Int J Biometeorol (2014) 58:1459–1470
DOI 10.1007/s00484-013-0748-6

ORIGINAL PAPER

Temporal dynamics of airborne fungi in Havana (Cuba) during dry and rainy seasons: influence of meteorological parameters

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Received: 10 May 2013 / Revised: 23 September 2013 / Accepted: 24 September 2013 / Published online: 20 October 2013

Abstract The aim of this paper was to determine for first time the influence of the main meteorological parameters on the atmospheric fungal spore concentration in Havana (Cuba). This city is characterized by a subtropical climate with two different marked annual rainfall seasons during the year: a "dry season" and a "rainy season". A nonviable volumetric methodology (Lanzoni VPPS-2000 sampler) was used to sample airborne spores. The total number of spores counted during the 2 years of study was 293,594, belonging to 30 different genera and five spore types. Relative humidity was the meteorological parameter most influencing the atmospheric concentration of the spores, mainly during the rainy season of the year. Winds coming from the SW direction also increased the spore concentration in the air. In terms of spore intradiurnal variation we found three different patterns: morning maximum values for *Cladosporium*, night peaks for *Coprinus* and *Leptosphaeria*, and uniform behavior throughout the whole day for *Aspergillus*/*Penicillium*."

Keywords Airborne fungal spore · Meteorological parameter · Dry and rainy season · Cuba

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Introduction

Analysis of the airborne dynamics of fungal spores is a valuable tool in the diagnosis and prevention of respiratory diseases. Because of their small size, aeroallergens can penetrate easily into the respiratory tract, prompting episodes of asthma and allergic rhinitis in sensitive people. These pathologies affect over 25 % of the population in the industrialized world (Kurup et al. 2002). In Cuba, it has been proved that allergenic processes have a higher prevalence in urban areas like Havana (Venero et al. 2009; Díaz et al. 2010). A number of epidemiological studies have drawn attention to the increasing prevalence and severity of respiratory diseases in recent decades due to climate change (Antico 2000; D'Amato and Cecchi 2008).

Weather conditions influence the biology of fungi (and consequently the production, release, dispersion and deposition of spores) as well as the diversity and number of airborne bioparticles (Şakiyan and Inceoglu 2003). Several authors have noted the influence of seasonal and daily variations of temperature and humidity, as well as the crucial effect of wind speed (vector of passive transport) and washout caused by rainfall, on the timing and magnitude of airborne spore counts (Burch and Levetin 2002; Stepalska and Wolek 2009; De Linares et al. 2010; Aira et al. 2012, 2013).

Although little research has specifically addressed airborne spore levels in Cuba, various studies have highlighted the marked influence of weather-related variables on fungal development in a range of substrates, and on the movement of spores once they become part of the bioaerosol, in other Caribbean countries (Rosas et al. 1992; Prospero et al. 2005). Aerobiological research in Cuba has focused on characterizing airborne fungal spores in different environments (Arnold et al. 1987; Herrera et al. 2003; Rojas et al. 2007; Almaguer et al. 2012; Rojas and Aira 2012) rather than on

examining the effect of meteorological factors on the timing and abundance of airborne spores. Systematic aerobiological monitoring has now made it possible to chart seasonal variations in fungal spore counts over the course of the year, and to determine the time of day at which peak counts are recorded. In the literature, many authors have suggested that intradiurnal differences in airborne spore counts depend on several variables: meteorological conditions, biogeographical location and distance from the source of production of fungal propagules (Hollins et al. 2004; Stennett and Beggs 2004).

Detailed analysis of weather-related data in conjunction with data on airborne spore dynamics is essential in order to estimate the potential impact of bioaerosol particles on human health in a given biogeographical area. Accordingly, this paper sought to determine, for the first time, the influence of major meteorological parameters on airborne spore counts in a subtropical climate with a distinct unimodal rainfall pattern, i.e., one dry season and one rainy season per year. Due to the volumetric method used, the findings have applications in various key fields including medicine (in order to prevent allergies) and agriculture (in order to prevent fungal diseases in plants).

Materials and methods

Study area and meteorological data

The study was conducted in the city of Havana (23°08′ N and 82°23′ W), located on the Northern coast of the island of Cuba (Fig. 1). This city is situated in the smallest province (721.01 km^2) of the island, but is the most populated with around the 20 % of the Cuban population. Vegetation in the city of Havana is shaped largely by human activity, with a prevalence of ornamental park-type plants, and anthropogenic plant communities in the form of lawns, gardens and a certain amount of urban agriculture in peripheral areas. Sampling was conducted over 2 years (November 2010 to October 2012). The subtropical climate of the island is characterized by the alternation of two weather seasons throughout the year—a dry (November–April) and a rainy (May–October) season—therefore two spore seasons were obtained for each year (Table 1). During the dry seasons total rainfall registered oscillated from 257.5 mm to 323.9 mm, whereas in the wet season rainfall exceeded 700 mm. The average of the maximum, mean and minimum temperatures is higher during the rainy seasons than in the dry period, with more than 5 °C of difference in the case of the minimum temperature. The same behavior was registered for relative humidity (70.5 % in dry season 1 vs 78.2 % in rainy season 2). The average wind speed was also higher during the dry season, with values around 14 km/h, which decrease by half during the rainy season. The predominant wind direction throughout the study period was northeast (NE). The daily values of meteorological variables were recorded by the meteorological station of Casablanca, which is located in Havana city (23°09′N and 82°20′W) at 50 m above sea level with a coastal location 4.39 km far from the Faculty of Biology in a straight line across the bay.

Aerobiological sampling

For the collection of the aerobiological data a volumetric sampler model Lanzoni VPPS-2000 (Bologne, Italy) was used. The sampler was located permanently on a terrace of the Faculty of Biology of the University of Havana, a central and busy area of the city. For sample and data processing the protocol proposed by the Spanish Aerobiology Network was followed (Galán et al. 2007). The Lanzoni VPPS-2000 flow

Fig. 1 Sampling location in Havana, northwestern Cuba

rate was adjusted to 10 l/min, a value similar to human breath. Spores were collected on Melinex adhesive tape and cut into daily segments (48 mm strips representing 24 h exposure). These segments were then mounted on microscope slides in glycerol jelly containing fuchsine (0.1 %) and protected with a cover glass. The spores were observed and identified using biological microscope at a magnification of 400X along two longitudinal traverses (Mallo et al. 2011). Spore counts were then converted into spores per cubic meter of air sampled (spore/m^3 day).

For taxonomic identification of spores, reference preparations from the collection of cultivations of the Department of Microbiology and Virology of the University of Havana as well as different specialized atlas books (among others: Ellis 1971, 1976; Käärik et al. 1983; Barnet and Hunter 1997; de Hoog et al. 2000; Pitt 2000; Kirk et al. 2001; Klich and Pitt 2002) were used.

Statistical methods

The data are presented and discussed considering the two weather stations observed in each year rather than taking into account a calender year. A Scheffé test was performed to identify potential differences between spore counts in dry vs rainy seasons. The Spearman test was used to discover potential correlations between mean daily airborne spore counts on given dates and major weather-related data on those dates and the days prior to them. The significance was calculated using IBM Statistics software version 2.0. To determine the influence of the wind direction, a one-way design permutational multivariate analysis of variance (PERMANOVA) was conducted. The eight wind directions were considered as groups and the spore concentration as observations. The significance was computed by permutation of group membership, with 10,000 replicates based on a Euclidean distance measure (Anderson 2001; McArdle and Anderson 2001). It was calculated using a PAST software version 1 (Hammer et al. 2001).

In order to obtain a model that reflects the intradiurnal fluctuations of the spore counts, during the sampling period, days without rainfall (in order to avoid the washing effect produced by rain only in particular hours of the day) in which the spore concentrations were higher than the mean were selected (Nilsson and Persson 1981). The resulting days were used to calculate the mean spore values every 2 h, thereafter expressing the data as percentages.

Results

A total of 293,594 airborne spores were counted over the 2-year study; spores belonged to 30 different genera (*Alternaria*, *Beltrania*, *Bipolaris*, *Cercospora*, *Chaetomium*, *Coprinus*, *Curvularia*, *Epicoccum*, *Fusarium*, *Ganoderma*, *Gliomastix, Helicoma, Helicomyces*, *Leptosphaeria*, *Monodictys*, *Nigrospora*, *Paraphaeosphaeria*, *Periconia*, *Pestalotiopsis*, *Pithomyces*, *Pleospora*, *Pseudocercospora*, *Pyricularia*, *Spegazzinia*, *Sporidesmium*, *Sporormiella*, *Stemphylium*, *Tetraploa*, *Torula* and *Venturia*) and five spore types (*Aspergillus/ Penicillium* type, *Cladosporium cladosporioides* type, *Cladosporium herbarum* type, Uredinales type and Xylariaceae type).

Spore types and genera accounting for over 1 % of the total spore count were more abundant during the dry season than in the rainy season, with the exception of *Coprinus, Lepthosphaeria* and *Ganoderma* spores (Table 2). The Scheffé test revealed statistically significant differences between the *Cladosporium*, *Lepthosphaeria* and *Coprinus* counts in rainy vs dry seasons, whereas no inter-season difference was found for *Aspergillus/Penicillum* or for total spore counts (Table 3). *Cladosporium* spores predominated over the rest in both seasons (68.49 % of the total spores in dry season and 50.55 % in rainy season). Moreover, the *Aspergillus/Penicillum* type achieved a remarkable representation followed by *Curvularia*, *Periconia*, *Nigrospora* and Uredospores. The maximum daily values of the spore types identified generally

Table 1 Climatic characterization of the dry and rainy seasons (November 2010–October 2012). Data from INSMET, Casablanca meteorological station

	Dry season 1	Rainy season 1	Dry season 2	Rainy season 2	1st year	2nd year
Rainfall (mm)	257.5	771.0	323.9	889.3	1,046.5	1,213.2
No. of rainy days	32	71	48	75	103	148
Maximum temperature (°C)	27.0	31.3	27.5	30.8	29.2	29.2
Minimum temperature (°C)	18.9	23.9	20.2	24.1	21.4	22.2
Mean temperature (°C)	22.8	27.2	23.5	26.9	25.0	25.3
Sunshine (hours)	8.2	7.9	8.1	7.5	8.1	7.8
Relative humidity (%)	70.5	75.5	72.3	78.2	73.1	75.3
Wind speed (km/h)	14.0	7.6	14.9	9.6	10.8	12.3
Predominant wind direction (%)	NE (43 %)	NE (44 %)	NE (45 %)	NE (42 %)	NE (43 %)	NE (44 %)

Table 2 Percentage of spore types identified during the dry and rainy seasons, daily maximum value and date

Spore type	Dry seasons Average values (%)	Rainy seasons	Daily maximum (spores/m^3)	Date
Cladosporium	68.49	50.55	4,337	23 May 2011
Coprinus	3.73	18.97	880	30 September 2011
Lepthosphaeria	8.99	15.28	576	21 May 2012
Asperg-Penicillium	7.27	6.46	384	11 April 2011
Curvularia	1.64	1.56	148	24 September 2011
Periconia	1.30	0.73	91	20 January 2011
Nigrospora	1.19	0.90	63	20 January 2011
Uredinales	1.15	0.19	33	23 February 2012
Gliomastix	0.82	0.28	88	6 November 2010
Chaetomiun	0.79	0.05	341	4January 2012
Monodictys	0.65	0.17	107	28 November 2010
Alternaria	0.63	0.62	56	2 October 2011
Bipolaris	0.55	0.51	76	30 June 2011
Xylariaceae	0.41	0.16	70	14 November 2010
Torula	0.38	0.18	35	3 November 2010
Fusarium	0.30	0.42	40	28 August 2011
Pithomyces	0.27	0.29	23	18 February 2012
Ganoderma	0.25	1.05	87	28 December 2010
Epicoccum	0.20	0.01	33	17 November 2010
Venturia	0.17	0.30	44	2 May 2012
Spegazzinia	0.15	0.06	21	23 November 2011
Cercospora	0.14	0.28	17	21 August 2011
Pseudocercospora	0.13	0.18	28	26 May 2011
Pestalotiopsis	0.11	0.07	8	11 September 2012
Pleospora	0.10	0.29	13	5 September 2012
Tetraploa	0.04	0.02	2	29 June 2012
Paraphaeosphaeria	0.04	0.08	9	31 May 2012
Stemphilium	0.03	0.01	44	4 September 2011
Helicomyces	0.02	0.07	9	29 July 2011
Sporidesmiun	0.02	0.03	4	14 September 2012
Beltrania	0.02	0.02	4	26 May 2012
Sporormiella	0.01	0.11	19	26 June 2011
Pyricularia	0.01	0.09	19	28 July 2011
Helicoma	0.01	0.01	2	21 February 2011

coincide with the presence of the most abundant types in the atmosphere. The maximum daily concentrations of *Cladosporium* (with 4,337 spores/m^3 recorded 26 May 2011), *Chaetomium* (341 spores/m^3, 4 January 2012) and *Monodictys* (107 spores/m^3, 28 November 2010) should be highlighted.

When the seasonal occurrence of the spores during the 2 years was analyzed (Fig. 2), it was observed that the highest concentrations were recorded between the months of November 2010 and October 2011. The maximum total monthly value of 31,663 spores was registered during January 2011 as well as the highest maximum daily concentrations (4,249 spores/m^3, 21 January). The peak daily concentration during the study period slightly exceeded this value (4,771 spores/m^3 registered on 26 May 2011). A prevalence of the higher daily concentrations was observed during the dry season in the 1st year of study, whereas during the 2nd year

Table 3 Scheffeè test values

Spore type	Rainy-dry season
Cladosporium	0.0157*
Lepthosphaeria	0.0000*
Coprinus	0.0000*
Aspergillus/Penicillum	0.6113
Total spores	0.8999

*$P<0.05$

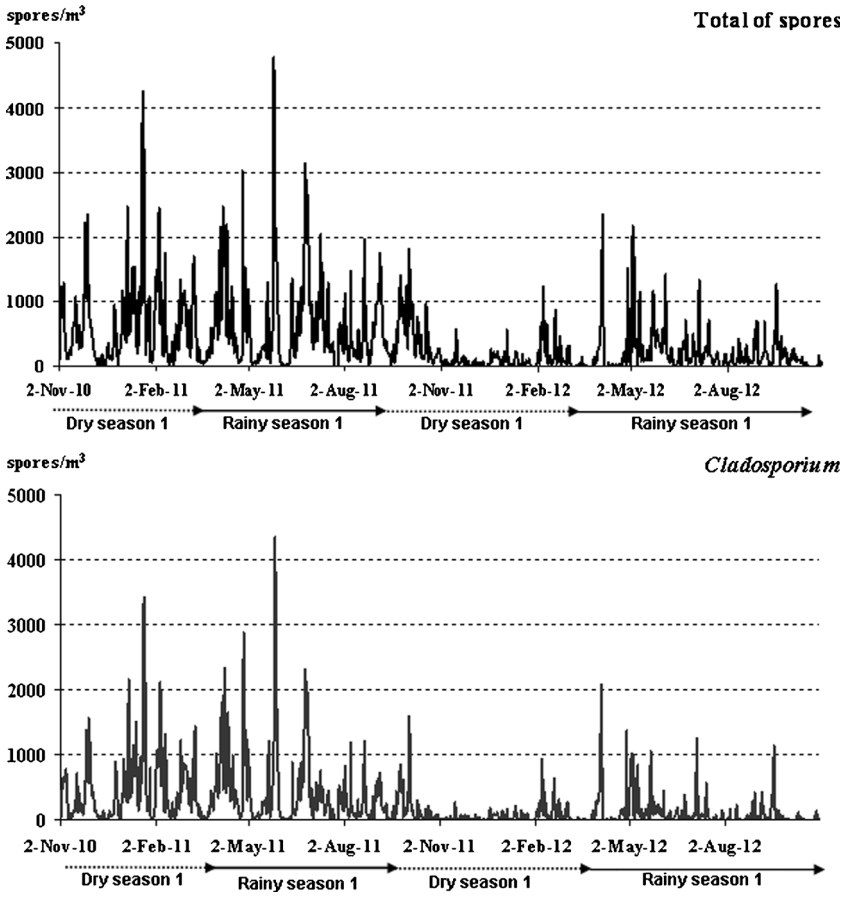

Fig. 2 Total spore concentration and *Cladosporium* spores during the dry and rainy seasons (November 2010–October 2012)

the spore concentration values were slightly higher in the rainy season.

The occurrence of *Cladosporium* spores (with a marked seasonal character) is similar to that observed for most genera and spore types (Fig. 3). By contrast, *Leptosphaeria* and *Coprinus* spores were most abundant during the rainy seasons (with daily peaks of 880 and 576 spores/m^3, respectively) and the *Aspergillus*/*Penicillium* type did not show clear seasonal variations (in spite of its marked decrease observed during the dry season of the 2nd year of the study).

With the aim of verifying the influence of the main meteorological parameters on the total airborne concentration of spores, bivariate correlations were conducted for the two seasons of each year and for each year of study (Table 4). Relative humidity was the parameter that was most influential for the atmospheric concentration of spores with a positive effect and high degree of significance (P <0.05), (mainly the relative humidity of the previous days in the case of *Aspergillus/Penicillum*, *Coprinus* and *Cladosporium*). The rainfall of the previous days also favors the presence of spores in the atmosphere. High maximum temperature values are optimal conditions for the appearance of spores in the atmosphere. The minimum and mean temperatures generally influence spore presence of the most abundant genera positively during the dry season and negatively during the rainy seasons. Sunshine hours and wind velocity induced a negative effect in the airborne presence of the spores (mainly the wind speed of the previous days). The Permanova test conducted showed the higher significant comparisons (P <0.05) with winds coming from the southern, western and southwestern directions (Table 5) for the total spore amount (southern wind 693 spores and southwestern wind 684 spores) and the predominant spore types (mainly *Cladosporium*: southern wind 420 spores and southwestern wind 416 spores/m^3).

Analysis of intradiurnal variations in total spore counts showed that peak counts for most airborne spore types were recorded between 11:00 a.m. and 12:00 noon (Fig. 4). This pattern was observed for *Cladosporium* spores, with a slight additional increase in the early morning, at around 5:00 a.m.–6:00 a.m., corresponding with the maximum concentration of *Coprinus* and *Leptosphaeria* in the atmosphere. *Aspergillus*/*Penicillium* spore counts fluctuated throughout the day, with no clearly discernible pattern.

Discussion

There has been little research into airborne fungal diversity and the influence of weather-related factors on spore counts in

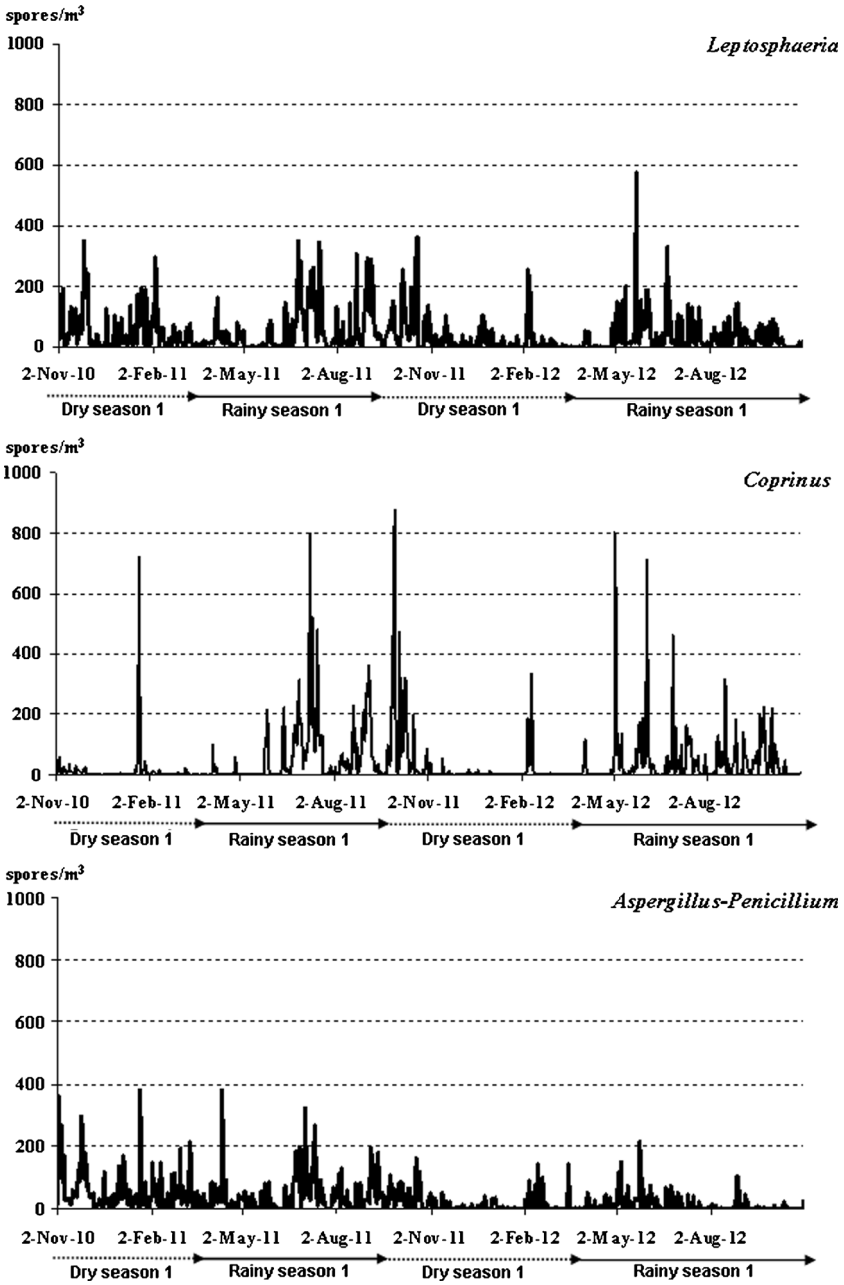

Fig. 3 *Leptosphaeria*, *Coprinus* and *Aspergillus/Penicillium* spore concentrations (spores/m^3) during the dry and rainy seasons (November 2010–October 2012)

the Caribbean area (Quintero et al. 2010). However, studies in other tropical areas report that airborne spore counts display seasonal behavior linked to the rainfall pattern (Hurtado et al. 1989; Chew et al. 2000; Levetin and Horner 2002). Data collection for the present study in Cuba was structured to reflect the island's unimodal rainfall pattern, with one dry season and one rainy season each year. High temperatures and elevated relative humidity favored significant airborne fungal spore counts throughout the year (Quintero Fossas 1964) in nearby areas such as Mexico city (Calderón et al. 1997) and Puerto Rico (Rivera-Mariani and Bolaños-Rosero 2012). Moreover, the influence of wind on the spore concentration in the atmosphere is discussed since some studies indicate that high wind speed favors the suspension of microorganisms and others have stated that fungal concentration decreases due its atmospheric dilution effect (Sabariego et al. 2000; Jones and Harrison 2004; Stennett and Beggs 2004). Wind speed had a negative effect on airborne spore levels in Havana, particularly on total and *Coprinus* spore counts. Aira et al. (2009) noted a correlation between low wind speeds and high basidiospore counts, while increased wind speeds can induce water loss from the basidiocarp and thus the suppression of spore production (Grinn-Gofroń and Strzelczak 2011). Given Havana's coastal location, the

Table 4 Correlations between concentrations of spores and the main meteorological parameters during the dry and rainy seasons. 1st year, $N=364$; 2nd year, $N=366$; rainy season 1 (RS1), $N=184$; rainy season 2 (RS2), $N=182$; dry season 1 (DS1), $N=180$; dry season 2 (DS2), $N=182$. *RH* Relative humidity

	Cladosporium						*Lepthosphaeria*						*Coprinus*						*Aspergillus/Penicillum*					
	1st year	2nd year	RS1	RS2	DS1	DS2	1st year	2nd year	RS1	RS2	DS1	DS2	1st year	2nd year	RS1	RS2	DS1	DS2	1st year	2nd year	RS1	RS2	DS1	DS2
Rainfall	0.086	−0.033	0.256*	−0.006	0.069	−0.009	0.246*	0.255*	0.282*	0.173*	0.215*	0.061	0.079	0.039	0.034	−0.010	0.028	0.084	0.097	0.013	0.218*	0.028	0.067	0.067
R-1	0.088	0.141*	0.036	0.142	0.176*	0.123	0.303*	0.126*	0.251*	0.110	0.300*	0.092	0.168*	0.093	0.565*	0.045	0.052	0.064	0.009	−0.019	−0.091	−0.022	0.064	−0.033
R-2	0.055	0.062	0.064	0.048	0.104	0.050	0.166*	0.025	−0.038	−0.034	0.198*	0.001	0.203*	0.189*	−0.001	0.159*	0.191*	0.171*	0.022*	0.045	−0.077	−0.040	0.078	0.074
R-3	0.064	0.036	0.179*	0.033	0.062	0.018	0.254*	0.058	0.018	−0.065	0.302*	0.058	0.214*	0.117*	−0.006	0.009	0.208*	0.105	0.157*	0.124*	−0.031	0.049	0.276*	0.151*
RH	0.204*	0.179*	0.466*	0.229*	−0.003	0.095	0.525*	0.372	0.557*	0.389*	0.509*	0.304*	0.299*	0.239*	0.183*	0.174*	0.284*	0.184*	0.190*	0.090	0.247*	0.017	0.163*	0.118
RH1	0.224*	0.197*	0.470*	0.219*	0.041	0.140	0.458*	0.312	0.406*	0.251*	0.500*	0.274*	0.343*	0.258*	0.183*	0.241*	0.370*	0.196*	0.208*	0.056	0.241*	−0.048	0.217*	0.111
RH2	0.221*	0.135*	0.431*	0.077	0.085	0.140	0.363*	0.234	0.254*	0.145	0.431*	0.183*	0.380*	0.285*	0.200*	0.239*	0.436*	0.256*	0.205*	0.114*	0.203*	−0.003	0.261*	0.206*
RH3	0.147*	0.078	0.318*	−0.026	0.050	0.113	0.302*	0.207	0.217*	0.067	0.340*	0.183*	0.323*	0.247*	0.170*	0.209*	0.348*	0.191*	0.220*	0.164*	0.197*	0.139	0.307*	0.179*
Max Temp	0.181*	0.123*	0.433*	0.130	0.228*	0.017	0.226*	0.169*	0.299*	0.067	0.034	−0.062	0.222*	0.188*	0.028	−0.061	0.048	0.009	0.109*	−0.011	0.176*	−0.062	0.153*	−0.107
Max T1	0.139*	0.061	0.368*	0.059	0.196*	−0.088	0.231*	0.137*	0.261*	0.043	0.105	−0.132	0.239*	0.206*	0.084	0.019	0.054	0.023	0.110*	−0.033	0.154*	−0.064	0.207*	−0.170*
Max T2	0.088	0.011	0.292*	0.019	0.159*	−0.193*	0.149*	0.067	0.116	−0.041	0.009	−0.262*	0.214*	0.177*	0.081	−0.068	−0.028	−0.013	0.063	−0.083	0.094	−0.115	0.141	−0.262*
Max T3	0.103	−0.024	0.304*	0.009	0.214*	−0.285*	0.143*	0.046	0.023	−0.065	0.111	−0.304*	0.231*	0.125*	0.047	−0.090	0.045	−0.135	0.068	−0.021	0.081	0.020	0.196*	−0.231*
Min Temp	−0.029	0.039	0.308*	0.086	−0.140	−0.265*	0.160*	0.147*	0.244*	0.090	−0.175*	−0.300*	0.262*	0.202*	0.012	0.078	0.008	−0.149*	0.034	0.000	0.128	−0.064	0.037	−0.157*
Min T1	−0.041	0.026	0.280*	0.046	−0.133	−0.282*	0.143*	0.175*	0.111	0.091	−0.083	−0.217*	0.255*	0.194*	0.099	0.083	−0.090	−0.188*	0.026	−0.012	0.089	−0.058	0.080	−0.217*
Min T2	−0.052	0.018	0.219*	−0.037	−0.057	−0.227*	0.145*	0.171*	0.047	0.027	0.008	−0.183*	0.247*	0.200*	0.066	0.105	−0.101	−0.175*	0.031	−0.001	0.132	−0.007	0.019	−0.229*
Min T3	−0.080	0.026	0.116	−0.032	0.019	−0.199*	0.176*	0.137*	0.077	−0.011	0.099	−0.266*	0.273*	0.212*	0.131	0.095	−0.047	−0.125	0.016	0.022	0.101	0.079	0.005	−0.234*
Mean Temp	0.034	0.041	0.355*	0.086	−0.009	−0.227*	0.155*	0.118*	0.250*	0.055	−0.203*	−0.327*	0.252*	0.190*	−0.002	0.010	0.024	−0.132	0.053	−0.008	0.142	−0.048	0.073	−0.181*
Mean T1	0.015	−0.012	0.342*	−0.004	−0.067	−0.332*	0.148*	0.114*	0.197*	0.029	−0.161*	−0.333*	0.239*	0.172*	0.058	0.065	−0.078	−0.213*	0.051	−0.024	0.133	−0.059	0.085	−0.233*
Mean T2	−0.004	−0.011	0.283*	−0.020	−0.018	−0.317*	0.127*	0.119*	0.090	0.004	−0.110	−0.307*	0.220*	0.150*	0.074	0.002	−0.172*	−0.259*	0.032	−0.057	0.121	−0.073	0.020	−0.338*
Mean T3	−0.018	−0.008	0.225*	−0.036	0.055	−0.293*	0.137*	0.105*	0.040	−0.015	−0.002	−0.336*	0.242*	0.152*	0.096	−0.014	−0.109	−0.243*	0.019	−0.028	0.090	0.040	0.022	−0.347*
Sunshine	0.022	−0.035	0.023	−0.131	0.005	0.031	−0.325*	−0.275*	−0.159*	−0.242*	−0.416*	−0.275*	−0.099	−0.115*	−0.149*	−0.130	−0.072	−0.094	−0.059	−0.073	−0.030	−0.035	−0.090	−0.086
S1	0.016	−0.094	0.108	−0.092	−0.087	−0.082	−0.255*	−0.247*	−0.002	−0.227*	−0.400*	−0.244*	−0.145*	−0.153*	−0.119	−0.045	−0.161*	−0.169*	−0.054	−0.074	0.018	−0.029	−0.124	−0.091
S2	0.059	−0.055	0.156*	−0.044	−0.044	−0.049	−0.233*	−0.198*	0.023	−0.172*	−0.382*	−0.196*	−0.200*	−0.159*	−0.054	−0.228*	−0.271*	−0.140	−0.132*	−0.164*	0.029	−0.146	−0.287*	−0.167*
S3	0.060	−0.027	0.178*	0.031	−0.060	−0.045	−0.191*	−0.130*	−0.003	−0.114*	−0.307*	−0.118	−0.165*	−0.105*	−0.032	−0.328*	−0.237*	−0.042	−0.137*	−0.127*	0.050	−0.101	−0.320*	−0.133
Wind speed	−0.025	−0.075	−0.178*	−0.086	−0.074	0.001	−0.162*	−0.135	−0.097	−0.140	−0.089	0.022	−0.278*	−0.197*	0.015	−0.029	−0.221*	−0.121	−0.005	−0.003	−0.040	−0.140	−0.006	0.156*
WS1	−0.072	−0.101	−0.269*	−0.088	−0.083	−0.047	−0.203*	−0.143	−0.197*	−0.128	−0.104	−0.001	−0.307*	−0.172*	−0.018	−0.145	−0.267*	−0.051	−0.055	0.012	−0.133	−0.125	−0.020	0.175*
WS2	−0.043	−0.084	−0.235*	−0.022	−0.049	−0.062	−0.233*	−0.099	−0.204*	−0.048	−0.166*	0.035	−0.313*	−0.147*	−0.128	−0.129	−0.229*	−0.015	−0.060	0.070	−0.036	0.048	−0.164*	0.157*
WS3	0.013	−0.055	−0.174*	0.002	0.026	−0.024	−0.197*	−0.133*	−0.112	0.021	−0.155*	−0.062	−0.289*	−0.127*	−0.080	−0.123	−0.200*	0.018	0.003	0.027	0.064	0.046	−0.121	0.075

*$P<0.05$ (Spearman test)

Table 5 Mean spores concentrations and wind direction over 2 years. The number of days for each wind direction are indicated in parenthesis

	N (107)	NE (314)	E (127)	SE (25)	S (92)	SW (24)	W (12)	NW 21)
Total spores	399.3	305.3	332.1	549.5	693.1*	683.7*	520.9	408.8
Cladosporium	415.2	205.6	235	344.3	420.1*	416.1*	310.9	251.5
Leptosphaeria	31.2	25.8	32	82.4*	103.4*	103.4*	58.7	47.1
Coprinus	19.0	25.8	48.3	42.5	51.0*	51.0	65.4*	42.6
Aspergillus/Penicillium	29.2	20.1	30.9	24.1	43.4*	43.4*	26.6	38.0

*$P < 0.05$ (significant comparisons PERMANOVA results)

airborne mycobiota may be affected by Saharan dust clouds that shift every year to the Caribbean (Shinn et al. 2000; Prospero and Lamb 2003) by means tropical cyclones in western Cuba or by the arrival of cold fronts with N-NW trajectories (Hernández 2002). There has been little research into the influence of wind direction on spore counts in the Caribbean area. However it is well-known that fungi can be transported hundreds of kilometers, as the case of *Peronospora tabacina* from Cuba to the Southeastern United States (Aylor 2003; Lopetegui et al. 2006). Here, the highest counts were recorded with southerly and south-westerly winds, which perhaps favored the transport of spores from rural areas where many different crops are grown. However, north-easterly winds predominated throughout most of the year (314 days); these blow in from the sea, and therefore carry few spores.

The concentration of spores in a certain period can be highly variable from one location to another as it is related directly to the bioclimatic characteristics of the sampling environment: vegetation, climate, location and type of

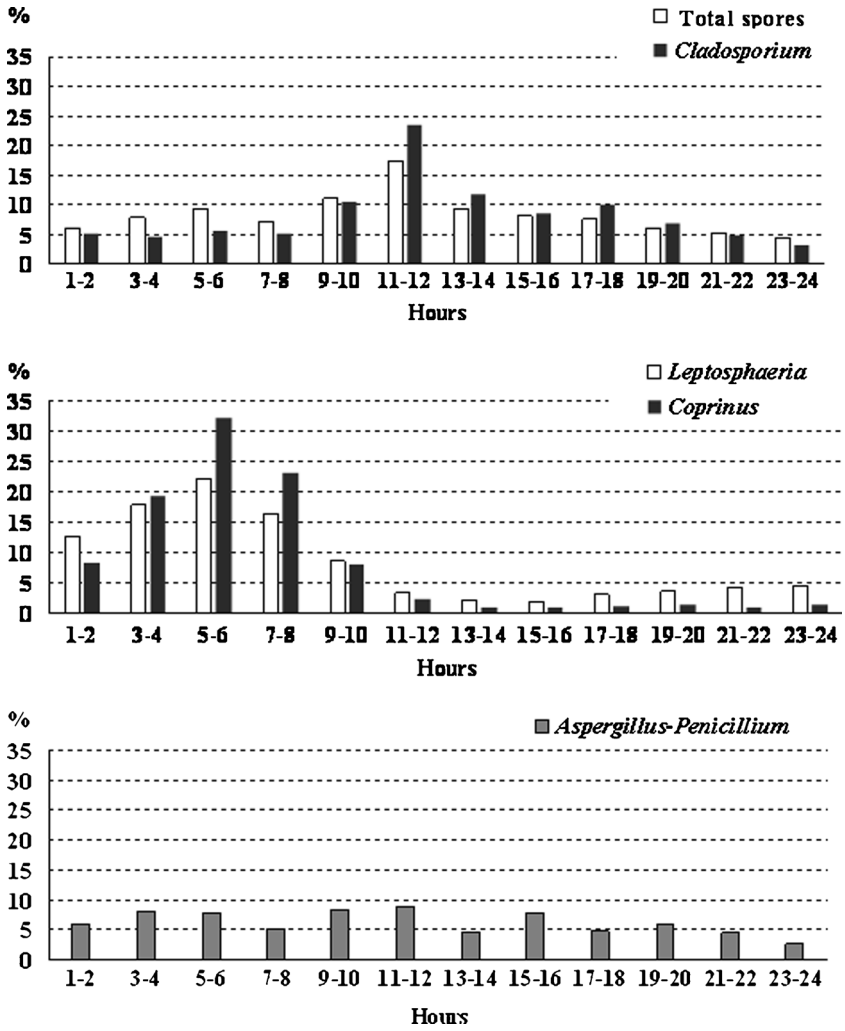

Fig. 4 Models of intradiurnal behavior of total spores, *Cladosporium*, *Leptosphaeria*, *Coprinus* and *Aspergillus/Penicillium* spores during two years (November 2010–October 2012)

sampler used, topography, microclimates, etc. (Morris et al. 2008). Furthermore, the atmospheric pollution degree and the vegetation of the environment (which acts as a substrate for the development of many fungi) could change substantially due to fire or human activity among other factors. The findings revealed that, despite similar weather conditions in the 2 study years, spore counts declined by almost one-third in the 2nd year of study (November 2011–October 2012). The meteorology in both years was quite similar in terms of maximum and average temperature. However, the 2nd year was slightly wettest [in terms of total rainwater collected (1,046.5 mm vs 1,213.2 mm) and number of days in which rainfall was recorded (103 days vs 148 day)] and the minimum temperature, relative humidity and wind speed were also higher. The statistical correlations shown the great influence of the relative humidity in the total spores amount of the most represented genus (*Cladosporium*, *Lepthosphaeria*, *Coprinus* and *Aspergillus*/*Penicillium*) and the effect of the maximum temperature.

During the 2 years analyzed in this study 293,594 spores were registered belonging to 30 genera and five spore types. Spore counts were higher during the dry season in the 1st year, and during the rainy season in the 2nd; similar findings have been reported in other tropical areas of the northern hemisphere (Halwagy 1994; Shaheen 1992; Dames and Cadman 1994; Calderón et al. 1997). The predominance of *Cladosporium* conidia has also been highlighted in a number of countries with temperate climates (Hussain et al. 2013; Tashiro et al. 2013).

For the conidia liberation into the atmosphere it is necessary a certain degree of environmental dryness induced by temperature increases (Corden et al. 2003) during the dry period registered in Cuba from November to April. In tropical latitudes, relative humidity has a greater influence than temperature on the airborne spore counts. Humidity values of around 70–80 % particularly favor the release of basidiospores and ascospores (Ianovici and Tudorica 2009; Tang 2009). Quintero et al. (2010) report that humid winds trigger the release of spores into the atmosphere, while winds and storms act as dispersal mechanisms. They also note that heavy rain has a washout effect, clearing the air of fungal spores, although subsequent elevated humidity prompts an increase in airborne spore counts. Nevertheless, during the subsequent hours under high humidity conditions the concentration of fungal aerosols increased. This fact means that spore counts may thus differ considerably during and after rain events. In Havana, moreover, the highest counts were recorded in the early morning; this may be linked to the elevated humidity at this time of day. Burge and Rogers (2000) report other bimodal mechanisms in which the precipitation and humidity could act as inducers or repressors of sporulation (depending on its different intensity). Besides, many airborne spores originate from plant surfaces whose humidity may favor spore release through evapotranspiration (Gottwald et al. 1997; Burge and Rogers 2000; Levetin and Horner 2002; Quintero et al. 2010).

Cladosporium conidia were abundant in the months before and after peak temperatures. Unfortunately, these findings cannot be compared with other published data, since research into airborne fungal spores in Cuba has focused mainly on characterizing viable propagules (Aira et al. 2002; Almaguer et al. 2012) or have used different methods (Cadrecha and Fernández de Castro 1955; Arnold et al. 1987; Herrera et al. 2003; Borrego and Perdomo 2012). Nevertheless, daily concentrations ranging from 288 and 51,112 spores/m^3 around the month of May have been reported in Puerto Rico, mainly for basidiospores (Rivera-Mariani and Bolaños-Rosero 2012).

The marked seasonal behavior of *Cladosporium* conidia recorded here (verified by their prevalence during the dry months of the 1st year) has also been highlighted in several studies carried out in European countries under a range of bioclimatic conditions, in which peak values were recorded during the warmer months of spring and summer (Kasprzyk et al. 2004; Aira et al. 2012). A strong statistical correlation was found between relative humidity levels and airborne conidia counts. Humidity is therefore a prerequisite for the release of these spores into the atmosphere (Hollins et al. 2004). The present study, like earlier research in Havana (Rojas et al. 2007), recorded a marked prevalence of *Cladosporium* at humidity values above 75 %. In the 1st year of study, the number of days with relative humidity values higher than the 75 % were lower than in the 2nd year (78 days vs 95 days). This difference was most evident during the dry season of the 1st year, so that the positive effect of relative humidity can be enhanced under dry periods.

Some studies conduced in tropical climates indicate the quantitative importance of the *Aspergillus* and various Ascomycetes and Basidiomycetes spores in the aeromycobiota (Calderón et al. 1995; Rivera-Mariani et al. 2011). Findings also revealed high counts for *Lepthosphaeria* ascospores as well as for *Coprinus* and *Ganoderma* basidiospores, particularly during the rainy season, presumably because these weather conditions favored the development of reproductive structures and subsequent spore release (Panda et al. 2009). Similar results regarding the timing of Ascomycetes and Basidiomycetes airborne spore counts have been reported in Mexico (Rosas et al. 1998), for *Leptosphaeria* spores in the Caribbean area (Puerto Rico) and in temperate climate (Poland) (Quintero et al. 2010; Dawidziuk et al. 2012) and *Coprinus* and *Ganoderma* in Portugal (Oliveira et al. 2005), Australia (Mitakakis and Guest 2001) and India (Jothish and Nayar 2004). Regarding this meteorological factor, Levetin and Horner (2002) described mechanisms by means of which rain can contribute positively to the representation of dry and wet spores in the air. Dry spores, which are often found on leaves, may be dispersed when raindrops impact on the leaf surface. Although this

spore type includes *Cladosporium* and *Penicillium*/*Aspergillus*, the later did not display marked seasonal behavior as noted by Hoa et al. (2005) in the Taiwan area with a climate similar to that of Cuba.

Results for intradiurnal variations in spore counts showed that daily *Cladosporium* counts peaked between 11:00 a.m. and 12:00 noon. At this moment of the day a high degree of solar radiation was recorded, which can increase dry conditions and the liberation of spores (Stepalska and Wolek 2009). Otherwise, the amount of *Leptosphaeria* and *Coprinus* spores increases, especially during the early morning hours where moisture conditions are favorable for the liberation of their spores. Different daily spore-count patterns are observed for 'dry' and 'wet' fungal spores in temperate zones: dry spores such as *Cladosporium* are released from the fungus under decreasing humidity and increasing airflow conditions (Grinn-Gofrón and Rapiejko 2009; Stepalska and Wolek 2009), Different daily spore-count patterns are observed for 'dry' and 'wet' fungal spores in temperate zones: dry spores such as *Cladosporium* are released from the fungus under decreasing humidity and increasing airflow conditions. Relative humidity was the most important variable influencing the atmospheric spore content whereas the impact of air temperature, dew point, air pressure, wind speed and wind direction was lower (Kasprzyk et al. 2011).

Conclusions

Cladosporium was the largest single contributor to total airborne spore counts in Havana. The most abundant spore types were recorded mainly during the dry season, whereas *Lepthosphaeria*, *Coprinus* and *Ganoderma* spores predominated in the rainy season. Relative humidity is the most influential parameter in the atmospheric concentration of spores, mainly during the rainy season of the year. Although NE winds predominate in the atmosphere of Havana, winds coming from the SW direction increased the spore concentration in the air. The intradiurnal spore variation shows three different patterns: morning maximum values for *Cladosporium*, night peaks for *Coprinus* and *Leptosphaeria*, and a uniform behavior throughout the day for *Aspergillus*/*Penicillium*.

Acknowledgments This research was partially supported by the USC-Banco de Santander program.

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