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Original Article

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Temporal variability in the allergenicity of airborne *Alternaria* spores

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Abstract

The concentration of fungal spores in the air is traditionally considered as a proxy of allergen exposure. However, *in vitro* experiments have shown that the allergenicity of *Alternaria* spores varies depending on ecophysiological and developmental factors. Despite the potential clinical significance of these findings, it has never been verified in outdoor environments. This study, therefore, aims to investigate variability in the amount of the major allergen (Alt a 1) released from *Alternaria* spores in outdoor air. During the 3-year monitoring study (2014–2016), the median seasonal allergenicity of *Alternaria* spores exceeded 8.6×10^{-3} pg Alt a 1/spore. The most allergenic spores were collected during the driest and the most polluted season (with respect to seasonal concentrations of ozone, sulphur dioxide, and particulate matter). Within the season, daily spore allergenicity ranged from 2.4 to 34.7×10^{-3} pg Alt a 1/spore (5th-95th percentile). No repeatable effects of weather and pollution on short-term variations in *Alternaria* spore allergenicity were found. However, during the episodes when high-potency spores were recorded, the air masses arrived from eastern directions. Contrary, the spores with the lowest allergenicity were related to western winds. This suggests that factors such as source area (habitat types) and species diversity could be responsible for the varying exposure to *Alternaria* allergens. Our findings show that high and low-potency spores are recorded in the air; therefore, the airborne concentrations of fungal spores alone may not be sufficient to provide allergy sufferers and healthcare professionals with information about allergen exposure.

Key words: fungal allergy, immunoenzymatic analysis, Alt a 1, allergen, mould, potency.

Introduction

The *Alternaria* sp. genus belongs to the Pleosporaceae family (Ascomycota phylum), which is one of the most important allergenic fungi.[1,2] The mean rate of sensitization to *Alternaria* sp. in patients with suspected inhalant allergy exceeded 8.9% in Europe.[3] Exposure to *Alternaria* has been recognized as a risk factor for the development, persistence and severity of asthma.[4–6] The genus *Alternaria* sp. consists of multiple saprophytic, endophytic and pathogenic species that can be found in all habitable regions. Many species belong to the most notorious and invasive pathogens of innumerable plants species, including important crops such as potatoes and cereals.[2,7] The opportunistic human infections caused by *Alternaria* (alternariosis) has also been diagnosed.[8]

Alternaria sp. produces spores that are usually distinctively club-like, multicellular, and septate, and produced in single or branching chains on commonly short, erect conidiophores.[9] *Alternaria* spores are large (usually between 50 and 100 μm) compared to some other anemophilous pollen and fungal spores but are often found in high quantities in the air.[7] Outdoor concentrations of airborne fungal spores have traditionally been considered as a proxy of allergen exposure, and this parameter was used in epidemiological studies aimed at establishing the effect of fungi on the prevalence of allergic respiratory disease.[10–13]

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However, the causal relationship between airborne spore concentrations and clinical manifestations of allergic disease, especially allergic rhinitis, has not been definitively established as the data are inconclusive and contradictive.[12,14]

Alternaria spores contain a number of allergens belonging to different protein families, for example, heat-shock proteins, dehydrogenases, transferases, and ribosomal proteins.[15] Alt a 1 is a major allergen of *Alternaria* spores that reacts with more than 90% of atopic human sera,[1] and it has been shown that outdoor concentrations of Alt a 1 significantly correlate with clinical symptoms of patients sensitized to *Alternaria*.[16] Alt a 1 has a unique, dimeric β-barrel structure[17] and is predominantly located in the melanin layer of the spore wall.[18] It possibly serves as a transporter of small ligands (a methylated flavonoid) inhibiting plant root growth and detoxifies reactive oxygen species. It was suggested that the release of Alt a 1 blocks plant defences and consequently favours fungal entry into the plant.[19]

In vitro studies have shown that considerable variations in allergen content exist between components of the same isolates (spore vs hyphae), strains of the same fungus and fungal species of the same genus.[20–22] In addition, not all spores release allergens, and the amount of allergen released greatly depends on the stage of spore germination, spore viability and the culture conditions.[20,22,23] Hypothetically, the allergenicity of spores may also change in response to various environmental factors, such as weather conditions and pollution, as described previously for pollen grain allergens.[24–26] In addition, many fungi, even those that are taxonomically unrelated, contain similar proteins with shared allergenic components and show low to high levels of cross-reactivity.[1,27]

These observations put into question the usefulness of traditional monitoring based solely on spore concentration data, and does not consider variability in the allergenicity of airborne spores. There is a risk that spore-based forecasts may not accurately reflect the real threat of fungal allergens. Presumably, the lack of precise information about spore allergenicity might also be responsible for the inconsistencies observed in epidemiological studies, where only airborne spore data were used. To shed a light on that issue, we carried out a 3-year monitoring study of atmospheric concentrations of *Alternaria* spores and the main *Alternaria* allergen (Alt a 1). The concomitant measurements of airborne spore and spore allergens has been conducted in the past,[16,28–30] but, to the best of our knowledge, this is the first study that has quantitatively determined daily and seasonal variations in the allergenicity of airborne fungal spores.

Methods

Airborne *Alternaria* spore data

Alternaria fungal spores in outdoor air were studied during three seasons (2014-2016) using a Hirst type volumetric trap[31] located in the northern part of Poznań, Poland (52.47N 16.92E) (Fig. 1).

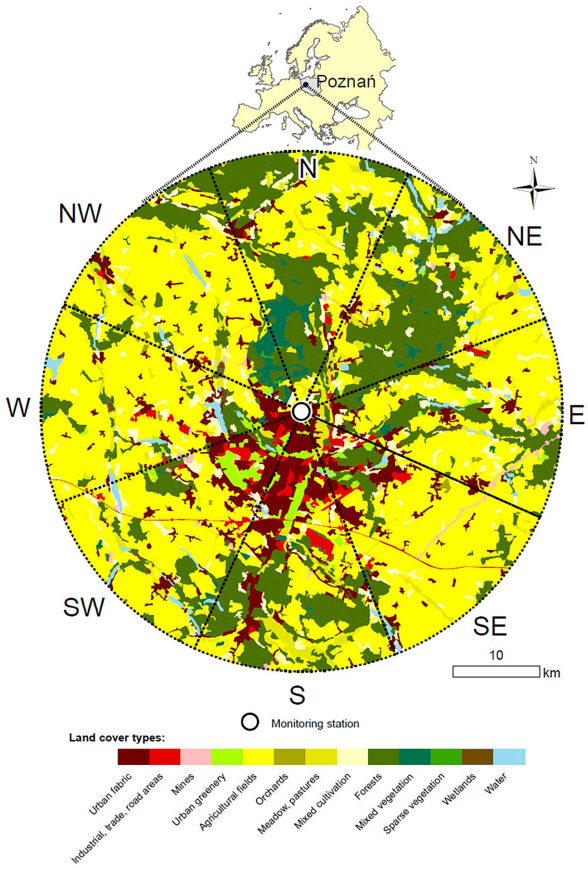

Figure 1. The location of the spore-monitoring site and land cover types (based on Corine Land Cover data, acquired from http://clc.gios.gov.pl/) within 30 km distance of the site. It is assumed that the amount of airborne particles recorded using volumetric trap is in general considered to reflect the overall particles load within a distance of about 30 km.[52] This Figure is reproduced in color in the online version of *Medical Mycology*.

Poznań is the largest city of Wielkopolska – an agricultural region dominated by the cultivation of cereals (~1000000 ha), rapeseed (~115000 ha), maize (~68000 ha), and beetroot (~40000 ha).[24] The vast crop areas and temperate climate make this region a suitable habitat for *Alternaria*. According to the latest study, airborne *Alternaria* spore concentrations in Poznań were the highest among many sites in Central and Eastern Europe.[32] *Alternaria* spores were collected each year from 10th July to 19th September ($n = 72$ days per season), that is, during the main sporulation season of *Alternaria* in Poland. The spore trap was sited at roof level (18 m a.g.l.). Air with fungal spores was sucked into the trap at a rate of 10 l per min through an orifice (2 mm × 14 mm). Spores were impacted on transparent adhesive tape supported on a clockwork-driven drum that moved past an orifice at 2 mm per hour. Deposited fungal spores were mounted on a glass microscope slide and examined by light microscopy (magnification 400 ×) along two longitudinal transects. The sampling time was 12:00-12:00 hours but is described as a "daily average" throughout. The daily average *Alternaria* spore concentrations were expressed as the number of spores in

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1 cubic meter of air (spore m^{-3}). The method of collecting and quantifying spores by Hirst type trap is currently considered as the gold standard in aerobiological studies.[23]

Quantification of airborne spore allergenicity

A three-stage ChemVol® cascade impactor (Butraco Inc., Son, Netherlands) supplied with a high-volume (400 l/min) vacuum pump (Digital High Volume air pump DHM60, Ludesch, Austria) was used to quantify the major allergen of *A. alternata*, Alt a 1. The device was located next to the Hirst volumetric spore trap (2 m away), and the sampling time was adjusted to spore collection time (12:00–12:00). The ChemVol consists of three stages collecting airborne particles with the following aerodynamic diameter: 1st stage: >10 μm, 2nd stage: 2.5–10 μm, and 3rd stage: 0.12–2.5 μm. The fungal spores were deposited on polyurethane filters (impacting substrate). After 24 hours, the filters were removed and cut into three equally sized pieces. As *Alternaria* fungal spores were almost exclusively found in the 1st and 2nd stages, only filters from these two stages were used for quantifying spore allergenicity. The airborne material collected on the filters was examined according to the protocol described in Buters, Weichenmeier.[33] Filters were extracted in the dark over a 4-hour period using 0.1 M ammonium bicarbonate buffer. Extracted material after centrifugation and lyophilization was dissolved in 1/10 of the original volume in phosphate-buffered saline (PBS). Quantification of Alt a 1 concentrations was performed using ant-Alt a 1 monoclonal antibody 2C10 and biotinylated monoclonal antibody 3B6 supplied by Indoor Biotechnologies Ltd, Cardiff, UK (Alt a 1 ELISA kit, EL-AA1). The "sandwich type" enzyme-linked immunosorbent assay (ELISA) was used following the protocol supplied by the reagents supplier. Two modifications were applied: (i) streptavidin-peroxidase (Sigma-Aldrich S5512) was used as an enzyme; (ii) 3.3′5.5′-tetramethylobenzidine (Sigma-Aldrich T0440) was used as a substrate and due to the rapid colorization reaction, the process was stopped after 3 minutes. The reaction was stopped by adding 2.5 M H_2SO_4 (5 N). The concentration of Alt a 1 in the sample was determined by reading the absorbance at 450 nm. The detection limit of ELISA was 0.05 ng/ml. All samples were determined in duplicate. In every season the interassay variability was below 20%. The reported values for each day are the means of two filter parts. If the coefficient of variance between values from two filter segments was >25%, the concentration of Alt a 1 in the third segment of the filter was determined and included in the analysis. Daily average concentrations of Alt a 1 were expressed as pg m^{-3}.

Finally, to calculate the daily allergenicity of airborne *Alternaria* spores the mean daily amount of released Alt a 1 (quantified by ChemVol trap and ELISA) was divided by the mean daily *Alternaria* spore concentration (quantified by Hirst trap and microscope analysis). The allergenicity of *Alternaria* spores were expressed as pg Alt a 1/spore.

Weather and pollution data

Daily and hourly weather data (daily mean temperature (°C), dew point temperature (°C), relative humidity (%), wind directions) was obtained from the main official meteorological station situated at the Poznań Ławica airport (app. 5 km south from spore-monitoring trap), belonging to the network of stations of the national Institute of Meteorology and Water Management. Mean daily air pollutant levels (carbon monoxide [CO], sulphur dioxide [SO_2], nitrogen dioxide [NO_2], ozone [O_3], and particulate matter [PM_{10}]) recorded in Poznań were extracted from the Polish Inspectorate for Environmental Protection database.

Statistical analysis

In order to obtain more robust results (due to the uncertainties in airborne fungal spore concentrations and allergen determination), only days with daily average *Alternaria* spore levels >100 s m^{-3} were included into statistical analysis (54 days in 2014, 45 days in 2015, and 66 days in 2016). As the aerobiological data did not follow a normal distribution (Shapiro-Wilk test, $P < .05$) a nonparametric Spearman's rank correlation analysis was used to examine relationships between daily average spore concentrations, spore allergenicity and weather/pollution data. Differences between the median seasonal sum and allergenicity of *Alternaria* spores were analyzed using the Kruskal-Wallis rank sum test with *P*-value adjusted for multiple comparisons (Benjamini & Hochberg method). Differences between the median allergenicity level of spores transported from western (SW-W-NW) and eastern (SE-E-NE) wind sectors has been calculated by Mann-Whitney *U* test. The density function was calculated using Gaussian kernel density estimator with default smoothing bandwidth (*x*-intervals) according to Silverman.[34] The statistical analysis were performed using the computing environment R.[35]

Results

The seasonal sum of *Alternaria* spores varied from 14025 to 46322 (in 2015 and 2016, respectively) (Table 1). The median seasonal spore concentrations in 2016 was 43.6% ($P = .023$) and 86.0% ($P < .001$) higher than in two previous seasons (2014 and 2015, respectively) (Fig. 2A). Two distinct peaks in the seasonal distribution of *Alternaria* spores concentrations were observed: the first at the end of July/beginning of August and a second lower peak in the middle of September. The day-to-day variations in spore concentration were positively related to mean daily temperature (Fig. 3).

The median allergenicity of *Alternaria* spores during all seasons studied exceeded 8.6×10^{-3} pg Alt a 1/spore (Fig. 4). The highest seasonal spore allergenicity was observed in 2015 (9.8×10^{-3} pg Alt a 1/spore) and was almost 25% higher than in 2016

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Table 1. Characteristics of monitoring seasons 2014–2016 (10th of July to -19th of September).

	2014	2015	2016
Alternaria spore and allergen data			
Seasonal sum of spore (spore)	24118	14025	46322
Maximum seasonal daily spore concentration (spore/m^3)	1307	657	3766
Median spore allergenicity (Alt a 1 pg/spore)	9.3×10^{-3}	9.8×10^{-3}	7.9×10^{-3}
Variation in allergenicity, 5th–95th percentile (Alt a 1 pg/spore)	$2.3–19.1 \times 10^{-3}$	$5.2–34.7 \times 10^{-3}$	$3.7–14.5 \times 10^{-3}$
Weather data			
Seasonal mean daily temperature (°C)	18.6	19.9	18.9
Seasonal mean daily humidity (%)	71.0	60.9	70.1
Seasonal mean daily dew point temperature (°C)	13.7	11.3	12.7
Pollution data			
Seasonal mean daily SO_2 concentration (μg/m^3)	0.9	1.7	1.1
Seasonal mean daily NO_2 concentration (μg/m^3)	17.6	20.9	20.8
Seasonal mean daily CO concentration (μg/m^3)	260.9	305.9	347.9
Seasonal mean daily PM_{10} concentration (μg/m^3)	21.6	31.1	28.6
Seasonal mean daily O_3 concentration (μg/m^3)	52.7	66.2	50.6

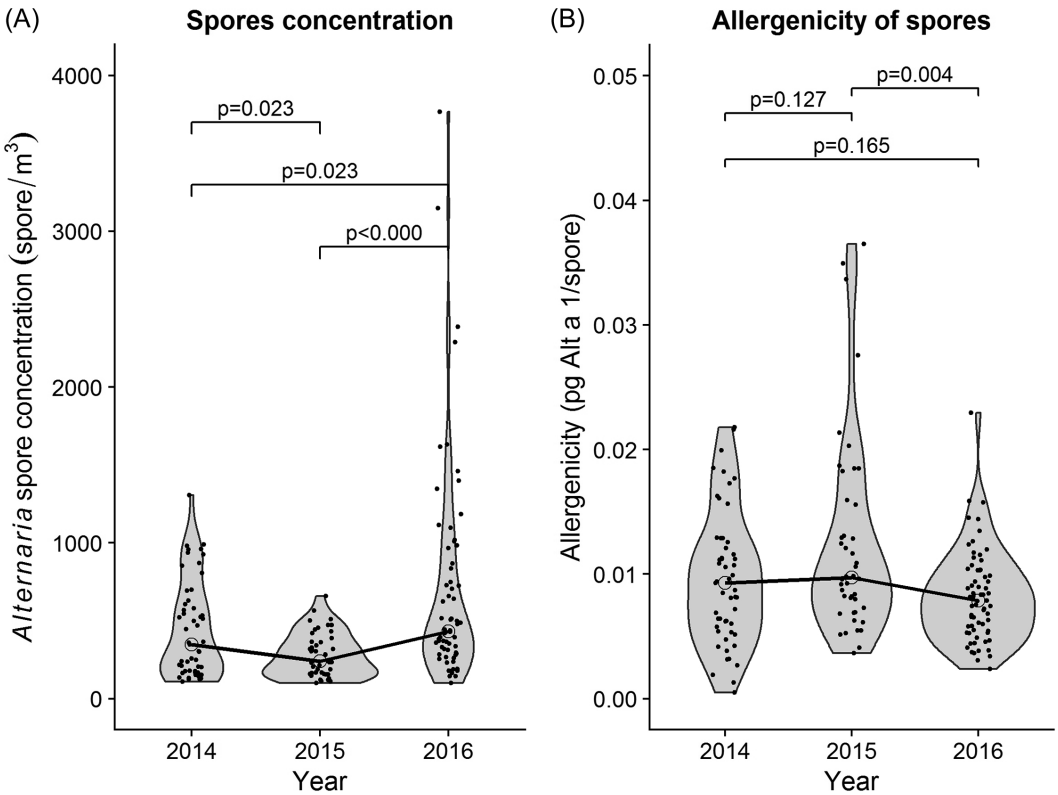

Figure 2. Comparison of seasonal sum of (A) *Alternaria* spores and (B) *Alternaria* spore allergenicity between years studied (median seasonal sum of spores and median spore allergenicity are marked and connect by black lines).

($P = .005$) (Fig. 2B). Within particular seasons, up to eightfold differences in day-to-day variability in spore allergenicity were recorded (5th–95th percentile) (Table 1). The lowest daily allergenicity was observed in the 2014 season (2.3×10^{-3} pg Alt a 1/spore), while the highest was recorded in the 2015 season (34.7×10^{-3} pg Alt a 1/spore). Daily variation in the allergenicity of *Alternaria* spores showed different pattern than seasonal variation in spores concentration. It was highlighted by negative relationship between these two variables (Fig. 3). In addition, in certain seasons, daily variations in spore allergenicity showed significant ($P < .05$) correlations with several meteorological and pollution factors, including relative humidity ($r = 0.30$), dew point ($r = 0.33$), ozone ($r = -0.33$), CO ($r = 0.32$), and PM_{10} ($r = 0.38$) levels (Table 2). To verify whether the

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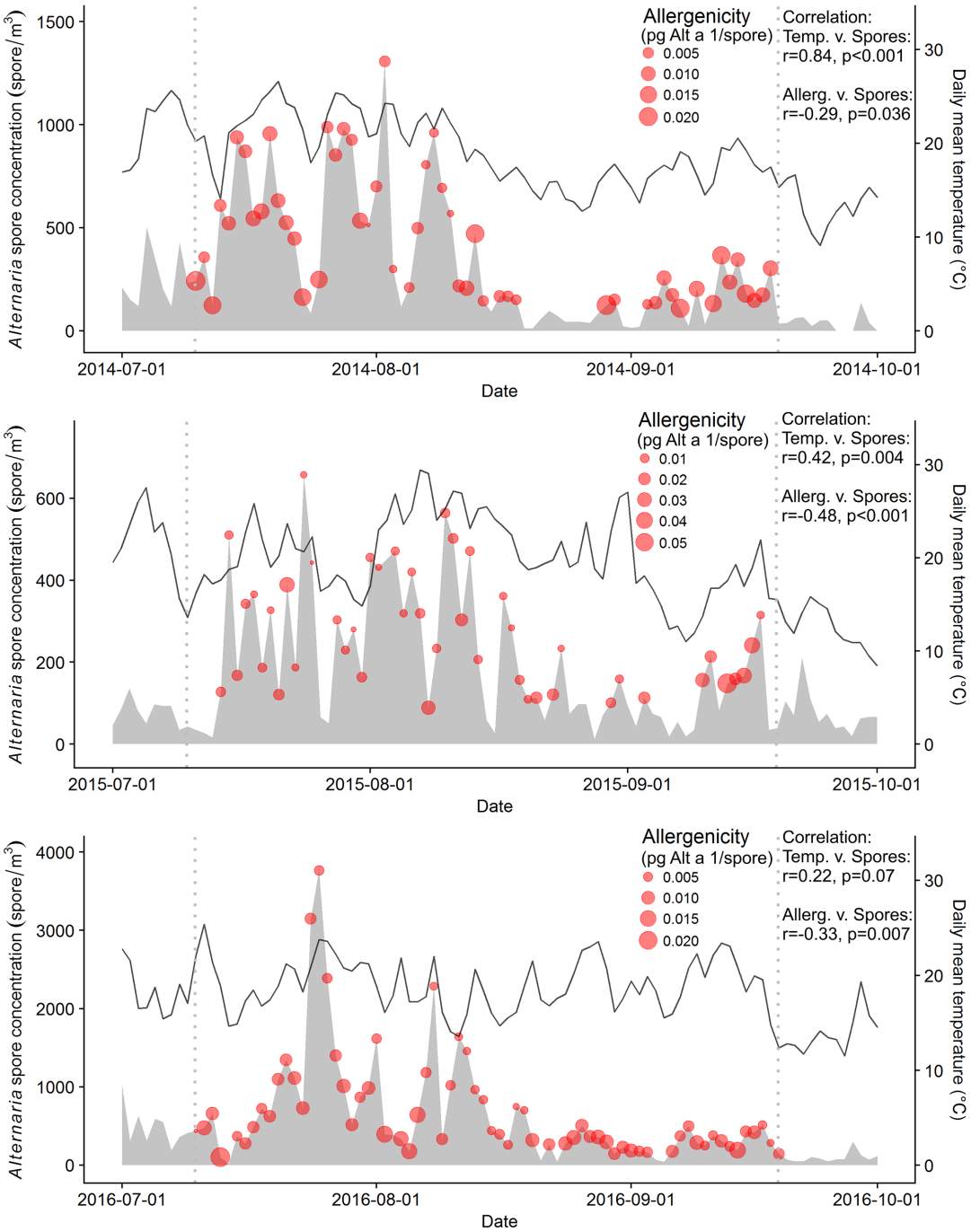

Figure 3. Daily variation in *Alternaria* spores concentration in relation to daily mean temperature during three years studied. The size of the dots represents the level of daily spore allergenicity; correlation coefficients indicate the relationship between daily mean temperature (Temp.) and daily spore concentrations (Spores), and between daily spore concentrations and daily allergencity level (Allerg.). Dotted horizontal lines represent the limits (start and end days) of allergen monitoring season. This Figure is reproduced in color in the online version of *Medical Mycology*.

direction of arriving air masses may affect spore allergenicity two episodes with the highest and two episodes with the lowest spores allergenicity has been selected: 1st episode (1–9 August 2014; potency = 4.1×10^{-3} pg Alt a 1/spore), 2nd episode (11–17 September 2014; potency -13.5×10^{-3} pg Alt a 1/spore), 3rd episode (10–16 September 2015; potency -32.9×10^{-3} pg Alt a 1/spore), and 4th episode (10–18 August 2016; potency -4.4×10^{-3} pg Alt a 1/spore) (Fig. 5). The wind rose analysis showed that during two episodes with the lowest spores allergenicity the winds arrived from western direction (SW-W-NW), while the high-potency spores were mainly recorded when winds come from eastern direction (NE-E-SE) (Fig. 6A). Differences in

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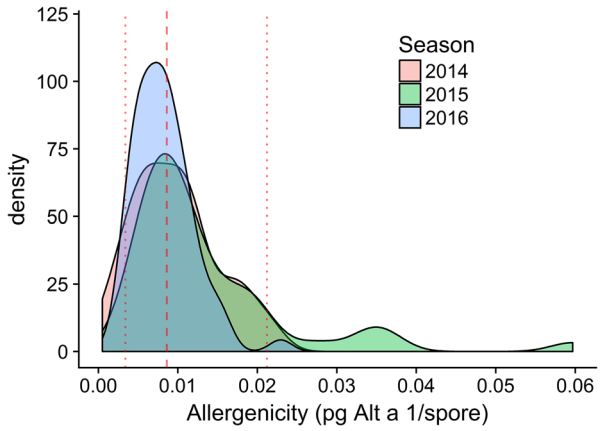

Figure 4. Frequency density of daily mean allergenicty level of *Alternaria* spores recorded during three seasons (the dashed line represents the median allergenicity level, while dotted lines represent the lower and upper range, i.e., 5th–95th percentiles, calculated for 2014–2016). This Figure is reproduced in color in the online version of *Medical Mycology*.

Table 2. Correlation between daily mean *Alternaria* spore allergenicity (pg Alt a 1/spore) and daily mean weather and pollution data (only days with spore concentrations >100 spore/m^3 are included).

Data	Factor	Seasons		
		2014	2015	2016
Weather	Daily mean temperature	−0.16	−0.12	0.18
	Daily mean humidity	0.14	**0.30**	**0.27**
	Daily mean dew point temperature	−0.02	0.13	**0.33**
Pollution	Daily mean SO_2 level	0.00	0.04	−0.07
	Daily mean NO_2 level	0.18	0.26	0.03
	Daily mean CO level	0.27	**0.32**	0.19
	Daily mean PM_{10} level	**0.38**	−0.07	0.06
	Daily mean ozone level	−0.15	**−0.33**	0.21

Significant ($P < .05$) correlations are bolded.

the allergenicity level of spores transported from western and eastern directions were statistically significant during selected episodes ($P = .012$) (Fig. 6B).

Discussion

Our study showed that the natural potency of airborne *Alternaria* spores can considerably differ between days, ranging from 2.3 to 34.7 × 10^{-3} pg Alt a1/spore. The observed variation is similar to those reported in pollen grains, where 7–17-fold differences in pollen allergen release were observed.[36–38] The allergy forecasts are solely based on the spore concentration data and miss the "varying-potency" aspect. These findings put into question the concept of clinical threshold levels, that is, the reference value of aeroallergens that are believed to evoke allergy symptoms. It is often accepted that the first symptoms of *Alternaria* occur when the number of *Alternaria* spores in the air exceeds 100 spores/m^3.[39] However, the allergenicity of spores vary, and so this value should be treated with extreme caution. The impossibility of finding a "universal" concentration at which an aeroallergen is able to induce symptoms has been already emphasized, for both spores[40] and pollen grains.[41]

The highest *Alternaria* spore allergenicity was observed in the season with the warmest temperatures, driest conditions and had the highest levels air pollution (ozone, PM_{10} and sulphur dioxide). The effect of environmental conditions on the allergenicity of fungal spores is not well studied. For instance, the impact of temperature has only been investigated with respect to *Aspergillus fumigatus* (another clinically important fungal species), showing that the increasing air temperature significantly decreased (even 12-fold) spore allergenicity.[42] However, Alt a 1 belongs to different protein family than the main allergen of *A. fumigatus* (Asp f 1).[1,19] As a result, it is not clear whether it would respond similarly to increasing temperature. In our study, no significant effects of temperature on daily variations in *Alternaria* spores allergenicity were observed. On the contrary, elevated *Alternaria* spore concentrations correlated with high daily mean temperatures, which supports results from previous studies.[32,43–45] Exposure of *Alternaria* spore to simulated sunlight was investigated by Mitakakis, O'Meara.[45] The authors found that simulated sunlight reduced the metabolic activity and germinability of spores but the proportion of allergen released remained unaffected. On the other hand, a significant impact of carbon dioxide (CO_2) level on sporulation and allergen production has been reported.[46] The study showed that elevated CO_2 concentrations increased the host plant leaf biomass and carbon-to-nitrogen ratio, and consequently higher *Alternaria* spore production. Interestingly, the allergen content per spore decreased during the experiment. The effect of air pollution on fungal spore allergenicity was, like temperature, only studied in *A. fumigatus*. The laboratory experiments revealed that the short exposure times (<12 h) of O_3 and NO_2 increase the allergenicity of *A. fumigatus* spores, while the effect weakens after longer exposure periods possible due to protein deamination.[41] In our study, several significant correlations between daily variation in *Alternaria* spore allergenicity and pollution/weather data have been observed; however, these relationships were not repeatable in every season.

Interestingly, the wind analysis revealed stable association between wind direction and the level of spore allergenicity. It was particularly noticeable when episodes characterized by the highest and the lowest spore allergenicity were investigated. The high-potency spores were recorded when air masses arrived from eastern directions, while low-potency spores were associated with western winds. The analysis of diversity and abundance of potential habitats suitable for *Alternaria* (within 30 km from the monitoring station) did not, however, showed any striking differences between west and east areas (Table S1). The agricultural fields, meadows, pastures and orchards were similarly

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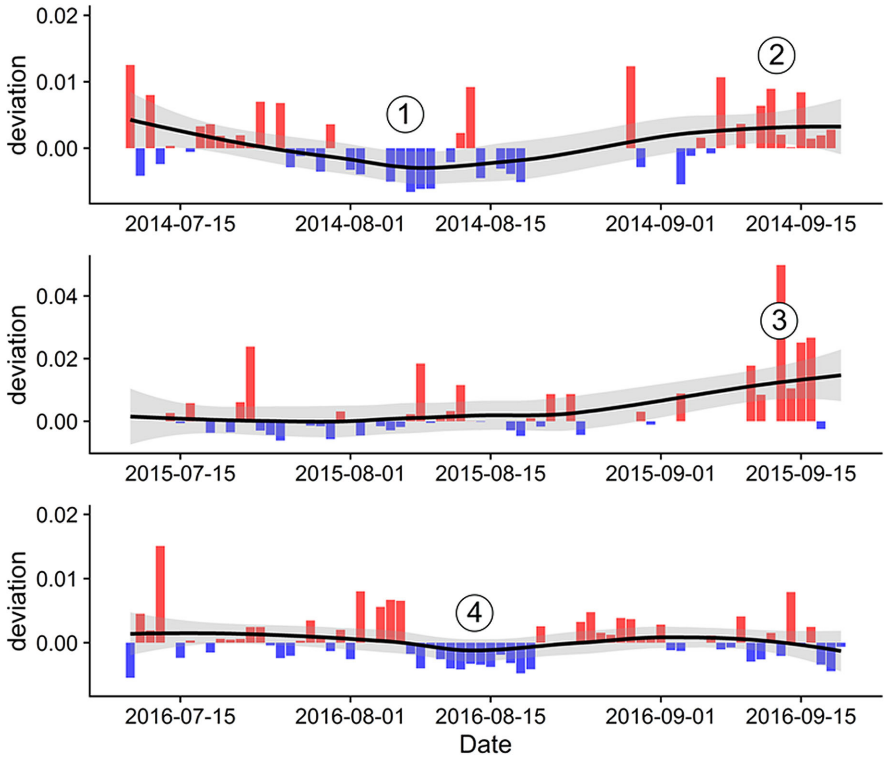

Figure 5. Deviation from the median allergenicity level in every season studied (numbers from 1 to 4 indicate four episodes with the highest (2 and 3) and the lowest (1 and 4) allergenicity of spores). This Figure is reproduced in color in the online version of *Medical Mycology*.

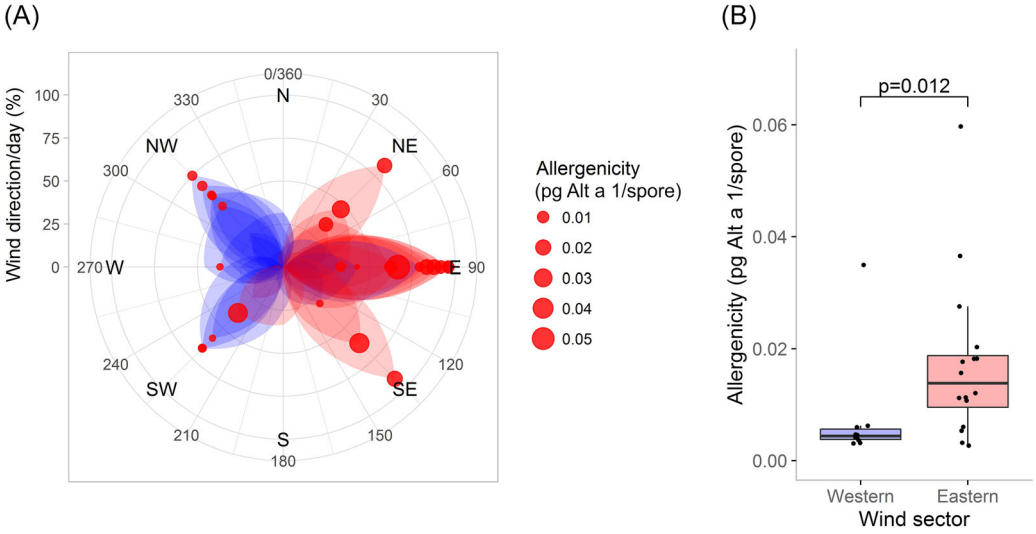

Figure 6. Wind rose analysis showing the main wind direction during four episodes with the highest and the lowest *Alternaria* spores allergenicity between 2014 and 2016 (see Fig. 5) (A). The boxplots show the comparison of median allergenicity levels of spores transported from eastern and western directions (B). This Figure is reproduced in color in the online version of *Medical Mycology*.

distributed. The most distinct differences were observed with relation to forests which were much more abundant in north-eastern site (covered by Zielonka Forest Landscape Park). Presumably, more detailed investigation of crops range and variety grown in both areas would have supplied some clue. Many *Alternaria* species are considered to be important pathogens of certain crops, for example, *A. brassicae* and *A. brassicicola* affect oilseed rape and cabbage, *A. solani* infects potato and tomato plants, while other species are saprotrophic or ubiquitous organisms, for example, *A. alternata*.[47] As the release of Alt a 1 is an important moment in plant infection process[19] the different living strategies or different host plants of pathogenic *Alternaria*

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species may hypothetically be related to the different amount of Alt a 1 produced by spores. Unfortunately, there is lack of information about the species-specific differences in spore potency within *Alternaria* genus, as well as the distribution, abundance and phenological appearance of certain *Alternaria* species. Routine aerobiological monitoring, based on spore morphology, is not able to distinguish between different *Alternaria* species, while molecular DNA-based detection and identification of airborne fungal spores is still limited.[48,49] Nevertheless, DNA-based studies are promising as it has been shown that it is possible to distinguish two morphologically similar species of *Leptosphaeria*, that is, *L. maculans* and *L. biglobosa*.[50] *Leptosphaeria* is an interesting taxa, as it is closely-related to *Alternaria,* sharing 85.8% sequence similarity to Alt a 1. The allergens released from *Leptosphaeria* spores could potentially enrich the total allergen pool measured in the air. Although *Leptosphaeria* spores are not generally counted during routine aerobiological monitoring, which is mainly focused on more clinically relevant species. With this in mind, it should be noted that other airborne fungal fragments, like hyphae or dissected spores that also contain allergens,[51] are not counted during aerobiological monitoring.

The current study is limited temporally and spatially. We have examined the allergenicity of *Alternaria* spores at just one location in Central Europe during only three seasons. However, to our knowledge, this is the first study that has investigated fungal spore potency for such a long period. The degree of daily variation was similar during the three seasons studied, which ensured that this is a natural and stable variation in spore allergenicity at this particular site. Based on previous findings derived from pollen allergen studies[36–38] we assume that high day-to-day variation in spore potency should also be expected in other geographical locations. The spatial variation in spore allergenicity should also be verified. The latest results related to allergenic pollen show distinct geographical variations in pollen allergenicity. For instance, the allergenicity of grass pollen varied from 1.7 to 4.5 pg Phl p 5/pollen (in Finland and UK, respectively), indicating the role of species distribution and geoclimatic adaptation in governing the variation in pollen allergenicity.[36] Similarly, we believe that the information about fungi distribution, biodiversity and ecological requirements are key to understanding the nature of observed changes in *Alternaria* spores allergenicity.

The health risk posed by airborne *Alternaria* allergens is a combination of at least two factors: (1) the number of spores in the air and (2) the amount of allergen per spore. This study shows that the amount of Alt a 1 released per spore may vary, especially in relation to intraseasonal fluctuations where at least several fold differences in spore allergenicity were observed. Although morphologically similar, not all *Alternaria* spores are the same. Taking into account this important phenomenon and the observation that *Alternaria*-sensitized patients reacts to outdoor Alt a 1 levels, it is recommended to include allergen measurement methods into routine aerobiological monitoring to improve overall quality of allergy forecasts.

Supplementary Material

Supplementary data are available at *MMYCOL* online.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and the writing of the paper.

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Supplemental Digital Content

Table 1S. Analysis of land cover types (sq. km) within a distance of 30 km from spore monitoring station in Poznań (see Fig. 1). Western sector is related to the appearance of low-potency spores, while eastern with high-potency spores.

Land cover types	Sectors					
	Western			Eastern		
	SW	W	NW	NE	E	SE
Urban fabric	48.75	26.70	19.70	11.06	20.17	37.13
Industrial areas	13.84	5.14	1.03	1.70	2.39	13.29
Mines	2.96	0.89	1.32	0.39	3.78	2.09
Urban greenery	15.42	2.75	1.76	2.06	2.58	5.27
Agricultural fields	192.17	239.13	226.49	123.01	201.47	233.53
Orchards	0.26	1.19	0.87	0.00	0.48	1.29
Meadows, pastures	8.69	10.13	14.39	20.79	10.43	10.38
Mixed cultivation	12.76	18.96	14.97	17.93	23.96	12.55
Forest	47.58	35.72	54.62	159.94	77.63	35.69
Mixed vegetation	2.66	1.09	12.75	6.62	2.30	0.26
Sparse vegetation	0.00	0.00	0.85	0.00	0.00	0.00
Wetlands	1.46	2.39	1.03	0.60	1.69	0.00
Waters	7.45	8.56	2.79	8.58	5.73	2.70