

Grass Pollen, Aeroallergens, and Clinical Symptoms in Ciudad Real, Spain

F Feo Brito,¹ P Mur Gimeno,² J Carnés,³ E Fernández-Caldas,³ P Lara,¹ AM Alonso,¹ R García,¹ F Guerra⁴

¹Allergy Section, General Hospital, Ciudad Real, Spain

²Allergy Unit, Hospital Santa Bárbara, Puertollano, Spain

³Research & Development Department, Laboratory LETI, S.L., Madrid, Spain

⁴Department of Allergy and Pathology, Medical School Unit, Hospital Universitario Reina Sofía, Córdoba, Spain

■ Abstract

Background: In allergic individuals, onset of symptoms is related to atmospheric pollen grain counts and aeroallergen concentrations. However, this relationship is not always clear.

Objectives: To analyze the correlation between grass pollen grain and aeroallergen concentrations in Ciudad Real, Spain, during the year 2004 and establish their association with symptoms in patients with allergic asthma, rhinitis, or both.

Methods: Two different samplers were used to assess allergen exposure: a Burkard spore trap to collect pollen grains and a high-volume air sampler to collect airborne particles. Individual filters were extracted daily in phosphate-buffered serum and analyzed by enzyme-linked immunosorbent assay based on serum containing high titers of specific immunoglobulin (Ig) E to grasses. The study population comprised 27 grass-allergic patients whose symptoms and medication were recorded daily.

Results: Grass pollens were detected between April 28 and July 18. There was a positive correlation between pollen grain counts and symptoms ($r=0.62$; $P>.001$). Grass aeroallergens were detected not only during the grass pollination period, but also before and after this period. There was also a very significant correlation between aeroallergen levels and symptoms ($r=0.76$; $P<.0001$). The threshold level for grass pollen was 35 grains/m³.

Conclusions: Grass-related allergenic activity is present throughout the year, demonstrating the existence of aeroallergens outside the pollen season. Symptoms in allergic patients may be related to airborne particle concentrations. This fact should be taken into account in the clinical follow-up and management of allergic patients.

Key words: Aeroallergen. Allergen quantification. Asthma. Grass. Grass threshold. Pollen count. Rhinitis.

■ Resumen

Antecedentes: En personas alérgicas los síntomas se relacionan con el recuento de pólenes atmosféricos. Sin embargo, no siempre es buena la correlación entre granos de polen, concentración de aeroalérgenos y síntomas.

Objetivos: Nuestros objetivos han sido analizar la correlación entre concentraciones de granos de polen y alérgenos de gramíneas durante el año 2004 en Ciudad Real (España), y establecer su asociación con los síntomas de pacientes con rinitis y/o asma.

Métodos: Se utilizaron dos tipos diferentes de captadores: un colector Burkard para cuantificar los granos de polen y un captador de partículas Air Sentinel para la recogida de aeroalérgenos. Los filtros fueron extraídos a diario e individualmente en tampón PBS y analizados mediante ELISA-inhibición por medio de un suero con IgE específica elevada para gramíneas. Se incluyeron 27 pacientes monosensibles a gramíneas, que diariamente registraron síntomas y fármacos consumidos.

Resultados: Los pólenes de gramíneas se detectaron exclusivamente entre el 28 de abril y el 18 de julio. Encontramos una correlación positiva entre granos de polen y síntomas ($r=0.62$, $P<0.0001$). Los alérgenos de gramíneas se detectaron no sólo en primavera sino también después de la estación polínica. La correlación entre aeroalérgenos y síntomas resultó muy significativa ($r=0.76$, $P<0.0001$). El umbral para polen de gramíneas se estableció en 35 granos/m³ de aire.

Conclusiones: La actividad alérgica de las gramíneas se expresa durante todo el año, demostrándose la presencia de alérgenos fuera de la estación polínica. Los síntomas de los pacientes pueden estar relacionados con los alérgenos de gramíneas. Esta posibilidad debía ser considerada en el seguimiento de los pacientes alérgicos a gramíneas.

Palabras clave: Aeroalérgenos. Asma. Cuantificación de alérgenos. Contajes de polen. Gramíneas. Rinitis. Umbral de gramíneas.

Introduction

In recent years, the number of patients suffering from rhinitis and allergic asthma has increased significantly, with an estimated prevalence close to 40% in older children [1]. There is considerable evidence that pollens are responsible for this increase, particularly in urban areas of the industrialized world [2,3]. The role of pollen grains as inducers of seasonal allergic rhinoconjunctivitis has been clearly demonstrated, but their implication in asthma remains controversial. Aerodynamic studies suggest that airborne particles larger than 10 μm in diameter, such as allergenic pollen grains, may be too large to enter small and medium-sized airways and cause asthma [4,5].

Onset of symptoms in sensitized patients correlates with atmospheric pollen grain counts, although this relationship is not always completely clear. Humidity or rain can cause the rupture of pollen grains, releasing hundreds of small starch particles into the air; these particles have high allergenic potential and are capable of entering the respiratory tract [6].

Since the seventies, when Busse et al [7] described the existence of allergenic particles in ragweed pollen grains, several authors have confirmed that not only pollen grains but also other parts of plants, such as leaves and stems, can be a source of allergenic particles [8-10]. The authors suggested that grass allergens can be found in the atmosphere outside the pollen season, thus explaining why the correlation between symptoms and pollen grain counts is not as strong as expected [11,12].

The objectives of this study were to analyze the correlation between grass pollen grain and aeroallergen levels during 2004 (January 1 to December 31) and to establish the relationship between those levels and symptoms in patients with allergic asthma, rhinitis, or both in the province of Ciudad Real, Spain.

Methods

Patient Population

The study population comprised patients with seasonal rhinitis, asthma, or both—onset of symptoms is mainly between April and June [13]—and who were screened at the allergy outpatient clinic of Hospital Santa Bárbara (Puertollano, Spain) and Hospital General Ciudad Real (Ciudad Real, Spain). The inclusion criteria were as follows: *a*) seasonal rhinitis and/or asthma of at least 2 years' duration based on the ARIA criteria [14] and GINA criteria [15]; *b*) monosensitization to grass pollen; *c*) minimum of 5 years' residence in the study area; and, *d*) age between 14 and 49 years. The exclusion criteria were as follows: *a*) perennial rhinitis and/or asthma; *b*) nasal polyposis; *c*) sensitization to pollens other than grass pollen; and *d*) inability to complete the questionnaire.

All patients included in the study were followed up during 2004, with particular emphasis given to onset of symptoms outside the pollen season.

Skin prick testing was performed with a battery of common aeroallergens consisting of standardized extracts of mites, pollens, moulds, and cat and dog dander (Laboratories ALK-

Abelló, Madrid, Spain). The inclusion criterion was a positive skin prick test result (>3 mm wheal diameter) to grass pollen extracts (*Lolium perenne*, *Dactylis glomerata*).

Symptoms and Medication Diary Cards

All patients were given diary cards on which they recorded their daily consumption of antiallergic medication, and their conjunctival, nasal, and bronchial symptom scores according to the following scale [16]: 0, no symptoms; 1, mild symptoms (slight nasal obstruction, slightly runny nose, or occasional sneezing or itching of the eyes); 2, moderate symptoms (moderate nasal obstruction, moderately runny nose, some sneezing and congestion, some ocular itching, or mild asthma); 3, severe symptoms (complete nasal obstruction, almost continuously runny nose, frequent sneezing or ocular symptoms, or asthma attacks). Graded symptoms were summarized with a weighted score for the drugs used as follows: 0, no drugs; 1, oral antihistamines, β_2 -adrenergic agonists, or both; 2, nasal or bronchial corticosteroids; 3, systemic corticosteroids. Of the 418 patients screened, we enrolled 27 grass-allergic patients during the study period.

Pollen Counts

Pollen grain counts were measured using a Burkard spore-trap (Burkard Manufacturing Co., Rickmansworth, Herts, UK) placed at a height of 15 m in Ciudad Real during the sampling period (January 1 to December 31, 2004). The sampling airflow rate was 10 L/min, and the size of the orifice of the spore-trap was 14 \times 2 mm. Pollens were caught on 24-mm wide transparent tape coated with a thin film of petroleum jelly. This tape was mounted on a cylinder rotating at a speed of 2 mm/h. To study the pollens caught over a 24-hour period, a 48-mm sweep (2 mm/h \times 24 h) was performed with the oil-immersion lens (Prior Scientific Instruments Ltd., Cambridge, UK) (\times 10 ocular, \times 100 objective, and field diameter of 0.18 mm) (Figure 1).

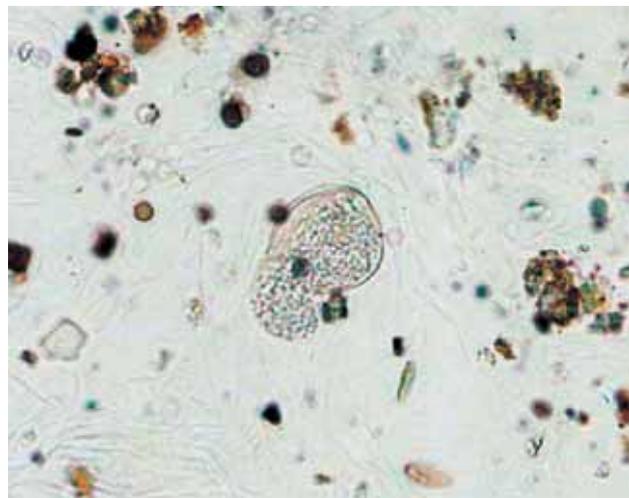


Figure 1. Light microscope micrograph of a grass pollen grain in which the exine has ruptured due to the effect of atmospheric humidity, thus releasing a large quantity of starch grains.

Air Sampling

A volumetric air sampler (Air Sentinel, Quan-Tec-Air Inc., Rochester, Minnesota, USA) adapted for outdoor use [17] was used for aeroallergen collection and run continuously during 2004. The sampler was placed 5 m above street level in the center of Ciudad Real. The airflow rate was 10 m³/h. Airborne particles were collected by polytetrafluoroethylene (PTFE) filters (Quan-Tec-Air Inc.), which are 99.9% efficient at 0.3 µm. The sampling time for each filter was 24 hours, that is, 240 m³ of air per sample. Filters were replaced at approximately the same time each day. After extraction, the filters were sealed in plastic bags and stored at 4°C.

Filter Extraction and Allergen Quantification

A total of 366 PTFE filter membranes were individually placed in tubes containing 0.5 mL of 0.01M phosphate-buffered saline (PBS). Each tube was stirred until the filter was completely wetted before being left overnight in a rotary mixer (Labinco B.V., Breda, The Netherlands) at 4°C. Samples were then centrifuged and the supernatant was collected and processed immediately to avoid loss of allergenic activity.

Total allergenic activity and allergen content were measured in eluted samples using validated enzyme-linked immunosorbent assays. A standard grass mixture extract containing *Phleum pratense*, *D. glomerata*, *Festuca elatior*, *L. perenne*, and *Poa pratensis* was used to coat flat-bottomed microtiter plates (Immulon IV, Chantilly, Virginia, USA) at a final concentration of 1 µg/well. The plates were incubated overnight at room temperature.

Samples were taken from the filter and several dilutions were made of the grass extract. All dilutions were incubated for 2 hours at room temperature with pooled serum obtained from allergic patients who were monosensitized to grasses and had high titers of specific immunoglobulin (Ig) E to grass pollen allergens (dilution 1/10). One positive and several negative controls were also included. The dilutions were then transferred to the grass-coated microplates and incubated overnight. After washing, 100 µL of peroxidase-labelled antihuman IgE (Ingenasa, Madrid, Spain) was added and the preparation was allowed to stand for 30 minutes at room temperature. After a second washing step, the plates were developed for 30 minutes and stopped with 1N H₂SO₄.

The percentage of inhibition was calculated using the reference inhibition curve determined from the standard grass mixture. Allergen levels were extrapolated using the standard curve and based on the inhibition capacity; the final results were expressed in µg of allergen/mL.

We defined the threshold level for grass pollen and grass allergen concentrations as the level at which participants who were clinically sensitive to grass pollen developed symptoms (minimum symptom score of 1) [18].

Meteorological Data

Detailed daily records of temperature, humidity, and wind speed and direction were obtained from the meteorological observatory in Ciudad Real (39° N, 41° E, 630 m above sea level).

Statistical Analysis

Correlations between symptoms, pollen grain concentrations, and aeroallergen levels were analyzed using the Spearman rank correlation and multiple linear regression. Correlation coefficients and *P* values were obtained for each analysis. Two analyses were performed, the first using data obtained throughout the year, and the second using only data from the pollen season (April 28 to July 18).

Multiple linear regression analysis was used to determine the relationship between atmospheric variable—rainfall, humidity, and temperature—and the pollen and aeroallergen levels.

Results

Study Population

The study population comprised 27 patients (13 men and 14 women) with a mean (SD) age of 27.7 (11.3) years (range, 10–51 years). They were all sensitized to grass pollen and had allergic upper respiratory tract symptoms. All patients suffered from rhinoconjunctivitis (mild rhinitis in only 5 patients) and 15 (55.6%) suffered from asthma (5 patients with uncontrolled asthma, 8 with partially controlled asthma, and 2 with controlled asthma) (Table 1).

All patients experienced symptoms during the pollen season, although 5 patients (numbers 1, 6, 10, 16, and 22) experienced symptoms outside the pollen season (mainly October and November) and these symptoms were almost always severe (4/5). January, February, and December were the months with the lowest numbers of symptomatic patients (Table 2).

Pollen Counts

In 2004, the principal grass pollination period ran from April 28 to July 18. The peak grass pollen concentration was detected on June 5, with 585 grains/m³. The total amount of grass pollen grains documented for the whole year was 4116 grains. No grass pollen grains were detected during the rest of the year.

The threshold level was 35 grains/m³ for grass pollen and 18 ng/m³ for grass allergens.

Quantification of Aeroallergens

Variable amounts of aeroallergens were detected throughout the year. The maximum values coincided with the pollen season; the highest count (62 ng/m³) was detected on May 22. Large quantities of grass aeroallergens were detected before and after the pollen season, particularly in autumn.

Correlations Between Symptoms, Pollen Counts, and Aeroallergen Levels

A moderate correlation was found between pollen grain and airborne aeroallergen concentrations ($r=0.52$, $P<.001$) and a positive correlation was found between the presence of symptoms and pollen grains ($r=0.62$, $P<.0001$). Grass pollen

Table 1. Characteristics of the 27 Monosensitized Patients

Patient No.	Age, y	Sex	Symptoms	Classification
1 ^a	43	F	R, C	SPR
2	28	F	R, C	SIR
3	21	M	R, C, A	PCA
4	35	F	R, C	MPR
5	34	F	R, C	MIR
6 ^a	10	M	R, C, A	UA
7	27	F	R, C	MPR
8	16	F	R, C, A	CA
9	11	M	R, C, A	PCA
10 ^a	36	F	R, C, A	UA
11	49	M	R, C, A	UA
12	35	F	R, C	MPR
13	14	M	R, C, A	PCA
14	17	F	R, C, A	PCA
15	30	M	R, C, A	CA
16 [*]	25	F	R, C	SPR
17	47	M	R, C, A	PCA
18	15	M	R, C	SPR
19	51	F	R, C	SPR
20	33	M	R, C, A	PCA
21	24	F	R, C	SPR
22 [*]	23	M	R, C, A	PCA
23	18	M	R, C, A	PCA
24	26	F	R, C	SPR
25	19	F	R, C	MPR
26	29	M	R, C, A	UA
27	31	M	R, C, A	UA

Abbreviations: A, asthma; C, conjunctivitis; CA, controlled asthma; MIR, mild-intermittent rhinitis; MPR, mild-persistent rhinitis; R, rhinitis; SIR, moderate-severe intermittent rhinitis; SPR, moderate-severe persistent rhinitis.
^aPatients with symptoms outside the pollen season.

Table 2. Grass Pollen Grains, Aeroallergens, and Symptoms in Each Month of the Year 2004.

Month	Pollen, Grains/m ³ , Range	Aeroallergen Levels, µg/m ³ , Range	Patients With Symptoms No. (%)	Mean Symptom Score	Maximum Number of Days With Symptoms
January	0-2	0-0.0064	2 (7.5%)	0.04	25
February	0-2	0-0.0017	2 (7.5%)	0.03	6
March	0-9	0	5 (18.5%)	0.07	31
April	0-19	0-0.0006	18 (66.7%)	0.38	30
May	0-330	0.009-0.064	27 (100%)	1.12	31
June	1-585	0.004-0.04	27 (100%)	0.67	30
July	0-18	0.004-0.016	6 (22.2%)	0.04	11
August	0-3	0-0.002	6 (22.2%)	0.07	19
September	0-5	0-0.0018	6 (22.2%)	0.11	30
October	0	0.0001-0.027	5 (18.5%)	0.09	23
November	0	0-0.017	5 (18.5%)	0.05	23
December	0	0-0.012	2 (7.5%)	0.01	4

grains were only detected during spring and early summer. However, some patients reported symptoms all year round, particularly in summer and autumn (Figure 2).

A higher level of significance was obtained when symptoms were correlated with grass aeroallergen levels ($r=0.76$, $P<.0001$) (Figure 3).

Multiple linear regression analysis for the whole year revealed significant differences between presence of symptoms and pollen grains ($P<.001$), aeroallergens ($P<.001$), and wind speed ($P<.01$). When this analysis was restricted to the pollen season (April 28 to July 18), differences were only detected between presence of symptoms and pollen grains ($P<.001$).

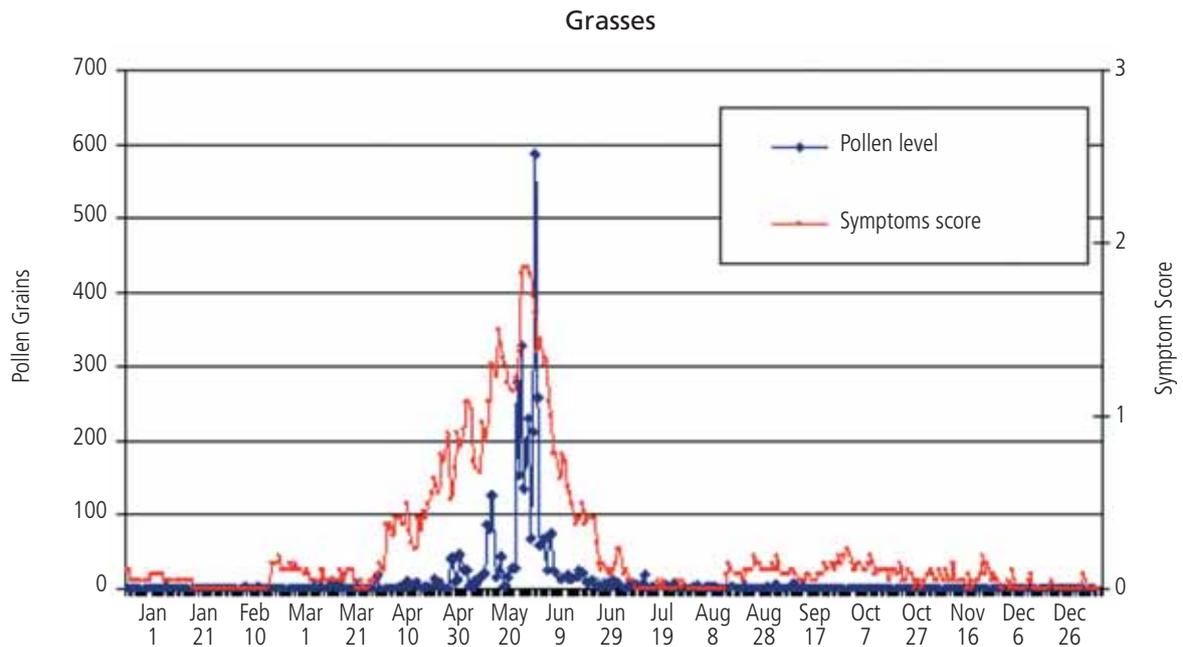


Figure 2. Correlation between pollen levels and symptoms in the study population ($r=0.62$).

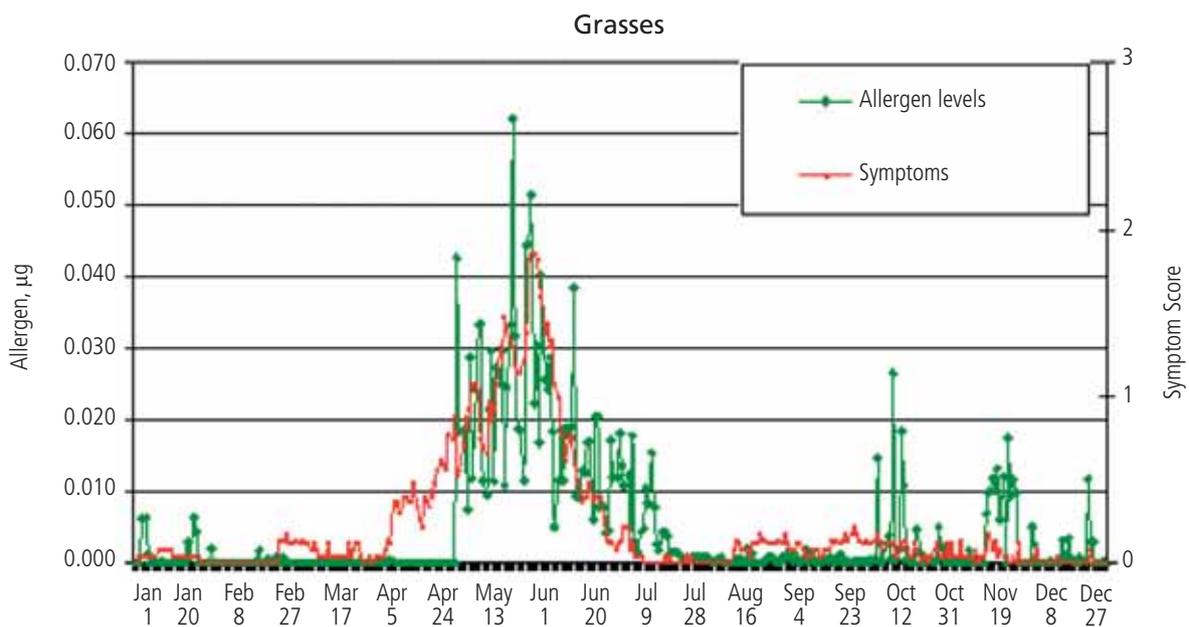


Figure 3. Correlation between aeroallergen levels and symptoms in the study population ($r=0.76$).

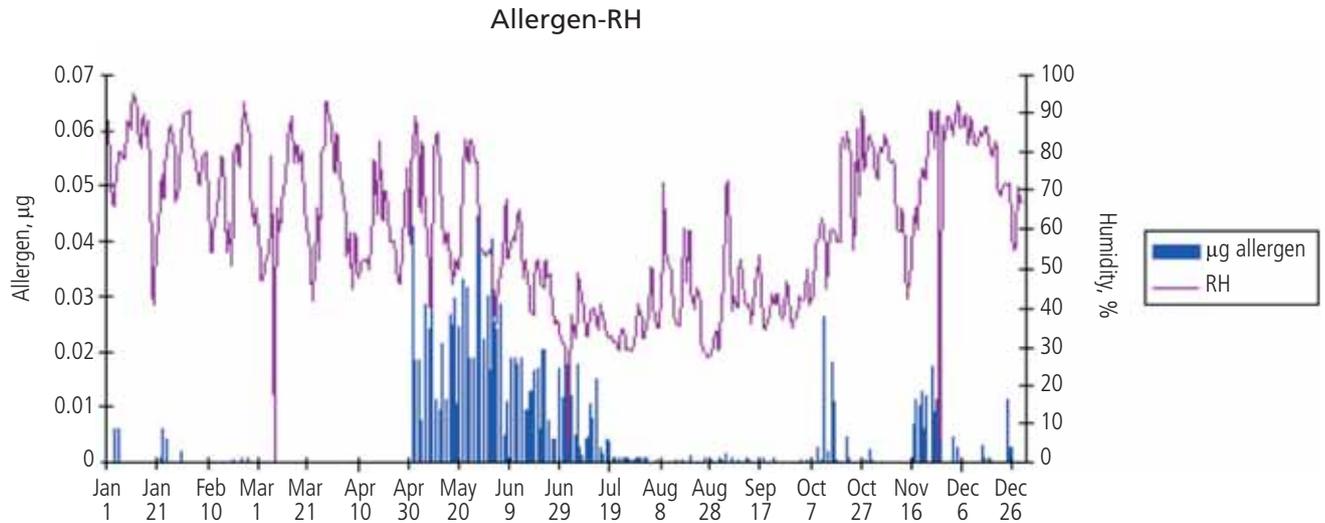


Figure 4. Correlation between allergens and relative humidity.

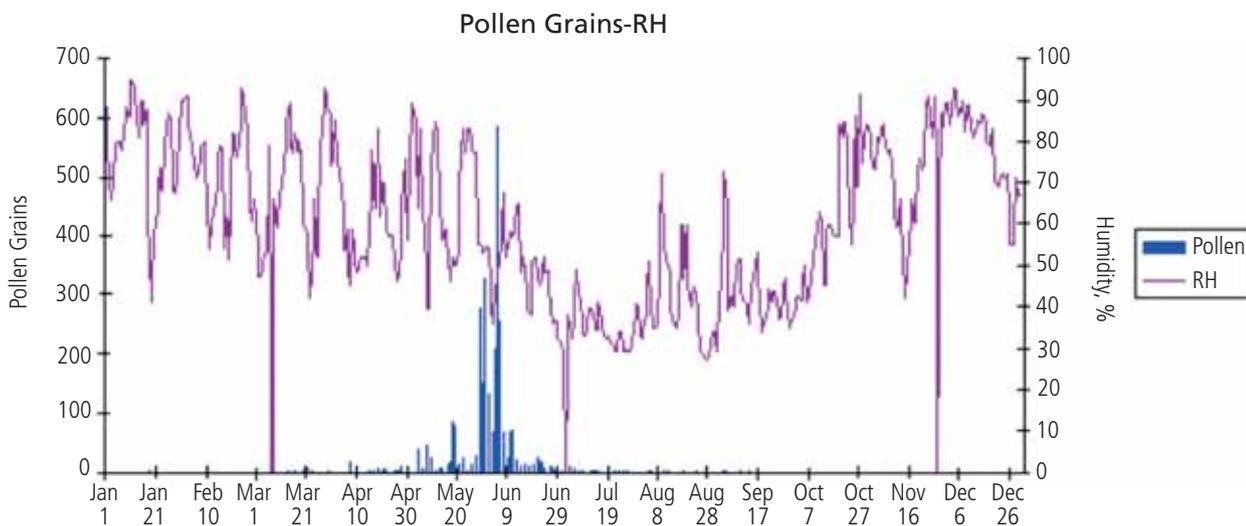


Figure 5. Correlation between pollen grains and relative humidity.

and aeroallergens ($P < .001$). When symptoms were compared with temperature and humidity, P values of .075 and .083, respectively, were obtained.

Correlation Between Meteorological Variables, Allergen Levels, and Pollen Counts

Multiple linear regression analysis for the whole year revealed significant differences between allergen or pollen content and temperature ($P < .001$) and humidity ($P < .001$) (Figures 4 and 5). Other variables, such as wind speed or rainfall, showed no significant differences.

Discussion

Ciudad Real is a city in southern central Spain (190 km south of Madrid), where olive and grasses are the sources of the most relevant pollen allergens. Ciudad Real is situated in a windy area and has a continental climate, with hot, dry summers and cold winters. Rainfall is low and occurs mainly during autumn. The mean annual values of grass pollen grains between 1987 and 2004 were 2913 grains/m³, and more than 71% of patients with symptoms of allergy are sensitized to grasses. Olive and Chenopodiaceae are also responsible for a large number of sensitizations. Most patients are sensitized to all 3 allergens,

and only 3% of the population is sensitized to grasses only [13]. Although pollen grains are responsible for sensitization in patients living in areas where this allergen is prevalent, our experience suggests that, in some cases, these symptoms occur not only during the pollen season, but also during the rest of the year, even when pollen grains are not detected in pollen counts.

Several investigators have compared pollen counts with aeroallergen levels. Fernández-Caldas et al [19], Rantio-Lehtimäki et al [20], and Cabrera et al [21] analyzed red oak, birch, and grass pollen allergens, respectively. They concluded that pollen counts and pollen aeroallergen activity were not closely correlated and detected allergenic activity when pollen grains were absent, both before and after the pollen season. These studies evaluated aerobiological parameters only, and did not discuss the clinical implications of these findings. However, D'Amato et al [16] investigated symptoms during the pollen season in Naples (123 days from May 1 to August 31) and compared the results with those for *Parietaria* pollen grain and pollen aeroallergen levels. They found a significant correlation between both parameters and the symptom scores of allergic patients.

In the present study, analysis of the atmosphere in Ciudad Real over the whole of 2004 enabled us to detect grass aeroallergens outside the pollen season. The original finding of this study is that the presence of symptoms correlated with pollens and aeroallergen concentrations, thus providing a clinical profile that has not been analyzed elsewhere. Our results demonstrate that the highest levels of pollen grains and aeroallergens coincided with the pollen season, thus revealing a very significant correlation between both parameters. However, we also detected allergenic particles outside the pollen season. The values were particularly high in autumn and early winter, although a significant positive correlation was obtained throughout the year. As the clinical association is much closer with aeroallergens than with pollen grains, we believe that aeroallergen levels should be established as the reference value for the follow-up of grass-allergic patients. Our hypothesis is that pollen grains can rupture and release particles that enter the smaller airways, thus inducing symptoms during periods when pollen grains are absent. The role of nonvegetative parts of the plant cannot be ruled out. These aeroallergens remain airborne for a long period, and are probably bound to different particles in the air or in the plant. According to this hypothesis, some vegetable organs or plant structures could act as sources.

Grass pollen levels in Ciudad Real show marked variability, and almost 4-fold interannual differences have been recorded (1200 grains in 1995 and 4500 grains in 1996) [13]. The high pollen counts detected in 2004 induced severe symptoms during the pollen season in 63% of the patients evaluated. Furthermore, 5 patients experienced symptoms not only during the pollination period, but also during the months of October and November, coinciding with the presence of grass aeroallergens. These patients presented a more severe clinical condition, suggesting that their threshold for onset of symptoms was lower than in the rest of the patients. The symptoms of these patients could be attributed to cross-reactivity with other pollens present in the atmosphere at that time. However, a cross-reactivity study between grass (*Trisetum paniceum*) and *Chenopodium album*, *Olea europaea*, and *Platanus hybrida* confirmed that none of the

latter 3 pollen extracts reached 50% inhibition, whereas grass (*T. paniceum*) reached 80% inhibition at a concentration that was approximately 10 times lower [21]. More than 30 years ago, Davis and Smith [18] established the threshold level of grass pollen grains for onset of symptoms as 50 grains/m³.

The amount of pollen collected in different cities worldwide has not increased in recent decades. However, the prevalence and severity of allergic rhinitis and allergic asthma have grown substantially [22]. One possible cause is air pollution, which could increase the allergenicity of pollen grains [23,24]. Accordingly, a smaller quantity of pollen grains would be needed to induce respiratory symptoms. Erbas et al [25] demonstrated that a concentration of 30 grass pollen grains/m³ was sufficient to induce symptoms in most grass-allergic asthmatics in Melbourne, Australia. These findings are consistent with ours, as all our patients experienced symptoms of allergic rhinitis or asthma at a level of 35 grains/m³ (grass pollen) or 1.84 µg/m³ (grass aeroallergens).

Although a low-level relationship between asthma symptoms and the effect of weather has been posited for some populations [26], the influence of meteorological conditions on pollen transport has not been well documented. Previous studies have suggested that relative humidity and atmospheric pressure are the most important meteorological variables in admissions due to asthma among adults [27] and even among children [28], and other studies have implicated fog or rain [29]. We found that higher temperature and humidity increased pollen counts and allergen levels. Both pollen grains and allergen content are directly associated with symptoms, suggesting a cascade reaction in which temperature and humidity stimulate the release of pollen grains and, consequently, allergen particles into the air; both of these particles are responsible for more severe symptoms. The relationship between symptoms and atmospheric variables was not statistically significant ($P=.075$ and $P=.083$), probably due to the small sample size.

In summary, we demonstrated the presence of aeroallergen particles in the atmosphere throughout the year. Although the highest levels of aeroallergens are detected during the pollen season and these peaks coincide with those of pollen grains, a significant aeroallergen concentration can be detected during early summer and even into the autumn, suggesting that these particles are in the environment for a long period. Our clinical data allow us to conclude that the presence of aeroallergens is sufficient to induce symptoms. Aerobiological information systems based on pollen and spore counts should therefore be supplemented with information on aeroallergens. Further studies must be conducted in order to determine the threshold of sensitization or the capacity to stimulate an allergic response.

Acknowledgments

Financial support for this project was provided by a grant from the Castille-La Mancha Institute of Health Sciences (GC 04010).

We would like to thank Pascual Roman Duran Lorente, Head of the Meteorological Office in Ciudad Real, for his valued collaboration in supplying the climatic data for our region.

References

1. Asher MI, Montefort S, Björkstén B, Lai CKW, Strachan DP, Weiland SK, Williams H. Worldwide time trends in the prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and eczema in childhood: ISAAC Phases One and Three repeat multicountry cross-sectional surveys. *Lancet*. 2006;368:733-43.
2. D'Amato G, Cecchi L, Bonini S, Nunes C, Annesi-Maesano I, Behrendt H, Liccardi G, Popov T, van Cauwenberge P. Allergic pollen and pollen allergy in Europe. *Allergy*. 2007;62:976-90.
3. Eder W, Ege MJ, von Mutius E. The Asthma Epidemic. *N Engl J Med*. 2006;355:2226-35.
4. Wilson AF, Novey HS, Berke MD, Surprenant EL. Deposition of inhaled pollen and pollen extract in human airways. *N Engl J Med*. 1973;288:1056-88.
5. Hoehne JH, Reed CE. Where is the allergic reaction in ragweed asthma? *J Allergy Clin Immunol*. 1871;48:36-9.
6. Knox RB. Grass pollen, thunderstorms and asthma. *Clin Exp Allergy*. 1993;23:354-9.
7. Busse WW, Reed CE, Hoehne JH. Where is the allergic reaction in ragweed asthma? Demonstration of ragweed antigen in airborne particles smaller than pollen. *J Allergy Clin Immunol*. 1972;50:289-95.
8. D'Amato G, De Palma R, Verga A, Martucci P, Liccardi G, Lobefalo G. Antigenic activity of nonpollen parts (leaves and stems) of allergenic plants (*Parietaria judaica* and *Dactylis glomerata*). *Ann Allergy Asthma Immunol*. 1991;67:421-4.
9. Vinckier S, Smets E. The potential role of orbicules as vector of allergens. *Allergy*. 2001;56:1129-36.
10. Fernández-Caldas E, Bandele EO, Dunnette SL, Swanson MC, Reed CE. Rye grass group allergen content in leaves from seven different grass species. *Grana*. 1992;31:157-9.
11. Solomon WR. Airborne pollen: A brief life. *J Allergy Clin Immunol*. 2002;109:895-900.
12. Spieksma FThM, Kramps JA, van der Linden AC, Nikkels BH, Plomo A, Koerten HK, Dijkman JH. Evidence of grass-pollen allergenic activity in the smaller micronic atmospheric aerosol fraction. *Clin Exp Allergy*. 1990;20:273-80.
13. Feo Brito F, Galindo PA, García R, Gómez E, Fernández F, Fernández-Pacheco R, Delicado A. Pólenes alergénicos en Ciudad Real: Aerobiología e incidencia clínica. *Rev Esp Alergol Inmunol Clín*. 13:78-95.
14. Bousquet J, Reid J, van Weel C, Baena Cagnani C, Canonica GW, Demoly P, Denburg J, Fokkens WJ, Grouse L, Mullol K, Ohta K, Schermer T, Valovirta E, Zhong N, Zuberbier T. Allergic rhinitis management pocket reference 2008. *Allergy*. 2008;63:990-6.
15. van Weel C, Bateman ED, Bousquet J, Reid J, Grouse L, Schermer T, Valovirta E, Zhong N. Asthma management pocket reference 2008. *Allergy*. 2008;63:997-1004.
16. D'Amato G, Gentili M, Russo M, Mistrello G, Saggese M, Liccardi G, Falagiani P. Detection of *Parietaria judaica* airborne allergenic activity: comparison between immunochemical and morphological methods including clinical evaluation. *Clin Exp Allergy*. 1994;24:566-74.
17. Swanson MC, Agarwal MK, Reed CE. An immunochemical approach to indoor aeroallergen quantitation with a new volumetric air sampler: studies with mite, roach, cat, mouse, and guinea pig antigens. *J Allergy Clin Immunol*. 1985;76:724-9.
18. Davies RR, Smith LP. Forecasting the start and severity of the hay fever season. *Clin Allergy*. 1973;3:263-7.
19. Fernández-Caldas E, Swanson MC, Pravda J, Welsh P, Yunginger JW, Reed CE. Immunochemical demonstration of red oak pollen aeroallergens outside of the oak pollination season. *Grana*. 1989;28:205-9.
20. Rantio-Lehtimäki A, Viander M, Koivikko A. Airborne birch pollen antigens in different particles sizes. *Clin Exp Allergy*. 1994;24:23-8.
21. Cabrera M, Martínez-Cóccera C, Fernández-Caldas E, Carnés Sánchez J, Boluda L, Tejada J, Subiza JL, Subiza J, Jerez M. *Trisetum paniceum* (Wild Oats) pollen counts and aeroallergens in the ambient air of Madrid, Spain. *Int Arch Allergy Immunol*. 2002;128:123-9.
22. D'Amato G, Liccardi G, D'Amato M, Cazzola M. The role of outdoor air pollution and climatic changes on the rising trends in respiratory allergy. *Respir Med*. 2001;95:606-11.
23. Knox RB, Suphioglu C, Taylor P, Desai R, Watson HC, Peng JL, Bursill LA. Major grass pollen allergen Lol p 1 binds to diesel exhaust particles: implications for asthma and air pollution. *Clin Exp Allergy*. 1997;27:246-51.
24. P Mur Gimeno, F Feo Brito, C Martínez, A Tobías, L Suárez, F Guerra, PA Galindo, E. Gómez. Decompensation of pollen-induced asthma in two towns with different pollution levels in La Mancha, Spain. *Clin Exp Allergy*. 2007;37:558-63.
25. Erbas B, Chang JH, Dharmage S, Ong EK, Hyndman R, Newbiggin E, Abramson M. Do levels of airborne grass pollen influence asthma hospital admissions? *Clin Exp Allergy*. 2007;37:1641-7.
26. Epton MJ, Martin IR, Graham P, Healy PA, Smith H, Balasubramaniam R, Harvey IC, Fountain DW, Hedley J, Town GI. Climate and aeroallergen levels in asthma: a 12 month prospective study. *Thorax*. 1997;52:528-34.
27. Qasem JA, Nasrallah H, Al-Khalaf BN, Al-Sharifi F, Al-Sherayfee A, Almathkouri SA, Al-Saraf H. Meteorological factors, aeroallergens and asthma-related visits in Kuwait: a 12-month retrospective study. *Ann Saudi Med*. 2008;28:435-41.
28. Priftis KN, Paliatatos AG, Panagiotopoulou-Gartagani P, Tapratzi-Potamianou P, Zachariadi-Xypolita A, Nicolaidou P, Saxoni-Papageorgiou P. Association of weather conditions with childhood admissions for wheezy bronchitis or asthma in Athens. *Respiration*. 2006;73:783-90.
29. Villeneuve PJ, Leech J, Bourque D. Frequency of emergency room visits for childhood asthma in Ottawa, Canada: the role of weather. *Int J Biometeorol*. 2005;50:48-56.

■ *Manuscript received August 25, 2009; accepted for publication October 26, 2009.*

■ Francisco Feo Brito

Sección de Alergología, Hospital General
C/ Tomelloso, s/n
13005 Ciudad Real, Spain
E-mail: feobrito@hotmail.com