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ATMOSPHERIC CONCENTRATIONS OF *CLADOSPORIUM* SPP. AND *ALTERNARIA* SPP. SPORES IN ZAGREB (CROATIA) AND EFFECTS OF SOME METEOROLOGICAL FACTORS

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Abstract: The aim of the study was to analyse the relationship between meteorological conditions and *Alternaria* and *Cladosporium* spore concentrations in the air of Zagreb in August 2002 and August 2003. These months were chosen because they represented climatic extremes. A 7-day VPPS 2000 Hirst volumetric pollen and spore trap was used for spore sampling. Spores marked as 'others' (ascospores, basidiospores, *Epicoccum*, *Ustilago*, *Pithomyces*, *Helminthosporium*, *Stemphylium*, *Torula*, *Botrytis*, *Didymella*) were found to have predominated in the month of August in both 2002 and 2003 with 91.1% and 70.5%, respectively. Because of favourable weather conditions (higher air temperature and minimal precipitation) in August 2003, the concentrations of *Alternaria* and *Cladosporium* spores were 3.4-fold those recorded in the same month in 2002. Also, the peak daily concentrations of these spores were measured on days without precipitation and with higher air temperature. Intradiuranal variation in the *Alternaria* and *Cladosporium* spore concentrations was identical in 2002 and 2003 (lowest in 2-hour interval between 06:00–08:00, and highest between 10:00–12:00). In spite of the three-fold increase in the *Cladosporium* spore concentration in August 2003, the borderline concentration of 3,000 spores/m^3 air that is associated with the occurrence of allergic reactions was only exceeded on a single day. Air concentration of *Alternaria* spores exceeded borderline value of 100 spores/m^3 air on as many as 17 days, suggesting that at that time of the year the risk of allergic reaction was only present in individuals allergic to this spore type.

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INTRODUCTION

Fungal spores are an ever-present component of the atmosphere with concentrations known to fluctuate according to meteorological conditions. The distinction between dry-air spores and wet-weather air spores is well known. Dry-air spores include *Cladosporium*, *Alternaria*, *Epicoccum*, *Drechslera*, *Pithomyces* and *Curvularia* [10, 7, 16]. Members of dry-air spores are found in greatest abundance in the atmosphere characterised by low humidity, generally during warmer afternoon hours [12].

The atmospheric incidence of *Alternaria* has been the subject of numerous studies on the phytopathogenic and/or saprophytic action of this fungal genus on plants. Due to its high colonizing capacity and its ability to degrade sugars, pectins and lignin, it is commonly found on plants, paper, leather, upholstery and foods. It is therefore a typically cosmopolitan genus. In addition to

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being a plant pathogen, the significance of *Alternaria* lies in its ability to trigger respiratory diseases and allergic processes that are occasionally accompanied by bronchial asthma. All of this is favoured by its virtual perennial presence in the atmosphere, inside buildings and in home dust [1, 9], where it fosters growth of other major allergens such as dust mites [8]. Seemingly, *Alternaria* act synergistically with other fungi and Poaceae pollen, with which they share antigens on their cell walls. *Cladosporium* is a common fungus that is a known and documented aeroallergen, which is usually associated with plants, wood products and leather goods. The spores are easily made airborne and as such are a common cause of respiratory problems [14]. Sporulation and spore dispersal depend on biological, climatic and physical processes [7]. If the relationship between meteorological conditions and fungal spore concentrations could be conclusively established, this would provide a huge benefit to patients with known allergies to fungal spores. The knowledge of this relationship would allow allergy patients to take preventive measures prior to the predicted high concentrations of particular allergens, thereby reducing needless suffering.

The effect of climate on spore concentrations in this area is less well known. In this study we compared fungal spore concentrations between August 2002–August 2003 in Zagreb, Croatia, according to some meteorological data recorded in the area. These months were chosen because they represented climatic extremes, i.e. August 2002 having unusually high precipitation levels, and August 2003 being an exceptionally dry month. A specific aim of the study was to determine how the difference in rainfall and temperature between these two months affected spore concentrations.

MATERIAL AND METHODS

A 7-day VPPS 2000 Hirst volumetric pollen and spore trap (Lanzoni, Bologna, Italy) was used for spore sampling [5, 13]. The trap was placed on the 19.7-m high roof of the Grič Observatory in the centre of the City of Zagreb (45°49' N and 15°59' E, 157 m above the sea level). Zagreb is an urban area in the central part of Croatia with a population of approximately 1,000,000 and continental climate.

Melinex tape, greased with a thin layer of silicone solution was changed twice a week. The exposed tape was cut in 48-mm segments representing 24-h periods. These segments were mounted on microscope slides and stained with basic fuchsin in a gelwatol mounting medium. The slides were analysed under a light microscope, magnification ×600. Spore counts were converted into atmospheric concentrations and expressed as spores per m^3 of air.

Meteorological data were obtained from the National Weather Bureau. In our study we used the meteorological daily averaged air temperatures and daily amounts of precipitation. Temperature was measured at 3 meteorological terms (at 07:00, 14:00 and 21:00 local time) and values were averaged as weighted mean, whereas daily precipitation was measured at 07:00 and represented the amount of precipitation from 07:00 on the day before till the time of measurement. The analysis method was Mann-Whitney Test (non-parametric independent 2-group comparisons).

RESULTS

August 2002 and August 2003 were primarily chosen for the difference in rainfall and temperature (Tab. 1). In August 2002, the monthly total spore count was 92,949 and in August 2003 it was 92,020, yielding no significant difference. However, the percentage of *Cladosporium* showed a significant difference, with 7.4% (monthly total 6,813) in August 2002 and threefold that number, 24.4% (monthly total 22,682) in August 2003. A similar difference was also recorded for *Alternaria* spores. In August 2003, the percentage of these spores in the overall spore count was 3.4-fold (5.1%; monthly total 4,706) that measured in August 2002 (1.5%; monthly total 1,408). In both 2002 and 2003, the atmosphere was generally found to be predominated by the spores marked as 'others' (ascospore, basidiospore, *Epicoccum*, *Ustilago*, *Pithomyces*, *Helminthosporium*, *Stemphylium*, *Torula*, *Botrytis*, *Didymella*) rather than *Cladosporium* and *Alternaria* spores (Fig. 1). In 2002 and 2003, the recorded percentage of the former was 91.1% and 70.5%, respectively. The percentage of *Alternaria* spores (16.9% and 17.3%) *vs.* *Cladosporium* spores (83.1% and 82.7%) was near identical in the two study years (Fig. 1). In 2002, *Alternaria* spores had their monthly peaks on 9, 20 and 30 August, with a concentration range from 112–216 spores/m^3 air, whereas in 2003 peak concentrations were recorded on 4 and 6 August, with a considerably higher concentration range from 404–430 spores/m^3 air. Monthly peaks of the

Table 1. Meteorological conditions during August in Zagreb, Croatia.

Parameter	2002	2003
Temperature (°C)	21.6	25.8
Precipitation (mm)	151	17.4
Number of days with rain	15	7

Table 2. Presence of *Alternaria* and *Cladosporium* spores in August 2002 and August 2003.

	Alternaria sp.		*Cladosporium* sp	
	August 2002	August 2003	August 2002	August 2003
Monthly total	1,408	4,706	6,831	22,682
Peak day	20	4	9	4
Concentrations on peak day	216	430	935	3,244
Number of days with >100 *Alternaria* m^3; 3,000 *Cladosporium* m^3	4	17	0	1

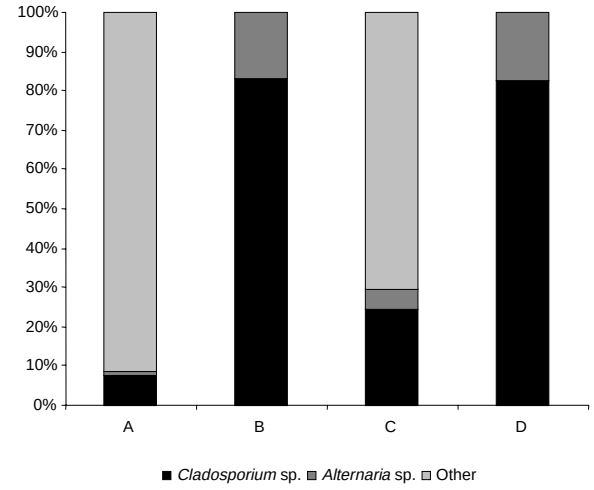

Figure 1. Percentage of *Cladosporium* spores, *Alternaria* spores and other spore types in Zagreb atmosphere: A. in August 2002, C. in August 2003. Percentage of *Cladosporium* spores *vs.* *Alternaria* spores: B. in 2002, D. in 2003.

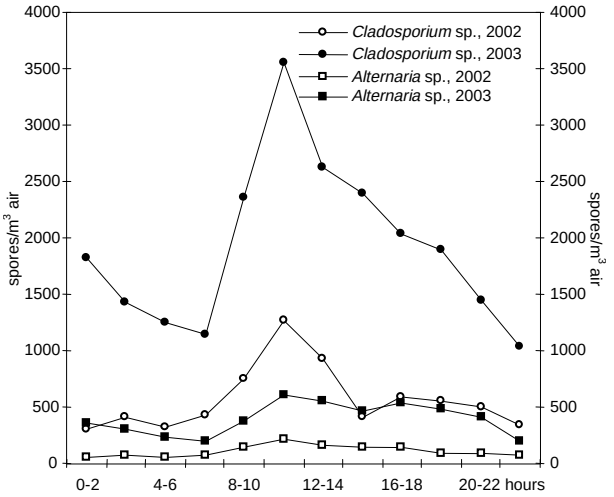

Figure 3. *Alternaria* spp. and *Cladosporium* spp. intradiurnal pattern in August 2002 and August 2003.

Cladosporium spore concentrations were observed on 3 and 9 August 2002 (715 and 935 spores/m^3 air), and on 4 August 2003 (3,244 spores/m^3 air). In August 2002, the air concentration of *Alternaria* spores exceeded the borderline level of 100 spores/m^3 on only 4 days, and in August 2003 on as many as 17 days. In August 2002, the air concentration of *Cladosporium* spores did not exceed the level of 3,000 spores/m^3, whereas in August 2003 a higher level was recorded on one day only (Tab. 2). The effect of rainfall on the *Alternaria* and *Cladosporium* spore concentrations can best be illustrated by 10 and 22 August 2002 and 16 August 2003. These days showed a decrease of spore concentrations in response to precipitation.

A strong association also existed between average daily temperature and average daily *Alternaria* and *Cladosporium* spore concentrations (Fig. 2 and 4). We found correlation between high precipitation and low *Alternaria* spore concentration ($p<0.000106$) and higher air temperature and high *Alternaria* spore concentration ($p<0.007764$). Also we showed that there are statistically significant differences between parameters (temperature, precipitation, *Alternaria* and *Cladosporium* spore concentrations) in 2002 and 2003 (Tab. 3, Fig. 5). Intradiurnal variation in the *Alternaria* spore concentrations was quite comparable between the two years. In August 2002, a rather uniform pattern of spore concentrations not exceeding 100 spores/m^3 air was observed during the night and morning hours (to 06:00), to start rising between 06:00 and 08:00, reaching maximal values between 10:00 and 12:00 (200 spores/m^3 air), then declining to below 100 spores/m^3 air by 24:00. In August 2003, the concentration of *Alternaria* spores in night hours (between 24:00–02:00) exceeded the value of 300 spores/m^3 air to decline thereafter to reach lowest value by 06:00, yet exceeding 200 spores/m^3 air. Likewise 2002, then the concentration rose to peak

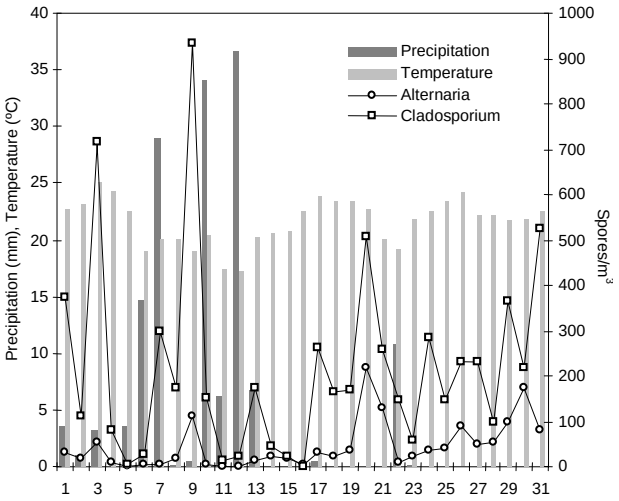

Figure 2. Daily values of some meteorological parameters and *Alternaria* spp. and *Cladosporium* spp. spore counts in Zagreb atmosphere during August 2002.

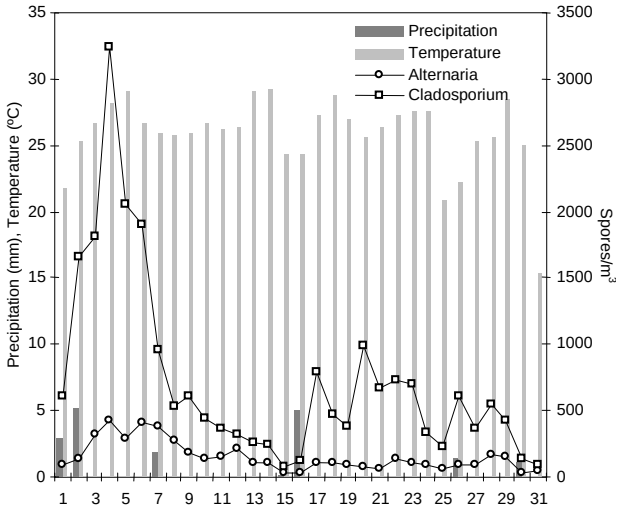

Figure 4. Daily values of some meteorological parameters and *Alternaria* spp. and *Cladosporium* spp. spore counts in Zagreb atmosphere during August 2003.

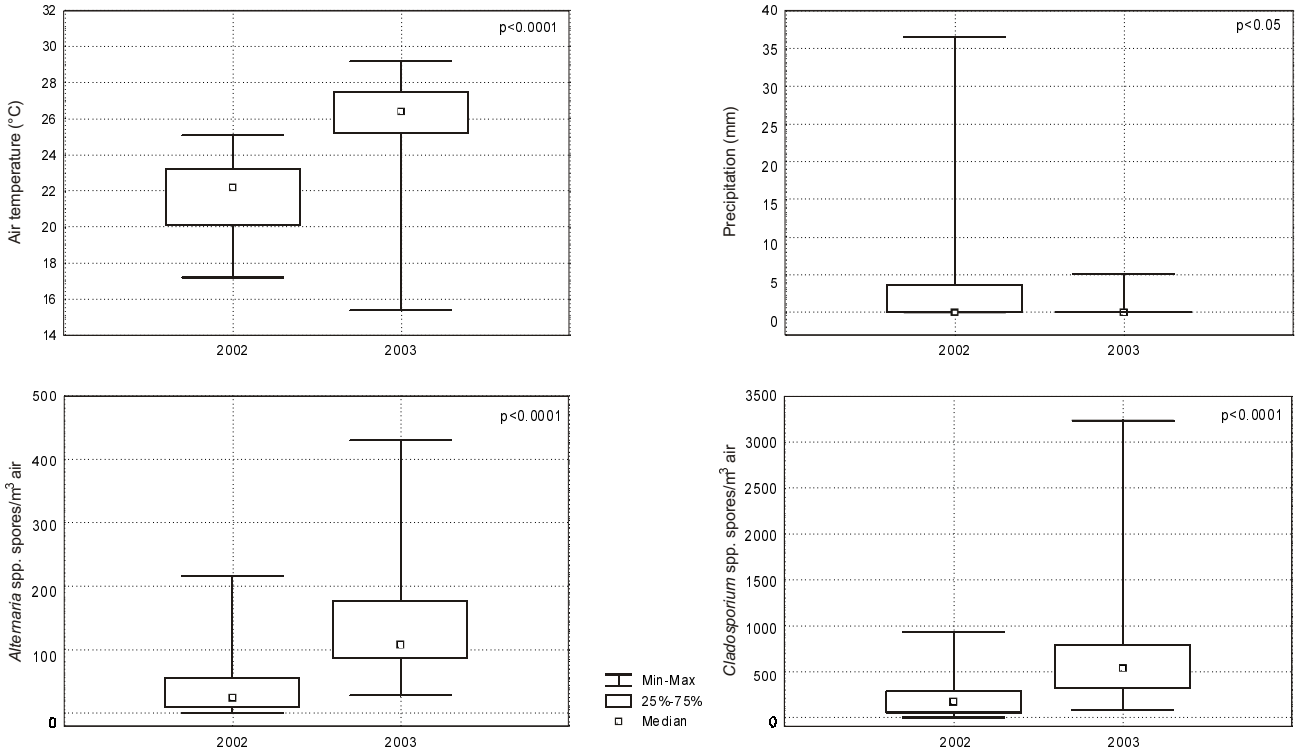

Figure 5. Differences between parameters (temperature, precipitation, *Alternaria* and *Cladosporium* spore concentrations) in Zagreb, August 2002 and August 2003. Mann-Whitney U-test.

between 10:00–12:00 (600 spores/m^3 air), then decreased abruptly by 14:00 and increased slightly to reach peak again between 16:00–18:00 (>500 spores/m^3 air). After 18:00 the concentrations decreased to reach a value of 200 spores/m^3 air around 24:00. Very similar intradiurnal concentration patterns were also observed for *Cladosporium* spores (Fig. 3).

DISCUSSION

Unlike Ankara, in Turkey, the atmosphere in the City of Zagreb in August 2002 and August 2003 was not predominated by *Alternaria* and *Cladosporium* spores [15]. In Zagreb, these spores accounted for only 8.9% (August 2002) and 29.5% (August 2003) of overall spore count present in the air, thus the results obtained for our geographical-climatological area being inconsistent with those reported by Kramer *et al.* [11] on *Cladosporium* and *Alternaria* spores to form the majority of airborne spores in the air. According to statistical analysis, the significantly higher percentage proportion of these spores in August 2003 was consequential to higher temperature and very low precipitation as compared with August 2002. Hasnian [4] found strong correlation between temperature and *Cladosporium* spore concentrations. Day to day variation in spore concentrations was also chiefly due to the effects of rainfall. A significant difference in spore concentrations before and after precipitation has also been reported [7, 11]. Rain influences air spores by removing them from the air [6]. These climatic conditions caused spores to reach maximum values in August 2003. In Ankara, maximal concentrations of *Alternaria* and *Cladosporium* spores were also recorded in August [15]. In Stockholm, Sweden, the highest daily *Cladosporium* spore concentration occurred on 29 July 1986, and highest daily *Alternaria* spore concentration on 7 August 1984 [7]. Temperature seemed to be the best predictor of *Alternaria*, because their daily concentration curve followed temperature

Table 3. Summarized results of the statistical analyse (Zagreb, August 2002-2003).

Variable	2002 Median (range)	2003 Median (range)	Mann-Whitney U Test p-level
Temperature	22.2000 (17.20000-25.100)	26.4000 (15.40000-29.200)	0.000000
Precipitation	0.0000 (0.00000-36.600)	0.0000 (0.00000-5.200)	0.022139
Alternaria	24.0000 (0.00000-216.000)	108.0000 (28.00000-430.000)	0.000001
Cladosporium	168.0000 (0.00000-935.000)	535.0000 (80.00000-3244.000)	0.000012

curve, so that highest air spore concentrations in Zagreb corresponded to highest daily temperatures in both 2002 and 2003. However, previous studies carried out by Timmer *et al.* [16] and Katial *et al.* [10] found no significant relationship between *Alternaria* and meteorological factors. Our results on intradiurnal pattern of the *Cladosporium* spore air concentration in 2-hour interval (between 06:00–08:00) are consistent with those reported for Tulsa, Oklahoma, USA, with only minor difference observed in highest spore concentrations that occurred between 10:00–12:00 in Zagreb, and around 14:00 in Tulsa [17]. The concentrations of 3,000 *Cladosporium* spores/m^3 air and 100 *Alternaria* spores/m^3 air were set as threshold values for clinical significance [2, 3]. In our research, we observed *Cladosporium* to be present in this amount on only one day in August 2003. *Alternaria* spores exceeded their borderline value on only 4 days and 17 days in August 2002 and August 2003, respectively, suggesting that the risk of allergic reactions in that time of the year is only present for individuals allergic to *Alternaria* spores.

CONCLUSIONS

• The 3.4/3.3-fold increase in the air concentrations of *Alternaria* and *Cladosporium* spores in August 2003 as compared with August 2002 was consequential to high air temperature and minimal precipitation in 2003.

• Day to day variation in *Alternaria* and *Cladosporium* spore concentrations was also chiefly due to the effects of rainfall. A significant difference in the spore concentrations was recorded before and after precipitation.

• Temperature can induce the presence of *Alternaria* spores, as the curve of their daily concentrations corresponded to temperature curve.

• Intradiurnal concentrations of *Alternaria* and *Cladosporium* spores were lowest between 06:00–08:00, and highest between 10:00–12:00.

• In August 2003, the concentration of *Cladosporium* spores exceeded the borderline concentration of 3,000 spores/m^3 air (potential to trigger allergic reactions) on one day only. The concentrations of *Alternaria* spores exceeded the borderline value of 100 spores/m^3 air on as many as 17 days.

• Accordingly, in August 2003, only individuals allergic to *Alternaria* spores were at a risk of allergic reaction.

• These conclusions have been made on the basis of 1 month during 2 years; future studies during a longer period will be necessary to gain a clearer insight into relationship between weather and spore concentration in the air.

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