

Airborne basidiospores of *Coprinus* and *Ganoderma* in a Caribbean region

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Abstract The present study sought to examine *Coprinus* and *Ganoderma* airborne spore counts, analysing seasonal variations, the influence of meteorological variables and intraday behaviour with a view to charting the aerobiological dynamics of both genera in Havana (Cuba) during a 2-year period (November 2010–October 2012). A Hirst-type volumetric air sampler was used as sampling methodology. The spores registered in the air were identified and counted following the model proposed by the Spanish Aerobiological Network based on two longitudinal transects along the slides. *Coprinus* spores outnumbered those of the *Ganoderma* (28,468 annual total spores vs. 1,921 spores). Moreover, both genera were recorded in the atmosphere on a large number of days

over the two-year study period, with daily maximum concentrations in the rainy months. The daily maximum value for *Coprinus* was 880 spores/m³ (30 September 2011), whereas for *Ganoderma* 44 spores/m³ (4 September 2011). The analysis of the Spearman correlation coefficient showed a significant positive correlation of the airborne *Coprinus* and *Ganoderma* spores with the temperature, relative humidity and rainfall, whereas the degree of association is negative with the wind speed. Regarding the intraday behaviour, both basidiospores were more abundant in the atmosphere during the night, with a maximum peak at 5–6 a.m.

Keywords *Coprinus* · *Ganoderma* · Basidiospores · Havana · Cuba

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1 Introduction

Over recent years, a number of mycological studies have used volumetric methods to determine the airborne phytopathogenic spore content of cropland in Cuba (Almaguer et al. 2012). To date, however, little research has addressed other local airborne mycobiota, particularly in the island's urban areas. The basidiomycetes *Coprinus* and *Ganoderma* are widespread in Cuba (Minter et al. 2001). In Havana city, the presence of 7 *Coprinus* species and 8 *Ganoderma* species has been reported (Pérez and

Vilaró 2000; Cabarroi et al. 2008). Both genera produce ballistospores, which are actively projected from the basidium, a discharge mechanism favoured by elevated humidity levels. In both cases, and particularly in *Ganoderma*, the basidiocarp produces abundant basidiospores (Webster and Webber 2007).

In many parts of the world, urban outdoor air is reportedly rich in basidiospores, which can be dispersed by the wind over long distances (Grinn-Gofron and Strzelczak 2011). In tropical environments, allergy sufferers tend to be more sensitive to basidiospores than to mitospores, suggesting that they play a greater role in allergy development (Rivera-Mariani et al. 2011a). Research in these areas indicates that *Coprinus* and *Ganoderma* spores can directly aggravate respiratory diseases (Rivera-Mariani and Bolaños-Rosero 2012) since their small size allows them to penetrate deeper into the airways (Levetin and Horner 2002). Around 20–30 % of patients with respiratory allergy symptoms exhibit positive skin tests for these genera; of these, 10–48 % are positive for *Ganoderma* (Singh et al. 1995). In Cuba a higher prevalence being recorded in urban areas (Venero et al. 2009; Díaz et al. 2010).

Despite the abundance of airborne *Coprinus* and *Ganoderma* basidiospores, most aerobiological studies using non-viable air sampling methods have focussed on mitosporic fungi and especially on genera of allergenic importance such as *Cladosporium* and *Alternaria* (Aira et al. 2012a, b). In the Caribbean area, airborne basidiospores of *Coprinus* and *Ganoderma* have been detected in Mexico (Calderón et al. 1995) and Puerto Rico (Quintero et al. 2010), but have not previously been reported in Cuba. The present study therefore sought to examine *Coprinus* and *Ganoderma* airborne spore counts, analysing seasonal variations, the influence of meteorological variables and intraday behaviour with a view to charting the aerobiological dynamics of both genera in Havana (Cuba). It is hoped that the findings will be of value to clinicians, for the prevention, diagnosis and treatment of spore allergies.

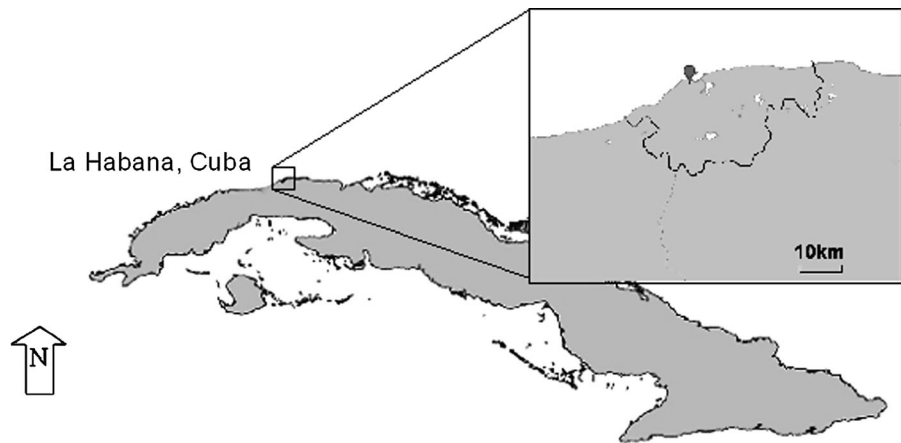
2 Materials and methods

The study was carried out in the city of Havana, located on the Northern coast of the Cuba Island (Fig. 1). Sampling was continuously conducted during 2 years, from 2 November 2010 to 31 October 2012. The

Havana climate is subtropical with a dry period (from November to April) and a marked rainy season (from May to October). The sampling period was characterized by an average minimum temperature of 21.8 °C, a maximum temperature of 29.1 °C, a mean temperature of 25.1 °C and a relative humidity of 74 %. During the dry seasons of both years, a total of 275.5 and 323.9 mm of rainfall were collected and the mean temperatures were 22.8 and 23.5 °C, respectively. During the rainy seasons, the amount of rainfall increased to 771 and 889.3 mm and the mean temperatures were 27.2 and 26.9 °C, respectively. Average wind speed was 29.9 km/h, with daily minimums near to calms (0.5 km/h) and maximum values of 132 km/h.

The spore concentration in the air was monitored by using a Hirst-type sampler, located on the rooftop of the Faculty of Biology (University of Havana) 35 m above ground level (23°08'N and 82°23'W). The equipment was calibrated for 10 l of air per minute, and the spores impacted a Melinex tape coated with a 2 % silicone solution. For the collection and processing of aerobiological samples, the methodology proposed by Galán et al. (2007) was used. In order to ensure the correct identification of the spores, a previous training qualitative study of all spore types present in the air (which could be recognized by their morphology using microscopy with 1,000× magnification) has been conducted. During this previous study, a list of the taxa that offered all the guarantees for a correct taxonomic identification was developed. The spores were counted along two longitudinal sections of microscopic slide with a magnification of 400×, using the 1,000× one in some cases in order to achieve a correct recognition. *Coprinus* and *Ganoderma* spores were identified taking into account its morphological differences (Aira et al. 2009; Bhosle et al. 2010), and the results were expressed as number of spores/m³ of air.

The Spearman test was used to determine the correlations between the spore daily concentrations in the air on a given day and the main meteorological factors. The meteorological variables evaluated were the following: minimum, maximum and mean temperature (°C), relative humidity (%), rainfall (mm) and wind speed (km/h). The significance was calculated using an IBM Statistics software version 20. The daily values of the meteorological variables were recorded by the meteorological station of Casablanca (placed 4.39 km from the sampler) and supplied by the Cuban Institute of Meteorology (INSMET).

Fig. 1 Location of Havana city in Cuba Island**Table 1** Total spores amount of each main spore type, percentage and number of days of its presence in the atmosphere during all the sampling period and each year of study

	Period of study			First year		Second year	
	Total spores	%	No. days	%	No. days	%	No. days
<i>Cladosporium</i>	184,254	62.75	629	66.13	353	51.06	276
<i>Leptosphaeria</i>	31,133	10.60	590	9.04	322	1,604	268
<i>Coprinus</i>	28,468	9.69	469	7.7	268	17.08	201
<i>Asperg-Pen</i>	20,521	6.98	477	7.19	307	6.28	170
<i>Curvularia</i>	4,354	1.48	532	1.44	325	1.63	207
<i>Nigrospora</i>	3,192	1.08	443	1.12	147	0.97	296
<i>Periconia</i>	2,945	1.00	400	1.01	261	0.96	139
<i>Gliomastix</i>	2,079	0.70	285	0.83	225	0.28	60
<i>Ganoderma</i>	1,921	0.65	360	0.63	230	0.72	135
<i>Alternaria</i>	1,777	0.60	378	0.61	243	0.58	130
Other basidiospores	1,672	0.57	315	0.54	185	0.66	130
Other spores identified	11,277	3.90	600	3.87	309	3.73	291
Total spores	293,594	–	–	–	–	–	–

Bold values correspond to fungi discussed in this paper

In order to obtain a model that reflects the intraday fluctuations of the spore counts, only the days without rainfall in which the concentrations were higher than the mean during the sampling period were selected (Nilsson and Persson 1981). The resulting data set was used to calculate an “ideal day” with the average values of the spore concentrations every 2 h, thereafter expressing the data as percentages.

3 Results

A total amount of 293,594 spores were observed in the atmosphere of Havana city during the 2 years of study

(Table 1). Quantitatively the more important taxa were *Cladosporium* (62.75 %), *Leptosphaeria* (10.60 %), *Coprinus* (9.69 %) and *Aspergillus/Penicillium* (6.98 %). With lower percentages than the 1 % were identified other genera such as *Curvularia*, *Nigrospora*, *Periconia*, *Gliomastix*, *Ganoderma* and *Alternaria*. As the goal of this work was the study of the most important airborne basidiospores, only additional information about the most abundant ascospores in the atmosphere will be reported by means of its categorization into two ascospores groups: “*Leptosphaeria*” and “others spores identified” (in which *Pleospora*, *Chaetomium*, *Paraphaeosphaeria*, *Venturia*, *Sporormiella* and *Xylariaceae* spores were included).

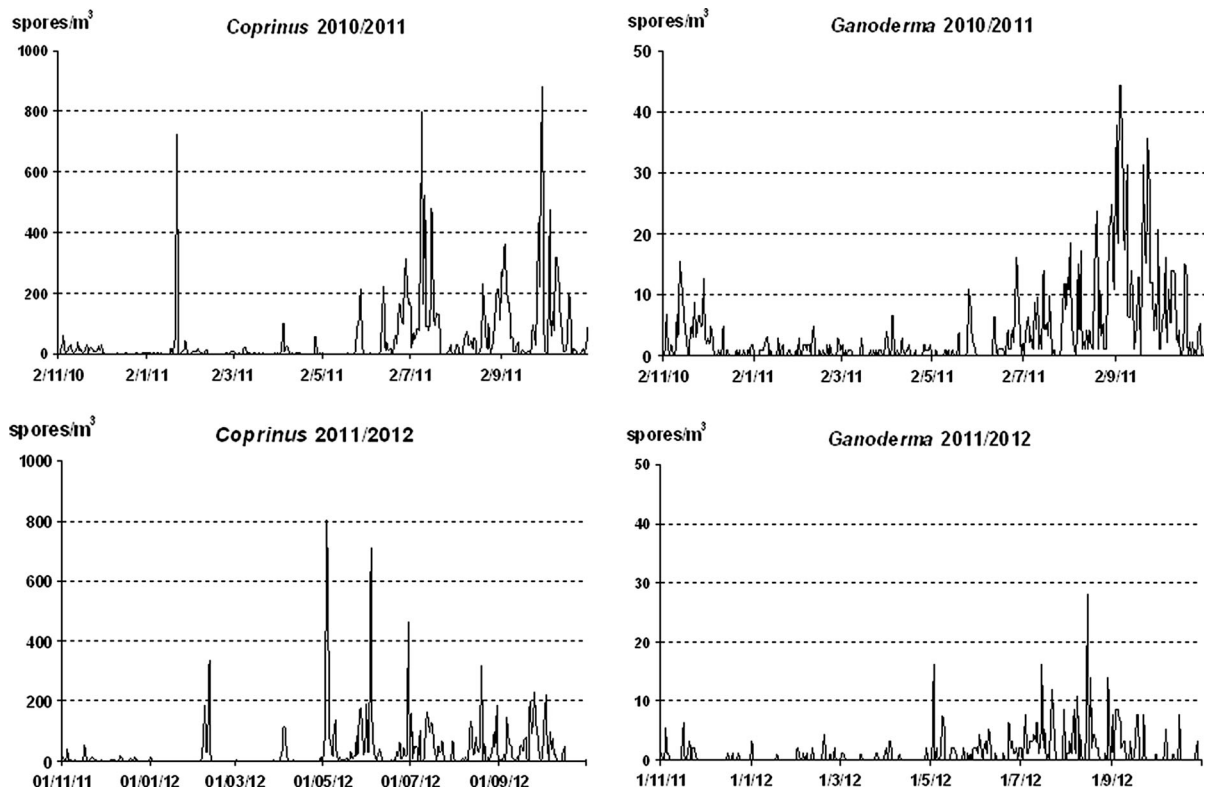


Fig. 2 Evolution of the concentrations of *Coprinus* and *Ganoderma* spores during each year of study (first year November 2010–October 2011 and second year November 2011–October 2012)

The total count for *Coprinus*, *Ganoderma* and other basidiospores, uredospores and teliospores (grouped under the basidiomycetous spore type due to their sporadic and occasional presence) was 32,061 spores, thus accounting for 10.92 % of the total spores identified. *Coprinus* and *Ganoderma* spores outnumbered those of the other basidiomycetous spore types identified and were recorded in the atmosphere on a large number of days over the two-year study period: 469 for *Coprinus* and 360 for *Ganoderma*.

Seasonal variations in *Coprinus* and *Ganoderma* spore counts over the two-year period are shown in Fig. 2. In the first year, *Coprinus* counts were higher from late May to mid-October. The mean daily concentrations registered values around 200 spores/m³ the most part of the days, reaching the daily maximum peaks the January 22 (724 spores/m³), July 9 (798 spores/m³) and September 30 (724 spores/m³). Likewise, the *Ganoderma* daily spore concentration started to rise from the end of May, in spite of the higher values were recorded during the months of August and

September. The daily mean peak concentration was registered the September 5 with 44 spores/m³.

However, a slight advance was observed in the sporulation period for both genera during the second year. Peak daily *Coprinus* counts were recorded during the first 10 days of May, when concentrations reached 802 spores/m³. Thereafter, counts declined continuously until early October, by which time they had dropped to 200 spores/m³. The first high *Ganoderma* counts were also observed during the first 10 days of May, although the largest share of total spore content was registered in August, a peak daily count of 28 spores/m³ being recorded on 15 August).

In order to determine the influence of the main weather variables in the airborne *Coprinus* and *Ganoderma* spore content, a Spearman correlation test analysis was carried out (Table 2). In general, highly significant Spearman coefficients were registered (p level <0.01). Only the correlation between the rainfall and *Coprinus* showed a lower significance (p level <0.05), whereas with *Ganoderma*, a significant

Table 2 Correlations between concentrations of *Coprinus* and *Ganoderma* spores and the main meteorological parameters

	<i>Coprinus</i>	<i>Ganoderma</i>		<i>Coprinus</i>	<i>Ganoderma</i>
<i>Minimum temperature</i>			<i>Relative humidity</i>		
First year	0.398**	0.353**	First year	0.466**	0.400**
Second year	0.438**	0.377**	Second year	0.458**	0.204**
Period of study	0.394**	0.311**	Period of study	0.425**	0.232**
<i>Maximum temperature</i>			<i>Rainfall</i>		
First year	0.385**	0.387**	First year	0.274**	0.271**
Second year	0.433**	0.429**	Second year	0.128*	0.024n.s.
Period of study	0.407**	0.406**	Period of study	0.191**	0.128**
<i>Mean temperature</i>			<i>Wind speed</i>		
First year	0.391**	0.377**	First year	−0.426**	−0.402**
Second year	0.450**	0.421**	Second year	−0.415**	−0.288**
Period of study	0.408**	0.376**	Period of study	−0.426**	−0.361**

Spearman's test (* $p < 0.05$; ** $p < 0.01$; n.s. not significance). First year $N = 364$; second year $N = 366$; period of study $N = 730$

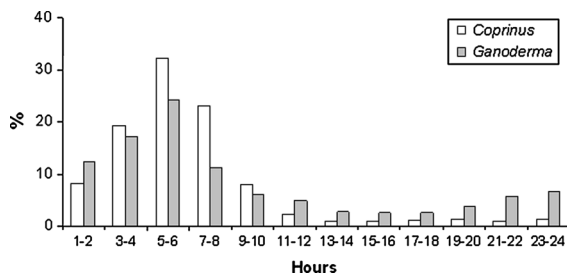


Fig. 3 Models of intradiurnal behaviour of *Coprinus* and *Ganoderma* spores during 2 years (November 2010–October 2012, 77 days for *Coprinus* and 60 days for *Ganoderma*)

coefficient was not obtained. The most important parameter for the airborne *Coprinus* spores presence in the atmosphere was the relative humidity, reaching a coefficient value of 0.466 during the first year of the study and 0.458 during the second year. Moreover, the temperature (minimum, maximum and mean) showed a positive significant correlation with the airborne spore content, especially during the second year of study. In the case of *Ganoderma*, the relative humidity and the temperature (mainly the maximum and mean values) were the most important meteorological parameters again. It is important to highlight the negative effect that wind speed exerts on the spore concentration of both genera, mainly for *Coprinus*.

Finally, the intraday spore variation showed a similar behaviour for both genera (Fig. 3). The atmospheric spore content was more abundant during the night, registering the higher values in the 5–6 a.m.

bihourly interval (period in which it is recorded the 39 % of the *Coprinus* spores and the 24 % of the *Ganoderma* spores), whereas the lower values between the 13 and 20 h.

4 Discussion

Although *Cladosporium* is the main component of airborne mycobiota in various parts of the world (Ho et al. 2005; Aira et al. 2012a; Hasnain et al. 2012), basidiospores (and particularly *Coprinus*) are a key feature of the Havana bioaerosol. Given their specific morphological attributes, *Coprinus* and *Ganoderma* basidiospores can be readily distinguished from other airborne particles (Hasnain and Khatija 2005). In view of the widespread sensitivity of allergy sufferers to these genera, the monitoring of spore counts is clearly of clinical interest.

There has been no previous research into airborne basidiospore dynamics in Cuba, but Quintero et al. (2010) report that these spores are very common in Puerto Rico (a nearby island with similar climate conditions), where *Ganoderma* spores in particular are a constant feature of the bioaerosol. Airborne spores of both genera have been detected in Mexico (Calderón et al. 1995), where *Coprinus* is more abundant and frequent, accounting for over half of all identified basidiospores. Research in the city of La Plata by Mallo et al. (2011) has highlighted the major contribution of *Coprinus*—and to a lesser extent

Ganoderma—spores to the urban bioaerosol. Similar findings have been reported in other parts of the world.

It has been suggested that several *Ganoderma* species display higher spore production and greater capacity for basidiospore release than *Coprinus*; however, the data obtained here indicated higher airborne spore counts for *Coprinus*. One reason for this may be that *Coprinus* abounds in the lawns and gardens of Havana's urban areas, while *Ganoderma* is a lignicolous basidiomycete more commonly found in areas with an abundance of trees (Cabarroí et al. 2008). Hasnain et al. (2004) has reported that regional variations in airborne basidiospore counts are influenced by the local distribution of basidiomycete sources. Differences found here in the basidiospore dispersion rate may thus reflect the differing typology of the basidiocarp and the position of the hymenium in these two fungi, as well as the deliquescent nature of the mature *Coprinus* carpophore.

Spores of both genera were recorded in the atmosphere throughout most of the year, the highest counts being observed during the rainy months. A similar seasonality has also been detected in Mexico (Calderón et al. 1995), USA (Craig and Levetin 2000), Australia (Mitakakis and Guest 2001), India (Jothish and Nayar 2004), Southern Spain (Morales et al. 2006) and Portugal (Oliveira et al. 2005). In non-tropical regions, by contrast, airborne *Ganoderma* spores have been reported from June to November, with mean daily peaks generally occurring from late July to mid-October (Kasprzyk et al. 2011).

A number of studies have observed high basidiospore counts during tropical rainy seasons, noting that precipitation favours basidiocarp development and spore release due to the resulting high humidity and abundant water availability (Quintero et al. 2010). Here, *Ganoderma* spores displayed similar behaviour in both study years, with a single peak in September during the rainy season. By contrast, *Coprinus* spore counts displayed two peaks during the rainy season of the second year (May and June) and three in the first year: two during the rainy season (July and September) and the other in the dry season (January).

Several authors (Craig and Levetin 2000; Oliveira et al. 2005) report high airborne *Coprinus* spore counts (with more than 1,000 spores/m³), while the behaviour of *Ganoderma* airborne spores exhibits greater variability. Even so, maximum daily counts recorded here for both spore types were higher than those detected by

Calderón et al. (1995) in more urbanized areas of Mexico city (80 spores/m³ for *Coprinus* and 10 spores/m³ for *Ganoderma*) or the reported by Morales et al. (2006) in the South Spain (545 spores/m³ for *Coprinus* and 21 spores/m³ for *Ganoderma*).

Weather conditions can affect spore production processes directly, and also indirectly through their effect on the substrates colonized by the fungus. The Spearman rank test revealed, for both genera, a positive correlation between airborne spore counts and temperatures, relative humidity and rainfall. This may account for the *Coprinus* peaks recorded during warm, rainy months and the high seasonal *Ganoderma* counts observed over the same period. Several authors have noted that heavy rainfall and elevated relative humidity favour basidiospore production and release, since fungal development takes place during rainy days (Craig and Levetin 2000; Hasnain et al. 2004). These two factors, both linked to substrate water availability, thus influence airborne spore counts regardless of the climatic region studied.

A negative correlation was observed between wind speed and *Coprinus* and *Ganoderma* spore counts, perhaps attributable to Havana's coastal location, and its consequent exposure to the effects of a constant, fairly intense sea breeze. A number of studies have noted that high basidiospore counts tend to be associated with low wind speeds, perhaps due to the dilution effect of high-speed winds on bioaerosol particles. Moreover, higher wind speeds also increase water loss in the basidiocarp, leading to the suppression of spore production (Grinn-Gofron and Strzelczak 2011).

Research suggests that spore counts also fluctuate in the course of the day (Okten et al. 2005). Studies in Kraców (Poland) have reported the highest wet spore counts during the predawn hours (Stępalska and Wołek 2009). Here, airborne spore counts increased at night. This is probably also due to the high relative humidity at this time of day, and its critical impact on spore release mechanisms; a strong correlation was observed between spore counts and humidity levels. A similar intraday distribution pattern, with high *Coprinus* spore counts at night and peaks at 4 a.m., has been reported in Spain (Aira et al. 2009). This pattern differs considerably from that reported for anamorphic fungi, which record the highest counts in the afternoon.

Higher airborne basidiospore counts may be linked to a greater incidence of chronic respiratory diseases

such as asthma or rhinitis, which can occur in response to the inhalation of allergenic spores by sensitive populations (Bush and Portnoy 2001; Newhouse and Levetin 2004; Semik-Orzech and Barczyk 2008). Published data on the role of basidiomycetes in respiratory allergies are more limited than that available for mitosporic fungi or other allergens. Less research has been carried out in tropical latitudes than in other climate zones, even though—as the present study shows—basidiomycetes are a major component of tropical bioaerosols. Studies involving respiratory allergy sufferers in San Juan (Puerto Rico) have shown that these patients display greater reactivity to basidiospores than to mitosporic fungi. It has also been demonstrated that *Coprinus* can trigger reactivity and nasal symptoms in patients with asthma or allergic rhinitis (Rivera-Mariani et al. 2011a, b; Rivera-Mariani and Bolaños-Rosero 2012). A number of authors have also highlighted the allergenic capacity of *Ganoderma*, which triggers cross-reactivity with the spores of other Basidiomycetes (Lehrer and Hughes 1994; Brander and Pichler 1999; Helbling et al. 2002). Published research also points to a link between respiratory allergies and elevated relative humidity (Taylor and Jonsson 2004; Nasser and Pulimood 2009), which favours the release of basidiospores such as *Coprinus* and *Ganoderma* into the atmosphere.

5 Conclusion

The present findings enhance our knowledge of the behaviour of airborne *Coprinus* and *Ganoderma* basidiospores. In this study, carried out in Cuba, *Coprinus* spore counts were higher than those of *Ganoderma*. Both genera are more abundant in the atmosphere during the rainy seasons. Temperature, relative humidity and rainfall showed a positive correlation with the spore presence in the atmosphere, while the winds speed exerts a negative effect. Taking into account the intradiurnal behaviour, both basidiospores were more abundant in the atmosphere during the night hours. Although the spores of the two genera are recognized as important aeroallergens, there are no previous studies in Cuba about its allergenic incidence. Therefore, the information included in the present paper will be very useful to study their relationship with the respiratory allergic diseases in sensitive individuals.

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