AEROMYCOLOGICAL MONITORING OF CLADOSPORIUM SPORES IN TIMISOARA

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ABSTRACT (online version)
Cladosporium is reported to be the most common airborne fungus in temperate zones. These spores were chosen for study on the basis of their dominance in fungal aeroplankton populations and documented involvement in triggering respiratory allergy symptoms. Generally, they were present at higher concentrations in late spring and summer, than in autumn and winter. Based on our results, clinicians should consider the spore type described herein as a possible cause of severe asthma attacks during periods of warm, dry summer weather. During the 2 months of this study the Cladosporium spores count exceeded the threshold value in Timisoara for one day. The maximum value for Cladosporium was observed on June 20, 2008 (3134.8 spores/m³). The intradiurnal variations recorded during the monitored period have been studied. The present study will contribute to our knowledge of airborne spores in Timisoara.

KEY WORDS: Cladosporium, mycoflora, aeromycological monitoring

INTRODUCTION
Aeromycology is a scientific discipline focused on the transport of airborne spores in outdoor and indoor environments. Fungal spores comprise the greatest portion of suspended biological material in the air (Salvaggio & Aukrust, 1981). The source of fungal spores and hyphal fragments is often unknown (Gregory, 1973), unlike that of pollen grains, which are easily traced back to their plant species.

During the last decades, allergic diseases have been constantly increasing in incidence and prevalence and consequently an increasing attention was paid to aeromycological monitoring of fungal spores in various parts of the world. Due to the increasing awareness of the relationship of airborne fungi to allergies in patients with asthma, many biologists and allergists have begun to examine the distribution and type of fungal spores and conidia in both indoor and outdoor environments (Miller, 1992). Allergy is defined as the exaggerated response of the immune system to foreign proteins (Blumenthal & Rosenberg, 1999). In certain atopic individuals, the sensitivity to airborne fungi is manifested as bronchial asthma, and the allergens are usually the airborne conidia of common soil fungi and those that live on the surfaces of leaves (phylloplane fungi). Sensitivity may also develop in normal individuals who are chronically exposed to the conidia of certain fungi at work, resulting in such disorders as farmer’s lung and teapicker’s disease (Rippon, 1982). Since only select individuals develop allergy in spite of the extensive exposure to allergens, evidence for genetic predisposition for development of atopic disease in these patients is likely (Kurup et al, 2000). It has recently come to the attention of the scientific community that fungi and their
spores are associated with indoor air quality problems. Also, fungal contamination in indoor environments has been shown to produce allergies in occupants of these buildings - sick building syndrome (McGrath et al., 1999). A large number of airborne fungi have been found to be responsible for the deterioration of organic material in indoor environments. Inside food grain godowns the airborne fungal spores get settled on stored bags, grains and walls. During loading, unloading and cleaning of such godowns these spores are dispersed in the air (Zahid et al., 1997). In libraries, books and periodicals get deteriorated, discoloured and damaged due to various fungal types. Deterioration of library materials has caused great concern to workers at different places (Gallow, 1963; Gutcho, 1974). Allergic symptoms in bakers have been found to be associated with moulds also (Singh & Singh, 1994). Fungi also causes heavy loss to bakery products and raw materials (Jain, 2000).

The characteristic features and size of the spores determine how deep they may penetrate into the respiratory tract, whereby the exact site of allergic response can be determined. Spores larger than 10 µm diameter are deposited in the nasopharynx causing rhinitis; spores smaller than 5 µm penetrate to the alveoli causing alveolitis. Spores <10 µm size mostly deposit in the bronchi and bronchioles causing asthma (Lacey et al., 1972). After inhalation, the spores release some biochemical reactions which induce the synthesis of specific IgE antibodies causing clinical symptoms of hypersensitivity in the form of respiratory allergic disease (Barui Chandra & Chanda, 2000). The transport and ultimate settling on the surfaces is affected by: the physical properties of particles and/or of droplets (size, density and shape); the environmental parameters and the bioreceptivity of the surface itself and nutrient availability (Urzì & Realini, 1998).

Cladosporium species live as saprophytes or as parasites on many kinds of plants. Many surveys of the occurrence of Cladosporium spores in different regions of the world clearly show their dominance in comparison with other spores (Mitakakis et al., 1997). Studies on the presence of spores in the atmosphere of Timisoara were started by Ianovici & Faur in 2001 using Lanzoni sampler (Ianovici & Faur, 2003). They found that the dominant air fungal microflora consist of Cladosporium and Alternaria. The above communications report results which revealed the presence of various types of fungal spores in the environment of Timișoara which is in accordance with results reported for other countries. The monitoring of fungal spores in Timisoara revealed the summer as the most favourable season for Cladosporium occurrence. Cladosporium type can be categorized as dry-air spora. Cladosporium belongs to the psychrophilic group of microorganisms which require low temperature, sometimes below 0°C, with an upper limit about 20°C and relative humidity about 70% for optimal growth (Shaheen, 1992; Stepalska et al, 1999). The aim of this study was to characterize the aerobiological behavior of airborne spores from Cladosporium in the city of Timisoara (Romania) for June.

**MATERIAL AND METHOD**

The study was conducted in Timisoara, Romania, in 2007-2008. Airborne spore was performed using a 7-day Hirst-type volumetric trap (VPPS-2000, Lanzoni) set on the roof of the West University in Timisoara, approximately 20 m
above ground level, and calibrated to sample air at 10L/min. The sampling site is above the adjacent buildings, and the circulation of the air currents was unobstructed in all directions. Sampler drums were changed weekly. The tapes were cut into strips 48 mm long, each representing 24 h exposure. Before exposure, the slides were covered with glycerine jelly mixed with basic fuchsin. The qualitative and quantitative compositions of the samples were determined under a light microscope. Spore counts were done at 2-h intervals and total daily counts were converted to numbers per cubic metre of air. The identification and counting of spores were limited to genus levels. All slides identified in this study are kept at West University, Department of Biology in Timisoara (Romania). Grant Smith (Sampling and identifying allergenic pollens and molds – An Illustrated Identification Manual for Air Samplers, 1990) was used as reference book for the identification and description of the fungal spore types.

RESULTS AND DISCUSSIONS

Species of Cladosporium are the most common saprophytic fungi living on organic materials worldwide. They produce dry spores 3-5 µm in size depending on species, which can be easily spread in the air by wind. Earlier studies on airborne mycoflora in Timisoara identified Cladosporium species as the dominant airborne fungi. The dominance of this genus in comparison with the other spores analyzed has been observed in many locations, including Denmark (Larsen & Gravesen 1991), Spain (Infante-Garcia-Pantaleon et al. 1992), Italy (Cosentino et al. 1990; Filippello Marchisio et al. 1997), Austria (Ebner et al. 1992), Sweden (Hjelmroos 1993), Finland (Kurkela, 1997), Poland (Grinn-Gofron & Mika, 2008), Croatia (Šegvić Klarić & Pepeljnjak), Jordan (Shaneen 1992), India (Chakrabatory et al. 2003), Canada (Li & Kendrick 1994) and Australia (Mitakakis & Guest 2001).

The taxa analyzed belong to the class of Deuteromycetes. Cladosporium airspores were found to be present regularly (frequency 100% of days). Concentrations of Cladosporium spores in the research station are shown Fig. 1-2. The dynamics of the spore season was very similar in each of the 2 years of the study period.

In June there was an small increase in spore concentration. The highest daily Cladosporium spore concentration during 2007 was counted on June 17. In this month the highest daily count of Cladosporium was 3444,2/m³. The maximal daily concentrations of Cladosporium spores during 2008 were recorded on June 20 (3134,8/m³). A total of 26158,59 spores from Cladosporium in 2007 and 29245,1 in 2008 were identified in the atmosphere of Timisoara. High temperature when combined with a sufficient amount of precipitation seemed to optimize the sporulation conditions for Cladosporium spores. The level of the spore count for Cladosporium was high in terms of number of spores.
The concentration of 3000 spores/m³ of air was determined as threshold value for clinical significance (Bagni et al., 1977). A potential allergen threat occurs when the concentration of *Cladosporium* spores reaches 2800 per 1 m³ of air (Rapiejko et al. 2004). In our research we observed *Cladosporium* to be in these amounts in the Timisoara air for 1 day for month.

The intra-diurnal variations recorded during the monitored period have been studied. The value for each hour is calculated by dividing the sum of the values of each hour by the number of days in which *Cladosporium* was present. Intradiurnal concentrations of *Cladosporium* spores were lowest between 20:00–4:00 and highest between 10.00-12.00. Intradiurnal concentrations of *Cladosporium* spores were highest in 2008 (113,79 spores/m³) (fig.3).
Figure 3. *Cladosporium* intra-diurnal patterns (2-h running mean)

It is not certain that all variation in spore concentrations can be explained by meteorological variables only. Spore concentrations may also depend on the state of the host and the weatherings on the host plants. The increase in fungal spore concentrations also be related to the maturing of tree foliage, grasses and local crops. Further, more extensive studies are necessary in order to evaluate the effects of local vegetation and climate on the fungal flora (Grinn-Gofron & Mika, 2008).

**CONCLUSIONS**

Our study examined only part of microfungal flora in a selected area. *Cladosporium* spores which could be detected in high numbers in the atmosphere of Timisoara, may cause asthma and allergic rhinitis in susceptible humans. We determined an increase in the total number of spores in 2008.

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