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FUNGAL SPORES IN THE ATMOSPHERE OF RZESZÓW (SOUTH-EAST POLAND)

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Abstract: Continuous aerobiological monitoring has been conducted in Rzeszów from 2000-2002, using the volumetric method. On each microscope slide 1 horizontal band was analyzed, divided into 24 fields corresponding to hours. For the analysis, 10 easy to identify fungal spores were chosen from ones regarded as allergenic: Alternaria, Botrytis, Cladosporium, Drechslera (type), Epiccocum, Ganoderma, Pithomyces, Polythrincium, Stemphylium, and Torula. The results were statistically tested using the χ^2 test as well as the Kruskal-Wallis test and ANOVA. The results were used to develop a calendar of the occurrence of fungal spores in Rzeszów. The spores occurred in the air throughout the whole year, but maximum concentrations were usually reached in July and August. Two groups of taxa were distinguished. *Alternaria*, *Cladosporium*, *Botrytis*, *Epicoccum*, *Ganoderma* spp. and Drechslera belong to the first group, and their spores and conidia were characterised by high frequency and abundance in the air. Low SFI values (Seasonal Fungal Index) and frequency of below 50% occurred in the second group of taxa, i.e. *Pithomyces*, *Polythrincium*, *Stemphylium* and *Torula* spp. Conidia of *Cladosporium* spp. were the most frequent, SFI values were very high and average annual concentrations did not differ significantly throughout the 3 years of study. The research confirmed the overlap of the period of maximum concentration of allergenic spores and the period of the domination of Poaceae and *Artemisia* pollen in the air.

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INTRODUCTION

Fungi spores are among the most commonly encountered airborne particles. In nature, they are always a component of bioaerosol which is why their occurrence in the air has been frequently studied [1, 7, 19, 21, 23]. Until now, not many fungi could be identified by using the morphology of their spores. Fungi can have a complicated life cycle, producing a few kinds of spores which are often difficult to interpret [13]. Depending on the method of study used (by volumetric or culture plate method), the list of taxa which can be correctly identified varies [23]. Studies on the occurrence and spread of fungal conidia and spores in the air are concentrated on taxa caused plant diseases, and these results have practical application in agriculture [7, 9, 14]. Some fungi and their spores have a negative effect on human health and are a frequent cause of allergies and immunotoxic diseases (e.g. sick building syndrome), producing carcinogenic and teratogenic mycotoxins [3, 8]. Most aeromycological studies, therefore, concentrate on these taxa [5, 12, 22, 25]. Recently, such studies have found another practical application, i.e. monitoring buildings where art objects, which could be endangered by biodegradation, are stored [6].

In Poland, such research has been carried out both in indoor environs [11, 18] and outdoors [10, 20, 25, 26]. To

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date, there is no data on the occurrence of airborne fungal spores, which include a reasonably large list of spore types, and has been used constantly for a few years. The aim of this study was to develop a calendar of the occurrence of fungal spores and conidia in the air of Rzeszów. The study included 10 chosen spore types. The research made it possible to check if frequency distributions of chosen spore types and their mean annual sums significantly differed during the 3 years of study (2000-2002).

MATERIAL AND METHODS

Rzeszów (50º01'N, 22º02'E) is located in the valley of the Wisłok river at the altitude of 200-215 m. The climatic conditions of the region are shaped by polar-maritime air masses, and to a large extent, by polar-continental air masses. Summers are hot and autumn is warm and sunny. Winters are not very severe. The mean annual precipitation is 734 mm, the mean annual temperature 7.5ºC. The mean July and January temperatures are 17.6ºC and –4.6ºC respectively. The average growth period is 215-220 days [17]. Flora and vegetation is strongly influenced by man. In the landscape of Rzeszów park, vegetation dominates, other vegetation types include municipal lawns, private gardens and roadsides.

The sampling of airborne fungal spores was carried out from February–December, 2000-2002. The volumetric method was applied. The trap was installed on the roof of a building, at the height of 12 m. The sampling was taken constantly and spores were collected on special tape coated with a thin film, a mixture of vaseline and parafin wax in toluene. The tape were changed weekly and cut into 7 segments, which were mounted on slides with glycerine jelly; thus, a newly prepared microscope slide was studied every day [15]. Fungal spores were counted from a single horizontal band, 48 mm long [23], divided into 24 fields corresponding to consecutive hours. The number of spores was multiplied by a factor to give the number of spores/1 m^3/24 h [15].

The identification of spores was focused on 10 easily distinguished spores and conidia belonging to taxa of varying taxonomic rank: *Alternaria* (*Nees*), *Botrytis* (*P. Micheli ex Pers.*), *Cladosporium* (*Link*), *Epiccocum* (*Link*), *Ganoderma* (*P. Karst*), *Pithomyces* (*Berk. & Broome*), *Polythrincium* (*Kunze*), *Stemphyllium* (*Wallr.*), *Torula* (*Pers.*) and Drechslera (type). Distinguishing the conidia of *Bipolaris* (*Shoemaker*), *Drechslera* (*Ito*), *Exserohilum* (*K.I. Leonard & Suggs*), *Helminthosporium* (*Link*) using an optic microscope is extremely difficult; they were therefore considered as belonging to the Drechslera type. The analysed taxa are regarded as allergenic [3, 13], and often occur in the air in high or very high concentrations [1, 23]. Keys and atlases [2, 24] were used for identification, as well as a few comparative specimens.

The annual sum of fungal spores was expressed as SFI (Seasonal Fungal Index). The χ^2 test was used to test the hypothesis about the lack of difference in the distribution frequencies among 10 distinguished spore types in consecutive years. Mean concentrations of spores in 2000-2002 were compared. The first stage of the analysis was the application of Leven's test to check the uniformity of variance. When variances were different, the Kruskal-Wallis test was used; when they were equal, ANOVA was applied. The significance level of $\alpha \leq 0.05$ was used.

RESULTS

Among the distinguished spore types, each year conidia of *Cladosporium* spp. reached the highest values of SFI and had the highest frequency (ca. 90%). Other taxa, among which conidia or spores occurred regularly (over 50% frequency) with high concentrations, were *Alternaria*, *Botrytis*, *Epicoccum*, *Ganoderma* spp. and Drechslera. Seasonal sums (SFI) and frequencies of other conidia and spores were much lower (Tab. 1), with the exception of *Torula* spp., whose conidia occurred in low but frequent concentrations (Tab. 1, Fig. 1). The abundance distributions of the distinguished taxa differed significantly

Table 1. Results of descriptive statistics.

	SFI			Percentage in total seasonal sum			Frequency (%)		
	2000	2001	2002	2000	2001	2002	2000	2001	2002
Alternaria	8,550	11,342	10,359	1.8	2.1	1.9	69.5	68.8	58.1
Botrytis	2,508	10,575	5,952	0.5	1.9	1.1	40.9	49.4	67.2
Cladosporium	428,852	488,186	487,412	93.1	89.4	90.6	99.7	90.6	88.9
Drechslera	6,409	2,712	2,076	1.4	0.5	0.4	59.9	53.0	54.8
Epicoccum	6,285	11,622	6,503	1.4	2.1	1.2	65.0	53.9	63.1
Ganoderma	5,356	17,486	22,762	1.2	3.2	4.2	53.3	56.1	56.6
Pithomyces	347	575	546	0.1	0.1	0.1	24.3	30.0	19.4
Polythrincium	379	698	546	0.1	0.1	0.1	23.3	28.5	18.8
Stemphylium	488	824	366	0.1	0.2	0.1	27.4	33.9	20.3
Torula	1,650	2,043	1,590	0.4	0.4	0.3	60.2	53.6	50.8

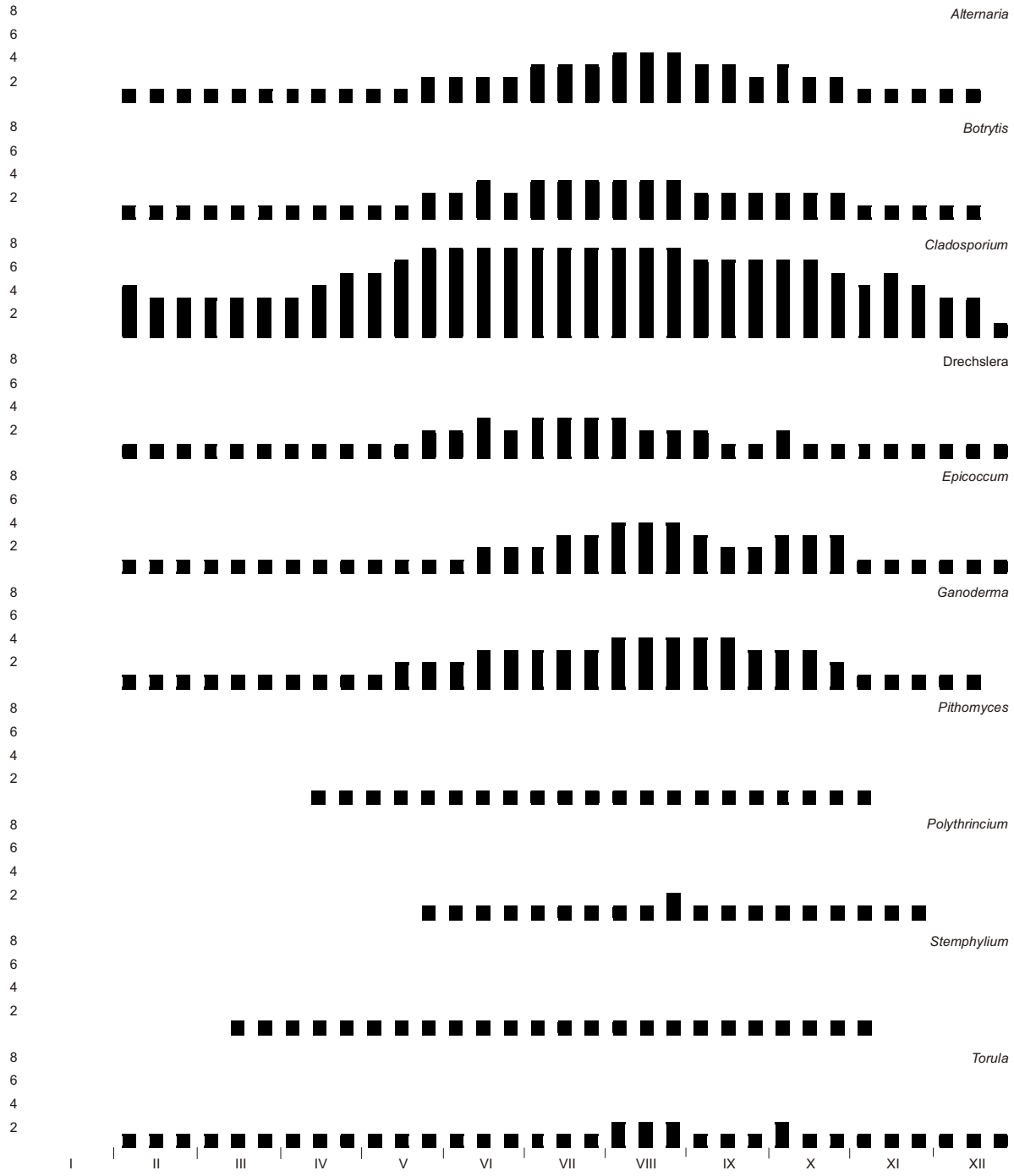

Figure 1. Fungal calendar for Rzeszów (2000-2002); ranges 1-8 correspond to average fungal spore concentration (s/m^3): 1-10; 11-30; 31-90; 91-270; 271-810; 811-2430; >2431.

between years (χ^2 test, $\alpha \leq 0.05$). Also, mean annual conidia concentrations of all the taxa, except for *Cladosporium* and *Epicoccum* spp., significantly differed between years (Tab. 2).

On the basis of the constructed calendar, we can conclude that airborne fungal spores occur in Rzeszów throughout nearly the whole year. Their concentrations in spring, late autumn and winter are low. The maximum concentrations occur in summer. The taxon that has the longest period of occurrence and the highest concentration is *Cladosporium*. In February, March, November and December concentrations of its conidia are usually low, the maximum falls in June, July and August. The maximum concentration of conidia of Drechslera type occurs in June and July; however, its numbers are much lower than those of the former type. The maxima of concentration of the other spore types usually occur in August; however, the values differ dramatically. The taxon whose spore concentration peaks the latest, i.e. in August and the first 20 days of September, is *Ganoderma* spp. (Fig. 1).

Table 2. The results of Kruskal-Wallis test (H) and ANOVA (F).

Taxa	H or F values	Statistical significance
Alternaria	H = 14,899	***
Botrytis	H = 31,536	***
Cladosporium	F = 0.061	NS
Drechslera	H = 35,046	***
Epicoccum	H = 1,451	NS
Ganoderma	H = 6,983	*
Pithomyces	H = 7,581	*
Polythrincium	H = 7,861	*
Stemphylium	H = 17,221	***
Torula	H = 7,330	*

*$\alpha \leq 0.05$, *** $\alpha \leq 0.001$, NS – not significant.

DISCUSSION

The taxa chosen for analysis belong to the group of anamorphic fungi (also called conidial or mitosporic), except for *Ganoderma* spp., which belongs to Basidiomycotina. The selection of taxa was arbitrary; it therefore cannot be concluded that anamorphic fungi dominate in the spectrum of airborne spores. Numerous papers confirm their high frequency and concentration in the air; however, according to many authors, airborne Basidiomycotina spores are equally common [1, 4, 7, 21]. Airborne conidia of *Cladosporium* spp. were the most frequent and most abundant (Tab. 1). Most aeromycological papers report this taxon in both temperate and hot climates; however, contributions of its conidia to annual sums range between 40–90%, and depend on the list of identified taxa and the chosen method [1, 7, 19, 21].

In Rzeszów, similarly to other cities in Poland [22, 25] and Europe [1, 23], maximum spore concentrations of most taxa occur in July and August. According to Nikkels *et al.* [23], the results from various countries of Europe are so similar that the network of permanent aeromycological monitoring stations should not be so dense for aeropalinological studies. However, it must be borne in mind that in particular years, daily concentrations of spores and SFI differ greatly [1, 5]. These parameters are influenced by the weather [26], and by habitat factors (e.g. availability of substrates [1]), often difficult to quantify or even determine. In Rzeszów during the 3 years of study, the distribution of conidia and spore frequencies and mean annual concentrations were, statistically, significantly different (Tab. 2). These differences applied to the taxa that had the largest share in the total annual sum, i.e. *Alternaria*, *Botryris*, *Ganoderma* spp.

The calendar of the occurrence of airborne fungal spores and the current data on the content of these spores should become a basic tool of any allergologist practitioner. In everyday practice, only the data on the occurrence of 2 taxa are usually taken into account: *Alternaria* and *Cladosporium* spp. It is these taxa which most commonly cause allergies, and according to Haines *et al.* [13] and Bush and Portnoy [2] the conidia and spores of all the taxa studied in this work cause allergies. In Rzeszów, the concentrations of the spores often exceeded threshold values, provoking allergy symptoms [12], and their maximum concentrations coincided with the occurrence of in the air Poaceae and *Artemisia* pollen (Fig. 1), [16], which are the commonest cause of pollen allergies. Such co-occurrence of aeroallergens increases the risk of disease [23].

CONCLUSIONS

1. Spores of the studied fungal taxa occur in the air throughout nearly the whole year; however their daily concentration varies considerably.
2. The maximum spore concentration of most taxa occurred in August, and their concentrations exceeded threshold values provoking allergy symptoms from June to September.
3. Conidia belonging to *Cladosporium* spp. are the commonest in the air; they occur in the highest concentrations and their mean year concentrations do not differ between years.
4. On the basis of frequency and SFI values, 2 groups of taxa can be distinguished. The spores and conidia of *Alternaria*, *Cladosporium*, *Botrytis*, *Epicoccum*, *Ganoderma* spp. and Drechslera (type) had high frequency (over 50%) and high or very high concentrations, whereas *Pithomyces*, *Polythrincium*, *Stemphylium* and *Torula* spp. had low frequency and concentrations of their conidia.

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