Comparison of Atmospheric Fungal Spore Concentrations between Two Main Cities in the Caribbean Basin

Félix E. Rivera-Mariani, PhD*; Michel Almaguer, PhD†;
María Jesús Aira, PhD‡; Benjamín Bolaños-Rosero, PhD§

Objective: Fungal spores are ubiquitous in the atmosphere worldwide, but their distribution is not homogeneous at different locations. Most studies have compared airborne fungal spores ecology in temperate zones, but less is known about the tropics.

Methods: This study compared, through statistical analysis of archived datasets, the predominant fungal groups, patterns and meteorological variables affecting airborne fungal spore concentrations between two major cities in the Caribbean (Havana and San Juan) during the year 2015.

Results: In Havana, the predominant fungal group was *Cladosporium* while in San Juan were basidiospores. Our data provide evidence of differences and similarities in the monthly distribution of airborne spores in Havana and San Juan, but *Cladosporium*, ascospores y basidiospores had comparable hourly patterns in both cities and were affected by the same meteorological variables.

Conclusion: Our study provides additional evidence to help design allergy interventions. [*P R Health Sci J 2020;39:235-242*]

Key words: Fungal spores, Meteorological parameters, Allergy, Caribbean

Fungal spores are ubiquitous in the atmosphere worldwide (1–3). This widespread distribution aids fungal spores' colonization potential on plants, soil, litter, decaying organic matter, among others. For example, approximately 45% of the bioaerosols in tropical rainforests are due to fungal spores (4). As a result, fungal spores represent a principal source of biogenic aerosols could potentially to affect human respiratory health (5–8).

Environmental mycology studies in tropical and subtropical zones are less frequent than in temperate ones (9–11). Nevertheless, meteorological variables influencing the airborne fungal spores in temperate regions, including rainy seasons, high humidity and temperatures, are also present in tropical and subtropical settings such as in countries of the Caribbean basin (9,12–14). Therefore, there is a need to further understand how meteorological, and possibly geographical, and topographical, variables influence fungal spores' diversity in tropical and subtropical regions.

In Cuba, studies have addressed the fungal aerobiology in Havana's through a Hirst-type volumetric sampler (15–17). For example, in Havana, *Cladosporium* spores have been identified among the most predominant outdoors; however, basidiospores, such as those from *Coprinus* and *Ganoderma*, are also important fungal propagules. In indoor studies with viable methods, *Aspergillus* and *Penicillium* have been predominant (12,18,19). However, outdoor studies in Havana have been less common and employed methodologies different to indoors studies.

In Puerto Rico, mainly in the San Juan area, the concentrations of outdoor pollens and fungal spores have also been studied. A study by Quintero et al (14) identified seasonal patterns of pollen and fungal spores, and also evidenced the predominance of ascospores and basidiospores. In indoor studies (through viable methods) identified predominance of *Aspergillus* spp, *Penicillium* spp, and xerophilic fungi (20,21). These studies evidence the diverse fungal ecology of outdoors compare to indoors in San Juan, PR.

In recent decades, fungal exposure in different settings has received public health awareness. Indoor exposure to mold has been associated with adverse human respiratory health effects, such as asthma (22). Fungal spores such as those from *Cladosporium*, *Aspergillus*, and *Alternaria*, have the potential to secrete allergenic compounds; for this reason, they have also been associated with respiratory allergies (13,23).

*College of Biomedical Sciences, Larkin University, Miami, Florida, USA; †Department of Microbiology, Biology Faculty, University of Havana, Cuba; ‡Department of Botany, Pharmacy Faculty, University of Santiago, Santiago de Compostela, Spain; §Department of Microbiology, School of Medicine, University of Puerto Rico Medical Sciences Campus, San Juan, Puerto Rico

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Address correspondence to: Benjamîn Bolaños-Rosero, PhD, Department of Microbiology and Medical Zoology, School of Medicine, University of Puerto Rico Medical Sciences Campus, PO Box 365067, San Juan, PR 00936. Email: benjamin.bolanos@upr.edu

In tropical environments, sensitization to airborne basidiospores, ascospores, and fungal fragments seems to be more prevalent than to asexual spores in subjects with active allergies (24–26). Proteins from spores of the basidiomycete *Ganoderma applanatum* were shown to have allergenic potential (27). Recently, we identified positive associations between increases in outdoor mold and PM10 exposures and outpatient asthma-related healthcare use (28). Thus, it is essential to further understand the ecology of potentially allergenic fungal spores in tropical and subtropical regions, such as the Caribbean basin.

In this study, we compared monthly and daily concentration patterns of fungal aeroallergens in the atmosphere of two main cities in the Caribbean basin (Havana, Cuba, San Juan, PR) during the year 2015. We also compared how meteorological variables were influencing the concentrations of fungal aeroallergens in both cities.

Materials and methods

Study area

The studies were performed in Havana (LH), Cuba and San Juan (SJ), Puerto Rico, which are among the most densely populated cities in the Caribbean basin (Fig. 1). Cuba, an archipelago of islands, islets, and keys, has an area of 1250 x 100 km. Cuba's flora is very rich and extremely diverse, including various palm trees and Cuban marsh species (29). The city of Havana, the largest city with approximately 2 million inhabitants, is on the northern coast and at 24 m above sea level. Nearby the location of the volumetric sampler, there is no dense vegetation besides inner-city gardens and organoponics (29,30). There are other agricultural areas, such as Artemisa and Mayabeque (25 km south of the volumetric sampler's location), and western green areas nearby (ONEI, http://www.onei.cu/): Pinar del Rio (12 120 m^2), Artemisa (8 959 m^2), Havana (27 483 m^2) and Mayabeque (10 023 m^2).

Cuba's climate is subtropical with two weather seasons based on rainfall: a dry (November to April) and rainy season (May to October). Havana's climate exhibits mid-continental characteristics: somewhat cold winters and warm, humid summers. Also, there is significant yearly swings in temperature, and most of the precipitation occurs in the warmer months (INSMET, http://www.insmet.cu/asp/genesis.asp?TBO=PLANTILLAS&TB1=CLIMAC&TB2=/clima/ClimaCuba.htm).

San Juan, the capital of Puerto Rico, is located on the North-Eastern coast. It is characterized by urban land cover, moist soils, and dense flora. Puerto Rico's vegetation zones range from dry to semi-deciduous forests in patches and bands on the north, east, and southwest coast. There are also moist forests covering most of the island (31). Its unique topographical and geological characteristics favor a high diversity of forest and ecosystems (32).

Overall, Puerto Rico is characterized by a tropical climate with moderate temperature and high precipitation (33). Temperatures exhibit small seasonal variation. During November to April, cold fronts from the Eastern US seaboard penetrates South, which can induce significant precipitation. San Juan is influenced by Atlantic Ocean Easterly trade winds that together with tropical climate, moist soils, and dense vegetation, induce a microclimate might affect airborne particulates' aerodynamics (34).

During the year of study (2015), San Juan reported slightly higher temperatures and relative humidity. Nevertheless, Havana reported higher maximum values for relative humidity and San Juan reported higher minimum. For both cities, the wind direction was blowing mainly from South East to South-South-East directions (Table 1).

Sampling and microscopic examination methodology

The study was carried out during the year 2015. In Havana, we implemented the methodology proposed by the Spanish Aerobiology Network (35): sampling at 10 liters of air/min (simulating human breathing rate) with a volumetric Hirst-type Lanzoni VPPS 2000 (Lanzoni s.r.l., Bologna, Italy). This equipment was located on the rooftop of the Biology Faculty at the University of Havana (23°08"12" N and 82°23'3" W) and 35 m above sea level. The sampler worked continuously for a week. In this sampler, fungal spores were collected in a cylindrical drum by impacting the surface of a melinex film coated with a thin layer of a 2% silicone. The drum was exchanged weekly. The exposed tape was cut into pieces (representing 24hrs of sample) and mounted on separate glass slides. Microscopic examination and counting of the fungal spores were performed on each preparation along two continuous longitudinal traverses. Spores were identified based on morphology (36).

The microscopical examination was performed on a bright-field optical Nikon Optiphot II microscope (Nikon Manufacturing, Tokyo, Japan) at 400X. In some cases, 1000X was used to achieve better fungal spore recognition. Results were expressed as spores/m^3 of air for daily mean spore

Table 1. Descriptive statistics (minimum, average, and maximum values) for meteorological variables in Havana (LH), Cuba and San Juan (SJ), Puerto Rico for the year 2015.

	Minimum (LH)	Minimum (SJ)	Average (LH)	Average (SJ)	Maximum (LH)	Maximum (SJ)
Minimum temperature (ºC)	3.9	21.7	21.0	24.5	26.5	28.3
Average temperature (ºC)	13.4	25.0	25.3	27.8	30.6	31.7
Maximum temperature (ºC)	17.8	27.2	30.7	31.0	38.2	35
Minimum relative humidity (%)	18.0	39.0	51.8	60.9	91.0	88
Average relative humidity (%)	51.0	60.0	78.4	75.6	95.0	94
Maximum relative humidity (%)	65.0	74.0	94.3	89.6	100.0	100
Wind degrees (°)	10.0	33.0	143.6	99.6	360.0	345
Mean wind speed (kph)	8.0	3.2	35.9	13.5	118	27.4
Precipitation (mm)	0	0	3.1	3.5	63.5	46.2

concentrations. However, monthly and annual values were expressed without units because it was reported as a sum of mean daily concentrations (35). The daily meteorological values were recorded by the meteorological station of Casablanca (4 km from the sampler) (INSMET, http://www.insmet.cu/asp/genesis.asp?TBO=PLANTILLAS&TB1=CLIMAC&TB2=/clima/ClimacCuba.htm).

In San Juan, airborne spores were collected using the Hirst-type Burkard sampler (Burkard Scientific Ltd, Uxbridge, UK) (18). This equipment was located on the rooftop of the Medical Sciences Campus of the University of Puerto Rico (18°23'48" N and 66°4'30" W) and 60 m above sea level sampling at 10 liters of aire/min. The sampler worked continuously at 10 L of air/min. Fungal spores were impacted on a microscopic slide coated with a thin layer of 2% silicone grease. The slide was changed daily and mounted on polyvinyl alcohol (PVA) mounting media. The microscopic examination was along 12 traverse fields, which constitute 2-hrs intervals and is a microscopical approach proposed by the British Aerobiology Federation (37). Microscopical examination was performed on a bright-field optical microscope Nikon Eclipse 80i (Nikon Manufacturing) at 1000X.

The daily values of the meteorological variables were from recorded by San Juan Airport Station (12 km from the sampler) of the National Oceanic Atmospheric Administration (NOAA) and extracted directly from the NOAA National Climate and Data Center through the rnoaa R package (38).

Table 2. Proportion of predominant fungal groups, from total spore concentration, in Havana (LH), Cuba and San Juan (SJ), PR for the year 2015

Predominant fungal groups			
Havana (LH)	**Total spores (%)**	**San Juan (SJ)**	**Total spores (%)**
Cladosporium	63.5	Basidiospores****	55.5
Ascospores*	16.9	Ascospores**	25.5
Basidiospores***	10.7	*Aspergillus/Penicillium*	5.1
Aspergillus/Penicillium	6.9	*Cladosporium*	5.1

*Ascospores in Havana include *Leptosphaeria*, *Paraphaerosphaeria*, Xylariaceae, *Venturia* and non-identified ascospores. **Ascospores in San Juan include *Leptosphaeria*, *Leptosphaerulina*, *Amphisphaeria*, Xylariaceae, Diatrypaceae, and non-identified ascospores. ***Basidiospores in Havana include *Coprinus* and *Ganoderma*. ****Basidiospores in San Juan include *Agrocybe*, *Pleurotus*, *Trametes*, *Coprinus*, *Agaricus*, and non-identified basidiospores

Statistical analysis

The statistical analysis was performed in R (version 3.6; R Core Team, Viena, Austria), and graphs and geospatial maps generated using the ggplot2 and ggmap packages (39–42). For inter-city comparison, the proportions from the total concentration for each hourly, monthly, and predominant fungal spore groups were analyzed to help normalize for differences in microscopic examination between Havana and San Juan (Table 2). The means were reported for meteorological data. The Spearman rank-order correlation coefficient was implemented to evaluate meteorological variables, previous-day spore concentration, and log2-transformed spore concentrations. Linear regression models included only variables that showed as statistically significant correlations. Models were further refined with all-subset regression (43,44). Comparison of linear regression models was carried out graphically. Statistical significance was considered at a $p < 0.05$.

Results

Cladosporium was the predominant fungal group in Havana: basidiospores were predominant in San Juan. *Cladosporium* in Havana was 11 times higher than San Juan, while basidiospores four times greater in San Juan. Both cities reported comparable levels for ascospores and *Aspergillus/Penicillium*. In San Juan, the proportion of *Aspergillus/Penicillium* and *Cladosporium* were similar. These results suggest that for the year 2015, a combination of dry and wet spores predominated in the atmosphere of Havana, but for San Juan it was mainly wet spores.

Both cities differed in hourly and monthly proportions of fungal spores. In Havana, hourly proportions were higher between the 6:00 and the 16:00 hours (Fig. 2A). In San Juan (Fig. 2B), the hourly proportions were higher between 20:00 hour of the previous day and

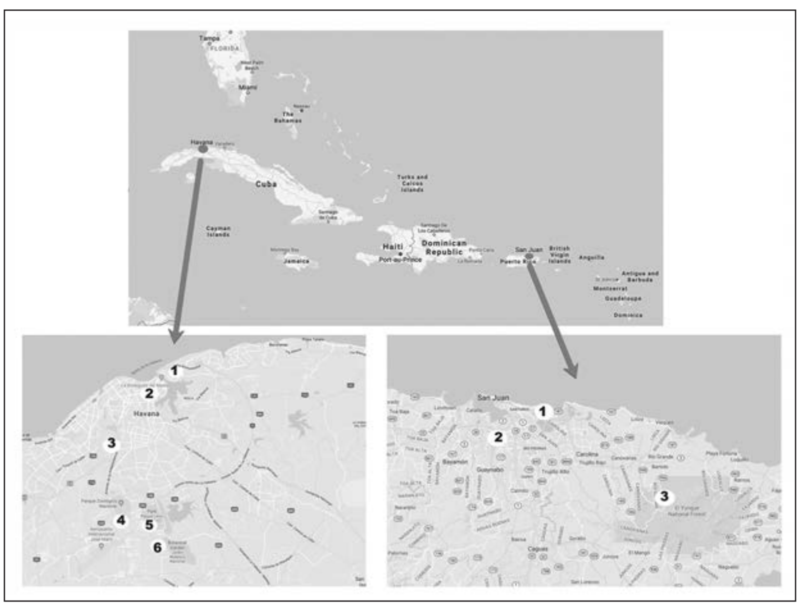

Figure 1. Spatial terrain maps of Havana (LH), Cuba and San Juan (SJ) Puerto Rico. A localization of the samplers in Havana and San Juan. B Havana, 1. Casablanca Meteorological Station (4 km); 2. Sampler; 3. Urban Forest (5km). C San Juan, 1. National Weather Service Meteorological Station (12 km); 2. Sampler; 3. El Yunque National Rainforest (28km).

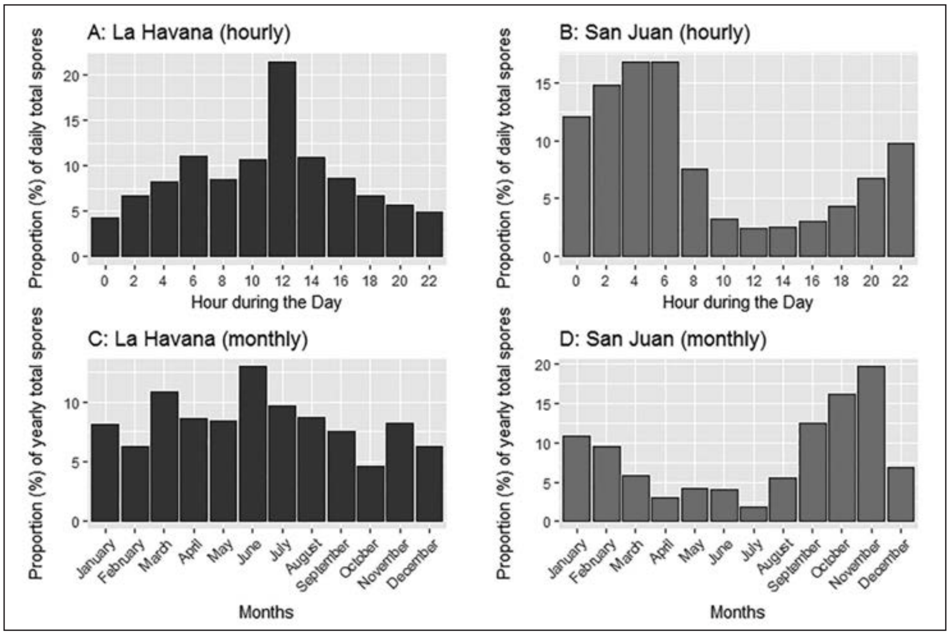

Figure 2. Different patterns of hourly and monthly proportions total fungal spores in Havana (LH), Cuba (A-C) and San Juan (SJ), Puerto Rico (B-D). The hourly and monthly proportions of spores represented by each 2-hour interval (A, B) and month (C, D), respectively, were calculated by dividing the yearly fungal spore concentration for a corresponding 2-hour interval or month by the total spores reported in Havana and San Juan for the year 2015. The findings in this figure suggests that there are different hourly and monthly patterns of total fungal spores in Havana compared to San Juan.

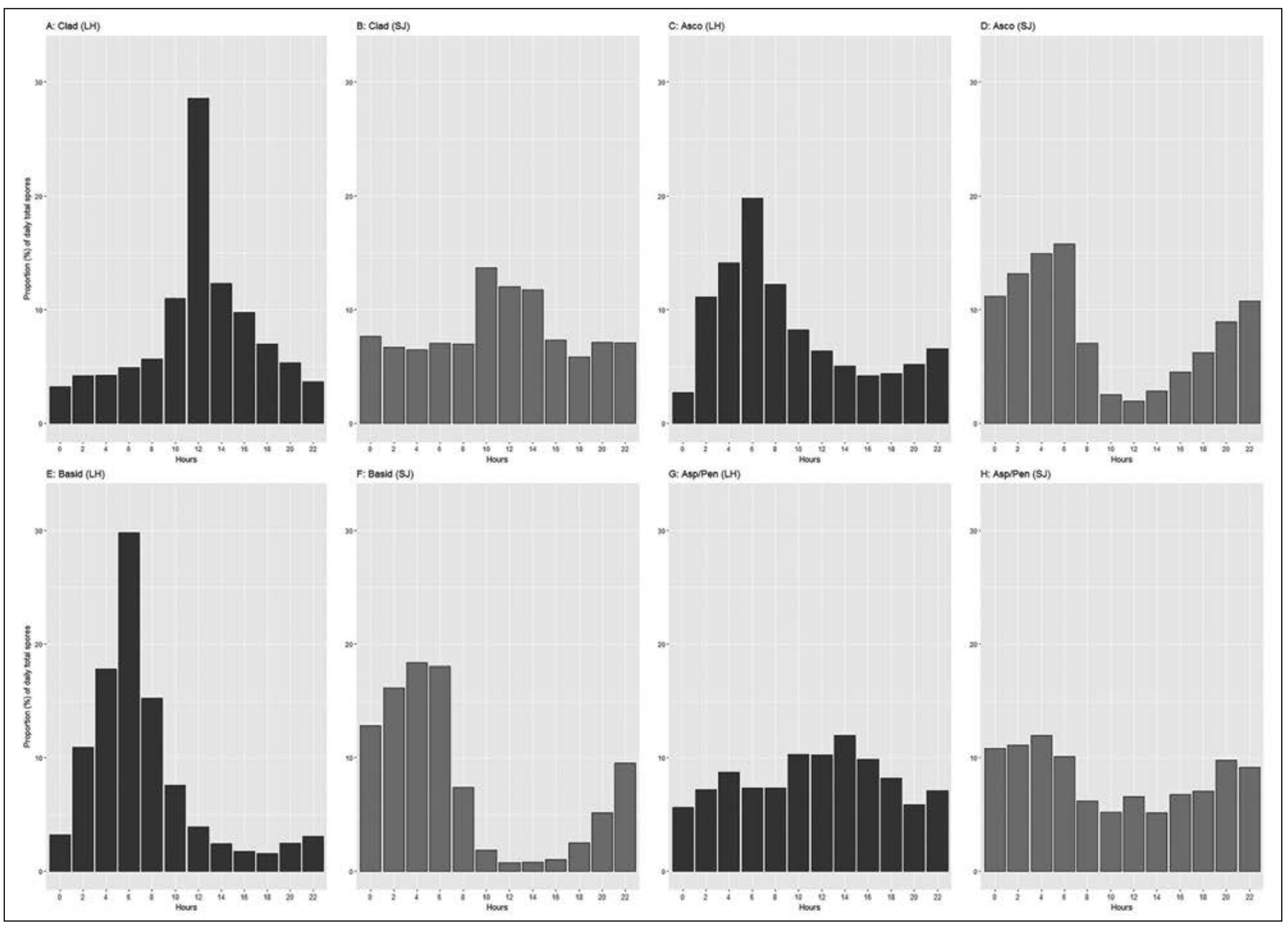

Figure 3. Different patterns of proportion of hourly concentrations of predominant fungal spores in Havana (LH), Cuba and San Juan, Puerto Rico. The hourly proportions of spores represented by each 2-hour interval were calculated by dividing each predominant fungal group's total spore concentration reported for a corresponding 2-hour interval by the total yearly fungal spore concentration reported for the predominant fungal group in the year 2015. The findings in this figure suggest that for each of the predominant fungal spore groups, (i.e. Clados, *Cladosporium*; Basid, basidiospores; Asco, ascospores, Asp/Pen, *Aspergillus*/*Penicillium*), there are different hourly patterns in Havana compared to San Juan.

the 8:00 hour of the next day. Havana reported higher monthly proportions between March and August (Fig. 2C), while in San Juan it was between September to November (Fig. 2D).

Cladosporium 2-hr interval proportions in Havana and San Juan (Fig. 3A-B) followed similar patterns. In both cities, the highest concentrations were in the middle of the day (10:00 to 16:00 hours). Ascospores (Fig. 3C-D) and basidiospores (Fig. 3E-F) in both cities showed comparable patterns: highest concentration proportions in early morning and late night. Nevertheless, 2-hr patterns of *Aspergillus/Penicillium* between both cities were different. Except for *Aspergillus/Penicillium,* these results suggest that similar fungal groups share comparable hourly concentrations patterns between Havana and San Juan.

As shown in figure 4, for both cities the log2-transformed spore concentration from the previous day had the most relative weight on total spore concentrations. Nevertheless, the San Juan model explained nearly 50% more the variability than the Havana. The differences in r^2, but similar relative weights of predictor variables suggest that other variables, or possible interactions between variables, play a role in total spore concentrations in Havana.

In addition to total spore concentrations, linear regression models were also evaluated for predominant fungal spore groups (Fig. 5). The variability explained by the linear regression models in Havana ranged from 4.2 to 51.8%: in San Juan, between 25.3 to 63.1%. In both cities, the basidiospore models explained the highest variability (51.8% in Havana and 63.1% in San Juan). On the other hand, Havana and San Juan differed in the fungal group regression models with the lowest r^2: 4.2% for *Aspergillus/Penicillium* in Havana and 25.3% for *Cladosporium* in San Juan. Apart from mean wind speed (important in San Juan), models included similar predictor variables. The exception, again, was for *Aspergillus/Penicillium*, in which for the San Juan model included more predictor variables. These findings suggest that similarities between Havana and San Juan in hourly concentration patterns for *Cladosporium*, ascospores, and basidiospores may be explained by a different subset of predictor variables.

Discussion

This study evaluated, for the year 2015, the predominant fungal groups, monthly and hourly patterns of fungal spore concentrations in the atmosphere of two cities in the Greater Antilles of the Caribbean: Havana, Cuba, and San Juan, Puerto Rico. We also evaluated the relationship between meteorological variables and fungal spore concentrations. Both cities had distinct total fungal spore and monthly patterns, but most predominant fungal groups had comparable hourly patterns. The similarity in hourly patterns could be explained by levels of predominant fungal groups being influenced by similar predictor variables, while differences justified by different proximities to inoculum sources. To our knowledge, this is the first study to compare the atmospheric fungal spore concentrations and predominant fungal groups between two cities in the Caribbean basin.

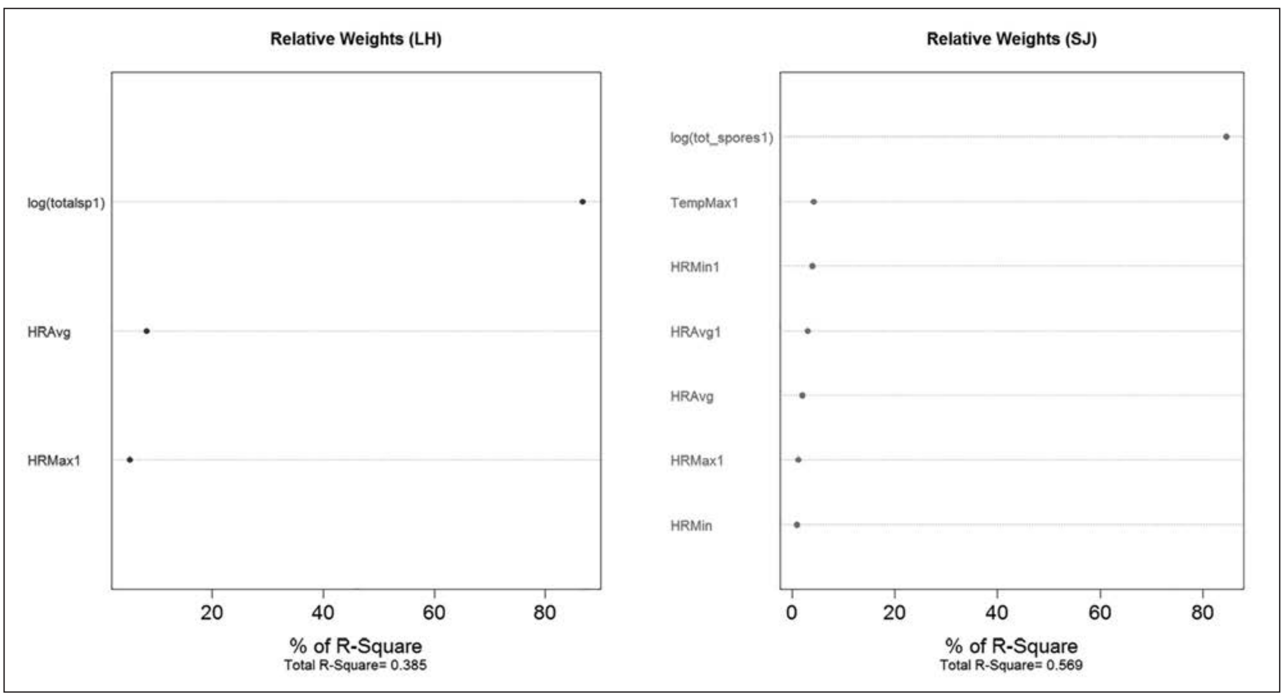

Figure 4. The total fungal spore accumulated on a previous day has the highest predictive weight on the total fungal spore concentrations for any given day in Havana, Cuba and San Juan, PR. Relative weights of predictor variables on total spore concentrations in Havana (LH), Cuba (A), and San Juan (SJ), Puerto Rico (B). The findings in this graph suggest that among predictor variables in the linear regression models in Havana and San Juan, the total spores accumulated on a previous day has a significantly higher predictive value than meteorological variables.

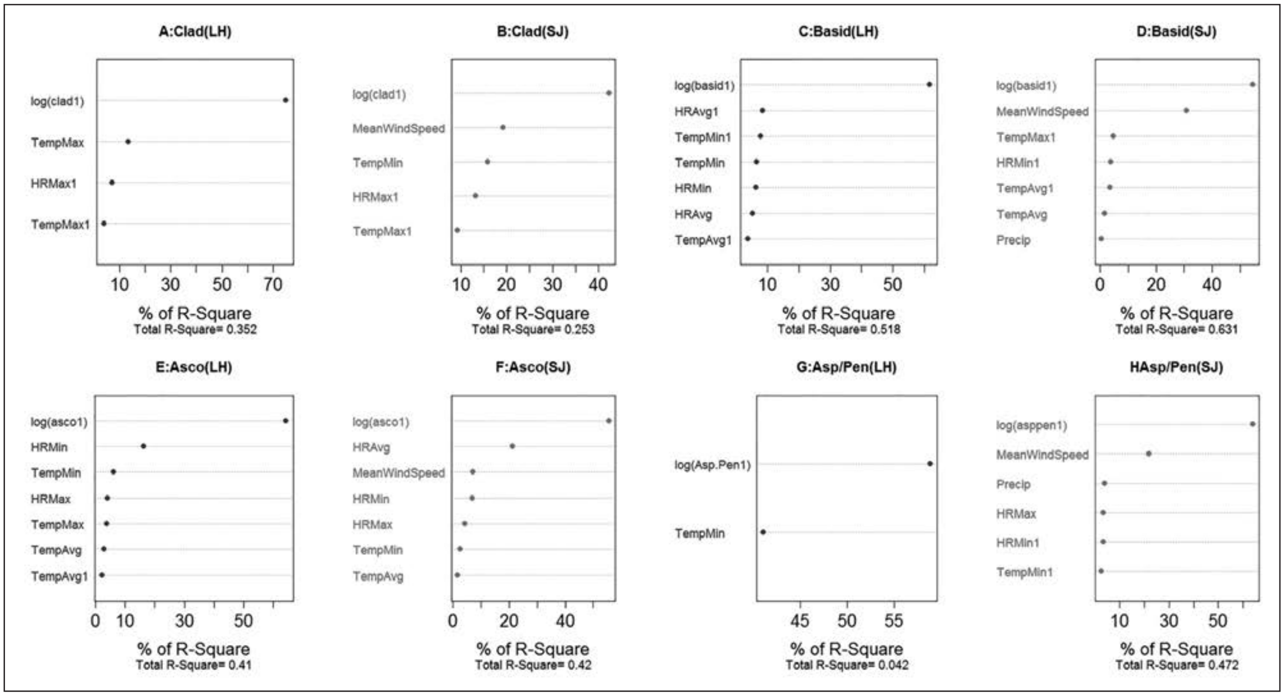

Figure 5. The predictive weight of meteorological variables on spore concentration of predominant fungal groups differs between Havana, Cuba and San Juan, Puerto Rico. Predominant fungal spore groups in Havana and San Juan are influence by fungal spores recorded in a previous day but differ in the contribution of meteorological variables. Relative weights of predictor variables on predominant fungal groups in Havana, Cuba (LH) and San Juan, Puerto Rico (SJ). (Clados, *Cladosporium*; Basid, basidiospores; Asco, ascospores, Asp/Pen, *Aspergillus*/*Penicillium*). The findings in the figure suggest that although spore concentrations from a previous day had the highest weight, Havana and San Juan differ on the contribution of meteorological variables on predominant fungal spore concentrations.

Predominant fungal spores in both cities differed in the proportion they contributed to the yearly total fungal spore. The predominance of *Cladosporium* spores in the current study has also been demonstrated previously in Havana (16,17). In San Juan, the high proportion of basidiospores in the atmosphere have also been previously corroborated (14). Although with different proportions as those found in Havana, *Cladosporium* was also among the fungal groups mostly encountered in San Juan in the current study. Differences in the proportion of predominant fungal spores could be explained by differences in topography and vegetation between Havana and San Juan. Another possible explanation could be differences in microscopical magnifications: in Havana, microscopic preparations were examined at 400X, while in San Juan at 1000X. Spores from some basidiomycetes, such as those of *Coprinus* and *Ganoderma*, could be identified at 400X given their pigmentation and characteristic ornamentation. In the case of other basidiomycetes, such as *Agrocybe*, *Pleurotus*, among others, 1000X would be needed given their hyaline morphology.

Almost identical groups of predictor variables explained similar fungal spore. This finding further supports possible roles of topographical and geographical in the proportion of predominant fungal spores. In all regression models, the concentration of spores from the previous day was the stronger predictor. This type of relationship is a common trait (i.e., autocorrelation) of time-series data. Also, the absence of forces (e.g., rain, wind gust) that would affect their aerodynamics would allow fungal spores to remain suspended long enough and thus influencing the next-day concentration (44). Except for wind speed, which was an important meteorological variable in San Juan, temperature and humidity were influential predictors. Also, it is known that San Juan is affected by the easterly trade winds. In San Juan, wind speed has been shown to be negatively correlated with fungal spore concentrations (14). With regards to hourly patterns, in both cities, *Cladosporium* proportions increased during the middle of the day, and ascospores and basidiospores mainly in the early morning. Higher concentrations of *Cladosporium* at noon and ascospores and basidiospores at dawn has also been previously demonstrated in Havana and San Juan, respectively (14,21). Interestingly, previous studies have also evidenced the lack of distinct patterns for *Aspergillus/Penicillium* seen in Havana in the current study.

Both cities differed in the hourly and monthly patterns of fungal spores. The hourly fungal spores were associated with spores group that predominated in both cities: in Havana, the hourly proportions of fungal spores were similar to those of *Cladosporium*, and in San Juan to basidiospores and ascospores. The monthly proportions of fungal spores, including higher between March and August, reported for Havana in the current study have been reported previously (17,45). In Havana, during these months temperature begins to increase. The start of the rainy season begins in May and thus an increase of relative humidity. In San Juan, although the rainy season also starts in May, there was a very long drought reported throughout the first half of the study year (https://pr.water.usgs.gov/drought/): during these months, San Juan reported the lowest proportions of fungal spores.

From a human health standpoint, we believe it is relevant that similar fungal groups (with comparable patterns) were prevalent in the atmosphere of both cities. In a study in San Juan, with 33 subjects with asthma and allergic rhinitis, there was higher than 90% prevalence of serum immunoglobulin E (IgE) reactivity to airborne ascospores and basidiospores (26). In other studies, crude extracts have provided significant evidence of serological reactivity to basidiomycetes (25,27). In these studies, sensitization prevalence to crude extracts against spores of *Ganoderma* was comparable to those of mites (an important indoor allergen) and proteins with allergenic potential were identified characterized biochemically. Recently, we identified positive associations between increases in outdoor mold and PM exposures and outpatient asthma-related healthcare use in San Juan, PR (28). In Cuba, similar studies to those performed in San Juan, PR are scarce. Díaz et al. reported the prevalence of sensitization to *Penicillium* (50%), *Cladosporium* (32%), and *Alternaria* (18%), but no association with atopic status. Therefore, the finding that basidiospores and ascospores were among the predominant fungal groups in the atmosphere of Havana further supports possible role of these fungi in the incidence of allergic respiratory diseases. Also, it supports the design of studies to evaluate the allergenicity of these fungal spores among the Cuban population.

Conclusion

In summary, for the year 2015, the atmosphere of Havana, Cuba, and San Juan, Puerto Rico had distinct patterns of total hourly and monthly fungal spores concentrations. Different groups predominated, but shared comparable hourly concentrations and influenced by similar sets of meteorological variables. To our knowledge, this is the first study in which predominant fungal spores, hourly and monthly patterns, as well as relevant meteorological variables influencing fungal spores' concentrations has been examined and compared between two cities in the Caribbean basin. Furthermore, the findings of predominant fungal groups identified in Havana, for which serological and immunological studies have been performed in San Juan, warrants clinical and epidemiologic approaches to assess the potential human health adverse effects from exposure to these fungal bioaerosols in Havana and possibly other cities in the Caribbean basin.

Resumen

Objetivo: Las esporas fúngicas son ubicuas en la atmósfera terrestre, pero su distribución no es homogénea. La mayoría de los estudios comparando la ecología de los aerosoles de esporas fúngicas se han hecho en países templados y poco se conoce de lo que sucede en regiones tropicales. Métodos: Este estudio compara, mediante análisis estadísticos de archivos de bases de datos, los grupos de hongos predominantes, patrones y variables meteorológicas que afectan las concentraciones de esporas fúngicas entre dos ciudades de la cuenca del Caribe (La Habana y San Juan) durante el año 2015. Resultados: En La Habana, el grupo predominante de hongos es *Cladosporium*, mientras que, en San Juan predominan las basidioesporas. Nuestros resultados evidencian las diferencias y similitudes en la distribución mensual de aerosoles fúngicos en ambas ciudades, sin embargo, *Cladosporium*, ascosporas y *basidioesporas* tienen patrones horarios similares al ser afectadas por las mismas variables meteorológicas. Conclusión: Nuestro estudio provee evidencia adicional que ayuda en el diseño de intervenciones para el manejo de alergias.

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