

First volumetric record of fungal spores in the atmosphere of Montevideo City, Uruguay: a 2-year survey

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Abstract In Uruguay, aeromycological studies are restricted to a gravimetric analysis performed from December 1942 to March 1944 in Montevideo where spores of Pucciniaceae, *Alternaria* and *Helminthosporium* were the only specimens identified. Daily monitoring of airborne fungal spores was carried out for the first time in Montevideo, from April 2012 to March 2014, using a Rotorod sampler in order to evaluate the seasonal variation and influence of meteorological parameters. A total of 548,309.68 spores/m³ were recorded which belong to anamorphs of Higher Fungi (69.18 %), Phyla Ascomycota (12.62 %), Basidiomycota (8.01 %), Oomycota (0.37 %) and Myxomycota (0.06 %). Airborne spores occurred in Montevideo throughout the whole year. However, a seasonal pattern was revealed, with the highest concentrations recorded in autumn and summer. The most abundant spore types were *Cladosporium* (53.22 %), *Alternaria* (6.62 %), *Didymella* Group (5.86 %), *Leptosphaeria* Group (4.37 %) and *Coprinus* (4.3 %). Temperature appeared to be the most influential meteorological factor correlating significantly and positively with total spore, *Cladosporium*, *Alternaria* and *Didymella* Group abundance. Relative humidity influenced positively total spore, *Cladosporium* and *Didymella* Group concentrations while a weak negative association was

obtained for *Alternaria*. Wind speed correlated negatively with total spore, *Cladosporium*, *Alternaria* and *Didymella* Group. Precipitation showed a negative influence on *Alternaria*, while positive correlations were observed for *Didymella* Group. For the first time, fungal spores considered allergenic were recorded in Montevideo atmosphere and the risk of exposure would have been high from December to June. However, long-term sampling is needed to define seasonal prevalence patterns and the influence of meteorological conditions on spore abundance.

Keywords Fungal spores · Aerobiology · Meteorological parameters · Montevideo · Uruguay

1 Introduction

Fungal spores are a major contributor to the spectrum of airborne allergens (Oliveira et al. 2009a). Since they play an important role in respiratory diseases such as allergic rhinitis and asthma (Horner et al. 1995), it is useful to investigate the seasonal distribution of airborne spores for allergic patients in order to take necessary precautions when spores concentration is high. In southern South America, aeromycological studies have been performed for a long time in several cities in Argentina (Iovannitti and Tiraboschi 1985; Quiroga de Pascual and Nobile 1985; Mangiaterra et al. 1993; Esquivel et al. 2003; Bianchi and

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Olabeuena 2006; Negrin et al. 2007; Nitiu et al. 2010; Mallo et al. 2011), Brazil (Gambale et al. 1977, 1981; Gambale 1980; Purchio et al. 1984; Schoenlein-Crusius et al. 2001; Mezzari et al. 2002; Távora et al. 2003; Menezes et al. 2004; Bernardi and Nascimento 2005; De Antoni Zoppas et al. 2006; Bernardi et al. 2006; Gonçalves et al. 2010) and Chile (Ibáñez et al. 2001). However, in Uruguay, aerobiological studies are very scarce and restricted to gravimetric analysis performed from December 1942 to March 1944 (Vaz Ferreira 1946) and volumetric pollen studies during 2000–2001 (Tejera 2002; Tejera and Beri 2003, 2005), both in Montevideo. In relation to airborne fungal spores, the only previous study was the one conducted by Vaz Ferreira (1946) where spores of Pucciniaceae, *Alternaria* and *Helminthosporium* were the only specimens identified. The purpose of this study was to identify and quantify fungal spores, for the first time in the atmosphere of Montevideo, over a 2-year period in order to evaluate the seasonal variation and influence of meteorological parameters on airborne fungal spore concentration.

2 Materials and methods

2.1 Area of study

Montevideo (34°56'S–56°09'W) is the capital and most populated city in Uruguay. It hosts 40 % of the country's population and is located on the Río de la Plata coast (Fig. 1). Uruguay has a temperate-sub-tropical humid climate, with hot summers and no dry/wet seasons ("cfa" type according to Kottek et al. 2006). The absence of important orographical systems and a small country size make climate fairly uniform nationwide. Montevideo has a total annual precipitation of 1060 mm, a mean annual temperature of 16.6 °C and a mean relative humidity of 75 % (Wells and Daborn 1998). The region belongs to the Pampa Province where grasslands are the dominant vegetation (Cabrera and Willinks 1973). The urban flora is mainly composed of cultivated trees, shrubs and herbs, or grows spontaneously along abandoned lots (Lombardo 1979, 1982, 1983, 1984).

2.2 Methods

Daily fungal spore concentration was recorded using a Rotorod sampler Model 40 (Sampling Technologies Inc., Minnetonka, MN) over a 2-year period, from April 2012 to March 2014. Sixty-nine days were lost over the whole period due to technical failure of the sampler and saturation of rods with aerosols. The sampler was installed on the roof of the Faculty of Science (34°52'56.8"S; 56°06'58.5"W) in an open area at a height of 12 m above ground level. The sampler was programmed at 10 % of the cycle, rods whirled for 1 min in every 10 min and the rest of the time remains folded and static. Samples were mounted on a specially designed polystyrene microscope slide provided with transversal grooves and stained with Calberla's solution. Spores were counted and analyzed using a photomicroscope Olympus CX41 (Olympus Corporation, Tokyo) at a magnification of 400× along one longitudinal transect to estimate daily spore concentration. Fungal spores are more abundant in the air than pollen grains; thus, their analysis is more time-consuming than pollen and sometimes a smaller area of the slides (Burge 1992; Sterling et al. 1999) or rods (e.g., Friesen et al. 2001; Adhikari et al. 2003) are counted in order to estimate daily concentrations. Therefore, the decision of counting 33 % of the rod area was taken in this study.

Spores were identified using specialized bibliography (Bassett et al. 1978; Nilsson 1983; Barnett and Hunter 1987; Smith 1990; Lacey and West 2006). Due to the complexity of identifying some fungal spores, morphological groups defined by Nitiu et al. (2010): *Didymella* Group, *Didymosphaeria* Group, *Lep-tosphaeria* Group and *Helminthosporium* Group were employed (see Table 1 for morphological types included in each group).

Fungal spore counts were converted into spores per cubic meter of air sampled and monthly, and total spore values were estimated by adding daily spore concentration values. Representativeness of each spore type in the total record was analyzed considering the following categories: *abundant*, for those spore types exceeding 5 % of the total spore concentration; *moderate*, ranging from 1 to 4.99 %; and *low*, below 0.99 %. Relative frequency was calculated as per-

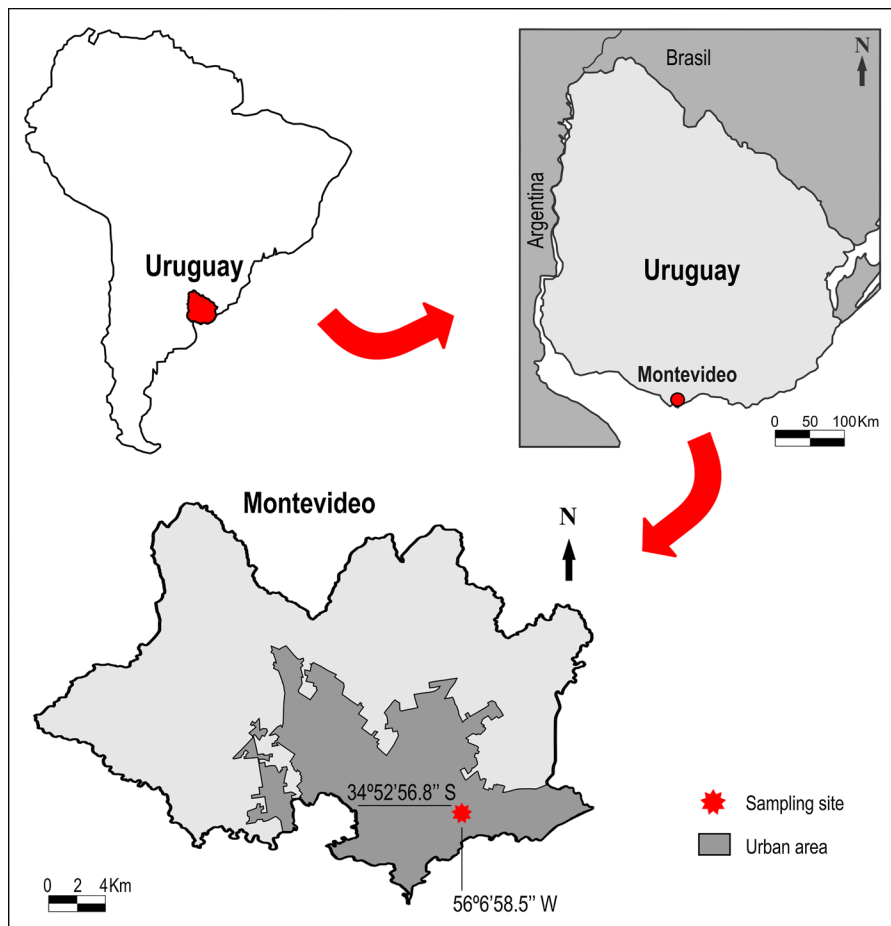


Fig. 1 Location of the sampling site in Montevideo, Uruguay

centage of days the spore type was recovered. Three categories were considered: *very frequent* (75.1–100 % of sampled days), *frequent* (36.6–75 %) and *unfrequent* (0.1–36.5 %).

In order to explore the influence of meteorological parameters (average, maximum and minimum temperatures, relative humidity, wind speed and precipitation) on total spore concentration, statistical correlation between the variables was studied. The Shapiro–Wilk test was used to evaluate normality of spore concentration and meteorological data. Since data were not normally distributed, the Spearman's rank correlation analysis was applied. This analysis was performed with lags up to 3 days for meteorological variables. Furthermore, the analysis was also carried out to examine dependences of *Cladosporium*, *Alternaria* and *Didymella* Group spore concentration on meteorological variables. These genera were

selected both for their allergenic capacity and for the high abundance in the atmosphere. Meteorological data from the nearest meteorological station at Carrasco International Airport (34°50'S, 56°00'W) were provided by the National Meteorological Service (DNM). Missed sampling days were excluded from all analyses.

3 Results

A total of 548,309.68 spores/m³ were recorded in the 2-year period (April 2012–March 2014), with a mean concentration of 840.97 spores/m³, which belong to anamorphs of Higher Fungi (69.18 %), Phyla Ascomycota (12.62 %), Basidiomycota (8.01 %), Oomycota (0.37 %) and Myxomycota (0.06 %). Fifty-six spore types were identified, 21 from

Table 1 Fungal spore types identified in the atmosphere of Montevideo from April 2012 to March 2014

Division/group	Spore type	Total concentration (spores/m ³)	Percentage of representation	Category	Frequency
Anamorph	<i>Alternaria</i>	36,281.47	6.6170	A	***
	<i>Arthrinium</i>	316.52	0.0577	L	*
	<i>Botrytis</i>	1692.75	0.3087	L	**
	<i>Cercospora</i>	4476.07	0.8163	L	**
	<i>Cerebella</i>	7432.11	1.3555	M	**
	<i>Cladosporium</i>	291,840.41	53.2255	A	***
	<i>Curvularia</i>	2852.66	0.5203	L	**
	<i>Epicoccum</i>	12,084.66	2.2040	M	***
	<i>Fusarium</i>	762.05	0.1390	L	*
	<i>Gliomastix</i>	388.44	0.0708	L	*
	<i>Helicomycetes</i>	134.89	0.0246	L	*
	<i>Helminthosporium</i> Group ^a	6369.62	1.1617	M	***
	<i>Nigrospora</i>	3375.02	0.6155	L	**
	<i>Oidium</i>	1162.13	0.2119	L	**
	<i>Pithomyces</i>	2679.54	0.4887	L	**
	<i>Polythrincium</i>	1696.25	0.3094	L	**
	<i>Spegazzinia</i>	263.11	0.0480	L	*
	<i>Stachybotrys</i>	101.00	0.0184	L	*
	<i>Stemphylium</i>	2184.35	0.3984	L	**
	<i>Tetraploa</i>	130.69	0.0238	L	*
	<i>Torula</i>	3084.13	0.5625	L	**
Ascomycota	<i>Acrodictys</i>	45.20	0.0082	L	*
	<i>Ascobolus</i>	42.19	0.0077	L	*
	<i>Caloplaca</i>	556.61	0.1015	L	*
	<i>Ceratosporeum</i>	1.00	0.0002	L	*
	<i>Chaetoconis</i>	5.98	0.0011	L	*
	<i>Chaetomium</i>	1901.98	0.3469	L	**
	<i>Chaetosphaerella</i>	158.94	0.0290	L	*
	<i>Diatrypaeaceae</i>	1265.99	0.2309	L	*
	<i>Didymella</i> Group ^b	32,135.87	5.8609	A	***
	<i>Didymosphaeria</i> Group ^c	2689.53	0.4905	L	**
	<i>Fusariella</i>	18.99	0.0035	L	*
	<i>Kassariosphaeria</i>	2.13	0.0004	L	*
	<i>Leptosphaeria</i> Group ^d	23,961.72	4.3701	M	***
	<i>Leptosphaerulina</i>	634.60	0.1157	L	**
	<i>Melanospore</i>	8.58	0.0016	L	*
	<i>Navicella</i>	2.18	0.0004	L	*
	<i>Paraphaeosphaeria</i>	2235.28	0.4077	L	**
	<i>Pleospora</i>	1946.89	0.3551	L	**
	<i>Sporormiella</i>	822.71	0.1500	L	*
	<i>Sordaria</i>	85.33	0.0156	L	*
	<i>Xylariaceae</i>	660.05	0.1204	L	*
	<i>Zygophiala</i>	25.44	0.0046	L	*

Table 1 continued

Division/group	Spore type	Total concentration (spores/m ³)	Percentage of representation	Category	Frequency
Basidiomycota	<i>Agaricus</i>	8.36	0.0015	<i>L</i>	*
	<i>Agrocybe</i>	1319.65	0.2407	<i>L</i>	**
	<i>Coprinus</i>	23,567.06	4.2981	<i>M</i>	***
	<i>Ganoderma</i>	2280.21	0.4159	<i>L</i>	**
	<i>Gymnopilus</i>	1692.45	0.3087	<i>L</i>	**
	<i>Laccaria</i>	382.04	0.0697	<i>L</i>	*
	<i>Scleroderma</i>	12.26	0.0022	<i>L</i>	*
	<i>Tilletia</i>	13.65	0.0025	<i>L</i>	*
	<i>Urediniospores</i>	6843.03	1.2480	<i>M</i>	***
	<i>Ustilago</i>	7784.85	1.4198	<i>M</i>	**
Oomycota	<i>Albugo</i>	394.56	0.0720	<i>L</i>	*
	<i>Peronospora</i>	1638.57	0.2988	<i>L</i>	**
Myxomycota	Myxomycetes	326.85	0.0596	<i>L</i>	*
Unidentified pigmented spores		45,376.624	8.2757		
Unidentified hyaline spores		8154.47	1.4872		

Representativeness: *A* abundant (>5 % of the total spore concentration); *M* moderate (1–4 %); *L* low (<0.99 %). Frequency (% of days the spore type was recovered): *** very frequent (75.1–100 %), ** frequent (36.6–75 %), * unfrequent (0.1–36.5 %)

^a *Helminthosporium* Group: hyaline to pigmented distoseptated phragmoconidia. Spore types included in this group: *Bipolaris*, *Drechslera*, *Exosporium*, *Exserohilum*, *Helminthosporium* and *Sporidesmium*

^b *Didymella* Group: hyalines or slightly colored didymospores

^c *Didymosphaeria* Group: pigmented didymospores

^d *Leptosphaeria* Group: hyaline to pigmented phragmospores. *Paraphaeosphaeria* was not include in this group due to its easy identification

anamorphs of Higher Fungi, 22 from Ascomycota, 10 from Basidiomycota, two from Oomycota and one from Myxomycota (Table 1). Anamorphs of Higher Fungi and Phylum Ascomycota were the most relevant groups, either by concentration or amount of spore types identified.

Airborne fungal spores were recorded throughout the whole year, with a wide fluctuation in the daily values (Fig. 2). A clear seasonal pattern was observed in both sampled years. Airborne fungal spores reached their highest concentration in autumn (2012–2013: 92,304.02 spores/m³; 2013–2014: 104,340.22 spores/m³) and summer (2012–2013: 80,805.87 spores/m³; 2013–2014: 117,605.51 spores/m³), while the lowest concentration occurred in winter (2012–2013: 56,213.29 spores/m³; 2013–2014: 31,054.81 spores/m³) and spring (2012–2013: 39,925.45 spores/m³; 2013–2014: 18,026.89 spores/m³) (Fig. 3). Total monthly concentrations remained low from July to

November and an increase in spore abundance was observed in December (Fig. 4). During the 2012–2013 period, the highest spore levels were noted in May, followed by June, April, February and March. During the second year, the highest concentrations were found, in descending order, in February, May, January, April and March. It is worth mentioning that low levels recorded in March 2014 may be due to the fact that 17 days were missed because of technical failure of the sampler. It is important to notice that the same seasonal pattern was observed in both studied periods where May and February registered peaks of total monthly concentration.

The analysis of monthly spore concentration according to their respective Phyla revealed a clear seasonal trend in both years of sampling for anamorphic spores (Fig. 5). In 2012–2013 the lowest concentrations were recorded in winter and early spring (from July to October), while maximum levels

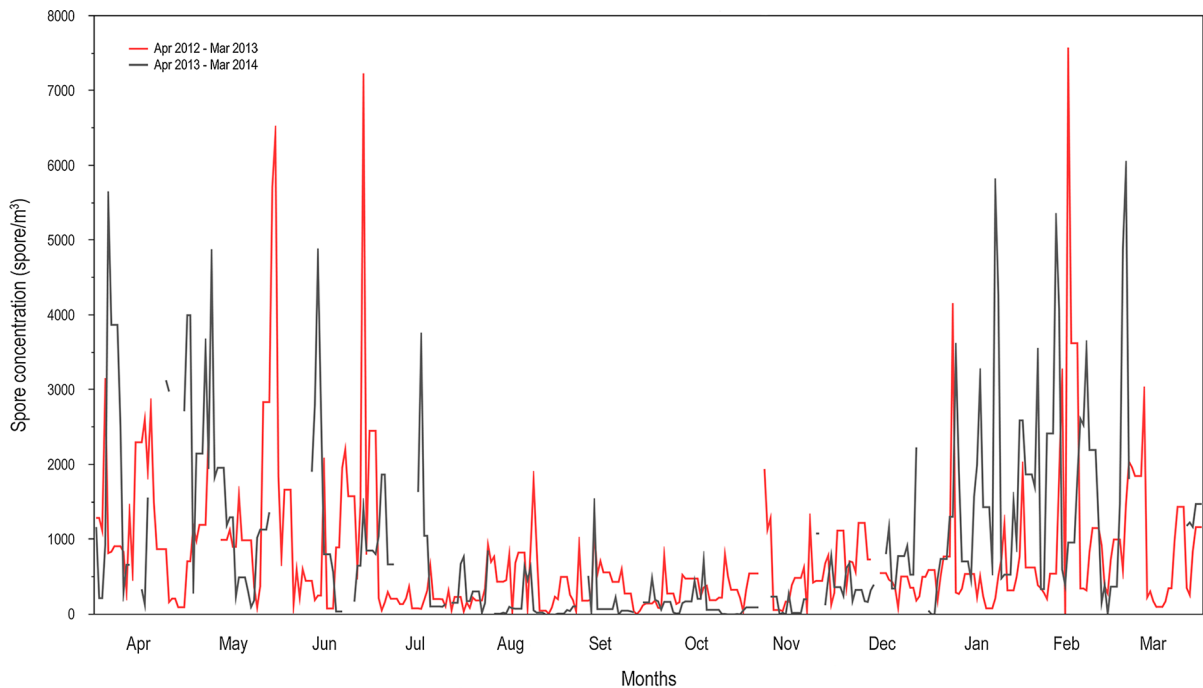


Fig. 2 Total daily concentration of airborne fungal spores in Montevideo from April 2012 to March 2014

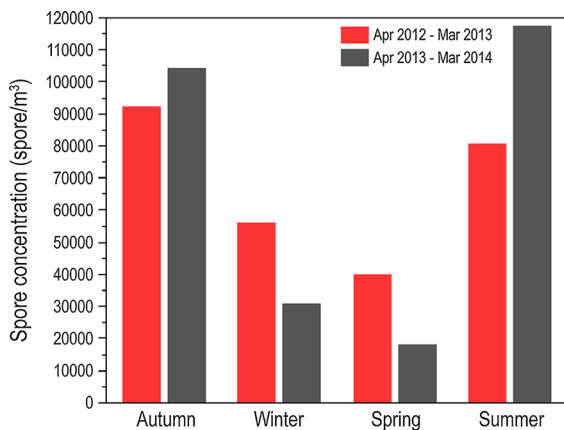


Fig. 3 Total seasonal concentration of airborne fungal spores in Montevideo from April 2012 to March 2014

occurred in autumn (from April to June) and summer (February and March). Likewise, in 2013–2014 the minimum values were observed in winter and spring (August to November), while the highest abundances were noted in autumn (April and May) and summer (January and February). Anamorphic spores were represented mainly by *Cladosporium* and secondarily by *Alternaria*.

A similar seasonal pattern was observed for members of the Phylum Basidiomycota, mainly represented by *Coprinus*, *Ustilago* and *Urediniospores*, which showed lowest levels in winter, while the highest abundances were reached in autumn and summer. In 2013–2014 an important peak of concentration was recorded in February. Spores of the Ascomycota Phylum showed no clear seasonal periodicity during 2012–2013. However, during 2013–2014 the lowest concentrations occurred in winter and spring (from August to November) and maximum levels were noted in summer (February). The Phylum Ascomycota is mainly represented by the *Didymella* and *Leptosphaeria* Groups. The Phyla Myxomycota and Oomycota had a very scarce representation and exhibited no evident seasonal pattern. To sum up, anamorphic spores and Phylum Basidiomycota were the groups which showed clear seasonal patterns in both sampling periods.

The analysis of representativeness showed that three spore types corresponded to the abundant category: *Cladosporium*, *Alternaria* and *Didymella* Group. These morphological types accounted for 65.7 % of the total record. Seven spore types were moderate and constituted 16.05 % of the total sampled

Fig. 4 Total monthly concentration of fungal spores in the atmosphere of Montevideo for both studied years (April 2012–March 2013/April 2013–March 2014). Note that 17 days were missed because of technical failure of the sampler during March 2014

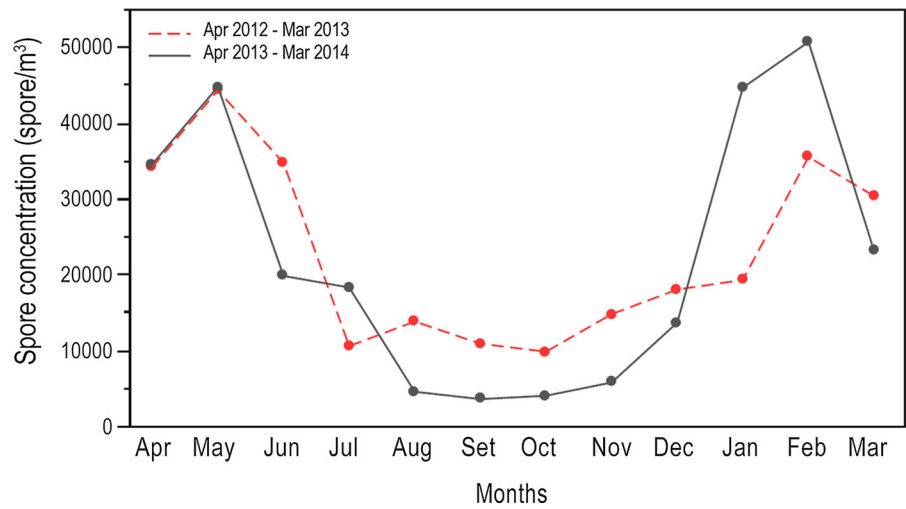
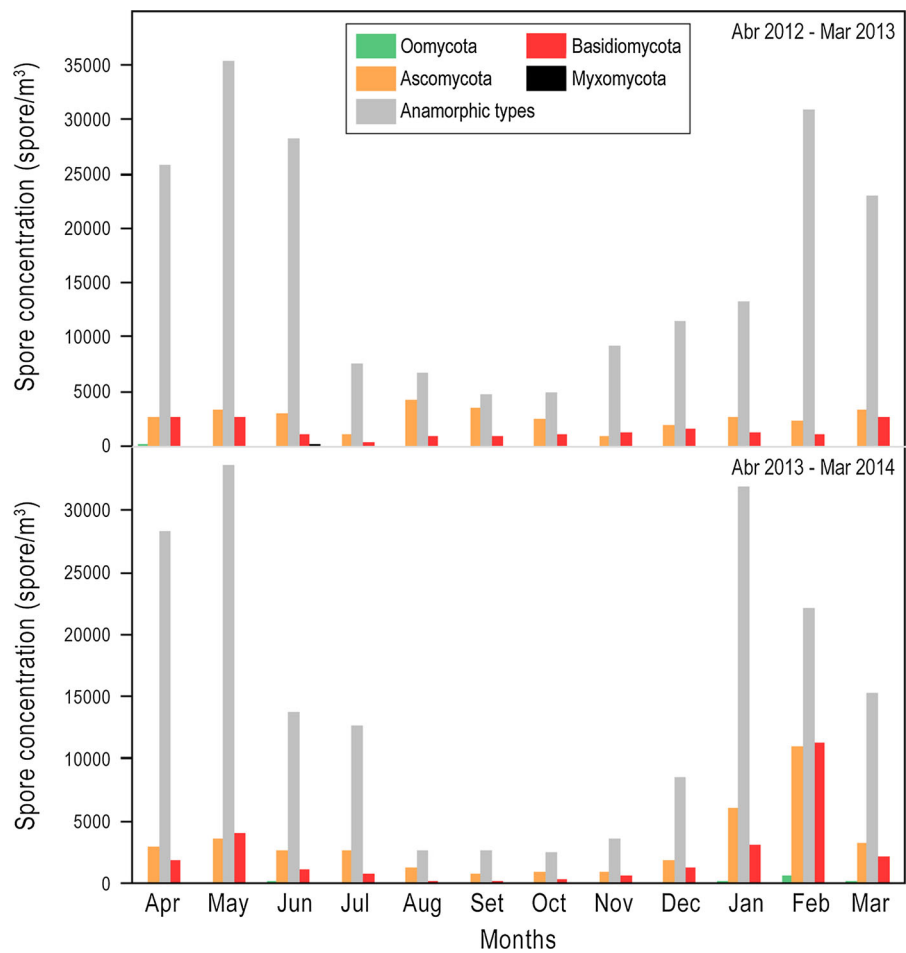


Fig. 5 Monthly concentration of airborne fungal spores belonging to Oomycota, Ascomycota, Anamorphic types, Basidiomycota and Myxomycota in Montevideo for both studied periods (April 2012–March 2013/April 2013–March 2014)



spores, being *Leptosphaeria* Group, *Coprinus* and *Epicoccum* the most important. Finally, the remaining 46 taxa corresponded to the *Low* category and represented 8.5 % of the total record. The most significant ones were *Cercospora* and *Nigrospora*.

In terms of relative frequency, eight taxa were very frequent types: *Cladosporium* (96 %), *Alternaria* (94.5 %), *Didymella* Group (91.4 %), *Leptosphaeria* Group (90.3 %), *Epicoccum* (90 %), *Coprinus* (89.8 %), *Helminthosporium* Group (83.1 %) and *Urediniospores* (78.6 %). Twenty morphological types were frequent, among which *Nigrospora* (74.1 %), *Paraphaeosphaeria* (68.9 %) and *Pithomyces* (67.5 %) were dominant. Twenty-eight spore types were unfrequent and the most prominent taxa were *Fusarium* (30.6 %), *Xylariaceae* (29.9 %) and *Sporomiella* (29.5 %).

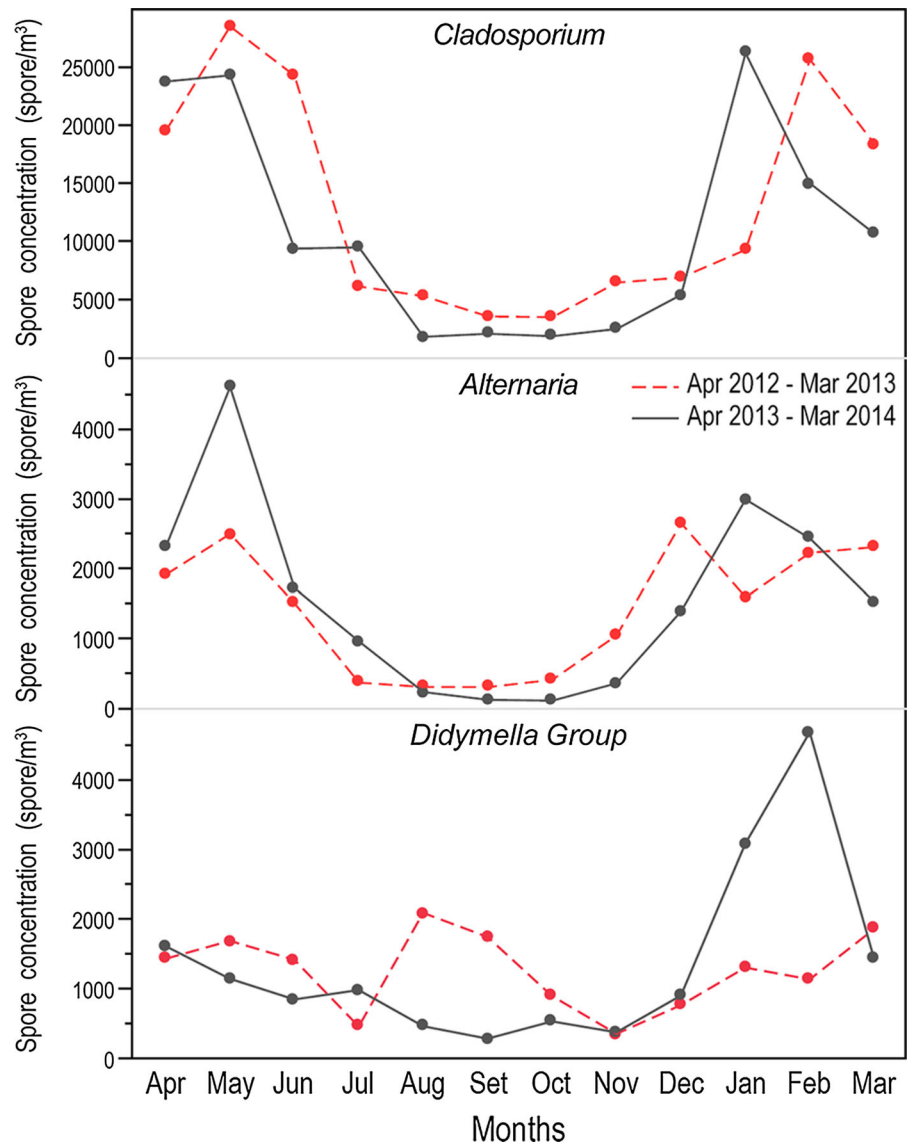
Cladosporium was the dominant component during the monitoring period, representing 53.22 % of the total record. The concentration of *Cladosporium* spores exhibited maximum values from April to June 2012, and during February and March 2013 (Fig. 6). During the period 2013–2014 very high concentrations of *Cladosporium* were recorded in April, May and January. In addition, *Cladosporium* reached levels considered allergenic, above 3000 spores/m³ according to Gravesen (1979), from January to July. On the other hand, *Alternaria* appeared as the second most frequent genera, accounting for 6.62 % of the total spectrum. *Alternaria* spores presented a peak of concentration in May for both monitoring years, and a second peak was observed in December 2012 and January 2014 (Fig. 6). Allergenic levels of *Alternaria*, above 100 spores/m³ (Gravesen 1979), were frequently registered from November to July. Finally, *Didymella* Group was the third most prevalent component, representing 5.86 % of the total sampled spores. This morphological group exhibited quite different patterns of monthly concentration when comparing both sampling periods (Fig. 6). On the one hand, during the period 2012–2013 maximum values were observed in August, September and March. However, these values were slightly higher than those recorded from April to June. On the other hand, during the period 2013–2014 very high concentrations were registered in January and February, while minimum levels were observed from August to November. In relation to allergenic levels of *Didymella*, there is no agreed upon threshold concentration

for this genera (Nasser and Pulimood 2009). In sum, *Cladosporium* and *Alternaria* showed similar seasonal patterns for both sampling periods. However, this behavior was not observed for *Didymella* Group.

The analysis of Spearman's rank correlation between total daily spore concentration and meteorological parameters for April 2012–March 2013, April 2013–March 2014 and the whole sampling period (Table 2) showed that the highest significant positive correlations were reached by maximum, minimum and average temperatures (both on the same day and 1, 2 and 3 days prior). Correlation levels for maximum temperature were similar between consecutive periods and the whole period but differed between lags. In the case of the average and minimum temperatures, a weaker positive correlation was observed. In addition, correlation values for average, maximum and minimum temperatures decreased with successive lags. Relative humidity weakly influenced spore abundance, except for the period April 2013–March 2014, when highly significant correlations were observed for the same day, and 1 and 2 days prior. Significant negative correlations between spore abundance and wind speed were observed, especially during April 2013–March 2014. No clear correlation was observed between spore content and precipitation. In conclusion, significant correlations were observed between total daily spore concentration and temperature, humidity and wind velocity.

The analysis of Spearman's rank correlation between *Cladosporium* spore concentrations and meteorological variables (Table 3) revealed, as well as in the case of total spore concentration, highly significant positive correlations for maximum, minimum and average temperatures (both on the same day and 1, 2 and 3 days prior) but of slightly lower strength. As in the case of total spore concentration, relative humidity weakly influenced spore abundance, except for the period April 2013–March 2014. *Cladosporium* abundance was significantly and negatively associated with wind speed. Precipitation showed no significant correlation with *Cladosporium* spore concentration. On the other hand, *Alternaria* concentrations were significantly and positively correlated with temperature parameters, such as maximum temperature (Table 4). A weak negative association was obtained for relative humidity in the period 2012–2013. A negative relationship occurred between *Alternaria* concentration and wind speed, but

Fig. 6 Monthly distribution of *Cladosporium*, *Alternaria* and *Didymella* Group in the atmosphere of Montevideo from April 2012 to March 2014. Note that 17 days were missed because of technical failure of the sampler during March 2014



the most significant correlation coefficients were obtained for lag 1 and 2. In addition, significant negative relationships occurred between precipitation and *Alternaria* content in April 2012–March 2013 for 1, 2 and 3 days prior. Finally, *Didymella* Group concentrations were significantly and positively correlated with temperature parameters, except for the period April 2012–March 2013 when significant correlations only occurred for maximum temperature (on the same day and 1 day prior) and average temperature (on the same day; Table 5). Highly significant positive correlations were obtained for relative humidity, especially during the period

2013–2014. Significant negative relationships occurred between *Didymella* Group abundance and wind speed during April 2013–March 2014 and the whole sampling period. Significant positive correlations were observed between precipitation and spore abundance during April 2013–March 2014 and the whole sampling period, especially for lag 1. To sum up, significant correlations were obtained between *Cladosporium*, *Alternaria* and *Didymella* Group concentrations and temperature, humidity and wind velocity. In addition, significant correlations were observed between *Alternaria* and *Didymella* Group abundances and precipitation.

Table 2 Spearman's rank correlation coefficients between total daily spore concentration and meteorological variables

Period	Average temperature				Maximum temperature			
	Same day	Lag 1	Lag 2	Lag 3	Same day	Lag 1	Lag 2	Lag 3
April 2012–March 2014	0.37***	0.36***	0.32***	0.30***	0.45***	0.44***	0.33***	0.30***
April 2012–March 2013	0.36***	0.36***	0.30***	0.22**	0.42***	0.46***	0.34***	0.23**
April 2013–March 2014	0.37***	0.36***	0.35***	0.38***	0.47***	0.41***	0.31***	0.37***
Period	Minimum temperature				Relative humidity			
	Same day	Lag 1	Lag 2	Lag 3	Same day	Lag 1	Lag 2	Lag 3
April 2012–March 2014	0.34***	0.30***	0.31***	0.26***	0.14*	0.17**	0.21***	0.15*
April 2012–March 2013	0.30***	0.26***	0.24**	0.17*	−0.005	0.008	0.114	0.079
April 2013–March 2014	0.37***	0.34***	0.36***	0.36***	0.32***	0.36***	0.33***	0.16
Period	Wind speed				Precipitation			
	Same day	Lag 1	Lag 2	Lag 3	Same day	Lag 1	Lag 2	Lag 3
April 2012–March 2014	−0.19**	−0.32***	−0.27***	−0.14*	0.07	0.002	−0.04	−0.05
April 2012–March 2013	−0.07	−0.21**	−0.28***	−0.24**	−0.06	−0.08	−0.17*	−0.15
April 2013–March 2014	−0.30***	−0.42***	−0.24**	−0.04	0.20*	0.06	0.13	0.07

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Table 3 Spearman's rank correlation coefficients between daily *Cladosporium* spore concentration and meteorological variables

Period	Average temperature				Maximum temperature			
	Same day	Lag 1	Lag 2	Lag 3	Same day	Lag 1	Lag 2	Lag 3
April 2012–March 2014	0.32***	0.32***	0.29***	0.27***	0.40***	0.40***	0.30***	0.27***
April 2012–March 2013	0.31***	0.33***	0.28***	0.20*	0.36***	0.41***	0.32***	0.21**
April 2013–March 2014	0.33***	0.32***	0.29***	0.31***	0.44***	0.38***	0.27**	0.32***
Period	Minimum temperature				Relative humidity			
	Same day	Lag 1	Lag 2	Lag 3	Same day	Lag 1	Lag 2	Lag 3
April 2012–March 2014	0.30***	0.26***	0.27***	0.23***	0.14*	0.17**	0.21***	0.11*
April 2012–March 2013	0.27***	0.24**	0.22**	0.14	−0.004	0.005	0.10	0.06
April 2013–March 2014	0.33***	0.27**	0.30***	0.31***	0.30***	0.34***	0.33***	0.15
Period	Wind speed				Precipitation			
	Same day	Lag 1	Lag 2	Lag 3	Same day	Lag 1	Lag 2	Lag 3
April 2012–March 2014	−0.21***	−0.34***	−0.26***	−0.14*	0.03	−0.05	−0.02	−0.05
April 2012–March 2013	−0.12	−0.25**	−0.28***	−0.26***	−0.10	−0.10	−0.13	−0.15
April 2013–March 2014	−0.29***	−0.42***	−0.23**	−0.03	0.17*	−0.01	0.11	0.06

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

4 Discussion

Daily monitoring of airborne fungal spores using a Rotorod sampler was carried out for the first time in Montevideo, over a 2-year period, in order to

evaluate the seasonal variation and influence of meteorological parameters on fungal spore concentration. Airborne spores occurred in Montevideo throughout the whole year; however, maximum concentrations were recorded in autumn and

Table 4 Spearman's rank correlation coefficients between daily *Alternaria* spore concentration and meteorological variables

Period	Average temperature				Maximum temperature			
	Same day	Lag 1	Lag 2	Lag 3	Same day	Lag 1	Lag 2	Lag 3
April 2012–March 2014	0.35***	0.36***	0.34***	0.35***	0.45***	0.44***	0.35***	0.33***
April 2012–March 2013	0.43***	0.44***	0.39***	0.35***	0.49***	0.52***	0.42***	0.32***
April 2013–March 2014	0.26**	0.26**	0.27***	0.34***	0.39***	0.34***	0.27**	0.33***
Period	Minimum temperature				Relative humidity			
	Same day	Lag 1	Lag 2	Lag 3	Same day	Lag 1	Lag 2	Lag 3
April 2012–March 2014	0.28***	0.27***	0.31***	0.32***	−0.16**	−0.06	0.03	−0.02
April 2012–March 2013	0.31***	0.30***	0.30***	0.30***	−0.34***	−0.30***	−0.16*	−0.12
April 2013–March 2014	0.23**	0.23**	0.29***	0.32***	0.05	0.20*	0.24**	0.09
Period	Wind speed				Precipitation			
	Same day	Lag 1	Lag 2	Lag 3	Same day	Lag 1	Lag 2	Lag 3
April 2012–March 2014	−0.11*	−0.27***	−0.23***	−0.13*	−0.04	−0.17**	−0.08	−0.06
April 2012–March 2013	−0.04	−0.20**	−0.24**	−0.23**	−0.12	−0.26***	−0.19*	−0.20**
April 2013–March 2014	−0.21*	−0.35***	−0.20*	−0.03	0.03	−0.10	0.06	0.08

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Table 5 Spearman's rank correlation coefficients between daily *Didymella* Group spore concentration and meteorological variables

Period	Average temperature				Maximum temperature			
	Same day	Lag 1	Lag 2	Lag 3	Same day	Lag 1	Lag 2	Lag 3
April 2012–March 2014	0.24***	0.23***	0.19***	0.19**	0.31***	0.28***	0.18**	0.19**
April 2012–March 2013	0.18*	0.14	0.09	0.04	0.26***	0.22**	0.11	0.05
April 2013–March 2014	0.32***	0.33***	0.31***	0.34***	0.38***	0.35***	0.26**	0.33***
Period	Minimum temperature				Relative humidity			
	Same day	Lag 1	Lag 2	Lag 3	Same day	Lag 1	Lag 2	Lag 3
April 2012–March 2014	0.24***	0.21***	0.19***	0.18**	0.31***	0.35***	0.25***	0.16**
April 2012–March 2013	0.14	0.11	0.05	0.04	0.21**	0.26***	0.19*	0.16*
April 2013–March 2014	0.36***	0.32***	0.33***	0.32***	0.45***	0.46***	0.34***	0.17
Period	Wind speed				Precipitation			
	Same day	Lag 1	Lag 2	Lag 3	Same day	Lag 1	Lag 2	Lag 3
April 2012–March 2014	−0.20***	−0.27***	−0.24***	−0.05	0.13*	0.20***	0.03	0.01
April 2012–March 2013	−0.06	−0.12	−0.22**	−0.08	0.08	0.15	−0.10	−0.02
April 2013–March 2014	−0.33***	−0.43***	−0.25**	−0.03	0.17	0.26**	0.18*	0.06

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

summer. Most spores identified belonged to anamorphs of Higher Fungi and the most abundant spore types were *Cladosporium*, *Alternaria*, *Didymella* Group, *Leptosphaeria* Group and *Coprinus*. Spore concentration levels for total spore,

Cladosporium, *Alternaria* and *Didymella* Group showed significant correlation with temperature, humidity and wind velocity for at least one sampling period. In addition, temperature appeared to be the most influential meteorological factor.

Airborne fungal spores occurred in Montevideo throughout the whole year. However, a seasonal pattern was revealed, with the highest spore concentrations recorded in autumn and summer, while minimum abundances were observed in winter and spring. Similar results were obtained for some locations of this South-American region, including Sao Paulo (Gambale 1980), Pelotas (Bernardi et al. 2006) and Santiago de Chile (Ibáñez et al. 2001), where the highest spore concentration were observed in autumn. On the other hand, a summer peak in spore concentrations was reported for other cities of the region, such as Caxias do Sul (De Antoni Zoppas et al. 2006) and La Plata (Mallo et al. 2011). It was observed that in temperate climates the highest spore concentrations occur during summer and late fall (Kasprzyk 2008). The summer peak in spore concentration might be due to favorable temperature (Grinn-Gofroń 2008). The still high content of spores observed in autumn could be explained by the decay of high amounts of vegetable matter occurring during this period (Levetin and Horner 2002; Grinn-Gofroń 2008). In winter and early spring, the substrate for spawn development is scarce and as a result airborne spores are absent or their concentrations are low (Kasprzyk 2008).

The vast majority of spores recorded in this study corresponded to anamorphs of Higher Fungi. This result is consistent with several studies which demonstrate that these spore types are more prevalent in temperate regions, while Basidiomycota and Ascomycota spores predominate in tropical areas (Rivera-Mariani and Bolaños-Rosero 2012). The most abundant spore types were *Cladosporium*, *Alternaria*, *Didymella* Group, *Leptosphaeria* Group and *Coprinus*. *Cladosporium* was the main component of the spore spectrum, and its dominance over other spores was noted in several regions with different weather conditions (Herrero et al. 1996; Sabariego et al. 2000; Ibáñez et al. 2001; Bernardi and Nascimento 2005; De Antoni Zoppas et al. 2006; Díez et al. 2006; Kasprzyk and Worek 2006; Negrin et al. 2007; Grinn-Gofroń 2008; Oliveira et al. 2009a; Mallo et al. 2011; Gadekar 2014). In this study *Alternaria* appeared as the second most important genera. The same results were obtained in cities of the region, such as Pelotas (Bernardi and Nascimento 2005), La Plata (Negrin et al. 2007) and Santiago de Chile (Ibáñez et al. 2001). Although spores of *Alternaria* reached much lower values than those of *Cladosporium*, seasonal variation

of these genera indicate that they have a similar behavior, with maximum values in autumn and late spring–summer. The third most prevalent component corresponded to *Didymella* Group. As mentioned before, this morphological group exhibited quite different patterns of monthly concentration when comparing both sampling periods. During the period 2013–2014, the occurrence of high *Didymella* spores levels in the atmosphere throughout summer (January and February) is consistent with previous studies (Harries et al. 1985; Frankland and Gregory 1973; Corden and Millington 1994; Richardson 1996; Stępańska and Wołek 2005; Grinn-Gofroń 2008; Grinn-Gofroń and Mika 2008; Stępańska et al. 2012). However, during the period 2012–2013 no clear seasonal pattern was observed since maximum values were recorded in winter (August and September) and summer (March). In addition, these values were slightly higher than those recorded in autumn (from April to June). The inconsistencies observed when comparing both sampling periods may be due to the fact that different genera could be included in this morphological group.

Temperature appeared to be the most influential meteorological factor correlating significantly and positively with total airborne spore levels. This was also revealed by authors, such as Troutt and Levetin (2001) and Levetin and Dorsey (2006). According to the results obtained in the present study, temperature was the most important meteorological factor not only for total spore concentration but also for *Cladosporium* and *Alternaria* abundance. Previous studies confirmed the positive influence of temperature on *Cladosporium* and *Alternaria* spore concentrations (Rodríguez-Rajo et al. 2005; Oliveira et al. 2009b; O'Connor et al. 2014). In addition, Sabariego et al. (2000), Grinn-Gofroń et al. (2011) and Recio et al. (2012) found significantly positive correlations with average, maximum and minimum temperatures, agreeing with this study. *Didymella* Group concentrations were significantly and positively correlated with temperatures parameters, except for the period April 2012–March 2013 when significant correlations only occurred for maximum temperature (on the same day and 1 day prior) and average temperature (on the same day). In accordance with these results, Stępańska and Wołek (2005), Grinn-Gofroń (2008), Grinn-Gofroń and Mika (2008) and Stępańska et al. (2012) observed positive correlations with minimum temperature while

O'Connor et al. (2014) found positive correlations with average temperature.

Relative humidity weakly influenced total spore and *Cladosporium* concentrations, except for the period April 2013–March 2014, when highly significant positive correlations were found. In addition, highly significant positive correlations were obtained between *Didymella* Group abundance and relative humidity. However, a weak negative association was obtained for *Alternaria* abundance in the period April 2012–March 2013. An inverse relation between relative humidity and *Cladosporium* and *Alternaria* spore concentrations was observed in other studies (Fernández et al. 1998; Sabariego et al. 2000; Grinn-Gofroń 2008; Grinn-Gofroń and Rapiejko 2009; Oliveira et al. 2009b; Grinn-Gofroń et al. 2011; Recio et al. 2012). In agreement with the results obtained in this study, Katial et al. (1997) and Ho et al. (2005) found *Cladosporium* to be positively correlated with relative humidity. Considerable reductions in relative humidity causes major releases of *Cladosporium* and *Alternaria* spores (Jones et al. 2004) since they are members of the “dry air spora” (Burge 1986) which tend to be found in higher concentrations during warm, dry weather conditions with high wind velocities (Troutt and Levetin 2001). On the other hand, previous studies confirmed the correlation between *Didymella* spores and relative humidity (Wahl and Kersten 1991; Richardson 1996; Sousa et al. 2008; Oliveira et al. 2009b; Stępańska et al. 2012; O'Connor et al. 2014). These “wet air spores” are released into the atmosphere by processes related to humidity conditions or rain (Stępańska and Wotek 2009).

Significant negative correlations between wind speed and total spores, *Cladosporium*, *Alternaria* and *Didymella* Group concentrations were observed in this study. Grinn-Gofroń et al. (2011) found low negative correlations between *Cladosporium* and *Alternaria* counts and average and maximum wind speed. In addition, Munuera et al. (2001) observed that low wind velocities favoured high *Alternaria* spore counts. In contrast to these results, positive correlations with wind velocity have been described in many sites throughout the world. Since *Cladosporium* and *Alternaria* spores are released by the action of wind it would be expected that their dispersion was favoured by increasing wind velocities. However, this effect was not observed in this study. In relation to *Didymella*, Stępańska et al. (2012) observed that wind

speed was correlated positively with spore number. However, the vast majority of studies found no significant correlations between wind speed and spore concentration.

Finally, significant negative relationships occurred between *Alternaria* concentration and precipitation of the 1, 2 and 3 previous days in April 2012–March 2013. Because a negative association was also obtained for relative humidity during this sampling year, it could be argued that rainfall could have caused an increase in relative humidity which would have led to a decrease of *Alternaria* content in the following days. Negative correlation between *Alternaria* and precipitation was also observed by Herrero et al. (1996), Mitakakis et al. (1997), Corden and Millington (2001), Oliveira et al. (2009b) and O'Connor et al. (2014). In addition, rain could stimulate sporulation and release of fungal spores; however, excessive rain tends to remove spores from the atmosphere (Burge 1986). This mixed effect of precipitation may explain conflicting results obtained. On the other hand, significant positive correlations were observed between *Didymella* Group spore abundance and precipitation during April 2013–March 2014 and the whole sampling period, especially for lag 1. Previous studies confirmed the positive correlation between *Didymella* spores and rainfall (Harries et al. 1985; Corden and Millington 1994; Grinn-Gofroń 2008; Grinn-Gofroń and Mika 2008; Sousa et al. 2008; Oliveira et al. 2009b; O'Connor et al. 2014).

Variation in airborne spore diversity and concentration cannot be explained exclusively by meteorological variables. Others factors, such as, the availability of organic matter for the development of the fungus and circadian cycle of light and darkness (Kasprzyk 2008) play an important role. In addition, human activity could alter the seasonal distribution and diversity of the captured fungal bioaerosols. Disturbance regimens, such as mowing, excavation and harvesting can enhance the aerosolization of specific phylloplane genera and result in elevated counts (Sreeramulu 1958; Pawsey and Heath 1964; Corden and Millington 2001). Urbanization seems to affect negatively spores records since they are usually lower in urban than in rural atmospheres (Simeray et al. 1993; Kasprzyk and Worek 2006; Oliveira et al. 2009b). Moreover, some meteorological factors, such as rainfall and relative humidity, have mixed effects on fungal concentrations.

Several spore types identified in the air of Montevideo (e.g., *Cladosporium*, *Alternaria*, *Coprinus*, *Epicoccum*, *Ustilago*, *Helminthosporium*, *Nigrospora*, *Torula*, *Curvularia*, *Ganoderma*, *Stemphylium*) have been reported as allergenic or with the potential of being allergenic (Burge 1985). Threshold concentrations for evoking allergic symptoms are estimated to be 100 spores/m³ for *Alternaria* and 3000 spores/m³ for *Cladosporium* (Gravesen 1979). These values were registered in this study from November to July for *Alternaria* and from January to July for *Cladosporium*. Nevertheless, threshold levels of *Alternaria* were recorded on a large number of days, while *Cladosporium* was found at allergenic levels only in a few days. For this reason, the risk of allergy due to airborne *Alternaria* spores in Montevideo would be high from November to July. On the other hand, Targonski et al. (1995) reported that the risk of asthma related deaths was increased 2.16 times when the total spore count was 1000 spores/m³. In this study daily levels of spores were frequently over 1000 from December to June; therefore, during this period the risk of severe asthma could have been high.

5 Conclusion

A great diversity of airborne fungal spores was registered in Montevideo throughout the whole year. However, a seasonal pattern was revealed, with the highest spore concentrations recorded in autumn and summer, while minimum abundances were observed in winter and spring. The predominant airborne fungi were *Cladosporium*, *Alternaria*, *Didymella* Group, *Leptosphaeria* Group and *Coprinus*. *Cladosporium* and *Alternaria* had a similar spore seasonal pattern with the highest counts in autumn and late spring–summer. On the other hand, *Didymella* Group exhibited quite different patterns of monthly concentration when comparing both sampling periods. During the period 2013–2014, maximum values were registered in summer. However, during the period 2012–2013 no clear seasonal pattern was observed since maximum levels were recorded in winter and summer. In addition, these values were slightly higher than those recorded in autumn.

Temperature was the most influential meteorological factor correlating significantly and positively with total spore, *Cladosporium*, *Alternaria* and *Didymella*

Group abundance. Relative humidity influenced positively total spore and *Cladosporium* concentrations during the period April 2013–March 2014, while a weak negative association was obtained for *Alternaria* abundance in April 2012–March 2013. In addition, highly significant positive correlations were obtained between *Didymella* Group abundance and relative humidity, especially during the period 2013–2014. Significant negative correlations between wind speed and total spore, *Cladosporium*, *Alternaria* and *Didymella* Group concentration were observed. Significant and negative relationships occurred between *Alternaria* concentrations and precipitation of the 1, 2 and 3 previous days in April 2012–March 2013. Significant positive correlations were observed between *Didymella* Group spore abundance and precipitation during April 2013–March 2014 and the whole sampling period, especially for lag 1.

Alternaria reached allergenic threshold levels on a large number of days from November to July. Therefore, during this period the risk of exposure to *Alternaria* spores in Montevideo would be high. In addition, the risk of exposure to fungal spores that could cause severe asthma would have been high from December to June due to the important level of spores recorded during this period.

Finally, long-term sampling is needed to define seasonal prevalence patterns and obtain a better correlation between fungal spore abundance and meteorological variables.

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