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SHORT COMMUNICATION

The usefulness of aerobiological methods in monitoring lycopod sporulation

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Background and aims – The purpose of the study was to determine whether the concentration of lycopod spores present in aeroplankton reflects their release during sporulation and whether it is a good indirect tool to estimate the condition of lycopod populations in Poland.
Methods – The study was conducted in Sosnowiec, S Poland. The airborne spores were continuously captured by the volumetric spore trap during a period of eleven years (1998–2008). Plant pollen grains and spores from the trap were macroscopically identified, counted and expressed as daily pollen grain and spore concentrations in 1 m^3 of air.
Key results – The average monthly sum of captured lycopod spores varied in the analysed period of time from 0.3 to 4.1 spores per year. Their yearly concentration was small in comparison to the ascertained pollen grains and did not exceed 0.5% of all airborne particles captured in a given year. In addition, they showed seasonal fluctuations with the highest concentration of spores usually detected in July. This finding is in accordance with the typical time of lycopod sporulation. In addition, we detected the presence of lycopod spores outside the typical summer sporulation time, i.e. during months of winter and early spring. In our opinion, their presence is related to the phenomenon of lengthened (winter) sporulation of clubmosses.
Conclusions – Our results showed only limited transportation of lycopod spores by wind. Therefore, the condition of lycopod populations in Poland can hardly be estimated using aerobiological methods. Nevertheless, the seasonal dynamics of lycopod spore concentration can be recorded by aerobiological methods.

Key words – *Lycopodium*, spore dispersal, winter sporulation, aerobiology, seasonal dynamics of sporulation.

INTRODUCTION

Lycopodium s.l. is a genus in the family Lycopodiaceae (clubmosses), represented by species with a regular alteration of generations. A gametophyte (haploid generation) usually develops as a few millimetre-long prothallium and ultimately produces gametes for generative reproduction, whereas a dominant diploid sporophytic generation forms long creeping plagiotropic shoots or rhizomes with short orthotropic branches (Øllgaard 1990, Tutin et al. 2010, Rimgailė-Voicik et al. 2015). The sporophyte generation of lycopods is reproduced by numerous spores which are small, having a diameter of c. 28–43 µm (Wilce 1971), and spread by wind (Oinonen 1967). The whole lifecycle of lycopods is prolonged and lasts several years (Øllgaard 1990, Naujalis 1995).

In Central Europe, lycopods mostly occur in pine forests, where they can form large patches. For example, populations of *Lycopodium annotinum* cover from 500 m^2 up to 18 000 m^2 and even more in mountain areas (Witting et al. 2007), whereas patches of *L. clavatum*, *Diphasiastrum complanatum* and *D. tristachyum* usually range from 200 to 600 m^2 (Naujalis 1995). In Poland, lycopod populations are generally of similar size, but they can also be much smaller and cover areas of only c.1 m^2 (e.g. Śliwińska-Wyrzychowska & Książczyk 2009, Kiedrzyński et al. 2015).

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In recent decades, *Lycopodium* species have become threatened in Europe because of a decrease of favourable habitats, a long-lasting lifecycle and generative propagation, the vulnerability of gametophytes to harsh environmental conditions as well as due to extensive commercial exploitation. Similarly in Poland, the number of populations and individuals at particular localities has recently been significantly reduced (Piękoś-Mirek & Mirek 2003), presenting a risk for species persistence. Lycopods are an ancient group of vascular plants (Kenrick & Crane 1997) which due to their specific biology and interactions with specialised mycobiota are unique components of forest ecosystems. Their presence reflects the long term stability and well-preservation of particular forest complexes. Monitoring and conservation of existing populations is thus crucial to maintain biodiversity on the micro- and macroscale. Previous studies have shown that based on the dynamics of pollen grains and/or spore concentrations in the air it is possible to infer the local abundance of species (Webb et al. 1981, Hicks 2001). Therefore, it appears worthwhile to analyse the potential of aerobiological methods (measuring aerial lycopod spore concentrations) to estimate the condition of lycopod populations in Poland. The data on natural dispersal of lycopod spores is however extremely rare (Kasprzyk 2004, Gómez-Noguez et al. 2017) as aerobiological analyses mainly focus on allergenic properties of airborne particles. Because spores of lycopods do not have such properties, there are no special aerobiological reports devoted to their presence.

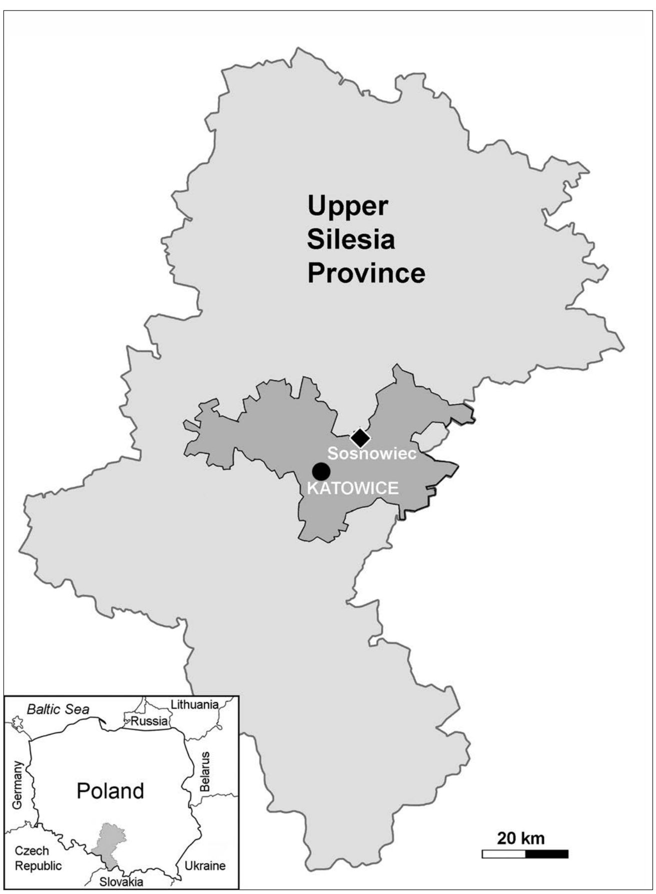

Figure 1 – Localisation of the aerobiological station in the Upper Silesia Province in Poland. Dark grey colour within the Upper Silesia Province marks the area of the Silesian conurbation.

Therefore, the main aims of our study were to analyse whether aerobiological analyses are sensitive enough (i) to mirror the seasonal variation in production and release of lycopod spores and (ii) to detect the decreasing population abundance of lycopods in Poland in the last decades as established in direct field observations (Piękoś-Mirek & Mirek 2003). To answer these questions, we analysed the dynamics of concentrations of lycopod spores captured in an aerobiological station located in Sosnowiec (S Poland) within a period of eleven years.

MATERIAL AND METHODS

The study was conducted in the city of Sosnowiec, Upper Silesia Province, S Poland (fig. 1). The city is located in the heavily urbanised and industrialised region of the Silesian conurbation, characterised by the highest degree of anthropogenic transformation in Poland. The coordinates of the aerobiological station are 50°17′50″N, 19°08′20″E.

Sosnowiec is situated in a temperate climate zone and the weather here is mostly (63.5% of the year) determined by polar maritime air masses (Niedźwiedź 2003). The average annual temperature is 8.1°C. The warmest month is July (+17.2°C) whereas the coldest is January (-1.2°C). The average annual precipitation is about 700 mm and predominant directions of winds are NW, W and SW. All these parameters are given for the years 1951–2007 (Łupikasza & Widawski 2008).

In our analyses, we used the data of the Allergen Research Center in Warsaw collected during the nationwide monitoring of pollen grains from 1998 to 2008. As monitoring is usually not performed in November and December, such data was not available and thus not included in our analyses. Airborne pollen grains and spores were continuously collected with the Hirst type volumetric spore trap (Hirst 1952; Burkard Scientific, UK), placed 20 m above the ground. Captured spores and pollen grains were microscopically identified (Stachurska et al. 1970). Lycopod spores were categorised only to the family level. The daily total concentration of spores and pollen grains was expressed as a number of airborne particles per m^3 of air and, accordingly, the monthly and annual total sums of spores and the monthly averages of spores per year were calculated. The methods were standardised according to Galán et al. (2014).

RESULTS AND DISCUSSION

We analysed the changes in the concentration of airborne lycopod spores collected by the trap over the period of eleven years (1998–2008). In general, the concentration of lycopod spores was low in comparison to ascertained pollen grains of spermatophytes (table 1) and consequently, its percentage in the total pool of airborne particles did not exceed 0.1%. This is not surprising regarding the relatively low number and abundance of lycopod species in comparison to the richness of seed plants (Christenhusz & Byng 2016). However, when compared to the total number of captured spores of the

Table 1 – Total annual sum of airborne lycopod spores in relation to the total annual sum of pollen grains and spores of monilophytes detected in successive years in the aerobiological station in Sosnowiec, S Poland.

Year	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008
Annual sum of pollen grains (spermatophytes)	22097	40552	51085	40682	37827	72128	53114	30872	68035	57311	85519
Annual sum of monilophyte spores	90	164	128	35	134	108	293	123	158	46	51
Annual sum of lycopod spores	15	41	15	12	7	20	29	9	18	3	11
Ratio of lycopod spores to pollen grains and monilophyte spores [%]	0.07	0.10	0.03	0.03	0.02	0.03	0.05	0.03	0.03	0.01	0.01
Ratio of lycopod spores to monilophyte spores [%]	17	25	12	34	5	19	10	7	11	7	22

monilophytes (ferns), participation of lycopod spores in the aeroplankton was quite significant (5–34%). Interestingly, both fern and lycopod spores are mostly dispersed locally to the distance of c. 5–8 m from a source of release (Bainbridge & Stedman 1979, Aylor & Ferrandino 1989, Peck et al. 1990, Aylor & Flesch 2001). Regarding their similar dispersal abilities, the better performance of lycopods in the analysed aerobiological samples can be attributed to differences in the habitat preferences and local species abundance. For instance, most fern species in the region occur in forest complexes, whereas a representative of lycopods, *Lycopodiella inundata*, can additionally form large plots in open and anthropogenically modified areas (Śliwińska-Wyrzychowska & Korzonek 2010). This possibly increases spore accessibil-

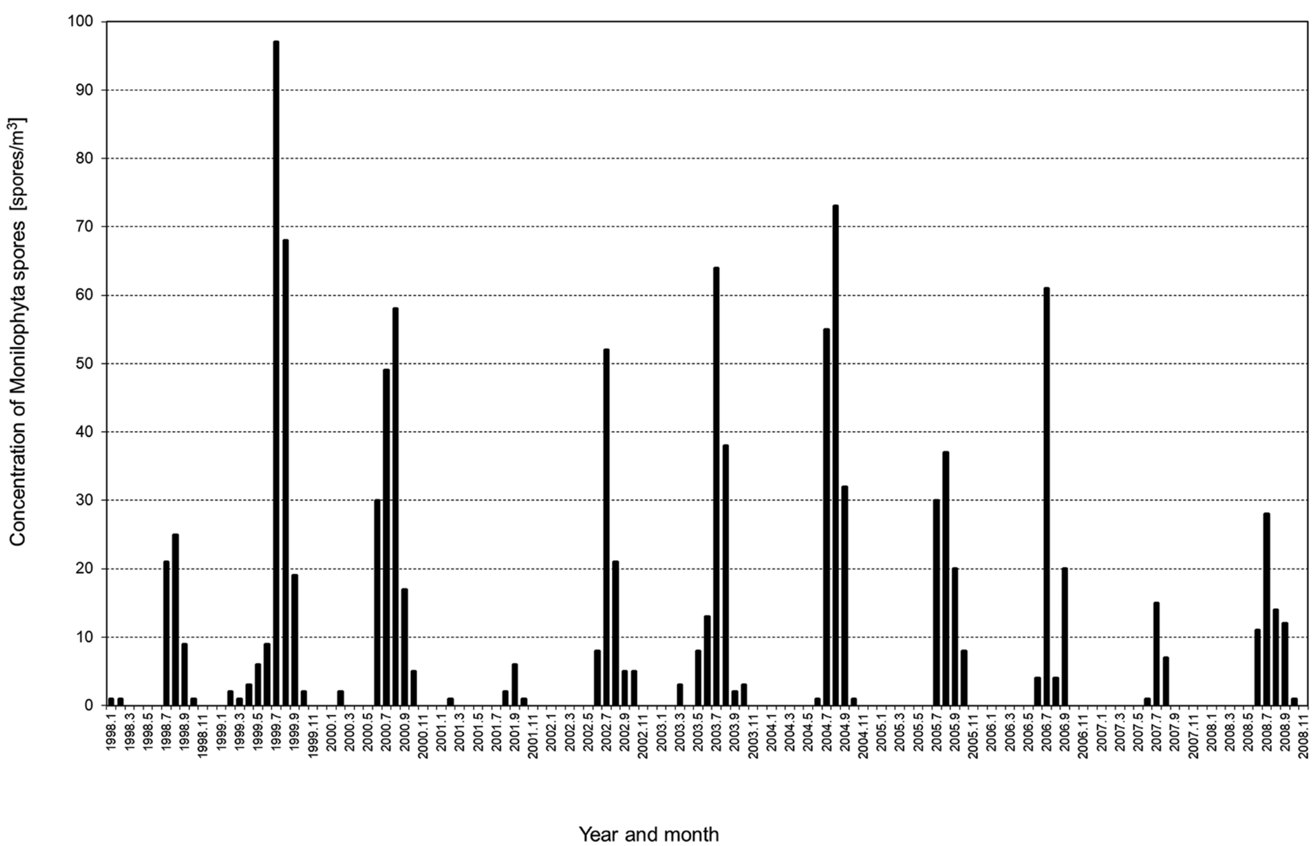

Figure 2 – Concentration of monilophytes spores [spores/m³] recorded in aeroplankton in successive months of the analysed years 1998–2008 in the aerobiological station in Sosnowiec, S Poland.

Table 2 – Monthly sums of airborne lycopod spores (spores/m^3) recorded for the period of eleven years (1998–2008). Asterisks mark the months with the maximal concentrations in a given year. n/a: months for which observations were not performed.

	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008
January	0	0	n/a	n/a	n/a	0	0	1	0	0	0
February	0	0	1	1	0	0	0	0	0	0	0
March	1	0	0	1	0	1	2	0	0	0	3
April	0	1	0	0	0	0	0	0	0	0	3
May	0	9	0	2	0	2	5	0	2	0	3
June	2	1	1	0	0	3	0	0	1	1	1
July	6*	22*	2	3	3*	3	2	5*	12*	2*	0
August	4	6	3	2	2	6*	9	3	3	0	1
September	2	2	8*	3	0	2	1	0	0	0	0
October	0	0	0	0	2	3	10*	0	0	0	0
November	n/a	n/a	n/a	n/a	0	0	n/a	n/a	n/a	n/a	n/a
December	n/a	n/a	n/a	n/a	n/a	0	n/a	n/a	n/a	n/a	n/a
Total	**15**	**41**	**15**	**12**	**7**	**20**	**29**	**9**	**18**	**3**	**11**
Monthly average per year	**1.50**	**4.10**	**1.66**	**1.33**	**0.70**	**1.66**	**2.9**	**0.90**	**1.80**	**0.30**	**1.10**

ity to air currents and, together with a large amount of spores produced, accounts for a higher participation of airborne lycopod spores.

A seasonal pattern of spore concentration (table 2) was observed with the highest values usually detected in July. This result is consistent with the typical time of lycopod sporulation from July to September (Schmeil et al. 2009). Interestingly, single lycopod spores were additionally detected from January to May, i.e. not during the typical time of sporulation (table 2). Their presence can possibly reflect three different phenomena, i.e. spore long-distance transport, redeposition and/or lengthened, so-called winter sporulation. The long-distant transport of pollen grains and spores is a well-documented phenomenon (e.g. Tryon 1986, Kato 1993, Rousseau et al. 2006), however, simultaneous lycopod sporulation in whole Europe and limited, mostly local lycopod spore dispersal by wind, as confirmed by our results, excludes the long-distance transport as the probable reason. The other explanation assumes the resuspension of spores, which released normally in summer and alight on soil, are again lifted up after winter and then they can be captured by the trap. This is a known mechanism enriching the air in particles after the end of sporulation or blossoming season (e.g. Mandrioli et al. 1980) and therefore it should also concern the monilophytes, with spores of similar dispersal abilities to lycopods. However, over the analysed period, fern spores were not detected in the early months of the year (fig. 2) indicating that redeposition is rather a marginal source of spores during winter and spring. The last and most probable explanation refers to the phenomenon of lengthened (winter) sporulation (Sonnberger et al. 2008, Bogdanowicz & Śliwińska-Wyrzychowska 2013), which is related to the formation of strobili in late summer and autumn. Consequently, these strobili release spores in winter and spring, and thus spores can be detected in aerobiological traps from December to May. The process repeatedly occurs in Polish and German populations of lycopods (Sonnberger et al. 2008), although it comprises only a small number of all strobili produced over the year.

Importantly, spore dispersal distance is related to numerous cooperating factors including the height of spore/pollen grain source of release, the density of surrounding vegetation, the settling velocity of spores/pollen grains, as well as other physical and climatic conditions, such as wind strength and predominant directions, precipitation etc. (Rodríguez de la Cruz et al. 2009, Heydel et al. 2014, Johansson et al. 2014, Gómez-Noguez et al. 2017). For instance, the average distance from the source of release, established in experiments with artificial *Lycopodium* spore dispersal, is usually only up to 8 m due to both the settling velocity of spores and limited wind currents in crop canopy (Bainbridge & Stedman 1979, Aylor & Ferrandino 1989, Aylor & Flesch 2001). This is in agreement with the data on seed and spore dispersal in the dense forest understoreys, typical lycopod habitats, where due to reduction in the wind speed the spread of diaspores is rather limited (Heydel et al. 2014, Johansson et al. 2014). Even if the lycopod spores are released in an open area, their transport remains only local, to the distance of approximately 1 km, as evidenced by experiments in an aerodynamical tunnel (Chamberlain 1967).

The significance of the proximity of the spore source to the spore trap for the captured spore concentration (Fernández-Rodríguez et al. 2014) has been confirmed by a higher average yearly sum of lycopod spores detected in our study (16.4 spores calculated based on data in table 1) in comparison to the results of similar studies conducted in Rzeszów, S Poland, where potential habitats for lycopods were distant more than 10 km away from the traps (only 6.4 spores; calculated based on table 1 in Kasprzyk 2004). In our study, the aerobiological station is directly located in the heavily urbanised and industrial area and the large coniferous forest complexes, being potential sources of lycopod spores, are in close vicinity (c. 6 km), and within the predominant direction of the wind (Łupikasza & Widawski 2008). Furthermore, there are additional sources of spores in the vicinity of our traps as clubmosses have been reported on post-industrial fields, such as closed quarries, sand and gravel pits (Śliwińska-Wyrzychowska & Korzonek 2010 and references therein).

Not only the distance between trap and spore source is of importance for spore capture but also the height of the trap above ground. It has been evidenced for herbaceous plants that the vertical mixing of their pollen grains in the air is not always efficient and leads to significant differences in the ascertained pollen grain concentrations decreasing with trap elevation (Hart et al. 1994). This can be the reason for the relative low concentration of lycopod spores obtained during our study, and in aerobiological samplers in general. Therefore, the location of the aerobiological station, in terms of its vicinity to source plants and the height at which the trap is placed, is of importance regarding the relatively limited, local lycopod spore dispersal.

The average monthly spore concentration fluctuated between years, ranging from 0.3 to 4.1 spores (table 1) and presented a subtle tendency to decrease from 1998 to 2008. However, this latter trend was not statistically significant, as shown by regression analysis $y = -0.0105x + 2.3304$ $R^2 = 0.017$, due to a high variation in the average monthly spore concentration among years. As this finding is contradictory to the observed decline of lycopod populations in Poland based on direct field observations (Piękoś-Mirek & Mirek 2003), it suggests that for lycopod species the aerobiological studies do not fully reflect the conditions of the respective lycopod populations.

CONCLUSIONS

Our analyses showed that the dispersal of lycopod spores by wind is mostly local as proven by their very low concentration in aerobiological traps in relation to the distance from the spore source. This finding combined with a high annual fluctuation of detected spore concentration renders the use of spore concentration as estimate for the well-being of lycopod populations difficult. Thus, only long-term in situ surveys of local populations will give sufficient insight. In addition, to make conclusions about the general condition of a lycopod species, the data from the whole area of the species´ distribution should be used. This is however a limiting factor as the standardised aerobiological traps are usually located in urbanised areas, out of lycopod habitats. Furthermore, the lycopod spores are usually neglected in the long-term monitoring of the airborne allergen particles as they do not have such allergic properties. Despite such limitations, our results proved the usefulness of the aerobiological approach to analyse the seasonal dynamics of spore concentration, which corresponded to the main time of lycopod sporulation in summer. Spores detected out of this time, during winter and early spring, could be an outcome of redeposition to some extent and possibly mostly of winter sporulation of lycopods.

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