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ORIGINAL RESEARCH PAPER

A fungal spore calendar for the atmosphere of Szczecin, Poland

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Abstract

A calendar of fungal spore seasons for Szczecin during 2013 was established using a 7-day volumetric Lanzoni trap. Thirty-five spore taxa were identified. The dominant spore types detected were *Cladosporium* (66%), *Didymella* (29%), *Alternaria* (1.67%), and *Leptosphaeria* type (1.21%). The spores were present throughout the study year. However, there was a wide daily fluctuation in the concentration values with a tendency towards an increase during the summer months. Seasonally, the spore levels of *Cladosporium*, *Alternaria*, and *Leptosphaeria* type peaked in summer (June–September), while those of *Didymella* mainly in July. Most of the other spore types had the highest concentrations in summer but occurred in the air from spring to late fall.

Keywords

aerobiology; air monitoring; fungi; spore calendar

Introduction

Air-borne particles can have many effects, including plant, animal and human diseases, allergies, plant pollination, and colonization of new habitats. Fungi, as one of the main component of air, tend to be especially difficult to study since their production, maturation, release, and concentration in the air depend on multiple biotic and abiotic factors. In Szczecin, there have been few studies on atmospheric mycobionta. Aeromycological research in outdoor environments has been carried out in Szczecin only for selected allergenic taxa (*Alternaria*, *Cladosporium*, *Ganoderma*, *Didymella*) [1–4]. The main goal of this study was to create a spore calendar for 2013 and show the percentage of all identified fungal spores. In the previous work [5], the annual and seasonal variations between 2004 and 2006 were examined, separating 10 spore types from the total spore numbers. This was not a quantitative investigation of the range of airborne spores in the atmosphere of Szczecin. Thus, a daily spore calendar was established for Szczecin, Poland, studying the year 2013 in detail and using a Lanzoni spore trap.

Material and methods**Daily spore counts**

Daily airborne spore counts were collected between January and December 2013 using a Lanzoni 7-day volumetric trap (Lanzoni model 2000, Bologna, Italy). The flow rate was adjusted to 10 L/min. Spores were trapped onto adhesive tape and cut into daily segments (48 mm strips representing 24 h of exposure). The segments were

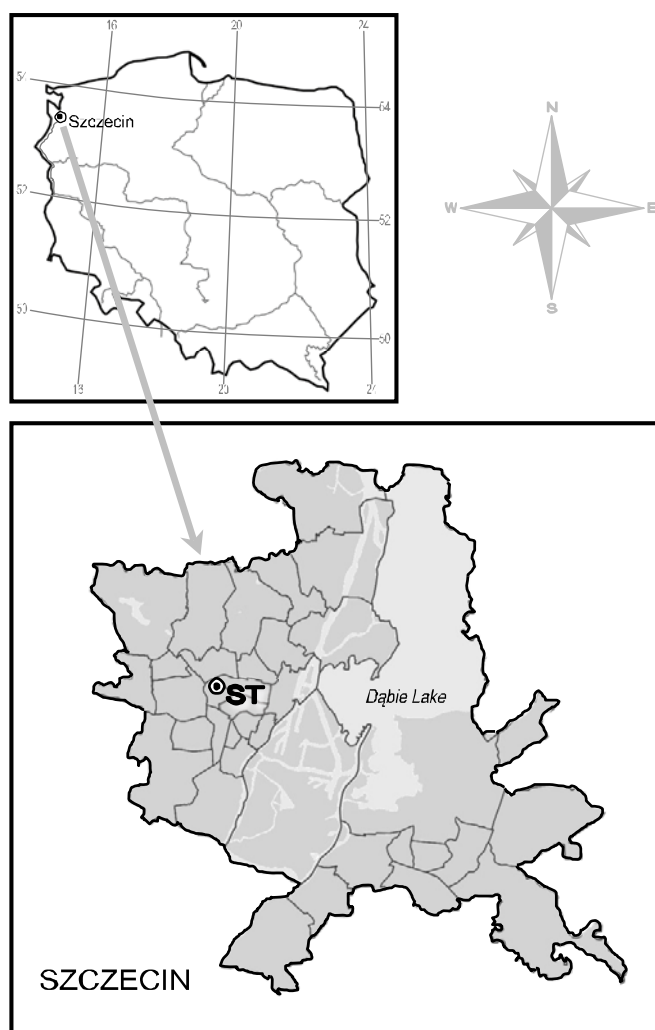


Fig. 1 The locality of the monitoring site at Faculty of Biology, University of Szczecin (149 m a.s.l.; 53°26'260 N, 14°32'95099 E).

mounted on microscope slides in glycerol jelly containing fuchsine (0.1%) and protected with a cover glass. The spores were observed and identified using a Zeiss Primo Star microscope at a magnification of 400× along one longitudinal traverse. The spore concentrations were estimated as the number of spores per cubic meter of air sampled according to the British Aerobiology Federation [6].

Location of the Lanzoni spore trap

The air sampler was placed on the roof of the Faculty of Biology building at the University of Szczecin at 21 m above ground level, near the city center. The area is completely urbanized with green spaces (Kasprowicza Park) and abundant trees.

Szczecin is situated in the Odra River valley in the northwest part of Poland (53°26' N, 14°32' E; Fig. 1). The altitude varies between 0.1 m under sea level and 148 m above sea level. The present "Baltic" climate in Szczecin is influenced by the air masses from over the Northern Atlantic and is characterized by mild winters and cool summers. The average relative humidity is 84%, the average air temperature ranges from 8 to 8.4°C. The average annual rainfall total is 550 mm and within a year there are approximately 167 days with precipitation.

Spore identification

The spore categories include the following 35 types: *Acrocybe*, *Alternaria*, *Aspergillus/Penicillium*, *Bispora*, *Cercosporium*, *Chaetomium*, *Cladosporium*, *Coprinus*, *Curvularia*, *Didymella*, *Diplocladium*, *Drechslera* type, *Epicoccum*, *Entomophthora*, *Fusarium*, *Ganoderma*, *Leptosphaeria* type, *Myxomycetes*, *Oidium*, *Periconia*, *Phaeosphaeria*, *Phaeosphaeria*, *Pleospora*, *Polythrincium*, *Puccinia*, *Sporormiella*, *Spegazzinia*, *Stachybotrys*, *Stemphylium*, *Tilletia*, *Tilletiopsis*, *Torula*, *Ulocladium*, *Ustilago*, and *Venturia*.

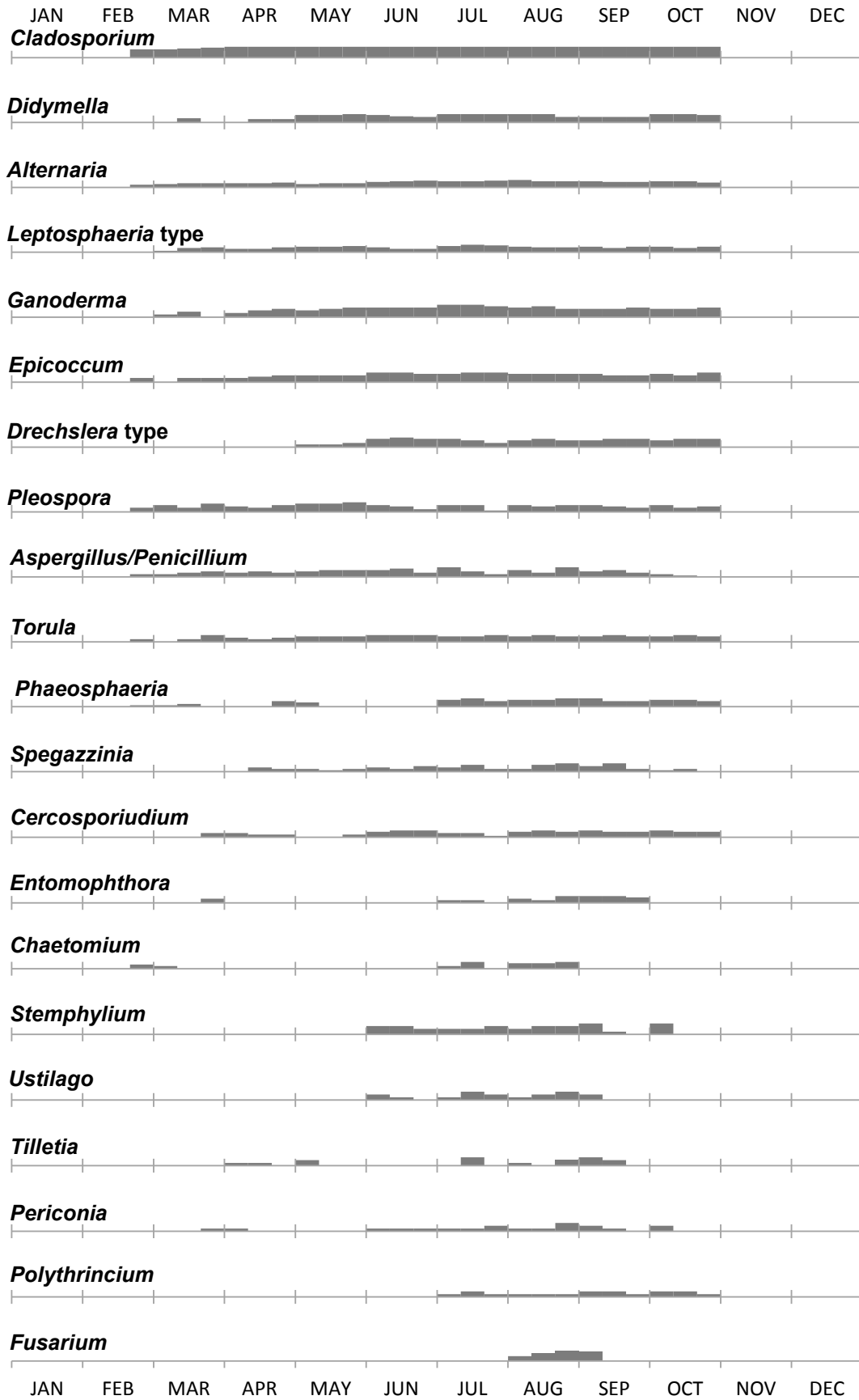
Fungal spores that did not fit into the above categories were designated as "other spores", which include partial or unidentifiable fungal spores. In order to verify the accuracy of the calculations performed under the microscope, most of the samples were reviewed using a microscopic camera connected to a computer screen. For the analysis of microscopic fungal spore images, the software Carl Zeiss AxioVision SE64 was used.

Results

Thirty-five spore taxa were identified (Tab. 1) and on the basis of this the spore calendar was created (Fig. 2). The cumulative annual total of daily spore counts for 2013 was 595199. Spores of the genus *Cladosporium* dominated, providing 66% of the total count, about two times more than the next most abundant genus. The six most frequently detected spore groups, accounting for 98.75% of the total count, were *Cladosporium*, *Didymella*, *Alternaria*, *Leptosphaeria* type, *Ganoderma*, and *Epicoccum* (Fig. 3) The remaining 29 spore types were present in low numbers, seven less than 0.15% and 22 less than 0.1% of the annual total.

Tab. 1 List and percentage contribution of airborne fungal spores in the atmosphere of Szczecin, 2013.

Spore type	Annual count	Percentage total
<i>Cladosporium</i>	392670	66.00
<i>Didymella</i>	175401	29.00
<i>Alternaria</i>	9979	1.67
<i>Leptosphaeria</i> type	7207	1.21
<i>Ganoderma</i>	3608	0.61
<i>Epicoccum</i>	1543	0.26
<i>Drechslera</i> type	856	0.14
<i>Pleospora</i>	824	0.14
<i>Aspergillus/Penicillium</i>	812	0.14
<i>Torula</i>	490	0.10
<i>Phaeosphaeria</i>	456	0.10
<i>Spegazzinia</i>	324	0.10
<i>Cercosporiudium</i>	321	0.10
<i>Entomophthora</i>	138	<0.10
<i>Chaetomium</i>	111	<0.10
<i>Stemphylium</i>	77	<0.10
<i>Ustilago</i>	42	<0.10
<i>Tilletia</i>	33	<0.10
<i>Phaeosphaeria</i>	32	<0.10
<i>Periconia</i>	31	<0.10
<i>Polythrincium</i>	31	<0.10
<i>Fusarium</i>	30	<0.10
<i>Acrocybe</i>	28	<0.10
<i>Bispora</i>	27	<0.10
<i>Coprinus</i>	22	<0.10
<i>Oidium</i>	20	<0.10
<i>Curvularia</i>	19	<0.10
<i>Diplocladium</i>	17	<0.10
<i>Myxomycetes</i>	16	<0.10
<i>Puccinia</i>	10	<0.10
<i>Sporormiella</i>	7	<0.10
<i>Stachybotrys</i>	7	<0.10
<i>Tilletiopsis</i>	5	<0.10
<i>Ulocladium</i>	3	<0.10
<i>Venturia</i>	2	<0.10
Total	595199	100.00



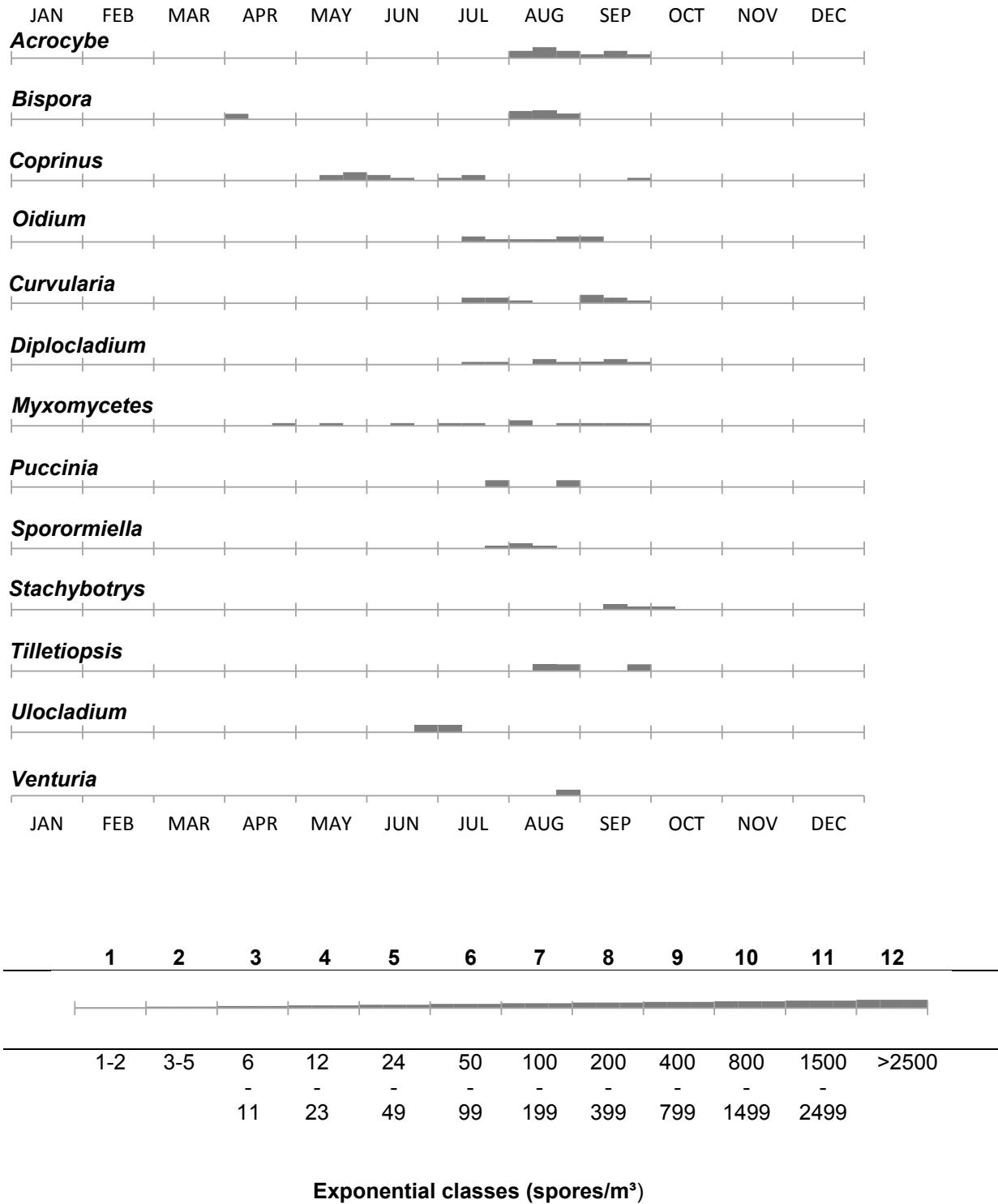


Fig. 2 A fungal spore calendar for the atmosphere of Szczecin, Poland, for the year 2013.

The average daily total fungal spore count for 2013 was 1630 spores m³ of air. Most spore types were present almost throughout the year. *Cladosporium* spores were abundant the entire year with a peak spanning late spring, summer and early fall. The spore concentration of *Alternaria*, *Didymella*, *Epicoccum*, *Stemphylium*, *Pithomyces*, and *Torula* peaked mainly in summer, *Ganoderma*, *Leptosphaeria* type, *Drechslera* type, *Pleospora*, *Parapheosphaeria*, *Polythrincium* in late summer and fall. The other taxa appeared sporadically from spring to fall.

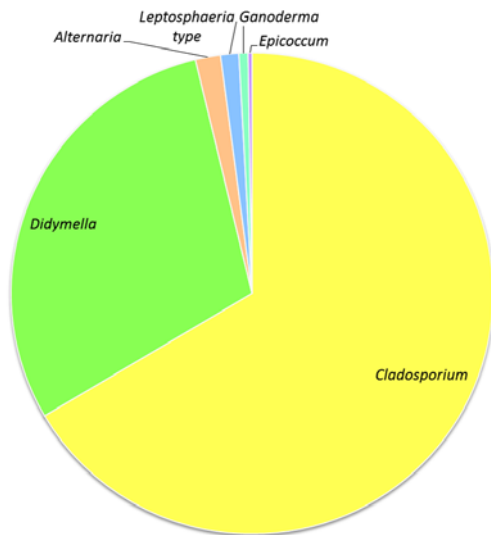


Fig. 3 The percentage of frequently found fungal spores in the area.

Discussion

Monitoring of fungal spore concentrations in the air is important from the aerobiological, agricultural, and medical point of view. Aerobiologists can compare the qualitative and quantitative analyses of aero-plankton in different locations. Moreover, the effects of biotic and abiotic factors on the changes in the concentrations of individual taxa can be indicated. In agriculture, effects of air monitoring are very important in order to study of the life cycle of parasites and to develop plant protection plans. From the human health point of view, the indication of exceeded threshold concentrations for allergenic taxa (among others, *Alternaria* and *Cladosporium*) helps in the diagnosis and treatment of inhaled allergens.

Currently, aerobiological monitoring is carried out on all continents and covers from a few to a dozen types of spores. In Australia (Melbourne) 29 genera and five spore groups were identified. The dominant spore types detected were *Cladosporium*, *Leptosphaeria*, and *Coprinus* [7]. *Cladosporium* spore levels peaked in spring and summer, *Leptosphaeria* toward summer and fall, whilst *Coprinus* fluctuated all year round.

Troutt and Levetin [8] described six spore types and three spore groups in the air of Tulsa (United States of America). The three most abundant spore types, which occurred in the air, were as follows: *Cladosporium*, *Alternaria*, and *Epicoccum*, and the most important meteorological factors influencing the presence of airborne spores were air temperature and dew point. Airborne spore monitoring in South America is represented by four locations: Argentina (La Plata) [9], Uruguay (Montevideo City) [10], Chile (Santiago) [11], and Cuba (Havana) [12]. The most taxa were described from Argentina (79) and Uruguay (56), followed by Cuba (34), and only 14 in Chile. In every monitoring station, the most numerous taxon was *Cladosporium*, followed by the following taxa: in three stations *Leptosphaeria*, in two *Alternaria* and *Coprinus*, while in single points *Agaricus*, *Didymella*, and *Aspergillus/Penicillium*.

In Asia, monitoring stations are located in Turkey (Gaziantep) [13], India (Kerala) [14], and Taiwan (Hualien) [15]. In the above-mentioned locations, 47, 28, and 16 taxa were described, respectively. As in the previously described areas, the most abundant taxon was *Cladosporium*, followed by, depending on the station, *Ustilago*, *Alternaria* (Turkey), *Ganoderma*, *Aspergillus/Penicillium* (Taiwan), and *Apergillus/Penicillium* (India).

In West Africa, three taxa were isolated from desert samples: *Cladosporium*, *Alternaria*, and *Aspergillus* [16], while on the Arabian Peninsula up to 30 taxa with the most numerous spore types belonging to *Cladosporium* and *Alternaria* genera [17].

In Europe, the largest number of monitoring stations is found compared to other continents. The biggest aerobiological monitoring networks are in Spain, Portugal, the United Kingdom, and Poland. For example, in Catalonia (Spain) 20 fungal taxa were identified with the most numerous *Agrocybe* and *Cladosporium* type [18]. In Portugal (Porto) 22 spore types were noted in 2003, and *Cladosporium* and *Ganoderma* reached the highest concentrations. The remaining spore types did not exceed 3% of the total annual sum [19].

The first investigations into airborne fungal spores in Poland were conducted in two cities: Cracow and Rabka [20], where 15 types of fungal spores were identified. Spore monitoring is conducted in Cracow to this day, and the last analysis of the content of spores in the air was published by Stępalska and Wołek in 2005 [21]. They described 13 spore taxa during one fungal season in which *Cladosporium* occurred as the most numerous fungal spore. Ten taxa were identified in Rzeszów during 2-year monitoring, and *Cladosporium*, *Alternaria*, *Epicoccum*, and *Torula* reached the highest concentrations [22]. In northwest Poland (Szczecin), detailed fungal monitoring has been carried on since 2004 [23]. The highest concentrations were found for *Cladosporium*, *Alternaria*, *Ganoderma*, and *Didymella*.

In recent years, the content of airborne fungal spores has been reported from Slovakia and Romania [24,25]. Ščevková et al. [24] described annual trends for three spore

taxa: *Alternaria*, *Epicoccum*, and *Stemphylium*. Ianovici [25] analyzed concentrations of five spore types: *Cladosporium*, *Alternaria*, *Epicoccum*, *Pithomyces*, and *Torula*. In both publications, the authors put special emphasis on climate change and concluded that temperature increase affects the concentration levels of airborne spores.

Comparing the results of this study, it can be concluded that in every continent *Cladosporium* is the dominant spore type in most monitoring stations. In our investigation, *Cladosporium* was present almost year round, except for the time with snow cover. The period in which the highest concentrations are noted, regardless of the continent, always occurs in the summer. This is associated with the respective air temperature and relative humidity, which can increase mycelium growth and spore release. Due to its allergenic properties, *Alternaria* is also a frequently monitored spore type. The higher prevalence of *Alternaria* sensitization is possibly explained by *Alternaria* allergens cross reacting with a range of genera, including *Cladosporium*, *Stemphylium*, *Epicoccum*, *Fusarium*, *Drechslera*, and *Culvularia* [26].

Didymella and *Leptosphaeria* spores, identified and recorded in Szczecin, represent proportionately “high” counts, compared to studies from other countries; this observation might be attributed to the humid and mild climate in Szczecin. Both types are classified as “wet spores”, so the meteorological parameters in northwest Poland are suitable for their development.

The basidiospores of *Ganoderma*, commonly known as a bracket fungus or wood decay fungus, are an important and prevalent group of airborne fungal spores worldwide. Studies from various parts of the world have clearly implicated *Ganoderma* spores as aeroallergens [27], and they have been implicated in the elicitation of respiratory allergic diseases. The high concentration *Ganoderma* spores in the air of Szczecin is the result of the proximity of two major forest complexes: Beech Forest and Wkrzańska Forest.

Other types of spores occurred seasonally in low concentrations. Most of them are also recorded in most aerobiological stations across the world, but only a few achieve relatively high concentrations. The highest spore concentration peaks were coincident with the peaks of some allergenic pollen types. In Poland, an increase in allergy symptoms related to pollen during summer is observed. At the same time, high concentrations of fungal spores occur, mainly *Cladosporium* and *Alternaria*, which can interact with airborne pollen aggravating allergic problems.

In further study, we intend to consolidate these results in order to develop a useful database that could be used to assist clinicians to identify and control fungal allergy symptoms as well as by farmers to prevent plant diseases.

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Kalendarz zarodnikowania grzybów w atmosferze Szczecina (Polska)

Streszczenie

Kalendarz sezonu zarodnikowania dla miasta Szczecin (2013) został opracowany przy wykorzystaniu aparatu wolumetrycznego Lanzoni 2000. W wykazie uwzględniono 35 zidentyfikowanych taksonów, z czego dominującymi rodzajami były *Cladosporium* (66%), *Didymella* (26%), *Alternaria* (1.67%) i *Leptosphaeria* (1.21%). Zarodniki były obecne w powietrzu przez cały rok badań, ale wyraźne zwiększenie ich stężenie notowano w czasie miesięcy letnich. Sezonowo, liczebność *Cladosporium*, *Alternaria* i *Leptosphaeria* osiągnęła najwyższą wartość w lecie i na początku jesieni (czerwiec–wrzesień) a *Didymella* głównie w lipcu. Dla większości pozostałych rodzajów najwyższe stężenia zaobserwowano latem, ale zarodniki występowały w powietrzu od wiosny (marzec) do późnej jesieni (listopad).