

Airborne fungal spores in urban and rural environments in Poland

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Received: 7 February 2005 / Accepted: 20 April 2006
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Abstract The concentrations of airborne fungal spores were measured during 2001–2002 in two sites in Poland—one in the city and the other in the countryside. The sites differed in habitat characteristics, such as urbanisation level, vegetation and microclimate. The aim of the study was to determine if, and in which way, land use type would affect spore occurrence. The volumetric method was used, and ten easily identifiable spore types were sampled and analysed: *Alternaria*, *Botrytis*, *Cladosporium*, *Epiccocum*, *Ganoderma*, *Pithomyces*, *Polythrincium*, *Stemphylium*, *Torula* and *Drechslera*. The season of spore occurrence was determined using the 90% method. The fungal spores studied were very frequent in the air (in most instances at a frequency higher than 50%). The most common spores were those of *Cladosporium*, with a frequency range of 83.1–90.5%. In both years the proportion of *Cladosporium* spores was statistically significantly higher in the city. In both 2001 and 2002 the total seasonal sum of all the spores was higher in the countryside than in the city as was the Seasonal Fungal Index (SFI) values and average concentrations of *Botrytis*, *Ganoderma* and *Torula*.

These latter three genera are usually represented as pathogens of plants. The mean spore concentrations of most taxa were significantly higher in the rural environment. Correlation coefficients between daily concentrations at both sites for most of the taxa studied were significant, but with lower correlation values between variables. Such results indicate that the values from the sites are weakly interdependent. The study confirms that land use type may very likely have an impact on the course of spore occurrence, the mean daily concentrations of spores as well as SFI values.

Keywords Aerobiology · Aeromycology · Conidia · Fungal spore concentration · SE Poland · Urban and rural environments

1 Introduction

The presence and concentration of airborne fungal spores, in both indoor and outdoor environments, have been the subject of numerous studies in various geographical regions (Adams, 1964; Burch & Levetin, 2002; Calderon, Lacey, McCartney, & Rosas, 1997; Diaz, Iglesias, & Jato, 1997; Icenhour & Levetin, 1997; Kasprzyk, Rzepowska, & Wasylów, 2004; Mitakakis & Guest, 2001; Stępalska & Wołek, 2005) and in various contexts, including those of agrobiolgy (Diaz et al., 1997; Frenguelli, 1998; Hirst, 1991;

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McCartney, 1994) and medicine (Corden, Millington, & Mullins, 2003; Crook, 1994; Gravesen, 1979; Icenhour & Levetin, 1997; Myszkowska, Stępańska, Obtułowicz, & Porębski, 2002). More recently, airborne fungal spores have been investigated with reference to the conservation of works of art (De Nuntiis et al., 2003). However, despite long-standing knowledge of the presence of airborne spores, data on the quantitative and qualitative features of this natural bio-aerosol is still incomplete. There is no universal study method that combines continuous measurements with the reliable and full identification of all the taxa encountered (D'Amato & Spieksma, 1995; Nikkels, Terstegge, & Spieksma, 1996).

While the number of environmental factors that are known to affect the daily and seasonal rhythms of the occurrence of airborne fungal spores keeps increasing, it remains difficult to estimate the relative importance of each factor. Fungi are heterotrophic organisms. They obtain nutrients from dead or living organisms (being saprotrophs and parasites, respectively). To develop and complete their life cycle they are dependent on a ready access to organic material, which is more abundant in rural areas than in urban environments. Other important factors influencing the occurrence of the spores in the air are weather and microclimatic factors. Cities have a specific microclimate which can be characterised by, among others, higher daily temperatures and intradiurnal temperature differences and a specific wind direction (Unger, 1999).

Most aerobiological research, including that of aeromycology, is conducted in cities (Corden et al., 2003; Nikkels et al., 1996), with very little data originating from rural settings (Simeray, Chaumont, & Leger, 1993). Consequently, it is very difficult to compare the urban and rural environments with respect to the presence and concentration of airborne fungal spores (Diaz et al., 1997). Bearing this in mind, this study was designed in such a way that measurements were made at sites in the same bio-climatic region but differing in one respect: one site was in a city and the other was in the countryside. The main aim of the study was to determine if and how the dominant land use type affects the presence of fungal spores in the air.

2 Materials and methods

This study was conducted in SE Poland (Podkarpacie region). The close proximity of the Carpathians has a major influence on the climate of this region: the summers are long and hot followed by warm, sunny autumns; the winters are not too severe, with more frost and snow in the outskirts of the region. In Rzeszów, the mean annual precipitation is 633 mm, and the mean annual temperature 8.1°C. The warmest month is July (18.3°C), and the coldest is January (−2.1°C) (Gus, 2001). The average vegetation growth period ranges from 215 to 220 days.

Spores were sampled at two sites, 10 km apart. The first was located in the city of Rzeszów, and the other site was situated in the countryside, in Krasne. Rzeszów is an average-sized city with a typical urban structure. The urbanisation index (built-up area/total area; Calderon et al., 1997) is $UI = 0.54$. The city is located in the valley of the Wisłok river. Vegetation in the city has been strongly transformed by human activities and anthropogenic plant communities dominate. A park type of vegetation is the most predominant form, with other major kinds of vegetation being municipal lawns, private gardens and roadside ruderal vegetation. Krasne, on the other hand, is a village with a landscape typical of the Podkarpacie region. Its most characteristic feature is the lack of large monocultures. Fields do not exceed a few hectares and are intermingled with farming premises surrounded by gardens. The dominant crop is wheat and rape. Small clusters of trees are frequent in the fields. Other common features are hay meadows as well as cherry and apple orchards. There are no forests in the close vicinity of Krasne, and the nearest one is a pine-oak forest 10 km north. The urbanisation index for Krasne is $UI = 0.09$. Data on the land use type were obtained from the local city and municipal administrations of Rzeszów and Krasne.

In Rzeszów measurements were taken from 1 January 2001 up to 31 December 2002. In Krasne, due to technical reasons, sampling began on 26 April 2001 and continued up to the end of 2002. Volumetric methods using Hirst-type spore traps (model VPPS 2000; Lanzoni s.r.l., Bologna, Italy) were used. The traps were located approximately

12 m above the ground. Fungal spores were identified and counted from one horizontal transect 48 mm long. A special conversion factor was used to transform the absolute numbers of spores into the daily average fungal spores/m³ of air. Identification was restricted to the following taxa: *Alternaria*, *Botrytis*, *Cladosporium*, *Epicoccum*, *Ganoderma*, *Pithomyces*, *Polythrincium*, *Stemphylium* and *Torula*. As it is very difficult to distinguish the spores of *Bipolaris*, *Drechslera*, *Exserohilum* and *Helminthosporium* using an optic microscope, these spores were lumped into one type—*Drechslera*. The identification of the spores was based on keys and atlases (Barnett & Hunter, 1972; Grant Smith, 1990) as well as comparative material.

Due to the delay in starting the spore monitoring in Krasne in 2001 relative to Rzeszów the comparative statistics presented in Table 1 refer to the period between 26 April and 31 December of each year. The seasonal totals of average concentrations of studied fungal types were expressed as Seasonal Fungal Index (SFI). Daily concentrations of the selected taxa were expressed as a 5-day moving average (Figs. 1–3).

The *z* test (*u* test) was used to verify the following null hypothesis: the percentage values of total spore numbers of each examined spore type do not differ between rural and urban areas (Wołek, 1992). Subsequent steps of the statistical analyses were performed according to the procedure proposed by Trigo, Toro, Recio, and Cabeduzo (2000). Statistical tests were applied for the seasons determined with the 90% method (Nilsson & Persson, 1981). The beginning of the season was defined as the day when 5% of the seasonal cumulative spore numbers were reached; the end of the season was defined as the day when 95% of the seasonal cumulative numbers was reached. After the normality of the distribution had been checked using the Kolmogorov-Smirnov normality test, the Spearman test was applied to determine the strength of the relationship between data from the study sites. The average daily concentrations of fungal spores in corresponding seasons (90% method) were then compared. When the Spearman correlation coefficient was statistically significant, the Wilcoxon test was used. The Mann-Whitney *U* test was used when the samples were independent (Trigo et al.,

Table 1 Descriptive statistics of airborne fungal spores in the 2001 and 2002 seasons

	Seasonal Fungal Index (SFI)		Percentage of seasonal sum		Percentage of frequency of season		Peak day spore/m ³	
	Rzeszów	Krasne	Rzeszów	Krasne	Rzeszów	Krasne	Rzeszów	Krasne
2001								
<i>Alternaria</i>	11,132	8,415	2.1	1.5	78	73.4	530	363
<i>Botrytis</i>	10,554	19,512	2	3.6	63	75.4	418	726
<i>Cladosporium</i>	478,717	474,909	89.3	87	89	91.5	20,555	14,337
<i>Drechslera</i>	2,597	6,003	0.5	1.1	61	78.2	70	273
<i>Epicoccum</i>	1,1595	11,591	2.2	2.1	68	78.6	555	339
<i>Ganoderma</i>	1,7462	20,877	3.3	3.8	71	73.8	459	504
<i>Pithomyces</i>	558	480	0.1	0.1	38	36.7	18	36
<i>Polythrincium</i>	698	759	0.1	0.1	38	33.9	30	33
<i>Stemphylium</i>	806	621	0.2	0.1	42	33.9	37	36
<i>Torula</i>	1,975	2,628	0.4	0.5	63	66.9	60	123
2002								
<i>Alternaria</i>	10,281	28,614	2	5.3	68.4	71.6	615	873
<i>Botrytis</i>	5,639	13,528	1.1	2.5	69.2	66.8	213	475
<i>Cladosporium</i>	473,432	445,641	90.5	83.1	82	84.8	14,028	13,467
<i>Drechslera</i>	1,968	1,863	0.4	0.3	62	49.2	90	77
<i>Epicoccum</i>	6,407	16,416	1.1	3.1	73.2	70.8	204	700
<i>Ganoderma</i>	22,718	26,484	4.3	4.9	70.8	66	654	642
<i>Pithomyces</i>	543	765	0.1	0.1	24.8	30.4	42	40
<i>Polythrincium</i>	546	990	0.1	0.2	24.4	36.8	33	42
<i>Stemphylium</i>	342	666	0.1	0.1	23.2	42	39	26
<i>Torula</i>	1,272	1,465	0.2	0.3	50	53.2	48	57

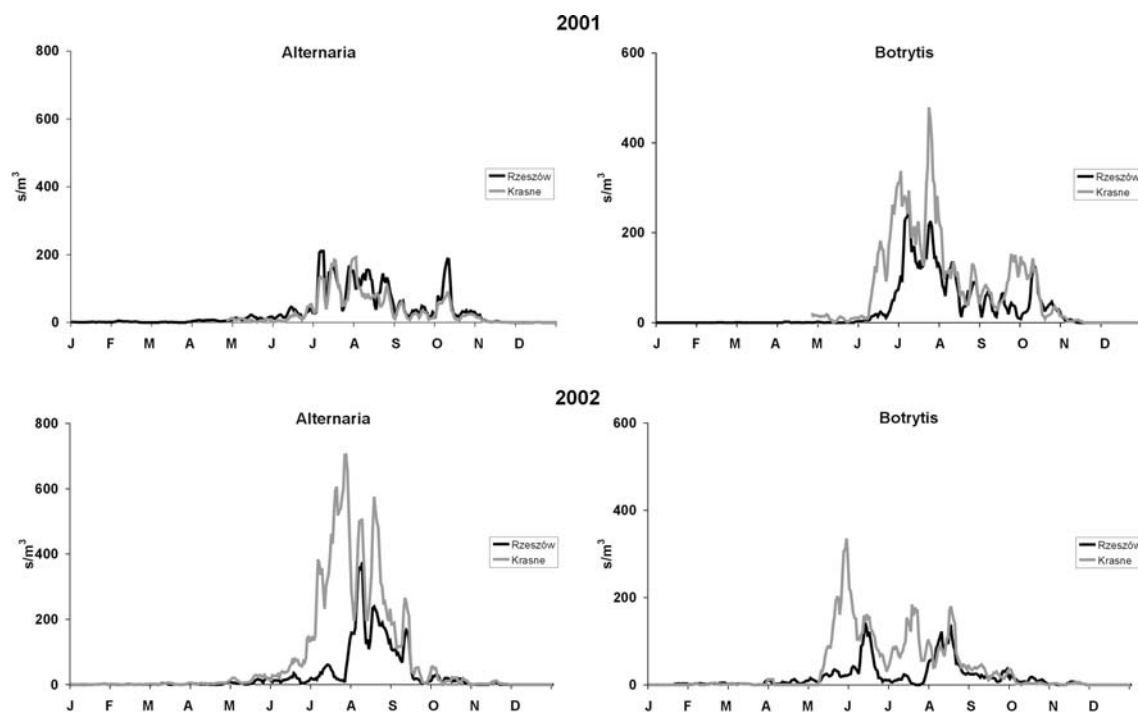


Fig. 1 The distribution of daily (5-day moving average) fungal spore concentrations of *Alternaria* and *Botrytis* in 2001–2002

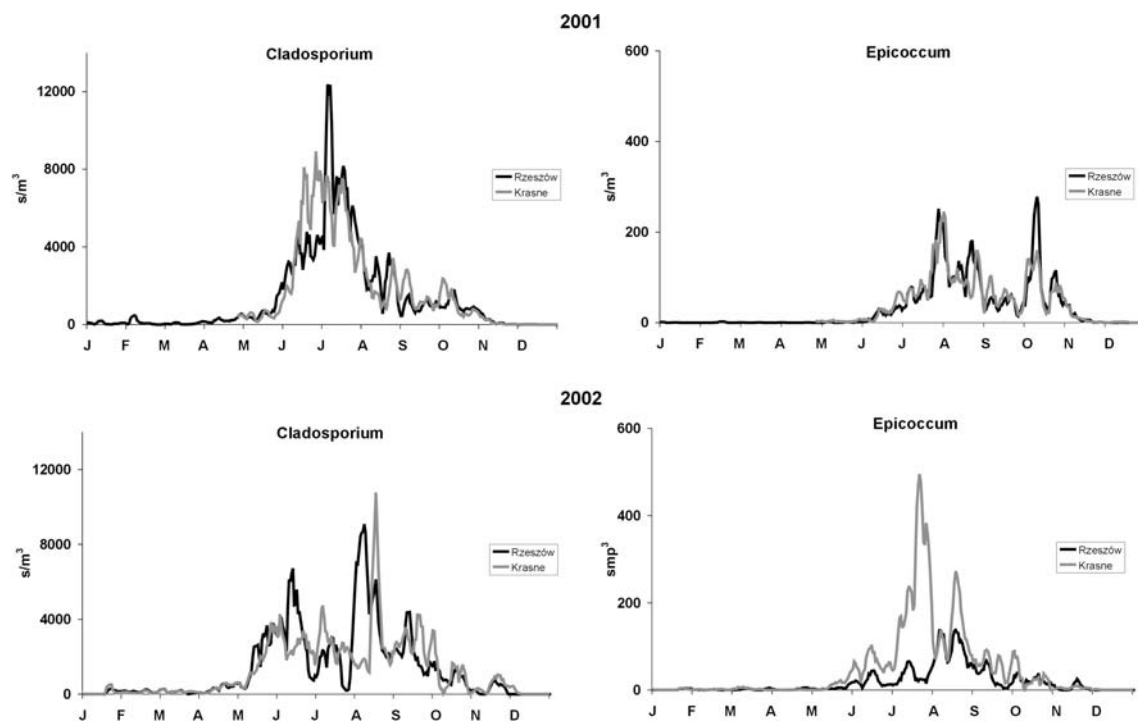


Fig. 2 The distribution of daily (5-day moving average) fungal spore concentrations of *Cladosporium* and *Epicoccum* in 2001–2002

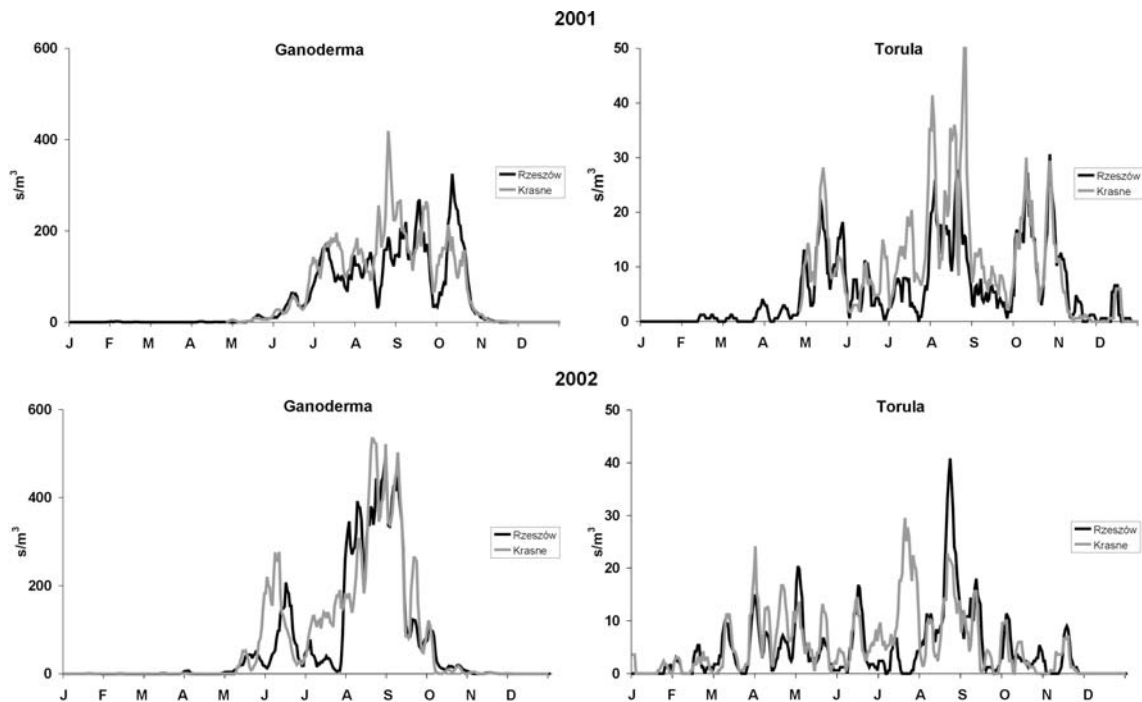


Fig. 3 The distribution of daily (5-day moving average) fungal spore concentrations of *Ganoderma* and *Torula* in 2001–2002

2000). Statistical tests were applied separately for each season using the STATISTICA program ver. 7.1 (Statistica, Tulsa, Okla.). In all tests the standard $\alpha < 0.05$ level of significance was used.

3 Results

The observed fungal spore types were sampled in the air for nearly the whole season (from 26 April up to 31 December) in each year at both sites. In 2001 the frequencies of spores (f) exceeded 60%, with the exception of *Pithomyces*, *Polythrincium* and *Stemphylium*; during 2002 the values were slightly lower. The most common spores were those from the genus *Cladosporium*, which occurred at a frequency of 82–91.5% (Table 1).

In both years the seasonal totals of average concentrations of all fungal spores were higher in the rural environment. However, when the SFI values for each fungal type were examined in more detail, the pattern became more complicated. In the first year of the study (2001) the SFI values of *Alternaria*, *Epicoccum*, *Pithomyces* and

Stemphylium were slightly higher in the urban environment. During 2002, however, most of the taxa were characterised by higher SFI values in the rural area. *Cladosporium* spores dominated at both sites throughout the study period. The percentage of the total seasonal *Cladosporium* spore count ranged from 83.1 to 90.5%. In Krasne, in addition to *Cladosporium*, *Botrytis*, *Epicoccum* and *Ganoderma* reached relatively high concentrations (over 2%); in Rzeszów it was *Alternaria* and *Ganoderma* (Table 1). In both years the percentages of seasonal spore sums of *Cladosporium* were significantly higher in the city, while the percentages of *Botrytis*, *Ganoderma* and *Torula* spores in total seasonal sums were significantly higher in the rural area (u test; $\alpha < 0.05$).

The periods of occurrence of spores in the air were similar in both sites, although differences in daily spore concentrations did occur (Figs. 1–3). In 2001 daily spore concentrations at the two sampling point were significantly correlated (Spearman correlation coefficient), with the exception of *Pithomyces* and *Polythrincium*. The highest correlation coefficient was observed for

Table 2 Results of Spearman correlation between daily fungal concentrations at the two sites

2001	Number of samples	Spearman correlation coefficient (R)	2002	Number of samples	Spearman correlation coefficient (R)
<i>Alternaria</i>	145	0.680***	<i>Alternaria</i>	86	0.315**
<i>Botrytis</i>	111	0.450***	<i>Botrytis</i>	115	0.137 ns
<i>Cladosporium</i>	107	0.555***	<i>Cladosporium</i>	149	0.114 ns
<i>Drechslera</i>	164	0.478***	<i>Drechslera</i>	126	0.276**
<i>Epicoccum</i>	120	0.562***	<i>Epicoccum</i>	119	0.368***
<i>Ganoderma</i>	122	0.372***	<i>Ganoderma</i>	104	0.652***
<i>Pithomyces</i>	146	0.089 ns	<i>Pithomyces</i>	69	0.225*
<i>Polythrincium</i>	110	0.098 ns	<i>Polythrincium</i>	74	0.323*
<i>Stemphylium</i>	179	0.244***	<i>Stemphylium</i>	145	0.101 ns
<i>Torula</i>	189	0.361***	<i>Torula</i>	213	0.307***

* $\alpha \leq 0.05$, ** $\alpha \leq 0.01$, *** $\alpha \leq 0.001$; ns, not significant

Alternaria. In 2002 no correlations between daily spore concentration at the two sample sites were observed for *Botrytis*, *Cladosporium* and *Stemphylium*; correlation coefficients for the other taxa were significant but with lower correlations between variables (Table 2). The mean concentrations of *Cladosporium* spores did not differ between sites in both years. In 2001 no differences between sites were observed for *Epicoccum*, *Pithomyces*, *Polythrincium* and *Stemphylium*; in 2002 there were no differences for *Drechslera* and *Ganoderma*. In both years the mean concentrations of *Botrytis* and *Torula* spores were significantly higher in the rural environment. In 2001 mean concentrations of *Ganoderma* and *Drechslera* type spores were also significantly higher in the rural area, while in the second year this was the case for most of the taxa sampled (Table 3).

Table 3 Results of the Wilcoxon's (z) and U-Mann-Whitney (Z) tests

Taxon	2001	2002
<i>Alternaria</i>	$z = 4.261***$	$z = -7.798***$
<i>Botrytis</i>	$z = -5.025***$	$Z = -7.962***$
<i>Cladosporium</i>	$z = 0.597$ ns	$Z = -0.486$ ns
<i>Drechslera</i>	$z = -7.320***$	$z = -0.665$ ns
<i>Epicoccum</i>	$z = 0.429$ ns	$z = -8.044***$
<i>Ganoderma</i>	$z = -2.866**$	$z = -1.459$ ns
<i>Pithomyces</i>	$Z = 1.323$ ns	$z = -2.591**$
<i>Polythrincium</i>	$Z = -0.400$ ns	$z = -3.780***$
<i>Stemphylium</i>	$z = 1.422$ ns	$Z = -5.094***$
<i>Torula</i>	$z = -3.532***$	$z = -2.227*$

* $\alpha \leq 0.05$, ** $\alpha \leq 0.01$, *** $\alpha \leq 0.001$; ns, not significant

4 Discussion

The hypothesis proposed in the introduction was that the fungal spore concentrations would be higher in the countryside than at the urban site, particularly for taxa known to be plant pathogens, such as *Alternaria solani*, *A. tenuis* and *Botrytis cinerea*, *B. alli* and *B. byssoidea*. This turned out to be the case: the total spore counts in both years were significantly higher in the rural environment. Similar results were obtained by Simeray et al. (1993) who compared values of colony forming units (C.F.U.) in the rural and urban air. However, based on the results of this 2-year study, it is difficult to specify a particular taxon characteristic of any of the two studied habitats. Spore concentrations are known to show a considerable daily, seasonal and annual variability (Kasprzyk et al., 2004; Stępańska & Wołek, 2005). It would appear, however, that *Botrytis*, *Ganoderma*, *Torula* and *Polythrincium* spores occur at higher concentrations in air of the rural environment. *Botrytis allii* is a pathogen of onions, while *B. cinerea* can attack cabbage, bean, lettuce, wine grapes. Other *Botrytis* species cause grey mould in vegetables (tomato, cucumber, red pepper, etc.) and flowers (narcissus, tulip, peony). *Ganoderma* generally grows on deciduous trees, and *Torula* occurs abundantly in the dead parts of herbaceous plants, in wood and in soil or as cereal pathogens (Barnett & Hunter, 1972; Diaz, Iglesias, & Jato, 1998). In the second year of study total spore sums (SFI) of *Alternaria* and *Epicoccum* were more than twofold higher in rural

environment than in the city. *Alternaria* is a common pathogen of cereals, vegetables and weeds. *Epicoccum* is commonly found on dead parts of numerous plants, seeds and soft fruits (Adams, 1964; Barnett & Hunter, 1972). The low urbanisation index of the Krasne region indicates that there is more organic material in this area than in the city; this would provide the substrate for these fungi to grow as parasites or saprophytes, thereby providing the most likely reason why their spores are commoner in the air of the rural environment.

Cladosporium spores occurred equally frequently and abundantly in both habitats, although their proportion in seasonal sums was significantly higher in the city. Many authors emphasise that this taxon is cosmopolitan and colonises a variety of substrates. Its spores occur in abundance in the air of many geographical regions (Adams, 1964; Calderon et al., 1997; D'Amato & Spiekma, 1995; Hasnain, 1993; Kasprzyk et al., 2004; Nikkels et al., 1996; Simeray et al., 1993; Stepalska & Wołek, 2005).

The temporal pattern of the occurrence of any one of these fungal species was usually similar. The differences between sites were based on differences of concentration. These results suggest that, although the sites of our study were located in relatively close proximity, we cannot predict the concentrations and the seasonal course of spore occurrence at one site by monitoring the other. As such, they confirm the preliminary hypothesis that daily concentrations, mean concentrations and seasonal sums for most of the studied taxa are, as a rule, lower in city air than in areas with a lower urbanisation level. Land use type and the flora, vegetation and microclimate associated with it seem to play an important role in determining the composition and concentration of the fungal airborne spore population, which was confirmed by Calderon et al. (1997). However, this hypothesis should be confirmed by long-term aeromycological studies, completed by detailed meteorological and climatic data, and the differences in flora and vegetation should be taken into account.

Acknowledgements I would like to thank Professor Kazimierz Szczepanek and Dr. Danuta Stepalska, Institute

of Botany, Jagiellonian University, Cracow, for their valuable comments on the manuscript. The research was financially supported by KBN grant nr. 6 PO468720.

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