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AIRBORNE ALLERGENIC POLLEN IN NATURAL AREAS: HORNACHUELOS NATURAL PARK, CORDOBA, SOUTHERN SPAIN

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Abstract: The present study shows the results of monitoring the atmospheric pollen in the atmosphere of an area of natural vegetation, the Hornachuelos Natural Park, Cordoba, southern Spain, during a six years (1998-2003). Special attention was paid in the seasonal and intra-diurnal characteristics of airborne allergenic pollen. During this period, 31 pollen types were described, some of them rare in aerobiological analysis. High concentrations of allergenic pollen from entomophilous species and from areas at a long distance were found. Significant differences between pollen spectrum and pollen concentration of the natural study area and the surrounding cities were detected. Intra-diurnal pattern from trees surrounding the trap presented a clear peak at midday/afternoon. Pollen from taxa comprising many species and from species at far locations showed a smoother intra-diurnal pattern. The correlation with meteorological parameters was positive with maximum and mean temperatures, and negative with humidity and rainfall.

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Key words: aerobiology, pollen, allergy, natural areas, rural areas, intra-diurnal pattern, meteorology.

INTRODUCTION

Atmospheric pollen is a major cause of allergies, especially in developed countries [8, 29]. In each geographical area there are different species that compound a characteristic airborne pollen calendar controlled by the meteorological conditions changeable in areas and years. The release and dispersion of pollen depends on the microclimates which explain the differences observed regarding the onset of flowering within the same species [12, 17]. Aerobiological studies are of a great interest from an ecological and agricultural point of view, but they have a special interest for clinicians and allergic patients in order to establish a chronological correlation between the air pollen concentration and hay-fever and asthma symptoms [8]. Plant phenology is genetically determined and controlled by meteorological parameters. Therefore, to evaluate the pollen allergenic risk of a given area it is necessary to have both a deep knowledge of the local vegetation and of the floral phenology and influence of weather variations.

In order to obtain reliable aerobiological information for a whole area, an appreciate number of aerobiological monitoring stations are necessary to cover all different climatic and/or phytogeographic areas. Monitoring stations are usually placed in urban areas because of their higher concentration of inhabitants; little information is available, however, about pollen allergy sources in rural and natural areas. Very few scientific studies have been carried out on pollen aerobiology or floral phenology in rural areas devoted to agriculture [1, 6, 7, 27, 28]. Aerobiological studies on natural vegetation areas are especially scarce, probably due to the technical difficulties in the location and

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management of the traps. Therefore, there is an important lack of information about the aerobiological dynamic of airborne pollen in natural and semi-natural low-populated areas. Several studies have reported lower rates of allergic sensitization and allergies in children living in rural as compared to urban communities [3, 4]. This has been attributed to the lower levels of air pollution in rural areas [9]. Nevertheless, biological and geographical differences, such vegetal species spectrum, landscape composition, urbanization, human impact, etc., between natural and urban areas, lead us to hypothesize that allergenic pollen composition, timing and dynamics may be different, even from areas located in a given bioclimatic region and may lead to specific pollen allergy problems differing from the nearest urban areas.

The objective of this study was to determine the airborne pollen spectrum, pollen concentration and the time (season, daily and hourly details) when the most allergenic airborne pollen types are present in the atmosphere of the Hornachuelos Natural Park in the Province of Cordoba, southern Spain. Information on the aerobiological behaviour of pollen grains in a natural vegetation area was recorded during 6 years in order to detect possible differences with surrounding urban areas in the same bioclimatic region. The presented results will improve prevention and managing of allergy problems in natural vegetation areas of southern Spain.

MATERIAL AND METHODS

The study was carried out in "El Cabril", a 1,200-ha reserve located in the northern part of the Hornachuelos Natural Park in the Province of Córdoba (southern Spain), 38º4'N, 5º24'W. The average elevation of the area ranges from 450-600 m a.s.l. This area has a sub-humid Mediterranean climate with virtually no rainfall in summer (June–August). The mean 50-year data indicate annual rainfall of 700 mm and a mean temperature of 16.8ºC [31]. Meteorological data were taken by using a meteorological station located 10 m from the pollen trap.

A pollen sampler (Burkard® Hirst type) was placed 2 m above ground on a hill 450 m above a valley, 1 km from a centre for Residual Storage (200 workers) and 15 km from the nearest village. Nevertheless, in a 50 km radius, 18 villages are placed, 15 of them in the Province of Cordoba. The population living and working in this area is estimated at 40,000 inhabitants (Diputacion of Cordoba, http://www.dipucordoba.es).

The trap was placed in an area of native vegetation, where the holm-oak (*Quercus ilex* subsp. ballota (Desf.) Samp.) is the dominant tree species. Shrub formations are composed by *Erica arborea* L., *Quercus coccifera* L., *Olea europea* var. *sylvestris* L., *Pistacea lentiscus* L. *Genista hirsuta* Vahl., *Cistus monspeliensis* L., *Cistus albidus* L., *Cistus ladanifer* L. and *Lavandula stoechas* L. *Herbaceous* species correspond to the genus *Trifolium* spp., *Plantago* spp., *Briza* spp. *Avena* spp., *Bromus* spp., and *Poa* spp.

Table 1. Percentage total pollen concentration by taxon.

Pollen type	%
Quercus	59.81
Poaceae	13.20
Olea	10.99
Plantago	4.71
Pinus	1.85
Rumex	1.52
Cistus	1.25
Cupresaceae	1.10
Urticaceae	0.96
Chenopodiaceae	0.71
Fraxinus	0.55
Urtica membranaceae	0.55
Compositae	0.51
Brassicaceae	0.43
Myrtaceae	0.37
Alnus	0.33
Cyperaceae	0.26
Morus	0.18
Castanea	0.15
Mercurialis	0.14
Populus	0.12
Artemisia	0.10
Ericaceae	0.07
Platanus	0.06
Ulmus	0.03
Echium	0.02
Casuarina	0.01
Corylus	0.01
Labiateae	0.01
Tamarix	0.01
Salix	0.01
Total number of identified taxa	31

Table 2. Percentage average monthly pollen concentration.

Month	%
January	0.69
February	1.12
March	16.56
April	40.60
May	27.18
June	10.20
July	1.38
August	0.69
September	0.32
October	0.31
November	0.64
December	0.25

The area has a high degree of natural conservation: 60% of the Natural Park is occupied by autochthonous vegetation, 32% is occupied by "dehesas", a semi-natural Quercus meadow formation devoted to sheep and pig breeding. The rest of the landscape is composed of *Pinus* reforested areas (4.5%) and *Olea* crop areas (3%) [31].

Pollen capture and analysis were carried out by following the rules proposed by the Spanish Aerobiology Network (REA) [16]. The characteristics of the pollen season of the most important allergenic taxa were studied. Principal Pollen Period (PPP) season was defined by a first day, on which at least one pollen grain/m^3 was reached, with subsequent days containing one or more pollen grains/m^3. The end of season day is considered as the first of 5 consecutive days without pollen grains [17]. The method proposed by Galan *et al.* [15] was used to study intra-diurnal pollen variation.

Finally, the correlation between pollen concentrations during the PPP and the main meteorological parameters was studied by means of a Spearman correlation test.

RESULTS

The pollen samplings started in April 1998 and continued until 2003. During the 6-year study, a total of 362,193 pollen grains were counted. 1998 and 1999 were the years in which the highest number of pollen grains was detected, 62,296 and 60,157 respectively (Fig. 1). 31 pollen types were registered (Tab. 1). The best-represented tree pollen type throughout the entire study period was Quercus, which attained the 59.8%. Regarding the herbaceous pollen taxa, the most abundant was Poaceae 13.2%. Other pollen types present in the Park's atmosphere were *Olea*, *Plantago*, *Pinus*, *Rumex*, *Cistus*, Cupressaceae, Urticaceae, Chenopodiaceae, *Fraxinus*, *Urtica membranaceae* and Compositae.

The greatest concentrations in relation to the annual total were registered in spring (Tab. 2). Highest concentrations were obtained in April, accounting for 40.6% of the total average. Winter months were the months with the lowest pollen values, frequently including days with an absence of atmospheric pollen.

A pollen calendar was prepared for the Hornachuelos National Park using the data obtained during the 6-year period. In relation to the order in which taxa appear in the atmosphere, the fist pollen grains in January correspond to Cupressaceae. Two peaks were recorded, the first in January, and the second in March. The temperature increase in the second fortnight of February favours the onset of the flowering of early spring tree taxa *Fraxinus*, *Morus* and *Quercus*. *Quercus* PPP lasted until June, with a second peak in May. The presence of *Cistus* pollen is remarkable, being the first time that this pollen type is registered in such as high concentration (117 gr/m^3 in the peak day of 1998). *Pinus* is the most abundant aloctonous tree species as a result of the reforestations carried out from the fifties onwards. This taxon has the main peak in April, and the PPP lasts until June. Olea pollination starts in April and lasts until June.

Herbaceous pollen grains appear in March and last until autumn. Urticaceae pollen is present during spring and summer. In the same period but in lower concentration,

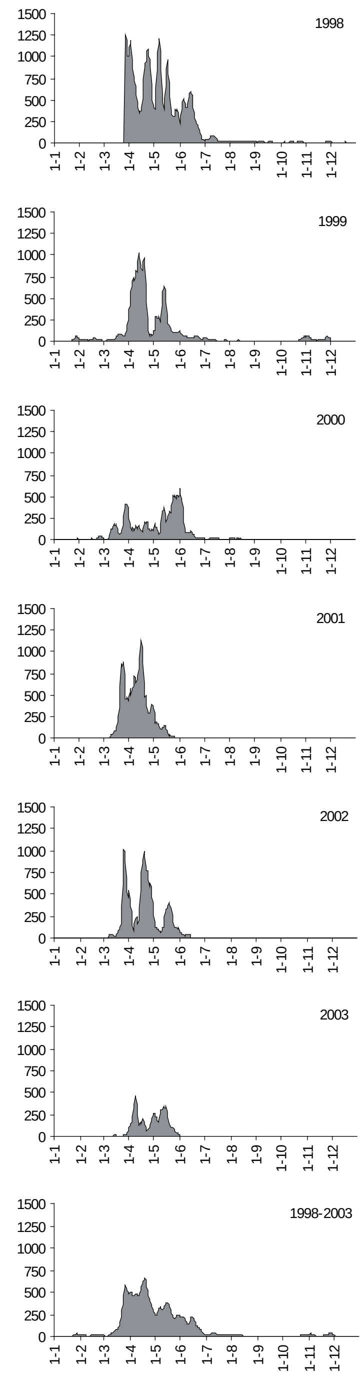

Figure 1. Annual variation of the pollen concentration and average of the six years, 1998-2003.

pollen from *Urtica membranaceae* was detected. *Plantago* pollen appears from March – August. A higher concentration was detected for Poaceae pollen which starts in May and finish in August. Rumex pollen last from March – August. Other allergic taxa found in the area came from the entomophilous species: *Echium*, Compositae, Ericaceae, Brassicaceae, *Tamarix* and Labiateae. Most of these taxa are rarely found in urban or rural aerobiological studies. Pollen from species located at a long distance was also

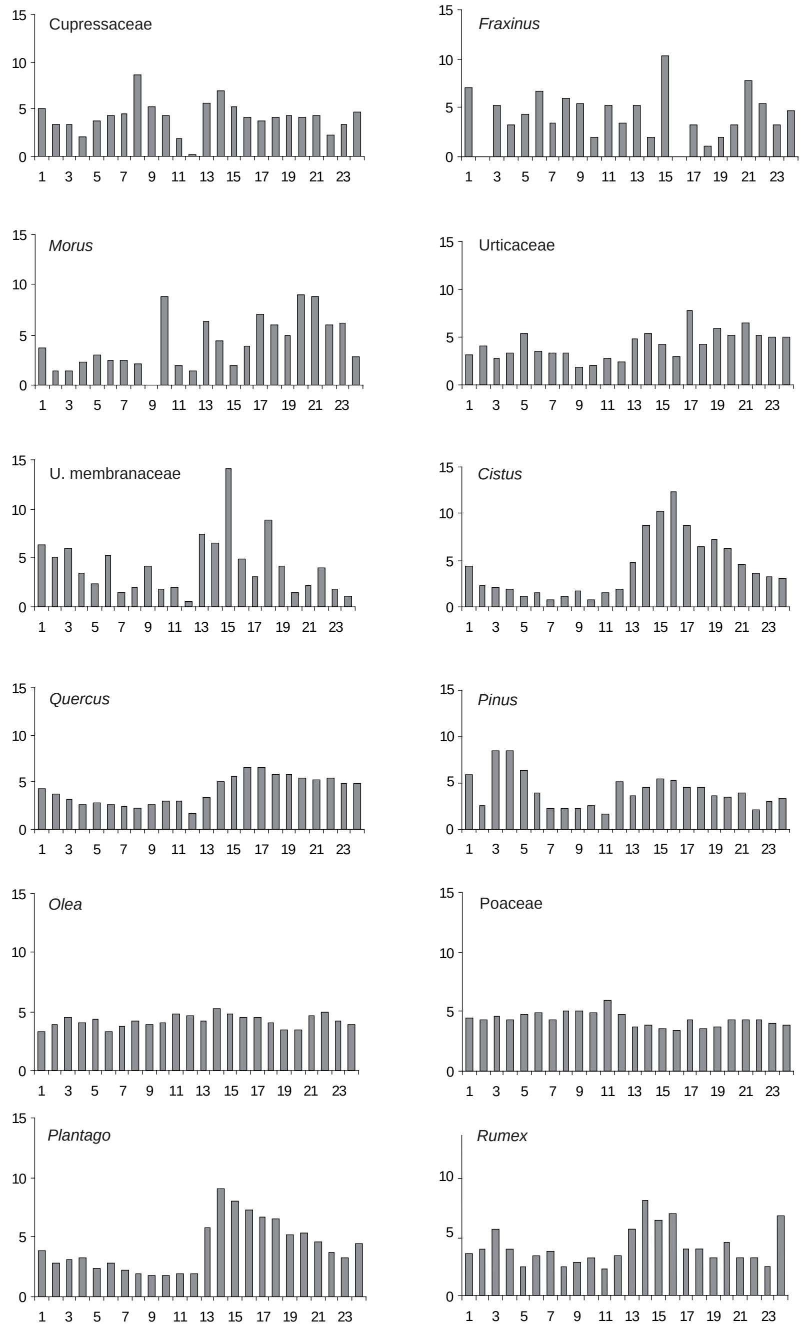

Figure 2. Intra-diurnal variation of the most important pollen types (average of study years, 1998-2003).

detected: *Castanea*, Myrtaceae, Cyperaceae, *Mercurialis*, *Artemisia* and *Salix*.

A study of the intra-daily variation of pollen (Fig. 2) reveals 2 behaviour patterns. The first is followed by tree/shrub species located near to the trap and shows a single well defined peak through the day, usually in the afternoon: *Quercus*, *Pinus*, *Cistus* and *Morus*. The rest of taxa present a non defined pattern except for *Plantago* which shows a clear peak in the afternoon. Poaceae and *Rumex* pollen presents a slight peak at midday.

Finally, the correlation between pollen concentration during the PPP and the main meteorological parameters (maximum, minimum and mean temperature; rainfall, relative humidity, and sun hours) was significant in a large number of cases (Tables 3 and 4). In view of the results of Table 3 that indicated negative influence of temperature and sun hours on pollen concentrations of several taxa, separate analyses of the Pre-Peak period (PP) were performed (Tab. 4). The parameters that exerted the greatest positive influence on the Hornachuelos Natural Park were

Table 3. Coefficients of correlation between pollen concentration of the Principal Pollen Period (PPP) and main meteorological parameters by using Spearman correlation test (*95%, **99% of signification).

	Cupress.	*Fraxinus*	*Morus*	Urticaceae	U. mem.	*Cistus*	*Quercus*	*Pinus*	*Olea*	Poaceae	*Plantago*	*Rumex*
Mean T°	0.201	0.402*	0.612**	0.054	0.085	0.435**	-0.268**	-0.028	0.047	0.248**	-0.112	0.181**
Max T°	0.230*	0.507**	0.594**	0.033	0.115	0.409**	-0.248**	0.016	0.016	0.238**	- 0.091	0.173**
Min T°	0.224*	0.279	0.459**	0.101*	-0.001	0.325**	-0.315**	-0.077	0.076	0.219**	0.138*	0.183**
Rainfall	-0.034	-0.271	-0.288*	-0.142**	-0.207**	-0.302**	-0.056	-0.041	0.055	-0.161**	-0.056	-0.113
Humidity	0.064466	0.377*	-0.461**	-0.027	-0.129*	-0.450**	0.228**	-0.081	0.026	-0.216**	0.004	-0.078
Sun hours	0.306284	0.256	0.321	0.023	0.028	0.062	-0.351**	0.265	0.002	0.508**	0.031	0.465**

Table 4. Coefficients of correlation between pollen concentration of the Pre-Peak period and main meteorological parameters by using Spearman correlation test (*95%, **99% of signification).

	Urtica-ceae	*Quercus*	*Pinus*	*Olea*	Poaceae	*Plantago*
Mean T°	0.197**	0.209*	-0.021	0.319**	0.471**	0.022
Max T°	0.227**	0.208*	0.033	0.267**	0.433**	0.040
Min T°	0.110	0.182*	-0.044	0.371**	0.427**	-0.009
Rainfall	-0.249**	-0.182*	-0.050	0.041	-0.168**	-0.165*
Humidity	-0.302**	-0.169*	-0.331*	-0.335**	-0.351**	-0.032
Sun hours	0.067	0.340	0.226	0.197	0.562**	0.165

mean and maximum temperature, while the greatest negative influence was exerted by humidity and rainfall.

DISCUSSION

During the study period, an average of 37,000 pollen grains per year was attained, which is lower than the value recorded in other urban areas of southern Spain [5, 10, 14, 33]. Nevertheless, recorded concentrations are far higher than those attained in rural and urban areas located in other Spanish bioclimatic regions [2, 6, 21, 35, 38].

During the year, pollen concentration in the area gradually increased, starting from February, and attaining the maximum peak in April due to the high concentration of *Quercus* pollen. Pollen spectrum of the study area notably differed from those of southern Spain where *Olea europaea* traditionally dominates the spring atmosphere. Results differ from nearby areas such as Cordoba, Seville, Jaen and Granada, where *Olea* is the predominant pollen type which reaches the peak in May [11].

Quercus pollen is a main allergen source in the northern hemisphere, with cross-reactivity occurring between pollen within the same order [25, 26]. Several studies consider *Quercus* pollen type responsible of allergy in areas with abundant *Quercus* vegetation [23, 36, 37]. Poaceae pollen is detected in the study area in higher concentration than in urban southern areas. The high allergenic prevalence of this taxon and the presence of great concentration of *Quercus* and Poaceae pollen enable us to indicate pasture and mixed forest-pasture areas of southern Spain as high risk areas for allergy sufferers in spring and summer.

Entomophilous pollen types, rarely described in aerobiological studies performed in Europe, were detected in the area. *Cistus* pollen, in spite of its high enthomophilous character, was detected in the highest concentration ever described [20, 34]. Other entomophilous taxa, such as Compositae, Ericaceae, *Tamarix* and *Echium* were frequent in the atmosphere of the natural studied area but not in European urban atmospheres. The abundance of individuals of this genus in the surroundings of the trap and the absence of buildings may favour the dispersion of these pollen types. All these pollen types have been described as allergenic by different authors [19, 22, 24, 32]. Therefore, more attention must be paid to these taxa, frequently obviated by allergologists in Europe, especially in managing of allergy problems in natural and rural areas.

Long distance transport phenomena were registered during the study. Pollen from *Castanea*, Myrtaceae, Cyperaceae, *Mercurialis*, *Artemisia* and *Salix* was detected. Populations of this species are located far of the trap. The nearest *Castanea* populations were recorded 200 km away in the Natural Parks of Sierra Norte (Sevilla) and Sierra Aracena (Huelva) [18]. This is supported by the medium and long distance transportation capacity of *Castanea* pollen highlighted by several authors [13, 30].

The study of the intra-diurnal pollen variation shows 2 different patterns. In the case of *Quercus*, *Pinus*, *Cistus* and *Morus*, a single well defined peak could be influenced by the fact that it is the only species responsible for pollen emission. On the other hand, taxas located far of the trap, *Olea*, or composed of many species in the area, Poaceae, showed a smother pattern. Norris-Hill [27] also detected differences between the Poaceae intra-diurnal pattern of a rural and an urban area of Wales in the United Kingdom, due to the proximity of pasture areas to the rural trap, and the wider species spectrum in those areas. Comparing the hourly pattern with the results found by Galan *et al.* [15] in the city of Cordoba, 150 km away, we have noted many differences. Galan *et al.* [15] indicated an evening peak for *Quercus*, probably due to the distance from the city to the holm-oak populations. These authors detected only one midday peak, the Cupressaceae curve. In Hornachuelos area two peaks were detected, one at midday and other in the morning. The presence of a spring flowering autochthonous species, *Juniperus oxicedrus* L., could explain this fact. In the case of Fraxinus, the presence in the area of individuals of *Phillirea angustifolia* could also explain the different pattern from the Cordoba city study.

The oscillation in pollen concentrations through the study period were largely determined by meteorological variations. In relation to temperature, positive significant values were obtained in the PPP of Cupressaceae, *Fraxinus*, *Morus*, *Cistus*, *Poaceae* and *Rumex*. It was noticeable that daily temperature variables showed positive coefficients in the Pre-Peak analysis for taxa with long Pollen Periods, *Quercus*, *Olea*, whereas previous results for the whole season displayed negative or non-significant coefficients. The high length of the pollen season makes coincident the increase in temperature in late spring/early summer with the end of the season, when decreasing pollen concentrations were recorded due to the intrinsic phenology of the plants. Due to this fact, different meteorological factors influence in the 2 pollination periods. Results showed that different meteorological factors affect the two periods. In the Pre-Peak period, temperature variables positively affect the pollen liberation, whereas in the After-Peak period, humidity influences the atmospheric dynamics of the pollen already liberated.

Studies have demonstrated that urbanization and high levels of vehicle emissions and westernized lifestyle are correlated with the increasing frequency of pollen-induced respiratory allergy in urban areas [9]. People who live in cities tend to be more affected by pollen-induced respiratory allergy than those from rural areas. Nevertheless, different pollen spectrum and higher allergenic pollen concentration can provoke pollen allergy responses different from urban areas. Pollen grains from “natural” species may promote specific sensitization by modulating the allergenicity of airborne allergens. Several factors could influence new interactions in native vegetation areas, including the type of air pollutants, plant species, climatic factors, degree of airway sensitization, degree of exposure and hyper-responsiveness of exposed subjects who usually work outdoors.

CONCLUSIONS

The pollen spectrum of the Hornachuelos Natural Park differ greatly from other aerobiological studies in southern Spain.

Higher presence of *Quercus* and Poaceae atmospheric pollen enable us to indicate pasture and mixed forest-pasture areas of southern Spain as high risk areas to allergy sufferers in spring and summer.

New airborne pollen types, *Cistus*, *Echium*, Compositae, Ericaceae, Brassicaceae, Tamarix and Labiateae were registerd, all of them described as allergenic on humans.

Long distance events provoke the presence of *Castanea*, Myrtaceae, Cyperaceae, *Mercurialis*, *Artemisia* and *Salix* pollen.

The meteorological parameters that exerted a positive effect were maximum and mean temperatures. Humidity and rainfall exerted a negative influence during the pollen season period.

During the day, pollen from nearly trees present a peak at midday/afternoon. Pollen from herbaceous species comprising many species and species far located far away show a smoother pattern.

In natural vegetation areas, differences in the pollen spectrum, timing and concentration can influence new allergy interactions differing from those detected in surrounding urban areas.

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