AIRBORNE *ALTERNARIA* SPORES IN FOUR MONITORING STATIONS FROM ROMANIA IN 2005

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A study of the aerobiology of fungal spores is of direct relevance to the medical community due to their role in respiratory diseases such as asthma, allergic rhinitis, chronic bronchitis, and hypersensitivity pneumonitis. The spores of the mitosporic-fungi Alternaria and Cladosporium are some of the most abundant allergens world-wide and the prevalence of allergical reactions to them shows great interregional variations. Alternaria species are parasites or saprophytes on plants and are widely distributed. Alternaria alternata is considered to be one of the most important aeroallergens. Because of its importance to human health, many studies have examined Alternaria and in conjunction with other fungal spores or with pollen The aim of our study was to determine the concentrations of Alternaria spores in four urban areas in Romania: Braşov, Bucureşti, Craiova and Timişoara. This investigation is the first for România. The sites differed in habitat characteristics, such as urbanisation level, vegetation and microclimate. In this study, a volumetric method and a Lanzoni sampler were used. The daily and maximum hourly concentrations in spores/m^3 of Alternaria were recorded. The spore is present in large numbers throughout the summer. The higher fungal spore concentrations occur in Craiova. The recorded levels of airborne spores in Bucureşti and Craiova were close the concentrations that are accepted as threshold levels for provocation of clinical responses. The Alternaria conidia is a large spore which is easy to identify, perhaps daily spore concentrations should be made more readily available to clinicians and the general public in the România.

***Key words**: Alternaria, allergenic conidia*

Alternaria is largely a soil-borne fungus that lives on rotting organic matter including wood, leaf debris, and grasses. The amount of this fungus is related to the amount of dead vegetation available for it to consume, so its seasonal pattern reflects that of the local vegetation [1]. Currently, the study of *Alternaria* is very interesting both from an aerobiological and from a clinical point of view, due to the infecting and allergenic power of their conidia. The species of this genus are present in many cities, and though quantitatively it does not release the greatest numbers of propagula into the air, its qualitative importance is beyond question. From this point of view, Kim et al. (1996) consider *Alternaria* as an important fungal antigen; Cosentino et al. (1995) point to *Alternaria* as the fungus which produces the highest number of positive skin tests, followed by *Cladosporium*, *Aspergillus* and *Candida*; Tarik et al. (1996) and De Blay et al. (1996) show a

positive correlation between sensitization to this genus and the symptoms of asthma, eczema, and rhinitis; and Caretta (1992) describes it as being responsible for most respiratory allergies to fungi.

Alternaria and *Cladosporium* have been identified as the most abundant and frequent airborne allergens in several cities and their allergenic capacity has been reported by numerous authors [3; 28; 5; 8; 2]. Since the sporulation process and spore dispersion of fungi are greatly dependent on the climatic conditions in each area, many authors have correlated meteorological parameters with the presence and prevalence of airborne spores in the atmospheres of climatically different locations. The content of *Alternaria* spores in the atmosphere increases when maximum, mean and minimum temperatures rise and there are more hours of sunshine; these factors (together with relative humidity) played a major role in the dispersion of *Alternaria* [26].

Zureik et al. (2002) suggested fungal spores may be inhaled as fragments and other amorphous bio aerosols, and the fragmentation of *Alternaria* observed by Corden and Millington (2001) is likely to be increased during combining or mowing, making entry into the lung relatively easy, although Licorish et al. (1985) suggested that with vertical presentation the spore had a diameter small enough to allow it to easily enter the lungs. Fewer people are allergic to fungal spores than to pollen, but as mould spores have been implicated as a major risk factor for fatal asthma attacks in USA [30]. The effect of climate change and associated arable production should not be ignored. In Europe, the interest in the spores of this taxa, as allergenic agent, has been very variable in different countries. In the North of the continent their behaviour is well described, because of the high number of allergy symptoms they produce and their socio-economical consequences[14]. In the Mediterranean region, studies of the seasonal variations of *Alternaria* and *Cladosporium* have increased in the last few years [23; 10].

MATERIALS AND METHODS

The concentrations of airborne *Alternaria* spores from four urban areas in România (Braşov, Bucureşti, Craiova and Timişoara) were investigated. Lanzoni traps were used. All slides identified in this study are kept at West University, Department of Biology in Timisoara (România). All spore counts were obtained daily (7 days/week) at our institution during 15 May to 15 July 2005. Slides were covered with glycerine jelly mixed with basic fuchsin. The slides were examined daily under the light microscope. The numbers of spores found in the glass area were counted. The specific fungal spores were counted with ×400 magnification and the counts corrected to number of particles/m^3. The identification and counting of spores were limited to genus levels.

RESULTS AND DISCUSSION

For over 70 years it has been known that spores of the genus *Alternaria* cause asthma [15; 11; 20; 24; 31]. Last (1955) found high concentrations of *Alternaria* spores within the crop of a wheat field and Sreeramulu (1958) described a four fold increase in the concentration of *Alternaria* associated with mowing

grass. Also McCartney & Lacey (1992) found *Alternaria* spores were released from an oilseed rape crop in the daytime and Corden and Millington (2001) observed that bursts of *Alternaria* occur during periods of grass mowing, and at harvest time, especially when the weather has been warm and dry. As *Alternaria* is a saprophyte living on most vegetation, the volumes of dust generated by combine harvesters will include many *Alternaria* spores which will be dispersed over a wide area. Hyde & Williams were investigating *Alternaria* levels in Cardiff and they postulated that asthma attacks were more likely in the late summer especially near wheat fields or wheat threshing. In the USA the greatest number of *Alternaria* spores are found in grain growing areas such as the Midwest [24] and children in Australia had greater *Alternaria* sensitization when they lived in the drier inland regions [25], whilst in rural Australian towns *Alternaria* spore levels were directly influenced by spore concentration in the wheat crop outside the towns [22].

The totals of *Alternaria* spores in the air recorded in this study show a considerable variation. The mean spores count obtained during the period studied was 2925.4 spores /m^3, the lowest value being recorded in Timişoara with 642,6 spores. The highest level of conidia emission was recorded in Craiova with 5700,6. The daily mean concentrations of *Alternaria* spores fluctuated between 10.36 - 91.95 spores/m^3. The mean number of *Alternaria* conidia isolated in the study period was quite similar to that shown by Li and Kendrick in Ontario (61.6 conidia/m^3) and by Munuera and Carrion in Murcia (31 conidia/m^3). The highest concentration of *Alternaria* spores, equal to 674.9 spores /m^3/24 h was noted in Craiova for 9 July. Daily concentrations were very low in Braşov and Timişoara, only exceeding 20 spores/m^3 on a few occasions. Only few days (18 days in Craiova and 14 days in Bucureşti) the concentration of *Alternaria* exceed the 100 spores/m^3 of air established by Gravesen as the critical value for symptomatology. The literature has few references to the number of spores that are needed to provoke an allergic reaction in susceptible individuals [29]. There is considerable variation between those numbers that have been presented. One estimated threshold concentration of *Alternaria* spores needed to provoke an allergic response is 100 spores/m^3 of air [12]. Licorish et al. (1985), however, suggest that of the order of 104–107 spores need to be inhaled over a 24-h period. Hasnain et al. (1998) suggest that a mean daily concentration of 50 *Alternaria* spores/m^3 are needed to cause sensitisation, after which smaller concentrations can cause symptoms. Frankland and Davies (1965) suggested that when the *Alternaria* count reaches 50 spores/m^3 or above, patients sensitive to *Alternaria* develop symptoms. Targonski et al. (1995) suggested that the risk of asthma related deaths was increased 2.16 times when the total mould countwas 1000 spores/m^3 or above, therefore if daily levels of *Alternaria* spores are sometimes over 1000 then as *Alternaria* is known to be a high risk factor for severe asthma with resulting hospitalization [24].

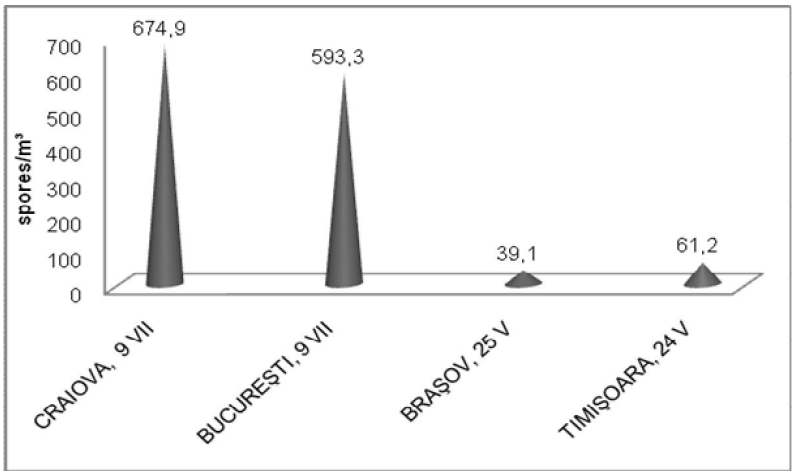

Figure 1 **The peak daily concentration of *Alternaria spores***

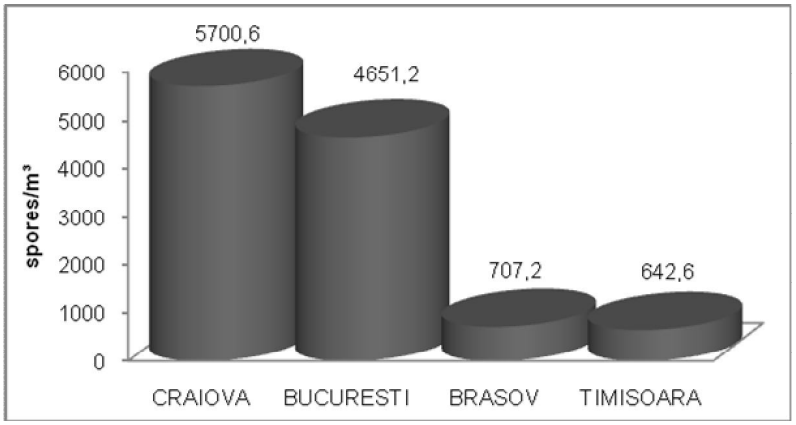

Figure 2 **Totals of A*lternaria* spores in the air recorded in four monitoring stations from Romania in 2005**

Daily distribution of airborne *Alternaria* conidia presents a minimum of conidia at 24.00–12.00 hours and a maximum at 14.00–16.00 hours (Braşov, Timişoara) and 18.00-22.00 hours (Craiova, Bucureşti). This distribution, with only one daily peak, coincides with that Hjelmroos (1993) detected for Stockholm, although not at the same time of day. *Alternaria* has an optimum growth when mean temperatures are between 22 and 28º C. As for the circadian cycle, it reflects the sensitivity of this fungi to weather fluctuations. Maximum values are found when relative humidity is between 30 and 50% and lowest values are found in the night, when relative humidity is between 65 and 80%. The same periodicity was found by Srivastava and Wadhwani, 1992; Hjelmroos, 1993; Fernandez et al., 1998.

Licorish et al. (1985) suggested that with vertical presentation the spore had a diameter small enough to allow it to easily enter the lungs. We find evidence microscopically of *Alternaria* spores broken into fragments with all or part of the distal portion sometimes separated from the rest of the spore. If these spores are

broken up, as could easily happen during combining or mowing, then lung entry by spore fragments would be relatively easy. *Alternaria* spores vary in size but Licorish et al. (1985) said it could be safely assumed that even the larger *Alternaria* spores could penetrate the lungs causing asthma. Because *Alternaria* is found in the air at the same time as grass pollen and manymore people are allergic to grass pollen, is the possibility of *Alternaria* being the culprit sometimes missed? A closer look at a patient's clinical history, or a rather longer period of seasonal symptoms, might indicate *Alternaria* sensitivity as suggested by Buisserett (1976).

CONCLUSIONS

Alternaria is found everywhere, it is an extremely common saprophyte found in the soil, on plants, and in the air. Sporulation and spore dispersal depend on biological, climatic and physical processes. *Alternaria* is one of the most common atmospheric mould spores found in the România, the greatest numbers being found in Craiova and Bucureşti areas. These differences could be due to not only meteorological factors, but also local factors which seem to have a great influence on the distribution of spores in the air.

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