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ANNUAL VARIATION OF FUNGAL SPORES IN ATMOSPHERE OF PORTO: 2003

Manuela Oliveira[1, 2], Helena Ribeiro[2, 3], Ilda Abreu[1, 2]

[1]Institute for Molecular and Cell Biology, Porto, Portugal
[2]Department of Botany, Faculty of Sciences, University of Porto, Porto, Portugal
[3]Autonomous Section of Agricultural Sciences, Faculty of Sciences, University of Porto, Vairão, Portugal

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Abstract: The seasonal distribution of fungal spore concentration in the city of Porto, Portugal, was continuously studied, from 1 January to 31 December 2003, using a 7-day volumetric Burkard trap. In Portugal, aerobiological studies are scarce, and to our knowledge there are no published data on the atmospheric concentration of fungal spores. The aim of this work therefore is to initiate the aeromycological studies in Porto. The total airborne spore concentration fluctuated between 2 and 2,198 spores/m^3 per day with an daily mean of 403 spores/m^3. The highest airborne spore concentration was found during the summer and the early autumn, while the lowest concentration was registered during the winter. A range of allergenic and phytopathogenic fungal spores was present in the atmosphere of Porto throughout the entire year, although in different concentrations. Among the 22 fungal spore types identified *Cladosporium* (74.5%), *Ganoderma* (11.7%), Aspergillaceae (2.9%), *Ustilago* (2.5%), *Coprinus* (1.5%), *Alternaria* (1.3%) and *Botrytis* (1.3%) were the most frequent. Seasonally, spore levels of *Cladosporium* and *Alternaria* peaked in the autumn and winter, *Ganoderma* peaked toward autumn, whilst spore levels of the basidiomycete *Coprinus* fluctuated throughout the year. The total spore concentration was negatively correlated with wind speed and positively correlated with temperature and relative humidity.

Address for correspondence: Ilda Abreu, IBMC, Rua do Campo Alegre 823, 4150-180 Porto, Portugal. E-mail: inoronha@ibmc.up.pt

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INTRODUCTION

Atmosphere contains gases, water droplets, microscopic and submicroscopic particles of pollen and dust, and microorganisms (bacteria, moulds, yeast and viruses) [1]. Fungal spores constitute a significant fraction of airborne bioparticles, being usual to find concentrations above 1,000 spores/m^3, several times more numerous than other airborne particles such as pollen grains [2].

In the field, agricultural operations such as watering, weeding and harvesting, create mechanical disturbances sufficient to liberate fungal spores into the atmosphere. The deterioration of stored material and the spoilage of foodstuffs are induced by growth of fungi which reach them from air [3].

In industrialized countries, up to 6% of the population suffers from allergy to fungal spores. The major allergic manifestations include asthma, rhinitis, bronchopulmonary mycoses and hypersensitive pneumonitis. The most frequent clinical symptoms are sneezing, nasal discharge, shortness of breath, urticaria angiedema and anaphylaxis [4].

Airborne fungi are also considered to act as indicator of the level of atmospheric bio-pollution. The presence of fungal propagules, volatiles and mycotoxins in the air can cause a health hazard in all segments of the population [5].

Aerobiological studies enable us to ascertain the concentration of the fungal spores present in the atmosphere [6]. These studies have been developed in many different parts of the world [6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17]. Fungi variety and concentration depend

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basically on numerous factors, including topography, time of day, meteorological parameters, seasonal climatic variation and type of vegetation [14, 15, 16, 17, 18].

In Portugal, aerobiological studies are scarce, and to our knowledge, there are no published data on the atmospheric concentration of fungal spores. Such studies are useful for clinicians and their patients who are allergic to fungal spores, and for farmers in order to avoid losses due to fungal plant diseases. The aim of this work therefore was to investigate the variations in the annual and seasonal distributions of airborne spores, initiating the aeromicological studies in Porto. Nevertheless, in Northern Spain (Galicia), namely in cities like Santiago de Compostela and Orense, quantitative and qualitative studies of fungal spores in the atmosphere have been conducted since 1993, in order to identify important clinical and phytopatological fungal types. The influence of the main meteorological factors on fungal spore concentration has also been studied [6, 19, 20, 21].

MATERIAL AND METHODS

Daily spore counts. Daily spore concentrations were sampled between 1 January–31 December 2003 using a Burkard 7-day volumetric trap (Burkard Manufacturing Co. Ltd., Rickmansworth, Hertfordshire, UK) with a flow rate of 10 litres per minute. Spores were trapped onto a melinex adhesive tape, and then cut into daily segments. The slides, with adhesive segments, were covered with fucsin-stained glycerol jelly and with a cover glass. The daily mean concentration of the number of fungal spores was carried out using an optical microscope at a magnification of ×400 along 2 full lengthwise traverses. Spore counts were then converted to correspond spores per cubic meter of air sampled per day.

Spore trap location. The air sampler was located in Porto on the roof of the Instituto de Botânica da Faculdade de Ciências (41º11' N, 8º39' W), approximately 20 m above ground level. Porto is situated on the right bank of Douro River, near the mouth, in northwest Portugal and is the second largest city. With around 300,000 inhabitants, it is the northern regional capital.

The most significant feature of the Porto weather is the annual rainfall level (1,236 mm) and its irregular distribution throughout the year, concentrated in winter and spring. Air temperature increases inversely to precipitation. Due to its maritime influence, Porto presents mild temperatures with an annual mean of 14ºC. No really cold season can be found in Porto, with being January the coldest month with an average of 9ºC. The mean summer temperature is about 18ºC, although high temperatures can be reached from May–September [22].

Meteorological data. The relationship between the daily spore concentration and the meteorological factors (temperature, relative humidity, rainfall, and wind speed) was established by means of Pearson correlation coefficient and Spearman rank correlation test, with a significance level of 1% and 5%.

Table 1. Fungal spores distribution in the atmosphere of Porto, during 2003.

Fungal spores types	Airborne concentration (%)
Alternaria	**1.30**
Aspergillaceae	**2.89**
Botrytis	**1.30**
Cladosporium	**74.49**
Coprinus	**1.42**
Corynespora	0.05
Didymella	0.06
Drechslera	0.14
Epicoccum	0.59
Fusarium	**1.01**
Ganoderma	**11.68**
Leptosphaeria	0.19
Oidium	0.02
Periconia	0.01
Pithomyces	0.19
Pleospora	0.34
Polythrincium	0.08
Rhizopus	0.18
Rust	0.59
Smuts	0.86
Torula	0.09
Ustilago	**2.53**

Table 2. Correlation between the total concentration of fungal spores present in the atmosphere and the meteorological factors in the city of Porto.

Meteorological factor	Pearson Coefficient	Spearman Coefficient
Mean temperature	0.24**	0.35**
Maximum temperature	0.24**	0.34**
Minimum temperature	0.26**	0.37**
Temperature amplitude	0.05	0.06
Mean relative humidity	0.08	0.07
Maximum relative humidity	0.11*	0.10
Minimum relative humidity	0.02	0.05
Relative humidity amplitude	0.03	0.02
Rainfall	-0.06	-0.07
Mean wind speed	-0.16**	-0.15**
Maximum wind speed	-0.16**	-0.13**

*Correlation is significant at the 5% level (2-tailed); **Correlation is significant at the 1% level (2-tailed)

RESULTS

Airspora components. During the year 2003, 22 genera of airborne fungal spores were identified (Tab. 1). *Cladosporium* provided 74.5% of the total identified spore concentration, being almost 7 times more frequent

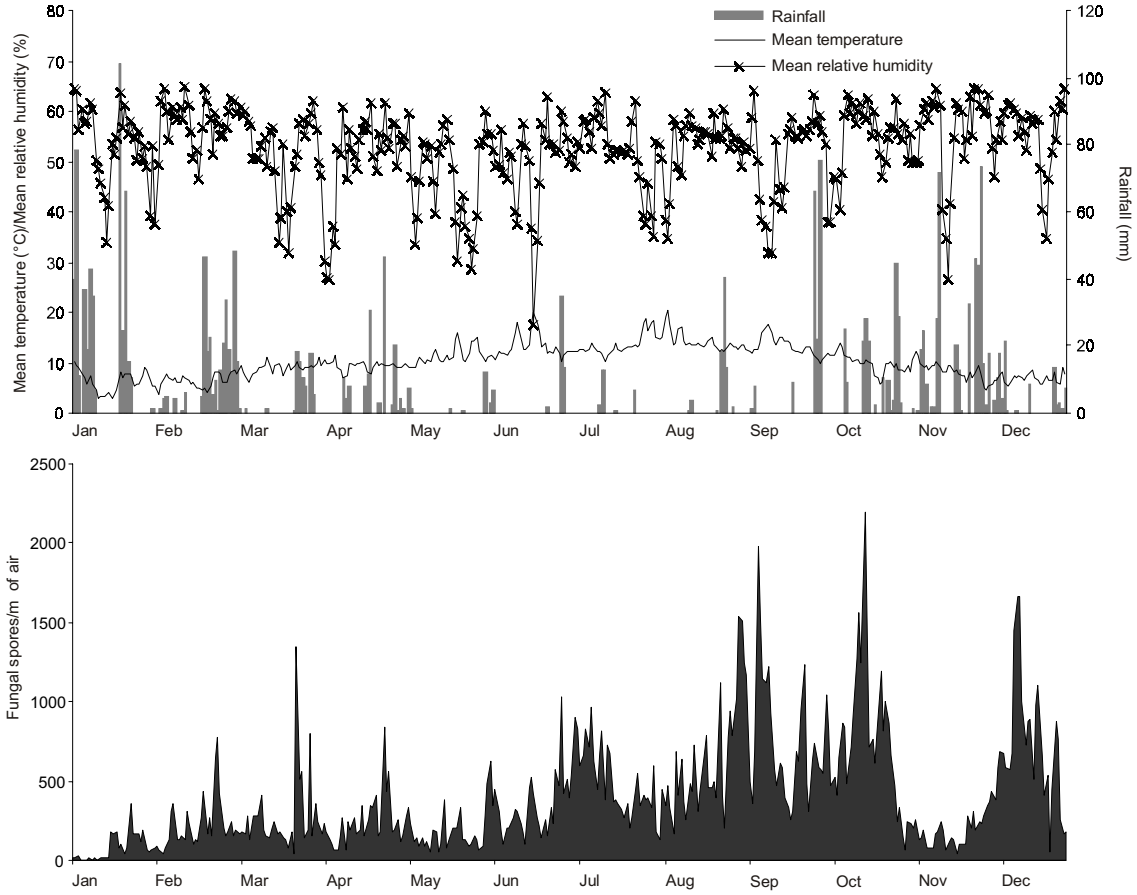


Figure 1. Daily meteorological factors and fungal spore concentration in Porto atmosphere.

than the second most abundant group (*Ganoderma*). The other most frequently detected spore groups were: *Alternaria*, Aspergilaceae (comprising the genera *Aspergillus* and *Penicillium*), *Botrytis*, *Coprinus*, *Epicoccum*, *Fusarium*, *Ganoderma*, 'rust' and *Ustilago*, representing 24.2%. The other 11 spore types were present in very low numbers, constituting the remaining 1.4% of the annual total airborne spore counts (Tab. 1).

Seasonal variation. The majority of spore types were present throughout the year. The average of daily total airborne fungal spores count was 402.7 spores/m^3 of air per day, ranging from a maximum peak of 2,198.8 spores/m^3 of air per day, detected on 9 September, and a lowest concentration of 2.0 spores/m^3 of air per day, detected on 8 January (Fig. 1).

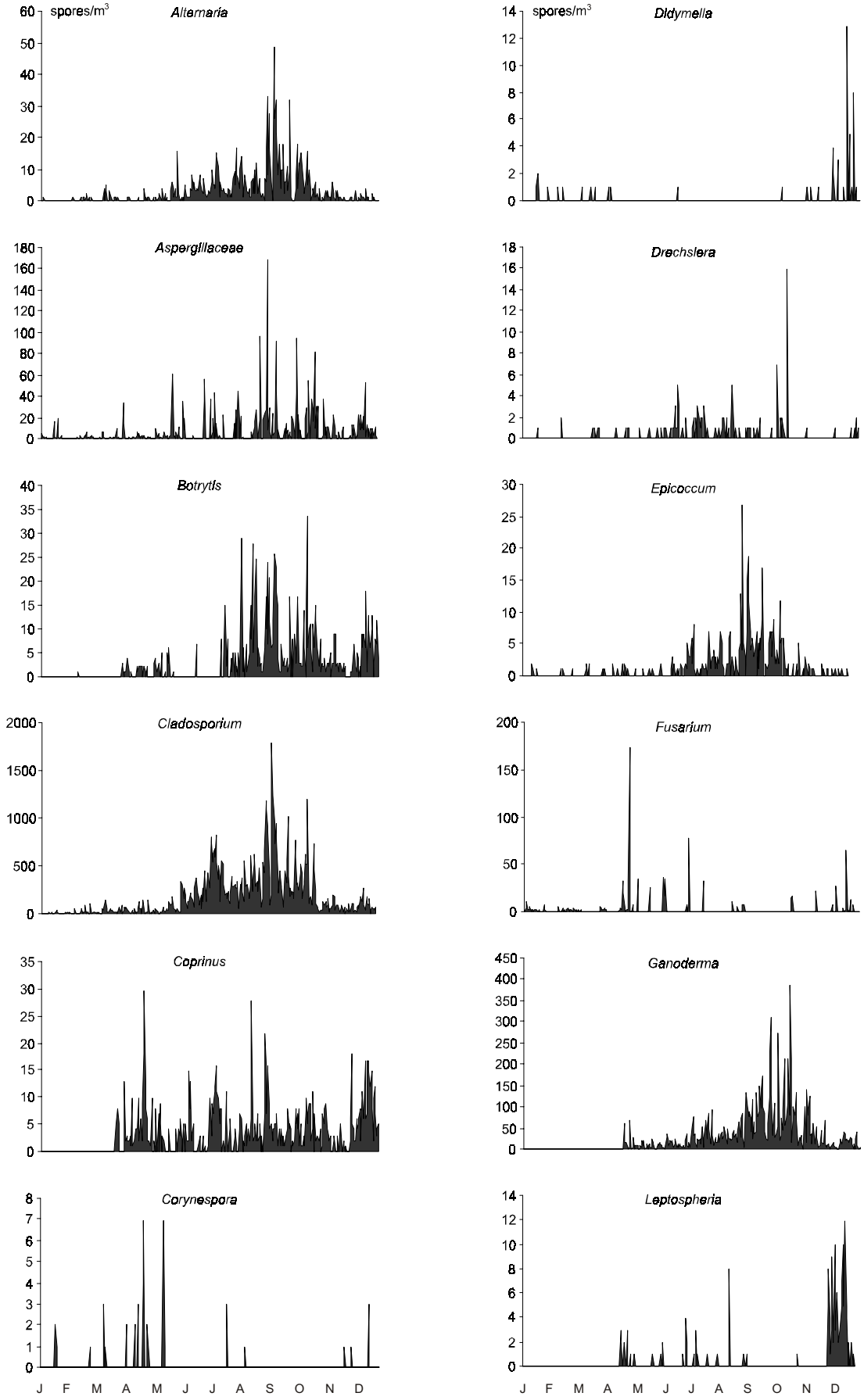
The highest airborne spore concentrations in Porto were observed during summer and late autumn (July-October) while the lowest concentrations were found during winter (January-February). Nevertheless, in February, a peak was registered that can be exclusively attributed to the presence of *Ustilago* spores in the atmosphere. Another peak was registered in December, due to an increase in the concentration of almost all fungal types (Fig. 2).

Clasdosporium was found throughout the year, while *Alternaria*, *Botrytis* and *Ganoderma* presented peaks spanning from summer to autumn. Spore densities of *Corynespora*, *Fusarium*, *Oidium*, *Periconia* and *Rhizopus* peaked in late spring. Spore densities of Aspergillaceae, *Epicoccum*, *Pithomyces*, 'rusts' and *Ustilago* peaked toward summer. The highest concentrations of *Drechslera*, *Pleospora* and *Ganoderma* spores were observed in autumn, whilst airborne spores of *Ustilago* were highest during winter. However, some spores present 2 similar peaks during the year: *Polyhthricium* peaked in spring and autumn, while *Coprinus* was found from spring to summer. Numbers of the *Dydimella*, *Leptosphaeria* and *Torula* fluctuated sporadically all year around (Fig. 2).

Correlations were established between the total spore counts and the meteorological factors in the atmosphere of Porto. The total concentration was negatively correlated with wind speed; while positively correlated with temperature and relative humidity (Tab. 2).

DISCUSSION

This aeromycological study is the first performed in Porto (Portugal) and, to our knowledge, there are no published data on the atmospheric concentration of fungal spores in our country. Twenty-two genera, belonging to various categories, were identified using a Burkard 7-day volumetric trap. The highest atmospheric concentrations of fungal spores were detected from July–October, the


spores/m3
Alternaria
Didymella
Aspergillaceae
Drechslera
Botrytis
Epicoccum
Cladosporium
Fusarium
Coprinus
Ganoderma
Corynespora
Leptospheria
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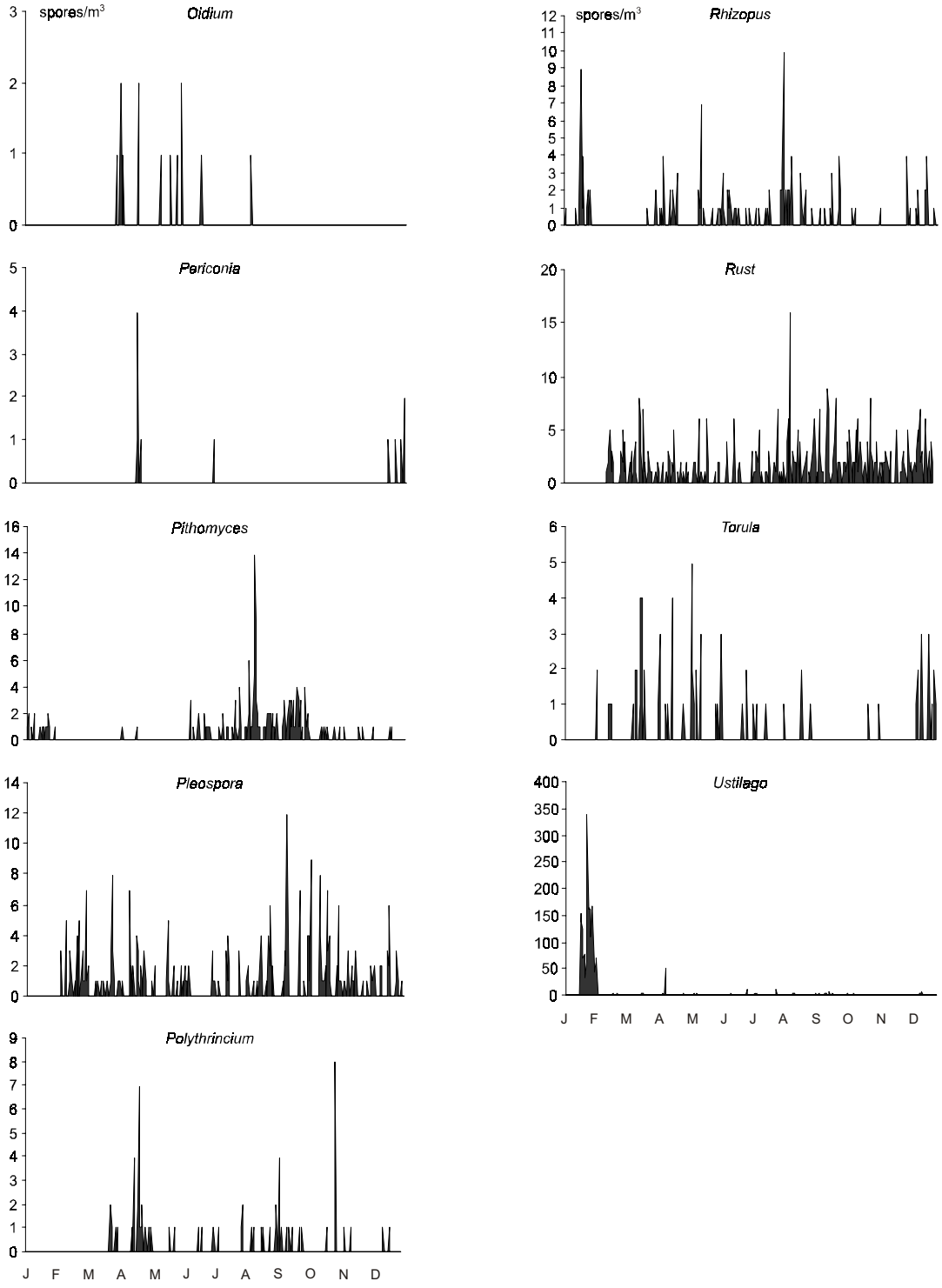

Figure 2. Daily concentration of some fungal spores present in Porto atmosphere.

lowest concentrations were found from January–February, and no spore-free season was observed during the study. This same occurrence pattern was previously described for different regions in Spain [6], Qatar [18] and Croatia [23]. Porto is an urban area with several gardens possessing ornamental trees and the majority of fungal spores present in the atmosphere are related to these plants. Indeed, the highest fungal spore concentrations can be related with the vegetative development of the plants in the surrounding area during spring. Also, the decaying leaves from these plants constitute a substrate where fungal spores can grow, possibly explaining the peaks that occur in autumn.

When compared to previous studies [24], in Porto, low spore concentrations were observed (maximum of 2,198.8 spores/m^3 of air per day), fact that may be due to the

geographic location of the sampler, mainly its proximity to the River Douro and the Atlantic Ocean that negatively influences the concentration of airborne bioparticules.

Although the majority of spores are present throughout the year, the most frequent genera studied were divided in 4 different groups accordingly to the main occurrence peak: the winter spores ('rusts' and *Ustilago*), the spring spores (*Corynespora*, *Fusarium*, *Oidium*, *Periconia* and *Rhizopus*), the summer spores (*Alternaria*, Aspergillaceae, *Epicoccum* and *Pithomyces*) and the autumn spores (*Drechslera*, *Ganoderma* and *Pleospora*).

During winter, very low fungal spore concentrations were observed. Nevertheless, in February a peak of *Ustilago* spores was registered, although in Spain this peak was only observed later, during spring and early summer [25, 26]. This temporal difference can be explained by the lack of cereal crops in our study area in opposition to the areas referred by the Spanish works.

Concerning the spring spores, also in Spain, similar peak dates for *Fusarium* [19] and *Oidium* [25] were determined.

During summer, *Alternaria*, Aspergillaceae and *Epicoccum* were the main spore types found. *Alternaria*, an important fungal spore from both clinical and phytopathological point of view [6, 16], presents its highest concentration, around 50 spores/m^3 of air per day, in September. This value is similar to those observed in Ourense [25]; however, this is very low when compared to the values registered in Córdoba and Cáceres, during late spring [25, 27] and in Melbourne, in the autumn [15]. The presence of *Alternaria* spores in the atmosphere spreads from late spring–early autumn. The same occurrence pattern was observed in other countries, such as Poland [11, 16] and the United Kingdom [13]. Aspergillaceae fungal spores were the fourth most abundant type in Porto atmosphere and similar results were observed in Qatar [23].

Cladosporium was present throughout the year, being the dominant fungal type. The dominance of this genus has also been observed in many countries, including Australia [15], Chile [14], Croatia [18], Qatar [23], Spain [25], Turkey [28] and the USA [29]. Nevertheless, the *Cladosporium* concentration registered in these works was much higher than in Porto, where the highest concentration was observed in September.

Besides *Cladosporium*, the main fungal type found during autumn was *Ganoderma*. The strong incidence of these spores can be related to the presence of decaying vegetal material during this season since *Ganoderma* spores are found growing on dead or living hardwood and Conifers [12].

Several authors [6, 30] found a negative correlation between fungal spores and temperature. It is common knowledge that fungal spores only develop under a certain temperature threshold, therefore with extreme high or low temperatures the spore concentrations decrease. The city of Porto is characterized by mild temperatures throughout the year which can explain the positive correlation with temperature. Nevertheless, the high temperatures of August and the cold weather of January correspond to a decrease in the spore concentration.

The positive correlation found with the relative humidity was previously described [6], and this fact can be explained by the importance of water in fungal spore discharge and dispersion.

The efficiency of the Hirst sampler varies with the wind speed, because an high wind speed accelerates the particles, such as fungal spores, deviating them from the trajectory from the inlet stream [31]. This can explain the negative correlation found between total spore concentration and wind speed.

The highest spore concentration peaks were coincident with the peaks of some allergenic pollen types. The spore peak observed in June-July matches the highest peak of Poaceae and with an Urticaceae peak. Also, the peak observed during September-October corresponds to *Plantago* and Chenopodiaceae-Amaranthaceae peaks.

In Portugal, an increase in allergy symptoms related to pollen during summer is observed. Interestingly, this occurs when the most significant bioparticles present in the atmosphere are the spores, mainly *Cladosporium*, which can interact with airborne pollen aggravating allergic problems.

Besides the importance of pollen monitoring, we must accentuate the importance of the fungal spores in the allergological field in order to put into action desirable preventive medical treatments.

CONCLUSION

Among the 22 genera of airborne fungal spores identified in the atmosphere of Porto during 2003, the most frequent spore type was *Cladosporium*, followed by *Ganoderma*, Aspergilaceae, *Ustilago*, *Coprinus*, *Alternaria*, *Botrytis*, *Epicoccum*, *Fusarium*, and 'rust'. Total atmospheric spore densities varied seasonally from peak counts in summer to early autumn, while the lowest values were found during winter.

Meteorological parameters influence the spore concentration, wind speed presents a negatively effect while temperature and relative humidity contribute to high spore concentrations.

In further works, we intend to consolidate these results in order to elaborate fungal spore calendars that may be used to assist clinicians to identify and control of fungal allergy symptoms, and by farmers in order to prevent plant diseases induced by these phytopathogens.

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