Chapter 20
Aerobiology of Pteridophyta Spores: Preliminary Results and Applications

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20.1 Introduction

Aerobiology is the science of airborne biological and non biological particles (viable or non viable), of their origin, transport and deposition in relation to meteorological conditions and their impact on human beings, animals or plants (Lebowitz and O'Rourke 1991; Spieksma 1992).

The field of aerobiology is interdisciplinary with applications in public health, agriculture, plant pathology, palynology and many others. Biology, plant physiology, mycology, meteorology and aerosol physics are the basic sciences for studying production, release, transport and deposition of airborne particles.

Some of airborne non biological particles could be considered as atmospheric pollutants, whose harmful effects on human health are well known (Kim et al. 2001; Cariñanos et al. 2007). The most studied airborne biological particles are pollen grains and fungal spores, with their implications on allergy, phenology, farming productivity, environment or even criminology widely addressed in the literature (Chuine et al. 1999; D'Amato 2000; Cunha et al. 2003; Bryant and Jones 2006; García-Mozo et al. 2006; Sánchez et al. 2009). Nevertheless, little is known about fern spores airborne behaviour and their possible applications on other fields, probably due to the scarce presence of Pteridophyta spores in the atmosphere in comparison with airborne levels of pollen grains and fungal spores (Favali et al. 2003).

On the basis of recent phylogenetic studies within vascular plants (Renzaglia et al. 2000; Pryer et al. 2004), lycophytes (including Lycopodiales, Selaginellales and Isoetales) have been separated from other groups included in the traditional group of Pteridophyta, such as Equisetales, Psilotales, Marattiales and Polypodiales. The latter ones, included in monilophytes group, are now related to seed plants (spermatophytes), both constituting the euphyllophytes clade. In this chapter, we follow the recent classification realised by Smith et al. 2006 for extant ferns, taking

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into account the historical assemblage of pteridophyte that includes together lycophytes and monilophytes due to their common features, i.e., spore-bearing.

We aimed to examine the main works carried out about fern spores aerobiology, in order to gain a better knowledge along with the current and future implications derived of these studies.

20.2 Methodology in Aerobiology Studies

There are different methods used to identify and quantify airborne pollen and spores content, being gravimetric and volumetric methods and their corresponding samplers, the most employed in aerobiological literature. The first one collects mainly airborne particles that fall by their own weight, whereas the volumetric traps capture these particles directly from the atmosphere.

The gravimetric method uses non-volumetric sedimentation traps, such as Tauber type (Tauber 1967), in which the number of grains obtained depends on the area of sedimentation, by means of adding a known spike of *Lycopodium* spores for estimation of absolute pollen and spore values. The samples are obtained monthly, weekly or even daily. Then, these samples are processed by acetolysis techniques and mounted in medium of gelatin-glycerol, counting after a fixed number of biological particles (Hall 1994). The results are expressed as particles trapped per temporal unit (day, week or month) and per square centimetre.

The volumetric method works mainly by volumetric suction samplers based on the impact principle (Hirst 1952). These samplers have two essential components: a vacuum pump whose suction flow rate is 10 L air/min, similar to the volume of air inhaled in humans; and a circular drum driven by a clockwork mechanism that rotates 2 mm every hour, ensuring continuous air sampling and the provision of hourly data. Thus, samples could be obtained weekly, daily or even hourly, and mounted in traditional-used medium of fuchsin-stained glycerine gelatin. The airborne data are expressed as particles per cubic metre of air and per day, after microscopic reading of samples and some standardised mathematical operations based mainly on the size of microscope field of vision used (Domínguez et al. 1991).

Both methods give similar quantitative and qualitative results for weekly total biological particles levels (Levetin et al. 2000), but volumetric method has more sensitive detection of these particles at low concentration (Latorre et al. 2008), an important aspect in allergic studies.

20.3 Aerobiological Notes of Pteridophyta

Pteridophyta includes more than 9,000 species in about 215 genera, distributed worldwide (Strasburguer et al. 2004) and attaining their greatest number and main development in the tropics and subtropics. The same pattern was registered in airborne spore content of Pteridophyte, where of the 49 taxa that have been distinguished in the few papers that reported data about aerobiology of ferns (Table 20.1), 42 belonged to researches carried out in tropical and subtropical zones of Asia. In that sense, we must point out the work of Yasmeen and Devi (1988) that informed about the airborne presence of 28 types of fern spores. There were only two studies dealing about atmospheric fern spores content in Africa (La Serna and Domínguez 2003; Njokuocha 2006), reporting 16 Pteridophyte spores. The number of spore types could be higher if more researches have been developed in that area, as also occurred in America, with three papers, especially in Buenos Aires and their surroundings (Majas and Romero 1992; Noetinger et al. 1994). On the other hand, five taxa were identified in the six works carried out in Europe. In other published papers about different aspects of palynology (Carson and Brown 1976; Calleja et al. 1993; Cabrales et al. 2003), fern spores were cited in the atmosphere of different tropical areas of Africa, Asia and America without any qualitative analysis.

The total number of fern spores depended on the method used and the spore type. According to annual total data reported in different years and different zones by means of gravimetric method (Fig. 20.1a), *Alsophila* and *Dicranopteris* were the main genera, with 300 and 140 spores per cm^2, respectively, between June 1974 and May 1975 in Taipei Basin (Chen and Huang 1980), followed by *Nephrolepis*, *Pteris* and Polypodiaceae with 49, 9 and 5 spores per cm^2. In Buenos Aires area, 107 spores per cm^2 of *Pteridium* were counted through year 1987 (Majas and Romero 1992), and 53 spores of *Equisetum* from July 1989 to June 1990 (Noetinger et al. 1994).

Volumetric method gave data about some fern taxa (Fig. 20.1b), like *Dicranopteris* with a total of 3,530 spores during year 2003 in Kinmen Island, Taiwan (Huang et al. 2008), the main fern taxa counted in Asia, followed by *Alsophila*, *Pteris* and *Lycopodium* with 40, 10 and 5 spores, respectively. A great number of spores of *Pteridium* were registered in an experimental farm of Great Britain (Lacey and McCartney 1994) between 20 August and 20 October, with around 40,000 spores. A lower annual average number of *Pteridium* spores were counted in other urban areas of Europe, such as Edinburgh (Caulton et al. 2000), with 118 spores during 1988–1997 period, and Salamanca (Rodríguez et al. 2009) with 67 spores between January 1998 and December 2007. In Rzeszów (Kasprzyk 2004) along a 6-year period (1997–2002), a mean annual total of 42, 12 and 5 spores of Polypodiaceae, *Equisetum* and *Lycopodium*, respectively, were registered.

The seasonal variations of airborne Pteridophyta spores levels were different depending on the area analysed (Fig. 20.2). In Asia, fern spore concentrations were continuous over the year in India (Yasmeen and Devi 1988), having higher levels between late Spring and early Autumn in Taiwan (Chen and Huang 1980; Chen and Chien 1986). In Nigeria (Njokuocha 2006), lower spore counts were registered between February and April, whereas in Buenos Aires (Majas and Romero 1992), lower levels were counted in January, March and between May and July. The presence of fern spores over all the months could be related with the high diversity and abundance of Pteridophyte species in tropics and their sequential development of sporangia, because in temperate areas (Nilsson and Praglowski 1974) there were

Table 20.1 Identified airborne taxa of Pteridophyte following classification proposed by Smith et al. (2006)

Division Tracheophyta								
Subdivision	Class	Order	Family	Genera	Africa	America	Asia	Europe
Lycophytina	Lycopsida	Lycopodiales	Lycopodiaceae	*Lycopodium*			2,4,5	7,13
	Selaginellopsida	Selaginellales	Selaginellaceae	*Selaginella*			17	
Euphyllophtina	Psilotopsida	Ophioglossales	Ophioglossaceae			14		
	Equisetopsida	Equisetales	Equisetaceae	*Equisetum*		10,14		7
	Polypodiopsida	Gleichniales	Gleichniaceae	*Dicranopteris*			2,3,4,5	
				Diplopterygium			2	
		Schizales	Anemiaceae	*Anaemia*			17	
			Lygodiaceae	*Lygodium*			2	
		Salviniales	Salviniaceae	*Azolla*		10		
		Cyatheales	Cyatheaceae	*Alsophila*			2,3,4,5	
				Cyathea			16	
		Polypodiales	Lindsaeaceae	*Lonchitis*	12			
				Tapeinidium			4	
			Dennstaedtiacaee	*Dennstaedtia*			2	
				Hypolepis	12		4	
				Microlepia			4,17	
				Pteridium	8	10	2,3	1,9,11,15
			Pteridaceae	*Acrostichum*			17	
				Adiantum	8	14	17	
				Cheilanthes			17	
				Notholaena			17	
				Pteris	12		4,5,17	
			Aspleniaceae	*Asplenium*	8,12		4,17	
			Thelypteridaceae		12		4,17	
				Cyclosorus	12		2,17	
			Woodsiaceae		12	14	4,17	
				Diplazium			2	

Blechnaceae		12	10,14	17	
	Stenochlaena			17	
Dryopteridaceae				2	
	Bolbitis			17	
	Cyrtomium			17	
	Dryopteris	8,12		17	13
	Elaphoglossum	12			
	Lithostegia			17	
	Polystichum			17	
Lomariopsidaceae	*Nephrolepis*	12	6,14	4,17	
Tectariaceae	*Tectaria*			17	
Davalliaceae	*Davallia*	8,12		4	
Polypodiaceae	*Colysis*			2,17	
	Drymoglossum			17	
	Drynaria			17	
	Elaphoglossum	12			
	Goniophlebium			17	
	Lemmaphyllum			2	
	Lepisorus			17	
	Mircrogramma	12			
	Microsorum			17	
	Polypodium	8	6,10	4,17	7,13

Caulton et al. (2000), Chen (1984), Chen and Chien (1986), Chen and Huang (1980), Huang et al. (2008), Hurtado and Riegler-Goihman (1986), Kasprzyk (2004), La Serna Ramos and Domínguez Santana (2003), Leitão et al. (1996), Majas and Romero (1992), Newson et al. (2000), Njokuocha (2006), Nilsson and Praglowski (1974), Noetinger et al. (1994), Rodríguez de la Cruz et al. (2009), Yang et al. (2003), Yasmeen and Devi (1988) (Underlined references correspond to studies carried out by means of gravimetric methods)

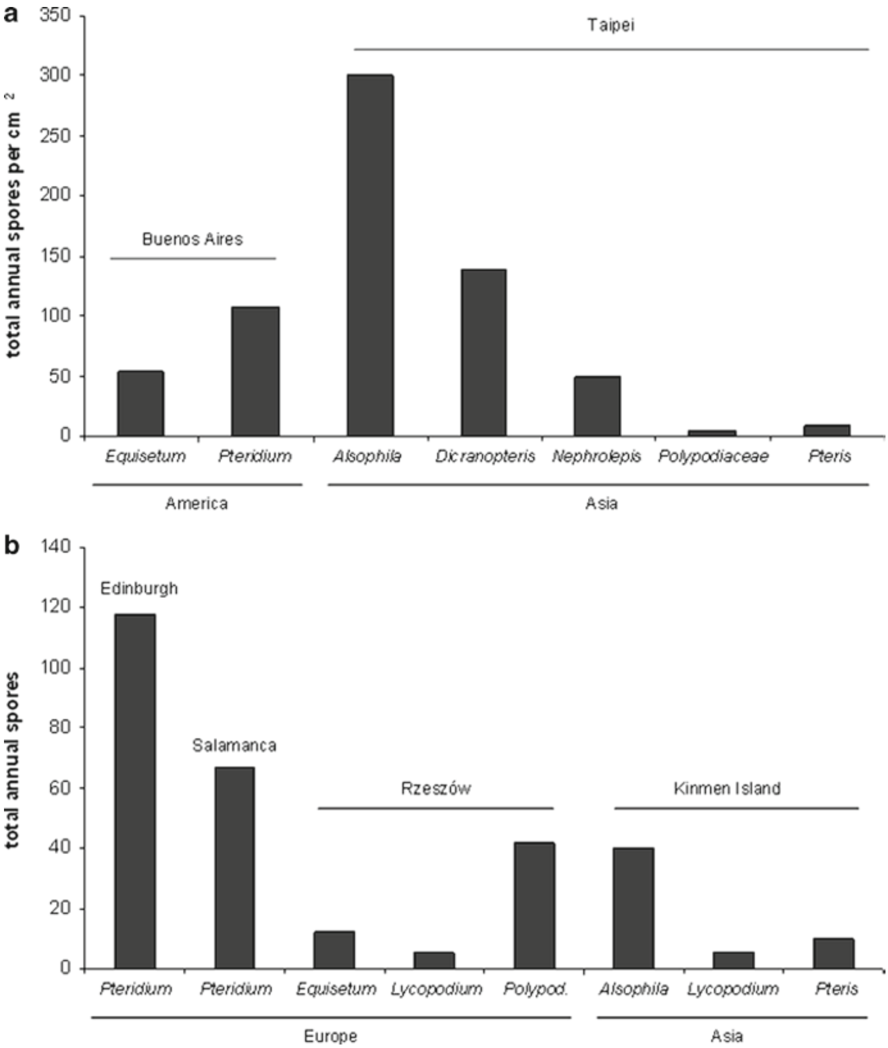

Fig. 20.1 Annual total records of some fern spores obtained by means of volumetric method (**a**), excluding *Dicranopteris*, or gravimetric method (**b**)

seasonal occurrence of fern spores between late Summer and early Autumn, specially in the case of *Pteridium* (Caulton et al. 2000), or even in early Spring in the case of *Equisetum* (Kasprzyk 2004).

Daily maximum concentrations of fern spores were reported in Asia (Chen 1984) for *Alsophila* (523 spores/cm^2, 25 July), *Dicranopteris* (148 spores/cm^2, 1 July) and *Pteridium* (82 spores/cm^2, 7 July) during the year 2003 by means of gravimetric method. The use of volumetric method registered some data about daily peaks of airborne spore levels, being 1,800 spores/m^3 of *Pteridium* during 4 September 1990, the highest daily value counted (Lacey and McCartney 1994). This value

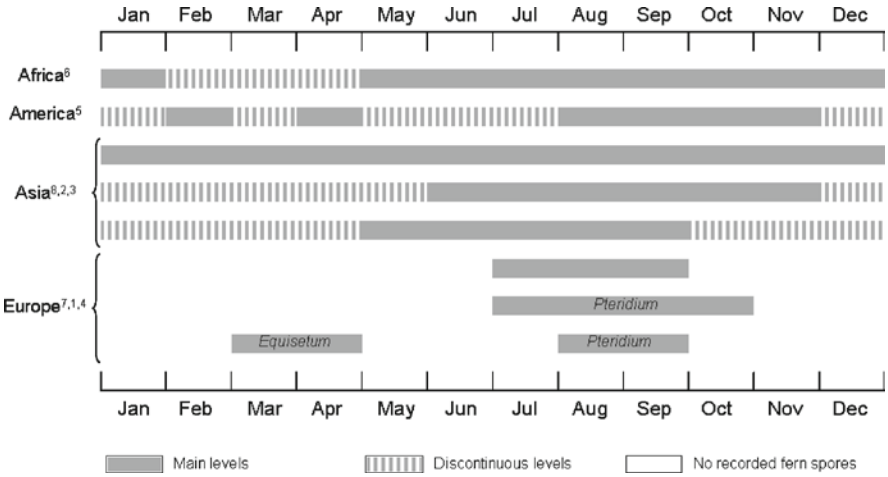

Fig. 20.2 Seasonal distribution of Pteridophyte spores (Caulton et al. 2000; Chen and Chien 1986; Chen and Huang 1980; Kasprzyk 2004; Majas and Romero 1992; Njokuocha 2006; Nilsson and Praglowski 1974; Yasmeen and Devi 1988)

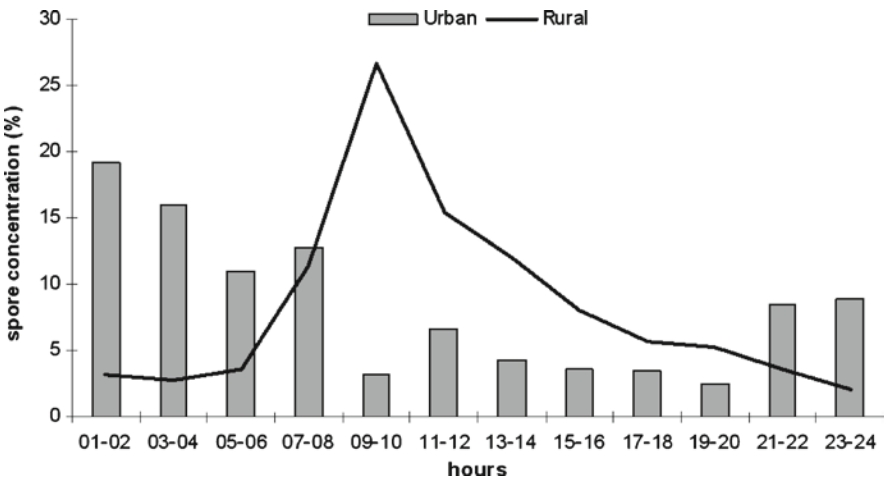

Fig. 20.3 Bi-hourly variation of bracken spore concentrations in urban and rural areas, mean daily counts (%)

could be explained with the close location of spore-trap to bracken thickets and could be also joined with intra-diurnal pattern, because in this rural area (Fig. 20.3) the levels were higher between 08:00 hours and midday, when sporing process occurs (Conway 1957). In urban areas of temperate zones, bracken and other ferns main populations are usually located far away (Favali et al. 2003), enabling higher airborne concentrations at night (Rodríguez et al. 2009), due to a probable medium-range transport according to different ways of atmospheric transport proposed by Rantio-Lehtimäki (1994). Regional transport was proved in *Castanea* pollen (Peeters and

Zooler 1988) and it was proposed for *Lycopodium* spores (Di-Giovanni et al. 1995), as well as other anemophilous pollen types.

20.4 Perspectives and Applications

Some of airborne biological particles present allergens, both included in the term "air pollution" and whose exposure represents a key factor among environmental determinants of allergy symptoms (Eder et al. 2006). The prevalence of pollen and fungal spores is increasing nowadays, especially in urban areas (Bush and Portnoy 2001; D'Amato et al. 2007). The importance of fern spores in allergic diseases was pointed out by Geller-Bernstein et al. (1987), by means of skin prick tests (SPT). Fern taxa with allergenic relevance are listed in Table 20.2, having extracts of spores in two species of *Dicranopteris* Bernh. (Chew et al. 2000), resam-fern (*Dicranopteris linearis* (Burm.f.) Underw.) and roadside fern (*Dicranopteris currani* Copel.) the highest frequency in SPT, with 34% and 31% percentages, respectively, followed by fishbone fern (*Nephrolepis auriculata* (L.) Trimen) and bird's nest fern (*Asplenium* nidus L.), with 30% and 25%, respectively, in Singapore. In addition, different works concerning the carcinogenicity of fern spores (Povey et al. 1996; Simán et al. 2000; Freitas et al. 2001) have indicated the risk for human health due to these spores which might be inhaled and ingested by humans (Wilson et al. 1998). Monitoring atmospheric fern spore levels is relevant in order to diminish exposure to fern allergens and to avoid the carcinogenic implications to human health.

In accordance with the clinical importance of atmospheric fern spore counts, climate change scenarios could be related to a greater production of airborne biological particles as pollen and fungal spores and propagules (Ziska et al. 2009) and might affect spore production in some fern spores, because in a model developed in some areas of Great Britain (Pakeman and Marrs 1996), bracken biomass (*Pteridium aquilinum* (L.) Kuhn.) would increase its presence and subsequently result in a greater spore liberation from sporangia.

Finally, some studies of modern biological particles spectra in caves can provide a reliable reflect of regional and local vegetation, directly comparable to the more

Table 20.2 Allergenic pteridophyte spores

Fern taxa	References
Acrostichum aureum L.	Yasmeen and Devi (1988), Bunnag et al. (1989)
Asplenium nidus L.	Chew et al. (2000)
Dicranopteris curranii Copel.	Chew et al. (2000)
D. linearis (Burm.f.) Underw.	Chew et al. (2000)
Lycopodium L.	Devi et al. (1989)
Nephrolepis auriculata (L.) Trimen	Chew et al. (2000)
Pteridium aquilinum (L.) Kuhn.	Chew et al. (2000)
Stenochlaena palustris (Burm.f.) Bedd.	Chew et al. (2000)

conventional spectra obtained from open sites (Coles and Gilbertson 1994; Navarro et al. 2002). In addition, fern spores have importance in a full interpretation and also in an accurate reconstruction of paleoenvironments (Barth et al. 2004; Zhu et al. 2008)

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