PRELIMINARY SURVEY OF AIRBORNE FUNGAL SPORES IN URBAN ENVIRONMENT

Nicoleta IANOVICI[1]

[1]West University, Timişoara
e-mail: nicole_ianovici@yahoo.com

Owing to their ubiquitous presence in nature, fungal spores represent an inevitable allergen source and are included among the most important agents responsible for provoking allergic manifestations. There have been many investigations in various parts of the world to determine the presence and sources of allergenic fungal species and to evaluate their seasonal variations. The airspora concentrations of outdoor environments depend on numerous factor including: time of day, meteorological factors, seasonal climatic factors, type of vegetation and human activity. The airborne spores from Timisoara city were investigated in 2006. Atmospheric samplings were conducted using Lanzoni VPPS trap. We have identified the following types of fungal spore with allergenic effect: Cladosporium, Alternaria, Epicoccum, Helminthosporium, Stemphylium, Curvularia and Fusarium/Leptosphaeria. The study showed that the most prevalent (major components) fungal spores in the air of Timisoara were those of the genera Alternaria spp. and Cladosporium spp. 57 spores with sporadic appearances were minor components of airplankton. Further research will focus on producing a more detailed calendar showing the incidence and types of fungal spore genera present in the Timisoara atmosphere, and to the level of mould spores in outdoor environments. The seasonal patterns of spore types follow the life cycle of the local vegetation. The present study will contribute to our knowledge of airborne spores in România. Such studies are useful for clinicians and their patients who are allergic to fungal spores.

***Key words**: fungal spores, aeroallergens*

Fungi are members of a distinct kingdom of life: eukaryotic, spore-producing, achlorophyllous organisms with absorptive nutrition that generally reproduce both sexually and asexually. They usually have filamentous branched somatic structures, known as hyphae, that typically are surrounded by cell walls [20]. Fungi are known to be ubiquitous in nature, growing where organic material is available. The release of fungal spores and consequently their concentrations in the atmosphere are the result of the action of many biological and environmental factors. Occurrence of fungal spores in the air is markedly seasonal because of their sensitivity to weather changes [9;4;13;5]. Surveys of spore concentrations have been carried out in many places in the world [12;19;24;9;6;2;14;17;22]. The role of fungal spores in producing respiratory disease was already suggested by the late 1800s when Blackley described chest tightness sympton associated with *Penicillium*. Even though they represented only a small proportion of the airborne particles present in the atmosphere, spores can be the cause of allergic responses in susceptible humans, and pollinosis is now a general public health problem. Outdoor spores are mainly present on decaying leaf materials, dung, straw, grass piles and in damp areas and they are spread into atmosphere during some activities performed by using grass cutting machines, rakes and plows. Therefore, sensitive people had better keep themselves away from such activities, keep their doors and windows closed and use filtered air conditions during risky months [8]. The aim of this study was to identify as much spore types as possible in our region.

MATERIALS AND METHODS

The sampling station for all aeroallergens is located on the roof of West University of Timişoara, România. Airborne spores were monitored from January 2006 through to October 2006. The slides were examined daily under the light microscope. 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 DISCUSSION

Fungi are among the most common organisms in nature. Therefore, fungal spores are abundant in the indoor and outdoor atmospheres, and are inescapable in any frost-free environment [23]. The fungi have a great capacity to colonise every type of substrate and to grow in extreme environmental conditions. It is therefore usual to find a great quantity of spores in the atmosphere. Fungi have a short asexual reproduction cycle taking a few days and they produce vast numbers of spores. The most studied and understood group of fungi is the anamorphic fungi. Many taxa of this group have a distinctive appearance, making identification possible, at least to the genus level [22]. During the last decades, allergic diseases have been constantly increasing in incidence and prevalence and consequently an increasing attention was paid to aerobiological monitoring of airborne fungal spores in various parts of the world [7]. The range of the fungi in outdoor air in the present study is almost the same as those previously reported in many European and North American countries and Israel [1; 14; 2]. The most abundant airborne spores are *Cladosporium, Alternaria, Epicoccum, Helminthosporium* type*, Fusarium/Leptosphaeria* type*, Stemphylium* and *Curvularia.* These represent a group of taxa of cosmopolitan fungi that can exploit virtually any organic substrate.

Cladosporium Link 1816 *is* an important component of the airborne population of spores, and is known to be allergenic [15]. In Denmark, the weekly clinical symptoms and medication scores were positively correlated with airborne spore counts of *Cladosporium* [16]. Many authors emphasise that *Cladosporium* is cosmopolitan and colonises a variety of substrates. It is also capable of growing on refrigerated foods. Its spores occur in abundance in the air of many geographical regions. Spores of the genus *Cladosporium* are from 2-12 to 3-21/µm, ovoid to cylindrical or irregular olivaceous-brown to hyaline and non septate or with one to three septa spores. They are smooth or verrucate. They may be collected as simple spores or in pairs but are more frequently seen in groups of four or five spores. *Cladosporium* spp. are causative agents of skin lesions, keratitis, onychomycosis, sinusitis and pulmonary infections. *Cladosporium sp.* is the common cause of allergenic health effects such as Type I allergies and Type III hypersensitivity pneumonitis [26, 21; 25].

Alternaria Nees 1817 is known to be one of the main genera that are implicated for respiratory allergy [3]. Alternaria is a common pathogen of cereals, vegetables and weeds [10]. *Alternaria* spore type shows great morphological variability. Generally, conidia are muriform, several celled with traverse and longitudinal septa, straight, dark, solitary or in chains of two or few conidia, with smooth to minutely verrucose surface. It is known to be allergenic and can cause Type I allergies and Type III hypersensitivity pneumonitis. It may also cause nasal and subcutaneous infections in immunocompromised individuals [26; 21].

Epicoccum Link 1815 is commonly found on dead parts of numerous plants, seeds and soft fruits [10]. *Epicoccum* is widely distributed and commonly isolated from air, soil and foodstuff. It is found also in some animals and textiles. Young conidia are round, nonseptate, and pale in color. Mature conidia (15-25 μm in diameter) are rough, verrucose to warty, and brown to black in color. Besides, mature conidia contain multiple transverse and vertical septa and have a funnel-shaped base and attachment scar that is formed from aggregated conidiophores on the sporodochium. The most common health effects are those that cause allergies such as Type I allergies [26; 25].

Helminthosporium type spores included phragmospores of the genera *Helminthosporium* Link 1809, *Drechslera* S. Ito 1930, *Bipolaris* Shoemaker 1959, *Exosporium* Link 1809, *Exserohilum* K.J. Leonard & Suggs 1974 and *Sporidesmium* Link 1809, that were included in the same group because of the difficulty of differentiating them using a light microscope. Conidia are cylindrical with rounded ends or subcylindrical, curved, straight or ellipsoidal, 50-170/μm long, hyaline to dark brown, sometimes with the cells unequally coloured, the end cells being paler than those intermediate. They show from one to 12 pseudosepta or septa and are mostly smooth, rarely verrucose. They frequently have a prominent, dark brown scar at the base. The most common health effects are allergenic and include Type I allergies. The clinical spectrum is diverse, including keratitis, endophthalmitis, endocarditis, endarteritis, osteomyelitis, meningoencephalitis, peritonitis, otitis media and fungemia as well as cutaneous and pulmonary infections and allergic bronchopulmonary disease. Can infect both immunocompetent and immunocompromised host. As well as being isolated as saprophytes on plants, *Helminthosporium* type may be pathogenic to Poaceae and also to animals, such as the dog [26, 21; 25].

The *Fusarium/Leptosphaeria* group type included spores of deuteromycetes and ascomycetes with great morphological variability. *Leptosphaeria* Ces. & De Not. 1863 is brownish, multiseptate, slightly curved spores with one cell larger than the others. This type included spores of *Melanomma* Nitschke ex Fuckel 1870 and *Phaeosphaeria* I. Miyake 1909 with yellow to brown, fusiform spores with traverse septa. Macroconidia with three to five septa, fusiform with thin walls were classified as *Fusarium* Link 1809. They are hyaline and reproduced in big numbers. Most species of this group are saprophytic and colonize organic materials and soil, but some are pathogens, mainly affecting Poaceae. They occur most frequently when vegetation is well developed [24]. *Leptosphaeria* spp. are the among the causative agents of human mycetoma and phaeohyphomycosis. *Fusarium* is one of the emerging causes of opportunistic mycoses. As well as being common plant pathogens, *Fusarium* spp. are causative agents of superficial and systemic infections in humans. Keratitis, endophthalmitis, otitis media, onychomycosis, cutaneous infections particularly of burn wounds, mycetoma, sinusitis, pulmonary infections, endocarditis, peritonitis, central venous catheter infections, septic arthritis, disseminated infections, and fungemia due to *Fusarium* spp. have been reported. *Fusarium* produce mycotoxins. The importance of the presence of these spores is related to their capability of provoking asthma and other respiratory diseases in humans [26, 21; 25].

Stemphylium Wallr. 1833 is the anamorph of *Pleospora* Rabenh. ex Ces. & De Not. 1863. Conidia (12-20 x 15-30 µm) are solitary, light brown to black in color, and rough- or smooth-walled. They are oblong or subspherical and rounded at the tips. These conidia have transverse and vertical septations (= muriform conidia) and there is a typical constriction at the central septum. *Stemphylium sp.* rarely grows in the indoor environment. *Stemphylium* is widely distributed on decaying vegetation and in the soil. *Stemphylium* may cause phaeohyphomycosis in humans. It is responsible for producing Type I allergies [26; 21; 25].

Curvularia Boedijn 1933 is a dematiaceous filamentous fungus. The conidia (8-14 x 21-35 µm), which are also called the poroconidia, are straight or pyriform, brown, multiseptate, and have dark basal protuberant hila. The septa are transverse and divide each conidium into multiple cells. Most species of *Curvularia* are facultative pathogens of soil, plants, and cereals. *Curvularia* spp. are among the causative agents of phaeohyphomycosis. Wound infections, mycetoma, onychomycosis, keratitis, allergic sinusitis, cerebral abscess, cerebritis, pneumonia, allergic bronchopulmonary disease, endocarditis, dialysis-associated peritonitis, and disseminated infections may develop due to *Curvularia* spp. *Curvularia* has recently emerged also as an opportunistic pathogen that infects immunocompromised hosts. The most common health effects are allergenic and include Type I allergies and allergic fungal sinusitis [25; 26].

Other fungal spore types that were identified in air samples included those belonging to the family Xylariaceae and the genera *Aspergillus* P. Micheli ex Haller 1768, *Agrocybe* Fayod 1889,*Amphisphaeria* Ces. & De Not. 1863, *Arenariomyces* Höhnk 1954, *Ascobolus* Pers. 1796, *Asperisporium* Maubl. 1913, *Bispora* Fuckel 1870, *Botrytis* Pers. 1794, *Caloplaca* Th. Fr. 1860, *Capronia* Sacc. 1883, *Ceratosporium* Schwein. 1832, *Cercospora* Fresen. 1863, *Cercosporella* Sacc. 1880, *Chaetoconis* Clem. 1909, *Chaetosphaerella* E. Müll. & C. Booth 1972, *Chaetomium* Kunze 1817, *Comoclathris* Clem. 1909, *Corynespora* Güssow 1906, *Delitschia* Auersw. 1866, *Dendryphiella* Bubák & Ranoj.1914, *Diatrype* Fr. 1849, *Diplodia* Fr.1834, *Farlowiella* Sacc. 1891, *Fusichalara* S. Hughes & Nag Raj 1973, *Fusariella* Sacc. 1884, *Fusicladium* Bonord.1851, *Ganoderma* P. Karst. 1881, *Helicoma* Corda 1837, *Massaria* De Not. 1844, *Massarina* Sacc.1883, *Massariosphaeria* (E. Müll.) Crivelli 1983, *Microsporum* Gruby 1843, *Monodictys* S. Hughes 1958, *Mytilidion* Sacc.1875, *Nigrospora* Zimm.1902, *Lophiostoma* Ces. & De Not.1863, *Oidium* Sacc.1880, *Paraphaeosphaeria* O.E. Erikss. 1967, *Periconia* Tode1791, *Peronospora* Corda 1837, *Pestalotiopsis* Steyaert 1949, *Piricauda* Bubák1914, *Pithomyces* Berk. & Broome 1875, *Polythrincium* Kunze 1817, *Pseudocercospora* Speg.1910, *Puccinia* P. Micheli ex Haller 1768, *Pithomyces* Berk. & Broome 1875, *Penicillium* Fr. 1832, *Sordaria* Ces. & De Not. 1863, *Sporormiella* Ellis & Everh. 1892, *Stachybotrys* Corda 1837, *Tilletia* Tul. & C. Tul. 1847, *Torula* Pers. 1794, *Trichodelitschia* Munk 1953, *Ulocladium* Preuss 1851, *Uromyces* (Link) Unger 1832. Spores were present in large numbers throughout the summer. During the autumn the relative humidity of the air decreases, thus, allowing other, relatively more thermophilic and xerophilic genera of fungi, e.g. *Epiccocum, Drechslera* and *Stemphylium* to grow [3].Spore dispersion is affected by passive or active discharge mechanisms, which depend upon meteorological parameters as well as other biological variables [11].We do not think that variation in spore concentrations could be explained by meteorological variables alone.Spore concentrations may also depend on the state of the host and how the weather effects the host. Increases in fungal spore concentrations could be related to the maturing and senescence of tree foliage, grasses and to some extent local crops [22].

CONCLUSIONS

64 fungi in outdoor air in the present study is reported. *Cladosporium* was the most abundant fungal spore type collected throughout the period of study reaching. Since knowing the local inhalant allergens facilitates diagnosis and treatment of these pathologies, our objective is to contribute with information of existing air mycobiota in Timişoara's atmosphere. This study was financed by the project PREVALERG, granted by the M.E.C.T. (2007-2009).

BIBLIOGRAPHY

1. Ahlström, K, Käärik, A., 1977 - *A study of airborne fungal spores with the aid of the FOA slit-sampler,* Grana; 16, 133–137.
2. Barkai-Golan, B.R., Frank, M., Kantor, D., Karadavid, R., Toshner, D., 1997 - *Atmospheric fungi in Desert Town of Arad in the coastal plain of Israel.* An of Allergy, 38, 270–274.
3. Beguin, H., 1995 - *Mould biodiversity in homes II. Analysis of mattress dust*, Aerobiologia,11, 3-10.
4. Corden, J.M., Millington, W.M., Mullins J., 2003 - *Long-term trends and regional variation in the aeroallergen Alternaria in Cardiff and Derby UK – are differences in climate and cereal production having an effect?*, *Aerobiologia,* 19, 191–199.
5. Craig, R.L., Levetin, E., 2000 - *Multi-year study of Ganoderma aerobiology,* Aerobiologia, 16, 75–81.

6. Dames, J. F., Cadman, A. 1994 - *Airspora of Durban: A sub-tropical, coastal South African city. II. Fungal spore component,* Grana, 33, 346–348.
7. Erkara Potoglu, I., Asan, A., Yilmaz, V., Pehlivan, S., Okten, S.S., 2008 - *Airborne Alternaria and Cladosporium species and relationship with meteorological conditions in Eskisehir City, Turkey*, Environ Monit Assess, DOI 10.1007/s10661-007-9939-0.
8. Fernandez, D., Valencia, R.M., Molnar, T., Vega, A., Sagues, E., 1998 - *Daily and seasonal variations of Alternaria and Cladosporium airborne spores in Leon (North-West, Spain), Aerobiologia, 14,* 215-220.
9. Hjelmroos, M., 1993 - *Relationship between airborne fungal spore presence and weather variables,* Grana, 32, 40–47
10. Kasprzyk, I., Worek, M., 2006 - *Airborne fungal spores in urban and rural environments in Poland,* Aerobiologia, 22,169–176
11. Katial, R.K., Zhang Y., Jones, R.H., Dyer, P.D., 1997 - *Atmospheric mold spore counts in relation to meteorological parameters*, Int J Biometeorol, 41,17–22.
12. Kramer, C.L., Paday, S.M., Rogersen, C.T.,1959 - *Kansas aeromicology* VIII: *Phycomycetes*, Transactions of the Kansas Academy of Science, 63, 19–23
13. Kurkela, T., 1997 - *The number of Cladosporium conidia in the air in different weather conditions*, Grana 36, 54–61
14. Larsen, L.S., 1981- *A 3-year-survey of microfungi in the air of Copenhagen 1977–1979,* Allergy 36, 15–22
15. Levetin, E, Shaughnessy, R, Fisher, E, Ligman, B, Harrison, J, Brennan, T., 1995 - *Indoor air quality in schools: exposure to fungal allergens,* Aerobiologia,11, 27-34
16. Malling, H.J., Dreborg, S., Weeke, B., 1987 - *Diagnosis and immunotherapy of mould allergy,* Allergy, 42, 305-314.
17. Mitakakis, T., Ong, E.K., Stevens, A., Guest, D., Knox, R.B.,1997 - *Incidence of Cladosporium, Alternaria and total fungal spores in the atmosphere of Melbourne (Australia) over 3 years,* Aerobiologia, 13, 83–90
18. Nikkels, A.H., Terstegge, P., Spieksma, F.Th.M., 1996 - *Ten types of microscopically identifiable airborne fungal spores at Leiden, The Netherlands.* Aerobiologia, 12, 107–112.
19. Palmas, F., Cosentino, S.,1990 - *Comparison between fungal airspore concentration two different sites in the South of Sardinia,* Grana, 29, 87–95.
20. Portnoy, J.M., Charles, S.B., Kevin, K., 2008 - Importance of Mold Allergy in Asthma, Current Allergy and Asthma Reports, 8, 71–78.
21. Reineria Diaz, M., Iglesias, I., Jato, V., 1998 - *Seasonal variation of airborne fungal spore concentrations in a vineyard of North-West Spain***,** Aerobiologia, 14, 221-227.
22. Stepalska, D., Wołek, J., 2005 - *Variation in fungal spore concentrations of selected taxa associated to weather conditions in Cracow, Poland, in 1997*, Aerobiologia, 21,43–52.
23. Waisel, Y., Eli, G., Glikman, M., Epstein, V., Brenner S**.**, 1997 - *Airborne fungal spores in the coastal plain survey of Israel: A preliminary,* Aerobiologia, 13, 281-287.
24. Wynn-Williams, D.D.,1991 - *Aerobiology and colonization in Antarctica – The BIOTAS Pragramme*. Grana, 30, 380–393.
25. http://www.doctorfungus.org.
26. http://www.quantuslabs.com.