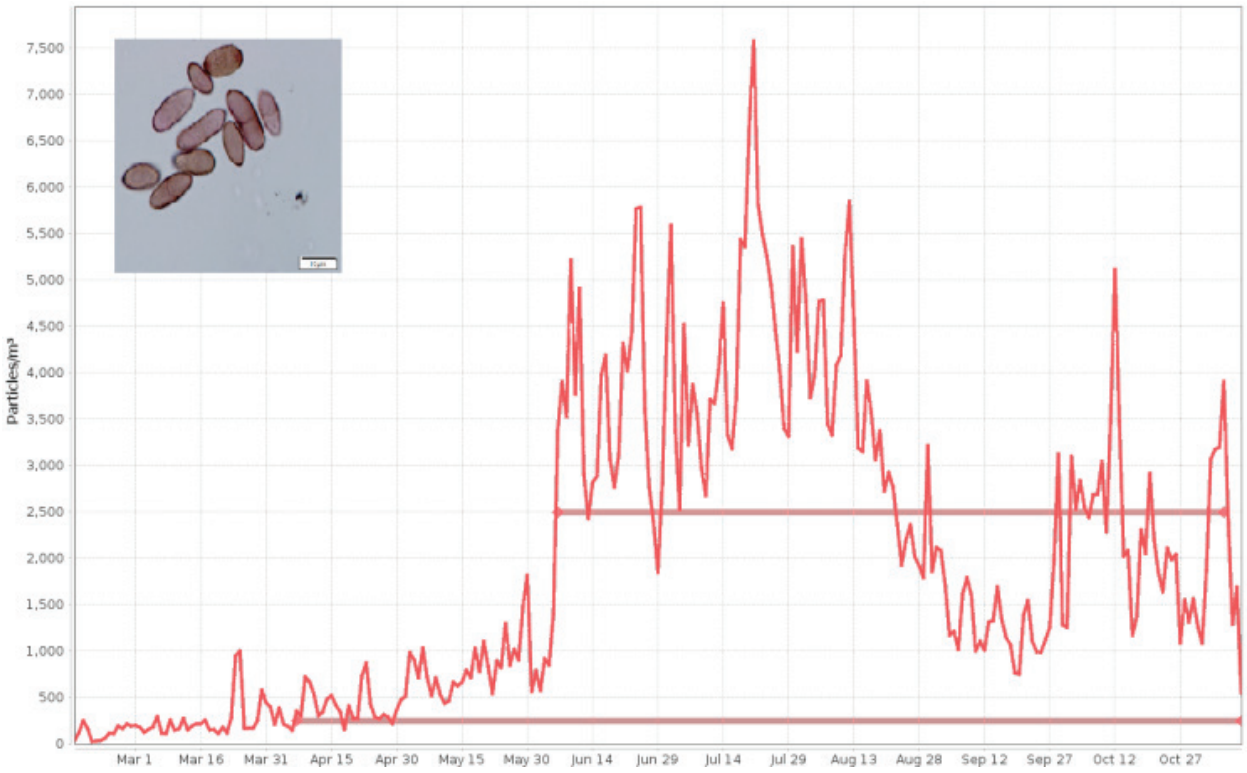

Fig. 8. The average seasonal dynamics of *Cladosporium* during 2011–2021

The average total number of collected spores was 407,633.6 spores/m^3/season. At the same time, the amount of spores per season varied from 10,357.1 spores/m^3/season in 2015 to 1,157,729.6 spores/m^3/season in 2020. The average number of days with a clinically significant concentration of more than 2,500 spores/m^3 was 47.8 ± 42.7 days.

The intervals during the registration of *Cladosporium* spores for the monitoring period were as follows: in 2021, 6 intervals were observed, in 2015 – 5 intervals, in 2019 – 4 intervals, in 2014, 2016, 2017 and 2020 – 2 intervals for each year, in 2018 – 1 interval. In 2011–2013, spores of these fungi were observed continuously throughout the season (Table 7).

Table 7
Characteristics of *Cladosporium* sporulation in 2011–2021

Year	Characteristics of the sporulation season							
	the start of the season, date	the end of the season, date	duration of the season, days	the day of peak sporulation	max, spores/m^3 (peak value)	Σ, spore/m^3/season	number of days with concentration >2500 spores/m^3	the number of intervals in spore registration
2011	05.04	10.10	188	14.07	16491.0	476060.0	65	0
2012	04.04	10.10	189	07.10	5311.2	262718.0	24	0
2013	23.03	21.10	212	30.05	7769.2	204658.4	17	0
2014	18.03	12.10	208	02.07	4753.7	121091.3	4	2
2015	02.03	04.06	94	29.04	366.7	10357.1	0	5
2016	04.03	18.10	228	01.07	5817.3	170239.7	19	2
2017	13.03	22.10	223	29.07	5892.0	241465.8	28	2
2018	29.06	29.08	61	09.08	15407.5	323056.8	46	1
2019	30.03	21.10	205	07.06	20059.4	917695.9	112	4
2020	23.03	19.10	210	20.07	35937.3	1157729.6	116	2
2021	23.03	25.10	216	30.08	16289.6	598897.0	95	6
Average for 2011–2021 (x ± SD)	–	–	184.9 ± 55.0	–	12190.4 ± 10068.5	407633.6 ± 354588.0	47.8 ± 42.7	–

The *Alternaria* fungal season in 2011–2021 began in March and lasted until the end of October (Fig. 9, Table 8). The highest concentrations of *Alternaria* spores were observed from the beginning of July to the mid of October. The average duration of the season of these fungi was 151.0 days. It ranged from 67 days in 2018 to 196 days in 2017.

Table 8
Characteristics of *Alternaria* sporulation in 2011–2021

Year	Characteristics of the sporulation season							
	the start of the season, date	the end of the season, date	duration of the season, days	the day of peak sporulation	max, spores/m^3 (peak value)	Σ, spore/m^3/season	number of days with concentration >100 spores/m^3	the number of intervals in spore registration
2011	05.05	05.10	153	14.07	1015.0	17325.0	61	0
2012	05.05	10.10	158	27.09	667.9	23200.0	79	0
2013	24.04	21.10	180	25.07	501.9	14669.3	50	0
2014	26.04	05.10	162	06.09	252.5	10422.9	41	2
2015	04.03	09.06	97	09.06	10.5	202.7	0	5
2016	29.06	11.10	104	19.07	491.4	13631.5	43	2
2017	31.03	13.10	196	16.09	679.0	18942.1	63	2
2018	28.06	03.09	67	26.07	346.3	9984.5	39	1
2019	14.04	20.10	189	23.07	640.8	24888.4	92	4
2020	14.04	22.10	191	03.10	943.2	22918.3	75	2
2021	10.05	21.10	164	24.07	995.1	26982.3	78	6
Average for 2011–2021 (x ± SD)	–	–	151.0 ± 42.9	–	594.9 ± 318.2	16651.5 ± 7932.7	56.5 ± 25.7	–

The average *Alternaria* peak value during this period was 594.9 spores/m^3. During the observation period, the peak concentrations of *Alternaria* spores were noted in September 2017 with a value of 679.0 spores/m^3, in October 2020 – with a value of 943.2 spores/m^3, in July 2021 – with a value of 995.1 spores/m^3. The peak for this fungal type ranged from the lowest value of 10.5 spores/m^3 in June 2015 to the highest – of

1015.0 spores/m^3 in July 2011. The average total number of collected spores was 16,651.5 spores/m^3/season. At the same time, the seasonal amount of spores varied from 202.7 spores/m^3/season in 2015 to 26982.3 spores/m^3/season in 2021. The average number of days with a clinically significant concentration was 56.5 ± 25.7 days.

The number of intervals for the spore registration during the monitoring season was as follows: 6 intervals were observed in 2021, 5 intervals in 2015, 4 intervals in 2019, 2 intervals in 2014, 2016, 2017 and 2020 each; 1 interval was registered in 2018. In 2011–2013, spores of *Alternaria* were observed continuously throughout the season (Table 8).

The season of *Epicoccum* in 2011–2021 lasted from the end of February to the beginning of November (Fig. 10, Table 9). *Epicoccum* spores were most often recorded from mid-July to early November. The average length of the season was 193.2 days. It ranged from 68 days in 2018 to 240 days in 2017 and 2019.

The average seasonal peak value for *Epicoccum* during the observation period was 242.9 spores/m^3. The highest peak concentrations of *Epicoccum* spores were recorded in 2012, 2017, and 2020 in October with values of 105.6, 327.2 and 782.7 spores/m^3, respectively. The peak value for this type of fungi ranged from the lowest amount of 9.9 spores/m^3 in April 2015 to the highest level of 883.3 spores/m^3 in October 2013.

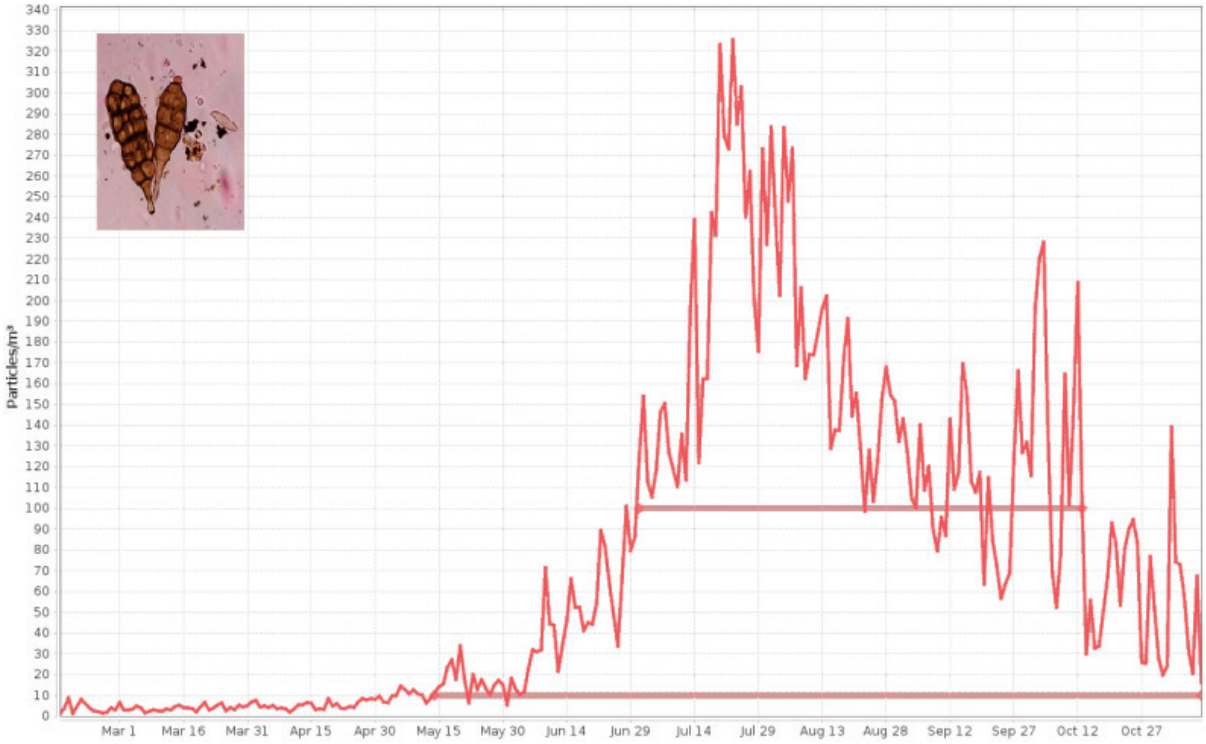

Fig. 9. The average seasonal dynamics of *Alternaria* during 2011–2021

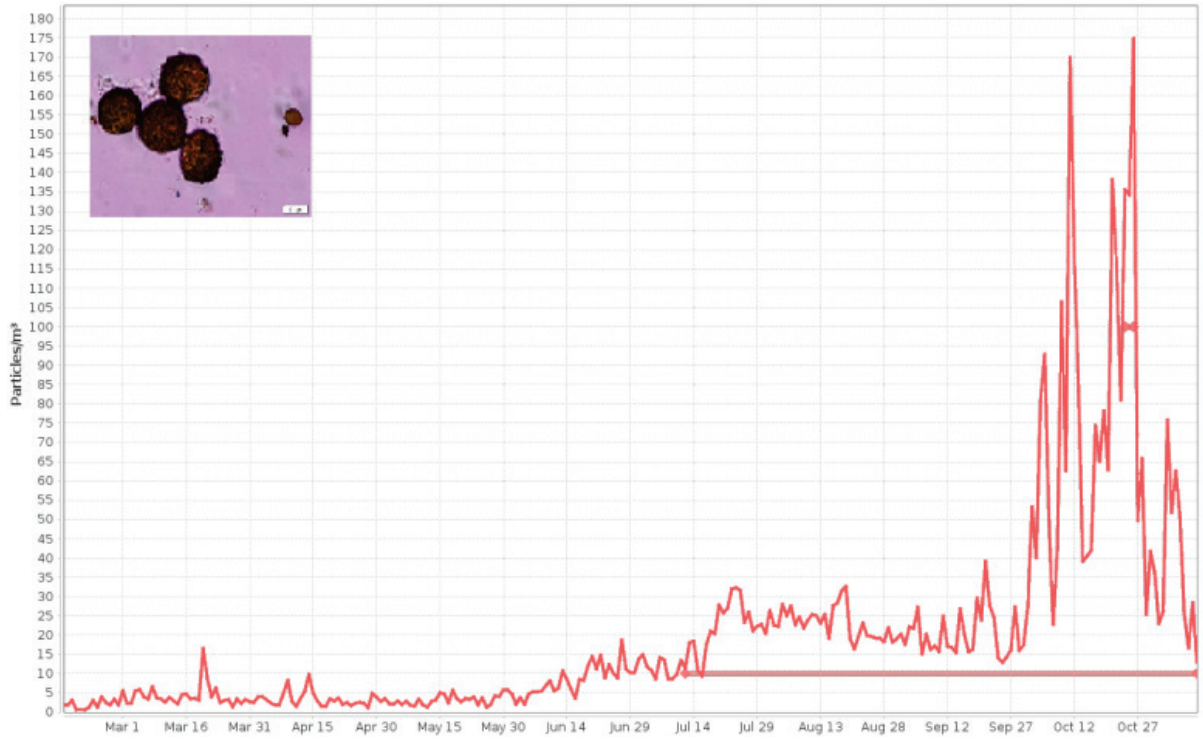

Fig. 10. The average seasonal dynamics of *Epicoccum* during 2011–2021

The average total number of collected spores was 3,343.3 spores/m^3/season. At the same time, the total seasonal amount of spores varied from 143.4 spores/m^3/season in 2015 to 8,069.1 spores/m^3/season in 2020. The average number of days with a clinically significant concentration of *Epicoccum* spores was 4.0 ± 6.1 days.

In 2011–2013, *Epicoccum* spores were observed continuously throughout the season without any gaps. In 2018 there was 1 interval. 2 intervals were recorded in 2014, 2016, 2017 and 2020 each. There were 4 intervals in 2019, 5 intervals in 2015 and 6 intervals in 2021 (Table 9).

Table 9
Characteristics of the *Epicoccum* sporulation in 2011–2021

Year	Characteristics of the sporulation season							
	the start of the season, date	the end of the season, date	duration of the season, days	the day of peak sporulation	max, spores/m^3 (peak value)	Σ, spore/m^3/season	number of days with concentration >100 spores/m^3	the number of intervals in spore registration
2011	31.03	20.10	203	02.10	125.0	4493.0	1	0
2012	24.03	25.10	215	08.10	105.6	3507.4	2	0
2013	11.04	27.10	199	26.10	883.3	5036.3	11	0
2014	27.02	09.09	194	10.07	11.1	210.8	0	2
2015	13.03	08.06	87	19.04	9.9	143.4	0	5
2016	05.03	24.10	233	18.08	101.2	2558.3	1	2
2017	02.03	28.10	240	26.10	327.2	3924.8	7	2
2018	28.06	04.09	68	22.07	56.2	1133.8	0	1
2019	08.03	03.11	240	03.11	173.5	4164.6	3	4
2020	05.04	29.10	207	10.10	782.7	8069.1	19	2
2021	10.03	04.11	239	12.10	95.7	3534.6	0	6
Average for 2011–2021 (x ± SD)	–	–	193.2 ± 59.8	–	242.9 ± 305.1	3343.3 ± 2305.4	4.0 ± 6.1	–

In 2011–2021 *Periconia* spores were registered from the beginning of March to the end of October (Fig. 11, Table 10). The highest concentrations of *Periconia* spores were observed from August to the end of October. The average season length was 169.9 days. It ranged from 61 days in 2018 to 225 days in 2020.

Table 10
Characteristics of the *Periconia* sporulation in 2011–2021

Year	Characteristics of the sporulation season							
	the start of the season, date	the end of the season, date	duration of the season, days	the day of peak sporulation	max, spores/m^3 (peak value)	Σ, spore/m^3/season	number of days with concentration >100 spores/m^3	the number of intervals in spore registration
2011	10.04	30.10	203	05.10	624.0	19326.0	62	0
2012	14.03	24.10	224	30.08	271.6	3043.1	1	0
2013	05.03	27.10	216	20.10	237.1	1839.3	3	0
2014	06.03	22.10	210	09.10	101.2	1617.6	1	2
2015	02.03	09.06	99	09.06	14.8	241.5	0	5
2016	03.07	21.10	110	18.08	90.7	1626.9	0	2
2017	30.06	25.10	117	19.10	168.5	3808.5	5	2
2018	05.07	04.09	61	01.09	199.4	2574.7	2	1
2019	11.03	10.10	213	04.05	131.5	809.5	1	4
2020	07.03	19.10	225	11.06	35.8	875.5	0	2
2021	08.04	16.10	191	16.06	135.8	1997.5	2	6
Average for 2011–2021 (x ± SD)	–	–	169.9 ± 60.3	–	182.8 ± 166.1	3432.7 ± 5370.1	7.0 ± 18.3	–

The average peak value for *Periconia* during the study period was 182.8 spores/m^3. The observation period for *Periconia* in different years was marked by the following peak concentrations: in August 2012, the peak was registered with a value of 271.6 spores/m^3, in October 2013 – with a value of 237.1 spores/m^3, in September 2018 – with a value of 199.4 spores/m^3. In general, the peak for this type of fungi ranged from the lowest value of 14.8 spores/m^3 in June 2015 to the highest of 624.0 spores/m^3 in October 2011.

The average total number of collected spores was 3,432.7 spores/m^3/season. At the same time, the seasonal amount of spores varied from 241.5 spores/m^3/season in 2015 to 19,326.0 spores/m^3/season in 2011. The average number of days with a clinically significant spore concentration was 7.0 ± 18.3 days.

The intervals during the registration of *Periconia* spores for the monitoring season changed as follows: in 2021, 6 intervals were noted, in 2015 – 5 intervals, in 2019 – 4 intervals, in 2014, 2016, 2017 and 2020 – 2 intervals, in 2018 – 1 interval. In 2011–2013, *Periconia* spores were observed continuously throughout the season, without any gaps (Table 10).

The *Stemphylium* season in 2011–2021 was recorded from the beginning of March to the end of October (Fig. 12, Table 11). The highest levels of *Stemphylium* spores were noted from mid-May to mid-October. The average length of the season for this spore type was 152.4 days. It ranged from 67 days in 2018 to 217 days in 2020.

Table 11
Characteristics of the *Stemphylium* sporulation in 2011–2021

Year	Characteristics of the sporulation season							
	the start of the season, date	the end of the season, date	duration of the season, days	the day of peak sporulation	max, spores/m^3 (peak value)	Σ, spore/m^3/season	number of days with concentration >100 spores/m^3	the number of intervals in spore registration
2011	01.04	03.10	185	24.07	71.0	1275.0	0	0
2012	16.03	09.10	207	15.07	13.0	372.7	0	0
2013	20.05	26.10	159	09.07	13.6	315.6	0	0
2014	08.05	30.08	114	09.07	82.1	361.3	0	2
2015	06.03	05.06	91	30.04	3.1	33.4	0	5
2016	01.07	20.10	111	17.07	21.0	618.1	0	2
2017	30.03	19.10	203	27.07	36.4	1069.3	0	2
2018	28.06	03.09	67	22.07	34.0	716.8	0	1
2019	17.05	03.10	139	23.07	22.2	531.7	0	4
2020	19.03	22.10	217	11.10	21.6	595.3	0	2
2021	16.04	16.10	183	13.05	30.9	709.6	0	6
Average for 2011–2021 (x ± SD)	–	–	152.4 ± 51.2	–	31.7 ± 24.3	599.9 ± 348.9	0.0 ± 0.0	–

The average peak value of *Stemphylium* during study period was 31.7 spores/m^3. For almost the entire observation period, the peak concentrations noted for *Stemphylium* were insignificant. Thus, in 2011, 2017 and 2018, peaks were observed in July with values of 71.0 spores/m^3, 36.4 spores/m^3 and 34.0 spores/m^3, respectively. In general, the peak value for this fungi species ranged from the lowest peak of 3.1 spores/m^3 in April 2015 to the highest one of 82.1 spores/m^3 in July 2014.

The average total number of collected spores was 599.9 spores/m^3/season. At the same time, the seasonal total varied from 33.4 spores/m^3/season in 2015 to 1,275.0 spores/m^3/season in 2011. Days with a clinically significant concentration of more than 100 spores/m^3 were not observed during this period.

The number of intervals for spores registration during the monitoring period changed as follows: 6 intervals were observed in 2021, 5 intervals – in 2015, 4 intervals – in 2019, 2 intervals – in 2014, 2016, 2017 and 2020 each. In 2018, 1 interval was recorded. In 2011–2013, *Stemphylium* spores were observed continuously throughout the season (Table 11).

As we can see, for most basidiomycetes, which are known for their ability to form fruiting bodies with spores in autumn, consistently high concentrations were observed at this time of year, in particular, for *Agrocybe*, *Coprinus*, Uredinales, Ustilaginales and unclassified basidiospores. Their concentrations ranged from a few dozen to 400 spores/m^3.

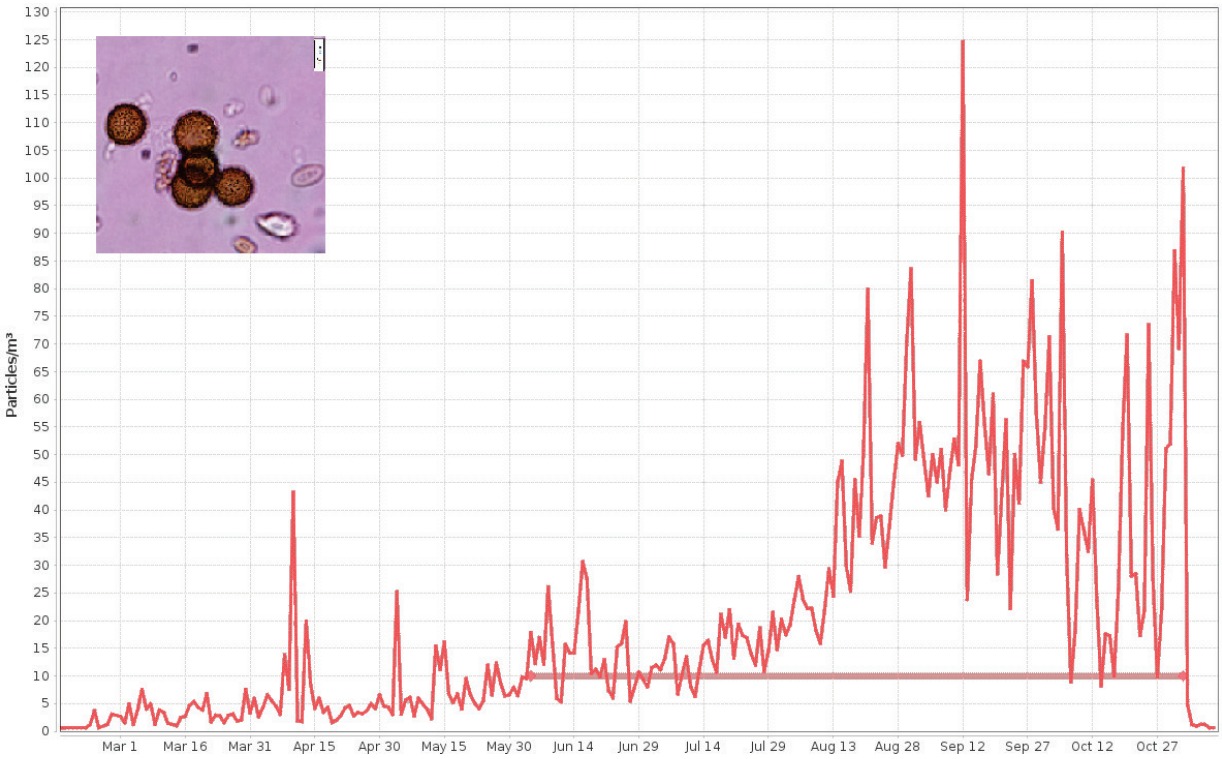

Fig. 11. The average seasonal dynamics of *Periconia* during 2011–2021

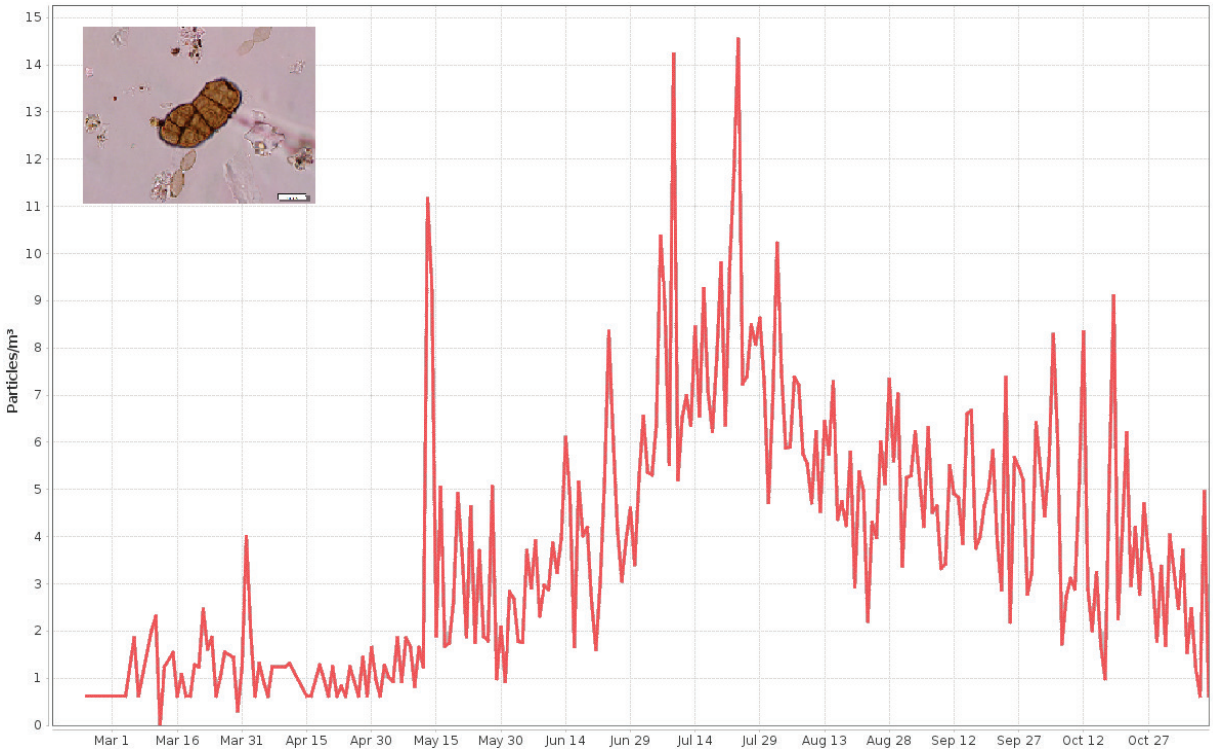

Fig. 12. The average seasonal dynamics of *Stemphylium* during 2011-2021

Cladosporium was the most abundant among ascospores. Its concentration exceeded 3,000 spores/m^3. Concentrations of about 100 spores/m^3 were common in autumn for *Alternaria*, *Epicoccum*, *Periconia*. Stemphylium spores were also present, though, their concentration was low, about 10 spores/m^3 (Fig. 13).

Discussion

The peculiarities of the influence of micromycetes on the human body are the subject of thorough scientific study all over the world. Our fundings, in general, correlate with the literature data. In particular, *Cladosporium* spores (71.88% of the total number of collected spores), *Coprinus* (8.84%), *Leptosphaeria* (3.88%), *Ganoderma* (3.43%) and *Alternaria* (2.79%) were the main constituents of the fungal component of the bioaerosol in Slovakia (Ščevková & Kováč, 2019). In Spain, *Cladosporium* (Reyes et al., 2016) dominated. *Cladosporium* (66%), *Alternaria* (1.67%) and *Leptosphaeria* (1.21%) dominated in Szczecin in Poland (Bednarz & Pawłowska, 2016). *Cladosporium* spores were noted as a primary airborne fungal component in both indoor and outdoor environments in Netherlands (Segers et al., 2023), Lithuania (Sauliene et al., 2023) and North-East Greece (Katsimpris et al., 2022).

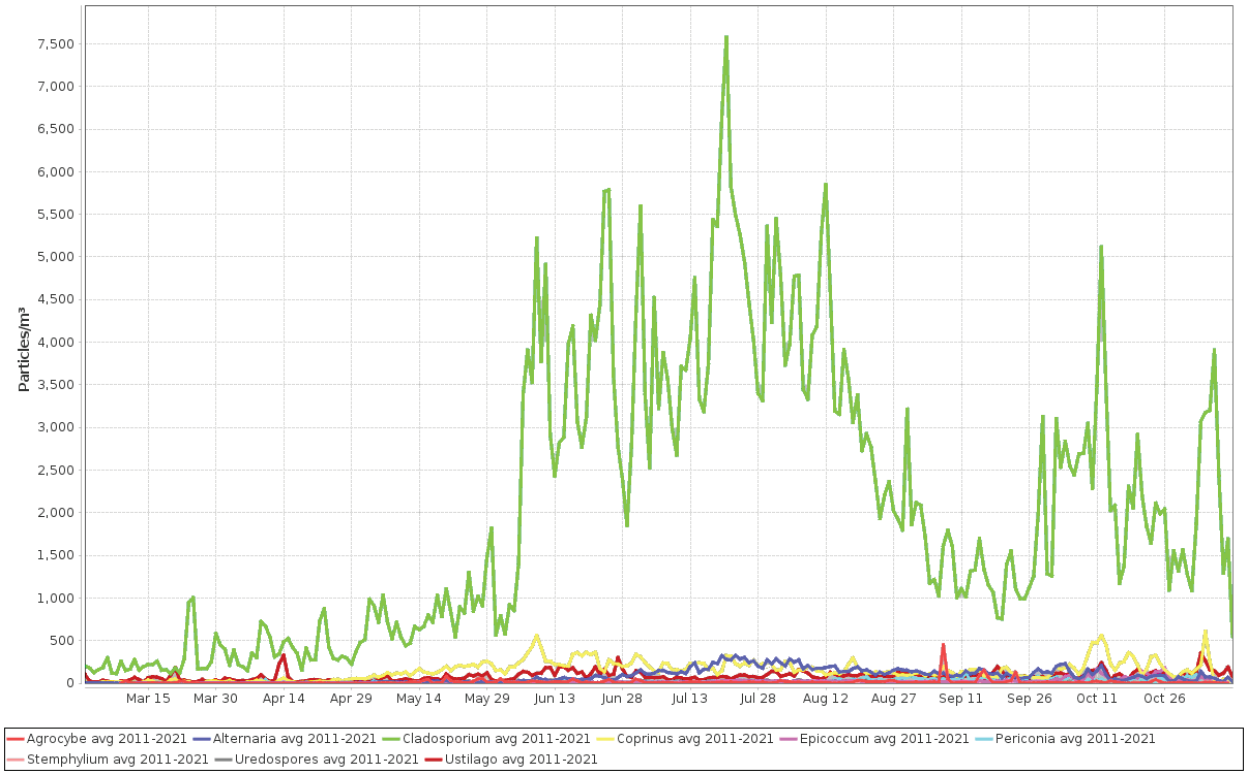

Fig. 13. The average seasonal dynamics of *Agrocybe*, *Alternaria*, *Cladosporium*, *Coprinus*, *Epicoccum*, *Periconia*, *Stemphylium*, Uredinales, *Ustilago* during 2011–2021

Cladosporium also dominated in all sampling heights in the Brazilian Atlantic Forests (Mantoani et al., 2023).

All aforementioned fungi are included in the list of the most significant in our study. The time of spread of fungal spores verified by us also agrees with the data of other scientific sources. In particular, the researchers note the presence of spores throughout the growing season from January to December, as well as high concentrations of *Epicoccum* in October and November. High concentrations of basidiospores are also noted in autumn, and *Cladosporium* and *Alternaria* in summer (Rodinkova & Jurjev, 2019), which supports our results perfectly.

Thus, in relation to the important immunopathologic role of fungi in chronic diseases (Shin et al., 2023) including the allergic ones both for humans (Knutsen et al., 2012; Forkel et al., 2021), and animals (Martins, 2022), other potential health impacts (Saad-Hussein & Ibrahim, 2020) and involvement of fungi in the global megatrends (Magyar et al., 2021), it is important to carry further studies on airborne diversity of fungi and their temporal and spatial variations over time. In addition, the need for this study is supported by the evidences of the impact of climate change on fungal sporulation, seen in our present work and noted by other researchers (Perrone et al., 2020; Hanson et al., 2022).

Conclusions

Spores of various taxonomic groups of fungi were observed throughout the growing season from March to October, with the highest concentrations in summer.

Thus, in June, July and August, peak concentrations of spores of *Ganoderma*, *Coprinus*, *Cladosporium* fungi were observed; in July, September and October – *Ustilago*; from July to November – *Alternaria*, *Epicoccum*; in October – *Basidiospores*, *Periconia*, *Stemphylium*, *Uredinales*; in September – *Agrocybe*. *Cladosporium* spore concentrations were the highest among all studied micromycetes.

However, significant sporulation persists even in the autumn period, which makes fungal spores a specific causative agent of airborne respiratory diseases at this time, especially against the background of low pollen concentrations.

Climate change can lead to a change in the duration of the growing season of fungi.

This information should be taken into account when predicting the symptoms of seasonal allergies among the population.

The study was performed within the scientific plan of Vinnitsa National Pirogov Memorial Medical University in the frame of the research topic "Development of scientific and methodological principles of long-term monitoring of biological pollution of the atmosphere" (registration number 0121U113516).

Authors have declared that they have no competing interests.

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