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Research Article

Analysis of the Seasonal Variations of the Concentration of air Functional Spores in the External Space of the School of Microbiology of the University of Costa Rica

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Abstract

Fungi are eucaryotic microoorganisms that reproduce by asexual or sexual spores. Fungal spores are involved in what is known as aerobiological process, which is influenced by several factors such as temperature, humidity, wind speed and precipitation. The aerial fungal concentration tends to be high in tropical countries. At the clinical setting, fungal spores play an important role in respiratory allergies due to a constant presence in the environment. These allergens are generally associated with several clinical symptoms, e.g., rhinitis, conjunctivitis, cough, and bronchial asthma. Thus, in the present study the external aerobiologic behavior of fungal spores in the School of Microbiology, University of Costa Rica from October 2014 to September 2015 was evaluated using an aerial volumetric monitor to determine the fungal concentration. The mean concentration of fungal aerial spores was 443.86 ± 201.68 spores/m^3. A total of 18 types of spores were identified as follows: Alternaria, ascospores, *Aspergillus/Penicillium*, basidiospores, *Botrytis, Cercospora, Cladosporium, Curvularia, Drechslera/Helminthosporium, Epicoccum, Erysiphe/Oidium, Fusarium, Nigrospora, Periconia, Pestalotia, Pithomyces, Stemphylium and Torula*. Asexual spores represented 53.19%, and sexual spores, 44.59%. The rest of the spores (2.22%) could not be identified. The asexual spores with the highest concentration in the external environment were those of *Cladosporium* (91 ± 22.36 spores/m^3), *Aspergillus/Penicillium* (71.24 ± 39.04 spores/m^3), *Nigrospora* (21.29 ± 3.62 spores/m^3), *Pestalotia* (17.62 ± 2.87 spores/m^3), *Erysiphe/Odium* (13.73 ± 1.75 spores/m^3) and *Epicoccum* (13.09 ± 2.07 spores/m^3). With respect to the influence of environmental factors, precipitation influence positively the concentration of asexual spores ($p<0.05$). The external aerial spore concentration in the School of Microbiology was highest in May and October and lowest in June and September. Fungal aerial spores counting is an important tool in the field of allergology since it provides valuable information about the fungi present in the locality and their seasonality.

Keywords: Allergy; Aerobiology; Aerial Fungal Spores; *Cladosporium; Aspergillus/Penicillium*

Abbreviations

SM-UCR: School of Microbiology, University of Costa Rica.

Introduction

There is a wide variety of microorganisms present in the air we breathe; of these, the largest group corresponds to fungal spores and, secondly, pollen grains are found. The most important aerial fungal spores outside the dwellings are those belonging to the genera: *Alternaria, Cladosporium, Curvularia, Drechslera, Epicoccum, Fusarium, Helminthosporium, Nigrospora and Stemphylium* [1], while *Aspergillus and Penicillium* are more abundant inside buildings [2]. These spores usually measure less than 20 micrometers, therefore, they are transported over long distances and at different heights [3]. The wind; for example, it acts as a dispersal factor, while rain exerts a cleansing effect on the atmosphere causing spore concentrations to decrease dramatically in a few minutes [2].

In recent decades, great efforts have been devoted to the study of aerobiological behavior and distribution in the atmosphere of fungal spores in order to establish the degree of exposure or risk to which guests are subjected susceptible to certain environments [4]. Due to the anatomical structure of the human respiratory tract during each inhalation, an increase in air velocity occurs when entering through the nose, trachea and bronchi, and subsequently decrease in its passage from the bronchi to the bronchioles. This causes the greatest impact to occur at the upper respiratory tract level. Since nasal hairs are inefficient intercepting large fungal spores, resulting in clinical pictures of rhinitis and bronchitis. Smaller spores, with diameters of 5 micrometers or less, can reach the terminal bronchiols and alveoli and cause pictures of bronchial asthma [5]. In addition, inhalation of aerial fungal spores can also lead to the development of mycosis [6].

Therefore, as Costa Rica is a tropical country, with a relative humidity of more than 60% and temperatures ranging from 18 to 30°C, conditions suitable throughout the year for reproduction and fungal growth, it is essential to study the concentration of fungal spores in an area that concentrates a large student population, as is the School of Microbiology of the University of Costa Rica (SM-UCR), where the number of people presenting allergic symptoms is relevant.

Materials and Methods

Taking the samples

The concentration of fungal spores was determined in one of the corridors on the first floor of the SM-UCR (GPS coordinates: Lat. 9° 93' 80.58'' North, Long. 84° 04' 90.95'' West), which was chosen as the one with the greatest exposure to air currents and increased attendance for students, teachers and officials, being in front of the main laboratory and the study and computer rooms.

The volumetric aerial sampling equipment "Burkard Personal Volumetric Air Sampler" by Burkard Manufacturing Co. Ltd. was used. It collects aerial fungal spores and deposits them directly on a slide covered with a thin and homogeneous layer of vaseline, at a speed of 10 liters per minute. According to the manual of the "Burkard Personal Volumetric Air Sampler", the optimal time for sample collection is 15 minutes per collection site. Samples were taken once a week, for a year. All samples were collected on Fridays of each week at a time between 11:00 am and 11:30 am.

Analysis of samples

The samples were analyzed under the light microscope at 400 x. A thorough count was performed of all spores deposited in the slide and each type of fungus was identified.

Weather analysis

Data on weather factors: relative humidity, temperature, rainfall and wind speed were provided by the National Meteorological Institute. An average of the daily data of seven days prior to the day of sampling was used to consider possible changes in environmental factors that could affect spore concentration.

Statistical analysis

The relationship between spore concentration variables and meteorological parameters was determined by using the Pearson correlation test and by linear regression. Statistical significance was defined with a value of p less than 0.05. Statistical analyses were performed with SPSS v 19 (SPSS Inc., Chicago) computer package.

Results and Discussion

Determination of the concentration of aerial fungal spores from October 2014 to September 2015

The concentration and distribution of aerial fungal spores was analysed in this study for one year of the SM-UCR central corridor. The importance of this work lies in determining the types of circulating fungal spores, to which students, teachers and administrative staff of the institution are exposed, as it is shown that there is a relationship between the respiratory allergic diseases and aeromicronology [5,6]. On the other hand, it will also help to suggest possible sources of contamination for classrooms, offices and teaching and research laboratories.

The average spore concentration during the year was 443.86 ± 201.68 spores/m^3. The highest fungal concentration was found in the months of May (783.6 ± 184.82 spores/m^3) and October (780.80 ± 310.81 spores/m^3). Conversely, the months with the least spores were June(192.5 ± 36.85 spores/m^3)and September (207.25 ± 29.29 spores/m^3) (Figure 1).

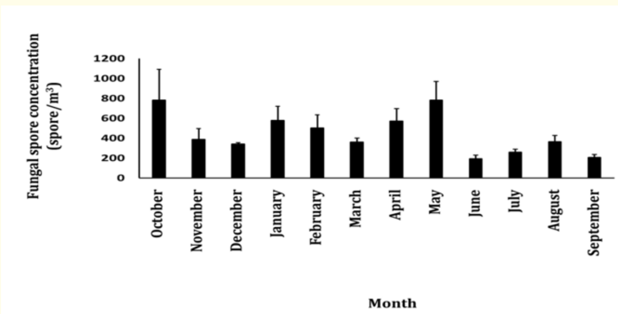

Figure 1: Concentration of fungal aerial spores per cubic meter in the corridor of the School of Microbiology, University of Costa Rica, October 2014 to September 2015.

These results are consistent with those found in another Costa Rican study conducted in schools in the first canton of Heredia province [7]. The concentration of spores in the outdoor air may vary depending on the type of vegetation, local microenvironment and human activity [6,7]. For example, our study found an average spore concentration of 443.86 ± 201.68 spores/m^3, at the School of Pharmacy of the Complutense University of Madrid, in 2000, it was 355.29 spores/m^3 [2], in the City of Murcia was 388 CFUs/m^3, in Taipei it was 2566.98 ± 3475.92 spores/m^3 [8], in rural areas of India the counts range from 82-2365 spores/m^3 [9] and on the outskirts of a hospital in Dijon, France concentration ranges from 49 CFU/m^3 in the winter and 168 CFU/m^3 in the fall [10]. On the other hand, the fungal concentrations in the internal environments of the buildings are usually higher since they are environments with little ventilation and this favors the accumulation of fungi in the environment [7].

Identification of fungal spores

During this analysis, a total of 18 types of environmental spores were identified: *Alternaria, Ascospores, Aspergillus/Penicillium*, basidiospores, *Botrytis, Cercospora, Cladosporium, Curvularia, Drechslera/Helminthosporium, Epicoccum, Erysiphe/Oidium, Fusarium, Nigrospora, Periconia, Pestalotia, Pithomyces, Stemphylium and Torula*. These types of spores were then classified according to their reproduction processes in sexual and asexual spores. 53.19% of spores were anamorphic and 44.59% teleomorphic. The remaining 2.22% corresponded to fungal spores which, on the basis of their morphological characteristics, could not be identified (Figure 2).

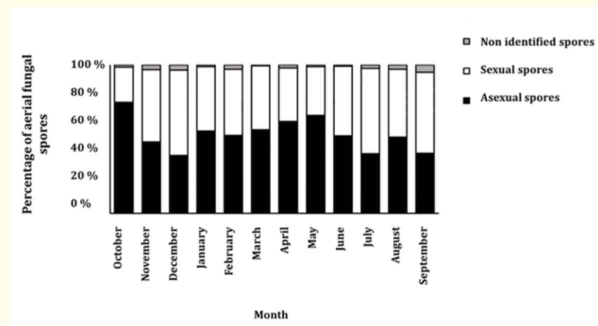

Figure 2: Percentage of the concentration of sexual, asexual and unidentified aerial fungal spores per cubic meter in the corridor of the School of Microbiology, University of Costa Rica, October 2014 to September 2015.

The group of sexual spores was formed taking into account the counts of ascospores (21.82%) and basidiospores (78.18%) (Figure 3). It is important to note that, in all months except November and May, statistically significant differences were found between the concentration of ascospores and basidiospores ($p<0.005$). In addition, no statistically significant differences in basidiospore concentration were found throughout the year ($F = 1.092$; $gl = 11$; $p = 0.397$). In the case of ascospores, fluctuation was found in their annual concentration ($F = 2.371$; $gl = 11$; $p = 0.027$).

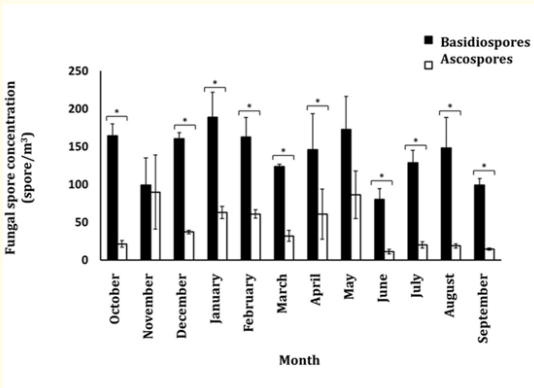

Figure 3: Concentration of sexual spores identified in the air of the central corridor of the School of Microbiology, University of Costa Rica, October 2014 to September 2015. *-p<0.05.

Throughout their life cycle, fungi are typically reproduced by spores that form in sexual and asexual processes, which are morphologically distinguishable and whose production, release and dispersion are conditioned by multiple factors, which impact on the success or failure of its permanence and distribution in the environment. Most fungi produce at least one type of specialized spore for air dispersion, due to the high effectiveness of this propagation method, which allows dispersion from a few centimeters to thousands of kilometers [4]. Asexual or anamorphic spores are usually produced externally individually or forming chains on a conidiophore allowing their release and drag through air currents. Therefore, they are responsible for the dispersion [11]. This could justify identifying more anamorphic spores than teleomorphic or sexual.

Of the total asexual spores identified, the ones that had a higher concentration in the period analyzed were: *Cladosporium* (91 ± 22.36 spores/m^3), *Aspergillus/Penicillium* (71.24 x 39.04 spores/m^3), *Nigrospora* (21.29 ± 3.62 spores/m^3), *Pestalotia* (17.62 ± 2.87 spores/m^3), *Erysiphe/Odium* (13.73 ± 1.75 spores/m^3)and *Epicoccum* (13.09 ± 2.07 spores/m^3) (Figure 4).

These results are consistent with the data reported in the world literature [2,8,12], as in this study, *Cladosporium* was also the most frequently isolated genus in countries such as Australia, Chile, Croatia, Spain (Madrid and Murcia), England, Ireland, Qatar, Turkey and the United States [2,12-14]. This ubiquot distribution is due to colonizing the surface of the leaves, stems and other organs of plants, mainly when they age [11]. In addition, it is important to note that it is a fungus that causes bronchial hyperreactivity and bronchial asthma [12,15].

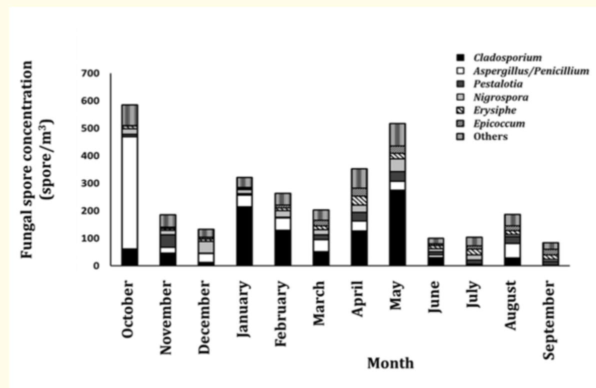

Figure 4: Concentration of asexual aerial fungal spores in the corridor of the School of Microbiology, University of Costa Rica, October 2014 to September 2015.

The second type of asexual spore identified (in quantity) was that of *Aspergillus/Penicillium*. As in the case of *Cladosporium*, these fungi are ubiquitous and their spores have been described as abundant throughout the year [3,6,16]. However, in Taiwan they have been seen to be more abundant during the spring months [8]. It is important to note that because of their small size (< 2 mm) they can remain in suspension in the air for a long period of time, thus increasing the likelihood of being inhaled [6]. With regard to clinical manifestations, in Costa Rica it is reported that 13.08% of atopic patients are allergic to this fungus. *Aspergillus* is the cause of bronchial asthma [5] and allergic alveolitis. The latter takes a cough, dyspnoea, fever, chills, myalgia and general discomfort, after 4 to 8 hours of exposure. If the patient continues to be exposed to this allergen, he or she may develop granulomatous disease or pulmonary fibrosis [17].

Aspergillus fumigatus, on the other hand, is the cause of bronchopulmonary aspergillosis. There are eight fundamental criteria for diagnosing this disease: episodes of bronchial obstruction (asthma), peripheral eosinophilia greater than 1000 cells/mm^3, positive Prick versus *Aspergillus* allergen test, aspergillus allergen, antigen agglutination- antibody with *Aspergillus* antigens against patient antibodies, high serum concentrations of total IgE, elevated serum levels of IgE and specific IgG against *A. fumigatus*, clinical history of pulmonary infiltrates and central bronchoectasis [17]. Also, exposure to high concentrations of *Aspergillus* has been associated with pulmonary haemorrhages in the paediatric population [18].

Third place, in terms of prevalence, is occupied by *Nigrospora* pores. In a study conducted in Spain, it was reported that their spores are common in the summer and autumn months, but it was not found that the weather variables influenced their presence in the atmosphere [19]. At the clinical level, it has been associated as an asthma and rhinitis cause [20].

The other genera most commonly found in air samples were *Pestalotia, Erysiphe/Oidium* and *Epicoccum*. These fungi are phytopathogenic [2,21,22]. In the case of *Pestalotia*, it is the cause of foliar spots or lesions in tropical fruits such as coconut, guava and azalea and on the leaves produces acuvulos with conidiophores that can release their conidia easily and be transported by the wind [21]. On the other hand, *Epicoccum* and *Erysiphe/Oidium* are on decaying wood [2,22]. Therefore, its prevalence in the external area of SM-UCR, could be due to the abundant vegetation, which is in constant decomposition covering the gardens of the enclosure.

On the other hand, in Costa Rica we do not have a high prevalence of *Alternaria*. So it is not an important allergen in our country [7]; however, it is important in other latitudes. In Spain, for example, 14% of the fungi identified belong to this genus [13] and in the United Kingdom it is the fourth most common type of spore in the air [14].

As already mentioned, asexual spores are produced by mitosis, in large quantities and their function is related to the dispersion of the species. On the other hand, sexual spores are latency and are formed by a meiosis process, after the union of two compatible thallus. These spores occur occasionally and their function in nature is to generate greater genetic variability [23]. Ascospores, for example, originate within a modified sac-shaped hifa, called asca. In addition, these asci can be naked or inside fruit bodies [24]. Therefore, the release of spores is the product of mechanical events such as the pressure exerted by raindrops, insects and other animals [25]. The opposite is true for basidiomycetes. Most of these fungi have evolved into the production of large amounts of sex spores (basidiospores) that are exposed to air, so they fulfill the roles of introducing genetic variability, latency survival and dispersion [11,23,24]. This could explain that more than two-thirds of circulating sexual spores were basidiospores. This finding is consistent with previous studies [7] where the incidence of basidiospores was higher than that of ascospores. In addition, it is important to note that 40.77% of atopic patients, with respiratory problems, of the Heredian Medical Clinic, Costa Rica, had allergies to basidiomycetes [5].

Influence of environmental factors on the different types of fungal spores identified

With regard to the influence of environmental factors temperature, humidity, precipitation and wind speed it was found that the concentration of sexual spores was not affected by these environmental factors ($p > 0.05$), as no differences were found between the concentrations of teleospores per month ($F = 1.147$; gl = 11; $p = 0.359$). On the contrary, the concentration of asexual spores presented a positive correlation with precipitation ($p < 0.05$). This is due to the influence these factors have on each of the separate types of asexual spores. For example, *Aspergillus/Penicillium* spores had a positive correlation ($p < 0.05$) with increased rain and negative ($p < 0.05$) with increased wind speed; those of *Alternaria* exhibited a positive ($p < 0.05$) with the increase in wind speed but negative ($p < 0.05$) with increased rain and relative humidity in the environment and *Cercospora* have a negative ratio ($p<0.05$) with increased rain and humidity in the environment (Table 1).

A statistically significant correlation between temperature variations and spore concentration was not found. This is because the temperature reported during the year, for the Canton of Montes de Oca, remained in a range of 18.1 to 23.0°C, which is within the optimal range of growth for mesophile fungi [26]. In other countries it has been reported that the ambient temperature influences the somatic growth and the production of spores, because they are countries that have all four seasons and their temperature variations are more than 40°C (3, 19). On the other hand, *Fusarium* spores had a negative correlation with the increase in temperature ($p < 0.05$) (Table 1), as in previous studies, possibly because they were susceptible to small changes in temperature [3].

Type of spore	Precipitation	Temperature	Wind	Moisture
Total	0.202	0.011	-0.72	0.046
Sexual spores	-0.552	-0.515	0.54	-0.434
Asexual spores	0.416*	0.174	-0.244	0.182
Ascosporas	-0.0535	-0.0351	0.37	-0.385
Basidiosporas	0.487	-0.501	0.524	-0.344
Alternaria	-0.693*	-0.072	0.617*	-0.767*
Aspergillus/Penicillium	0.767*	0.218	-0.593*	0.523
Botrytis	0.108	0.131	0.18	-0.201
Cercospora	-0.663	-0.173	0.531	-0.656*
Cladosporium	0.0403	0.167	0.486	-0.412
Curvularia	-0.147	0.282	0.074	-0.194
Dreschlera	-0.414	0.16	0.298	-0.469
Epicoccum	-0.198	0.356	0.259	-0.488
Eryiphe	0.044	0.446	0.035	-0.205
Furasium	-0.162	-0.619*	0.115	0.127
Nigrospora	-0.337	-0.286	0.273	-0.11
Periconia	0.407	0.036	-0.358	0.468
Mildew	0.14	-0.135	-0.078	0.122
Pestalotia	-0.11	0.399	-0.166	-0.127
Pithomyces	-0.237	0.235	0.35	-0.546
Stemphylium	0.08	0.405	-0.107	0.107
Torula	0.077	0.187	-0.039	0.032
Total	0.202	0.011	-0.72	0.046

Table 1: Correlation between weather factors and concentration of aerial fungal spores collected in the corridor of the School of Microbiology, University of Costa Rica, October 2014 to September 2015.

The correlation is significant at level 0.05.

The influence of precipitation and relative humidity on the production and dispersion of spores was analyzed. Both parameters are related to water availability, which is important for the growth of the fungus and as a mechanism for spore release. It is important to note that the year in which the study was conducted, it exhibited atypical behavior, with respect to the amount of rain that is expected to fall in a tropical country such as Costa Rica, so this could influence the prevalence of environmental fungal spores.

Previous research has suggested that atmospheric fungal concentration tends to be higher in temperate and tropical regions than in high latitudes and deserts, where fungal colonization is limited by adverse weather conditions [2,7]. However, it is important to note that heavy rains decrease the concentration of suspended particles [8]. In our study, the increase in the amount of rain positively influenced the concentration of asexual spores, especially those of *Aspergillus/Penicillium*, which has already been described in other studies [3,28]. On the other hand, in the case of *Alternaria* spores, their dispersion was adversely affected by rain and humidity. This is because particles measuring between 5 and 10 micrometer in diameter remain in suspension for longer periods than those measuring between 10 and 40 micrometer [29]. In the case of *Alternaria* spores, they measure from 4-12 x 18-68 micrometer [30] and, in addition, when moistened they gain weight so they fall to the ground and do not disperse as much [31,32].

Although the spores are dispersed through the air, no positive correlation was found between wind speed and fungal spore concentration, which is consistent with what Gioulekas *et al.,* previously reported [33]. It should be noted that, in the literature, there is a disagreement with regard to the effect of the wind as reports have been made showing a positive correlation [34] and in others a negative correlation [8]. This is because it has been seen that when the wind speed is greater than 5m/s the spore concentration increases, but when the speed is lower a dilution effect of the atmosphere occurs, resulting in lower concentrations of air pollutants such as fungal spores [8]. In this study, the average wind speed ranged from 1 to 3.6 m/s, so this atmospheric dilution effect could have been given.

Conclusion

Cladosporium and *Aspergillus/Penicilium* were the most frequently identified fungi from air samples, analyzed, and are able to grow and accumulate on walls, ceiling and air conditioners. The concentration of external air spores was higher in the months of May and October and minors in June and September. Therefore, it is recommended to repeat this type of sampling inside classrooms and laboratories in order to evaluate the contamination to which students and teachers of the School of Microbiology are exposed. The counting of aerial fungal spores is important from a public health point of view and their monitoring is essential in the prevention of allergies caused by environmental contamination of fungal spores.

In addition, it is recommended to carry out further studies at the same collection site that was used in this research. Because consecutive sampling is required for at least five years to obtain adequate comparison parameters to understand the true aerobiological behavior of allergens and thus provide information to help the allergen allergist in decision-making during the diagnosis and management of patients allergic to fungi.

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Conflict of interest

The authors declare that they have no conflict of interest in this article.

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