

See discussions, stats, and author profiles for this publication at: <http://www.researchgate.net/publication/263532639>

Study of airborne fungus spores by viable and non-viable methods in Havana, Cuba. Grana, 52(4), 289-298

ARTICLE *in* GRANA · DECEMBER 2013

Impact Factor: 1.06 · DOI: 10.1080/00173134.2013.829869

CITATIONS

3

READS

19

4 AUTHORS, INCLUDING:



F. Javier Rodriguez Rajo

University of Vigo

81 PUBLICATIONS **875** CITATIONS

SEE PROFILE

This article was downloaded by: [Uvi]

On: 07 October 2014, At: 04:55

Publisher: Taylor & Francis

Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



Grana

Publication details, including instructions for authors and subscription information:
<http://www.tandfonline.com/loi/sgra20>

Study of airborne fungus spores by viable and non-viable methods in Havana, Cuba

Michel Almaguer^a, María-Jesús Aira^b, Francisco-Javier Rodríguez-Rajo^c & Teresa-Irene Rojas^a

^a University of Havana, Department of Microbiology and Virology, Cuba

^b University of Santiago, Department of Botany, Santiago de Compostela, Spain

^c University of Vigo, Department of Plant Biology and Soil Sciences, Vigo, Spain

Published online: 20 Sep 2013.

To cite this article: Michel Almaguer, María-Jesús Aira, Francisco-Javier Rodríguez-Rajo & Teresa-Irene Rojas (2013) Study of airborne fungus spores by viable and non-viable methods in Havana, Cuba, *Grana*, 52:4, 289-298, DOI: [10.1080/00173134.2013.829869](https://doi.org/10.1080/00173134.2013.829869)

To link to this article: <http://dx.doi.org/10.1080/00173134.2013.829869>

PLEASE SCROLL DOWN FOR ARTICLE

Taylor & Francis makes every effort to ensure the accuracy of all the information (the "Content") contained in the publications on our platform. However, Taylor & Francis, our agents, and our licensors make no representations or warranties whatsoever as to the accuracy, completeness, or suitability for any purpose of the Content. Any opinions and views expressed in this publication are the opinions and views of the authors, and are not the views of or endorsed by Taylor & Francis. The accuracy of the Content should not be relied upon and should be independently verified with primary sources of information. Taylor and Francis shall not be liable for any losses, actions, claims, proceedings, demands, costs, expenses, damages, and other liabilities whatsoever or howsoever caused arising directly or indirectly in connection with, in relation to or arising out of the use of the Content.

This article may be used for research, teaching, and private study purposes. Any substantial or systematic reproduction, redistribution, reselling, loan, sub-licensing, systematic supply, or distribution in any form to anyone is expressly forbidden. Terms & Conditions of access and use can be found at <http://www.tandfonline.com/page/terms-and-conditions>

Study of airborne fungus spores by viable and non-viable methods in Havana, Cuba

MICHEL ALMAGUER¹, MARÍA-JESÚS AIRA², FRANCISCO-JAVIER RODRÍGUEZ-RAJO³ & TERESA-IRENE ROJAS¹

¹University of Havana, Department of Microbiology and Virology, Cuba, ²University of Santiago, Department of Botany, Santiago de Compostela, Spain, ³University of Vigo, Department of Plant Biology and Soil Sciences, Vigo, Spain

Abstract

The Havana aeromycobiota diversity was studied from November 2010 to October 2011 using two complementary volumetric methods. A total of 35 fungal genera were characterised, 26 of them were recognised only by non-viable methods, six with viable methodology and the other three with both sampling methods. Furthermore, 47 species were identified by cultivation and the spores collected with the non-viable methodology. These could not be included in a specific genus, and thus, were categorised into five fungal types. In general, the main, spread worldwide, mitosporic fungi also predominated the Havana atmosphere. The predominant species were *Cladosporium cladosporioides*, *Aspergillus flavus* and *Penicillium citrinum*. Moreover, several Zygomycetes (*Syncephalastrum racemosum*, *Rhizopus stolonifer* and *Rhizopus oryzae*), Ascomycetes (*Chaetomium globosum*) and Basidiomycetes such as *Coprinus* or *Ganoderma* were isolated. In the present paper, the review of the airborne fungi conducted in previous studies in Cuba was completed by the detection of two new genera and the first isolation of ten new records in the Cuban atmosphere. Most of the fungi detected showed a diurnal pattern with high spore peak at 11.00–12.00.

Keywords: Airborne fungi, spores, propagules, culture-based, direct microscopy, Havana, Cuba

The identification and enumeration of fungal spores in the air are of great interest, because they are potential allergens that can cause important respiratory problems (Toivola et al., 2002; Raphoz et al., 2010) or pathogens causing serious economic losses in crops (Fernández-González et al., 2012). Furthermore, some spore types could induce biodegradation processes in a wide range of materials (Mesquita et al., 2009; Guiamet et al., 2011).

In Cuba, the first environmental mycobiota studies were only focused on the relationship between fungi propagules and allergic processes. Fuentes (1958) conducted a review of the researches about these issues during the decade 1945–1955. Some of these studies stated a marked exacerbation of the clinical symptomatology during the highest airborne fungi concentration periods (Estrada de la Riva, 1951), which some authors located during winter (Cadrecha

& Fernández de Castro, 1955), while others report the influence of weather parameters in the airborne spore concentrations (Quintero Fossas, 1964). All aforementioned studies, as well as other recently conducted in different Cuban localities (Seidel & Räuber, 1970; Rojas et al., 2002), were based on a gravimetric methodology, which limits the expression of their results to the quantification of the identified colonies. The study by Herrera et al. (2003) in Santa Clara detected 24 types of spores using a gravimetric method with slides coated with an adhesive substance.

More recently, the use of viable volumetric methods allowed to identify the most common fungi in the atmosphere of Havana (Rojas et al., 2007) and to assess the activity of fungal plant pathogens that affect rice (Almaguer et al., 2012). However, outdoor studies are scarce, and most of them were focused

Correspondence: María Jesús Aira, Department of Botany, Faculty of Pharmacy, University of Santiago, 15782 Santiago de Compostela, Spain. E-mail: mariajesus.aira@usc.es

(Received 10 June 2013; accepted 9 July 2013)

© 2013 Collegium Palynologicum Scandinavicum

Table I. Total concentrations, relative density and frequency of the detected fungi.

Genus, species or sporal type	Spores			Propagules		
	Direct microscopy			Culture-based method		
	TC	RD	RF	TC	RD	RF
<i>Alternaria</i> Nees.	1396	0.6	49.4	—	—	—
<i>Beltrania</i> Penzig.	41	0.0	9.4	—	—	—
<i>Bipolaris</i> Schoemaker	1338	0.6	73.2	—	—	—
<i>Cercospora</i> Fres.	369	0.2	20.8	—	—	—
<i>Chaetomium</i> Kunze	165	0.1	13.9	—	—	—
<i>Coprinus</i> Pers.	17 244	7.6	73.4	—	—	—
<i>Curvularia</i> Boedijn	3286	1.4	40.8	—	—	—
<i>Fusarium</i> Link	1150	0.5	56.2	—	—	—
<i>Ganoderma</i> Karst.	1446	0.6	63.1	—	—	—
<i>Gliomastix</i> Gueg.	1895	0.8	61.6	—	—	—
<i>Helicoma</i> Corda	19	0.0	4.4	—	—	—
<i>Helicomycetes</i> Link	141	0.1	22.5	—	—	—
<i>Leptosphaeria</i> Ces. et De Not.	20 591	9.0	45.5	—	—	—
<i>Nigrospora</i> Zimm.	2557	1.1	81.9	—	—	—
<i>Paraphaeosphaeria</i> Erik.	153	0.1	20.5	—	—	—
<i>Periconia</i> Tode	2311	1.0	71.6	—	—	—
<i>Pestalotiopsis</i> Steyaert	206	0.1	31.8	—	—	—
<i>Phthomyces</i> Berk. et Broome	561	0.2	48.8	—	—	—
<i>Pleospora</i> Rabenh. ex Ces. et De Not.	325	0.1	29.6	—	—	—
<i>Pseudocercospora</i> Speg.	321	0.1	15.6	—	—	—
<i>Pyricularia</i> Sacc.	164	0.1	12.3	—	—	—
<i>Sporidesmium</i> Link	47	0.0	10.7	—	—	—
<i>Stemphylium</i> Wallr.	55	0.0	9.6	—	—	—
<i>Tetraploa</i> Berk. et Broome	51	0.0	12.9	—	—	—
<i>Torula</i> Pers.	576	0.3	39.7	—	—	—
<i>Venturia</i> Sacc.	452	0.2	20.2	—	—	—
<i>Cephalosporium</i> Corda	—	—	—	34	0.1	2.3
<i>Geotrichum</i> Link	—	—	—	34	0.1	2.3
<i>Gliocladium</i> Corda	—	—	—	34	0.1	2.3
<i>Memnoniella</i> Hohn.	—	—	—	34	0.1	2.3
<i>Nodulisporium</i> Preuss.	—	—	—	34	0.1	2.3
<i>Phoma</i> Sacc.	—	—	—	34	0.1	2.3
<i>Epicoccum</i> Link	327	0.1	24.4	34	0.1	2.3
<i>Monodictys</i> Hughes	1052	0.5	34.2	34	0.1	2.3
<i>Spegazzinia</i> Sacc.	193	0.1	27.4	34	0.1	2.3
<i>Aspergillus</i> / <i>Penicillium</i> -type	16 392	7.2	53.6	—	—	—
<i>Cladosporium cladosporioides</i> -type	148 717	65.3	96.7	—	—	—
<i>Cladosporium herbarum</i> -type	1982	0.9	52.9	—	—	—
Xylariaceae-type	940	0.4	36.2	—	—	—
Uredinales-type	1238	0.5	50.7	—	—	—
<i>Acremonium strictum</i> W. Gams	—	—	—	34	0.1	2.3
<i>Alternaria tenuissima</i> (Kunze ex Pers.) Wiltshire	—	—	—	1380	4.5	45.2
<i>Aspergillus candidus</i> Link	—	—	—	34	0.1	2.3
<i>Aspergillus clavatus</i> Desm.	—	—	—	34	0.1	2.3
<i>Aspergillus flavus</i> Link	—	—	—	1552	5.0	59.5
<i>Aspergillus foetidus</i> Thom et Raper	—	—	—	34	0.1	2.3
<i>Aspergillus japonicus</i> Saito	—	—	—	103	0.3	4.8
<i>Aspergillus niger</i> van Thieghem	—	—	—	1069	3.5	40.5
<i>Aspergillus niveus</i> Blochwitz	—	—	—	34	0.1	2.3
<i>Aspergillus ochraceus</i> G. Wilh.	—	—	—	34	0.1	2.3
<i>Aspergillus oryzae</i> (Alburg) Cohn.	—	—	—	138	0.4	9.5
<i>Aspergillus sidowii</i> (Bain. et Sart) Thom et Church	—	—	—	34	0.1	2.3
<i>Aspergillus terreus</i> Thom	—	—	—	34	0.1	2.3
<i>Bipolaris australiensis</i> (M.B.Ellis) Tsuda et Ueyama	—	—	—	103	0.3	4.8
<i>Bipolaris hawaiiensis</i> (M.B. Ellis) Uchida et Ueyama	—	—	—	34	0.1	2.3
<i>Chaetomium globosum</i> Kunze	—	—	—	34	0.1	2.3
<i>Chrysomilia sitophila</i> (Mont) Arx	—	—	—	724	2.3	21.4

(Continued)

Table I. (Continued).

Genus, specie or sporal type	Spores			Propagules		
	Direct microscopy			Culture-based method		
	TC	RD	RF	TC	RD	RF
<i>Cladosporium cladosporioides</i> (Fresen.) G.A. de Vries	—	—	—	20 177	65.3	95.2
<i>Cladosporium oxysporum</i> Berk. et M.A. Curtis	—	—	—	103	0.3	7.1
<i>Cladosporium sphaerospermum</i> Penz.	—	—	—	34	0.1	2.3
<i>Cladosporium tenuissimum</i> Cooke	—	—	—	69	0.2	4.8
<i>Curvularia aeria</i> (Bat., J.A. Lima et C.T. Vasconc.)	—	—	—	552	1.8	28.6
<i>Curvularia clavata</i> B.L. Jain	—	—	—	69	0.2	2.3
<i>Curvularia verruculosa</i> Tandon et Bilgrami ex M.B. Ellis	—	—	—	69	0.2	2.3
<i>Fusarium graminearum</i> Schwabe	—	—	—	34	0.1	2.3
<i>Fusarium lateritium</i> Nees	—	—	—	138	0.4	7.1
<i>Fusarium oxysporum</i> Schlecht.	—	—	—	69	0.2	4.8
<i>Fusarium semitectum</i> Berk. et Rav.	—	—	—	172	0.6	7.1
<i>Fusarium sulphureum</i> Schlecht.	—	—	—	103	0.3	2.3
<i>Nigrospora sphaerica</i> (Sacc.) Manson	—	—	—	34	0.1	2.3
<i>Paecilomyces variotii</i> Bain	—	—	—	34	0.1	2.3
<i>Penicillium chrysogenum</i> Thom	—	—	—	345	1.1	14.3
<i>Penicillium aurantiogriseum</i> Dierckx	—	—	—	172	0.6	7.1
<i>Penicillium brevicompactum</i> Dierckx	—	—	—	69	0.2	2.3
<i>Penicillium citrinum</i> Thom	—	—	—	1966	6.4	7.1
<i>Penicillium digitatum</i> (Per. Fr.) Sacc.	—	—	—	34	0.1	2.3
<i>Penicillium glabrum</i> (Wehmer) Westling	—	—	—	172	0.6	2.3
<i>Penicillium griseofulvum</i> Dierckx	—	—	—	34	0.1	2.3
<i>Penicillium oxalicum</i> Curri et Thom	—	—	—	103	0.3	2.3
<i>Penicillium purpurogenum</i> Stoll	—	—	—	34	0.1	2.3
<i>Penicillium waksmanii</i> K.M. Zalesky	—	—	—	310	1.0	7.1
<i>Rhizopus oryzae</i> Went & Prins. Geerl.	—	—	—	69	0.2	4.8
<i>Rhizopus stolonifer</i> (Ehrenb.) Vuill.	—	—	—	34	0.1	2.3
<i>Scopulariopsis candida</i> (Gueguen) Vuill	—	—	—	34	0.1	2.3
<i>Syncephalastrum racemosum</i> Cohn ex Schrot	—	—	—	69	0.2	2.3
<i>Trichoderma harzianum</i> Rifai	—	—	—	34	0.1	2.3
<i>Trichoderma viride</i> Pers. ex Gray	—	—	—	34	0.1	2.3
Total spores/isolates identified	227 702	—	—	30 903	—	—

Remark: C, total concentration; RD, relative density; RF, relative frequency.

on the assessment of the fungal destructive effect on cultural heritage (Borrego et al., 2010; Borrego & Perdomo, 2012; Rojas & Aira, 2012; Rojas et al., 2012) or about the air quality evaluation in homes (Aira et al., 2002). The use of volumetric systems for fungal propagules sampling represents a great advantage in relation to gravimetric methods since they allow to obtain quantitative results as colony forming units per volume of air (CFU/m³). Even though, in order to characterise the fungal biodiversity of a given area, we recommend its combination with non-viable volumetric methods (Magyar et al., 2009). Despite the fact this sampling system impedes fungus culture and, therefore, the recognition of other reproductive and vegetative structures, nowadays, volumetric spore collecting methods are widely used around the world as they allow to perform continuous sampling (Oliveira et al., 2009; Stepalska & Wolek, 2009; Mallo et al., 2011; Aira et al., 2013). Spore trap and culture methods are complementary, each with their own advantages and disadvantages.

The aim of this work was to characterise the atmospheric mycobiota of Havana by using two volumetric sampling methods, one based on the culture of fungi and other by direct identification of their spores in the light microscope.

Material and methods

The study was conducted in the city of Havana, located at the northern coast of Cuba at an elevation of 24 m above sea level. Its climate is subtropical with a dry (November–April) and a rainy season (May–October). The average annual temperature is 25.5 °C, the total annual rainfall is 1320 mm and the relative humidity is >80% most of the year. Havana is the largest and most populated city of Cuba. There are a lot of buildings around the sample site and most of the area in between is occupied by streets and gardens. The vegetation in the proximity of the trap is not dense and consists of a wide variety of trees, shrubs and ornamental plants.

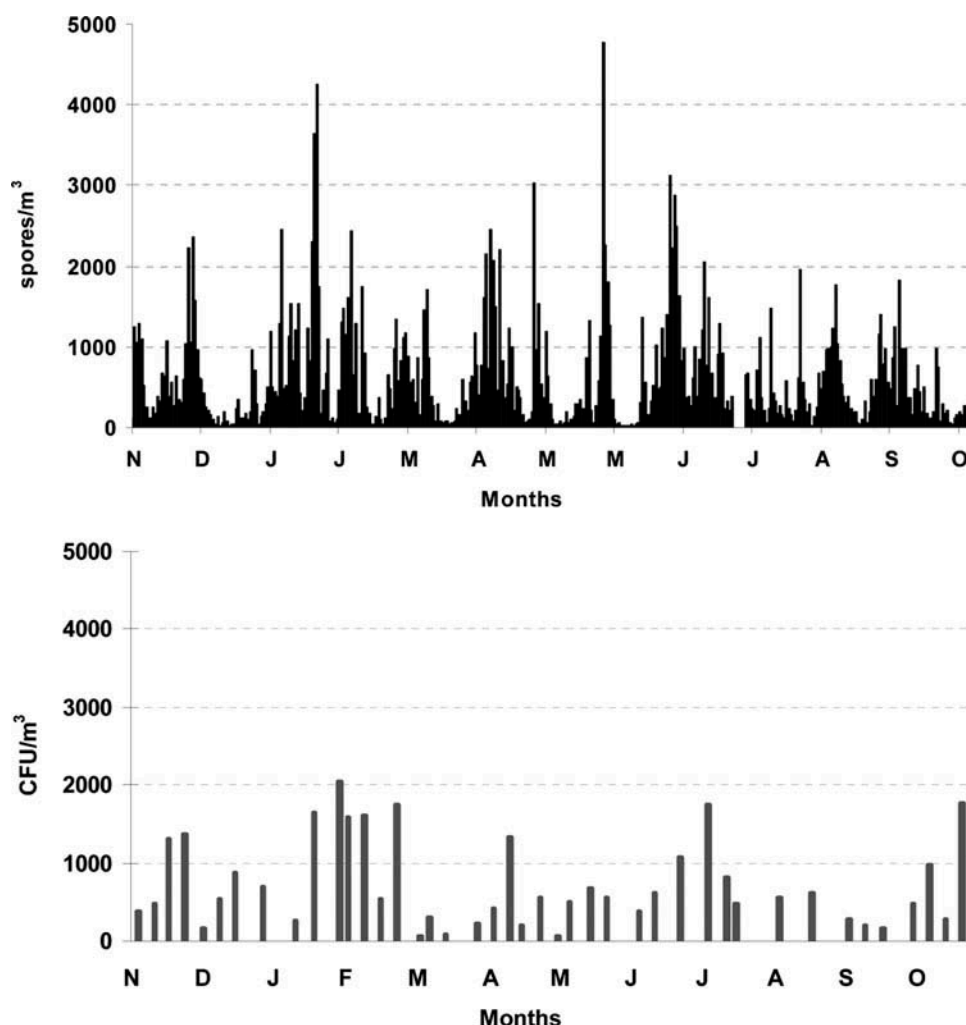


Figure 1. Daily mean spore concentration (spores/m³) and propagules (CFU/m³) identified from November 2010 to October 2011.

Two volumetric samplers were used for fungal collection. The spores for detection by direct microscopy were collected with a Hirst type sampler (Lanzoni VPPS 2000, Bologna, Italy). This sampler was located on the rooftop of the Biology Faculty of the University of Havana, 35 m above ground level (23° 08' N; 82° 23' W). Sampling was carried out continuously (operating 24 h/day) during one year, from 2 November 2010 to 31 October 2011. The Lanzoni equipment was calibrated for a ten litres of air per minute flow, and the spores impacted a Melinex tape coated with a 2% silicone solution. The spore count was performed using the methodology proposed by Galán et al. (2007). Results were then expressed as spores per cubic metre (spores/m³) of air for daily mean spore concentrations, and as total spores for monthly and annual values.

Cultivable airborne fungi were sampled using a slit-to-agar impactor aeroscope (Chirana, Ostrava, Czech Republic) with a suction capacity of 29 litres of air per minute; this method is comparable to

conventional techniques for viable spore sampling (Rojas et al., 2012). Air sampling was performed weekly at 11.00 local time. Samples were cultured in malt extract agar (MEA). Plates were incubated for seven days at 28 ± 1 °C. After incubation, fungal colonies growing on each Petri dish were counted, isolated and identified according to their microscopic features. Results were then expressed as CFU/m³.

The culture method allowed the viable spore sample and, consequently, the study of the fungal structures for a deep identification to genus or species level. Taxonomic identification of isolates was based on standard monographs for genera (Barnett & Hunter, 1997) and species (Ellis, 1971, 1976; Käärik et al., 1983; De Hoog et al., 2000; Pitt, 2000; Kirk et al., 2001; Klich & Pitt, 2002).

The spores observed by direct microscopy were identified at genus level and, for this purpose, the preparations from the reference collection of cultivations of the Department of Microbiology and Virology of the University of Havana were used as

well as specialised bibliography (i.e. Nikkels et al., 1996; Nitiu et al., 2010; Doctorfungus Corporation, 2001; Index Fungorum Partnership, 2004; amongst others).

In order to obtain a model reflecting diurnal fluctuations in total identified spore counts of the sampling period, we selected days on which spore counts were higher than the mean for the sampling period, and included the days without rainfall (Nilsson & Persson, 1981). The resulting days were used to calculate mean counts for every two hours, thereafter expressing the data as percentages. The daily values of the rainfall were recorded by the meteorological station of Casablanca, which is located close to the sampling area. The data were supplied by the Cuban Institute of Meteorology (INSMET).

Results

Qualitative data

Twenty six genera were identified based on the spores collected by the non-viable methodology (Table I). Most of the spores were from anamorphic fungi (i.e. *Alternaria*, *Beltrania*, *Bipolaris*, *Cercospora*, *Curvularia*, *Fusarium*, *Gliomastix*, *Helicoma*, *Helicomycetes*, *Nigrospora*, *Periconia*, *Pestalotiopsis*, *Pithomyces*, *Pseudocercospora*, *Pyricularia*, *Sporidesmium*, *Stemphyllium*, *Tetraploa* and *Torula*), but also some Ascomycetes (*Chaetomium*, *Leptosphaeria*, *Paraphaeosphaeria*, *Pleospora* and *Venturia*) and Basidiomycetes (*Coprinus* and *Ganoderma*) were found. Three genera were identified by means of cultivation (*Epicoccum*, *Monodictys* and *Spegazzinia*). Additionally, five fungal types have been detected with this methodology (i.e. *Aspergillus*/*Penicillium*-type, *Cladosporium cladosporioides*-type, *Cladosporium herbarum*-type, Xylariaceae-type and Uredinales-type). The similar features of these spore types made a precise identification difficult.

The cultivation-based methods allowed the identification of some genera difficult to recognise by direct microscopic observation (i.e. *Cephalosporium*, *Geotrichum*, *Gliocladium*, *Memnoniella*, *Nodulisporium* and *Phoma*). The growth of isolates and the development of vegetative or reproductive parts allowed their specific identification. In this work, the propagules of 47 species were identified mainly from mitospore fungi. Several Zygomycete species (e.g. *Syncephalastrum racemosum*, *Rhizopus stolonifer* and *R. oryzae*) as well as Ascomycete species (e.g. *Chaetomium globosum*) were isolated.

Most of the identified taxa have been previously reported in aerobiological studies conducted by viable methods in Cuba, either in outdoor or indoor environments. However, two genera (*Gliocladium*

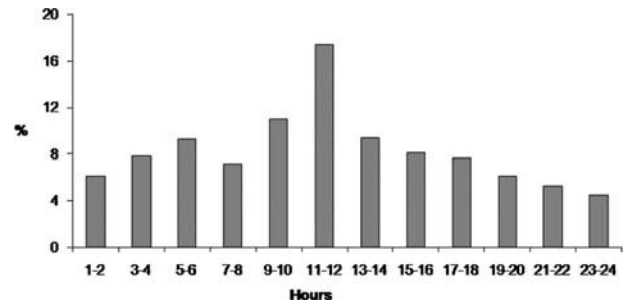


Figure 2. Model of intra-diurnal behaviour of total spores identified by direct microscopy.

and *Nodulisporium*) and ten species (*Acremonium strictum*, *Aspergillus foetidus*, *Cladosporium tenuissimum*, *Curvularia verruculosa*, *Fusarium graminearum*, *Penicillium digitatum*, *P. griseofulvum*, *Scopulariopsis candida*, *Trichoderma harzianum* and *T. viride*) represent new reports in the atmosphere of the country.

Quantitative data

The spores collected by non-viable method predominated, mainly the *Cladosporium cladosporioides*-type with a total of 148 717 spores. Moreover, *Leptosphaeria*, *Coprinus* and the *Aspergillus*/*Penicillium*-type registered a considerable incidence with an amount of total spores of 20 591, 17 244 and 16 392 spores, respectively. Other fungi present in the atmosphere of Havana with values higher than 1000 spores are *Alternaria*, *Bipolaris*, *Cladosporium herbarum*-type, *Curvularia*, *Fusarium*, *Ganoderma*, *Gliomastix*, *Nigrospora*, *Periconia*, *Monodictys*-type and *Uredinales*-type (Table I).

The importance of *Cladosporium cladosporioides* has been corroborated by the high number of isolates (20 177 CFU/m³) obtained by the cultivation-based method (Table I). This taxon shows great relative density (RD = 65.3) by both methods and a high frequency, with similar values (relative frequency, RF = 96.7 for direct microscopy and RF = 95.2 for cultivation-based method). Other relevant species are *Penicillium citrinum* (1966 CFU/m³), *Aspergillus flavus* (1552 CFU/m³), *Aspergillus niger* (1069 CFU/m³) and *Alternaria tenuissima* (1380 CFU/m³). They registered a RD with values ranging from 3.5 to 6.4 and have been isolated in almost half of the days (RF = 40.5–59.5), with the exception of *P. citrinum* (RF = 7.1).

With regard to the genera with concentration values of 1000 spores and higher (referred to the non-viable method), only *Alternaria*, *Bipolaris*, *Curvularia* and *Fusarium* were registered in a considerable number of isolates (Table I). All *Fusarium* species had similar RD (0.1–0.6) and RF (2.3–7.1).

Table II. Genera and fungal types detected by microscopy with one or two well-defined intra-diurnal spore peaks.

Genera	Spores peak			
	Hour	%	Hour	%
<i>Venturis</i>	1–2	25	–	–
<i>Pestalotiopsis</i>	1–2	15	–	–
<i>Pyricularia</i>	1–2	21	–	–
<i>Leptosphaeria</i>	5–6	22	–	–
<i>Ganoderma</i>	5–6	24	–	–
<i>Coprinus</i>	5–6	32	–	–
<i>Xylariaceae</i> -type	5–6	37	–	–
<i>Sporidesmium</i>	5–6	24	–	–
<i>Helicoma</i>	5–6	36	–	–
<i>Paraphaeosphaeria</i>	5–6	18	–	–
<i>C. cladosporioides</i> -type	11–12	20	–	–
<i>C. herbarum</i> -type	11–12	16	–	–
<i>Bipolaris</i>	11–12	20	–	–
<i>Nigrospora</i>	11–12	23	–	–
<i>Periconia</i>	11–12	21	–	–
<i>Alternaria</i>	11–12	13	–	–
<i>Uredinales</i> -type	11–12	17	–	–
<i>Pseudocercospora</i>	11–12	21	–	–
<i>Chaetomium</i>	11–12	67	–	–
<i>Curvularia</i>	11–14	15	–	–
<i>Monodictys</i>	11–14	22	–	–
<i>Stemphylium</i>	15–16	21	–	–
<i>Pithomyces</i>	17–18	22	–	–
<i>Helicomyces</i>	23–24	16	–	–
<i>Cercospora</i>	3–4	16	13–14	18
<i>Gliomastix</i>	3–4	10	13–14	15
<i>Spegazzinia</i>	3–4	16	13–14	34
<i>Epicoccum</i>	5–6	15	13–14	15
<i>Beltrania</i>	9–10	18	17–18	21

The same was observed for the *Bipolaris* species (RD = 0.1–0.3, RF = 2.3–4.8). *Curvularia aerea* was also a predominant species (RD = 1.8, RF = 28.6). All *Alternaria* isolates were identified as *A. tenuissima* (RD = 4.5, RF = 45.2). The remainder of the identified or isolated taxa were recorded at lower values (153–576 total spores) or represented by only a single isolate obtained by cultivation.

The prevalence of the higher daily concentrations was observed during the dry season (November–April; Figure 1). The peak daily concentration was 4771 spores/m³, registered on 26 May 2011. The high monthly average was obtained in January (31 663 spores; Figure 1), the month, in which also important daily peaks were recorded (i.e. 3646 and 4249 spores/m³ on 20 and 21 January, respectively). Other secondary peaks were registered on 26 April and 24 June (4020 and 3132 spores/m³, respectively).

The propagule concentration fluctuates along the year. The highest level was registered on 31 January 2011 with 2034 CFU/m³ (Figure 1). Other important values higher than 1700 CFU/m³ were detected on 25 February, 10 July and 28 October. The

intra-diurnal variation of the total identified spores shows a marked oscillation during the course of the day (Figure 2). The lowest levels were detected at dawn. One or two well-defined intra-diurnal spore peaks of different genera or spore types were present (Table II). Peak daily counts were recorded at noon (11.00–12.00), the moment, in which the sampling of the cultivation method was conducted. The most common taxa markedly peaking at 11.00–12.00 were *Cladosporium cladosporioides*-type, *Cladosporium herbarum*-type, *Bipolaris*, *Nigrospora*, *Periconia*, *Alternaria*, *Uredinales*-type, *Pseudocercospora*, *Chaetomium*, *Curvularia*, *Monodictys*, *Stemphylium* and *Pithomyces*. Only a few genera (i.e. *Gliomastix*, *Spegazzinia*, *Cercospora*, *Epicoccum* and *Beltrania*) had two concentration peaks during the day. Otherwise, the spores from *Helicomyces*, *Venturia*, *Pestalotiopsis*, *Pyricularia*, *Leptosphaeria*, *Ganoderma*, *Coprinus*, *Xylariaceae*-type, *Sporidesmium*, *Helicoma* and *Paraphaeosphaeria* showed a nocturnal pattern. Other fungi such as *Aspergillus*/*Penicilium*-type, *Pleospora* and *Tetraploa* did not present a well-defined pattern.

Discussion

This is the first study of fungal diversity in the Cuban atmospheric ecosystem, combining a methodology based on identification by direct microscopy with the isolation and identification of viable propagules. The combination of the two methodologies allows generalising conclusions about the genera and species that form the environmental mycobiota of Cuba. In this work, 35 genera have been characterised in the atmosphere of the Havana, 26 of which recognised by non-culture-based methodology, six by culture-based method and three with both sampling systems.

Previous studies only reported the presence of *Leptosphaeria* ascospores and some mitospores in the atmosphere of the Caribbean (Herrera et al., 2003). However, we detected the continuous presence of ascospores and basidiospores in the atmospheric bioaerosol throughout the year (Quintero et al., 2010). Rivera-Mariani and Bolaños-Rosero (2012) stated that this spore types could induce more allergic sensitisations than the mitospores in other Caribbean areas.

The genera detected exclusively after cultivation belong to Zygomycetes (*Rhizopus* and *Syncephalastrum*) or mitosporic fungi (*Acremonium*, *Cephalosporium*, *Chrysonilia*, *Geotrichum*, *Gliocladium*, *Memmoniella*, *Nodulisporium*, *Paeclomyces*, *Phoma*, *Scopulariopsis* and *Trichoderma*). Two of them were not previously identified in the air of Cuba: *Gliocladium* and *Nodulisporium*.

Syncephalastrum racemosum, *Rhizopus stolonifer* and *R. oryzae* have been previously reported in indoor environments of Cuba only, mainly in warehouses and stores (Borrego & Perdomo, 2012). *Trichoderma* was previously isolated from indoor and outdoor environments (Borrego et al., 2010). The presence of *T. harzianum* and *T. viride* has been now confirmed by means of our study. These fungi have a rapid growth, similar to *Chrysospora sitophila* (Arnold, 1991), and their spores are difficult to separate in microscopic preparations from other genera.

Epicoccum, *Monodictys* and *Spegazzinia* have been identified by both methods. *Epicoccum* and *Monodictys* are cosmopolitan fungi, which can develop in different environments. They have been cited in previous studies of the Cuban atmosphere (Estrada de la Riva, 1951; Fuentes, 1958; Arnold et al., 1987; Herrera et al., 2003; Almaguer et al., 2012; Rojas & Aira, 2012) and the world (Nikkels et al., 1996; Oliveira et al., 2009; Nitiu et al., 2010). *Spegazzinia*, an uncommon fungus in the atmosphere, has also been detected in our study.

The use of volumetric methods combined with direct microscopy identification presents unquestionable advantages in the studies of environmental mycology, mainly the possibility to know the intra-diurnal behaviour of spores. However, a fungal identification at species level could hardly ever be achieved (Mediavilla et al., 1998; Magyar et al., 2009; Nitiu et al., 2010) with this methodology. Therefore, isolation, culture and identification of viable propagules can be used to complete this information and to ascertain the species, which could have been included in each fungal type.

In this paper, five fungal types were defined (*Cladosporium cladosporioides*, *C. herbarum*, *Aspergillus/Penicillium*, Xylariaceae and Uredinales). The *C. cladosporioides*-type is represented by *C. cladosporioides*, *C. oxysporum* and *C. tenuissimum* because their conidia share similar characteristics (ellipsoid or lemon-shaped with a smooth wall). Spores included in the *C. herbarum*-type have greater similarity with those of *C. sphaerospermum* (ellipsoid or oblong shape, and thick or verrucose walls). Mediavilla et al. (1998) included *C. herbarum*, *C. sphaerospermum* and *C. macrocarpum* Preuss. among others in the *C. herbarum*-type. Therefore, it is possible that the species included in this spore type could be extended in future investigations. In this case, we have found that *C. cladosporioides* is more frequent registering higher concentrations than the *C. herbarum*-type. These results were corroborated by the cultivation-based method.

Cladosporium is a cosmopolitan genus of the atmospheric mycobiota (Abdel Hameed, 2005; Aira et al., 2012; Haas et al., 2013). Its presence has been

previously reported in Cuba (Arnold et al., 1987; Rojas et al., 2002; Herrera et al., 2003; Almaguer et al., 2012), but this is the first time that the two aforementioned spore types were defined and identified. The presence of *C. tenuissimum* in outdoor environments was reported for the first time. The importance of the identification and the monitoring of both types is given by the allergenic character of their spores (Abdel Hameed, 2005). In addition, many *Cladosporium* species are recognised as plant, animal or human pathogens and their important role in deteriorating processes of the manufactured goods, monuments and painting murals is proven (Martínez et al., 1987; Sharma et al., 2011; Huyan et al., 2012).

The *Aspergillus/Penicillium*-type includes small hyaline amero-spores, sometimes in chains without ramifications. Its generic differentiation is very difficult by non-viable methods (Pyrrri & Kapsanaki-Gotsi, 2007; Oliveira et al., 2009) and therefore, the combination with cultivation-based methods is important. The presence of *Aspergillus/Penicillium*-type has been previously reported from Cuba and in the atmosphere of other tropical countries (Khan et al., 1999; Afzal & Mehdi, 2002; Herrera et al., 2003). We identified 11 *Aspergillus* and ten *Penicillium* species. *Aspergillus flavus*, *A. niger* and *P. citrinum* were most concentrated or frequent. These species together with the rest of both genera were observed in indoor or outdoor atmospheres by the cultivation-based method (Rojas et al., 2002; Almaguer et al., 2012). Nevertheless, the non-frequent species *A. foetidus*, *P. digitatum* and *P. griseofulveum* are new citations for the Cuban atmosphere. These species have also been found in the air of other tropical regions (Adhikari et al., 2004; Abu-Dieyeh et al., 2010).

The Uredinales- and Xylariaceae-types have been previously reported from Cuba (Herrera et al., 2003) and other regions (Pyrrri & Kapsanaki-Gosti 2007; Oliveira et al., 2009; Quintero et al., 2010). *Xylaria* is the most representative genus of the Xylariaceae (the *Nodulisporium* anamorph phase corresponds to this genus). It is detected by the cultivation-based method and their citation is the first scientific detection in the air of Cuba.

From each *Alternaria* and *Nigrospora*, only one species was identified by the cultivation-based method: *Alternaria tenuissima* and *N. sphaerica*. *Alternaria* has been previously detected by the viable (Arnold et al., 1987; Rojas et al., 2002) and non-viable methods (Herrera et al., 2003), but this is the first time, they are identified at species level in Caribbean outdoor areas. *Alternaria tenuissima* has been reported in other parts of the world (Abu-Dieyeh et al., 2010) and *N. sphaerica* has been mainly

found in Cuban aerobiological indoor environments (Borrego & Perdomo, 2012).

The diversity of species in *Fusarium* has been previously documented in the Havana atmosphere (Arnold et al., 1987) and in a rural area close to Havana (Almaguer et al., 2012). However, in none of these studies, the presence of *Fusarium graminearum* was reported.

In our study, several viable species of *Bipolaris* and *Curvularia* were identified (*B. australiensis*, *B. hawaiiensis*, *C. aeria*, *C. clavata* and *C. verruculosa*). All have been cited in other atmospheric ecosystems of Cuba (Arnold et al., 1987; Almaguer et al., 2012), with the exception of *C. verruculosa*.

During the period of the present study, 227 702 spores and 30 903 CFU/m³ were registered. We observed a higher prevalence of spores during the dry season in contrast to other tropical areas of the Northern Hemisphere (Halwagy, 1994; Shaheen, 1992; Dames & Cadman, 1994; Calderón et al., 1997). For the conidia liberation into the atmosphere, a certain degree of environmental dryness is necessary, which can be reached during the dry period, registered in Cuban in November–April. Most of the genera detected presented dry conidia (*Cladosporium*, *Aspergillus* and *Penicillium*) and their presence in the air can be favoured by atmospheric dryness (Kasprzyk et al., 2004; Aira et al., 2012).

It has been reported that the spore concentration fluctuates also during the day (Okten et al., 2005). In this study, the increase of the spore content in the air takes place during the day, 11.00–12.00. This fact is associated with the dry conditions registered during the diurnal period as consequence of the strong tropical radiation and its important relationship with the release of dry spores (such as *Cladosporium*, the major component of the air in Cuba). Moreover, the most important concentrations of various fungal genera and species were detected during this time of the day. This fact proves that the hour between 11.00–12.00 is an accurate period to conduct the cultivation-based method sampling, because it is the time of the day with highest spore concentrations and viable airborne fungal diversity.

Conclusion

The combination of both methodologies (cultivation-based and visual identification by direct microscopy) is effective for a better knowledge of the fungal biodiversity in the atmosphere. The methods based on identification by direct microscopy are essential for the quantification of ascospores, basidiospores and other fungi that show specific difficulties for sporulation. The isolation of viable fungi from

the air complemented the impossibility of identifying some spores without peculiar characteristics, as well as spores that cannot germinate due to malformations or losses of parts in their structure. Most of the identified fungi are saprophytes of plants, soil or wood and their spores are potential allergens or phytopathogens. This information could be applied in several scientific disciplines, mainly in the medical and the agricultural fields.

Acknowledgements

The authors acknowledge funding support by the USC-Banco Santander programme.

References

- Abdel Hameed, A. A. (2005). Vegetation: A source of air fungal bio-contaminant. *Aerobiologia*, 21, 53–61.
- Abu-Dieyeh, M. H., Barham, R., Abu-Elteen, K., Al-Rashidi, R. & Shaheen, I. (2010). Seasonal variation of fungal spore populations in the atmosphere of Zarqa area, Jordan. *Aerobiologia*, 26, 263–276.
- Adhikari, A., Sen, M. M., Gupta-Bhattacharya, S. & Chanda, S. (2004). Volumetric assessment of airborne fungi in two sections of a rural indoor dairy cattle shed. *Environment International*, 29, 1071–1078.
- Afzal, M. & Mehdi, F. S. (2002). Atmospheric fungi of Karachi City, Pakistan. *Journal of Biological Science*, 5, 707–709.
- Aira, M. J., Rodríguez-Rajo, F. J., Fernández-González, M., Seijo, C., Elvira-Rendueles, B., Abreu, I., Gutiérrez-Bustillo, M., Pérez-Sánchez, E., Oliveira, M., Recio, M., Tormo, R. & Morales, J. (2013). Spatial and temporal distribution of *Alternaria* spores in the Iberian Peninsula atmosphere, and meteorological relationships: 1993–2009. *International Journal of Biometeorology*, 57, 265–274.
- Aira M. J., Rodríguez-Rajo F. J., Fernández-González, M., Seijo, C., Elvira-Rendueles, B., Gutiérrez-Bustillo, M., Abreu, I., Pérez-Sánchez, E., Oliveira, M., Recio, M., Morales, J. & Muñoz, A. F. (2012). *Cladosporium* airborne spore incidence in the environmental quality of the Iberian Peninsula. *Grana*, 51, 293–304.
- Aira, M. J., Rojas, T. I. & Jato, V. (2002). Fungi associated with three houses in Havana (Cuba). *Grana*, 41, 114–118.
- Almaguer, M., Rojas, T. I., Rodríguez-Rajo, F. J. & Aira, M. J. (2012). Airborne fungal succession in a rice field of Cuba. *European Journal of Plant Pathology*, 133, 473–482.
- Arnold, G. R. W. (1991). Contribución al conocimiento de los hongos del orden Mucorales (Zygomycetes, Zygomycotina) de Cuba. *Revista del Jardín Botánico Nacional de la Universidad de La Habana*, 12, 121–126.
- Arnold, G. R. W., Guerra, A. G. & Rodríguez, N. (1987). Presencia de hongos en el aire del INIFAT. Reporte de Investigación. *Revista INIFAT*, 43, 3–7.
- Barnet, H. L. & Hunter, B. B. (1997). *Illustrated genera of imperfect fungi*. New York: MacMillan Publisher Co.
- Borrego, S., Guamet, P., Gómez de Saravia, S., Batistini, P., García, M., Lavín, P. & Perdomo, I. (2010). The quality of air at archives and the biodeterioration of photographs. *International Biodeterioration and Biodegradation*, 64, 139–145.
- Borrego, S. & Perdomo, I. (2012). Aerobiological investigations inside repositories of the National Archive of the Republic of Cuba. *Aerobiologia*, 28, 303–316.

- Cadreja, J. & Fernández de Castro, J. (1955). Quantitative studies of airborne cultivable spores in Havana, Cuba. *Journal of Allergy*, 26, 150–152.
- Calderón, C., Lacey, J., McCartney, A. & Rosas, I. (1997). Influence of urban climate upon distribution of airborne Deuteromycete spore concentrations in Mexico City. *International Journal of Biometeorology*, 40, 71–80.
- Dames, J. F. & Cadman, A. (1994). Air-spores of Durban: A sub-tropical, coastal South African city. II. Fungal spore component. *Grana*, 33, 346–348.
- De Hoog, G. S., Guarro, J., Gené, J. & Figueras, M. J. (2000). *Atlas of clinical fungi*. Utrecht: Centraalbureau voor Schimmelcultures.
- Doctorfungus Corporation (2001). Fungi descriptions. <http://www.doctorfungus.org> (accessed 7 June 2013).
- Ellis, M. B. (1971). *Dematiaceous hyphomycetes*. Kew: Commonwealth Mycological Institute.
- Ellis, M. B. (1976). *More dematiaceous hyphomycetes*. Kew: Commonwealth Mycological Institute.
- Estrada de la Riva, J. (1951). Variaciones en la Micología ambiental de Cuba. Estudio Micológico y Clínico. *International Archives of Allergy and Applied Immunology*, 2, 360–370.
- Fernández-González, M., Rodríguez-Rajo, F. J., Escuredo, O. & Aira, M. J. (2012). Optimization of integrated pest management for powdery mildew (*Uncinula necator*) control in a vineyard based on a combination of phenological, meteorological and aerobiological data. *Journal of Agricultural Science*, doi: 10.1017/S0021859612000743
- Fuentes, C. A. (1958). Revisión de las investigaciones sobre Micología medica y de la literatura en Cuba durante el decenio de 1945 a 1955. *Mycopathologia et Mycologia applicata*, 9, 207–223.
- Galán, C., Cariñanos, P., Alcázar, P. & Domínguez, E. (2007). Manual de Calidad y Gestión de la Red Española de Aerobiología. Córdoba: Servicio de Publicaciones de la Universidad de Córdoba.
- Guíamet, P., Borrego, S., Lavin, P., Perdomo, I. & Gomez de Saravia, S. (2011). Biofouling and biodeterioration in materials stored at the historical Archive of the Museum of La Plata, Argentine and at the National Archive of the Republic of Cuba. *Colloids and Surfaces B: Biointerfaces*, 85, 229–234.
- Haas, D., Galler, H., Luxner, J., Zarfel, G., Buzina, W., Friedl, H., Marth, E., Habib, J. & Reinthaler, F. F. (2013). The concentrations of culturable microorganisms in relation to particulate matter in urban air. *Atmospheric Environment*, 65, 215–222.
- Halwagy, H. M. (1994) Fungal airspora of Kuwait City, Kuwait, 1975–1987. *Grana*, 33, 340–345.
- Herrera, L., Carrazana, D. & Quiñones, R. (2003). Los hongos anemófilos de la ciudad de Santa Clara, Cuba. *Centro Agrícola*, 3, 78–83.
- Huyan, X. H., Yang, Y. P., Fan, Y. M., Huang, W. M., Li, W. & Zhou, Y. (2012). Cutaneous and systemic pathogenicity of a clinical isolate of *Cladosporium sphaerospermum* in a Murine Model. *Journal of Comparative Pathology*, 147, 354–359.
- Index Fungorum Partnership (2004). Index fungorum. <http://www.indexfungorum.org> (accessed 7 June 2013).
- Käärik, A., Keller, J., Kiffer, E., Perreau, J. & Reisinger, O. (1983). *Atlas of airborne fungal spores in Europe*. Berlin: Springer-Verlag.
- Kasprzyk, I., Rzepowska, B. & Wasylów, M. (2004). Fungal spores in the atmosphere of Rzeszów (southeast Poland). *Annals of Agricultural and Environmental Medicine*, 11, 285–289.
- Khan, Z. U., Khan, M. A., Chandy, R. & Sharma, P. N. (1999) *Aspergillus* and other moulds in the air of Kuwait. *Mycopathologia*, 146, 25–32.
- Kirk, P. M., Cannon, P. F., David, J. C. & Stalpers, J. A. (2001). *Dictionary of fungi*. Wallingford: CAB International.
- Klich, M. A. & Pitt J. I. (2002). *A laboratory guide to the common Aspergillus species and their 19 teleomorphs*. Canberra: CSIRO, Division of Food Processing.
- Magyar, D., Frenguelli, G., Bricchi, E., Tedeschini, E., Csontos, P., Li, D.-W. & Bobvos, J. (2009). The biodiversity of air spora in an Italian vineyard. *Aerobiologia*, 25, 99–109.
- Mallo, C. A., Nitiu, D. S. & Gardella, M. C. (2011). Airborne fungal spore content in the atmosphere of the city of La Plata, Argentina. *Aerobiologia*, 27, 77–84.
- Martínez, P., Sánchez, A. I., Brizuela, A. L. & Cuello, S. (1987). Contaminación microbiana en pinturas murales de la Catedral de La Habana. *Revista Cubana de Biología*, 1, 73–75.
- Mediavilla, A., Angulo, J., Infante, F., Comtois, P. & Domínguez, E. (1998). Preliminary statistical modelling of the presence of two conidial types of *Cladosporium* in the atmosphere of Córdoba, Spain. *Aerobiologia*, 14, 229–234.
- Mesquita, N., Portugal, A., Videira, S., Rodríguez-Echeverría, S., Bandeira, A. M. L., Santos, M. J. A. & Freitas, H. (2009). Fungal diversity in ancient documents. A case study at the Archive of the University of Coimbra. *International Biodeterioration and Biodegradation*, 63, 626–629.
- Nikkels, A. H., Terstege, P. & Spijksma, F. T. M. (1996). Ten types of microscopically identifiable airborne fungal spores at Leiden, The Netherlands. *Aerobiologia*, 12, 107–112.
- Nilsson, S. & Persson, S. (1981). Tree pollen spectra in the Stockholm region (Sweden), 1973–1980. *Grana*, 20, 179–182.
- Nitiu, D. S., Mallo, A. C., Gardella-Sambeth, M. C. & Morbelli, M. A. (2010). Contribución a la identificación de esporas del reino fungi en la atmósfera de la Plata, Argentina. *Boletín de la Sociedad Argentina de Botánica*, 45, 301–308.
- Oliveira, M., Ribeiro, H., Delgado, J. L. & Abreu, I. (2009). Seasonal and intradiurnal variation of allergenic fungal spores in urban and rural areas of the North of Portugal. *Aerobiologia*, 25, 85–98.
- Okten, S. S., Asan, A., Tungan, Y. & Türe, M. (2005). Airborne fungal concentrations in east patch of Edirne City (Turkey) in autumn using two sampling methods. *Trakya University of Journal Sciences*, 6, 97–106.
- Pitt, J. I. (2000). *A laboratory guide to common Penicillium species*. Canberra: CSIRO, Food Science.
- Pyrri, I. & Kapsanaki-Gotsi, E. (2007). A comparative study on the airborne fungi in Athens, Greece, by viable and non-viable sampling methods. *Aerobiologia*, 23, 3–15.
- Quintero Fossas, J. M. (1964). *Atmospheric fungi*. University of Puerto Rico, PhD Diss.
- Quintero, E., Rivera-Mariani, F. & Bolaños-Rosero, B. (2010). Analysis of environmental factors and their effects on fungal spores in the atmosphere of a tropical urban area (San Juan, Puerto Rico). *Aerobiologia*, 26, 113–124.
- Raphoz, M., Goldberg, M. S., Garneau, M., Héguy, L., Valois, M. F. & Guay, F. (2010). Associations between atmospheric concentrations of spores and emergency department visits for asthma among children living in Montreal. *Archives of Environmental & Occupational Health*, 65, 201–210.
- Rivera-Mariani, F. E. & Bolaños-Rosero, B. (2012). Allergenicity of airborne basidiospores and ascospores: Need for further studies. *Aerobiologia*, 28, 83–97.
- Rojas, T. I. & Aira, M. J. (2012). Fungal biodiversity in indoor environments in Havana, Cuba. *Aerobiologia*, 28, 367–374.
- Rojas, T. I., Aira, M. J., Batista, A., Cruz, I. L. & González, S. (2012). Fungal biodeterioration in historic buildings of Havana (Cuba). *Grana*, 51, 44–51.

- Rojas, T. I., Llanes, N., Benítez, M., Aira, M. J. & Malagón, H. (2007). El género *Aspergillus* en la atmósfera de La Habana (Cuba). *Boletín Micológico*, 22, 41–46.
- Rojas T. I., Martínez, E., Gómez, Y. & Alvarado, Y. (2002). Airborne spore of *Aspergillus* in cultural institutions at Havana University. *Grana*, 41, 190–193.
- Seidel, D. & Räuber, A. (1970). Beobachtungen über den Flug von Pilzsporen auf Kuba. *Beiträge zur tropischen und subtropischen Landwirtschaft und Tropenveterinärmedizin*, 8, 43–50.
- Shaheen, I. A. (1992). Aeromycology of the Amman area, Jordan. *Grana*, 31, 223–228.
- Sharma, K., Agrawal, M. & Jaiswal, A. K. (2011). Bio-deterioration of ancient monuments: Problems and prospects. *Current Botany*, 2, 23–24.
- Stepalska, D. & Wolek, J. (2009). Intradiurnal periodicity of fungal spore concentrations (*Alternaria*, *Botrytis*, *Cladosporium*, *Didymella*, *Ganoderma*) in Cracow, Poland. *Aerobiologia*, 25, 333–340.
- Toivola, M., Alm, S., Reponen, T., Kolari, S. & Nevalainen, A. (2002). Personal exposure and microenvironmental concentration of particles and bioaerosols. *Journal of Environmental Monitoring*, 4, 166–174.